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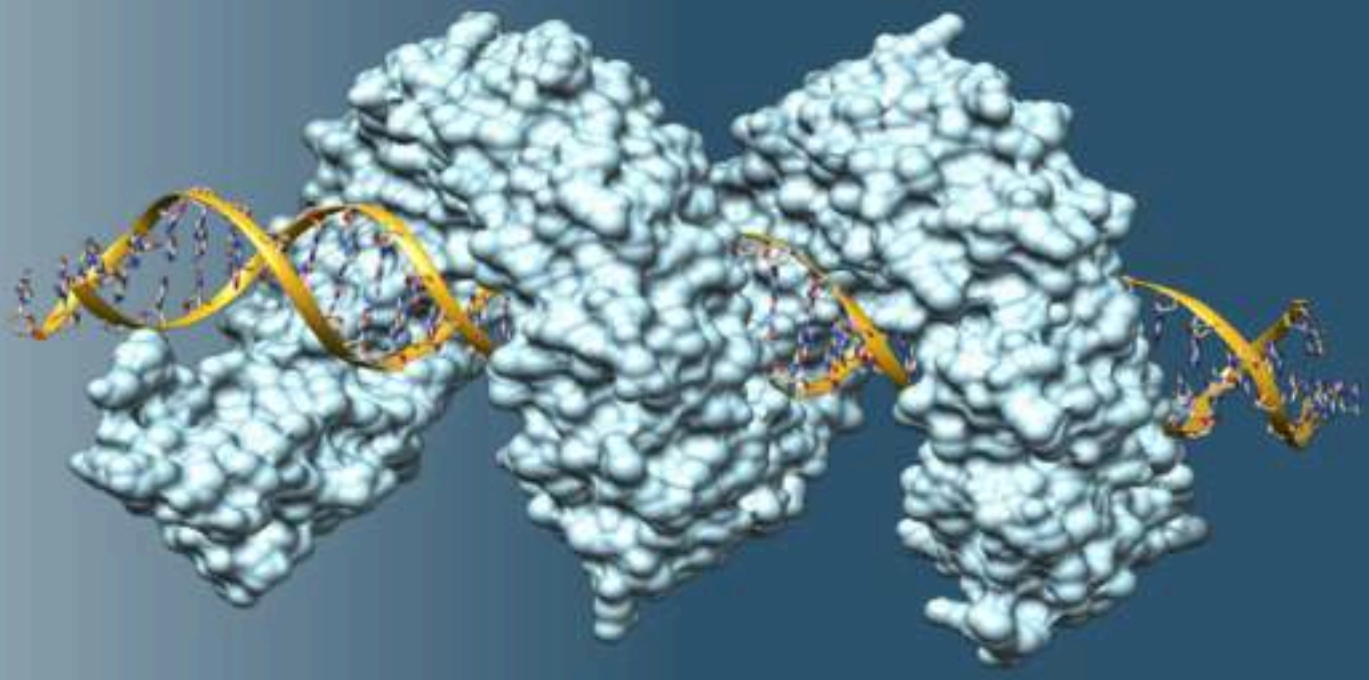
# BOOK OF ABSTRACT



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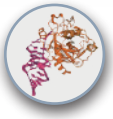
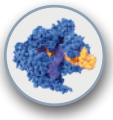


**Binding Parameters**
$k_{ON}$, $k_{OFF}$, $K_D$, avidity



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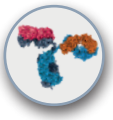

**Multi-specific Binders**
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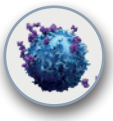
**Conformational Changes in Proteins**
Structure-activity-relationships



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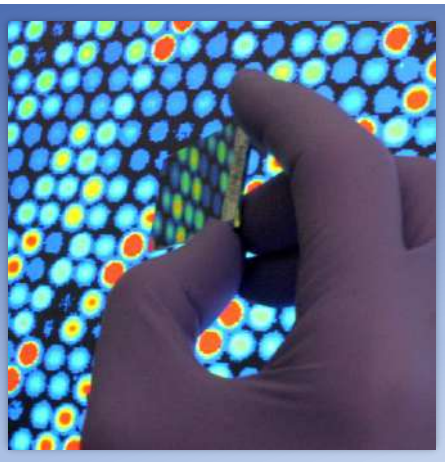

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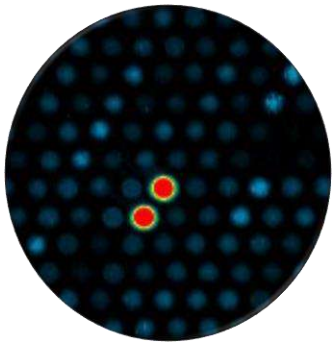

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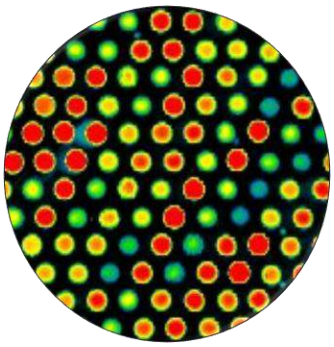

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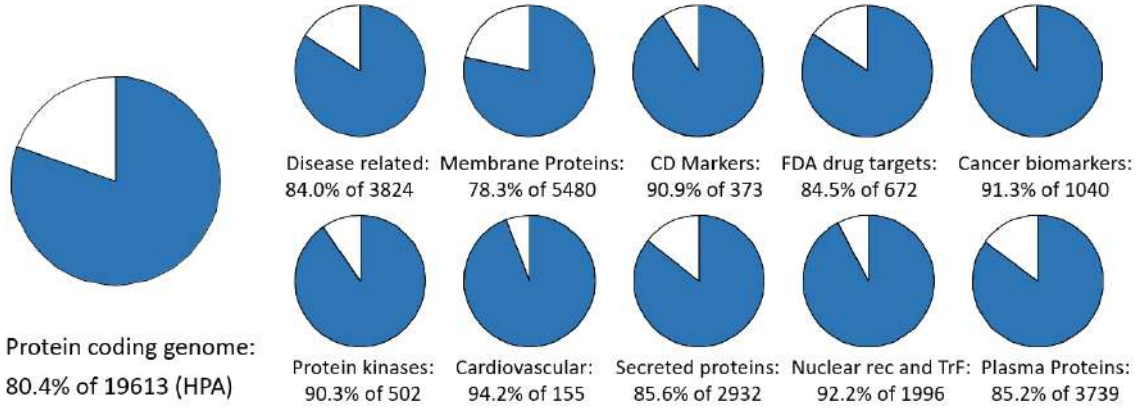


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# The Human Protein Atlas – implications for human biology, drug development and precision medicine

Tuesday, 22nd June - 13:15: Keynote 1 - Oral - Abstract ID: 51

***Prof. Mathias Uhlen*** [1]

***1.*** *KTH Science for Life Laboratory*

The Human Protein Atlas (HPA) program aims to map of all the human proteins in cells, tissues and organs using integration of various omics technologies, including proteomics, transcriptomics, antibody-based methods and AI-based systems biology. The current version (www.proteinatlas.org) consists of six separate parts and the database contains in total more than 20 million web pages with information on various aspect of the genome-wide analysis of the human proteins. All the data in the knowledge resource is open access to allow scientists both in academia and industry to freely access the data for exploration of the human proteome. The implications for the understanding of human biology in health and disease will be discussed with a focus on drug development and precision medicine.

*Selected publications:*

1. Uhlen et al (2015) “Tissue-based map of the human proteome” *Science* 347(6220):1260419.
2. Uhlen et al (2017) “A pathology atlas of the human cancer transcriptome” *Science* 357(6352): eaan2507
3. Uhlen et al (2019 “A genome-wide transcriptomic analysis of protein-coding genes in human blood cells” *Science* 366 (6472): eann9198
4. Sjöstedt et al (2020) “An atlas of the human, pig and mouse brain” *Science* 367 eaay5947
5. Mahdessian et al (2021) “Spatiotemporal dissection of the cell cycle with single-cell proteogenomics” Nature 590 (7847): 649-654.

# Large scale identification of SARS-CoV-2 motif based hijacking of human proteins

Tuesday, 22nd June - 13:45: Oral Session - Oral - Abstract ID: 85

***Ms. Caroline Benz*** [1], ***Dr. Thomas Kruse*** [2], ***Ms. Dimitriya Garvanska*** [2], ***Mr. Richard Lindqvist*** [3], ***Mr. Filip Mihalič*** [4], ***Dr. Fabian Coscia*** [2], ***Dr. Ravi Inturi*** [4], ***Dr. Ahmed Sayadi*** [1], ***Dr. Leandro Simonetti*** [1], ***Ms. Emma Nilsson*** [5], ***Dr. Muhammad Ali*** [1], ***Ms. Johanna Kliche*** [1], ***Ms. Ainhoa Moliner Morro*** [6], ***Prof. Andreas Mund*** [2], ***Mrs. Eva Andersson*** [4], ***Prof. Gerald McInerney*** [6], ***Prof. Matthias Mann*** [2], ***Dr. Norman Davey*** [7], ***Dr. Anna Överby*** [3], ***Prof. Jakob Nilsson*** [2], ***Prof. Ylva Ivarsson*** [1]

***1.*** *Department of Chemistry - BMC, Uppsala University, Box 576, Husargatan 3, 751 23 Uppsala, Sweden,* ***2.*** *The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Faculty of Health and Medical Sciences, Blegdamsvej 3B, 2200 Copenhagen, Denmark,* ***3.*** *Department of Clinical Microbiology, Umeå University, 90185 Umeå, Sweden,* ***4.*** *Department of Medical Biochemistry and Microbiology, Uppsala University, Box 582, Husargatan 3, 751 23 Uppsala, Sweden,* ***5.*** *Department of Clinical Microbiology, Umeå University, 90185 Umeå, Sweden,* ***6.*** *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden,* ***7.*** *Division of Cancer Biology, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK.*

The small proteomes of viruses make extensively use of short linear motif-based protein protein interaction to hijacking cellular process for their own purpose. We are using Proteomic Peptide Phage Display with a library representing intrinsically disordered regions of Sars-CoV2 and related human relevant species to identify attacked interactions. In the process we identified 269 peptide based interaction for 18 coronaviruses. One of those interaction is the human G3BP1/2 protein getting hijacked by a motif in the Sars-CoV2 nucleocapsid N protein (FxFG). This interaction supports viral replication by rewiring of endogenous G3BP1/2 interactions and influences stress granules formations. A peptide based inhibitor interacting with the same side has shown to block Sars-CoV2 infection which can be directly translated in a G3BP1/2 directed antiviral reagent

1. Kruse, T., Benz, C., Garvanska, D.H., Lindqvist, R., Mihalic, F., Coscia, F., Inturi, R., Sayadi, S., Simonetti, L., Nilsson, E., Ali, M., Kliche, J., Moliner Morro, A., Mund, A., Andersson, E., McInerney, G., Mann, M., Jemth, P., Davey, N. E., Överby, A. K. Nilsson, J. and Ivarsson Y. Large scale discovery of coronavirus-host factor protein interaction motifs reveals SARS-CoV-2 specific mechanisms and vulnerabilities. bioRxiv: https://doi.org/10.1101/2021.04.19.440086

# Novel 177Lu-Labeled PNA Probes for Affibody-Mediated PNA-Based Pretargeting

Tuesday, 22nd June - 14:00: Oral Session - Oral - Abstract ID: 108

***Dr. Kristina Westerlund*** [1], ***Ms. Hanna Tano*** [1], ***Ms. Maryam Oroujeni*** [2], ***Dr. Anzhelika Vorobyeva*** [2], ***Mr. Yongsheng Liu*** [2], ***Ms. Tianqi Xu*** [2], ***Dr. Daniel Vasconcelos*** [1], ***Prof. Anna Orlova*** [3], ***Prof. Amelie Eriksson Karlström*** [1], ***Prof. Vladimir Tolmachev*** [2]

***1.*** *Department of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, 106 91 Stockholm,* ***2.*** *Department of Immunology, Genetics and Pathology, Dag Hammarskjölds väg 20, Uppsala University, 751 85 Uppsala,* ***3.*** *Department of Medicinal Chemistry, Dag Hammarskjölds väg 14C, Uppsala University, 751 23 Uppsala*

Introduction:

Radioimmunotherapy (RIT) utilizes tumor-specific radiolabeled antibodies to deliver cytotoxic radiation to tumor cells. Direct RIT is however often limited by slow blood clearance or uptake of the radiolabelled targeting-agent in normal tissue. Pretargeting is a strategy to lower this unwanted radiation exposure by uncoupling the tumor-targeting step from the administration of the cytotoxic radionuclide (see Figure 1).

We have previously developed a pretargeting system based on high affinity PNA:PNA (peptide nucleic acid) hybridization in vivo. In an experimental therapy study, mice with human HER2 overexpressing SKOV-3 xenografts were treated with a combination of an anti-HER2 affibody-PNA conjugate, $Z_{HER2}$-*HP1*, and a complimentary and $^{177}$Lu-labeled PNA-based effector probe, $^{177}$Lu-*HP2*. Median survival of pretargeted mice was significantly longer (66 days) than in control groups injected with either PBS or the affibody-PNA conjugate alone (37 days), or with only $^{177}$Lu-*HP2*(32 days).

In an attempt to further improve our PNA-based pretargeting system we recently designed a second-generation of hybridization probes. The first-generation PNA-based probes contain 15 complementary PNA-bases and our hypothesis was that shorter PNA probes may provide an even better biodistribution in vivo. A new 15-mer primary probe, *HP15* was designed and conjugated to an anti-HER2 Affibody molecule, producing $Z_{HER2}$-*HP15*. Three complementary effector probes, *HP16*, *HP17* and *HP18* with increasing numbers (9, 12 and 15, respectively) of nucleobases were designed, synthesized and then characterized in vitro and in vivo (see Figure 2).

Methods:

We have produced and biophysically characterized PNA-based probes and Affibody-PNA chimeras. Pretargeting using the new $^{177}$Lu-labelled effector probes was studied in cell-based assays and in mice carrying human HER2 overexpressing SKOV-3 xenografts.

Results & Discussion:

The biodistribution of the new-generation of pretargeting probes were evaluated in SKOV-3 xenograft-bearing mice. When the mice had been preinjected with the tumor-targeting $Z_{HER2}$-*HP15*conjugate, kidney and tumor were the only organs with prominent uptake of the $^{177}$Lu-labeled effector probes (see Figure 3). The kidney uptake would thus be the dose-limiting organ in a therapeutic context. Out of the three new probes investigated the shortest effector probe, the 9-meric $^{177}$Lu-*HP16*, provided the highest tumor-to-kidney ratio, and is thus the most promising effector probe for efficient pretargeted radiotherapy.

Impact:

PNA-based pretargeting opens the way a for more efficient cancer therapy by delivering the cytotoxic radiometal to the tumor site while minimizing the exposure to healthy organs. As a result, higher doses of radiation can be administrated to the tumor without reaching dose-limiting toxicity.

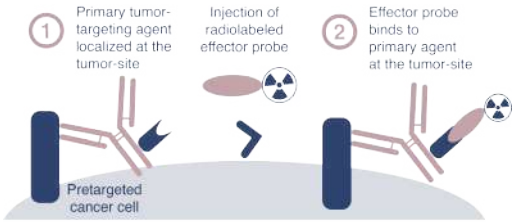


Figure 1: Schematic overview of the pretargeting concept

Figure 1.png

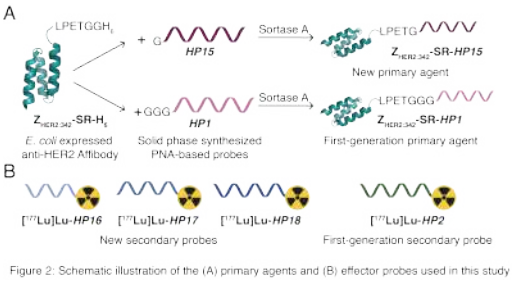


Figure 2: Schematic illustration of the (A) primary agents and (B) effector probes used in this study

Figure 2.png

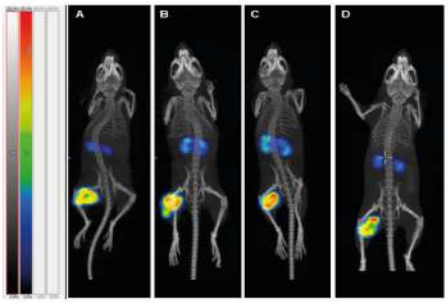


Figure 3: SPECT/CT imaging (maximum intensity projection) of HER2-expressing SKOV3 xenografts for pretargeting using (A) [$^{177}$Lu]Lu-HP16, (B) [$^{177}$Lu]Lu-HP17, (C) [$^{177}$Lu]Lu-HP18, and (D) [$^{177}$Lu]Lu-HP2 4 h after injection.

Figure 3.png

# Large-scale discovery and exploration of virus-host interaction motifs

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 88

***Mr. Filip Mihalič*** [1], ***Ms. Caroline Benz*** [2], ***Mr. Richard Lindqvist*** [3], ***Ms. Eszter Kassa*** [2], ***Ms. Marie Sander*** [4], ***Dr. Dilip Badgujar*** [2], ***Dr. Ravi Inturi*** [1], ***Dr. Leandro Simonetti*** [2], ***Dr. Ahmed Sayadi*** [2], ***Dr. Muhammad Ali*** [2], ***Dr. Doreen Dobritzsch*** [2], ***Dr. Ola Söderberg*** [4], ***Dr. Anna Överby*** [3], ***Dr. Per Jemth*** [1], ***Dr. Norman Davey*** [5], ***Prof. Ylva Ivarsson*** [2]

***1.*** *Department of Medical Biochemistry and Microbiology, Uppsala University, Box 582, Husargatan 3, 751 23 Uppsala, Sweden,* ***2.*** *Department of Chemistry - BMC, Uppsala University, Box 576, Husargatan 3, 751 23 Uppsala, Sweden,* ***3.*** *Department of Clinical Microbiology, Umeå University, 90185 Umeå, Sweden,* ***4.*** *Department of Pharmaceutical Biosciences, Uppsala University, Box 591, Husargatan 3, 751 24 Uppsala, Sweden,* ***5.*** *Division of Cancer Biology, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK.*

Short Linear Motifs (SLiMs) are 4-10 amino acid long stretches of protein sequences predominantly found in the intrinsically disordered regions of eukaryotic proteins. They serve as docking sites and are recognized by a larger globular protein domain of an interaction partner, playing crucial roles in processes such as cellular signalling, trafficking, transcription modulation and protein degradation. Owing to their simplicity and their intrinsically disordered nature, viral proteins often exploit SLiM-based interactions by mimicking the core recognition motif and consequently hijacking the relevant pathways, to promote viral survival, replication and egress.

To identify viral SLiM mimicry we used a novel proteomic peptide phage display (Pro-PD) library named RiboVD, displaying intrinsically disordered regions of 229 RNA-virus proteomes. The library was presented to 139 human protein domains and the resulting candidate ligands were rank ordered into high and low confidence sets. To validate the interactions identified by the RiboVD screen we have focused on three cellular processes, namely (i) the ESCRT pathway (ii) the clathrin mediated trafficking pathway and (iii) the PABP mediated translation regulation. Affinities between human protein domains and viral peptides were measured and compared to the native human interactions to assess the relationship between native and pathological interactions. Two globular domains were co-crystallized with viral peptide binders. Based on our results we propose several viral species that may exploit the ESCRT pathway for the viral particle egress. Moreover, we propose clathrin mediated trafficking as a hub of viral interference, implicating the N-terminal domain of clathrin and several of its adaptor proteins. Finally, we identified the C terminal domain of PABP as a broad target for viral interference and showed that the overexpression of a high-affinity peptide binder inhibits flavivirus replication in Vero cell cultures.

Using the described general strategy, we aim to obtain a global perspective of host-pathogen SLiM mimicry and identify new potential druggable targets creating the starting point for development of broad acting antiviral agents.

# Development and optimization of an enzymatic protocol for the quantification of salivary biomarkers

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 110

***Ms. Alonso Ornelas*** [1], ***Dr. Margarita Ortiz-Martinez*** [1], ***Dr. Mirna González-González*** [1], ***Prof. Richard Willson*** [2], ***Dr. Marco Rito-Palomares*** [1]

***1.*** *Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Nuevo Leon, Mexico.,* ***2.*** *University of Houston*

Early detection can often improve patient outcomes. In diabetes, detection in the early stages allows the correct management of the disease. Blood glucose and glycated hemoglobin are the most used tests for the diagnosis, prognosis, and control of diabetes. However, these methodologies present some limitations since they only reflect the glycemic status at a short time (24 hours) or in the long term (two-three months), respectively. In addition, they require blood extraction, which implies an uncomfortable and painful process. Methods based on novel metabolites have emerged, seeking to overcome these inconveniences. 1,5-anhydroglucitol is a relatively new biomarker that can provide more precise information on the glycemic status in the middle term (last days to weeks). Its quantification and validation in diabetes disease have been carried out using elaborate analytical techniques such as high-performance liquid chromatography, gas chromatography, and mass spectrometry in different biological samples, including saliva. However, there are no successful reports of its quantification in saliva using simple and inexpensive techniques such as enzymatic assays, which could be implemented in basically equipped laboratories. Therefore, this project aims to develop, optimize and validate a flexible enzyme platform that allows the quantification of biomarkers in saliva, including 1,5-anhydroglucitol. To do this, various parameters that have a significant impact on enzymatic determinations will be explored, such as pH, enzyme and substrate concentrations, and other methodological aspects such as incubation time and temperature. The preliminary results obtained so far in the standardization of the method show its ability to quantify glucose in saliva samples in a rapid and simple way, showing positive results between experimentally added and measured glucose. Once the development and optimization stages have been completed, the methodology will be validated using 500 samples from patients with known physiological and lifestyle characteristics and complete medical history. The aim of this research work is to develop a flexible, simple, robust, and economical methodology that can help in the diagnosis and control of diabetes through the detection of salivary biomarkers, with the ultimate goal of point of care testing in the near future.

# Biomimetic sensors for rapid and sensitive SARS-CoV-2 detection

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 16

***Dr. Sara Björk Sigurdardóttir*** [1], ***Dr. Xiaofeng Feng*** [1], ***Dr. Yulia Sergeeva*** [1], ***Dr. Börje Sellergren*** [1]

***1.*** *Department of Biomedical Sciences, Malmö University*

**Introduction:**The coronavirus SARS-CoV-2 has been identified as the cause of the severe respiratory disease COVID-19 pandemic. One of the most essential tools to combat the spread of the virus is diagnosis. The current testing strategies are based on either PCR of the RNA or antibody-antigen assays. While the PCR test is sensitive, it is time-consuming and costly. The rapid antibody-antigen tests are fast but lack sensitivity for the virus.[1] Rapid, sensitive, and low-cost diagnostic tools are therefore in high demand. This project aims to develop a novel type of rapid diagnostic tests based on biomimetic sensors using reversible Self-Assembled Monolayers (rSAMs).[2] These cell membrane mimics are modified with epitopes chosen from the binding region of the angiotensin-converting enzyme 2 (ACE2) receptor, which has been identified as the main cellular target for the virus.[3] Due to the lateral mobility of the rSAMs, these ligands can form clusters and bind to the analyte in a multivalent binding event, allowing for sensitive and selective detection of the virus.[2] Modification of the peptides could furthermore better detect other variants of the virus.

**Methods:** Three peptide epitopes were chosen based on the proposed binding of the wild-type virus to the human ACE2. The epitopes were synthesized with an additional propargylglycine as a bioorthogonal site for a copper-catalyzed azide-alkyne cycloaddition (CuAAC) and were in that way immobilized on a gold surface. Using surface plasmon resonance (SPR), the binding affinity of the SARS-CoV-2 receptor-binding domain (RBD) was measured towards each ACE2 epitope. Furthermore, the peptide epitopes were coupled to building blocks for the rSAMs to construct the biomimetic sensors, using one or more epitopes based on their binding affinities.

**Results and discussion:** The dissociation constants ($k_D$) for two of the epitopes were low, revealing a strong binding event towards the SARS-CoV-2 RBD. This is an excellent indication for the further use of these epitopes as ligands for our biomimetic sensors for SARS-CoV-2 detection.

**Impact:** The ongoing COVID-19 pandemic will be a real threat until herd immunity is reached worldwide. It is therefore vital to continue the development of tools to combat and contain the pandemic, and rapid, sensitive, and low-cost diagnostics will continue to be vital to that effort.

(1) Tang, Y. W. *et al. Journal of Clinical Microbiology* 58, (2020)

(2) Yeung, S. Y. *et al. Langmuir* 35, 8174–8181 (2019)

(3) Shang, J. *et al. Nature* 581, 221–224 (2020)

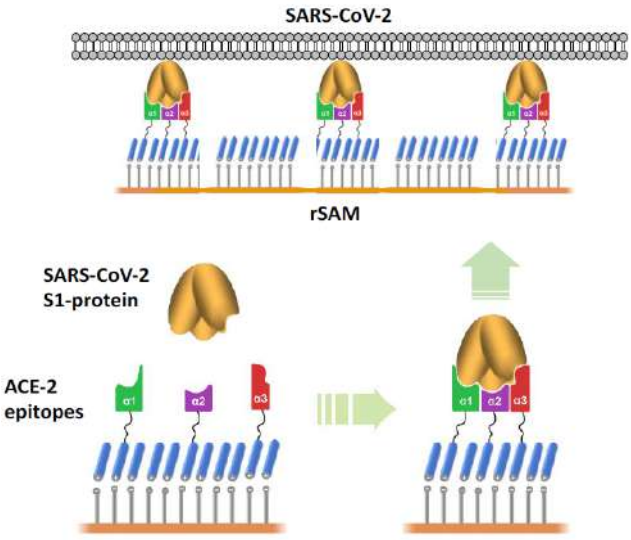


Figure 1 - a schematic of the design of the rsam biomimetic sensors for sars-cov-2 detection.png

# A new approach on targeting aberrant glycans in cancer cells

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 89

***Mr. Nuno Prego R.*** [1], ***Ms. Daniela F. Barreira*** [2], ***Ms. Rita A. Lourenço*** [2], ***Dr. Sara Lemos*** [3], ***Dr. Benedita Pinheiro*** [2], ***Prof. Angelina Palma*** [2], ***Prof. Filipa Marcelo*** [2], ***Dr. Leonardo Chicaybam*** [3], ***Prof. Paula A. Videira*** [4]

***1.*** *CellmAbs Biopharmaceuticals, Óbidos, 2510, Portugal Faculdade de Ciências da Universidade de Lisboa, Portugal,* ***2.*** *UCIBIO, Department of Life Sciences, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal,* ***3.*** *CellmAbs Biopharmaceuticals, Óbidos, 2510, Portugal,* ***4.*** *UCIBIO, Department of Life Sciences, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal CDG Professionals and Patient Associations International Network (CDG & Allies-PPAIN), Lisbon, Portugal*

**Introdution:**Cancer remains one of the major mortalities causes worldwide. Cancer cells are characterized by the expression of sialyl-Tn (STn) antigen, which is rarely expressed in healthy cells, thus a promising target for anti-cancer therapies. One of the challenges associated with glycan recognition is the interaction between monoclonal antibodies (mAbs) and target antigen, since it is different from typical protein-protein interactions, mainly because of: i) the target size - STn has low molecular weight; and ii) the binding and affinity, which depend on antigen presentation and glycan clustering and density.

**Methods:** We have developed a high specificity anti-STn antibody, the L2A5, using a unique set of processes, combining hybridoma and microarray technology. The murine IgM L2A5 antibody was reformatted into an IgG isotype, and into a chimeric human IgG. This chimeric antibody was then humanized, allowing us to obtain several human anti-STn antibody variants. After selecting several anti-glycan clones, we resorted to glycan arrays, Surface Plasmon Resonance (SPR), and flow cytometry with different cancer cell lines models expressing or not STn, to verify if the intensity of the binding of the variant antibodies to STn antigen was retained.

**Results & Discussion:**Our results confirmed that our mAbs retained the ability to interact with sialylated O-glycans, and that most antibodies possessed an increased and more selective binding pattern to STn. The kinetical evaluation of the antibody-antigen interaction (KD) indicated that all versions had prime affinity towards STn compared to the chimeric control. Lastly, the cellular binding assays performed with STn+ breast cancer cells showed a high binding mAbs' efficiency only to STn+ (positive) cells. When compared with other disclosed anti-STn antibodies, L2A5 mAbs presented better STn affinity and specificity. Interestingly, the variants with higher KD had better cell internalization profiles, increasing the level of penetration in solid tumours. By using our ScFVs in Chimeric Antigen Receptor T (CAR-T) cells or in a Bispecific T cell engager, we sought a mAb with medium range affinity, which can lead to increased persistence in the tumour, but also reduce the risk of earlier T cell exhaustion.

**Impact:** We were able to identify several L2A5 mAb variants with a broad range STn affinity. Thus, we can now build upon these different profiles, targeting tumour cells using various approaches and modalities. Moreover, due to similarities in the glycosylation pattern of cancer cells and viruses, we are also evaluating and exploring the antiviral potential of L2A5 mAbs.

# Preclinical Evaluation of the GRPR-Targeting Antagonist RM26 Conjugated to the Albumin-Binding Domain for GRPR-Targeting Therapy of Cancer

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 75

***Mr. Ábel Nagy*** [1]

***1.*** *Dept. of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH - Royal Institute of Technology, SE-106 91 Stockholm, Sweden*

Prostate cancer is one of the most frequently diagnosed cancers and leading causes of cancer-related death among men. Targeting overexpressed cancer cell targets is a popular strategy for cancer treatment. In targeted radionuclide therapy, a cytotoxic radioligand can be delivered through receptor-specific agents to the cancer cells, thus achieving selective killing of the malignant cells. Gastrin-releasing peptide receptor (GRPR) is over-expressed in various cancer types, including breast cancer and prostate cancer. GRPR is a potential candidate to target for diagnostic imaging of prostate cancer and, possibly, for therapeutic use.

Bombesin analogues have previously been developed to target GRPR. An antagonistic 9 amino acid peptide analogue called RM26 (D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-$NH_2$) has been extensively studied due to its high affinity to GRPR and favourable pharmacokinetics. A radiolabelled RM26 analogue has recently been evaluated for GRPR imaging in cancer patients with prostate cancer. Due to their small size, peptide-based therapeutics would however require multiple and frequent injections in order to avoid rapid blood clearance. By conjugation of RM26 to an albumin-binding domain (ABD), we aimed to extend the in vivo half-life of the peptide. ABD is a three-helix bundle protein derived from streptococcal protein G, which binds serum albumin, one of the most abundant and long half-life proteins in our body.

In our recently published work, the conjugate DOTA-ABD-RM26 was synthesised, characterised, radiolabelled with indium-111 and evaluated *in vitro* and *in vivo*. The labelled conjugate retained its binding specificity towards GRPR and demonstrated a significantly longer residence time in blood and in tumors than the parental RM26 peptide. However, undesirable elevated activity uptake in kidneys abolished its use for radionuclide therapy. This proof-of-principle study justified further optimization of the molecular design of the ABD-RM26 conjugate.

In a follow-up study, we aim to improve the biodistribution of the conjugate to reduce kidney uptake. New second generation constructs have been designed, produced and evaluated. The ABD part of the conjugate was redesigned for recombinant expression with an N-terminal stabilising cap, that might lead to higher thermal stability. The positions of RM26 and DOTA relative to ABD were also changed in order to achieve higher stability and better receptor binding. The new constructs showed improved helicity and thermal stability in circular dichroism experiments.

Preliminary *in vivo* studies confirmed a more favourable biodistribution profile compared to the first-generation construct, making it a promising agent for prostate cancer radionuclide therapy studies.

# Title: Tracking SARS-CoV-2 spike domain antibodies in plasma of convalescent COVID-19 patients by SPR

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 102

***Dr. Gabriela Canziani*** [1], ***Ms. Shiyu Zhang*** [1], ***Mr. Jackie Tang*** [1], ***Ms. Aakansha Nangarlia*** [1]

***1.*** *Drexel University*

Gabriela Canziani[1*], Shiyu Zhang[1*], Aakansha Nangarlia[1], Jackie Tang[1], Jennifer Connors[2], Gina Cusimano[2], Mariana Bernui[2], Matt Bell[2], Nicholas J Tursi[3], Ali R Ali[3], Ebony Gary[3], Michele Kutzler[2], Elias. K. Haddad[2], David B Weiner[3], Charles Cairns[4], Irwin Chaiken[1]

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[4]Drexel University College of Medicine, Medical Affairs

*equal contribution

The overall goal is to identify natural anti-SARS-CoV-2 protective responses in hospitalized and non-hospitalized COVID patients by tracking antibody specificity and durability. In this broad cross-sectional study, convalescent plasma collected at three- and six-months post-infection were diluted and individually screened using a Biacore S200 instrument by injecting aliquots over sensors derivatized with spike RBD, S1 and S2. We measured both diffusion-limited and kinetic binding of antibodies from each aliquot on surface densities of 50 RU/kDa and alternating flows of 5 and 50 uL/min. Calculated concentrations indicated immunodominance of RBD, as previously reported, but also significant responses to other epitopes on S1 and S2 (Fig.1). We tested the hypothesis that antibody target specificity, abundance and affinities are indicators of the immune response, while changes in binding kinetic (Fig.2) leading to affinity maturation and subclass switch indicate immune durability and correlate with pseudovirus infection inhibition potency. We have micro-affinity purified polyclonal antibodies from convalescent plasma to test antiviral potency of domain-specific fractions in pseudovirus neutralization assays *in vitro*. We speculate that blocking immunodominant epitopes on RBD, S1 and S2 exposed at different stages of infection can translate into both antibody-driven receptor binding inhibition and hindrance of spike conformational changes that lead to fusion and viral entry.

**Financial Support**

Cairns, and Haddad (05/2020-ongoing) *Supported by U19 Human Immunology Program* Grant COVID-19 Supplement. 5U19AI128910-04 & 3U19AI1289104SI

PA CARES Act (Kutzler, PI; 03/01-11/30 2020) *"This Project was financed [in part] by a grant through the federal Coronavirus Aid, Relief, and Economic Security (CARES) Act through the Commonwealth of Pennsylvania Department of Health and administered through a subgrant by the Department of Community and Economic Development."*

Drexel COVID-19 Response Fund, Chaiken, PI; 04/01-09/30 2020) *"Peer reviewed and selected: To identify inactivators of SARS-CoV-2 Spike active conformation"*

S10 NIH Equipment Grant (Chaiken, PI; 06/2019-06/2024) *"S200 state-of-the-art Biacore Biosensor in a Shared Resource at Drexel-SKCC/TJU"*

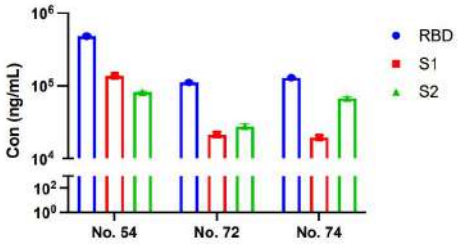


Figure1.jpg

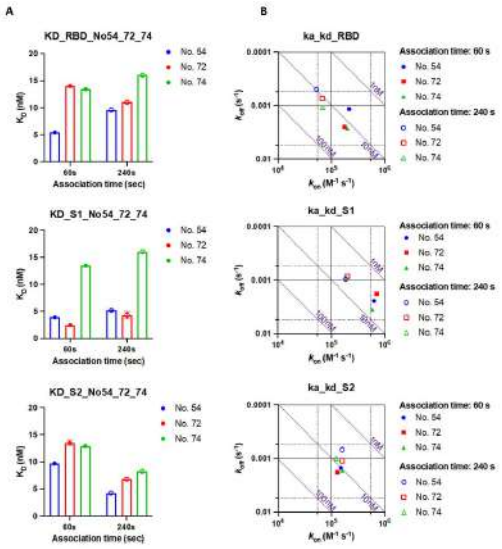


Figure2.jpg

# Development and characterization of bispecific protein binders targeting key players of the immune system based on a new ADAPT protein library

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 96

***Mr. Andreas Wisniewski*** [1], ***Dr. Emma von Witting*** [1], ***Dr. Sarah Lindbo*** [1], ***Prof. Sophia Hober*** [2]

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Biological drugs, like small scaffold proteins, are storming the pharmaceutical market due to their high specificity and applicability in diagnostics and therapy. Especially small engineered scaffold proteins are getting more attention as an alternative to the market-leading antibodies, thanks to their high stability, low immunogenicity and low cost of production. However, their small size also leads to fast blood clearance which can be an advantage for diagnostic purposes but is a disadvantage in a therapeutic setting. To overcome current downsides of the small size, a new protein library based on Albumin-derived affinity proteins (ADAPTs) was designed, originating from an albumin-binding site of streptococcal protein G. From this protein library, it is possible to achieve protein modules with ability to simultaneously bind to its intended target as well as to Human Serum Albumin (HSA), thereby increasing its half-life in the body (*Fig. 1*).

To achieve binding modules from the newly developed library several rounds of phage display selections were performed towards Tumor necrosis factor alpha (TNFa) and Interleukin 17c (IL17c), both being important proinflammatory cytokines and possibly interesting targets for therapeutical applications of autoimmune diseases. The output of the selections was analyzed using next generation sequencing (NGS) and promising candidates were cloned and produced in *Escherichia coli* (*E. coli)*, followed by detailed characterization of each candidate for target binding as well as stability using methods like Surface Plasmon Resonance (SPR), Circular Dichroism (CD). Further their oligomeric state was analyzed by Size Exclusion Chromatography (SEC). It was possible to generate binders that passed all characterization criteria, which then further showed simultaneous bispecificity to either TNFa or IL17c in combination HSA while being evaluated in a capture assay using SPR. Knowing all of this, each binder was then examined for their usefulness as a real therapeutic by successfully testing its ability to block the interaction of the cytokine and its specific receptor on SPR as well as in an actual *in vitro* setup.

This newly developed protein binders, showing high affinity towards their targets as well keeping its initial binding to HSA present another possibility to combine the advantages of small engineered scaffold proteins with those of typical larger proteins allowing for more convenient production in bacteria leading to lower production costs and making them therefore ideal candidates future therapeutics.

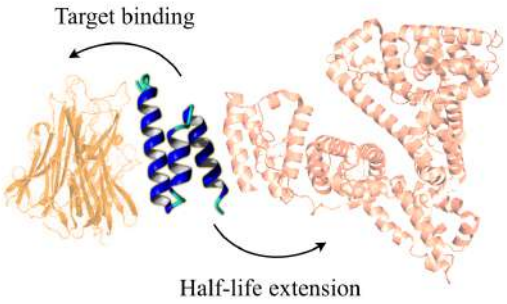



Fig. 1 - schematic overview of the adapts ability to not only bind to its target but the maintained half-life extension by interacting with hsa as well.jpg

# Towards a serum biomarker panel for muscle-protective interventional therapies in Duchenne muscular dystrophy

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 87

***Ms. Camilla Johansson*** [1], ***Mr. Fred Schnell*** [2], ***Ms. Jyoti D. Malhotra*** [2], ***Dr. Cristina Al-Khalili Szigyarto*** [1]

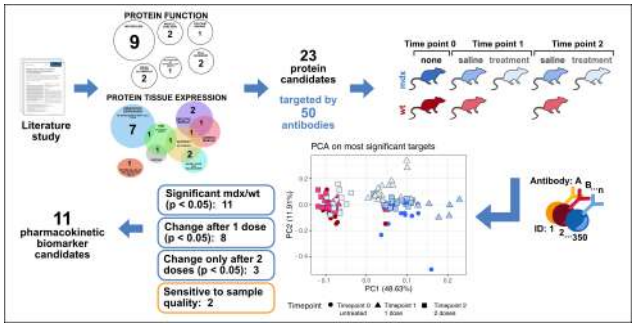
***1.*** *Department of Systems Biology. School of Chemistry, Biotechnology and Health. KTH Royal Institute of Technology, Stockholm,* ***2.*** *Sarepta Therapeutics, 215 First Street, Cambridge, MA 02142*

**Introduction:** A growing number of blood biomarker candidates for Duchenne muscular dystrophy (DMD), a progressive fatal muscle wasting disorder, have been reported in literature. While many previous studies have aimed at diagnostic biomarker discovery, we hypothesised that a biomarker panel, built on the xMAP immunoassay technology, could target a broader aspect of the DMD phenotype and facilitate monitoring of biological effect from muscle-protecting therapeutic intervention. This poster describes the first stage of the project, focusing on target selection and preliminary screening for treatment outcome biomarkers in mdx mice treated with an interventional therapy.

**Methods:** 75 serum biomarker candidates were selected from literature and scored based on prevalence, consistency, discovery method and antibody availability. The 23 top-scoring targets were selected and classified based on biological function (GO annotations) and tissue origin (Human Tissue Atlas, mRNA expression database). A multiplex antibody immunoassay was constructed towards the 23 targets using antibodies from the Human Protein Atlas and built on the xMAP technology. Antibodies raised against human protein fragments were selected on sequence similarity between human protein fragments and mouse analogous protein sequence. Serum from untreated dystrophin deficient mdx mice and wild-type controls, as well as mdx mice treated with either an interventional compound or saline solution with 2 doses at 2 timepoints, were screened. Median fluorescent intensities (MFI) were cleaned based on count and inter-target CV, log2 transformed and normalised using modified probabilistic quotient normalisation. Robustness against haemolysis was investigated by correlation to hemoglobulin.

**Results and Discussion:**23 protein targets were selected from literature, covering major biological processes known to be affected in DMD. 11 targets translated to mice using our assay, with regulation similar to what was reported in the literature on studies comprising patient samples. Upon muscle-protective treatment, all 11 proteins in mdx mice approached wild-type levels, 8 responded after only one treatment and 3 after two treatments. Interestingly, two inflammatory protein targets showed correlation with serum haemolysis due to sample handling errors, as proved by strong correlation to hemoglobulin levels and serum colour change.

**Impact:**When selecting antibodies raised against antigens with high sequence similarity between human and mouse analogues, 48% of targets were successfully translated to mouse models, despite the fact that there are some biological differences in phenotype between DMD patients and mdx mice.




Graphical abstract.png

# Electrochemical immunosensors for rapid diagnosis of Human Cytomegalovirus in urine samples

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 84

***Dr. Filipa Pires [1], Prof. Juan Carlos Vidal [2], Prof. Maria Julia Arcos-Martinez [3], Prof. Ana Cristina Dias-Cabral [1]***

***1.*** *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal,* ***2.*** *Analytical Spectroscopy and Sensors Group (GEAS), Institute of Environmental Sciences (IUCA), University of Zaragoza, c/Pedro Cerbuna 12, 50009, Zaragoza, Spain,* ***3.*** *Departamento de Química. Facultad de Ciencias. Universidad de Burgos, Plaza Misael Bañuelos s/n, 09001 Burgos, Spain*

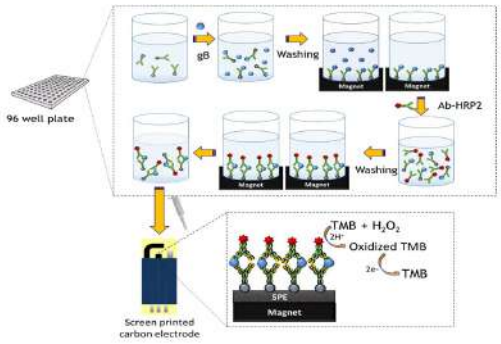
Today, the available methodologies to detect and quantify human cytomegalovirus (HCMV) are lengthily and/or expensive to be routinely applied in a point-of-care.

In a previous study, we have reported a magnetic particle-based enzyme spectrophotometric immunoassay for human cytomegalovirus quantification. In this work, we propose new electrochemical immunosensors for the diagnosis of this disease. This type of transduction improved the analytical properties with respect to the spectrophotometric one.

The approach is based on a sandwich-type immunoassay scheme with gB sandwiched between the primary monoclonal antibody (MBs-PrG-mAb1) and a secondary anti-gB antibody labelled with Horseradish peroxidase (Ab2-HRP), using TMB as substrate.

The method shown a linear dependence between gB concentration and the current intensity in a range of concentrations from 0.06 to 40 ng $mL^{-1}$ , with a detection limit of 60 ± 10 pg $mL^{-1}$ . The reproducibility was good, with a % RSD of 8.1. No cross-reactivity was observed with other viruses from the Herpesviridae family, namely varicella zoster and Epstein Barr viruses. Recovery percentages from spiked human urine samples (n=3) ranged from about 97 % to 117 %, showing the accuracy of the method.

The electrochemical immunosensor was validated in urine samples following the guidelines of the European Medicines Agency (EMEA-2014). The developed biosensor allows easy quantification of gB-HCMV in urine samples at relevant clinical levels and can be used in point-of-care tests for the rapid diagnosis of the human cytomegalovirus.




Dias-cabral.jpg

# Novel approach using aqueous two-phase systems in 3D culture for the neural differentiation of stem cells

Tuesday, 22nd June - 14:15: Flash Session 1 - Poster - Abstract ID: 103

***Ms. Karolina Chairez-Cantu*** [1], ***Dr. Mirna González-González*** [1], ***Dr. Marco Rito-Palomares*** [1]

**1.** *Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Nuevo Leon, Mexico.*

Introduction: Aqueous two-phase systems (ATPS) are classified as a type of liquid-liquid extraction technique that involves combinations of polymer-polymer or polymer-salts at certain concentrations. The recovery and purification of biomolecules of interest have been the main applications. However, novel biotechnological applications have emerged including 3D cultures using ATPS. Their construction involves the encapsulation of stem cells in polymer droplets dispersed by non-contact over a substrate mixed with another polymer. Thus, different types of ATPS-3D cultures can be given rise such as cell monoculture, exclusion micropatterning, co-culture, and micropatterning (Figure 1). Their greatest advantage is the generation of size-controllable spheroids distributed in defined patterns that enables the reproduction of cell niches mimicking *in vivo* microenvironments. The project aims to improve the construction of ATPS-3D cultures to differentiate induced pluripotent stem cells (iPSCs) into motor neurons.

Methods: Characterization of the differentiation from iPSCs to motor neurons using culture media supplemented with specific growth factors and a neural extracellular matrix in 2D cultures and ATPS-3D cultures. The cell differentiation efficiency between both cultures is analyzed by the detection of specific neuronal markers using immunocytochemistry and RT-qPCR. For the construction of ATPS-3D cultures, the selection of dextran, polyethylene glycol, UCON, and Ficoll polymers is considered.

Results & Discussion: It is foreseen that ATPS-3D cultures will result in higher differentiation efficiency than traditional 2D cultures, due to an enhanced intercellular communication promoting cell-cell and cell-substrate interactions, in which cell viability is not compromised (Figure 2). Factors including colony size, cell density, and interconnectivity during the construction of ATPS-3D cultures are important to evaluate as they directly impact neurite outgrowth and lineage commitment.

Impact: Stem cells are characterized for their remarkable differentiation potential that has led to the development of cell therapies to treat degenerative diseases. However, sufficient cell expansion and high cell differentiation efficiency are some drawbacks that hinder their translation from lab to clinical settings. Therefore, ATPS-3D cultures stand as a promising strategy to overcome the limitations of 2D cultures and scaffold-based 3D cultures, as this technology has also the potential to be easily scalable and robust for any cell lineage.

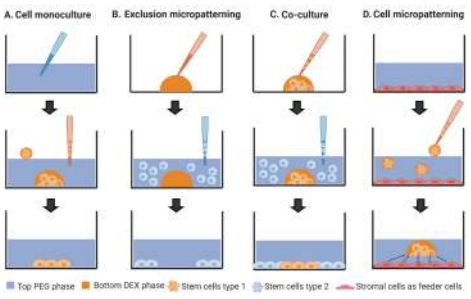



Figure1 affinity21 kcc.jpg

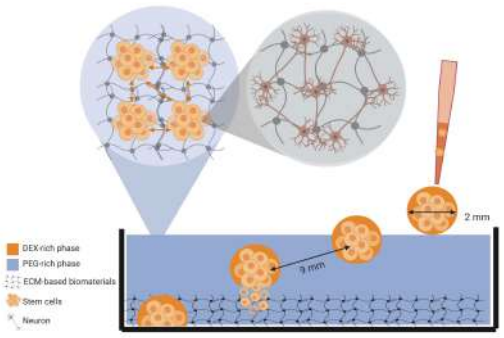



Figure2 affinity21 kcc.jpg

# Design meets evolution: the quest for modular binding of linear epitopes

Tuesday, 22nd June - 15:30: Keynote 2 - Oral - Abstract ID: 116

***Prof. Andreas Plueckthun*** [1]

***1.*** *University of Zurich, Dept. of Biochemistry*

The traditional generation of binding molecules is based either on immunization of animals to generate monoclonal antibodies, or on the selection of binders from large universal libraries. While well established by now, they share a fundamental and intrinsic limitation: for every new target, a completely new selection and quality control has to be started — it always starts from square one, and the number of useful target epitopes is essentially infinite. For folded 3D epitopes (conformational epitopes), this remains the state of affairs.

However, when it comes to linear epitopes, it should be possible to exploit the periodicity of peptide bonds, and create a completely modular system based on a binding protein that shares the same periodicity. If such modules can be created, this would eliminate and selection or design for an individual target altogether. Instead, it would permit researchers to immediately assemble a sequence-specific binder, from the previously verified parts. This would allow to assemble a binding protein only from knowing the sequence of the target protein.

We can report on significant progress toward this very challenging goal. Specific binding pockets for a range of different amino acid side chains have been created, and their correct assembly has been demonstrated. Using many orthogonal approaches of design, selection, evolution, biophysical testing, and structure determination, significant progress has been reached and will be summarized. It will become apparent that such a challenge can neither be met by design nor by selection alone, but likely by combinations of all these methods.

Ernst, P., Zosel, F., Reichen, C., Nettels, D., Schuler, B., and Plückthun, A. (2020). **Structure-guided design of a peptide lock for modular peptide binders**. *ACS Chem. Biol.* **15**, 457-468.

Hansen, S., Ernst, P., König, S. L. B., Reichen, C., Ewald, C., Nettels, D., Mittl, P., Schuler, B., and Plückthun, A. (2018). **Curvature of designed armadillo repeat proteins allows modular peptide binding**. *J. Struct. Biol.* **201**, 108-117.

Hansen, S., Tremmel, D., Madhurantakam, C., Reichen, C., Mittl, P. R., and Plückthun, A. (2016). **Structure and energetic contributions of a designed modular peptide-binding protein with picomolar affinity**. *J. Am. Chem. Soc.* **138**, 3526-3532.

# heliX®: Next-Generation Modular Biosensor for Interaction and Conformation Analysis

Tuesday, 22nd June - 16:00: Oral Session - Oral - Abstract ID: 33

***Dr. Daisy Paiva*** [1], ***Dr. Stefanie Mak*** [1]

***1.*** *Dynamic Biosensors GmbH*

switchSENSE® is an automated, fluorescence-based biosensor chip technology that employs electrically actuated DNA nanolevers for the real-time measurement of binding kinetics ($k_a$, $k_d$) and affinities (with $K_D$ values down to the fM range).

Fluorescent dyes located on the biosensor surface detect the interaction of ligand and analyte molecules in different ways. The fluorescence proximity sensing mode detects the binding of molecules in real-time through changes in the dye's local environment. Association and dissociation of analyte molecules can be observed in real-time. In addition, the high frequency dynamic electrical switching mode probes the hydrodynamic friction of analyte molecules and serves to determine the size and shape of biomolecules. When analytes bind to oscillating DNA nanolevers on the sensor spots, the nanolever movement is slowed by the additional friction imposed by the analyte, thereby revealing its size changes. The unique use of two different fluorophores makes it possible to monitor two independent signals from two interactions at the same time and on the same sensor spot. It also enables fluorescence resonance energy transfer (FRET) experiments, for binding and conformation analyses that require the resolution of intra-molecular distance changes with sub-nanometer resolution. The DNA-encoded anchor sequences present in the biochip surface allow the immobilization of a wide range of different molecules or even a combination of different molecules in varying ratios and densities. This technology is unlike existing methodologies in that it combines high sensitivity real-time kinetics with structural information on size, shape, and conformation providing a new depth and understanding of the interaction.

The seminar will highlight the broad range of applications of the switchSENSE® technology that is supported by the recently launched heliX® biosensor:

- Resolving the fastest kinetics with confidence using advanced microfluidics and 10 ms data collection. Taking advantage of improved signal stability for the characterization of high-affinity binders in long dissociation measurements.
- DNA/RNA Binding Proteins | Flexible exchange of DNA/RNA targets for binding and enzymatic activity studies in real-time.
- Conformational Changes | Screening and ranking of small molecule induced conformational changes by de novo real-time conformation referencing.


Switchsense fundamentals and applications.jpg

# Characterization of multispecific antibodies and implications for target selectivity: practical analysis of binding kinetics and avidity

Tuesday, 22nd June - 16:15: Oral Session - Oral - Abstract ID: 19

***Dr. Stefanie Mak*** [1], ***Dr. Joanna Deek*** [1], ***Mrs. Agnes Marszal*** [1], ***Dr. Kolio Raltschev*** [1], ***Dr. Alice Soldà*** [1], ***Dr. Nena Matscheko*** [1], ***Dr. Ulrich Rant*** [1]

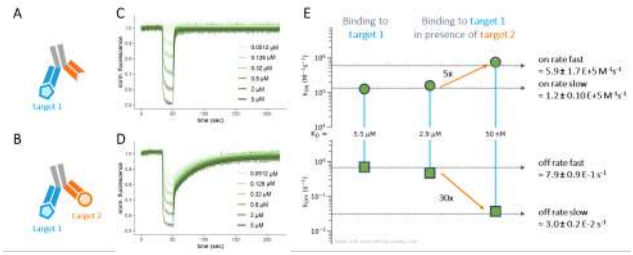
***1.*** *Dynamic Biosensors GmbH, Martinsried, Germany*

Bispecific antibodies are promising therapeutics that can be used for different applications. Prominent examples are activators of immune cells in cancer immunotherapy or cofactor mimetics in the treatment of haemophilia. The measurement of binding rates and avidity effects in the simultaneous engagement of two antigens is key for understanding and optimizing target selectivity of a bispecific antibody.

Here, we present the application of a novel type of biosensor that uses DNA-guided surface functionalization for the precise control over the relative abundance and spatial arrangement of two antigen species. The biosensor is equipped with dual-color fluorescence detection for the simultaneous single and dual-binding kinetic studies of bispecific antibodies. We determined real-time parameters (on and off rates) and equilibrium parameters (dissociation constants) of both individual and cooperative binding of different bispecific antibodies. We could cover a broad range of kinetic rates using a novel surface modification with Y-shaped DNA. A statistical comparison of those results showed that analysis of individual association and dissociation rates is not sufficient to predict cooperativity. Finally, by simulation of different antigen expression patterns on the biosensor surface, we could connect kinetic rates with the residence half time of a bispecific antibody on target and off target cell types.

Our results highlight how quantitative analysis of individual and cooperative target binding can be used to improve the engineering of bispecific antibodies. The individual kinetic rates of the bispecific antibody should be optimized to achieve optimal target selectivity and minimal off-target binding.

Figure 1: Example measurement for bispecific antibody. Schematic assay setup for determination of affinity association and dissociation rates (A) or for determination of cooperativity and avidity rates (B). Measurement curves for association and dissociation of bispecific antibody from one single target molecule (C) or from one target molecule in presence of the other target (D). Kinetic rate overview showing binding enhancement of both association and dissociation rates with dual target engagement.




Characterization of bispecific antibody.png

# Authentication of honey origins using next-generation sequencing of enriched pollen DNA and trace DNA from pollen-free honey

Tuesday, 22nd June - 16:30: Oral Session - Oral - Abstract ID: 34

***Ms. Dimple Chavan*** [1], ***Dr. Jay Adolacion*** [2], ***Dr. Mary Crum*** [3], ***Mr. Suman Nandy*** [3], ***Dr. Binh Vu*** [3], ***Dr. Katerina Kourentzi*** [3], ***Dr. Aniko Sabo*** [4], ***Prof. Richard Willson*** [5]

***1.*** *Department of Biology and Biochemistry, University of Houston, Houston, Texas,* ***2.*** *Department of Chemical Engineering, University of the Philippines, Diliman, Quezon City,* ***3.*** *Department of Chemical and Biomolecular Engineering, University of Houston, Houston, Texas,* ***4.*** *Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas,* ***5.*** *Department of Biology and Biochemistry and Department of Chemical and Biomolecular Engineering, University of Houston, Houston, Texas*

Adulteration and mis-labeling of honey to mask its true origin have become a global issue. Pollen microscopy, the current gold standard for identifying the geographical origins of honey, is time-consuming and requires expert personnel. Additionally, pollen microscopy cannot identify honey samples that have been filtered to remove the original pollen and/or spiked with pollen from more-remunerative plants. We have explored the DNA-based characterization of honey origins using deep sequencing targeting the nuclear ribosomal ITS2 region of plant genomic DNA, known to facilitate species-level discrimination of plants.

Using next-generation sequencing and clustering analysis, we are assembling country-specific plant DNA sequences obtained from NGS of plant genomic DNA isolated from 300 honey samples. We also have successfully isolated trace DNA and sequenced plant ITS2 from five different pollen-free, filtered honeys using three methods: (i) anti-dsDNA antibodies coupled to magnetic particles; (ii) batch adsorption on Q Sepharose anion exchanger; and (iii) batch adsorption on ceramic hydroxyapatite. The amplified ITS2 region of the captured pollen-free DNA was sequenced using next-generation sequencing and was found to be identical to plant ITS2 of pollen DNA from the same honey sample. Enrichment of trace pollen-free DNA from filtered honey samples opens a new approach to identify the true origins of filtered honey samples, and may suggest other applications of DNA-based product sourcing.

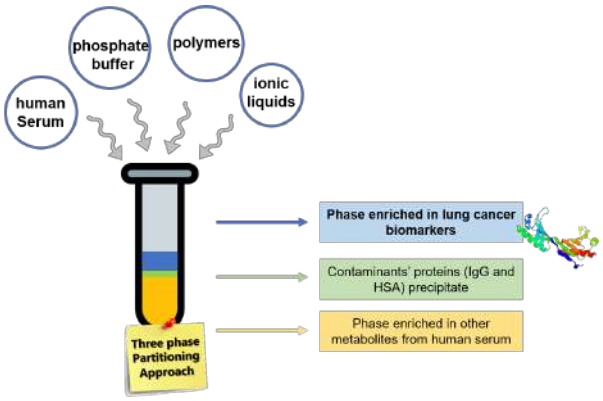
# Alternative approach for human serum pretreatment resorting to three-phase partitioning

Tuesday, 22nd June - 16:45: Oral Session - Oral - Abstract ID: 113

***Mrs. Marguerita Rosa*** [1], ***Dr. Francisca Silva*** [1], ***Prof. João Coutinho*** [1], ***Prof. Mara Freire*** [2]

**1.** *CICECO- Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal,* **2.** *ciceco*

Globally, lung cancer is one of the deadliest types of cancer, representing circa 18.4% of cancer deaths (GLOBOCAN 2018). This is a result of the long asymptomatic phase and lack of effective early-stage diagnosis methods. Currently available diagnosis techniques resort to X-rays or computed tomography scans, which may lead to a high occurrence of false positives and an avoidable radiation exposure. To tackle these issues, the identification and quantification of lung cancer biomarkers in human fluids, such as in serum, is a propitious approach. Most often, however, the reliability of diagnosis is limited by the low concentrations of biomarkers in human serum and their propensity to be masked by abundant proteins including immunoglobulin G (IgG) and human serum albumin (HSA). Thereby, a sample pretreatment step aimed to reduce or eliminate abundant proteins, while improving biomarkers detection is frequently considered necessary. This work proposes an alternative serum pretreatment approach based on three-phase partitioning (TPP) to simultaneously remove highly abundant serum proteins (IgG and HSA) and concentrate target biomarkers (cf. Fig. 1). TPP systems composed of biocompatible and cost-effective components including polymers and phosphate buffer as well as having ionic liquids as adjuvants were developed. Upon human serum addition, the proposed systems led to the formation of two aqueous phases, where the concentration of biomarkers might be achieved, and a solid interphase where most abundant proteins may be depleted. The main phenomena that lead to the TPP formation are the hydrophobicity of the used polymers and the salting-out effect of the phosphate buffer. Consequently, the exclusion of highly abundant human serum proteins from both the top (PEG-rich phase) and bottom (salt-rich) phases while precipitating at the system's interphase takes place. Initial depletion studies using commercial human serum revealed that, among all systems investigated, those containing 30 wt% polyethylene glycol 1000 $g.mol^{-1}$ (PEG 1000), 12 wt% phosphate buffer pH 7 ($K_2HPO_4/KH_2PO_4$), 5 wt% of tetrabutylphosphonium chloride ($[P_{4444}]Cl$) or tetrabutylammonium chloride ($[N_{4444}]Cl$), 10 wt% of serum and 43% water were the most efficient, with IgG and HSA depletion efficiencies higher than 85%. Although further investigation is in progress, the developed TPP systems hold potential as a cheaper and faster strategy to pretreat human serum samples, paving the way to an enhanced early-stage diagnosis of lung cancer.




Tpp affinity.png

# Beyond affinity: predicting protein-ligand binding kinetics

Tuesday, 22nd June - 17:30: Keynote 3 - Oral - Abstract ID: 45

***Prof. Rebecca Wade*** [1]

***1.*** *HITS and Heidelberg University*

The rates at which molecules associate and dissociate are important determinants of biological function. Growing evidence that the efficacy of a drug can be correlated to target binding kinetics has led to the development of many new methods aimed at computing rate constants for receptor-ligand binding processes [1,2], see also kbbox.h-its.org. Here, I will describe our recent studies to explore the determinants of structure-kinetic relationships [3,4] and to develop computationally efficient methods - employing molecular simulations and machine learning - to estimate protein-ligand binding kinetic parameters [5-8].

[1] Bruce NJ, Ganotra GK, Kokh DB, Sadiq SK, Wade RC. New approaches for computing ligand-receptor binding kinetics. *Curr Opin Struct Biol.* **2018**, 49: 1-10.

[2] Nunes-Alves A, Kokh DB, Wade RC. Recent progress in molecular simulation methods for drug binding kinetics *Curr Opin Struct Biol.* **2020**, 64:126-133

[3] Amaral M, Kokh DB, …Wade RC, Frech M. Protein conformational flexibility modulates kinetics and thermodynamics of drug binding. *Nat Commun.* **2017**, 8:2276.

[4] Berger B, Amaral M, Kokh DB, Nunes-Alves A, …. Wade RC, Knapp S Structure-kinetic relationship reveals the mechanism of selectivity of FAK inhibitors over PYK2, *Cell Chem. Biol.*, **2021**, doi: 10.1016/j.chembiol.2021.01.003

[5] Reinhardt M, Bruce NJ, Kokh DB, Wade RC, Brownian Dynamics Simulations of Proteins in the Presence of Surfaces: Long-range Electrostatics and Mean-field Hydrodynamics*J. Chem. Theory Comput.* **2021**, doi: 10.1021/acs.jctc.0c01312.

[6] Ganotra GK, Wade RC. Prediction of Drug–Target Binding Kinetics by Comparative Binding Energy Analysis. *ACS Med.Chem. Lett.* **2018**, 9: 1134–1139.

[7] Kokh DB, Amaral M,……Wade RC. Estimation of drug-target residence times by t-random acceleration molecular dynamics simulations, *J. Chem. Theory Comput.* **2018**, 14: 3859–3869.

[8] Kokh DB, Doser B, Richter S, Ormersbach F, Cheng X, Wade RC. A workflow for exploring ligand dissociation from a macromolecule: Efficient random acceleration molecular dynamics simulation and interaction fingerprint analysis of ligand trajectories*J. Chem. Phys.***2020**, 153: 125102.

# Evaluation of large library selections for high affinity binders by MACS/FACS serial sorts using E. coli surface display.

Tuesday, 22nd June - 18:00: Oral Session - Oral - Abstract ID: 76

***Mr. Luke Parks*** [1], ***Mr. Jacob Clinton*** [1], ***Mr. Charles Dahlsson Leitao*** [1], ***Prof. John Löfblom*** [1], ***Prof. Stefan Ståhl*** [1]

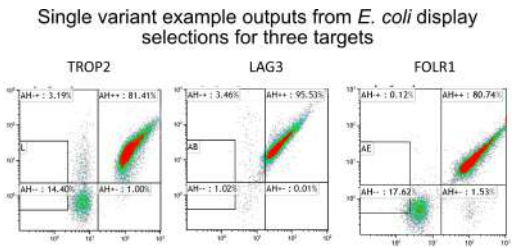
***1.*** *KTH Royal Institute of Technology*

Large library selections have become a cornerstone in the development of high affinity binders through directed evolution. Traditionally, phage display has been used with great success as it can present these large libraries for magnetic capture through MACS. Efficient characterization becomes an issue however, as phage ELISAs do not necessarily correlate with actual affinity and can lead to misrepresentation of results. Therefore, protein expression becomes the required method of elucidating variant properties. While picking enriched clones in theory should be the most efficient method of choosing the best candidates, instances of infrequently occurring clones with the most desirable properties are common. As such, an inordinate number of candidates need protein purification and characterization. Cell surface display mitigates these issues by the inherent nature of single cell evaluation by flow cytometry. Employing a cell based library gives the opportunity to not only rapidly and with high-throughput analyze single clones, but also allows for the specific sorting determination of individual cells by FACS during selections.

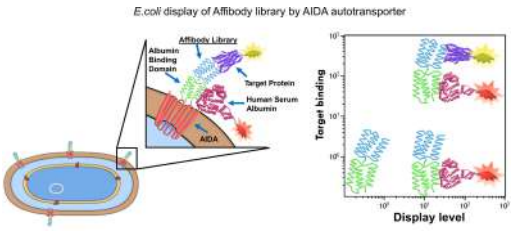
A large *E. coli* surface displayed naïve library of 1.57x10ˆ11 potential affibody variants was constructed. In this design, ABD fused affibodies were brought and mediated through the inner membrane by a signal peptide, and finally displayed on the outer membrane by AIDA-I. While the use of gating in FACS gives governable sorts, library sizes of 5x10ˆ8 are the largest feasible for FACS. Because of this MACS was utilized as an alternative in the first rounds of selection, followed by FACS in later rounds.

Therapeutic and diagnostically relevant protein targets were chosen (TROP2, LAG3, MSLN, FOLR1, and CD155). Starting with a library size of 2x10ˆ11, three rounds of MACS were performed followed by 2 rounds of FACS. The library was measured after every round by flow cytometry to determine enrichment. In the end, the output was plated to produce colonies of individual clones. These were taken and for each of the targets 64 single clones (320 total) were analyzed by flow cytometry over two days. This produced a sufficient coverage of each library output. The best identified variants were proceeded with for production and further analysis. Promising variants were found and will be utilized in additional studies before *in vivo* experiments.

This study highlights the potential for the utilization of *E. coli* display based methods for large libraries. Time and resource saving steps provide motivation for inclusion of cell display in the repertoire of competitive directed evolution methods.




Selection output graphs.png




Surface display and flow cytometry overview.png

# Nano-scale to mesoscale molecular mechanisms in protein protein-A affinity chromatography

Tuesday, 22nd June - 18:15: Oral Session - Oral - Abstract ID: 83

***Dr. Rupert Tscheliessnig*** [1], ***Dr. Goncalo Silva*** [2], ***Dr. Jacek Plewka*** [2], ***Prof. Helga Lichtenegger*** [3], ***Prof. Ana Cristina Mendes Dias Cabral*** [4], ***Prof. Alois Jungbauer*** [5]

***1.*** *Department of Biotechnology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria,* ***2.*** *Austrian Centre of Industrial Biotechnology, Muthgasse 18, 1190 Vienna, Austria,* ***3.*** *Department of Material Science and Process Engineering, University of Natural Resources and Life Sciences, Peter-Jordan Strasse 82, 1190 Vienna, Austria,* ***4.*** *Department of Chemistry, University of Beira Interior, R. Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal,* ***5.*** *University of Natural Resources & Life Sciences*

Protein from Staphylococcus aureus Affinity chromatography is a critical in the purification of antibodies and allows platform processing processes [1, 2]. The adsorption mechanism is complex and, up to date, described phenomenologically at best. Its power cannot be explained by ligand density alone. The morphology of the particles, the number of antibodies bound per ligand, and the spatial structure of the ligands were all evaluated using in-situ SAXS, scanning electron microscopy, and adsorption isotherm measurements. Despite numerous attempts in industry and academia to develop this affinity chromatography material, there is little understanding of ligand delivery available. In our pioneering works [3, 4], we present a systematic method to combine experimental techniques, particularly small-angle X-ray by molecular models. We discussed the tedious takes of data interpretation small density fluctuations and linked these to molecular modes by classical density functional theory and Langevin molecular dynamic simulations. Based on the in-situ measurements and adsorption isotherm tests combined with the extensive molecular modeling, we may infer that the antibody-staphylococcal protein A complex reorient and antibody adaptation to the ligand is one reason for the high binding potential of the TOYOPEARL® AF-rProtein A HC.

We propose experimental and theoretical routes to link experimental data to nanoscopic, mesoscopic modeling and propose molecular models that are suitable to rationalize the design of chromatographic media for protein A chromatography.

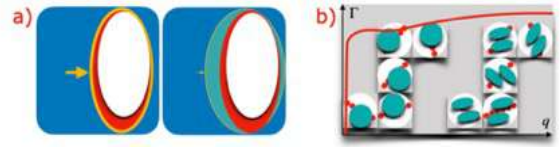
*Figure a) The bare resin is characterized by ellipsoid pair density distributions, left row. When antibodies saturate the resin, the pair density changes for a more spherical one. Water's surface energy is reducing. b) We suggest a two-state adsorption model based on our comprehensive studies on pair density fluctuations.*

[1] A.A. Shukla, J. Thömmes, Recent advances in large-scale production of monoclonal antibodies and related proteins, Trends in Biotechnology 28(5) (2010) 253-261.

[2] A.A. Shukla, B. Hubbard, T. Tressel, S. Guhan, D. Low, Downstream processing of monoclonal antibodies-Application of platform approaches, Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 848(1) (2007) 28-39.

[3] J. Plewka, G.L. Silva, R. Tscheließnig, H. Rennhofer, C. Dias-Cabral, A. Jungbauer, H.C. Lichtenegger, Antibody adsorption in protein-A affinity chromatography – in situ measurement of nano-scale structure by small-angle X-ray scattering, Journal of Separation Science 41(22) (2018) 4122-4132.

[4] G. L. Silva, J. Plewka, H. Lichtenegger, A.C. Dias-Cabral, A. Jungbauer, R. Tscheließnig, The pearl necklace model in protein A chromatography: Molecular mechanisms at the resin interface, Biotechnology and Bioengineering 116(1) (2019) 76-86. https://doi.org/10.1002/bit.26843.




Picture1.jpg

# Generation and characterization of Affibody affinity probes to Ephrin B3 in non-small cell lung cancer

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 29

***Dr. Johan Nilvebrant*** [1]

***1.*** *Div. of Protein Engineering, Dept. of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH - Royal Institute of Technology, SE-106 91 Stockholm, Sweden*

Introduction

Molecular probes allowing *in vivo* imaging to follow growth and therapy response in Non-Small Cell Lung Cancer (NSCLC) are urgently needed as targeted therapy gives heterogeneous response and tumor localization frequently impairs repetitive biopsy procedures. The Ephrin and Eph growth factor receptor system has recently attracted interest as a putative source of therapeutic and imaging targets in different tumor types. Here we have developed anti-Ephrin B3 affibody binders, aimed toward future imaging agents for NSCLC.

Methods

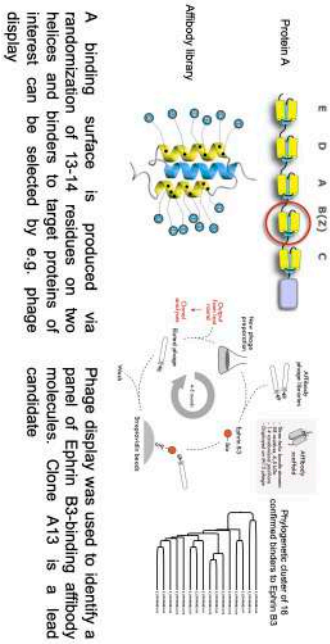
Small 58 amino acid three-helix affibody binders to Ephrin B3 were selected from a combinatorial library using phage display technology and thoroughly characterized.

Results and discussion

Biosensor assays of the binders to Ephrin B3 protein revealed a lead candidate denoted $Z_{EPHRINB3:A13}$ with low nanomolar affinity for both human and mouse Ephrin B3 protein. $Z_{EPHRINB3:A13}$ also showed a high Ephrin B3 selectivity when assayed against a panel of other Ephrins. $Z_{EPHRINB3:A13}$ was fluorescently labeled and used to profile NSCLC cell lines with different Ephrin B3 expression levels. Results revealed binding of $Z_{EPHRINB3:A13}$ in all cell lines but with different degree of binding. Experiments using Ephrin B3 siRNA-treated NSCLC EGFR-mutant PC-9 cells demonstrated that $Z_{EPHRINB3:A13}$-binding was specific for Ephrin B3. Moreover, $Z_{EPHRINB3:A13}$was found to also bind PC-9 cells post EGFR-TKI erlotinib or osimertinib treatment indicating a potential clinical utility for monitoring treatment response and refractoriness.

Impact

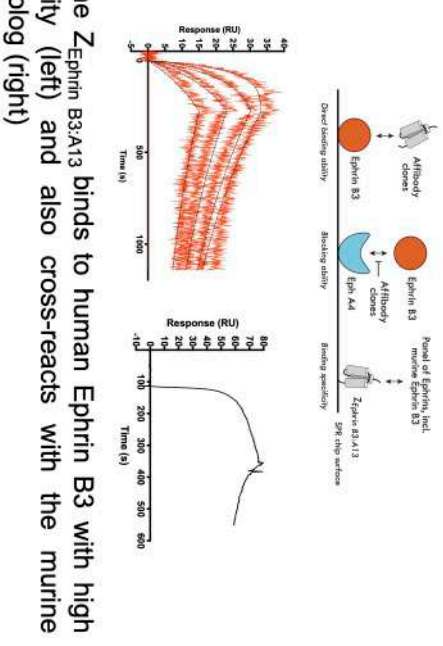
We report that affibody-based targeting of Ephrin B3 holds potential for future molecular imaging of NSCLC in a clinical setting.




A binding surface is produced via randomization of 13-14 residues on two helices and binders to target proteins of interest can be selected by e.g. phage display

Phage display was used to identify a panel of Ephrin B3-binding affibody molecules. Clone A13 is a lead candidate

Picture 1. affibodies and phage display.jpg

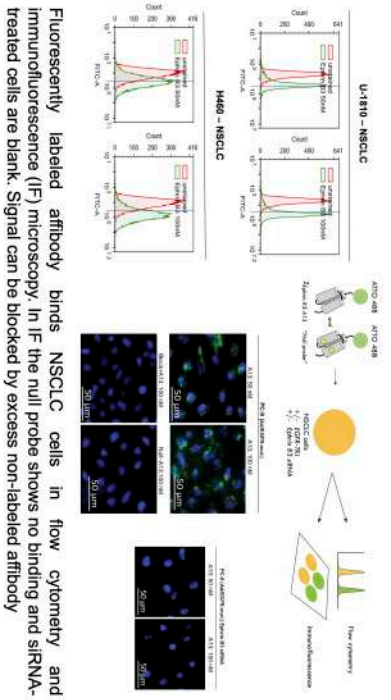



Clone $Z_{Ephrin\ B3:A13}$ binds to human Ephrin B3 with high affinity (left) and also cross-reacts with the murine ortholog (right)

Picture 2. in vitro binding assays.jpg

All alanine mutants retained secondary structure and stability. A few substitutions resulted in significant loss of binding to Ephrin B3 and when combined the resulting null-affibody does not show any binding. $Z_{Ephrin\ B3:A13}$ and null-probe were modified with C-terminal cysteine residues for site-specific fluorophore labeling

Picture 3. ala-scan and null-probe.jpg




Fluorescently labeled affibody binds NSCLC cells in flow cytometry and immunofluorescence (IF) microscopy. In IF the null probe shows no binding and siRNA-treated cells are blank. Signal can be blocked by excess non-labeled affibody

Picture 4. flow cytometry and immunofluorescence microscopy.jpg

# An easy-to-use high-throughput selection system for the discovery of recombinant protein binders

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 99

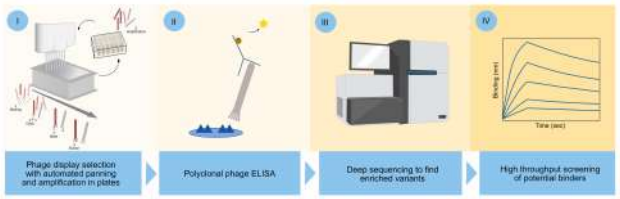
***Ms. Marit Möller*** [1], ***Ms. Louise Larsson*** [2], ***Dr. Magnus Lundqvist*** [2], ***Prof. Sophia Hober*** [3]

***1.*** *KTH,* ***2.*** *Div. of Protein Engineering, Dept. of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH - Royal Institute of Technology, SE-106 91 Stockholm, Sweden,* ***3.*** *Dept. of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH - Royal Institute of Technology, SE-106 91 Stockholm, Sweden*

Phage display is a popular and widely used technique for the discovery of recombinant protein binders from large protein libraries for therapeutic use. The protein library is hereby displayed on the surface of bacteriophages which can be amplified using bacteria, preferably *Escherichia coli*. Traditionally, the so-called panning procedure during which the phages are incubated with the target protein, washed and eluted is done manually thus limiting the throughput. High-throughput systems with automated panning already in use often require high-priced equipment and elaborate programming. We here present an easy-to-use high-throughput selection system which includes automated panning and cost-efficient equipment.

The high-throughput selection system was developed for automated selection in 96-well plate format using a magnetic bead handling device, enabling selection in solution with biotinylated target and streptavidin-coated magnetic beads for the capture. Furthermore, a protocol for the amplification of the phages in 24-well plate format was established, which simplifies the handling and reduces the material costs. Next generation sequencing with Illumina MiSeq of the selection outputs was integrated into the workflow for in-depth analysis of enriched variants. The selection system was tested with two different alternative scaffold protein libraries: The Albumin-Derived Affinity ProTein (ADAPT) library and the Calcium Regulated Affinity (CaRA) library. When comparing the newly established semi-automated workflow standard to the manual workflow, we found that the hands-on-time was drastically reduced while the selection outcome was very similar. Illumina MiSeq analysis showed similar enrichment patterns and the same binder sequences could be found among the top 100 most enriched variants. Additionally, by developing a protocol for high-throughput screening using bio-layer interferometry it was possible to accurately identify binders from lysates making it possible to avoid the time-consuming protein purification step.

The developed selection system has shown to be highly versatile with the potential to be integrated into any lab for the discovery of new protein binders.




High-throughput selection workflow.png

# A biorecognition approach for surface modification of cellulose-based hydrogels using versatile derivatives of carbohydrate-binding modules

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 101

***Dr. Mariana Barbosa*** [1], ***Mr. Hélvio Simões*** [1], ***Prof. Duarte Miguel F. Prazeres*** [1]

***1.*** *iBB - Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Tecnico, Universidade de Lisboa, and Associate Laboratory i4HB - Instituto for Health and Bioeconomy, Avenida Rovisco Pais, 1049-001, Lisboa, Portugal*

Functionalization of cellulose-based matrices with valuable biomolecules can be used to develop high-performance materials with improved functionalities that meet the requirements of specific end applications. The success of such tailored platforms depends, to a large extent, on efficiently master the assembly of relevant molecules into cellulose-based materials in such a manner that the intended functionality is achieved.

In this context, bi-functional biomolecular constructs were developed for the precise modification of cellulose hydrogels with relevant bioactive molecules. Accordingly, distinct recombinant fusions of carbohydrate binding modules (CBMs) and important biological entities were produced and purified resorting to biomolecular engineering techniques. In particular, CBM-based fusions were envisioned to enable the bridging of proteins, peptides, or oligonucleotides with cellulose hydrogels. The work focused on constructs that combine a family 3 CBM derived from the cellulosomal scaffolding protein A from *Clostridium thermocellum* (CBM3) with i) an N-terminal green fluorescent protein (GFP) domain (GFP-CBM3), (ii) a double Z domain that recognizes IgG antibodies, and iii) a C-terminal cysteine (CBM3C), to covalently link DNA strands or antimicrobial peptides (AMP). Pull-down assays were used to assess the ability of the CBM3 fusions to efficiently bind and/or anchor their counterparts onto the surface of cellulose hydrogels. Capture of GFP-CBM3 by cellulose was first demonstrated qualitatively by fluorescence microscopy. The binding of the fusion proteins, the capture of antibodies (to ZZ-CBM3) and the grafting of an oligonucleotide and an AMP (to CBM3C) were successfully demonstrated. Additionally, in vitro antimicrobial activity assays revealed that AMP-cellulose hydrogels exhibited potent bactericidal and antibiofilm activities against clinically relevant pathogens.

The bioactive cellulose platform described herein enables the exact anchoring of diverse biomolecules onto cellulose-based hydrogels and holds great promise towards development of effective and innovative medical diagnostic sensors or highly specialized biomaterials, such as antimicrobial materials, with potential biomedical interest.

# A novel Staphylococcal protein A based ligand: production, characterization and application

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 78

***Dr. Anna Schneider*** [1], ***Dr. Martin Kangwa*** [2], ***Prof. Hector Marcelo Fernández-Lahore*** [3]

***1.*** *Postdoctoral Fellow,* ***2.*** *Visiting Researcher,* ***3.*** *Associate Professor*

Due to constantly growing demand on monoclonal antibody production for therapeutic and research uses, antibody production has increased drastically over the past decades. Consequently, the high product requirements and standards require efficient production and purification techniques. In this study, in antibody purification, the affinity interactions of the engineered AviPure - ligand with a range of monoclonal antibodies (mAbs) and various fragments of crystallization (Fc) fragments were analyzed.

The affinity chromatography has been used with Staphylococcal Protein A (SpA) as main ligand. A novel SpA (AviPure thereafter) has been presented as a synthetic ligand analogue based on native SpA B domain, with a molecular weight of approximately 14 kDa.

A better understanding of the affinity nature of the interaction between mAbs to AviPure can help to guide in the design of enhanced synthetic ligands for mAbs purification. The interaction binding affinity of AviPure-IgG was evaluated using Surface Plasmon Resonance (SPR). Different experimental variants were chosen between IgG and selected ligands (Fig.1): A) AviPure with IgG, B) rSpA with IgG (control), C) AviPure with CHO-IgG, D) AviPure with IgG variants.

Based on the experimental results, it was shown, that AviPure can easily be immobilized on an adsorbent via the N-terminus or the cysteine-containing C-terminal. Additionally, higher capacity can also be achieved through novel design by evenly spacing and varying the number of binding domains and ligand.

The equilibrium dissociation constant ($K_D$) between the AviPure and mAbs was systematically measured using 1:1 (Langmuir) model and found to be $4.7\times10^{-8}$ M, with constant of dissociation at $k_d \leq 1.0\times10^{-3}$ $s^{-1}$ and $k_a$ being $3.1\times10^{4} M^{-1} s^{-1}$. When immobilized on Sepharose, the AviPure ligand density was 429 nmol/g moist weight resin and was able to effectively bind immunoglobulin and Fc fragment samples with higher affinity and the most effective flow rate when using ligand - Sepharose beads was at 75 cm/h giving the dynamic binding capacity of 53 mg/mL and 91% recovery of IgG.

Suitable ligands used in affinity purification should have a $K_D \leq 10^{-6}$M and a dissociation rate ($k_a$) averaging $10^{-3} M^{-1} s^{-1}$ with the $k_d$ ranging between $10^{3} – 10^{8} M^{-1}$. Therefore, the AviPure ligand can be used as an alternative to the standard protein A ligand in the purification of mAbs and Fc-fused proteins, which has the potential to address the demand for a more innovative, cost-effective mAb purification technology.

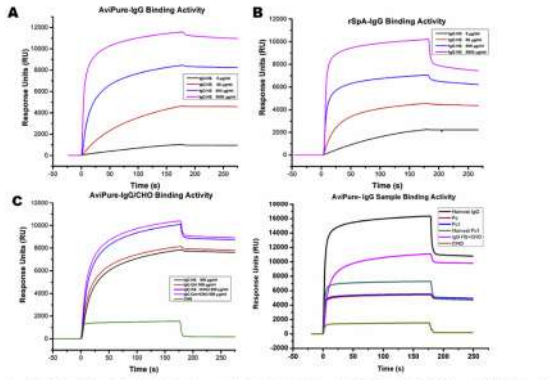



Fig. 1. An overlay of the SPR signals from the interaction between immunoglobulin and the selected ligands. A) AviPure with IgG, B) rSpA with IgG, C)AviPure with CHO-IgG, D) AviPure with IgG variants.

Fig.1.png

# Enhancing VOCs selectivity by designing new e-nose sensing materials for gas sensing

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 94

***Ms. Rita Oliveira*** [1], ***Mr. Gonçalo D.G. Teixeira*** [1], ***Mr. Gonçalo Santos*** [1], ***Ms. Cláudia Alves*** [1], ***Prof. Ana Cecília A. Roque*** [1]

***1.*** *Chemistry Department, NOVA School of Science and Technology, Campus Caparica, 2829-516 Caparica, Portugal*

Artificial olfaction mimics the sense of smell through the use of electronic nose devices (e-noses) that include an array of gas sensors associated with signal-processing tools. Those arrays include sensing materials which composition can be manipulated to provide recognition of extremely small targets as volatile organic compounds (VOCs). Proteins and peptides can be coupled to gas sensors to increase selectivity towards specific VOCs.

In this work we studied the incorporation of peptides onto gas sensing materials based on ionogels and hybrid gels. As a model, we selected the P1 peptide known to perform single carbon discrimination of benzene among its derivatives[1]. We designed two modified versions of P1 (P2 and P3 peptide) by adding non-canonical amino acids at the C-terminal with the purpose of promoting the location of the peptide in specific compartments of the hybrid gels.

Ionogels containing ionic liquid and the peptides within a gelatin matrix were spread in Gold Interdigitated Electrodes. We observed significant improvement of volatiles discrimination from P2 and P3 sensors, especially towards hexane and xylene, respectively.

Regarding the hybrid gels, we successfully incorporated the peptides into hybrid gels, and observed that P2 and P3 changes the morphology of the droplets within the hybrid gel sensors, through POM analysis. By taking advantage of the hybrid gel organization containing liquid crystal droplets, it was possible to assess the optical properties of our sensors. We found that not only the P1-hybrid gels were able to achieve a better performance regarding the overall classification of the volatiles, but also that it provided an enhanced discrimination towards benzene – and also hexane – as intended. As for the derivatives of P1 peptide hybrid gels, they presented a great discrimination towards ethanol.

1. Ju, S. *et al.* Single-carbon discrimination by selected peptides for individual detection of volatile organic compounds. *Sci. Rep.* **5**, 9196 (2015).

# The impact of N-glycans on the affinity of IgA2 and their implications on receptor binding

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 107

***Ms. Valentina Ruocco*** [1], ***Dr. Rupert Tscheliessnig*** [2]

***1.*** *BOKU,* ***2.*** *Department of Biotechnology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria*

The N-glycan in the Fc domain of IgGs plays a crucial role in the protein structure and modulates the affinity and binding to specific receptors [1]. Comparatively little is known about how N-glycans impact the protein affinity of IgAs. Our research hypothesis rests on a possible analogy in behavior between IgAs and IgGs N-glycans to affect the stability and conformation of IgA2 and that IgA2 N-glycans influence the affinity of FcRL4-receptor binding. In the presented work we focus on the form, structure of N-glycosylation of IgA2. We point out that the knowledge of glycan patterns of IgA2 will contribute to make them a new promising therapeutic class of antibody. Influencing IgA functions can provide several therapeutic possibilities, like IgA vaccination or IgA antibodies in cancer treatment. In addition, the usage of a glycoengineered plants helps to overcome the immunogenicity, benefiting from plants' undoubted strengths: rapidity, low production costs and safety, relevant bio economic aspects to fight the current SARS-CoV-2 pandemic in the global south. We screen different glycosylation patterns and elucidate the conformation in solution as well as the conformation and the affinity bound to the receptor FCRL4 by small-angle X-ray scattering (SAXS), surface Plasmon Resonance (SPR) and molecular modelling (MM). The IgA2 presented are SARS-CoV-2 neutralizing monoclonal antibodies: S309 and H4. They take an essential part in the molecular mechanism of the current SARS-CoV-2 pandemic. We express them in a glycoengineered line of *Nicotiana benthamiana*, able to generate human-type complex N-glycans; the human FcRL4-receptor is produced in HEK293 cells. Comparable approaches [2] elucidated the conformational characteristics of different IgG domains comparing the SAXS profile of glycosylated and de-glycosylated Fc region with profiles theoretically calculated from crystal structures with varying domain orientations. But they lack a detailed description of the molecular mechanism relevant for the particular affinity of IgA2 and FCRL4 receptor. We discuss SAXS measures on IgA2s in different glycoforms and complexed with the particular affinity receptor. Changes in the conformation of the different isoforms are addressed to N-glycans-induced modification, their characteristic affinity is discussed.

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# A Methyl Substituent Impacts on MIP-Morphology and Recognition - AA versus MAA

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 35

***Dr. Kerstin Golker*** [1], ***Dr. Gustaf Olsson*** [1], ***Prof. Ian Nicholls*** [1]

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Molecularly imprinted polymers (MIPs) are recognition materials widely used in sensing- and separating protocols. These materials can display pre-determined affinity and selectivity towards a target molecule of a degree normally expressed by their biological counterparts such as enzymes and antibodies.[1] To date, methacrylic acid (MAA) is the most often reported functional monomer in MIP-protocols using the non-covalent strategy, which raises the following questions:

1. Why has MAA the dominance it does in the molecular imprinting of small organic structures?
2. Is there a physical basis for MAA's apparent superiority to other functional monomers, not least other carboxylic acid containing functional monomers?

We here compared and contrasted MAA with the closely related acrylic acid (AA) in terms of polymer morphologies, recognition characteristics, and molecular level events in the corresponding pre-polymerization mixtures in a series of bupivacaine-imprinted co-polymers.[2] AA was considered of particular interest on account of its strong similarity to MAA, differing only by virtue of a methyl substituent. The effects were examined using full-scale molecular dynamics (MD) simulations of pre-polymerization mixtures, equilibrium rebinding studies on corresponding synthesized polymers and morphology characterization through nitrogen sorption measurements.

While results from MD simulations point to higher hydrogen bonding interaction frequencies in the pre-polymerization mixtures between AA and bupivacaine than between MAA and bupivacaine results from binding studies demonstrate lower binding capacities for the AA-polymers compared to the MAA-polymers. Further, the surface areas and pore volumes of the AA-polymers were on overall higher than those of the MAA-polymers. The analysis of the pore structures indicates a high fraction of ink-bottle shaped pores in the AA-polymers in contrast to slit-shaped pores in the MAA-polymers. The differences in binding behavior may therefore result from the differences in morphology, in particular pore-shape, affecting mass transfer and analyte access.

An explanation may be found in the reaction kinetics of these functional monomers that are commonly used in MIP protocols. We suggest that AA undergoes auto-accelerated polymerization as the lack of the methyl group present in MAA facilitates attack of the radical center. Monomer reactivity is an issue seldom considered in the design of MIP systems, nor the consequences of the kinetics of reaction on morphology and its influence on binding. The results presented highlight the complexity of the polymerization process and may lead to consider new interpretations of the mechanisms underlying recognition in other imprinted polymer systems.

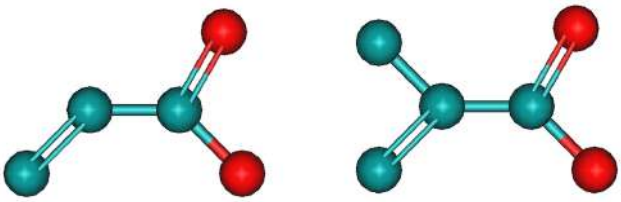


Figure 1. the impact of a methyl substituent on mip-performance was studied by comparing maa right structure with aa left structure .jpg

# Magnetically controlled drug delivery: Interaction of a cationic antimicrobial peptide with bare iron oxide nanoparticles.

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 3

***Ms. Chiara Turrina*** [1], ***Dr. Sebastian Schwaminger*** [1]

**1.** *Technical University of Munich, Bioseparation Engineering Group*

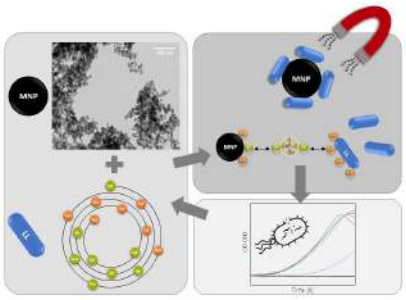
Magnetically controlled drug delivery is a new promising method for administering a pharmaceutical compound. It gives the opportunity for selective and targeted drug delivery. By the application of an external magnetic field, drug-loaded magnetic nanoparticles can be localized at the target side. Due to their huge surface area, superparamagnetic iron oxide nanoparticles (SPIONs) have the potential to carry a large drug dose that leads to a high local concentration at the target side. This provides a promising method for cancer treatment since the strong and harmful side effects of conventional chemotherapy can be reduced by lowering the dose of cytotoxic drugs. The SPIONs offers a non-remanent magnetic behavior at room temperature, non-toxicity and low-cost production. Particle characterization is made with infrared spectroscopy, transmission electron microscopy, X-ray diffraction, zeta potential measurements and the superconducting quantum interference device.

For our research, we chose the short cationic peptide lasioglossin isolated from bee's venom as a potential drug. It belongs to the group of antimicrobial peptides that are an alternative to conventional antibiotics. The peptide is not only showing high antimicrobial behavior against gram-positive and gram-negative bacteria, but it is also low haemolytic, thus shows a potency to kill various cancer cells [1].

Adsorption and desorption behavior of lasioglossin on bare nanoparticles is analyzed under various conditions. Considering the influence of the pH value, the buffer, the particle concentration-time and temperature. With photometric measurements, the drug loading of 23% is determined. The influence of the peptide binding is analyzed with infrared spectroscopy, dynamic light scattering and zeta potential measurements. Growth studies with *Escherichia coli*of the drug-loaded particles show high antimicrobial activity, determining that the peptide is still active when it is bound to the particles.

The work shows that binding and release of lasioglossin to SPIONs is possible due to reversible binding, while also the antimicrobial activity is preserved. Even if many more aspects need to be analyzed hereafter this work forms the basis for future usage of cationic antimicrobial drugs or similar peptide-based drugs in magnetically controlled drug delivery based on electrostatic-binding.

[1] Chem. Bio. Chem. 10 (2009) 2089




Graphical abstract.jpg

# Amino acid-based hybrid blocks for biommimetic protein imprinting

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 23

***Dr. Raquel Gutiérrez-Climente*** [1], ***Dr. Giang NGO*** [2], ***Mrs. Margaux Clavié*** [1], ***Dr. Jérémie Gouyon*** [1], ***Dr. Yoann Ladner*** [1], ***Prof. Catherine Perrin*** [1], ***Prof. Pascal Etienne*** [3], ***Prof. Pierre Martineau*** [2], ***Prof. Ahmad Mehdi*** [4], ***Prof. Martine Pugnière*** [2], ***Prof. Gilles Subra*** [1]

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By carrying out the polymerization in presence of a target molecule (Scheme), molecular imprinting offers the possibility to create biomimetic synthetic materials with molecular recognition properties similar to natural receptors in terms of affinity and selectivity[1,2]. Those materials are actually referred as MIP (Molecularly Imprinted Polymers) and they were first developed in silica matrix. At the beginning of the 1970s, the bigger versatility in terms of organic functional monomers triggered the transition to organic polymerization approaches[3]. Nevertheless, when complex and fragile templates such as protein are selected, the use of an inorganic polymerization approach presents several advantages compared with the traditional protocols of organic MIPs. For example, the sol-gel chemistry is performed under protein-compatible conditions in terms of solvent (water instead of traditional low polarity organic solvents) and temperature (room temperature instead of traditional 60°C or higher). It offers, therefore, a simple, versatile, cost-effective, biocompatible, and environmentally friendly alternative to organic MIPs.

However, two main challenges have to be tackled to get protein MIPs by sol-gel approach: the pH of the reaction and the lack of diversity of functional monomers[4]. We propose then a biomimetic approach using silylated amino acids (AA) as hybrid functional monomers. The basic protein cytochrome C (p.I 9.6) was chosen as a model to prepare MIPs on the surface of silica core-shell magnetic particles. We compared the efficiency of the MIPs obtained by polymerization of common cationic and hydrophobic precursors (3-aminopropyltriethoxysilane and n-propyltriethoxysilane) with the ones obtained from functional monomers mimicking the arginine and leucine. At last, we used tyrosine and serine monomers which are the most common AA found in antibody epitope for protein recognition[5]. The efficacy of all the types of MIPs was assessed by quartz-crystal microbalance (QCM-D). The promising results obtained by this biomimetic approach based on inorganic-bioorganic polymers, open the doors for the development of imprinted devices for specific protein recognition.

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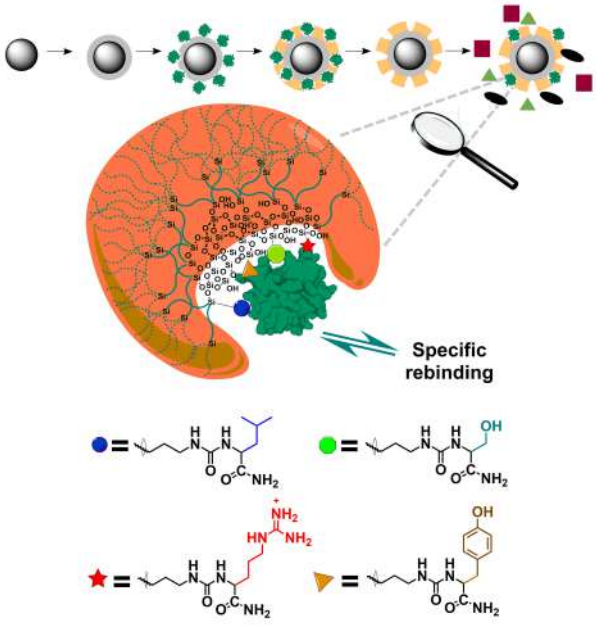


Graphical abstract10.png

# Rational selection of odorant-binding proteins for gas sensing applications

Tuesday, 22nd June - 18:30: Flash Session 2 - Poster - Abstract ID: 93

***Mr. Gonçalo D.G. Teixeira*** [1], ***Ms. Joana R.S. Calvário*** [1], ***Dr. Artur J. Moro*** [1], ***Prof. João C. Lima*** [1], ***Dr. Arménio J.M. Barbosa*** [1], ***Prof. Ana Cecília A. Roque*** [1]

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Electronic-nose devices simulate the human olfactory system, allowing the detection of Volatile Organic Compounds (VOCs), and the application in several fields, from rapid disease diagnostics to the detection of contaminated food, and air quality control. These devices comprise an array of sensors composed by different sensing materials. Incorporating Odorant Binding Proteins (OBPs) into gas sensing materials can increase VOC recognition and selectivity. OBPs are small soluble proteins found in vertebrates and insects, responsible for VOCs solubilization and transportation in the respiratory channel. OBPs variability in nature, thermal stability and ability to recognize different compounds make them great biological candidates to implement in gas sensing devices.

In this work, we propose the rational selection of 8 OBPs with unique and distinct characteristics for further improvement of VOC molecular recognition in gas sensing. The selection process was based in the development of a database with 39 available OBP structures, comprising their biochemical information (e.g. binding pocket size and composition), as well as the binding constants to experimentally tested ligands. Moreover, specific thresholds were used in the developed database to complete the selection process. Therefore, the 8 OBPs presented selectivity towards different chemical classes and VOC structures, variability in their binding pocket dimensions, and a set of 16 or more experimentally tested VOCs. In addition, complementary computational studies were performed to study the structural conformations of the selected OBPs, such as molecular dynamics simulations. We also report the production and purification of some of these proteins. In particular, OBP3 from rat (*R. norvegicus*) was selected to develop a proof-of-concept for its incorporation in a gas sensing system. OBP3 characterization was carried on using western blot, circular dichroism (Tm = 71,1±1,1ºC) and fluorescence displacement against 2,6-diisopropylphenol with 1-aminoanthracene fluorophore (Kd = 0.24 μM).

Overall, we highlight the selection process of 8 OBPs for gas sensing applications, which show variable binding, structural and selective characteristics, providing a new selective sensor array for VOC detection. Significant outcomes reported in OBP3 proof-of-concept, such as the easy production, purification and high thermal stability, support OBPs good adaptability in gas sensing systems.

# Strategies that enhance SLiM binding to EVH1 domains

Tuesday, 22nd June - 19:15: Keynote 4 - Oral - Abstract ID: 47

***Prof. Amy Keating*** [1]

***1.*** *MIT*

Like many other modular peptide recognition domains, EVH1 domains bind to short, linear motifs (SLiMs) in their partner proteins. EVH1 domains in the actin-polymerizing Ena/VASP protein family are important for proper subcellular localization. For example, these domains direct family members to lamellipodia and filopodia, where they promote cell motility and invasiveness. The Ena/VASP EVH1 domains bind a 5-residue SLiM that adopts a polyproline-II helix. The sequence preference of the motif can be summarized as [FWYL]Px(hyd)P, with hyd a hydrophobic residue. Because there are more than 5,000 instances of this motif in the human proteome, we wondered whether additional features of the motif-flanking sequence are also relevant for EVH1 molecular recognition. To answer this question, we conducted a whole-proteome screen for 36-residue binders of the EVH1 domain of ENAH. We identified many motif-containing hits, and biophysical analysis of selected partners revealed three mechanisms by which SLiM-flanking sequence can modulate peptide binding affinity. The highest affinity peptide ligand that we identified revealed a novel conformational specificity mechanism. We used this mechanism as the basis for designing the tightest and most selective known binder of ENAH, which is considered a challenging-to-inhibit prospective therapeutic target for invasive breast cancer. Given that SLiMs encode limited information, it is likely that most SLiM-binding proteins have evolved strategies for achieving heightened affinity and specificity, as we found in this work. Screening and analysis approaches like those we applied to ENAH in this study provide a route to efficiently discover new interaction modes and refine our understanding of broadly important domain-SLiM interactions.

# Intelligent gels for artificial olfaction

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 111

***Ms. Carina Esteves*** [1], ***Dr. Susana Palma*** [1], ***Dr. Efthymia Ramou*** [1], ***Ms. Rita Oliveira*** [1], ***Mr. Guilherme Rebordão*** [1], ***Mr. Joao J M Santos*** [1], ***Ms. Rita Alves*** [1], ***Prof. Ana Cecília A. Roque*** [1]

***1.*** *UCIBIO, Chemistry Department, School of Science and Technology, NOVA University of Lisbon*

Artificial olfaction is an emerging field aiming to mimic natural olfactory systems. Olfactory systems rely on a first step of molecular recognition where volatile organic compounds (VOCs) bind to an array of specialized olfactory proteins. As a result, electrical signals are transduced to the brain where pattern recognition is performed. An efficient approach in artificial olfaction combines gas-sensitive material arrays with dedicated signal processing and classification tools.

Supramolecular self-assembly provides the possibility to generate modular and tunable materials with stimuli-responsive properties. This field is attracting enormous interest as an approach to functional materials design, and has tremendous, mostly untapped opportunities in creating new types of sensors. We have recently developed the concept of hybrid gels (Hussain *et al.*2017). These materials result from the combination of functional components – liquid crystals for reporting, ionic liquid as solvent, biopolymer as matrix - which give rise to molecular recognition properties not seen in the individual components. Each component has its own role, yet in combination, they provide a molecular environment and compartmentalization that provides the selectivity required for sensing. When casting the hybrid gels as thin films, they exhibited dual optical and electrical stimuli-responsive properties in the presence of VOCs. In this work, films of hybrid gels were studied as gas sensing materials in a custom-built electronic nose. Several features were extracted from the signals obtained upon VOC exposure, and then used to implement a dedicated automatic classifier based on support vector machines for data processing (Esteves *et al.*2019). As an optical signature could be associated to each VOC, the developed algorithms classified eleven distinct VOCs with high accuracy and precision (higher than 98%), using optical signals from a single film composition [3]. This shows an unprecedented example of soft matter in artificial olfaction, where gelatin hybrid gels can provide enough information to accurately classify VOCs with small structural and functional differences. Furthermore, we show that the developed device can be used in the quantification of ethanol in automotive fuel (Hussain *et al.*2017) or in fish spoilage monitoring (Semeano *et al.* 2018). The versatility shown by the developed opto-electronic gas sensing materials opens a wide range of applications in different areas such as medical diagnostics, food or agriculture.

# Glycolipid biosurfactants: alternative methodologies for separation and identification.

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 27

***Dr. Maïssa Dardouri*** [1], ***Ms. Rita Mendes*** [1], ***Mrs. Johannes Friezel*** [2], ***Prof. Judite Costa*** [1], ***Prof. Isabel Ribeiro*** [1]

***1.*** *Research Institute for medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Avenida Prof. Gama Pinto, 1649-003, Lisboa, Portugal.,* ***2.*** *Pharmaceutical Institute, Rheinische Friedrich-Wilhelms-Universität Bonn, An der Immenburg 4, 53121 Bonn, Germany.*

Glycolipid biosurfactants are surface-active molecules and among them sophorolipids (SLs) and rhamnolipids (RLs) have been at the center of scientific and industrial research. They have gathered the attention not only by their physicochemical properties but also by their anti-biofilm, anti-microbial, low toxicity, and biodegradability properties making them very promising molecules for different applications. These versatile properties arise from the different structures of biosurfactants but since they are produced as mixtures this represents a drawback towards specific applications in pharmaceutical, cosmetics and biomedical fields. This work, aims the separation and purification of SLs and RLs, by improving the developed methodologies. A reverse phase C18 solid phase extraction (SPE) showed a selective isolation and purification of the biosurfactants C18:0 and C18:1 lactonic diacetylated SLs, with a purity of 85.7% and 94% respectively. Moreover RhaC8:0C8:0, RhaRhaC8:0C12:0, RhaRhaC10:0C10:0 and RhaC10C10:1 RLs congeners were obtained with a purity of 99.56%, 89.9%, 86.6% and 81.2% respectively. Additionally, eight SLs and thirteen RLs congeners were identified by an alternative UPLC-MS/MS fast method. Hence, these optimized methods revealed a good alternative for the laborious, time consuming and the toxic conventional ones used for the glycolipids' separation and purification and could in fact easy the hard work of the researcher teams focused on the biosurfactants' production and purification.
**Acknowledgments**: Portuguese government, Fundação para a Ciência e Tecnologia (FCT) for PTDC/BTM-SAL/29335/2017, UIDB/04138/2020 and UIDP/04138/2020 (iMed.ULisboa) projects financial support.

# Elucidating the interaction of lactoferrin with V-ATPase towards understanding its mechanisms of action

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 58

***Mrs. Cátia Santos-Pereira*** [1], ***Mrs. Juliana F. Rocha*** [2], ***Mr. Henrique S. Fernandes*** [2], ***Prof. Lígia R. Rodrigues*** [3], ***Prof. Manuela Côrte-Real*** [4], ***Dr. Sérgio F. Sousa*** [2]

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**Introduction**: Lactoferrin (Lf), a bioactive milk protein, exhibits strong anticancer and antifungal activities [1,2]. The search for Lf targets and mechanisms of action is of utmost importance to enhance its effective applications. A common feature among Lf-treated cancer and fungal cells is the inhibition of a proton pump called V-ATPase. Lf-driven V-ATPase inhibition leads to cytosolic acidification, ultimately causing cell death of cancer and fungal cells [2–4]. Given that a detailed elucidation of how Lf and V-ATPase interact is still missing, in this work we aimed to fill this gap by employing a multi-level computational approach.

**Methods**: Molecular dynamics (MD) simulations of both proteins were performed to obtain a robust sampling of their conformational landscape, followed by clustering and protein-protein docking with the Haddock software. Subsequently, MD simulations of the docked complexes and binding free energy calculations were carried out to evaluate the dynamic binding process and build the final ranking.

**Results & Discussion**: This computational pipeline yielded a set of V-ATPase-Lf complexes that had Lf consistently docked in the same domain of V-ATPase, allowing to unravel the putative mechanism by which Lf inhibits V-ATPase and the identification of key binding residues.

**Impact**: Our results will certainly aid in the rational design of follow-up experimental studies, bridging in this way computational and experimental biochemistry.

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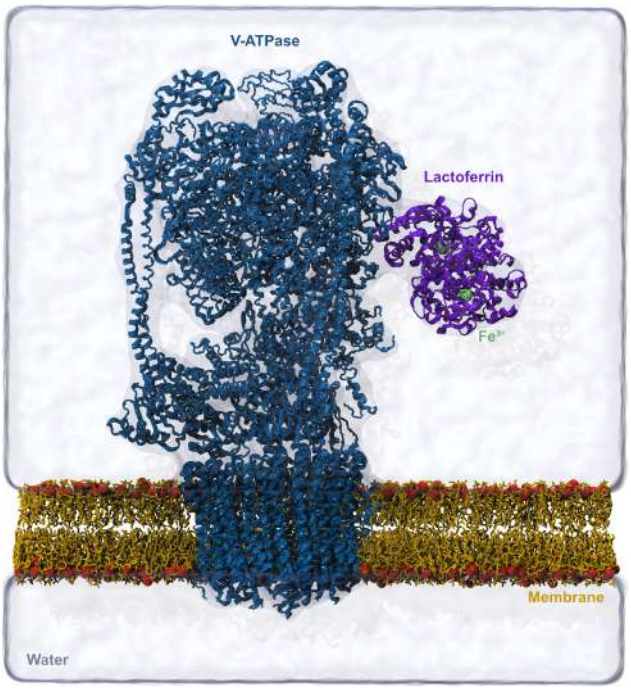


Graphical abstract.jpg

# Conformation of cellobiose dehydrogenase determined by small angle X-ray scattering

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 81

***Mrs. Bettina Motycka*** [1], ***Dr. Daniel Kracher*** [2], ***Dr. Roland Ludwig*** [1], ***Dr. Rupert Tscheließnig*** [1]

***1.*** *BOKU-University of Natural Resources and Life Sciences,,* ***2.*** *TU Graz*

Fungi are the most efficient wood degrading organism known. They are mainly using oxidoreductases, such as cellobiose dehydrogenase (CDH). It is a dynamic electron transfer enzyme present in the secretomes of many biomass-degrading fungi. CDH consists of a catalytic FAD-containing dehydrogenase domain (DH), tethered to a mobile heme *b*-containing cytochrome domain (CYT). Its native function is the reductive activation of the polysaccharide-degrading lytic polysaccharide monooxygenases (LPMOs) via the mobile CYT domain.[1] This bi-enzymatic system is essential for fungal biomass degradation and has potential applications in industrial biofuel production.

While a condensed structure of CDH is thought to favor interdomain electron transfer to the heme *b*, spatial separation of DH and CYT is required to interact with LPMO.[1] It is hypothesized that the orientations of the CDH domains are modulated by different ambient conditions, such as ionic strength and pH.

Using small angle X-ray scattering (SAXS), we elucidated the CYT and DH domain's conformation and interaction in CDH from *Crassicarpon hotsonii*and *Neurospora crassa* in solution. The idea was to determine the preference of the closed- and open-sate conformation of CDH regarding pH, ion concentration and oxidized/reduced cofactors. Our results suggest a difference in the conformation by changing the pH and that the domains are further apart in the presence of ions, but it seems to be in a more condensed structure when the CDH is in the reduced state. However, out of this result, we cannot make a structure prediction regarding the position of the two domains.

To have a reference how the scattering signal of a closed conformation looks like, we performed an amino acid substitution on both domains to introduce cysteines that offer an accessible SH group that can anchor a Maleimide-$PEG_x$-succinimidyl ester linker with a defined PEG length. By this bioengineering approach, we enforce the closed conformation of CDH irrespective of the protein environment. The Maleimide-PEG linker acts like a string that enforces constant distances of the two domains. With this approach, we will determine the cause of changes in the scattering signal regarding the conformational changes. The results will be essential to optimize biomass hydrolysis, biofuel production and develop LPMO-based biosensors for analytical applications.

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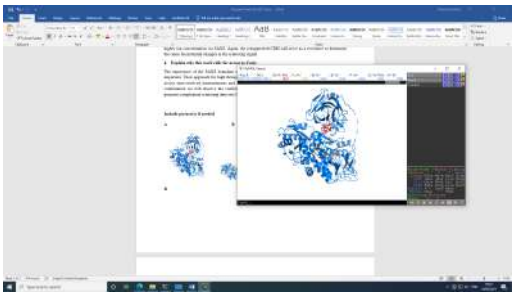
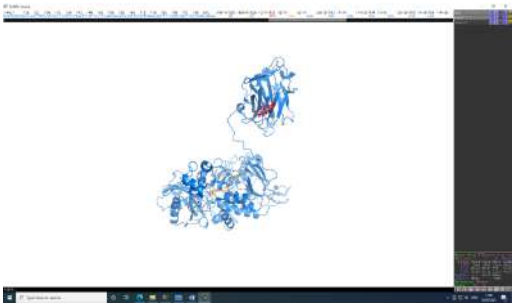
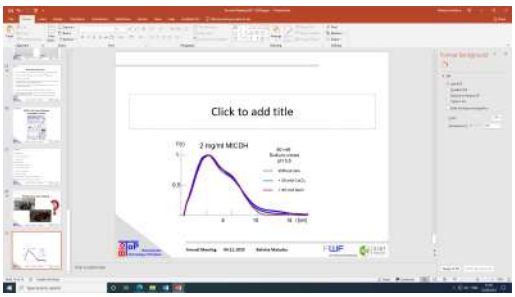


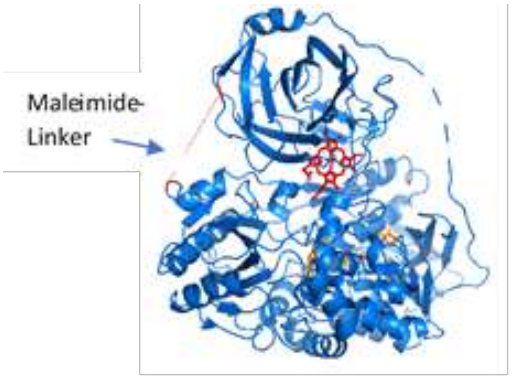
Cdh in the closed state.png



Cdh in the open state.png




Result pddf of mtcdh under different conditions.png




Shows where the cross-linking takes place.png

# In silico methodology for the identification of new drugs against biofilm formation in P. aeruginosa

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 77

***Ms. Tatiana Vieira*** [1], ***Ms. Rita Magalhães*** [1], ***Dr. Sérgio F. Sousa*** [1]

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*Pseudomonas aeruginosa* is an opportunistic Gram-negative pathogen. It causes acute and chronic infections especially in immune-compromised and hospitalized patients [1]. Infections caused by *P. aeruginosa* are very difficult to eradicate because the bacteria can be organized in structured microbial communities forming a biofilm. Biofilms are structured microbial communities of surface-attached cells embedded in a self-produced matrix of extracellular polymeric substances (EPS) [2] . They can be formed in a variety of environments and surfaces, such us living tissues, medical devices, industrial/potable water systems piping. They are responsible for 80% of all bacterial infections each year, in the US alone [3,4]. Therefore, controlling biofilm formation and development is of the utmost importance.

A biofilms structural database was created to quickly assess all the structural information on different protein structures involved in biofilm formation, development, and virulence available[5].

Here we report the optimization of a methodology using docking and virtual screening to identify new drugs against a specific quorum-sensing system in *P. aeruginosa*responsible for the transcription of virulence genes and biofilm development, the PQS system. Four large databases of compounds (such as IBS InterbioScreen, Mu.Ta.Lig Chemotheca, Chimiothèque Nationale and ZINC), were used for the VS stage after careful optimization and validation Molecular dynamics simulations were performed in the top 5 results of each database to further validate the docking results.

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This work was supported by national funds from Fundação para a Ciência e a Tecnologia [grant numbers UIDP/04378/2020 and UIDB/04378/2020, SFRH/BD/137844/2018, 2020.01423.CEECIND].

Some of the calculations were produced with the support of INCD funded by FCT and FEDER under project 01/SAICT/2016 number 022153 and projects CPCA/A00/7140/2020 and CPCA/A00/7145/2020.

# Protein A Z Domain-Nanoluciferase Fusion Protein for Generalized, Sensitive Detection of Immunoglobulin G

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 41

***Mr. Suman Nandy*** [1], ***Dr. Mary Crum*** [2], ***Ms. Katherine Wasden*** [1], ***Dr. Ujwal Patil*** [3], ***Dr. Binh Vu*** [4], ***Dr. Katerina Kourentzi*** [4], ***Prof. Richard Willson*** [4]

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**Introduction**

Detection and quantification of antibodies, specially Immunoglobulin G (IgG), is the cornerstone of ELISA, many diagnostics, and antibody-based drug development. The current state-of-the-art immunoassay techniques for antibody detection require species-specific secondary antibodies and carefully controlled bioconjugation techniques. However, reduced functionality because of poor conjugation efficiency not only increases the time and cost of the diagnostic process but also the risk of non-specific binding and false positives. We have developed a generic IgG quantification platform using a recombinant fusion protein comprising the IgG-Fc binding protein, Z domain of Staphylococcal Protein A, and the ultrabright bioluminescence reporter Nanoluciferase (Nluc).

**Methods**

The immunoassay was based on a sandwich immunoreaction of capture antibody/antigen, IgG, and the fusion protein. We optimized the detectability of antibodies by incorporating multiple repeats of the Z domain in the protein construct. Anti-SARS-nCoV2 Nucleocapsid protein (NP) IgG produced in rabbits and mice were studied as model antibodies. The Z domain-Nluc encoding genes were cloned into plasmid pET28 and expressed as $His_6$-tagged recombinant proteins in *E. coli*. Following cell culture, harvesting, and cell lysis the proteins were purified by immobilized nickel affinity and IgG-Sepharose column chromatography. The purified protein was then incubated with serially diluted antibodies captured in white microplates. Upon addition of Nanoglo™ substrate, the luminescence signal was measured with an Infinite® 200 PRO (Tecan) plate reader.

**Results & Discussion**

The assay platform was able to quantitate the antibodies at concentrations as low as 70 fM (~10 pg/mL) using standard laboratory equipment. The ELISA was carried out in two different formats, using the SARS-nCoV2 nucleoprotein as capture antigen or by using polyclonal chicken (IgY) as a capture antibody.

**Impact**

The proposed fusion protein has excellent performance for the detection of human, and rabbit IgG with a wide dynamic range and low detection limit. Given the protein's simple preparation, wide applicability for mammalian IgG, and the potential of ultra-low-level detection, it provides a generic and flexible platform for IgG-based diagnostic technologies.

# Nanobodies as highly affine reagents for site-directed antibody capture

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 95

***Mr. Michael Metterlein*** [1], ***Dr. Christian Linke-Winnebeck*** [1], ***Ms. Sabrina Wendler*** [1], ***Dr. Felix Hartlepp*** [2]

***1.*** *ChromoTek GmbH,* ***2.*** *ChrmoTek GmbH*

Classical methods to capture antibodies in biosensor or ELISA assays are mainly based on indirect capture using Protein A/G/L or direct capture using covalent coupling or biotinylation. However, all these methods bear disadvantages: Antibody capture by Protein A/G/L lacks consistent affinity to the various antibody species and subtypes, for example. Direct capture approaches are of extraordinary stability (covalent or femtomolar binding), but result in random orientation of antibodies.

To enable site-directed and high-affinity antibody capture, we have developed monovalent reagents that we call Nano-CaptureLigands™. These Nano-CaptureLigands consist of biotinylated alpaca single-domain antibody fragments (Nanobodies or $V_HHs$) that allow efficient subclass- and site-specific binding of human, mouse, and rabbit immunoglobulins. Nanobodies constitute the variable domain derived from camelid heavy-chain-only antibodies (HCAbs) and figure among the smallest known functional antibody fragments (~14 kDa). Like conventional antibodies, $V_HHs$ can bind to their antigen with high affinity, i.e. with dissociation constants ($K_D$) in the nanomolar to low picomolar range.

Our Nano-CaptureLigands are characterized by site-specific binding to the antibody Fc or Fab parts, which ensures homogenous presentation of antibodies (1) resulting in higher data quality and better reproducibility of experiments. Moreover, the high affinity (low nanomolar to picomolar) of our Nano-CaptureLigands is further enhanced by the avidity effect between the monovalent ligand and its dimeric antibody target. This allows efficient immobilization of antibodies even at low antibody concentrations. Furthermore, our Nano-CaptureLigands are compatible with crude antibody solutions (3), can provide high loading densities (4) and can be regenerated several times for multiple reuse (5). Our Nano-CaptureLigands are fully sequenced and extensively validated with regards to their specificity, stability and affinity. Their recombinant production in microorganisms ensures consistently high quality without batch-to-batch variation.

Using Bio-layer interferometry (BLI) and Enzyme-linked immunosorbent assays (ELISA), we here show that our Nano-CaptureLigands enable (a) the efficient capture of antibodies, (b) the valuable characterization of antibodies and (c) the quantitation of antibodies from crude mixtures.

Taken together, our Nano-CaptureLigands enable site-directed capture of antibodies in combination with outstanding efficiency of antibody immobilization in bioassays. Thereby, they constitute a new, innovative tool that combines the advantages of different classical capture methods. By integrating them into experimental workflows - from early stage to final candidate validation - they will contribute to the development of better understood antibodies.

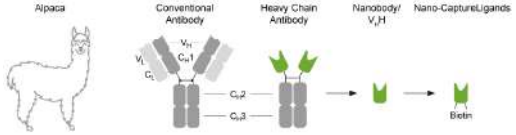
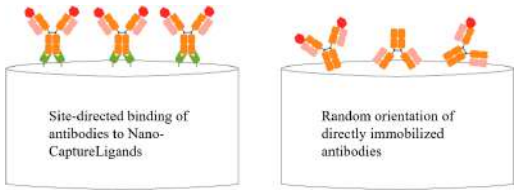
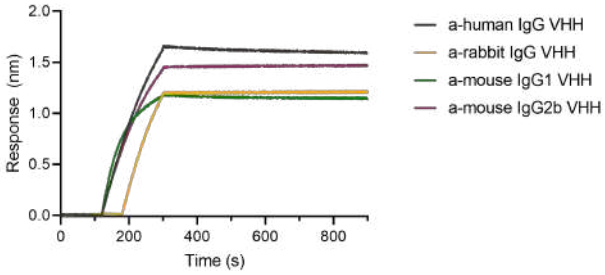



Illustration of nano-captureligands.jpg




Site-specific binding of nano-captureligands.png




High affinity of nano-captureligands.png

# Immobilization of L-asparaginase towards surface-modified carbon nanotubes

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 39

***Dr. Mafalda R. Almeida*** [1], ***Dr. Raquel O. Cristóvão*** [2], ***Ms. Maria A. Barros*** [2], ***Mr. João C.F. Nunes*** [1], ***Dr. Rui A. R. Boaventura*** [2], ***Dr. José M. Loureiro*** [2], ***Prof. Joaquim L. Faria*** [2], ***Dr. Márcia Neves*** [1], ***Prof. Valéria C. Santos-Ebinuma*** [3], ***Dr. Ana P. M. Tavares*** [1], ***Dr. Cláudia G. Silva*** [2]

***1.*** *CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro,* ***2.*** *Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua do Dr. Roberto Frias, 4200-465, Porto,* ***3.*** *Department of Engineering Bioprocess and Biotechnology, School of Pharmaceutical Sciences, UNESP-University Estadual Paulista, Araraquara*

L-asparaginase (ASNase, EC 3.5.1.1) is an enzyme that catalyzes L-asparagine hydrolysis into L-aspartic acid and ammonia and is mainly applied in pharmaceutical and food industries [1]. The ASNase currently commercialized for pharmaceutical purposes is produced from two main bacterial sources: recombinant *Escherichia coli* and *Erwinia chrysanthemi.* However, some disadvantages are associated with its free form, such as the shorter half-life [2]. Immobilization of ASNase has been proposed as an efficient approach to overcome this limitation [3]. In this work, a straightforward method, including the functionalization of multi-walled carbon nanotubes (MWCNTs) through a hydrothermal oxidation treatment with nitric acid, and the immobilization of ASNase by adsorption over pristine and modified MWCNTs was investigated. Different operation conditions, including pH, contact time, ASNase/MWCNT mass ratio, and the operational stability of the immobilized ASNase were evaluated. The characterization of the ASNase-MWCNT bioconjugate was addressed using different techniques, namely Transmission Electron Microscopy (TEM), Thermogravimetric analysis (TGA), and Raman spectroscopy. Functionalized MWCNTs showed promising results, with an immobilization yield and a relative recovered activity of commercial ASNase above 95%, under the optimized adsorption conditions (pH 8, 60 min of contact and $1.5´10^{-3}$ $g.mL^{-1}$of ASNase). The ASNase-MWCNT bioconjugate also showed improved enzyme operational stability (6 consecutive reaction cycles without activity loss), proving its suitability for application in industrial processes.

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**Acknowledges:**

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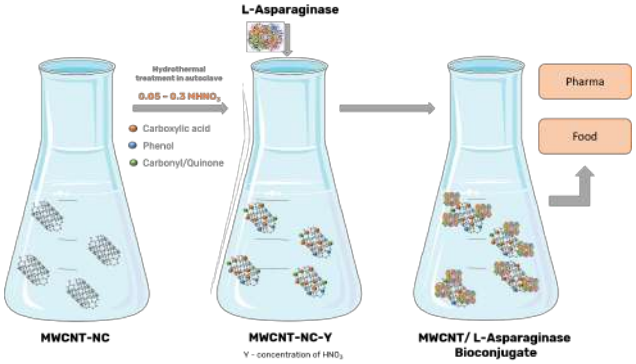


L-asparaginase immobilization.png

# Artificial receptors for affinity-driven pharmaceutical purification processes

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 11

***Prof. Teresa Casimiro*** [1], ***Dr. Raquel Viveiros*** [1]

***1.*** *CleanMIPTech group, LAQV-REQUIMTE, Chemistry department, NOVA School of Sciences and Technology, NOVA University of Lisbon, Portugal*

A simple, clean, and sustainable technology - supercritical carbon dioxide ($scCO_2$) - was applied in the development of amide-based artificial receptors for the pharmaceutical impurity, acetamide. $scCO_2$ has been already successfully applied in the synthesis of molecularly imprinted polymer (MIP) [1] where the final properties of the resulting materials show significant advantages such as the high-yield one-pot preparation of ready-to-use powders that are free-flowing and easy to handle. $ScCO_2$ has already been tested in the development of affinity polymers for drug delivery, removal of pollutants from fuels and environment, switchable sensors, separation of valuable targets from the environment and the selective removal of pharmaceutical impurities [2].

Artificial receptors as analogue template molecularly imprinted polymers (AT-MIPs) were produced in a $scCO_2$ using methacrylamide and ethylene glycol dimethacrylate as the functional monomer and the crosslinker, respectively. AT-MIPs were obtained in high yields as easy-to-handle and ready-to-use material. The materials were characterized by Fourier transform infrared spectroscopy, nitrogen adsorption porosimetry, scanning electron microscopy, and Morphologi G3 particle size analysis. The affinity properties of AT-MIPs for three amides (acetamide, benzamide, and pivalamide) were assessed by packing the affinity materials into blank columns and evaluating their profiles under static and dynamic modes. The size and shape of the cavities created on the artificial receptors had a strong impact on the material's performance. The results suggest a potential use of three-dimensional-shaped artificial receptors as efficient devices for the removal of amide-based compounds, whose dimensions are equal to or lower than those of the template molecule, from pharmaceutical crude mixtures.

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[2] https://sites.fct.unl.pt/clean-mip-tech/

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# Advanced bacteriophage-based bioligands for biosensing applications

Wednesday, 23rd June - 17:05: Flash Session 3 - Poster - Abstract ID: 42

***Ms. Inês Simões [1], Ms. Marta Patinha [1], Mr. João Lampreia [1], Mr. Jorge João [1], Prof. Ana Azevedo [1], Dr. Verónica Romão [2]***

***1.*** *IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal,* ***2.*** *INESC Microsistemas e Nanotecnologias, Lisbon*

Bacteriophages are viruses that are naturally able to target and infect bacteria with great specificity. This characteristic, associated to other attractive properties – robustness, easiness of production and low cost – makes them highly interesting as bioligands in biorecognition events for biosensing. The main limitation encountered in the use of such bioligands is the lysis of the target bacteria upon phage attachment, consequently hampering detection signal in a biosensor. As a solution, we propose the development of two alternative bacteriophage nanoligand production approaches. First, the obtention of isolated phage tails, cleaved from the heads, which eliminates the infection ability while retaining their recognition capability. Second, the production of ghost phages (without genetic content) and their conjugation with magnetic nanoparticles inside the capsid, to potentiate their application as capture and labelling agents, simultaneously. The most common labelling strategy using magnetic particles is through the attachment of the particles on the phage outer surface, which can also lead to loss of recognition capacity.

As disruption and conjugation methods, we used both osmotic shock and ultrasonication techniques. As characterization methods for both generated ligands it was used phage titration assays and Transmission Electron Microscopy (TEM).

To cleave the phage tails from the heads, a 25 W probe ultrasonication was performed. When submitted to TEM analysis using negative staining, the T4 phages appeared to be with their tails separated from their respective capsids and also with the base-plate and tail fibres visible, which may indicate that the recognition portion is intact and functional (Figure 1). Future work will address the phage tails isolation and their separation from the heads.

On the other hand, osmotic shock using 4M sodium acetate was used as a method for ghost phage production and conjugation with nanoparticles inside the capsid. When comparing the results from the osmotic shock performed in two phages of different sizes (~60x60 nm and ~120x86 nm), it was possible to conclude that the morphology of the phages influenced the loss of infection capacity, since it was observed a higher loss of infectivity in the larger phages (Table 1). Nevertheless, when treated along with the nanoparticles a lower percentage of loss of infectivity was observed in larger phages, indicating a protective effect.

Future perspectives lie in the characterization of the new nanoligads in TEM and bioassays, their purification and further testing in biosensors aiming to provide reliable pathogen identification tools to the in vitro diagnostics field.

**Table 1** - Large phage (T4 phage) and smaller phage (*P. aeruginosa* phage) osmotic shock for all tested conditions (**Phage Only** – samples with only phage in solution during the osmotic treatment; **Phage + Particles (Before)** – samples with phage and particles in solution during osmotic treatment; **Phage + Particles (After)** – samples with osmotically treated phage and nanoparticles added after). Obtained average (%) loss of infectivity values for each condition.

| | Loss of infectivity (%) | | |
|---|---|---|---|
| | **Phage Only** | **Phage + Particles (Before)** | **Phage + Particles (After)** |
| **Large phage (T4)** | 70% | 41% | 71% |
| **Small phage (*P. aeruginosa*)** | 34% | 85% | 52% |

Table 1.png

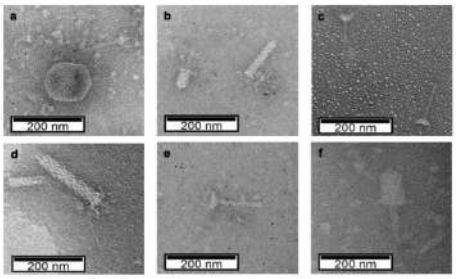



**Figure 1** – TEM images of specific *E. coli* T4 bacteriophage submitted to a 25 W probe ultrasonication stained with Uranyless (EMS). Images obtained at 40 000x magnification at a 200 nm scale at IST MicroLab. (a) phage capsid separated from the tail with 15 minutes of probe ultrasonication; (b) contracted and non-contracted tail separated from phage capsid with 15 minutes of probe ultrasonication; (c) tail with visible baseplates separated from phage capsid with 15 minutes of probe ultrasonication; (d) tail with visible tail fibers separated from phage capsid with 15 minutes of probe ultrasonication; (e) tail with visible baseplates separated from phage capsid with 20 minutes of probe ultrasonication; (f) tail with visible baseplates separated from phage capsid with 20 minutes of probe ultrasonication.

Figure 1.png

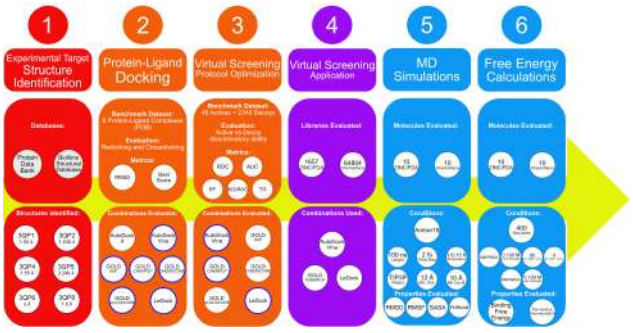
# Specialized multi-level computational approach for the identification of novel quorum sensing inhibitors

Wednesday, 23rd June - 17:50: Oral Session - Oral - Abstract ID: 66

***Mr. Fábio Martins*** [1], ***Dr. André Melo*** [2], ***Dr. Sérgio F. Sousa*** [1]

***1.*** *UCIBIO@REQUIMTE, BioSIM, Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto, Porto, Portugal,* ***2.*** *LAQV/REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto*

Biofilms are aggregates of microorganisms anchored to a surface and embedded in a self-produced matrix of extracellular polymeric substances. Bacteria within biofilms have multiple advantages when compared to their planktonic counterparts. As a consequence of these advantages, biofilm infections have been recognized as a serious threat to our society, being associated with 80% of all bacterial infections in humans. Quorum Sensing is an important process during biofilm maturation, in which cells communicate using auto-inducer signals. Because quorum sensing has specific protein targets, it is possible to design inhibitors to block this process.
Protein-Ligand molecular docking is a computational tool which predicts the binding pose and affinity of a ligand to a specific receptor or enzyme. During a virtual screening procedure, thousands of molecules are docked into a particular target and scored, giving an indication to which molecules are more probable to be binders. MM/PBSA and MM/GBSA are used to estimate the free energy of the binding of small ligands to biological macromolecules. These methods are based on molecular dynamics (MD) simulations of the receptor-ligand complex. In this work molecular docking, MD simulations and MM/PB(GB)SA were used to find promising compounds against CviR, the quorum sensing receptor from *Chromobacterium violaceum*. *C. violaceum* is a model organism for the study of quorum sensing. Autodock 4, Autodock Vina, GOLD and LeDock were the molecular docking programs used in this work. The ability to discriminate the active molecules within a large database was optimized by screening a library containing known active molecules and decoys. The optimized protocol was then applied to a ZINC/FDA Approved database and to the Mu.Ta.Lig Virtual Chemotheca. Finally, Molecular dynamics simulations of the most promising molecules in complex with CviR were performed using the Amber18 software. Using the last 40 ns of simulation, MM/PBSA and MM/GBSA calculations were done in order to estimate the affinity of each molecule towards CviR. This study yielded multiple promising compounds which in the future can be tested and validated experimentally.




Workflow.png

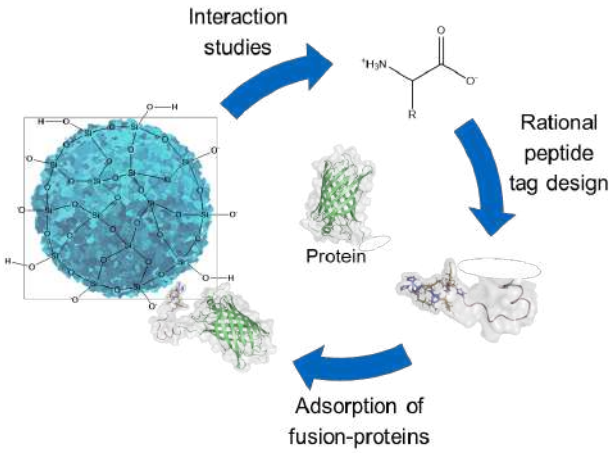
# New Silica-Binding Peptide Tag: Molecular Interactions at Silica Surfaces

Wednesday, 23rd June - 18:05: Oral Session - Oral - Abstract ID: 28

***Dr. Sebastian Schwaminger*** [1], ***Mr. Stefan Rauwolf*** [1], ***Prof. Sonja Berensmeier*** [1]

**1.** *Technical University of Munich, Bioseparation Engineering Group*

Production costs of therapeutic antibodies and recombinant proteins are determined mainly by the downstream process. Here, silica-based preparative chromatography offers a new, inexpensive and scalable method to reduce operation costs, especially when the bare silica acts as an affinity matrix, as in this case. Silica has been widely used as the backbone of chromatography resins due to its high mechanical strength, column efficiency, easy manufacturing in controlled size and porosity, and low-cost. Despite this fact, the mechanism of the interaction between silica and biomolecules in aqueous conditions is still not completely understood. We used chromatographic zonal elution experiments of the 20 amino acids in different aqueous systems. The pH, the ionic strength, and the valency of ions have been evaluated towards the binding affinity of different amino acids to the silica surface. Selected capped arginine and alanine were used to reveal the individual contribution of backbone and functional charged groups of the amino acids. Experiments and simulation indicated that the interaction of amino acids with silica is dominated by electrostatic interactions with the mechanism not being classical ion exchange but ion pairing. Based on the results from amino acid interactions a peptide tag was designed to purify fusion proteins with a bare silica chromatographic column with over 90 % purity in one run. The interaction of the new affinity tag is verified spectroscopically with infrared and X-ray photoelectron spectroscopy and by microscale thermophoresis. Our designed peptide-tag combined with the purification process allows bare silica to be exploited in preparative chromatography for downstream bioprocessing. This method is not only restricted to a large scale but also for everyday laboratory use.




Affinity-schwaminger-picture.png

# Decorating bacteria with self-assembled synthetic receptors

Wednesday, 23rd June - 18:20: Oral Session - Oral - Abstract ID: 49

***Dr. leila Motiei*** [1]

***1.*** *Weizmann Institute of Science*

Cell surface receptors (CSPs) constitute a wide range of membrane proteins that mediate the response of cells to changes in their environment. In recent years, much effort has been devoted to genetically modifying bacterial membranes with heterologous protein 'receptors' in order to provide them with new properties.[1] However, these methods cannot be used to incorporate synthetic molecules such as fluorescent probes, drugs and affinity tags on the bacterial surface. In this study, we demonstrate the possibility of decorating bacteria with artificial, self-assembled receptors that imitate the dynamic features of cell surface proteins.[2] More specifically, we have reported a method for decorating His-tagged outer membrane protein C (His-OmpC) with self-assembled synthetic receptors based on modified DNA duplexes (Figure 1a). The structure of the artificial receptors can be 'programmed' by a simple self-assembly process, which provides the means to reversibly change the properties of the cell. For example, we have demonstrated that this approach can be used to fluorescently label the His-tagged proteins with different colors, simply by changing the dye (Y) on Y-ODN-2 (Figure 1b). In addition, this approach allowed us to decorate the cell membrane with a thiol or folate group, and consequently enable the bacteria to bind to gold surfaces (Figure 1c) or cancer cells (Figure 1d), respectively. In summary, we have shown that, similar to the responses of natural CSPs, changes that occur on the artificial receptors can alter the properties of bacteria, such as their ability to glow, adhere to surfaces, or interact with proteins, or cells, properties that may be used in developing cell-imaging methods, living materials, and devices, as well as live cell-based therapeutics and diagnostic systems, respectively.

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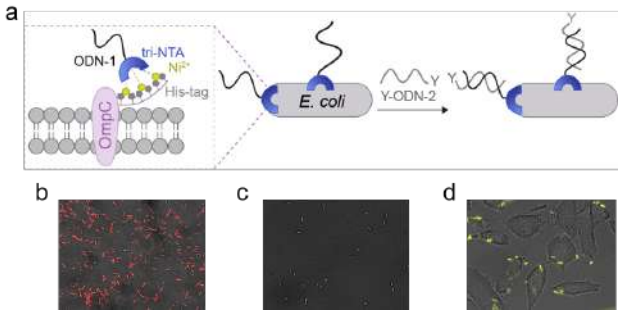



1.png

# Engineering ligands and receptors as next-generation protein therapeutics

Wednesday, 23rd June - 18:35: Keynote 5 - Oral - Abstract ID: 55

***Prof. Jennifer Cochran*** [1]

***1.*** *Stanford University*

We create engineered protein ligands and receptors with altered biochemical and biophysical properties with several goals in mind. These engineered proteins are being used as tools for studying the relationship between properties such as binding kinetics and affinity, and biological effects like cell signaling, proliferation, and migration. We are also developing these engineered proteins as therapeutics and diagnostic agents for applications including cancer and regenerative medicine. My presentation will highlight examples of engineered proteins from our lab with translational impact, in particular natural ligands and receptors engineered to function as agonists or antagonists for therapeutic applications.

# Occam's pharmacokinetics: dosing tumors from the inside out

Wednesday, 23rd June - 19:30: Keynote 6 - Oral - Abstract ID: 114

***Prof. K. Dane Wittrup*** [1]

***1.*** *MIT*

-Traditional oncological principles held that it was essential for an administered cytotoxic or signal-blocking drug to reach every single cancer cell in the body - an unattainable goal that almost inevitably results in the emergence of drug resistance. The advent of immune oncology has shifted the burden of tumor cell elimination to the adaptive immune system, which patrols everywhere in the body.

Thus, the job of an immunotherapy drug is to instruct the adaptive immune system - in other words, to act as a vaccine. This process is well understood to be a highly local one, leading to a groundswell of intratumoral immunotherapy efforts. We will present recent work from our lab developing new molecules and design principles for such intratumoral immune therapeutics.

# The Coming of Age of De Novo Protein Design

Wednesday, 23rd June - 20:00: Affinity Awards - Oral - Abstract ID: 115

***Prof. David Baker*** [1]

**1.** *University of Washington*

Proteins mediate the critical processes of life and beautifully solve the challenges faced during the evolution of modern organisms. Our goal is to design a new generation of proteins that address current-day problems not faced during evolution. In contrast to traditional protein engineering efforts, which have focused on modifying naturally occurring proteins, we design new proteins from scratch based on Anfinsen's principle that proteins fold to their global free energy minimum. We compute amino acid sequences predicted to fold into proteins with new structures and functions, produce synthetic genes encoding these sequences, and characterize them experimentally. SARS-CoV-2 provided a test of the relevance of these methods to real-world challenges. In this talk, I will describe the de novo design of SARS-CoV-2 candidate diagnostics, therapeutics, and vaccines: designed switches that luminesce in the presence of antiviral antibodies, designed 55-residue proteins that bind to the Spike with picomolar affinity and block viral infection, and nanoparticle immunogens which elicit much higher yields of neutralizing antibodies in animals than the Spike trimer that is the basis of most current vaccine trials. I will discuss the status of getting these into the clinic, and lessons for combatting future pandemics, and then describe several additional recent advances in de novo protein design, including the hallucination of new proteins using deep neural networks.

# Computational modelling of molecularly imprinted polymer enantioselectivity

Wednesday, 23rd June - 21:15: Oral Session - Oral - Abstract ID: 17

***Dr. Gustaf Olsson*** [1], ***Prof. Ian Nicholls*** [2]

***1.*** *Department of Chemistry and Biomedical Sciences, Linnaeus University,* ***2.*** *Bioorganic and Biophysical Chemistry Laboratory, Department of Chemistry and Biomedical Sciences, Linnaeus University*

**Introduction**

The concept of molecular imprinting involves the generation of ligand selective recognition sites in synthetic and molecularly imprinted polymers (MIP), a field which in recent years has undergone rapid development and expansion generating a need for fundamental understanding of mechanisms underlying material recognition properties.[1] Computer simulations have grown in popularity for these purposes, including molecular dynamics (MD) simulations of pre-polymerization mixtures.[2,3] Here we present insights from studies combining MD and docking simulations in efforts to correlate events occurring in MIP prepolymerization mixtures and polymer performance, including enantioselective recognition properties.

**Methods**

Initial all-atom, full-system MD simulations and analyses of MIP prepolymerization mixtures were conducted using the Amber suite of programs. Conditions and compositions replicated those of a study involving enantioselective and catalytically active transition state analogue imprinted MIPs (A).[4] A series of "model imprints" was generated by constraining the template microenvironments around template structures over a series of timepoints throughout the MD simulations, and removing the template structures to create virtual binding sites. These sites were then probed with the enantiomers of the template and related structures through docking simulations using AutoDock Vina.

**Results & Discussion**

Correlations with experimental data were observed that provided insight regarding MIP performance and recognition site heterogeneity (B). Importantly, pre-polymerization template complexation from simulations supported the experimentally observed previously observed enantioselectivity of MIPs.

**Impact**

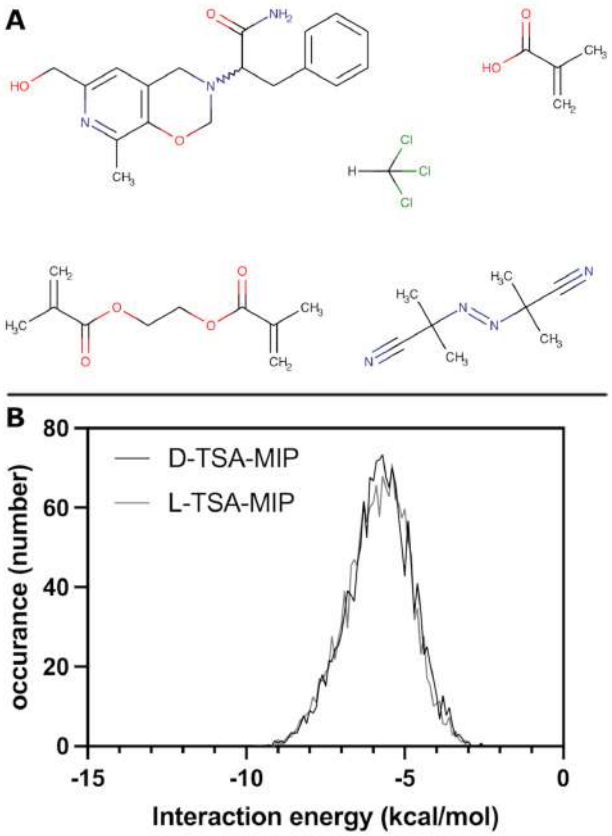
In addition to establishing the diagnostic and prognostic capabilities of MD simulations in MIP development, this approach should be useful for facilitating enantioselective enantioselective MIP design.

[1] Whitcombe, M.J.; Kirsch, N.; Nicholls, I.A. Molecular Imprinting Science and Technology: A Survey of the Literature for the Years 2004–2011. *J. Mol. Recognit.* **2014**, *27*, 297–401.

[2] Nicholls, I.A.; Olsson, G.D.; Karlsson, B.C.G.; Suriyanarayanan, S.; Wiklander, J.G. Theoretical and Computational Strategies in Molecularly Imprinted Polymer Development. In Polymer Chemistry Series; Kutner, W., Sharma, P.S., Eds.; Royal Society of Chemistry: Cambridge, **2018**; 197–226.

[3] Karim, K.; Breton, F.; Rouillon, R.; Piletska, E.V.; Guerreiro, A.; Chianella, I.; Piletsky, S.A. How to Find Effective Functional Monomers for Effective Molecularly Imprinted Polymers? *Adv. Drug. Del. Rev.* **2005**, *57*, 1795–1808.

[4] Svenson, J.; Zheng, N.; Nicholls, I.A. A Molecularly Imprinted Polymer-Based Synthetic Transaminase. *J. Am. Chem. Soc.* **2004**, *126*, 8554–8560.




Structures of components studied and example of observed binding site model heterogeneity in virtual imprinting sites.png

# Protein Biomarker Epitopes for Molecularly Imprinted Polymer Nanogels: 3D Structural Prediction and Binding Specificity Validated by Solution NMR Spectroscopy

Wednesday, 23rd June - 21:30: Oral Session - Oral - Abstract ID: 112

***Mrs. Alicia Alejandra Mier Gonzalez*** [1], ***Dr. Irene Maffucci*** [1], ***Dr. Bernadette Tse Sum Bui*** [1], ***Prof. Karsten Haupt*** [1]

**1.** *Université de Technologie de Compiègne*

Abnormalities in the expression of cell surface proteins or receptors are promising biomarkers of human diseases. However, the detection and quantification of these biomarkers are often challenging. Molecularly imprinted polymers (MIPs) are tailor-made synthetic receptors (antibody mimics), able to specifically recognize target molecules. They are synthesized by copolymerization of functional and cross-linking monomers in the presence of a template molecule, resulting in the formation of binding sites with affinities and specificities comparable to those of antibodies.

In the present project, we demonstrate the targeting of the protein Hepatitis A Virus Cell Receptor-1 (HAVCR-1) with MIP nanogels. This protein was selected because of its ubiquitous implication in numerous pathologies. MIPs were synthesized using a solid-phase approach in which a fragment of the protein was selected as epitope and immobilized on glass beads (solid support) via click chemistry. This configuration allows an oriented immobilization of the template upon which thermoresponsive MIP nanogels are synthesized. The binding sites of the resulting MIPs all have the same orientation, thus MIPs synthesized by the solid-phase approach can be considered analogous to monoclonal antibodies.

MIPs were found to bind the epitope with high (nanomolar) affinity and selectivity as demonstrated by equilibrium binding assays with the peptide fluorescently labeled. Specific binding of the epitope template to the MIPs was confirmed by NMR Saturation Transfer Difference and WaterLOGSY spectroscopies. The application of MIPs as diagnostic and therapeutic agents is being studied.

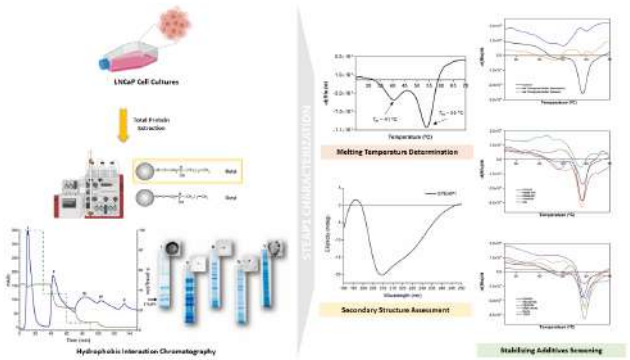
# A Molecular Perspective on the Bio-Structural Properties of the Human Native Six-Transmembrane Epithelial Antigen of the Prostate 1 (STEAP1)

Wednesday, 23rd June - 21:45: Flash Session 4 - Poster - Abstract ID: 57

***Dr. Jorge Ferreira*** [1], ***Dr. Marino Santos*** [2], ***Prof. Cláudio Maia*** [1], ***Prof. Teresa Santos Silva*** [2], ***Prof. Luís Passarinha*** [2]

***1.*** *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6201-506 Covilhã, Portugal,* ***2.*** *UCIBIO – Applied Molecular Biosciences Unit, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.*

The Six-Transmembrane Epithelial Antigen of the Prostate 1 (STEAP1) is an integral membrane protein composed of six-transmembrane helices located in both tight- and gap-junctions, cytoplasm and endosomal membranes, connected by intra- and extra-cellular loops (Hubert et al. 1999). This protein may play an important role as transporter protein, being involved in cell communication and in the stimulation of cell growth by increasing the levels of reactive oxygen species (Yamamoto et al. 2013; Grunewald et al. 2012). The recent cryo-EM structure of STEAP1 supports its functional role to recruit and orient intracellular electron-donating substrates to enable transmembrane-electron transport and the reduction of extracellular metal-ion complexes (Oosterheert and Gros 2020). The STEAP1 is particularly over-expressed in prostate cancer, in contrast with non-tumoral tissues and vital organs, contributing to tumor development and aggressiveness (Barroca-Ferreira et al. 2018). These features highlight the usefulness of STEAP1 as a promising biomarker or target for anti-cancer therapies (Moreaux et al. 2012). The reported studies lack experimental data on STEAP1 derived from its native source, its putative structure, and chemical modifications. These facts reinforce the relevance of exploring the expression of STEAP1 in its natural cancer microenvironment, and further purification and characterization. In this work, we successfully isolated the native full-length human STEAP1 protein from LNCaP prostate cancer cells in a fraction with a high degree of purity. For that, we evaluated the fractionation capacity of Butyl- and Octyl-Sepharose matrices by manipulating the ionic strength in both binding and elution phases. Interestingly, STEAP1 was not fully captured with 1.375 M (Butyl) and 1.2 M (Octyl) $(NH_4)_2SO_4$, being directly eluted in the flowthrough. Butyl-Sepharose demonstrated higher selectivity for host impurities removal from injected crude samples, which allowed an acceptable clearance of samples containing STEAP1, in a single step, similar to a negative chromatography strategy. The melting temperature ($T_m$) of purified STEAP1 sample was determined by Thermal Shift Assay, as approximately 55 ºC, confirming the stability of the target protein in the purification buffer, 10 mM Tris pH 7.8. Different stabilizing additives (e.g., Gly-Gly-Gly, PEG3350, DNA Library, Biotin, and TCEP) increased the $T_m$ value, suggesting their putative role as crystallization additives for further structural studies. Noteworthily, Circular Dichroism allowed to identify a predominant α-helical secondary structure of STEAP1, ensuring the protein structural stability.




20210503 graphical abstract 24th meeting of the international society for molecular recognition.png

# Structural analysis of protein and peptide ligands of the SARS-CoV-2 spike protein

Wednesday, 23rd June - 21:45: Flash Session 4 - Poster - Abstract ID: 74

***Mr. Carlos Costa*** [1], ***Dr. Arménio J.M. Barbosa*** [2], ***Dr. Margarida Dias*** [3], ***Prof. Ana Cecília A. Roque*** [4]

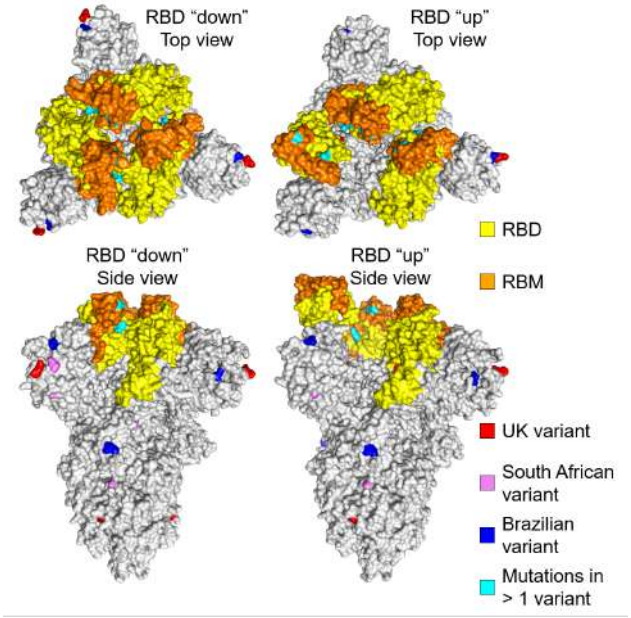
***1.*** *UCIBIO – Applied Molecular Biosciences Unit, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.,* ***2.*** *UIBIO - NOVA SST, University NOVA Lisbon,* ***3.*** *FCT-NOVA,* ***4.*** *Organisation*

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the deadly coronavirus disease 2019 (COVID-19) pandemic and a concerning hazard to public health. This virus infects cells through the binding of one of the spike proteins on its capsid, receptor-binding domain (RBD) region, to host human angiotensin-converting enzyme 2 (hACE2), initiating viral fusion. Approaches to halt SARS-CoV-2 from infecting cells involve hindering this interaction. One example are natural antibodies from immunised patients targeting the spike protein. Similarly, other ligands have been and are being developed which also bind to this viral protein. In this work, we analysed 131 structures of spike protein in complex with various ligands and provide an overview of the different hot spots for binding, binding affinities and contacting residues.

All 198 SARS-CoV-2 spike protein structures (UniProt P0DTC2) released on the Protein Data Bank (PDB) until 1st January 2021 were retrieved, 131 of which consisted of the spike protein complexed with a ligand. Molecular visualisation analysis was performed (PyMOL v2.4.0) and structures were aligned by the spike protein RBD (residues 319-541). From the superimposed structures, the location and recurrency of spike protein binding sites were assessed. Interacting residues were considered important for binding within a radius of 4.0 Å from the spike protein's residues. Ligands were categorised by type: hACE2, antibody Fab fragments, VHH fragments and *de novo* designed peptide scaffolds.

Some antibody Fab fragments interact with the spike protein in a region different from the hACE2 binding site, yet the vast majority compete for the same area, just as do all the other ligand types analysed. This is due to some degree of functional similarity found between the contacting residues on these ligands. From our analysis, we observe that smaller ligands like VHH fragments and *de novo* designed peptide scaffolds have higher affinities to the RBD and establish more contacts and hydrogen bonds per surface area.

This work summarises relevant details of the interactions the SARS-CoV-2 spike protein establishes with known ligands and the highlights found herein could be used as reference for future design of improved spike protein binders.




Sars-cov-2 spike protein with highlighted mutated residues of variants.png

# Altered Env conformational dynamics as a mechanism of resistance to peptide-triazole HIV-1 inactivators

Wednesday, 23rd June - 21:45: Flash Session 4 - Poster - Abstract ID: 73

***Ms. Shiyu Zhang*** [1]

**1.** *Drexel University*

We previously developed drug-like peptide triazoles (PTs) that target HIV-1 Envelope (Env) gp120, inhibit viral entry, and irreversibly inactivate virions. Here, we investigated potential mechanisms of viral escape from this promising class of HIV-1 entry inhibitors. HIV-1 resistance to cyclic (AAR029b) and linear (KR13) PTs was obtained by dose escalation in viral passaging experiments. High-level resistance for both inhibitors developed slowly (relative to escape from gp41-targeted C-peptide inhibitor C37) by acquiring mutations in gp120 both within (Val255) and distant to (Ser143) the putative PT binding site. The similarity in the resistance profiles for AAR029b and KR13 suggests that the shared IXW pharmacophore provided the primary pressure for HIV-1 escape. In single-round infectivity studies employing recombinant virus, V255I/S143N double escape mutants reduced PT antiviral potency by 150- to 3900-fold. Curiously, the combined mutations had a much smaller impact on PT binding affinity for monomeric gp120 (4- to 9-fold). This binding disruption was entirely due to the V255I mutation, which generated few steric clashes with PT in molecular docking. However, this minor effect on PT affinity belied large, offsetting changes to association enthalpy and entropy. The escape mutations had negligible effect on CD4 binding and utilization during entry, but significantly altered both binding thermodynamics and inhibitory potency of the conformationally-specific, anti-CD4i antibody 17b. Moreover, the escape mutations substantially decreased gp120 shedding induced by either soluble CD4 or AAR029b. Together, the data suggest that the escape mutations significantly modified the energetic landscape of Env's prefusogenic state, altering conformational dynamics to hinder PT-induced irreversible inactivation of Env.

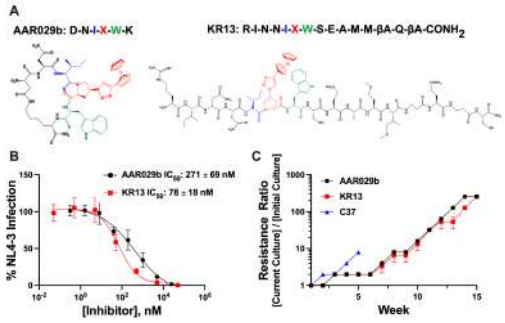



Fig 1. generating resistance to peptide triazole inhibitors of hiv-2 entry copy.jpg

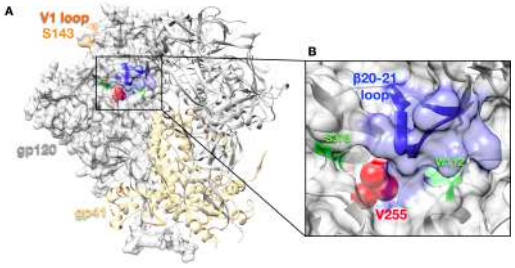



Fig 2. location of ser143 and val255 in the prefusogenic structure of a clade b hiv-1 env trimer.jpg

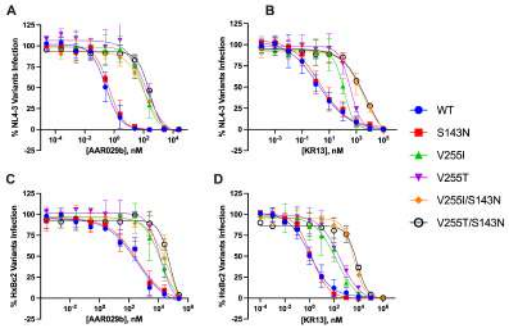



Fig 3. peptide triazole inhibition of hiv-1 pseudotyped with wild type or resistant envs.jpg

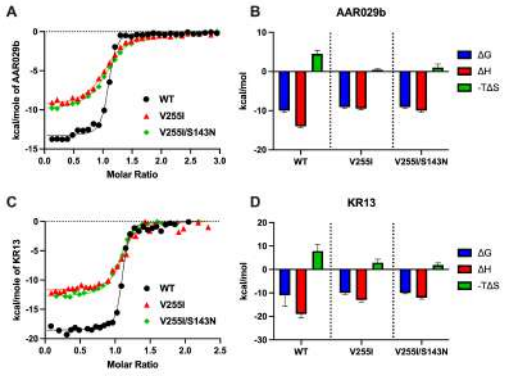



Fig 4. thermodynamic dissection of aar029b and kr13 binding to wild type and resistant gp120 by isothermal titration calorimetry.jpg

# Discovery of New Drug Candidates Against Biofilm Formation in P.aeruginosa through Molecular Docking, Virtual Screening, Molecular Dynamics and MM/GBSA Calculations

Wednesday, 23rd June - 21:45: Flash Session 4 - Poster - Abstract ID: 90

***Ms. Rita Magalhães*** [1], ***Ms. Tatiana Vieira*** [2], ***Dr. André Melo*** [3], ***Dr. Sérgio F. Sousa*** [2]

***1.*** *UCIBIO/REQUIMTE, BioSIM Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal,* ***2.*** *UCIBIO@REQUIMTE, BioSIM, Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto, Porto, Portugal,* ***3.*** *LAQV/REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto*

Biofilms are communities of bacteria enclosed in a self-produced matrix and attached to a surface that show high resistance to known antibiotics and natural host immune responses. Biofilm formation is complex and involves several mechanisms, such as quorum-sensing (QS), the cell-to-cell communication in bacteria. In gram-negative bacteria, QS typically involves a synthase that produces an autoinducer molecule that binds a receptor. QS allows for gene expression changes in a population density dependant manner. QS-inhibitors are molecules that disrupt the QS pathway without exerting evolutionary pressures, being therefore non-contributing to bacterial resistance. *P.aeruginosa* is a highly pathogenic bacteria responsible for up to 20 % of hospital bacterial infections capable of forming biofilm through QS. One of its main QS receptors, LasR, was the target of this study.

A computational workflow involving Molecular Docking, Virtual Screening, Molecular Dynamics, and Free Energy Calculations was developed to identify new LasR inhibitors. Docking was performed with AutoDock4, AutoDock Vina, LeDock, and GOLD (ChemPLP, GoldScore, ChemScore and ASP). Virtual Screening was done on a total of 294,498 compounds from five different libraries (Chemoteca, Chimiotheque, ZINC FDA Approved Drugs, Scubidoo and IBS Natural Compounds). Molecular Dynamics Simulations were performed on 30 compounds with Amber18 software for 100 ns to discriminate stable from unstable binders. MM/GBSA calculations were performed with Amber18 software on 300 structures from the equilibrated portion of the MD simulation to refine docking predictions and evaluate binding affinity. Simulations with the natural autoinducer and several experimentally tested actives were performed for comparison purposes.

After careful analysis of docking, MD and MM/GBSA results, seven compounds were selected as promising QSIs in *P.aeruginosa* LasR system. These chemically diverse and unique compounds show competitive binding affinity predictions with the natural autoinducer and currently known actives and are promising molecular templates for the experimental design of new inhibitors. The developed protocol proven capable of identifying likely binders and can now serve as inspiration for further experimental testing and confirmation.

Magalhães *et al*, Trends Biotechnol. 38 (2020) 9

Martins *et al*, Molecules. 26 (2021) 9

S. F. Sousa *et al*, Comb. Chem. High. T. SCR, 13 (2010) 5

This work was supported by the Applied Molecular Biosciences Unit -UCIBIO which is financed by national funds from FCT (UIDP/04378/2020 and UIDB/04378/2020) and was produced with the support of INCD funded by FCT and FEDER under the projects 01/SAICT/2016 nº 022153, CPCA/A00/7140/2020 and CPCA/A00/7145/2020. We thank FCT for the PhD grant 2020.09087.BD

# Irreversible Inactivators of HIV-1 and SARS-CoV-2 by Targeting Metastable Virus Spike Proteins

Wednesday, 23rd June - 21:45: Flash Session 4 - Poster - Abstract ID: 100

***Ms. Aakansha Nangarlia*** [1], ***Dr. Gabriela Canziani*** [1], ***Mrs. Farah Fazloon*** [1], ***Mr. Jackie Tang*** [1], ***Dr. Irwin Chaiken*** [1]

***1.*** *Drexel University*

Almost four decades ago, the HIV-1 pandemic swept across human populations, infecting 60 million people and causing over 25 million deaths globally. Today, HIV-1 is one of the leading causes of death in the world. The highly active antiretroviral therapy (HAART) has allowed us to prolong our patients' lives and control the disease's transmission, but it is no cure. To find a cure for HIV 1 and eradicate it, drugs need to target both infected cells and HIV-1 viruses. Seeing this as an unmet need, the Chaiken lab has devised HIV-1 envelope-targeting irreversible inactivators capable of doing just that. These envelope inactivators are capable of targeting the HIV-1 envelope protein, gp120, and cause self-destruction of the HIV-1 envelope in both infected cells and viruses. Their unique modes of action make them unique in the market. Our compounds include macrocyclic peptide triazoles, macrocyclic peptide triazole thiols, small CD4-mimetic dual action lytic inactivators, and lectin-dual action lytic inactivators. Each of these compounds has a unique mode of action but are all capable of causing irreversible inactivation. These findings have built the foundation for the development of potent HIV-1 inactivators and allowed us to expand our understanding of the mechanism of HIV-1 envelope transformation during infection.

At the onset of the COVID-19 pandemic, we saw the structural similarities between HIV-1 and SARS-CoV-2 viruses. We decided to test the hypothesis that our lectin-dual action lytic inactivators will inhibit SARS-CoV-2 infection and cause cell-free virolysis of SARS-CoV-2, similar to the effects observed in HIV-1. To our surprise, we discovered that the lectin alone, CVN could cause irreversible inactivation of both SARS-CoV-1 and SARS-CoV-2 pseudoviruses in *in vitro* assays. Upon testing the effects of other lectins on SARS-CoV pseudoviruses, we found partial irreversible inactivation with GRFT and no effect with MVN. These results suggest that the irreversible inactivation of virus is possible due to glycan engagement that disrupts the spike-membrane structure, perhaps due to spike conformational metastability. Furthermore, the irreversible inactivation function of CVN may be Pan CoV. Overall, the results suggest that glycans are potential targets for development of treatments for SARS-CoV-2/COVID-19.

# Membrane affinity for bacterial membranes: a patchy mechanism of disruption

Wednesday, 23rd June - 22:15: Keynote 7 - Oral - Abstract ID: 14

***Prof. Mibel Aguilar*** [1]

***1.*** *Monash University*

Peptide-membrane interactions can destabilise and damage the membrane which can lead to cell death. Characterisation of the molecular details of these binding-mediated membrane destabilisation processes is therefore central to understanding cellular events such as antimicrobial action. We have used optical biosensor and high resolution atomic force microscopy (AFM) techniques to allow biophysical analysis of membrane structure changes during antimicrobial peptide binding [1,2]. It is now evident that membrane interactions involve multiple kinetically distinct stages through measurement of birefringence in a real-time format. AFM topographic analysis revealed the formation of domains of different height and confirmed that these domains exhibited different susceptibilities to antimicrobial attack. The combination of these biosensors with other biophysical techniques now opens the door to redefining molecular mechanism of antimicrobial peptide action. This has provided new insight into the role of lipid domains in the response of bacteria to antimicrobial peptides.

1. Lee TH, Hirst DJ, Kulkarni K, Del Borgo MP and Aguilar MI, 'Exploring Molecular-Biomembrane Interactions with Surface Plasmon Resonance and Dual Polarization Interferometry Technology: Expanding the Spotlight onto Biomembrane Structure', Chemical Reviews, 118 (2018) 5392-5487.
2. Lee TH., Hall, K, and Aguilar MI, 'Antimicrobial Peptide Structure and Mechanism of Action: A Focus on the Role of Membrane Structure', Current Topics in Med Chem, 16 (2016) 25–39.

# Production and purification of non-mab proteins by platform processes

Thursday, 24th June - 13:10: Keynote 8 - Oral - Abstract ID: 10

***Prof. Alois Jungbauer*** [1]

***1.*** *University of Natural Resources & Life Sciences, Vienna*

The production of non-mAb biopharmaceuticals gained momentum and the so-called new formats are often overexpressed in E.coli. For these new therapeutic molecules, a platform purification process is not readily available. The conventional His tag often leads to reduced expression level and cleavage is expensive, slow, and often the required N-terminus is not achieved. An overview will be given which possibilities are available to tackle thus proteins production problem. Then an own expression and purification system based on a circular permutated Caspase will be shown. It generates an authentic N-terminus irrespective of the amino acid. For this purpose, and to enable production of the protease as an active monomer, the human Caspase-2 was circularly permutated. Circular permutation drastically increased manufacturability of the Caspase-2. Moreover, the sequence was optimized via random mutagenesis and molecular modelling in silico. The expression/purification tag increases the expression level and of soluble protein. The protein of interest can be purified by a two-step affinity chromatography. Examples with four different proteins of interest are shown.

# A practical self-cleaving affinity tag for research to manufacturing scales

Thursday, 24th June - 13:40: Oral Session - Oral - Abstract ID: 105

***Prof. David Wood*** [1], ***Dr. Izabela Gierach*** [2], ***Mr. Joseph Taris*** [1], ***Dr. Jackelyn Galiardi*** [1], ***Mr. Brian Marshall*** [1]

***1.*** *The Ohio State University,* ***2.*** *Protein Capture Science, LLC*

The purification of new recombinant proteins presents a number of challenges for both basic research and the development of new therapeutic candidates. At laboratory scales, these challenges are met through the use of affinity tags, which allow the rapid purification of new targets using established protocols with minimal optimization. For clinical applications however, the use of tags is forbidden due to their potential immunogenicity, thus leading to a requirement for multi-step processes based on conventional chromatography. The required multi-column processes can take months to develop, assuming they can be developed at all. These approaches have led to the emergence of two worlds in protein purification; one where affinity tags are used at research scale, and another where conventional chromatography is used for clinical applications. Most importantly, moving between these worlds carries risk since the effects of an affinity tag on its partner are difficult to predict.

We have developed a new platform to bridge these worlds through a practical self-cleaving affinity tag (Figure 1: Self-cleaving tag schematic), which enables high-throughput work at laboratory scale while also providing a manufacturing platform for clinical material. In practice, the user purchases the *i*CapTag affinity resin, which includes a split intein ligand covalently immobilized onto a conventional chromatography backbone. A 36 amino-acid tag is then fused to the N-terminus of the target, which is selectively captured by the *i*CapTag resin. Once the target is captured and purified, the tag is induced to self-cleave by a small shift in pH. The tagless and traceless target protein is thereby released from the column, while the tag remains bound by the affinity ligand. Finally, the tag can be removed by a simple low-pH wash, allowing the resin to be regenerated and reused multiple times. Overall, this resin has been designed for use in full-scale manufacturing, with attention to simple buffers, low cost, high binding capacity and reusability that are attractive for large-scale manufacturing. Most importantly, we have identified factors that influence the cleaving rate and can therefore provide reliable performance over a wide variety of target proteins. This technology is being commercialized by Protein Capture Science, LLC. This presentation will describe the system and its use, as well as provide several case studies for proteins produced in HEK, CHO and *E. coli*, including filgrastim (a biosimilar of Neupogen® – Figure 2), Epoetin Alpha (a biosimilar of Epogen® - Figure 3) and Covid-19 Spike RBD glycoprotein (Figure 4).

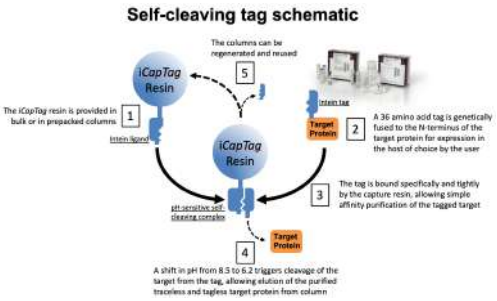
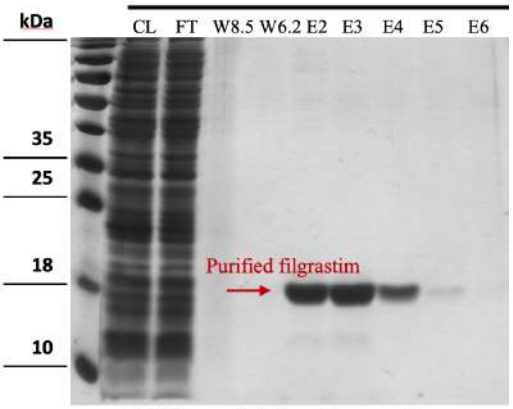


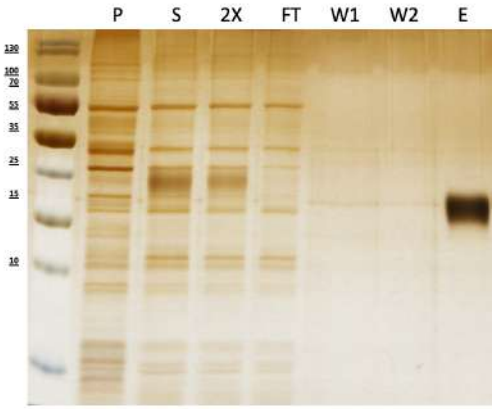

Figure 1 self-cleaving tag schematic.png




High sensitivity silver stain

**CL**: clarified lysate; **FT**: flow through; **W8.5**: wash with pH 8.5 buffer; **W6.2**: wash with pH 6.2 buffer; **E**: Elution fractions

Figure 2 purification of filgrastim from e coli.png




Purification of epoetin alpha from Expi293. Lanes: P= cell pellet; S = cell culture supernatant; 2X = ½ dilution of cell culture supernatant; FT = column flow-through; W1/W2 = column washes; E = eluted epoetin alpha.

Figure 3 purification of epoetin alpha from expi293.png

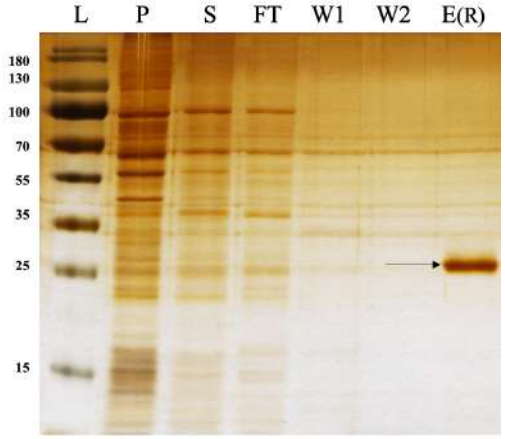



Figure 4: Purification of Covid-19 spike RBD from Expi293. Lanes: L = protein ladder; P = cell culture pellet; S = cell culture supernatant; FT = column flow-through; W1/W2 = column washes; E(R) = eluted spike RBD (reducing gel).

Figure 4 purification of covid-19 spike rbd from expi293.png

# Affinity Enrichment and LC-MS Analysis of Trastuzumab and Pertuzumab from Human Plasma Using Affimer® Reagents

Thursday, 24th June - 13:55: Oral Session - Oral - Abstract ID: 30

***Mr. Oladapo Olaleye*** [1], ***Mr. Baubek Spanov*** [1], ***Dr. Natalia Govorukhina*** [1], ***Dr. Robert Ford*** [2], ***Prof. Nico Van De Merbel*** [3], ***Prof. Rainer Bischoff*** [1]

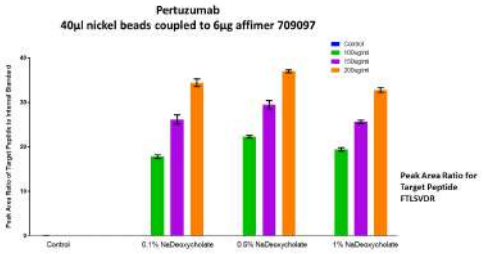
***1.*** *University of Groningen,* ***2.*** *Avacta Life Sciences,* ***3.*** *PRA Health Sciences*

Trastuzumab and pertuzumab are monoclonal antibodies used in the treatment of human epidermal growth factor receptor-2 (HER2) positive breast cancer. Biotransformation refers to chemical modifications that occur in therapeutic proteins after they have been administered to patients. Trastuzumab was reported in an earlier study to have shown altered detection in a validated ELISA after being received by breast cancer patients. Also, certain patients do not show the expected response to therapy after receiving trastuzumab.

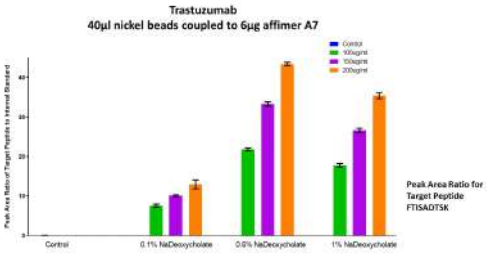
The ability to enrich these antibodies from plasma for further characterisation will provide more insight into modifications that occur in patients that receive them.

Affimer® reagents are alternative binding protein possessing two-variable binding loops and are based on human protease inhibitor stefin A or phytocystatin protein scaffolds.

In this study, an Affimer® bead-based method in combination with LC-MS (SRM mode) for the analysis of trastuzumab and pertuzumab from plasma is described. 16 anti-trastuzumab and 18 anti-pertuzumab Affimer® reagents were screened and the four-best performing affimers were selected for the enrichment process. The Affimer® reagents were engineered with His and cysteine tags and this enabled coupling to nickel and maleimide beads, respectively. Recovery values of about 80% were obtained for both trastuzumab and pertuzumab when spiked at 100, 150 and 200µg/mL concentrations in plasma after digestion in the presence of 0.5% sodium deoxycholate and 10mM DTT. Further work is being done presently to validate the method and to apply it to forced stability samples in human plasma as well as to clinical samples.




Peak area ratio for trastuzumab.jpg




Peak area ratio for pertuzumab..jpg

Trastuzumab Recovery

| | PAR for 100% Recovery | Average PAR 0.1% NaDe | Average PAR 0.5% NaDe | Average PAR 1% NaDe | PR 0.1% NaDe | PR 0.5% NaDe | PR 1% NaDe |
|---|---|---|---|---|---|---|---|
| 100ug/ml | 26.8 | 7.63 | 21.89 | 17.83 | 28.47 | 81.68 | 66.53 |
| 150ug/ml | 40.2 | 10.14 | 33.34 | 26.64 | 25.22 | 82.94 | 66.27 |
| 200ug/ml | 53.6 | 12.99 | 43.48 | 35.49 | 24.24 | 81.12 | 66.21 |

PAR – Peak Area Ratio
PR – Percentage Recovery
NaDe – Sodium Deoxycholate

Recovery for trastuzumab.jpg

Pertuzumab Recovery

| | PAR for 100% Recovery | Average PAR 0.1% NaDe | Average PAR 0.5% NaDe | Average PAR 1% NaDe | PR 0.1% NaDe | PR 0.5% NaDe | PR 1% NaDe |
|---|---|---|---|---|---|---|---|
| 100ug/ml | 23.2 | 17.77 | 22.25 | 19.43 | 76.59 | 95.91 | 83.75 |
| 150ug/ml | 34.8 | 26.17 | 29.45 | 25.61 | 75.2 | 84.63 | 73.59 |
| 200ug/ml | 46.4 | 34.13 | 37 | 32.83 | 73.56 | 79.74 | 70.75 |

PAR – Peak Area Ratio
PR – Percentage Recovery
NaDe – Sodium Deoxycholate

Recovery for pertuzumab.jpg

# Immobilization of Antibody-Binding Ligand onto Bare Iron Oxide Nanoparticles by an Affinity Peptide Tag

Thursday, 24th June - 14:10: Oral Session - Oral - Abstract ID: 92

***Ms. Yasmin Kaveh-Baghbaderani*** [1], ***Dr. Sebastian Schwaminger*** [1], ***Dr. Paula Fraga-García*** [1], ***Prof. Sonja Berensmeier*** [1]

**1.** *Technical University of Munich, Bioseparation Engineering Group*

Magnetic separation of antibodies is a promising alternative to preparative chromatography. For antibody capture, an antibody-binding ligand is immobilized on the magnetic bead material. The most popular materials so far are iron oxides coated with various polymers. Their surface groups are activated for performing the ligand coupling chemistry. Here, we present the immobilization of antibody-ligands directly onto the surface of cost-effective bare iron oxide nanoparticles (BION), without further modifications or activations. Therefore, we use a peptide tag with iron oxide affinity. As nanoparticles, they offer a high surface area of approx. 100 $m^2g^{-1}$ that is especially necessary for large proteins as antibodies. An optimized ligand consisting of eight polymerized B-domains of the common Protein A is bound onto the BION. The site-directed immobilization is promoted by a C-terminal affinity peptide tag comprising four units of arginine and histidine in an alternating sequence $(RH)_4$. Magnetic iron oxide nanoparticles can be synthesized easily and cost-effectively using the popular co-precipitation method. The successful immobilization of the peptide tagged ligand is proven. The resulting material can efficiently and reversibly capture polyclonal human IgG. The recovery is of up to 418 mg $g^{-1}$polyclonal IgG. Consequently, our affinity material is setting the benchmark in the state of the art regarding IgG binding capacities of magnetic nano- as well as microparticles. We examined the impact of the ligand density. Particles with a lower ligand density show a higher percentage recovery of IgG, which allows for a cost-effective design of the adsorbent. This result confirms the basic idea of affinity-immobilized ligands on BION as a promising magnetic adsorbent for antibody purification.

# Selective capture of RNA and clarification of pDNA using carbon nanotubes

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 104

***Mr. Pedro Ferreira [1], Dr. Cláudia G. Silva [2], Prof. Mara Freire [3], Dr. Ana P. M. Tavares [4], Dr. Fani Sousa [5]***

***1.*** *CICS-UBI - Health Sciences Research Centre,* ***2.*** *Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua do Dr. Roberto Frias, 4200-465, Porto,* ***3.*** *ciceco,* ***4.*** *CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro,* ***5.*** *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6201-506 Covilhã, Portugal*

Gene therapy approaches are performed by introducing exogenous nucleic acids such as plasmid DNA (pDNA), mRNA, small interfering RNA, and antisense oligonucleotides to modulate cell activity. These exogenous molecules arise from complex and multi-step processes, which to be considered as biopharmaceuticals, require effective manufacturing and quality control. One of the most crucial and time-consuming step is their purification since most of the impurities share similar characteristics. In order to solve this problem, and isolate pDNA from complex samples, a promising alternative based on the use of carbon nanotubes (CNTs) is here proposed. CNTs, composed of graphene sheets rolled up to form tubular, needle-shaped structures with nanometric scale in diameter, have been explored for diverse biological applications, due to their interesting size, shape, structure, and biocompatibility.

In this work, an effective method to isolate pDNA is described, based on the selective capture of RNA from complex extracts using CNTs. Multi-walled carbon nanotubes with different diameters were evaluated, while mixtures of RNA and pDNA were first prepared to confirm the ability of CNTs to interact with nucleic acids.

Obtained results reveal that the CNTs preferentially interact with RNA, through different types of interactions (electrostatic, van der Waals, hydrogen bond), being also verified that smaller CNTs are able to adsorb larger quantities of RNA, as expected by their larger surface area. Additionally, when adding a complex lysate sample, it was achieved a higher selectivity, as the RNA was completely bound to CNTs over DNA, that remained in solution. In general, CNTs enabled the recovery of 86% of pDNA, significantly reducing contaminating gDNA and eliminating about 80% of proteins in a first adsorption cycle. When performing up to three consecutive cycles, the purity of soluble pDNA was even improved, being also confirmed that pDNA maintained its stability along the capture process.

The results of this work show evidences that CNTs can be used for RNA capture from a complex sample, facilitating the purification of pDNA, that can be further used as biopharmaceuticals. Further developments to this method are required; however, obtained results suggest the potential of developing a simple, efficient, and reliable method for rapid purification of pDNA.

Acknowledgments: This work was supported by the project PTDC/BII-BBF/29496/2017 (PUREmiRSILs) funded by FEDER, through COMPETE2020 - POCI, and FCT/MCTES, and the projects UIDB/00709/2020, UIDB/50011/2020 and UIDP/50011/2020. The authors also acknowledge financial support from IF/01634/2015, IF/00514/2014 and HORIZON 2020, under TEAMING Grant agreement No 739572-The Discoveries CTR.

# Real-Time Detection of Monoclonal Antibodies in Protein-A Breakthrough and in Process Samples

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 37

***Mr. Atul Goyal*** [1], ***Dr. Ujwal Patil*** [2], ***Mr. Vijay Maranholkar*** [2], ***Dr. Binh Vu*** [1], ***Dr. Katerina Kourentzi*** [1], ***Prof. Richard Willson*** [3]

***1.*** *Department of Chemical and Biomolecular Engineering, University of Houston, Houston, USA,* ***2.*** *Department of Biology and Biochemistry, University of Houston, Houston, USA,* ***3.*** *Department of Chemical and Biomolecular Engineering and Department of Biology and Biochemistry, University of Houston, Houston, USA and Escuela de Medicina y Ciencias de la Salud ITESM, Monterrey, MX*

Measurement of antibody concentrations is ubiquitous in biopharmaceutical process development and manufacturing. Purification of therapeutic monoclonal antibodies (mAbs) usually involves a protein A affinity capture step. Because column breakthrough of antibody in complex, UV-absorbing culture fluid cannot be readily detected in real-time, processes are conservatively designed and column capacity often is underutilized, wasting adsorbent and reducing productivity. We have developed a fluorescence-based monitoring technology which allows mix-and-read mAb detection in cell culture fluid [1], which may be useful in at-line assays and in clone and culture development, and here report the use of reporters immobilized on agarose monolith supports for continuous detection of IgG in column breakthrough. The agarose monolith structure was optimized using residence time distribution measurement across a range of cooling rates. The column effluent was continuously contacted with immobilized fluorescein-labeled Fc-binding ligands to produce an immediately detectable shift in fluorescence intensity. The technology allows rapid and reliable monitoring of IgG in a flowing stream, without prior sample preparation. We observed significant shifts in fluorescence intensity at 0.05 g/L human IgG, sufficient to detect 5% breakthrough of a 1 g/L load within 4 minutes or 8 CV of the monolith at a flow rate of 0.5 mL/min. The fluorescence intensity response at different load concentrations is monitored and the differences in the response was observed, allowing calibration of fluorescence intensity with IgG concentrations.

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# A smartphone-readable lateral flow assay for POC monitoring of urine ALCAM in lupus nephritis

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 22

***Ms. Rongwei Lei*** [1], ***Dr. Binh Vu*** [1], ***Dr. Katerina Kourentzi*** [1], ***Prof. Chandra Mohan*** [1], ***Prof. Richard Willson*** [1]

***1.*** *University of Houston*

**A smartphone-readable lateral flow assay for POC monitoring of urine ALCAM in lupus nephritis**

Rongwei Lei[1], Binh Vu[2], Katerina Kourentzi[2], Chandra Mohan[1], Richard C Willson[1,2,3,4,]

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Introduction

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease that leads to chronic inflammation in multiple organs, including the kidneys. Lupus Nephritis (LN), the renal involvement of SLE, has drawn attention due to its high morbidity and mortality. However, the current standard diagnosis of LN is by renal biopsy. The invasive nature, inter-observer variability and attendant morbidity of this technique preclude serial biopsies and thus increase the risk of progression to end-stage renal disease. Here we report progress toward a point of care assay for the specific LN urinary biomarker-CD166/ALCAM and urinary normalizer-TNFRSF14/HVEM, which is used to correct for ALCAM dilution in the urine. In this research, green-emitting $SrAl_2O_4$: $Eu^{2+}$, $Dy^{3+}$ (SAO) phosphorescent nanoparticles were used as reporters for high-sensitivity LFA and compared to traditional gold nanoparticles.

Methods

Following milling, fractionation, silica-encapsulation, conjugation, and blocking of SAO, prepared phosphors were mixed with the sample and loaded onto LFA strips. An app-controlled smartphone was used to excite, image, and numerically-analyze the test results.

Results & Discussion

The limit of detection (LoD) of ALCAM and HVEM in urine was 0.5 ng/ml. Dried LFA strips and freeze-dried SAO storage stability were verified after being stored for five months at room temperature. The reproducibility of the preparation of LFA strips and nanoparticles was verified by three repeated standard curves.

Impact

This approach may greatly advance the management of SLE and lupus nephritis. The addition of markers like VCAM-1, Properdin, L-selectin, and Hemopexin to ALCAM could enhance the discriminatory power in active LN patients. HVEM was the most promising dilution normalizer for ALCAM, but studies are needed to verify the normalization ability of HVEM in adjusting other biomarkers across all ethnicities. Overall, we demonstrated a platform technology for quantitative, sensitive, and reproducible lateral flow tests using minimal equipment for self- or POC-detection of SLE flares.

# Supported Ionic Liquids (SILs) as functional materials for the removal of hazardous pharmaceuticals

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 31

***Dr. Márcia Neves*** [1], ***Mr. Rafael Francisco*** [1], ***Dr. Matheus Pereira*** [1], ***Dr. Ana Sousa*** [2], ***Prof. Mara Freire*** [1]

***1.*** *CICECO- Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro,* ***2.*** *Comprehensive Health Research Centre (CHCR), Department of Biology, University of Évora, Évora*

Environmental contamination by highly toxic pharmaceuticals is a matter of great concern. Cytostatic drugs, used to treat cancer, are amongst the most toxic pharmaceutical released into the aquatic environment. To mitigate this serious environmental and public health problem, several technologies have emerged for their removal envisioning their implementation in wastewater treatment plants [1-2]. Nevertheless, they are not completely efficient in the removal of pharmaceuticals, including cytostatics. Since urine only represents 1% of the volume of the total domestic wastewater [3], one of the best ways to avoid contamination with cytostatics is by removing them at the entrance point, i.e. from the urine. This could be achieved using silica particles functionalized with ionic liquids (IL), to be applied as a device in urinary settings.

Materials functionalized with ionic liquids, namely supported Ionic Liquids (SILs), can be envisaged as suitable tools for the removal of pharmaceuticals, since they join the advantages of solid supports and the tunable characteristics of ILs [4]. In this work, several SILs based on quaternary ammonium were synthetized using silica as the support material and changing the alkyl chain length of the quaternary ammonium. After characterization, the adsorption behavior of these SILs for distinct cytostatic drugs, viz. cyclophosphamide, mycophenolate acid and its pro-drug mycophenolate mofetil, 5-fluorouracil and its pro-drug, was evaluated by determining adsorption kinetics and isotherms. Studies of adsorption of each drug in continuous mode and in mimicked urine samples were conducted demonstrating the potential of SILs to be incorporated in a urinary device.

**Acknowledgements:** This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. This work was financially supported by the project POCI-01-0145-FEDER-031106 (IonCytDevice) funded by FEDER, through COMPETE2020- Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. R.F. acknowledges FCT for the PhD Grant SFRH/BD/138651/2018 and M.C.N. acknowledges FCT, I.P. for the research contract CEECIND/00383/2017 under the CEEC Individual 2017.

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# Gellan microspheres affinity towards biopharmaceuticals isolation from complex cell lysates

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 7

***Dr. Diana Gomes*** [1], ***Prof. Luís Passarinha*** [2], ***Prof. Ângela Sousa*** [1]

***1.*** *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6201-506 Covilhã, Portugal,* ***2.*** *UCIBIO – Applied Molecular Biosciences Unit, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.*

**Introduction**

Biopharmaceuticals as plasmid DNA (pDNA) and human soluble Catechol-*O*-methyltransferase (hSCOMT) are explored as targets in diseases' treatment. The biotechnological process to obtain its isolation involves several steps, which becomes time-consuming and expensive. Therefore, it is crucial to explore and develop new alternatives. Gellan gum is an anionic linear exopolysaccharide with great properties. Recently, it was used as a gel chromatographic matrix and in microspheres formulation to purify or capture proteins, respectively [1,2,3].

**Methods**

Different gellan microsphere formulations were produced through a water-in-oil emulsion [1] and some were functionalized with polyethylenimine (PEI). It was confirmed the microspheres formulation, the crosslinkers presence ($Cu^{2+}$, $Ni^{2+}$, $Mg^{2+}$) and the PEI functionalization, respectively, by semi-optical microscopy, SEM, and EDX analysis. After, binding and elution strategies were explored to maximize the pDNA and hSCOMT capture through a batch method.

**Results and Discussion**

Copper-crosslinked microspheres captured 15.61% of pDNA (≈15 fold more pDNA than in *Escherichia coli* lysate) with 2.42% of purity. This strategy is based on immobilized metal affinity chromatography by applying an elution step with 200 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. Copper-crosslinked microspheres functionalized with PEI improved pDNA recovery to 88.09%, with 3.18% of purity, by applying the same elution buffer with pH increased to 10.5.

Two strategies were established to capture hSCOMT from a *Komagaetella pastoris* lysate. Affinity strategy with magnesium-crosslinked microspheres recovered hSCOMT with 19% of bioactivity and a purification degree of 0.73% by elution with 250 mM NaCl, 500 mM $MgCl_2$, 10 mM MES, pH 5.2. The ionic strategy with nickel-crosslinked microspheres increased the hSCOMT bioactivity to 200% and purification degree to 77% by applying 100 mM NaCl, 10 mM MES, pH 6.2 as elution condition. It is one of the best results in the literature.

**Impact**

We demonstrated the gellan microspheres' affinity towards biopharmaceutical complex samples clarification, which can be applied in biotechnological platforms, reducing time, costs, and manipulations associated with following purification steps.

**Acknowledgments**

FEDER funds through POCI - COMPETE 2020 (Project POCI-01-0145-FEDER-007491) and national funds from FCT - Foundation for Science and Technology (Projects UID/Multi /00709/2019 and UID/Multi/04378/2019); UCIBIO is financed by National Funds from FCT/MCTES (UIDB/04378/2020); D. Gomes acknowledges PhD grant 2020.06792.BD supported by FCT.

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# Lab-on-valve platform for molecular recognition studies

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 67

***Mr. Bruno Gregório*** [1], ***Dr. Inês Ramos*** [1], ***Dr. Luís Magalhães*** [2], ***Dr. Rudolf Schneider*** [3], ***Dr. Marcela Segundo*** [1]

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Molecular recognition resorting to immunoaffinity and hybridization-based schemes is usually carried out on solid-phase surfaces. The immobilization of molecular recognition elements, such as receptors (affinity evaluation), antibodies (immunoassays), and nucleic acid probes (hybridization assays) on the surface of beads and further detection of target species by spectrophotometric, fluorescence, and luminescence measurements represent simple and versatile analytical workflows [1-3].

The implementation of the bead injection (BI) concept coupled to the lab-on-valve (LOV) creates a flexible platform for the automation and miniaturization of bioanalytical assays, permitting the manipulation of bead suspensions inside flow conduits [1]. The BI-LOV method implements renewable solid-phase supports for automation of molecular recognition schemes, preventing sorbent fouling phenomena and sample carry-over effects. In addition, the LOV platform presents several other advantages, namely short time-to-result intervals, low sample and eluent volumes that contribute to greener analysis, and minimal operator intervention thanks to LOV's automation features. Through computer programming, it is possible to perform bi-directional and stopped-flow operations, and to adapt the same manifold to different chemistries/separation features [2].

The present communication aims to demonstrate the implementation of BI-LOV for molecular recognition studies, highlighting its potential for studying antibody-antigen interaction for analytical applications.

**Acknowledgements:** The work was supported through the project UIDB/50006/2020, funded by FCT/MCTES through national funds. Bruno J. R. Gregório thanks FCT (Fundação para a Ciência e Tecnologia) and ESF (European Social Fund) through Programa Operacional Regional Norte for his PhD grant ref. SFRH/BD/137224/2018.

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# A molecular imprinting polymer (MIP) for the isolation of lupanine coupled to nanofiltration and solvent extraction

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 4

***Dr. Teresa Esteves*** [1], ***Dr. Flávio Ferreira*** [1], ***Ms. Ana Mota*** [1], ***Ms. Catarina Barbeitos*** [1], ***Ms. Késsia Andrade*** [2], ***Prof. Carlos Afonso*** [2], ***Prof. Frederico Ferreira*** [1]

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**Introduction**

Lupin beans are highly nutritious seeds, which bitter flavour is conferred by the presence of toxic alkaloids, namely lupanine, which is the starting material for the synthesis of sparteine, a known building block for the pharmaceutical industry. Lupanine has a complex chemical structure, making its synthesis quite challenging. The industrial process to make lupin beans edible, removing toxic alkaloids, uses high amounts of fresh water. We assessed nanofiltration and solvent extraction for the isolation of lupanine from industrial wastewaters (Fig. 1)[1]. However, the final purity (78%) needs improvement and we report the synthesis of a MIP for lupanine for improved isolation and purity.

**Methods**

Functional monomers were screened by computational design. The MIP for lupanine was synthesised by bulk polymerization using itaconic acid (IA) as functional monomer, EGDMA as cross-linker, and AIBN as initiator. The polymer was characterized by SEM, FTIR-ATR, BET, binding isotherm and kinetics studies. Batch binding experiments were performed with industrial wastewaters. Lupanine was quantified by HPLC.

**Results and Discussion**

The MIP presented higher lupanine binding (>95%) than the NIP (<60%) even in competitive solvents (Fig. 2), providing the most stabilized and favoured interactions by strong hydrogen bonds. BET showed that MIP has lower surface area and pore volume than NIP with a similar pore diameter indicating that lupanine binding on MIP relies on recognition driven by selective interactions, not on surface area. MIP adsorption follows the Freundlich isotherm, typical of MIPs obtained by bulk polymerization. Binding equilibrium is reached within 1 hour, following a pseudo-second order kinetic model. Recovery of lupanine proved to be efficient, with MIP robustness and recyclability observed over 5 consecutive cycles (Fig. 3).

**Impact**

Nanofiltration and solvent extraction allowed to concentrate and isolate a fraction rich in lupanine from industrial processing lupin beans wastewaters with 78% purity. Improved purification of lupanine is envisaged using the MIP synthesised with IA, which is a versatile building block produced from biological sources. By coupling the MIP step to the previous developed technologies, we aim to recover an added value product, from food industry waste, contributing to a more sustainable lupin beans processing.

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**Acknowledgements**

The authors acknowledge dedicated funding from Water JPI through the collaborative project ID 278 - Biorg4WasteWaterVal+ (WaterWorks 2014 ERA-NET Cofunded Call), and FCT through the Projects WaterJPI/0001/2014, WaterJPI/0002/2014 and WaterJPI/0003/2014 as well as Institute for Bioengineering and Biosciences (UIDB/04565/2021).

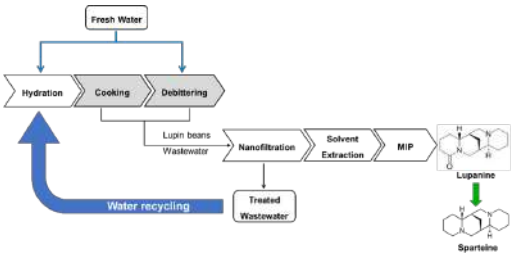



Figure 1 - lupin beans process and lupanine isolation.png

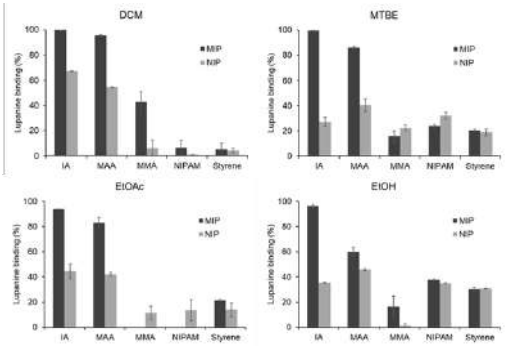



Figure 2 - binding of lupanine on mip.png

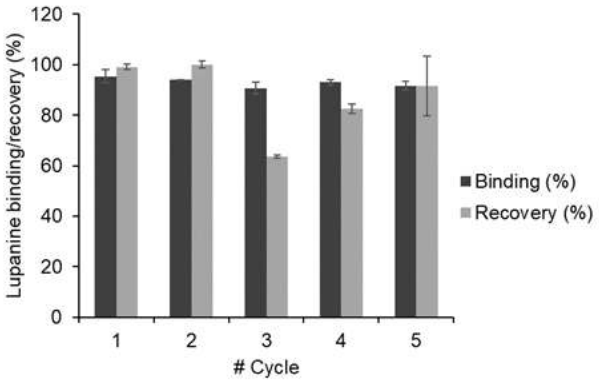



Figure 3 - lupanine binding-recovery on mip.png

# Green and cheap molecular recognition material for wastewater purification

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 106

***Mr. Ricardo Velez [1], Ms. Ana Furtado [1], Mr. Paulo Farinha [1], Dr. Raquel Viveiros [2], Prof. Teresa Casimiro [1]***

***1.*** *CleanMIPTech group, LAQV-REQUIMTE, Chemistry department, NOVA School of Sciences and Technology, NOVA University of Lisbon, Portugal,* ***2.*** *CleanMIPTech group, LAQV-REQUIMTE, Chemistry department, NOVA School of Science and Technology, NOVA University of Lisbon, Portugal*

Pharmaceutical pollution is becoming a global concern, especially due to its impact in the water and environment lifecycle. Drugs are released in high amounts to the environment during their consumption, between 30 and 90% of an oral dose is excreted in urine as an active substance. More than 600 different drugs have been detected in drinking water, wastewater, sewage sludge and soils. Typically, the wastewater treatment facilities are not equipped to remove these active ingredients from sewage. The improper disposal of drugs and the low efficiency in the removal of these compounds from wastewater treatment streams are leading to their persistence in the environment.

Mefenamic acid (MFA) is a non-steroidal anti-inflammatory drug indicated for relief of mild to moderate pain, and for the treatment of primary dysmenorrhea. This drug has been detected in wastewater treatment effluents with concentrations between 87-163 ng/L. However, these treatments are not specific and have not been efficient in the removal of MFA from wastewater streams. Affinity polymers with molecular recognition ability have been advanced for a wide range of processes where high-affinity, robustness and reusability are an added value.

Green and cheap polymers as molecularly imprinted polymers (MIPs) have been developed using supercritical carbon dioxide ($scCO_2$), a clean technology [1]. MIPs are obtained as dry-powders, in high yields, stable, ready-to-use, with controlled morphology, reusable, robust and with similar binding properties in comparison with natural molecules.

An adsorbent polymeric material with affinity towards MFA was developed in $scCO_2$, using 2-vinylpiridine as monomer, ethylene glycol dimethacrylate as crosslinker and AIBN as a free-radical initiation. The material was characterized by FTIR, average particle size, as well as, particle size distribution. The MFA-MIP and their control (non-imprinted polymer) were then loaded into a Solid Phase Extraction (SPE) column and evaluated as adsorption materials for MFA, mimicking a wastewater crude solution. MFA-MIP was able to absorb 26% more than the control material revealing high potential to be used as adsorbent in wastewater purification streams.

**References:** [1] https://sites.fct.unl.pt/clean-mip-tech/

**Acknowledgments:** The authors would like to thank financial support from Fundação para a Ciência e Tecnologia, Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES), Portugal through project PTDC/EQU-EQU/32473/2017 (by national funds through FCT/MCTES, PIDDAC). The Associate Laboratory Research Unit for Green Chemistry - Clean Technologies and Processes - LAQV is financed by national funds from FCT/ MCTES (UIDP/QUI/50006/2020 and UIDB/QUI/50006/2020) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER – 007265).

# Xanthene based Chemosensor for selective and sensitive Detection of organophosphate.

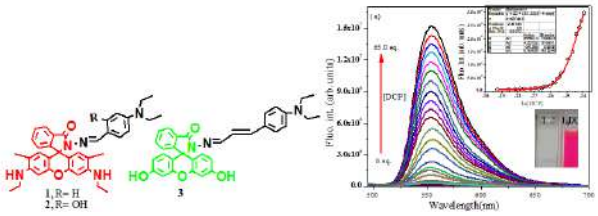
Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 53

***Mr. Kanhu Charan Behera*** [1]

***1.*** *Materials Chemistry Department, CSIR-Institute of Minerals and Materials Technology*

Nerve mimic agents are one of the most significant and deadly classes of chemical warfare agents and organophosphates. Organophosphates (OPs), which are all derived from phosphoric acid, a class of pesticide, frequently used as herbicides, pesticides, in agriculture as well as chemical warfare agents, e.g. Sarin, Soman, Tabun, Glyphosate, and VX, etc and several of which are acutely toxic and hazardous to the public. The main fonts of pesticide exposure to humans are through the air, water food chain, soil, flora, and fauna. moreover, acute poisoning generally affects farmers and industrial workers, chronic poisoning is more common in the general population. The major pathway of OPs poisoning involves inhibition of acetyl-cholinesterase, which particularly play a critical function in the transmission of nerve impulses observed in synaptic membranes, leads to surge/ accumulation of acetylcholine in the body which subsequently leads to continuous stimulation of glands, muscles, central nervous system, results in fetal death. OPs do however figure in much official causeforconcern importance list because of their toxicity, particularly to the aquatic environment. Ops incline not to persist or bioaccumulate in the environment. The widespread use of organophosphate is therefore leading to safety concerns. Diethyl chlorophosphate (DCP) serves as a nerve-agent mimic due to its low toxicity but similar activity to Sarin Hence, detection and quantification of organophosphates such as DCP and their degradation products highly desirable indispensable target.

Herein we designed and synthesized three xanthene-based **1**, **2,** and**3** chemosensory probes to achieve 'naked eye' for detecting DCP in the aqueous medium with adequate accuracy both in solution and vapor phase was revealed. The DCP selective binding as indicated by both the fluorescence enhancement and the chromogenic changes was clearly observable by the naked eye in ambient light and under UV light irradiation.




Kanhu . oral abstract.png

# Isolation of nucleic acids using chromatographic resins functionalized with ionic liquids

Thursday, 24th June - 14:25: Flash Session 5 - Poster - Abstract ID: 36

***Dr. Márcia Neves [1], Dr. Patrícia Pereira [2], Dr. Augusto Pedro [1], Dr. Fani Sousa [2], Prof. Mara Freire [1]***

***1.*** *University of Aveiro,* ***2.*** *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6201-506 Covilhã, Portugal*

Nucleic acids play relevant roles in biochemistry, biology, and medicine fields. Due to their properties and functions, nucleic acids are relevant biopolymers used in therapy and diagnosis, for which their purity and biological activity are of crucial relevance. For these applications, high-purity nucleic acids are required. The commonly used methods for nucleic acids purification include denaturant acrylamide gel electrophoresis and chromatographic strategies, such as affinity, ion-exchange, ion-pairing, reversed-phase, and gel filtration, which are laborious and time-consuming, and still result in RNA and DNA samples with low yields and purity levels. In this work, we took advantage of the ionic liquids (ILs) tailoring ability to design novel ligands with high affinity and selectivity towards specific nucleic acids, and investigated supported ionic liquids as novel columns to be used in chromatography aiming at developing cost-effective methods for nucleic acids purification. An initial screening with distinct IL chemical structures supported in silica was carried out, in which the IL 1-methyl-3-propylimidazolium chloride was identified as the most promising ligand. A chromatographic macroporous matrix was functionalized with the best IL and binding/elution studies were carried out. The IL acts as a multimodal ligand, acting by electrostatic or hydrophobic interactions which depend on the chromatographic conditions applied, with a remarkable dynamic binding capacity. The developed macroporous support allows the (one-step and sustainable) purification of nucleic acids from a bacterial lysate, namely small RNAs, ribosomal RNA, and genomic DNA, and can be regenerated and reused without compromising its separation performance.

# A New Tool Set for Examining Preferred Binding Domains of Proteins in Bioseparation Systems.

Thursday, 24th June - 15:35: Keynote 9 - Oral - Abstract ID: 48

***Prof. Steven Cramer*** [1]

***1.*** *Rensselaer Polytech Inst*

The identification of preferred binding regions and specific residues on proteins that interact with ligands and ligand coated surfaces is important for understanding the molecular basis for the purification of biological products from product related impurities. In this presentation we will discuss a variety of biophysical and simulation tools that can provide insights into these interactions. NMR with labelled proteins and ligand coated nanoparticles is employed to identify preferred binding domains for the Fc domain of antibodies and to examine the impact of multimodal ligand chemistry and ligand densities on this interaction. Umbrella sampling molecular simulations are then used to provide information on the contributions of ligand clustering to these interactions. Protein surface footprinting using covalent labeling followed by LC/MS analysis is then employed to identify preferred binding domains and interacting residues of several new protein biotherapeutics in multimodal systems as a function of pH. Finally, this data set is examined using a variety of simulation tools to shed light on the nature of selectivity in these challenging bioseparation systems.

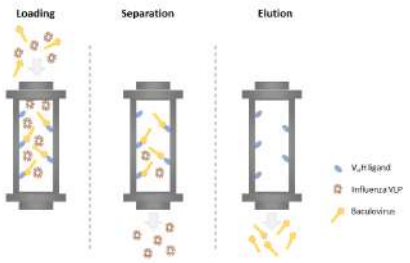
# An affinity approach for baculovirus clearance from viral-based bioprocesses

Thursday, 24th June - 16:05: Oral Session - Oral - Abstract ID: 46

***Ms. Rita Fernandes*** [1], ***Ms. Mafalda Moleirinho*** [2], ***Ms. Sofia Carvalho*** [2], ***Ms. Sandra Bezemer*** [3], ***Mr. Frank Detmers*** [3], ***Mr. Pim Hermans*** [3], ***Dr. Ricardo Silva*** [1], ***Dr. Cristina Peixoto*** [2]

***1.*** *IBET,* ***2.*** *IBET, ITQB,* ***3.*** *Thermo Fisher*

The biopharmaceutical industry is approaching a turning point where new therapeutic modalities such as virus-based biopharmaceuticals have been used in several applications such as vaccination, gene therapy and oncolytic therapy. The production of these biopharmaceuticals improved considerably over the last years however, main challenges are now focused on downstream processes. In insect-cell based biopharmaceuticals produced using the baculovirus expression vector system (BEVS) there is a concern regarding the co-production of baculovirus with the particles of interest. Their rod-shaped form and similar envelope to virus-like particles (VLPs), raises the difficulty when discriminating between both particles, increasing the purification complexity. This work presents a new purification strategy based on an affinity chromatography approach for baculovirus removal in VLPs manufacturing. Affinity ligands where discovered by phage display towards baculovirus particles and the most promising candidates were selected and immobilized in POROS® beads. The chromatographic performance of those resins was evaluated as well as the dynamic binding capacities. The impact of the residence time in the process performance was evaluated and the best ligands showed baculovirus removals above 70% and VLPs' recoveries above 60%. As a proof-of-concept, these ligands were also evaluated using two different biopharmaceuticals produced in BEVS: hepatitis C VLPs and adeno-associated virus (AAV). Afterwards, these resins proved to be robust to harsh elution and cleaning conditions, being used over 20 cycles without losing capacity. Overall, the work developed reports a new tool for baculovirus removal from viral-based bioprocesses, that can be used in the manufacturing of biopharmaceuticals produced using the BEVS.




Graphical abstract affinity2021.png

# Magnetic particles as affinity-based ligands: purification of single stranded DNA scaffolds for biomanufacturing DNA-origami nanostructures

Thursday, 24th June - 16:20: Oral Session - Oral - Abstract ID: 64

***Mrs. Ana Rita Santos*** [1], ***Mr. Rui Oliveira-Silva*** [1], ***Dr. Pedro M. R. Paulo*** [2], ***Prof. Duarte Miguel F. Prazeres*** [1]

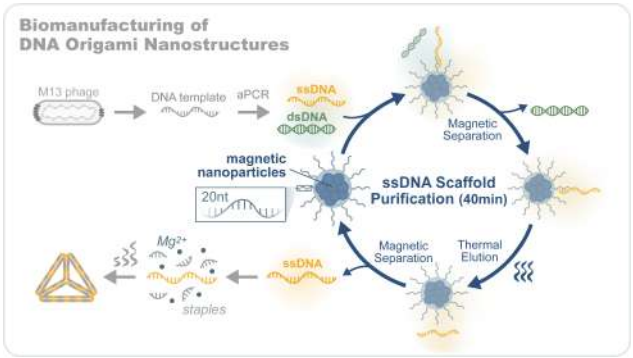
***1.*** *iBB - Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Tecnico, Universidade de Lisboa, and Associate Laboratory i4HB - Instituto for Health and Bioeconomy, Avenida Rovisco Pais, 1049-001, Lisboa, Portugal,* ***2.*** *Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal*

DNA nanotechnology encompasses the self-assembly of nucleic acids into complex nanostructures by exploring Watson–Crick base pairing. Usually, asymmetric PCR (aPCR) is used to generate 500-3500 nucleotide (nt) long, object-specific, single-stranded DNA (ssDNA) scaffolds using the DNA of the M13 phage as template. Scaffolds are purified by agarose gel extraction, a technique that is laborious, limited, not scalable, presents low recovery yields and a low-quality product. We present an affinity-based method using magnetic particles to purify ssDNA scaffolds from aPCR mixtures, which can be used in DNA-origami techniques.

aPCR, using the genome of the M13mp18 phage as template, generated 449 and 1000 nt-long single and double-stranded DNA (dsDNA). Carboxyl-modified magnetic particles were functionalized with a 20-nt oligonucleotide complementary to the 3' terminal of the 449 and 1000 nt-long ssDNA scaffolds. Hybridization between the ssDNA scaffolds in the aPCR mixture and the affinity beads was promoted at high LiCl concentrations. The dsDNA did not hybridize and could thus be separated from the magnetic beads. Following washing, magnetic beads were heated up to denaturation temperatures and ssDNA were recovered in the solution by magnetic separation.

The recovered ssDNA were used to assemble 31 and 63-bp edge length tetrahedrons using site-specific short oligonucleotides (staples), thermal annealing and high magnesium concentrations. Agarose gel electrophoresis and photochemical studies showed high assembly yield and purity.

In conclusion, affinity-based purification was successfully used to purify 550 ng of 449-nt and 880 ng of 1000-nt ssDNA fragments per aPCR reaction, which were subsequently folded into DNA-origami nanostructures.




Graphicalabstract.png

# A calcium-dependent Protein A-derived ligand for mild purification of antibodies and antibody fragments

Thursday, 24th June - 16:35: Oral Session - Oral - Abstract ID: 65

***Ms. Julia Scheffel*** [1], ***Dr. Sara Kanje*** [1], ***Ms. Emma Larsson*** [1], ***Ms. Malin Jönsson*** [1], ***Prof. Sophia Hober*** [1]

***1.*** *Dept. of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH - Royal Institute of Technology, SE-106 91 Stockholm, Sweden*

The gold standard for antibody purification is affinity chromatography based on Protein A, but a major issue associated with this method is the low pH required for elution. These acidic elution conditions can be detrimental to some antibodies, including Fc-fusion proteins, which may hinder the development of new Fc-containing proteins, despite promising therapeutic potential. To enable purification of all Protein A-binding antibodies or Fc-fusion proteins, regardless of their stability in a highly acidic environment, we have engineered a Protein A-derived domain that allows for considerably milder elution of the captured antibody. This domain, named $Z_{Ca}$, displays a calcium-dependent binding to the Fc region of IgG, which permits alteration of the antibody binding by depleting the calcium ion from an introduced calcium-binding loop (Figure 1). Calcium depletion from $Z_{Ca}$ can be accomplished merely by the addition of sodium chloride at low concentrations, even below physiological levels, which results in the efficient release of all captured antibody at neutral pH for subclasses IgG2 and IgG4 and at pH 6 for IgG1. This mild elution completely eliminates the formation of antibody aggregates in the capture step, in contrast to a conventional Protein A column where more than a third of the same antibody eluted at low pH formed aggregates (Figure 2). Further, multimerization of $Z_{Ca}$ has led to a binding capacity comparable to commercial Protein A resins for a tetrameric variant. In addition to providing a mild, sustainable and high-capacity antibody capture step, the $Z_{Ca}$ chromatography column provides high selectivity and recovery, equal to other resins such as the widely used MabSelect SuRe. Implementation of the ligand in a continuous antibody capture step has also proven the capability of the ligand to efficiently process high antibody titers throughout a large number of cycles. In addition to full-length antibodies, antibody fragments also represent promising candidates for therapeutic applications, but tend to be even more susceptible to aggregation. The concept of mild calcium-dependent purification has consequently also been applied to single-chain variable fragments (scFvs) through the development of new ligands based on $Z_{Ca}$ with novel specificity. To enable this, a phage display library was designed, from which a number of scFv-binding calcium-dependent variants were selected (Figure 3). These calcium-regulated affinity ligands offer minimal impact on the antibodies or scFvs to be purified. Potentially, this gentle purification strategy can enable the development of a broader range of antibodies, Fc-fusion proteins and antibody fragments in the future.

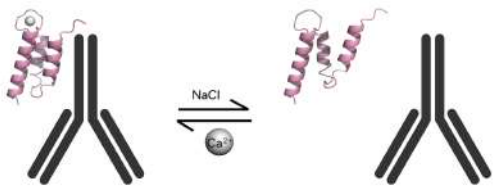



Figure 1. schematic of the calcium-dependent antibody binding of zca.jpg

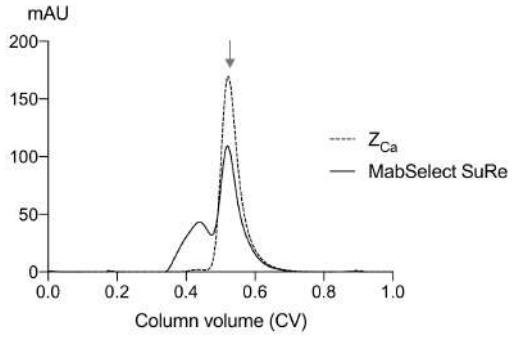



Figure 2. size-exclusion chromatography of zca and mabselect sure antibody eluates.jpg

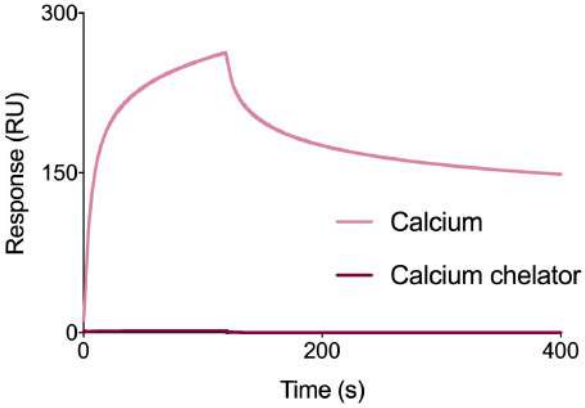



Figure 3. sensorgram of zca-based protein displaying scfv-binding only in the presence of calcium.jpg

# Introducing CaRA - A Multi-Purpose Library for Calcium-Regulated Affinity Domains with Novel Target Specificities

Thursday, 24th June - 16:50: Oral Session - Oral - Abstract ID: 109

***Ms. Malin Jönsson*** [1], ***Ms. Julia Scheffel*** [2], ***Ms. Emma Larsson*** [1], ***Ms. Marit Möller*** [1], ***Ms. Gabriella Rossi*** [1], ***Dr. Sara Kanje*** [1], ***Prof. Sophia Hober*** [1]

***1.*** *Dept. of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH - Royal Institute of Technology, SE-106 91 Stockholm, Sweden,* ***2.*** *Div. of Protein Engineering, Dept. of Protein Science, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH - Royal Institute of Technology, SE-106 91 Stockholm, Sweden*

Engineering naturally occurring proteins enables us to customize affinity domains according to our specific needs, tailoring them to become an important tool in a wide array of applications limited merely by our creativity. One of nature's ways to control protein activity is by creating a functional change through alteration of a protein's tertiary structure, a change which could be induced upon binding to a target molecule or through the interaction with ions that help stabilizing the protein's structure. The binding of metal ions makes metalloproteins a key component in some of the most fundamental biological processes like photosynthesis and oxygen transport. Calcium ions are known to allosterically control proteins through the EF-hand motif consisting of a helix-loop-helix structure which, upon attraction of calcium, undergoes an induced conformational change. Inspired by nature's elegant solution, we created a combinatorial library from which metal-binding protein domains with novel target specificities were selected (Figure 1). Previously, we successfully introduced a calcium-binding loop into a small protein domain derived from staphylococcal Protein A in an engineering effort to render its target-binding dependent on calcium. This domain served as the blueprint when designing our multi-purpose library for selection of calcium-regulated affinity – CaRA – proteins. To resemble the multifaceted usefulness of naturally occurring metalloproteins, we performed phage display campaigns towards a diverse set of targets relevant for various applications from bioprocessing to biological therapies. When evaluating the binding properties in the presence and absence of calcium using surface plasmon resonance, all discovered CaRA-variants displayed calcium-dependent binding and affinities in the nanomolar range. To exemplify, the sensorgram of $CaRA_{TNFa\_1}$ illustrates the difference in target interaction with and without calcium (Figure 2). The conferred metal-dependency can also be reflected in the structural stability of a protein, thus the isolated CaRA-variants thermal stability in the presence and absence of calcium was characterized. Some CaRA-variants displayed a large difference in the fraction of unfolded protein at each measured temperature with and without calcium present, indicating that the attraction of calcium to a high degree increases the thermal stability. For instance, $CaRA_{tPA\_3}$, showed a $\Delta T_m$ of 32 °C, resulting in a partly unstructured protein at room temperature if depleted of calcium, while a melting point as high as 59 °C was seen with calcium bound (Figure 3). CaRA is the first, to our knowledge, alternative scaffold library from which numerous calcium-dependent binders with novel target specificities have successfully been isolated.

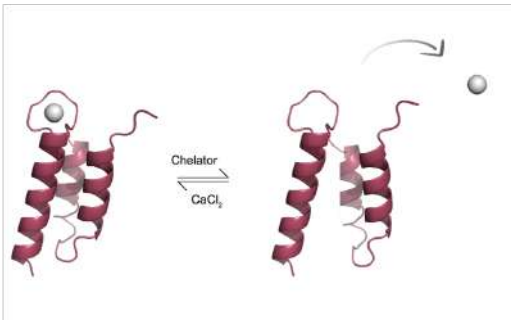



Figure1 mechanism of ca-dependency.png

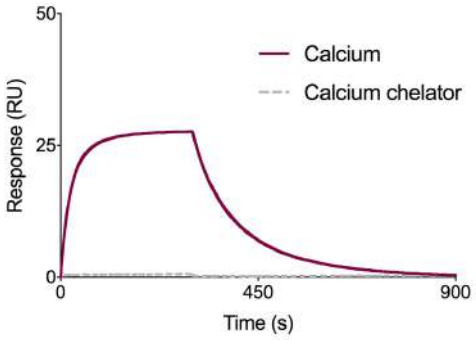



Figure2 sensorgram of caratnfa 1.jpg

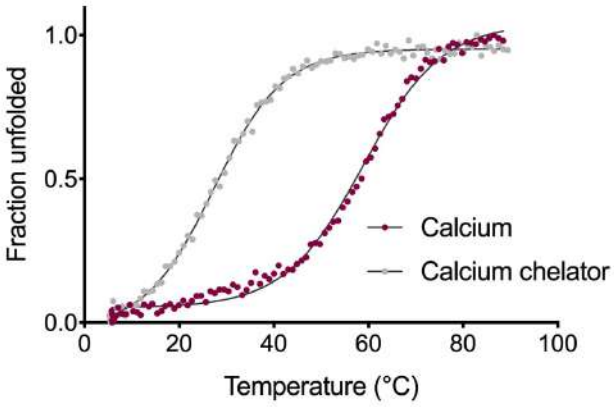



Figure3 variabletemperaturemeasurement of caratpa 3.jpg

# Affinity-triggered assemblies for cell differentiation and tissue engineering

Thursday, 24th June - 17:35: Oral Session - Oral - Abstract ID: 79

***Ms. Inês Padrão*** [1], ***Dr. Claudia Fernandes*** [1], ***Dr. Arménio J.M. Barbosa*** [2], ***Prof. Vitor Alves*** [3], ***Prof. Paula Gomes*** [4], ***Prof. Tiago Fernandes*** [5], ***Dr. Ana Pina*** [1], ***Dr. Margarida Dias*** [1], ***Prof. Ana Cecília A. Roque*** [6]

***1.*** *UCIBIO - NOVA School of Science and Technology,* ***2.*** *UCIBIO - NOVA SST, University NOVA Lisbon,* ***3.*** *LEAF, Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa,* ***4.*** *LAQV, REQUIMTE, NOVA School of Science and Technology,* ***5.*** *iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa,* ***6.*** *NOVA School of Science and Technology*

Affinity-triggered protein assemblies are a versatile and promising tool that can mimic the behavior of the extracellular matrix that supports cells, with applications in tissue engineering and stem cell research. The broad range of affinity pairs with different binding constants combined with the existence of diverse multivalency systems (e.g. multimeric proteins, branched polymers, etc) allows the formation of biocompatible and biodegradable affinity-triggered hydrogels with tunable porosity, mechanical properties and erosion rate [1]. In our lab, we have recently designed multicomponent assemblies based on the affinity pairs a) avidin-biotin with different multivalent systems and b) WW domain-proline-rich peptide.

The avidin-biotin affinity pair, which registers the highest affinity constant, was explored to create biocompatible hydrogels that support encapsulation of induced pluripotent stem cells that successfully differentiate into a neural cell line. Biotin is conjugated to polyethylene glycol with different multivalency and further mixed with avidin to yield hydrogels with different characteristics, such as resistance to erosion, influenced by the multivalent display selected. This system establishes an important first step towards the development of cell replacement therapies, where hydrogels could support and guide the effective integration of cells at the site of injury [2].

WW domains are small hydrophobic proteins binding to proline-rich peptides and, are typically biologically produced in the insoluble form which results in lower protein yields. To overcome this issue, an alternative approach is to engineer and chemically produce a 13-mer minimal version of the WW domain that retains the ability to establish interactions to target proline-rich peptides. Both ligand and target peptides are conjugated to multivalent polyethylene glycol, yielding two different components. Upon mixing together, a soft biocompatible affinity-triggered assemblies is formed with proven stability in stem cell culture media, and with mechanical properties in the same order of magnitude as for those hydrogels formed with the full WW protein in tandem repeats [3].

In the future, we believe that affinity-triggered systems can be useful for tissue regeneration, but also for other applications. Our research impact is visible in the development of a highly versatile strategy that can be applied to other affinity pairs, yielding biomaterials with tunable performance responding to different application needs.

References:

[1] DOI: 10.1016/j.biomaterials.2020.120563

[2] DOI: 10.1021/acs.biomac.0c00473

[3] DOI: 10.1002/biot.201800559

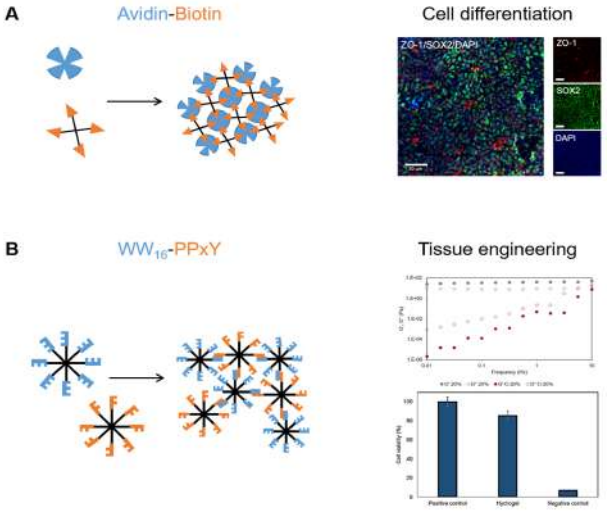



**Figure 1 –** Affinity-triggered assemblies. A) Avidin-biotin pair, hydrogel allows complete cell differentiation. B) WW-PPxY affinity pair, hydrogel allows cell growth with high cell viability.

Affinity2021.png

# Protein engineering of multi-functional biomaterials for regenerative medicine

Thursday, 24th June - 17:50: Keynote 10 - Oral - Abstract ID: 52

***Prof. Sarah Heilshorn*** [1]

***1.*** *Stanford University*

Stem cell transplantation is a promising therapy for a myriad of debilitating diseases and injuries; however, current expansion and transplantation protocols are inadequate. My lab designs biomaterials to overcome these challenges using biomimetic, protein-engineering technology. By integrating protein science methodologies with simple polymer physics models, we manipulate the polypeptide chain interactions and demonstrate the direct ability to tune the material properties including hydrogel mechanics, cell-adhesion, and biodegradation. These materials have allowed us to identify matrix remodeling as a previously unknown requirement for maintenance of stemness in neural progenitor cells within 3D expansion systems. Through a series of *in vitro* and *in vivo*studies, we demonstrate that protein-engineered hydrogels may significantly improve transplanted stem cell retention and regenerative function. Furthermore, many of the lessons learned about designing injectable biomaterials can be extended to design new bio-inks for 3D printing applications.

# Authors Index

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