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

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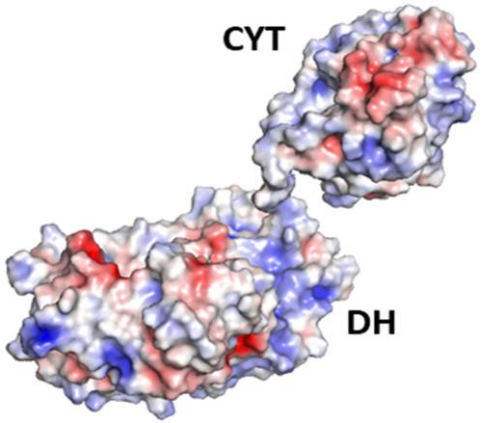
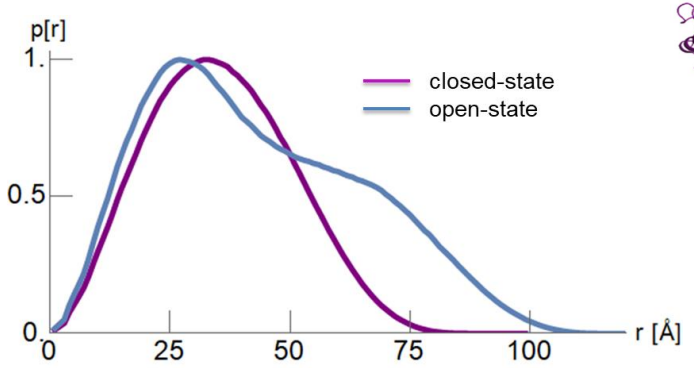
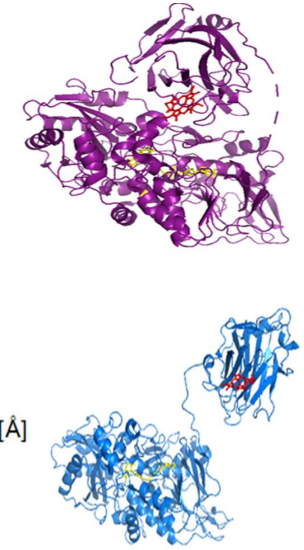


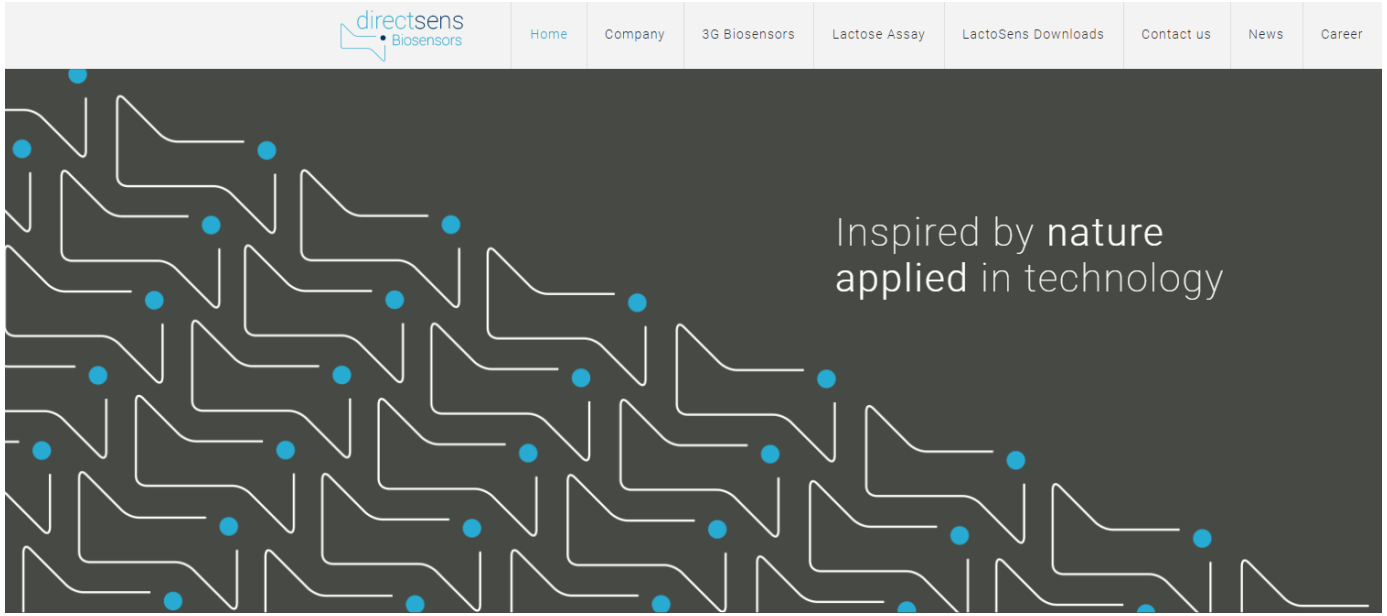




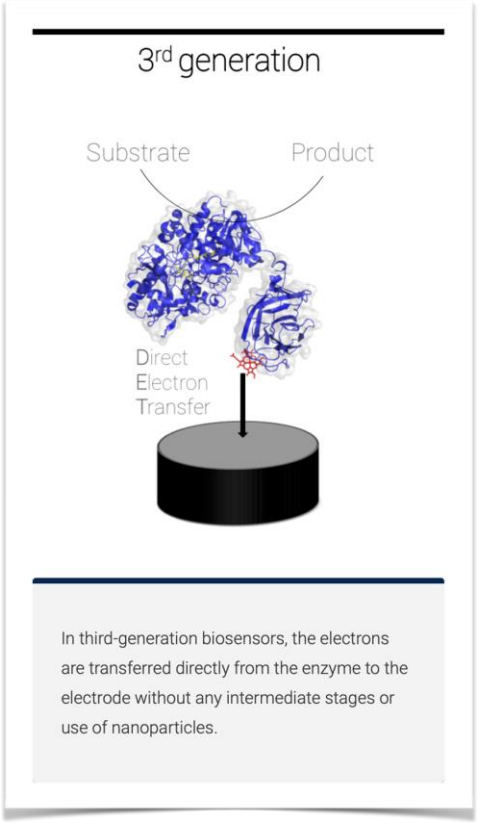
Application of cellobiose dehydrogenase

→ Affinity in diagnosis

Cellobiose dehydrogenase is the only enzyme capable of direct electron transfer that is commercially applied in biosensors.
They use the enzyme to detect a substance in a sample and convert it into a measurable signal.
For example food allergence: to detect extremely low concentrations of lactose in lactose free-milk products



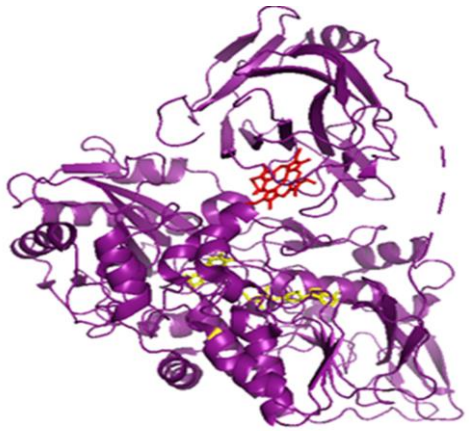
https://www.directsens.com/



Cellobiose dehydrogenase (CDH)

Cellobiose Dehydrogenase (CDH) 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). It‘s native function is the reductive activation of the polysaccharide-degrading lytic polysaccharide monooxygenases (LPMO) via the mobile CYT domain[1].
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 for the interaction with LPMO.

A



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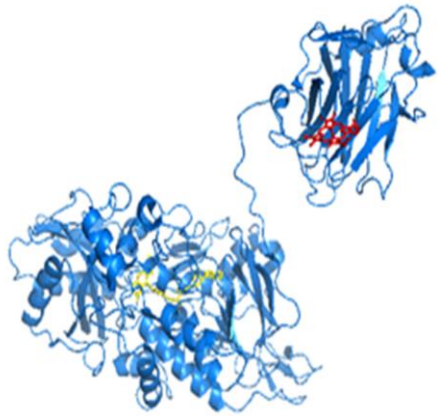
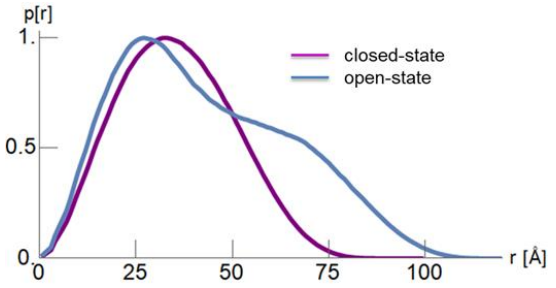


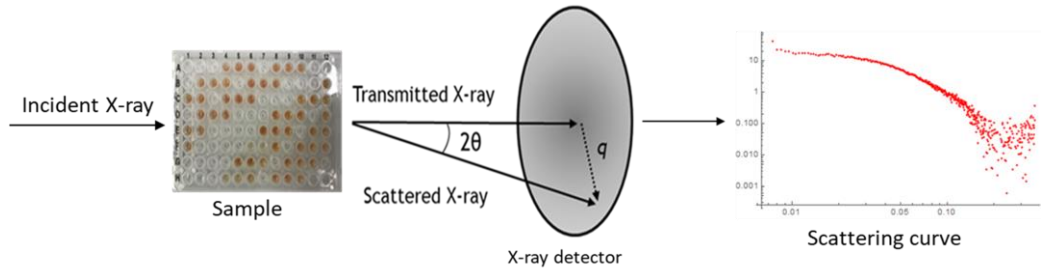
C

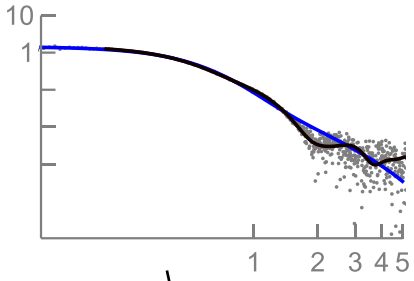


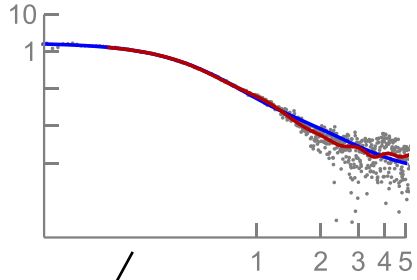
(A) Structure of cellobiose dehydrogenase from *Myriococcum thermophilium (MtCDH)* in the more compact or closed state. Colour-coding: heme *b* (red), FAD (yellow). **(B)** Structure of cellobiose dehydrogenase from *Neurospora crassa (NcCDH)* in the open state[2]. **(C)** Pair-distance distribution function of the models: distances are plotted against the distribution.

[1] Tan, T.-C. *et al. Nat. Comun.* **6**, 7542 (2015)
[2] RCBS Protein Data Bank (PDB), http://www.rcsb.org/

Small angle X-ray scattering (SAXS) results

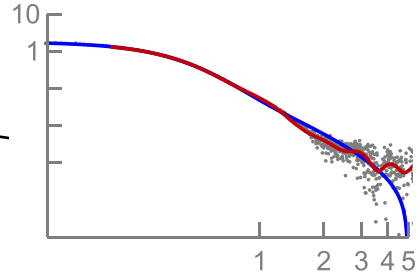


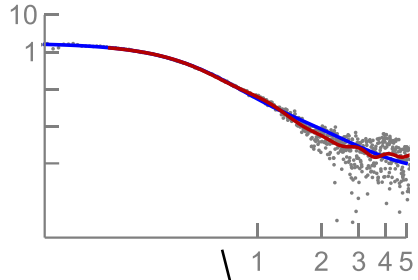


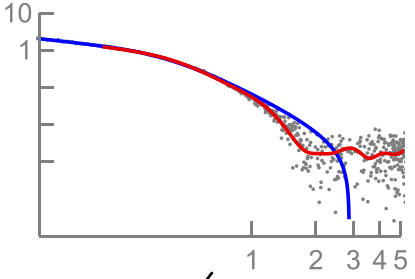


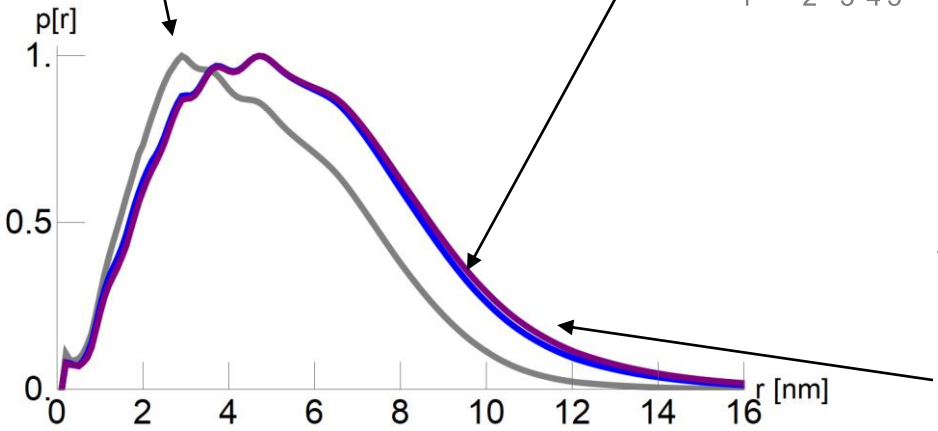
2mg /ml NcCDH

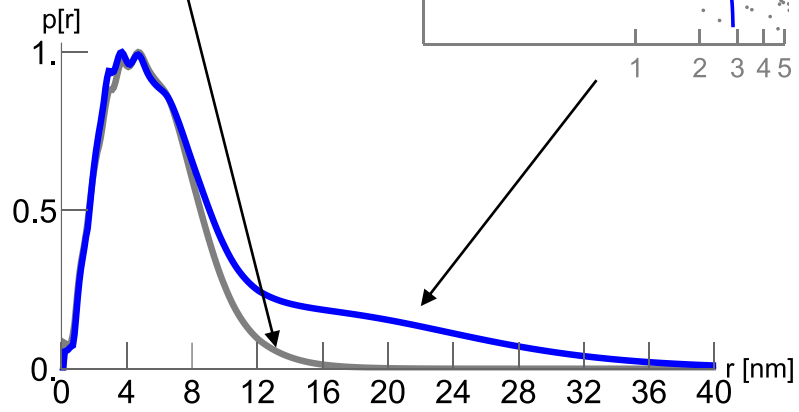
50 mM Sodium-citrate	50 mM Sodium-citrate pH 5.5
pH 4.5	Without ions
pH 5.5	+ 50 mM $CaCl_2$
pH 6.5	









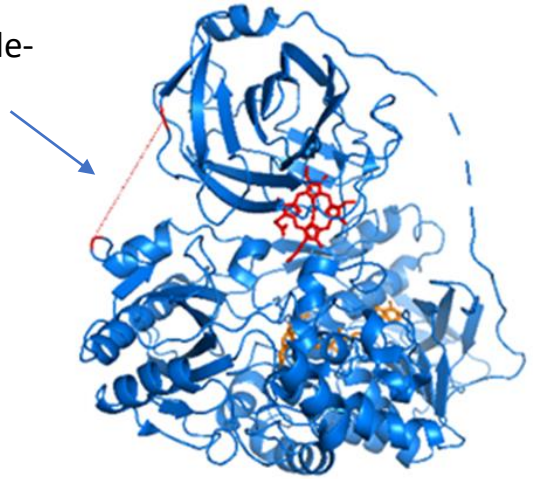


Pair distance distribution functions and the corresponding scattering curves. Experiment was 2mg/ml NcCDH in 50mM Sodium citrate buffer

Cross-linking of the two domains

→ Idea is to get a fixed closed conformation of the MtCDH, to use it as a reference for further experiments

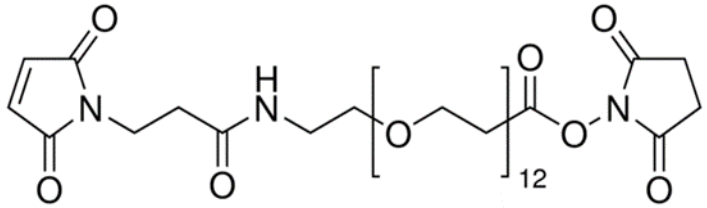
Maleimide-Linker



MtCDH variant: N27C, T701C

We performed an amino-acid substitution on both domains to introduce cysteins to offer an SH-group that can anchor the ester linker

linker



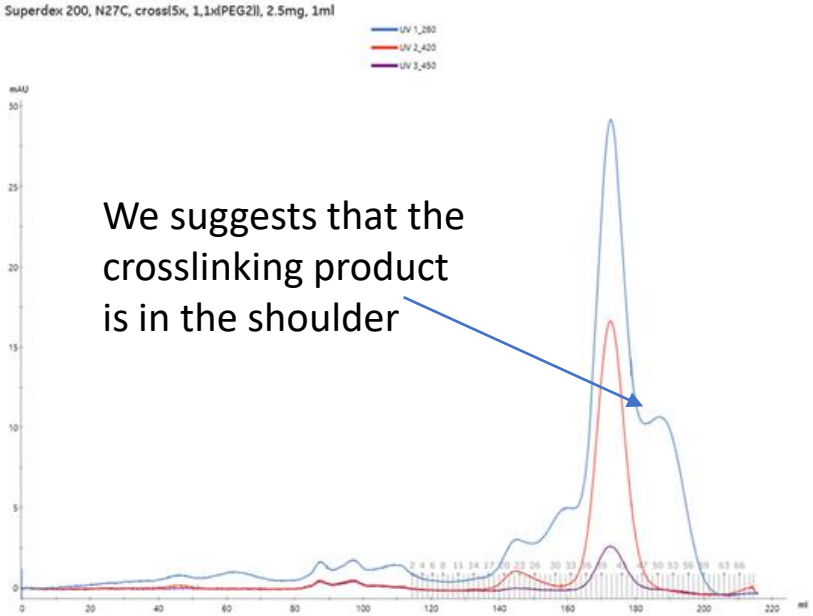
Maleimide-PEG$_6$-succinimidyl ester ($C_{26}H_{39}N_3O_{13}$)

- MW: 601,60 g/mol
- Spacer length: 31,7 angstroms

Maleimide-PEG$_2$-succinimidyl ester ($C_{18}H_{23}N_3O$)

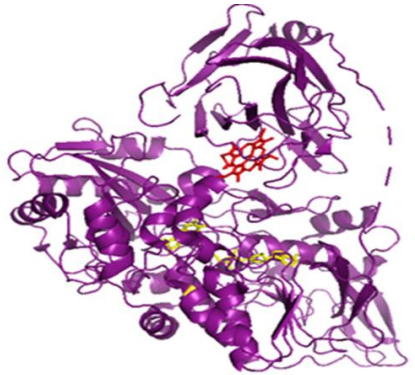
- MW: 425,39 g/mol
- Spacer length: 17,6 angstroms

SEC column: Superdex 200



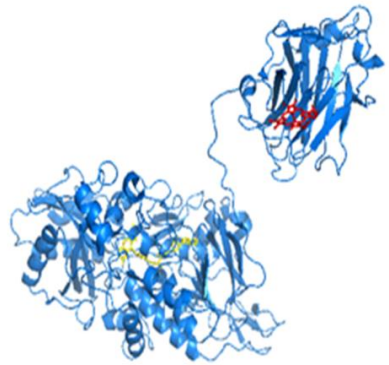
We suggests that the crosslinking product is in the shoulder

Conclusions

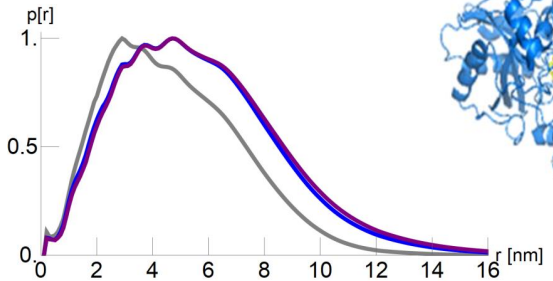


The mobility of the cytochrome domain of the CDH is important for its application in biosensors and biofuel cells. In this project SAXS is used to study the CDH conformation by different ambient conditions.

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 for the interaction with LPMO.



Our data suggest that the pH as well as the ionic strength affecting the mobility of CYT.



However, it is hard to make a prediction on the present conformation of the CDH. So the idea is to get a better idea by performing crosslinking.

We complement these experimental findings by molecular dynamics simulations and discuss molecular mechanism of this electron transfer system.