Molecular Mechanics of Enzymes

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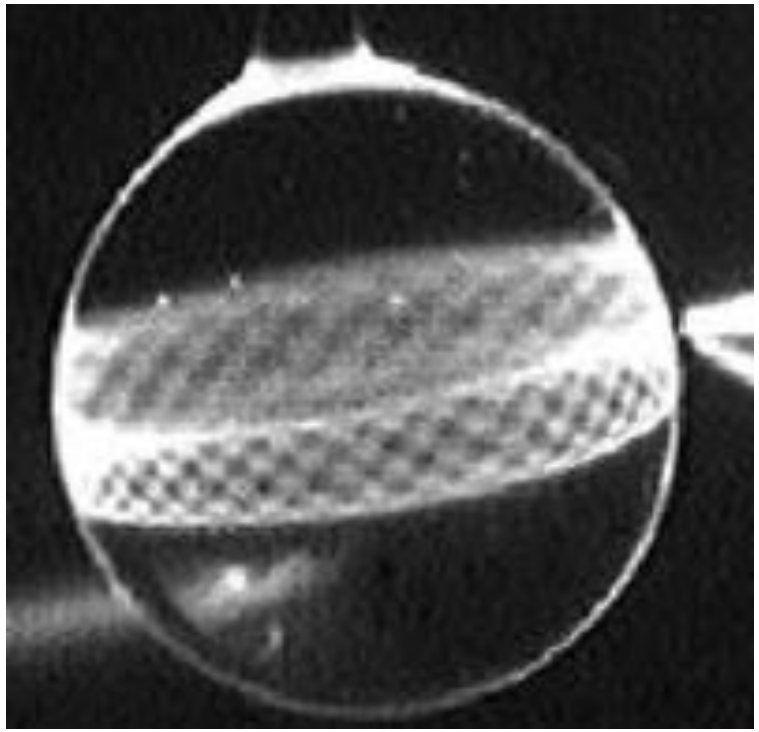
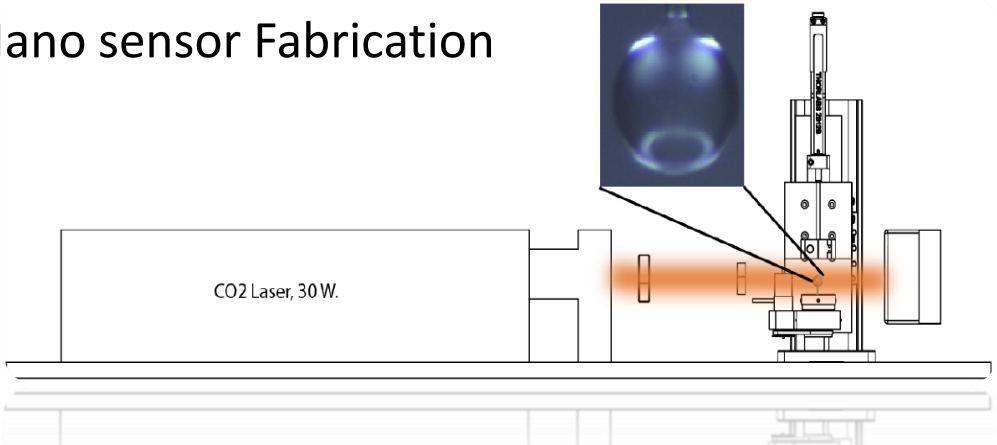


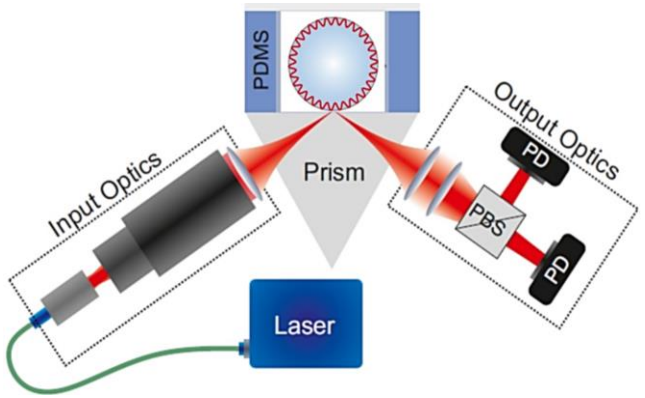
Optical Whispering Gallery Mode (WGM) based biosensors have been developed as a label-free system to study the dynamics and interactions of biomolecules. The principle of detection is based on monitoring the resonance shift of optical WGM in spheroidal resonators. Techniques based on WGM are non-invasive, label-free, ultra-sensitive and allow a real-time quantitative detection. Here, we present a single-molecule optical platform to probe single enzyme conformational change and activity.

Nano sensor Fabrication



Schematic of the setup to fabricate microresonators using a focused CO_2 laser. Zoom in shows a silica microsphere d ≈ 88 µm made at the tip of a single mode fibre by melting the fibre using a CO_2 laser.

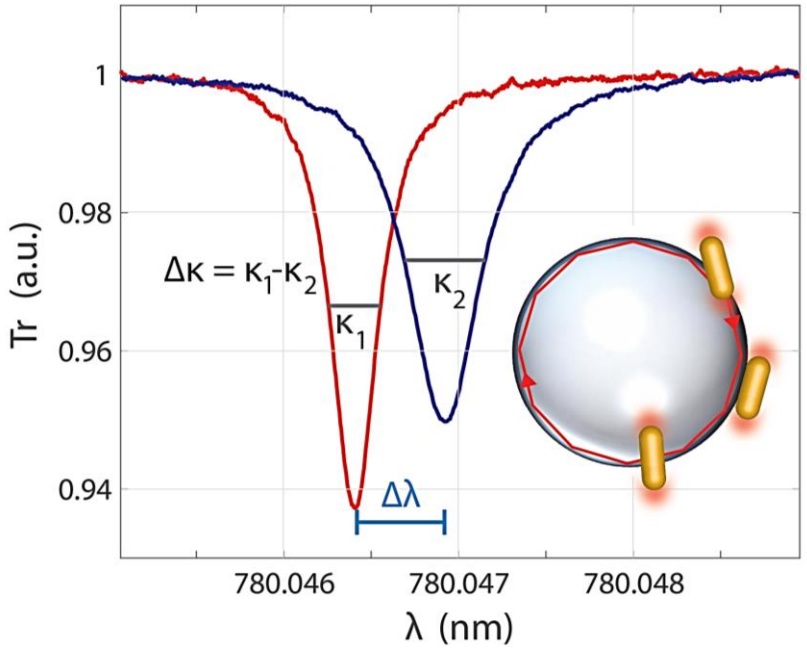
WGM Setup

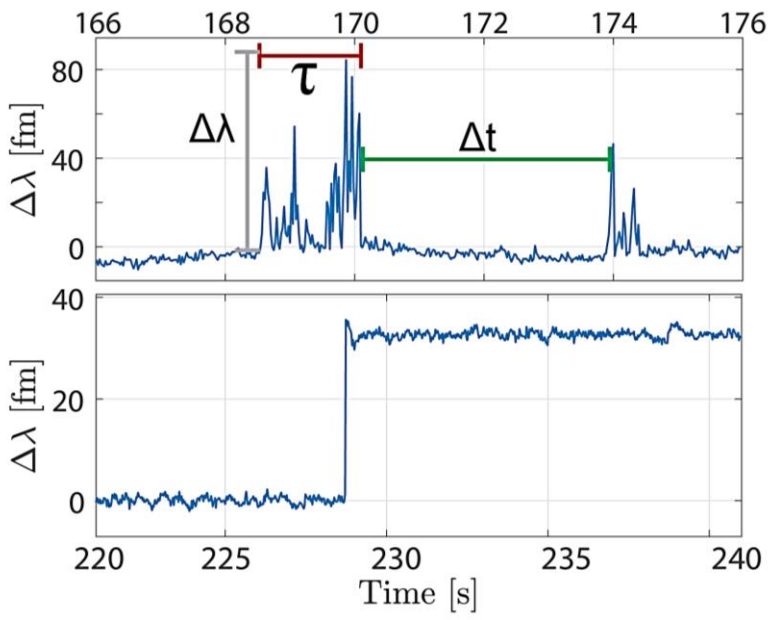


Schematic of the optical setup used for measurements. It consists of: a continuous wave (CW) laser equipped with optical elements, a prism providing evanescent waves for excitation of whispering gallery modes in a sensor, the optoplasmonic sensor (microresonator) and high-speed photodetector connected to a PC with software for data acquisition and processing.

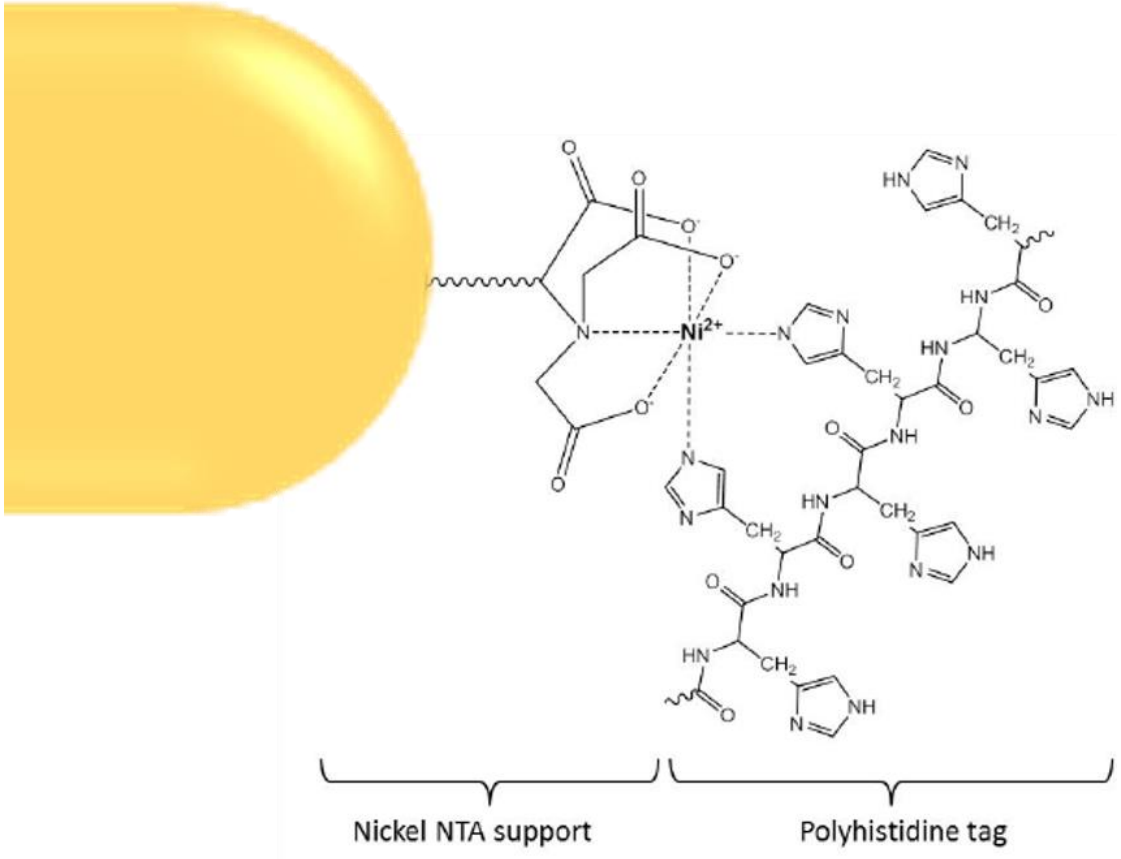
Sensor Signal

The sensor uses plasmonic gold nanoparticles coupled to optical whispering gallery modes (WGMs) to enhance the signal and probe the enzyme. The conformational change and the interaction of enzyme with its substrate are recorded as a shift in the WGM resonance frequency. On the top right an example of transient interaction, on the bottom right an example of permanent interaction.



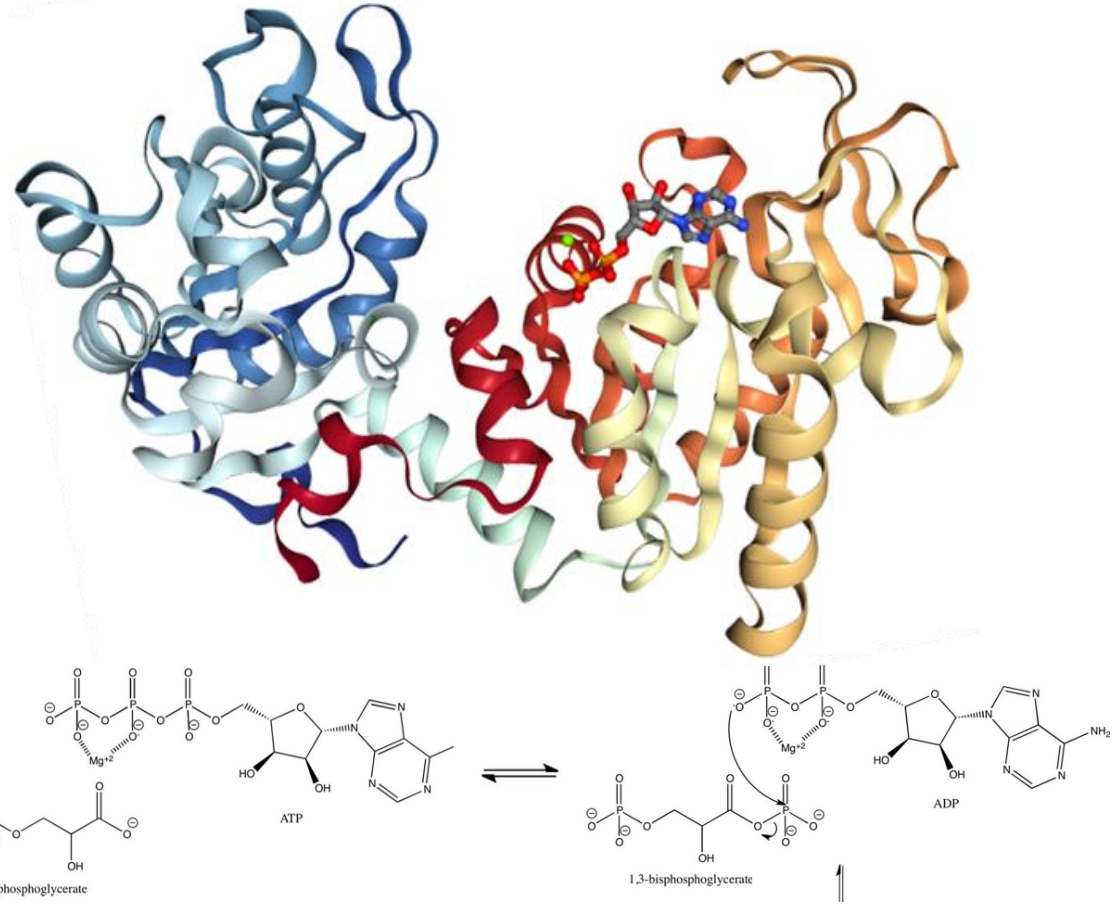


Protein immobilisation



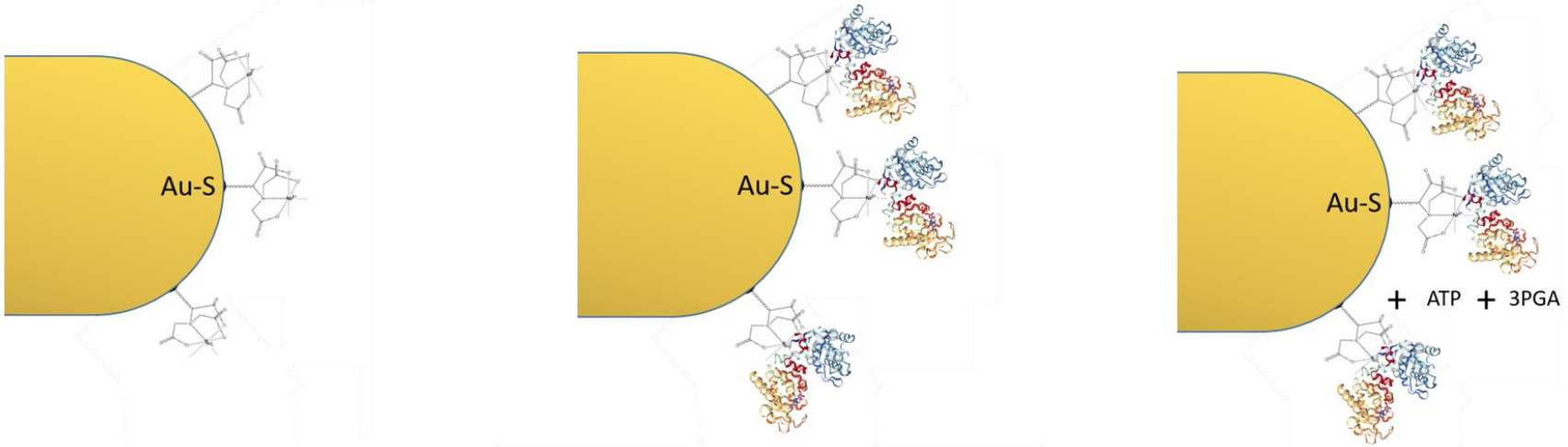
The immobilisation of the enzyme on the plasmonic gold nanoparticles (NP) is achieved by functionalising the NP with a linker which the polyhistidine-tag binds with micromolar affinity. The histidine ‘tag’ is introduced genetically at the proteins C-terminal to allow a specific orientation of the enzyme over the nanosensor.

3 Phosphoglycerate kinase

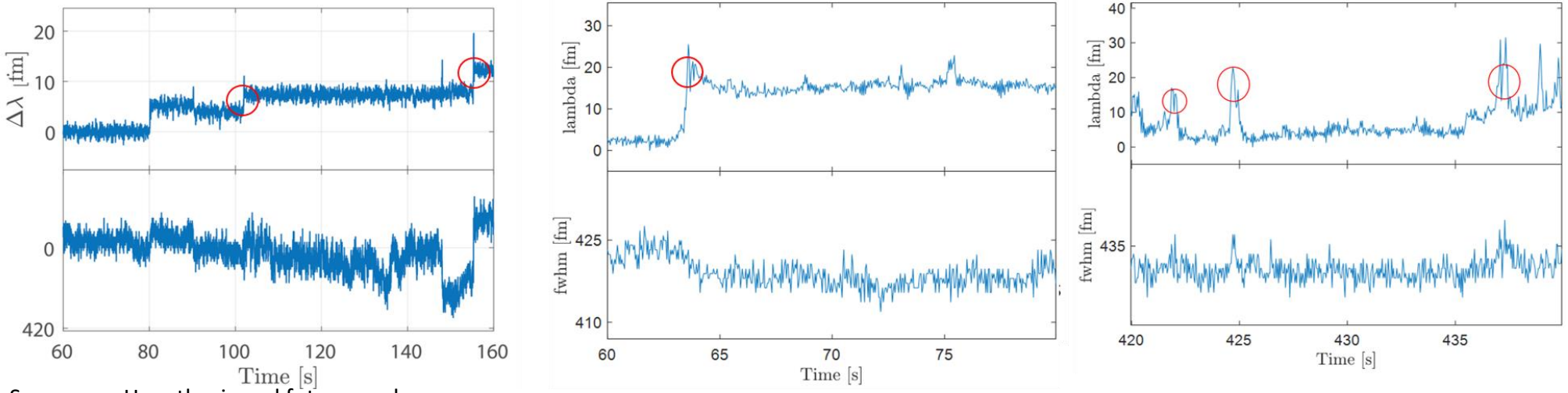


The enzyme used for these experiments is the 3 Phosphoglycerate Kinase, which has large domain conformational movement during turnover, referred to as a ‘hinge-bending’ enzyme. This figure show the 3PGK-ADP complex from *Geobacillus stearothermophilus* (PDB:1PHP).

WGM protein conformational change sensor



Frequency (corresponding wavelength) shifting and resonant linewidth (FWHM) changes at A) binding of NTA linker, B) binding interaction of 3PGK (the step indicates a long-term attachment of the enzyme to the gold nanoparticle), C) further signals observed upon the enzyme substrates, 3PGA and ATP, interaction. Control experiments have been performed not shown for brevity.



Summary , Hypothesis and future work

The 3PGK had been used as test-bed for the WGM. The WGM requires the use of gold nanoparticles in order to enhance the signals, for this reason the protein has been successfully immobilised on the plasmonic nanoparticles via the Ni-NTA linker. The protein activity was then tested on and off gold nanoparticles (not shown is this poster for brevity) before proceeding with the WGM experiments. Our current working hypothesis is that each double-peak (bottom-right panel) is associated with the movement of the enzyme as it opens or closes during the turnover when the substrate is given. We are in the process of data analysis in collaboration with the theory team to build a mathematical model that will allow us to associate the signal with the conformational changes.