4.6 INSTRUMENTATION TO UNDERSTAND THE CONNECTION OF PROTEINS AND ORGANISMS

Use of synchrotron radiation in life science research is expanding dramatically as researchers become aware of the capabilities of novel scattering and imaging modalities. These techniques range from solution scattering and fiber diffraction to phase-contrast and scanning imaging. In many cases, the APS is the only place in the western hemisphere with the capabilities to support this research. Future progress will be greatest at beamlines dedicated to a single experimental technique and equipped for the special needs of handling biological specimens. For example, high sensitivity of many specimens to radiation damage requires cryopreservation for measurement of relevant data. Studies of many variants under different conditions require high-throughput of specimens.

MICROCRYSTALLOGRAPHY

Microcrystallography was pioneered at the ID-13 of the ESRF. Several APS beamlines have already begun offering microcrystallography capabilities to users with beams sizes down to 5 microns. Other facilities around the world are also developing similar capabilities, but no beamline dedicated to macromolecular crystallography exists with a beam size smaller than 5 microns. (Smaller beams are available on a limited basis at the ID-13 of the ESRF.) The large unit cell parameters of crystals of proteins, viruses and macromolecular complexes constrains the optical design to be of low divergence. Typically, this implies long focal length optics resulting in a magnification of source motion due to imperfections in the optics. Maintaining the intersection of micron-sized beams and rotating, cryogenically cooled, micron-sized crystals is challenging. The APS renewal will allow the collection of data of high signal-to-noise ratio by improving the stability of the source, optical components, and endstation components by incorporating nano-positioning devices.

Efforts to validate the prediction of reduced radiation damage with micron sized beams mentioned in section 2.6 have been limited by the use of an energy-dependent, Fresnel Zone Plate focusing optic. Studies with a 1-micron, 15 keV beam show that the distribution of radiation damage about the 1-micron beam is anisotropic with more damage along the polarization vector. However, at 15 keV, the damage is observed to decay monotonically with distance from the beam center. No damage is observed beyond 4 microns (R. Fischetti, unpublished). The APS renewal will provide state-of-the-art, energy independent focusing optics allowing the high energy aspects of the APS to be exploited for further investigations of the possibility of reduced radiation damage with a 1-micron sized beam.

The planned improvements in photon beam stability outlined in section 3.4 will greatly enhance our ability to use small x-ray beams (of order 10 μm) to address common problems of crystal size and inhomogeneity. The improvements to stability and brilliance are essential for the development of microcrystallography using beams of ~ 1μm diameter.

Sample automounters which are critical to the efficient use of the APS beamlines allow screening of many crystals to find the “diamond in the rough”. However, the wide variety of sample automounters available on the beamlines reduces the ease of movement of users from one beamline to the next. The renewal will provide the possibility for standardization of relevant aspects of these systems.

The increased efficiency of pixel-array detectors and beamline automation will result in the acquisition of vast amounts of data in short time periods. The data will be accommodated by investments to improve our computer backbone, the addition of massive data storage arrays and through additional technical support. We will use the opportunity of the renewal, and the infrastructure investments, to assist older beamlines in improving their capabilities, and in providing leveraged support to many beamlines, e.g. night operators to assist MX users.

OTHER CAPABILITIES

Time resolved studies have great potential for the future. In the ultrafast section we discussed our plans to provide sliced ~1ps pulses to APS users and to upgrade existing ultrafast capabilities. Ultrafast studies of protein photochemistry have been very interesting, and their utility has been

increased by the development of optogenetics (Miller 2006), which permits the pumping of non-photo biochemical reactions using light. Figure 4.6.1 shows an example of light sensitizing a histidine kinase effector. Through this kind of approach, the time development of biochemical intermediate states might be studied with laser pump/x-ray probe techniques, as has been so effective for photoreactive proteins (Moffat 2001).

The imaging needs of the life sciences community will be addressed through the development of new beamlines that will form part of the imaging capabilities in support of our multi-scale characterization of materials (section 2.1) The three relevant capabilities will be:

- A 200-m-long beamline for high sensitivity, wide field x-ray phase imaging,
- A beamline with a 20-m-long experimental hutch for coherent diffraction imaging (CDI) accommodating next-generation focusing optics and high dynamic range area detectors, and
- Coherence-conditioning and focusing x-ray optics that provide well-defined wavefronts for CDI under both plane and curved-wave conditions.

Combined with upgrades to existing imaging capabilities, these new capabilities will provide an extraordinary set of tools for characterizing the hierarchical structures intrinsic to biological systems. The correlative microscopy capabilities will also be valuable.

Dedicated facilities for SAXS/WAXS with tunable x-ray energies (for anomalous diffraction) and time-resolved capabilities will provide a unique window into the dynamics of macromolecular systems. The APS renewal will enhance data quality and reduce the amount of protein needed for an experiment by providing improved beam stability and facilities optimized for solution SAXS/WAXS data collection. Large format detectors will allow the simultaneous collection of SAXS/WAXS data providing information on the secondary, tertiary and quaternary structure of proteins and macromolecular complexes. Automation of the sample delivery and cell cleaning process will allow the study of proteins under a much wider variety of conditions than is currently possible furthering our understanding of protein dynamics and drug/target binding.

Optogenetics – using light to probe biochemistry beyond photochemistry

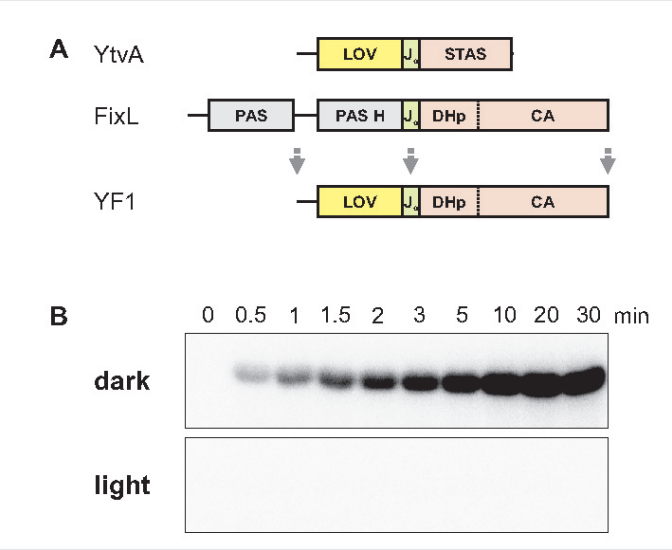

Fig. 4.6.1. *Design of mix-and-match proteins (Moeglich et al. 2009). A) The oxygen-sensitive PAS H sensor domain of one parent protein FixL is replaced by the blue-light-sensitive LOV sensor domain of the second parent protein YtvA, to produce the fusion protein YF1 whose histidine kinase effector domain DHp-CA is now light sensitive. B) Phosphorylation by the histidine kinase proceeds briskly in the dark but is completely abolished in the light.*