Soft Condensed Matter

As in the previous years, this chapter presents a diverse set of articles related to the Soft Matter field published during the last year. A particular feature of this selection of highlights is the maturity attained by different techniques which now reveal unprecedented details. The first article, by Kim *et al.*, shows the advancement of pump-probe solution scattering that has permitted the reconstruction of three-dimensional molecular envelopes of the microsecond range protein intermediates involved in the photocycle of photoactive yellow protein. The second example, by Bressel *et al.*, demonstrates that the insight gained from time-resolved SAXS experiments could be utilised for the rational design of self-assembled unilamellar vesicles with controllable size and long-term stability. The XPCS technique has reached a new level of sensitivity that allowed Orsi *et al.* to derive even the fourth-order time-correlation function from a two-dimensional gel of a Langmuir monolayer of nanoparticles formed at an air-water interface. Time resolved GISAXS enabled Vegso *et al.* to capture a transient compression phase in a Langmuir film of nanoparticles upon continuous increase of surface pressure.

The article by Liu *et al.* illustrates that the flurry of discoveries of new liquid-crystal phases with T-shaped "bolaamphiphiles" go uninterrupted. Weinhausen *et al.* demonstrate that nanodiffraction with a complex biological specimen does yield results. Finally, new light is shed on crystallisation pathways in globular proteins (Zhang *et al.*) and polymers (Cavallo *et al.*). Within the space restriction, there are some notable omissions in this chapter, these include two-state rapid assembly mechanism of SV40 virus-like particles [S. Kler *et al.*, *JACS* 134, 8823 (2012)] , conformational changes of haemoglobin observed within red blood cells following laser flash photolysis [A. Spilotros *et al.*, *Soft Matter* 8, 6434 (2012)], etc.

Among the technical developments, ID10 has completed a major refurbishment with two end-stations for soft interfaces and coherent scattering coming to successful operation. The preparatory work for UPBL9a (ID02) is progressing well. The existing ID02 beamline will be closed in July and the upgraded beamline is expected to be reopened in May 2014. The technical design report for UPBL9b (ID09 Time Resolved) has been completed. Finally, the formal collaboration contract between ESRF and ILL for the Partnership for Soft Condensed Matter (PSCM) has been signed. The Science Building is nearing completion on schedule and the full scale operation of the PSCM in expected to begin in the second half of 2013. At present, the PSCM is actively seeking collaborative partners from academia and industry. In addition, enhanced services are offered to industrial customers for selected techniques (SAXS/WAXS and microdiffraction) in an effort to promote better use of synchrotron radiation in soft materials industrial R&D.

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Unravelling the protein structural dynamics of photoactive yellow protein in solution using pump-probe X-ray solution scattering

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Photoreceptor proteins play crucial roles in receiving light stimuli that give rise to biological responses. The detailed time-dependent conformational transitions in their native aqueous environment have been elusive, however, even for a simple prototype photoreceptor, photoactive yellow protein (PYP). PYP is responsible for signal reception of the phototaxis response of the bacterium *Halorhodospira halophila*. It is one of the most studied biophysical systems and has served as a model for understanding the photoreception and the subsequent signal transduction at a molecular level **[1,2]**. Various experiments using spectroscopy, crystallography, and NMR have been performed to reveal the kinetic mechanism and the structure of intermediates involved in the photocycle (**Figure 73**). However, the global structures of intermediates in the solution phase have never been identified due to the lack of structural sensitivity, the restraint by crystal contact, and the limitation of time-resolution of the techniques used so far.

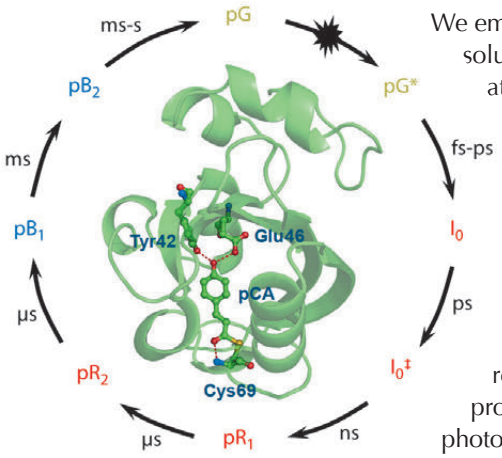

Fig. 73: General photocycle of photoactive yellow protein with corresponding time region [1]. On illumination with visible light, the ground state (pG) is set on a photoreaction pathway with the isomerisation of the chromophore region.

We employed the pump-probe X-ray solution scattering method established at beamline **ID09B** to overcome these limitations and unveil the structural dynamics of PYP in a wide time region from 3.16 μs to 300 ms (**Figure 74a**). The quantitative kinetic analysis was implemented to determine the number of kinetic components from the scattering curves. The results permit us to suggest the probable kinetic framework during the photocycle of PYP. Four intermediates and five time constants (10 μs, 279 μs, 1.3 ms, 23 ms, and 650 ms) during the photocycle were identified from the kinetic analysis. These rate-constants and the number of kinetic components are similar to the previous result observed with Laue crystallography [3] (**Figure 74b**). It implies that a common kinetic mechanism (parallel kinetics) can be operated in both the solution and crystalline phases. To describe the structural shape of an intermediate, the structural analysis, based on species-associated scattering curves determined from kinetic analysis, was performed. It permits us to visualise the global molecular shapes of all intermediates (pR_1, pR_2, pB_1, and pB_2) that show the gradual expansion of protein volume and the protrusion of the N-terminus with the progress of the photocycle (**Figure 74c**). The protrusion of the N-terminus becomes maximal in the pB_2 state, the so-called signalling state.

Our results from pump-probe X-ray solution scattering observations provide several insights into the structural dynamics of PYP. First, the protrusion of N-terminus during the photocycle of PYP is suppressed in the crystalline phase due to the crystal contact [3]. However, in the solution phase, the unrestrained N-terminus is already elongated in the pB_1 interediate, which is the precursor of the signalling state, and is maximised at the pB_2. Second, the rate-constant of the intermediate accompanying large conformational change such as the N-terminus protrusion in the solution phase show a dramatic difference from those in the crystalline phase. Third, the movement of the N-terminus in the solution phase is highly correlated with that of the chromophore (*p*-coumaric acid, pCA).

Using pump-probe X-ray solution scattering, we can directly observe a profound effect of the molecular environment on structural dynamics and the reaction rates of the PYP photocycle. This study is the first instance where the three-dimensional molecular envelopes of the protein intermediates are reconstructed with microsecond time resolution and should serve as a cornerstone for further structural studies of protein structural dynamics in solution.

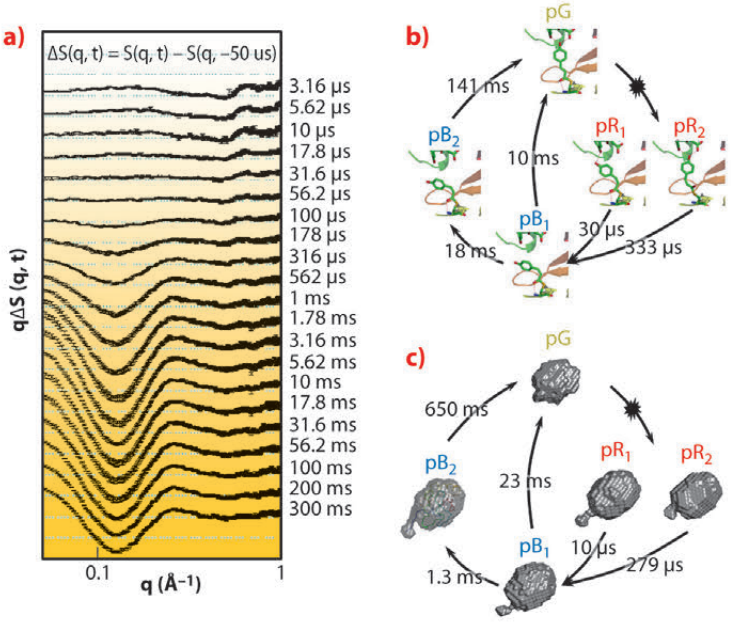

Fig. 74: a) Pump-probe X-ray difference scattering curves of the PYP photocycle. The photocycle of the photoactive yellow protein is triggered by blue light excitation (λ_{max} = 446nm). b) The kinetic framework and the rate-constants in the crystalline phase determined by time-resolved Laue crystallography [2]. The chromophore motion is visualised with atomic-resolution. c) Reconstructed molecular shapes of the intermediates and their associated rate-constants in the solution phase determined from pump-probe X-ray solution scattering.

References

[1] K. Hellingwerf, J. Hendriks and T. Gensch, *J. Phys. Chem. A* **107**, 1082 (2003).

[2] P. Ramachandran, J. Lovett, P. Carl, M. Cammarata, J.H. Lee, Y.O. Jung, H. Ihee, C. Timmel and J. van Thor, *J. Am. Chem. Soc.* **133**, 9395 (2011)

[3] H. Ihee, S. Rajagopal, V. Srajer, R. Pahl, S. Anderson, M. Schmidt, F. Schotte, P.A. Anfinrud, M. Wulff and K. Moffat, *Proc. Natl. Acad. Sci. USA* **102**, 7145 (2005).