Life Science Team Report
APS Renewal

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Utilization of synchrotron radiation by the life science community has been growing rapidly in recent years, driven by advances in x-ray science and a need for new approaches to solving the multitude of questions arising from the explosion of biological information coming out of genomic, proteomic and metabolomic projects. In many cases, life scientists have shared synchrotron facilities with materials scientists and chemists, utilizing multi-purpose beamlines that represent, at best, a compromise between what physical scientists require for their experiments and the needs of the life science community. This has limited the efficiency of experiments and the ultimate impact of the work. The community of biologists with compelling needs for synchrotron facilities has now grown to the point where the development of dedicated single-purpose beam lines will be required for further progress. As we outline here, the impact of experiments enabled by this development will be substantial and wide spread.

1. Executive Summary

Every organism is the outward physical manifestation (phenotype) of internally-coded inheritable information (genotype). An overarching challenge in biology and biomedicine is understanding the links between genotype and phenotype and predicting phenotypic effects of altered genotype. A major goal of modern biology is to reliably link the avalanche of data on genetic diversity and expression from molecular biology to the major conceptual problems in biology, ranging from basic research questions on biological diversity to applied concerns such as why one individual but not another becomes ill (injured) in response to the same stress. Resolving these genotype-to-phenotype questions requires techniques that bridge the scales of molecular biology with those of organisms. New capabilities spanning length scales from nm to cm are essential and will lead to breakthrough results in basic and applied biology and biomedical science. Synchrotron-based imaging techniques provide unmatched and relatively unexplored methods for understanding the connection of molecular-based information in the phenotype to the hierarchy of structural scales relevant to organismal function.

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. Although much progress has been made, this has often been done at multi-purpose beam lines that require special adaptations to accommodate biological materials, slowing some experiments and precluding others. Future progress will be greatest at beamlines dedicated to a single experimental technique and optimized for use on 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.

Much of the newly expanding efforts involve biomedical applications which take advantage of all available imaging and scattering modes. These include studies in development of contrast agents for biomedical imaging; the use of nanoparticles for image-guided therapeutics and diagnostics; fundamental studies of neurological diseases (e.g., Alzheimer's, Lou Gehrig's Disease); blood flow in capillaries during exercise; in heart during infarctions; and in brains during strokes.

Other applications being planned include study of air flow in lungs and among alveoli as regulated by chemicals or drugs and studies to determine the changes in air flow induced by asthma. Investigators are exploring the use of x-ray imaging to approach questions about the effect of microbial infection or food allergies on the morphology and flow of the gastrointestinal tract. Finally, fundamental questions about osteoporosis and the mechanisms of bone and tooth fracture can be answered using the advanced imaging facilities currently being discussed. In many cases the questions being asked require the penetrability and resolution available only at the APS.

The special requirements for preservation of molecular structure and handling of biological samples make the availability of dedicated beam lines a high priority for all of the relevant techniques. Beam lines for solution and fiber studies; scanning microscopy; coherent diffraction, phase contrast and multi-length scale tomographic imaging are very high priorities for the life science community and will have significant impact on multiple fields in the biological sciences. The APS is one of the best places on earth to stage these experiments and is well positioned to make a substantial impact on the biological sciences through their implementation and use.

2. Introduction

Macromolecular crystallography has had a huge impact on the biological sciences, leading to many significant insights into the function of a myriad of biological systems. The impact of other x-ray techniques on biology is developing more slowly due to factors including limited availability of appropriate experimental facilities. America has been slow to develop facilities dedicated to scattering or imaging of biological specimens, and synchrotrons in Europe and Asia have a considerable advantage in these areas, placing American basic scientists and engineers at a decided disadvantage in studying many fundamental biological questions.

A variety of factors contribute to make this an important time for development of new x-ray facilities for the study of biological materials. First, development of novel x-ray optics is paving the way for development of the bio-nanoprobe (BNP). Second, convergence of computational and experimental techniques is greatly enhancing the amount of information that can be extracted from phase-contrast imaging and solution scattering data. Third, development of the field of bio-nanotechnology as an interface between inorganic and organic sciences is providing new tools for probing intracellular structures. Such technological advances have increased the sensitivities and temporal resolutions of x-ray imaging of biological structures by orders of magnitude.

Enhanced capabilities for synchrotron-based imaging, scattering and nanoprobe quantification of biological structures and elements will impact virtually every aspect of biology from biomedicine and pharmacology to evolutionary biology. A number of imaging tools can probe objects at nanometer spatial resolution, but few can image the internal structures of three-dimensional objects from nanometer to centimeter scales. Synchrotron x-ray imaging has become such a tool and is uniquely suited for investigating a broad-range of objects in biological and materials sciences with complex hierarchical internal or buried structures that cannot be properly preserved or studied by sectioning, and dynamic and real-time studies of biological and materials processes intrinsic to physiological and biomechanical function.

3. Key Science Drivers

The impact of the proposed facilities would span virtually all of biology, from biochemistry and molecular biology, to ecology and evolution.

Evolutionary biology. Understanding the evolution of life's diversity, and how the planet will respond to global climate change requires detailed interrogation of diverse plant and animal systems. The following are among the questions that the new x-ray phase-contrast imaging methods will be essential to addressing: How did the initial diversification of metazoans proceed, for example, as represented by fossilized embryos from the late Neoproterozoic and earliest Phanerozoic? How did early angiosperms develop from earlier forms? How did physiological systems develop their present characters (e.g., insect vs mammalian respiration)? How did the two predominating biomineralization systems (calcium carbonate, calcium phosphate) develop, in particular, the apatite-collagen bio-nanocomposite system in bone? Not only do these (and other similar questions) have important intrinsic value, they also suggest directions for biomimetic materials design. For example, the many-spine architectures of the families of diadematoid sea urchins represent an evolutionary sampling of the "design space" for functional calcite structures (i.e., cell- controlled, low temperature ceramic processing pathways)

Quantitative analysis of energy-supply systems (metabolism). Many unanswered, fundamental questions exist in these areas that can be addressed with small animals of major agricultural, medical and ecological importance. For example, how do birds and insects achieve one-way flow through their valve-less respiratory systems? High temporal and spatial resolution of the volumes of the various regions of these systems is required to understand how these high-capacity respiratory systems function. A second fundamental question involves scaling of metabolism. The energetics of cardio-respiratory flow are hypothesized to drive the well-known but poorly understood scaling of metabolic rate in organisms, whereby smaller organisms have higher mass-specific metabolic rates, tissue mitochondrial contents and heart rates. This fundamental scaling pattern drives much variation in life history ranging from heart rate to fecundity and lifespan, yet the mechanisms driving this pattern remain highly controversial. X-ray phase-contrast imaging of flow through supply networks of animals promises the first technique for rigorously testing current supply-limitation theories of metabolic scaling.

Environmental stress tolerance. Organisms vary dramatically in their tolerance to extreme environments, with some being able to survive freezing and others tolerating body temperatures exceeding 50°C. Extreme environmental stresses are always associated with major structural changes (e.g. ice crystal formation during freezing, vacuole formation during drying, protein and lipid conformational changes during heat-damage) that are extremely challenging to visualize with conventional methods. Synchrotron-based, wide-field phase contrast imaging is the only technique with the spatial and temporal resolution necessary to address these questions. How organismal structures (resistant vs normal strains) respond to drying or temperature remains poorly understood, and understanding the role of specific phenotypic characters is key to developing a mechanistic understanding of environmental tolerance and the production of new plant, micro-organismal and animal strains resistant to environmental stress. The structural hierarchies in soil also are extremely important for plant stress tolerance, and remain poorly understood. A key policy goal for preventing food shortages associated with human population growth and global climate change is the development of new strains of drought, freeze, and heat-resistant plants, and synchrotron-based imaging can provide critical tools in these efforts.

Tissue mechanics. Nanocomposite-based biological support structures (e.g. bone, tendons, teeth, chitin, xylem, phloem, spider silk) exhibit combined strength and elasticity far exceeding engineered materials. Many important diseases (e.g. osteoporosis, joint injury) result from pathologies of bones and tendons. All of these support structures are complex nanocomposites possessing a hierarchy of structures of at least ten spatial scales. For example, bone is a nanocomposite of collagen and mineral (apatite). How the two very different phases function together to produce a tough, fracture-resistant material remains incompletely understood. Why

certain aged patients suffer fractures (osteoporosis) remains incompletely predicted by current measures (bone mineral density, bone microarchitecture), a critical issue since osteoporosis-related hip replacements are estimated to cost the U.S. over $16 billion yearly. How loading induced damage accumulates in bone remains to be quantitatively understood. The 3D network of the canaliculi-osteocyte system, thought to be the mechanical sensors controlling bone remodeling, remains largely unquantified: numerical modeling of real networks of these submicron diameter channels may help to explain how they function in this role. All of these areas require 3D x-ray imaging beyond that presently available at the APS. In teeth, the interior, mechanically tough dentin (another nanocomposite) and the exterior hard and chemically resistant enamel (very high density apatite) are joined by the dentinoenamel junction. The new imaging capabilities will greatly improve the understanding of how this natural graded interface functions and how it might be replicated in clad or coated engineering materials, including those used in biomedical implants. Imaging coupled with *in situ* loading and x-ray scattering quantification of internal strain within the tissues is an example of where synergistic increases in understanding will result.

In-vivo, longitudinal biomedical imaging. In modern medicine, it is important to quantify development of key microstructural features in the natural history of a given disease and to understand, and eventually to control, the molecular mechanisms and quantitative physiological responses of drugs for effectively treating the disease. Small animal models are now preeminent in these studies, and use of each individual animal as its own control (through longitudinal, i.e., repeated imaging over time) has been an important advance, helping to minimize the effect of inter-individual variance. Although the first *in vivo* microCT imaging of small animal models was done with synchrotron radiation in the 1990s, tube-based dedicated *in vivo* systems now dominate the applications. However, *in vivo* synchrotron-based phase-contrast imaging remain very important for many diseases requiring imaging with higher contrast, spatial and temporal resolution. In particular, local tomographic reconstruction has opened new doors for studying details that are inaccessible to tube-based systems. For example, effects of asthma drugs on tracheal pathways and surrounding muscles in mouse models may be observed quantitatively, leading to better numerical models of these processes and to more effective treatment for asthma and other airway constrictive diseases. Study of heart valve and blood vessel calcification also require high sensitivity imaging. Being able to apply high-resolution, high speed phase-contrast imaging to the major biomedical models (mice, rats) requires larger fields of view and a longer beamline than currently available at the APS.

Metals in biology. The biology of the past decades has significantly changed scope, and large compendia of data enabled us to study biological “meta units” such as the genome, proteome, metabolome and transcriptome. At this time, the term “metalome” is still used only rarely, and yet it is estimated that one-third of all known proteins contain metal cofactors and function as metalloenzymes. With current developments in genomics and proteomics, our knowledge of the enormous number of pathways in which metals and trace elements are necessary for life is ever increasing. However, our knowledge about the redistribution of metals and trace elements accompanying the development of different degenerative diseases (such as Alzheimer’s Disease, Lou Gehrig’s Disease, and others) is limited even though metals and metal chelators represent an increasingly important class of drugs used to treat a diverse variety of diseases. At present, synchrotron radiation based X-ray fluorescence microscopy (XFM) is the only available technique for quantitative elemental imaging of whole cells with submicrometer-resolution. In the field of pathology, XFM and BNP would enable studies of changes in metal and trace element abundance disease development in response to drugs. In the environment, many non-essential metals are toxic to cells even at extremely low concentrations (e.g. Cd, As, Pu, Cr, Hg, Pb). In order to determine the mechanism of toxicity, it is important to identify the specific cell types and

subcellular organelles that are targeted by these contaminants. To do so, an *in situ* probe with high spatial resolution and detection sensitivity is required.

Bio-nanotechnology. Two areas that would particularly benefit from BNP development are bio-nanotechnology and pathology. In the field of bio-nanotechnology, the BNP would allow us to ascertain targeting specificity of nanovectors (hybrid organic-inorganic particles usually designed for diagnostics, imaging or therapeutics), their intracellular stability and oxidation changes and their capacity to create multi-particle assemblies *in vivo*. The penetrance of XFM would allow every aspect of these analyses to be done inside cells or tissues. Observing nanovectors "in action" at the site of their activity in cells, made possible by using flash-frozen samples treated with hybrid nanoparticles, will yield critical data that can be used to streamline and guide nanovector design. Many of these nanomaterials are being used as contrast agents for MR, CT, and PET imaging; testing of these materials and their distribution in cellular systems prior to use in people is essential, and the XFM provides one of the few approaches for direct detection of these agents in cells. Coupling these studies with large-scale animal imaging, also proposed here, would provide synergistic links between subcellular and (organ) systematic scales.

Fibrous biomaterials. A huge proportion of biomass is fibrous, from plant cell walls to connective tissues to muscle, and detailed analysis of much of this material is beyond the purview of crystallography, NMR or electron microscopy alone. Fiber diffraction and ancillary techniques are making great strides towards a mechanistic understanding of the molecular mechanisms of cardiac and skeletal muscle contraction and regulation and of the construction of connective tissues and their metabolic processes in health and disease. Fiber techniques have the potential for providing greatly increased understanding of the molecular pathology of diseased aggregates in brain diseases of old age and from infectious particles. They are finding new applications in determining the structural changes that occur in plant structure designed for new food or fuel sources and in the design of new biomaterials for future sources of protective clothing and armor, food, fuel and medical treatments. Furthermore, because this technique utilizes the native crystallinity of samples, it is able to offer molecular resolution structural information free of artifacts and of direct relevance to the natural state of the system under study. Significant and focused efforts to develop better dedicated facilities that include capability for relatively large-scale cryo-preservation will contribute substantially to future progress.

Molecular structure and dynamics. Solution scattering is poised to produce remarkable insight into the structure and dynamics of proteins and protein complexes. Although providing lower resolution information than crystallography, the ease with which data can be collected on very large complexes in multiple physiological and functional states makes it an important tool that is being used by an increasingly large community. The coordination of computational methods with solution scattering is enhancing the amount of information that can be extracted from solution scattering patterns, and these data are increasingly being used as constraints or tests of the results of molecular dynamics or normal mode analyses of protein structure and dynamics. High impact studies will include those on membrane proteins; measurement of the amplitudes of normal modes of proteins in solution; the range of motion of enzymes undergoing catalysis; the measure of domain motions during substrate binding; and the progression of structure formation during protein folding. Efficient utilization of these methods requires the development of dedicated beam lines that can readily accommodate high throughput exchange of large numbers of specimens.

4. Significance of APS

The APS is the only source of high-energy x-rays in the Western hemisphere where facilities for x-ray scattering from and imaging of biological specimens can be staged to adequately address

these many scientific challenges. The APS provides sufficient flux of coherent x-rays photons to achieve the high sensitivity that the high-energy X-ray BNP and high-speed phase-contrast imaging require. The characteristics of undulator beam lines at the APS are close to ideal for solution scattering and fiber diffraction applications. The need for high contrast, spatial and temporal resolution for phase imaging and micro-CT make the APS the best place in the hemisphere for most high energy x-ray imaging techniques. In many cases, with development of appropriate instrumentation and facilities, the APS will continue to improve and add to its repertoire of important biological experiments that simply cannot be performed elsewhere.

5. Scientific Community

The scientific community impacted will involve two cohorts – those biologists that actually use the facilities developed at the APS and those that benefit from the results of these studies. The first community is relatively small in this country, being limited by the low availability of appropriate x-ray facilities here. The second community is vast, and includes biological researchers involved in studies of systems with scales that range from molecules to ecosystems. The second community includes many researchers with little experience in the use of synchrotron radiation and substantial need to work either in collaboration with synchrotron scientists or with substantial guidance from these workers.

There is a large and growing community of users that would benefit from the development of the new beamlines and instrumentation discussed here. Most of the current beamlines used in whole or in part for biological research are over-subscribed by three-fold or more, making it nearly impossible to recruit new users into the program. There is clearly a need within those communities for the expansion of existing facilities and beamlines to accommodate the user base. It should also be noted that new users in biological areas usually require extensive support during the initial phases of their research and therefore broad support from the APS will be needed to grow the community in a broader sense. Moreover, many of these new users will utilize synchrotron experiments as only a small fraction of their research programs, thus requiring continued collaboration from APS staff. In order to be successful, the APS will need to grow the life sciences expertise on its staff by recruiting scientists in these areas. The development of capabilities that could benefit all users, including data analysis tools, sample preparation methods, animal holding facilities and others would be useful for all beamlines.

6. Requirements and Capabilities

The APS is capable of supporting a vibrant life-science community engaged in a range of synchrotron-based studies with unique potential for study of biological systems across multiple length scales. The science drivers (Section 3) require development of several beam lines in order to achieve the impact described above, including dedicated beamlines for the bionanoprobe; for coherent diffraction imaging; for phase imaging; and for SAXS/WAXS. ,

Bionanoprobe. A hard X-ray bionanoprobe at the APS that will enable a) imaging of trace elements in samples as thick as 10 μm with a spatial resolution of better than 20 nm and b) detecting as few as 5 metal atoms in thin sections. The BNP would be applied in bio-nanotechnology studies and in biomedical research (e.g. studies in development and differentiation, neurobiology, molecular imaging, etc.). This instrument would be capable of microscopy, micro-spectroscopy and spectro-microscopy techniques (μ-XANES) and nano-CT, with an emphasis on X-ray fluorescence analysis of trace elemental content in biological samples. With the development of a complete toolbox of software utensils for data analysis and interpretation it would be well positioned to create a reference elemental database of living

organisms for the entire scientific community, and will significantly increase our understanding of life and disease on an unprecedented level.

Phase Imaging Capabilities. Advanced imaging capabilities including wide field x-ray phase imaging with high-definition phase sensitivity could utilize any of a number of beam line configurations: addition of a long (200 m) beam line or shorter lines using advanced optics. This would enable fast, high-resolution imaging on the major biomedical research models (rodents). Such a capability would greatly expand the community of biological users of APS, as biomedical researchers studying brain, heart, and bone function could use the APS to address important biomedical questions. X-ray phase (and absorption) imaging is now a standard tool at most synchrotron facilities and its user community is among the most diverse, including zoologists, entomologists, evolutionary biologists, and biomedical researchers. A dedicated bending magnet beam line could be configured optimally for phase and absorption micro-CT with capabilities for submicrometer voxel reconstructions. An insertion device line would be required for real-time phase radiography and micro-CT, with additional capabilities for nano-CT. Zoom-in capabilities are essential to allow moving from wide field-of-view to magnified observation.

Coherent Diffraction Imaging. The potential of coherent diffraction methods for study of biomolecular systems has been recognized only recently. Although its greatest potential may be realized at the LCLS and XFEL hard x-ray free electron laser projects, the APS represents a source with characteristics adequate for a substantial range of important experiments. Existing capabilities, enhanced by advanced detector systems and sample handling hardware are well positioned to make a substantial impact.

SAXS/WAXS/Fiber. The rapidly growing community using SAXS/WAXS or fiber diffraction could easily support 1- 2 dedicated beam lines even if they were outfitted with robotic specimen handling equipment for rapid data collection. Hardware should be capable of a range of specimen-to-detector distances for measurement of scattering at spacings from 1000 Å out to 2 Å spacing at the very least. A very high speed two-dimensional detector with a minimum of 2k x 2k elements should be available and configured for sub-microsecond time resolved studies.

Cryopreservation. Samples preserved at cryogenic temperatures are substantially more resistant to radiation damage than those at room or physiological temperatures. Cryo-freezing has had a substantial impact on macromolecular crystallography and is now almost universally used for crystallography at the APS. Non-crystalline samples are often much larger, requiring more elaborate techniques for freezing and more robust systems for maintaining cryogenic temperatures during experiments. Routine methods for freezing these larger specimens (e.g. high pressure freezing) will require access to specialized equipment.

Detectors. While many solution or fibrous specimens give rise to diffraction pattern well served by conventional large area CCD detectors developed for macromolecular crystallography, other classes of experiments require specialized detectors optimized for this application. Diffraction patterns characterized by closely spaced fine diffraction peaks superimposed on a high background require a detector with high dynamic range and very good spatial resolution. Detectors built using current large format CCD technology with small demagnification fiber optic tapers and custom phosphors (providing relatively small point spread functions and large active areas) are appropriate but expensive and read out is slow. Existing detectors, however, are not yet up to the challenge of time-resolved experiments in which sub-millisecond readouts are required. Detectors using hybrid technologies with CCD's and massively parallel CMOS readouts could potentially fill this void.

The Advanced Protein Crystallization Facility (APCF). The planned APCF building will place over 30,000 square feet of biological laboratories and offices immediately adjacent to the APS. This facility has the potential to act as a central focus of biological research at the APS. Properly implemented and managed, this building could grow to be the centerpiece of greatly expanded life-science research at the APS, providing both staff and visitors with the shared laboratory space and facilities required for their work. The concept of a 'biology village' is finding broad popularity at synchrotron facilities around the world. The APCF is poised to embody that concept for life science researchers at the APS.

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