

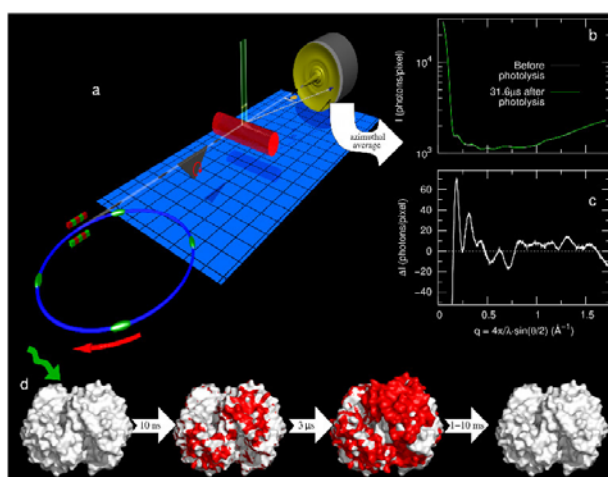
Static and Time Resolved (100ps) Wide Angle X-ray Scattering Probe Protein Structures and Motions

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Proteins are rather complex objects whose function is determined by the 3 dimensional arrangement of atoms. Consequently function regulation is often accomplished by structural changes. Wide Angle X-ray Scattering (WAXS) is becoming more and more important as tool to study proteins structures in their natural environment [1]. We have used our experience in tracking small molecule chemical reactions in solution [2], to develop strategies that can be used to monitor reversible and irreversible protein structural changes [3]. Data on Human Hemoglobin (Hb) are discussed in details.

Hb is responsible for oxygen delivery and it is known that cooperative oxygen binding/delivery arises from the interplay between different structures that have different affinities for ligands.



Ligated Hb has been crystallized in different structures using different crystallization protocols. Our **static data** permitted us to see which of these is closer to the solution one. These data are of impressive quality and will allow medium resolution structural modeling. **Time resolved data** of the ligated-to-unligated structural change have been recorded and they have shown:

1. The presence of a short lived (few microsecond) intermediate
2. A much faster formation of the equilibrium unligated structure ($\sim 3 \mu\text{s}$) than previously guessed from spectroscopy (absorption and Raman) based data.

The talk will discuss also the experimental challenges, the key elements of the beamline, and the future development in terms of data analysis (structural modeling) and of experimental capabilities (refurbished ESRF time resolved beamline and free electron laser).

References

- [1] - Makowski, L. et al. Molecular crowding inhibits intramolecular breathing motions in proteins. *J. Mol. Biol.* 375, 529-546 (2008).
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