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Structure, Dynamics, and Function of Biomolecules

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Abstract

This is the final report of a three-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). We enhanced Los Alamos' core competency in *Bioscience and Biotechnology* by building on our present strengths in experimental techniques, theory, high-performance computing, modeling, and simulation applied to biomolecular structure, dynamics, and function. Specifically, we strengthened our capabilities in neutron/x-ray scattering, x-ray crystallography, NMR, laser, and optical spectroscopies. Initially we focused on supporting the Los Alamos Neutron Science Center (LANSCE) in the design and implementation of new neutron scattering instrumentation, we developed new methods for analysis of scattering data, and we developed new projects to study the structures of biomolecular complexes. We have also worked to strengthen interactions between theory and experiment, and between the biological and physical sciences. We sponsored regular meetings of members from all interested LANL technical divisions, and supported two lecture series: "Biology for Physicists" and "Issues in Modern Biology." We also supported the formation of inter-disciplinary/inter-divisional teams to develop projects in science-based bioremediation and an integrated structural biology resource. Finally, we successfully worked with a multidisciplinary team to put forward the Laboratory's *Genome and Beyond* tactical goal.

Background and Research Objectives

At present, bioscience and biotechnology progress without a full understanding of the concepts and theories underlying the complex phenomena that are studied and used. Even so progress has been fast. More rapid progress toward directed goals will be possible with a more quantitative understanding of complex bio-systems derived from the continued integration of the biological and physical sciences. Los Alamos can unite theory and experiment, the life sciences, computer expertise, and the physical sciences for an unprecedented effort in this area. This competency development project works toward establishing common scientific and technical goals, increasing interdisciplinary interactions, and strengthening Los Alamos' bioscience and biotechnology competency in areas crucial to the Department of Energy's mission and that have high priority in the national agenda.

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Importance to LANL's Science and Technology Base and National R&D Needs

Bioscience and biotechnology have important applications in diverse areas that encompass health, manufacturing, and the environment. The Department of Energy (DOE) can play a major role in the development and use of biological tools, techniques, and materials in these areas. Within the DOE, Los Alamos has the capability of being a leader in this development. This project directly supports the Laboratory's core competency in *Bioscience and Biotechnology*, and is an integral part of the Laboratory's *Genome and Beyond* tactical goal. This work also complements the Laboratory's *Science Based Bioremediation* scientific thrust, which requires development of improved enzymes. The increased capabilities gained from this competency development will be crucial in the bioremediation endeavor.

Bioscience and biotechnology form a vast field. We have chosen to focus on structure, dynamics, and function of biomolecules. Los Alamos has considerable strength in this area with capabilities in the Chemical Science and Technology (CST), Life Sciences (LS), Physics (P), and Theoretical (T) Divisions, as well as in the Los Alamos Neutron Science Center (LANSCE). Further, full utilization of the data from the Human Genome Project, in which Los Alamos plays a pivotal role, will ultimately depend upon understanding the structures of the proteins that are coded in our genomes, and whose function (or dysfunction) give rise to the molecular pathologies of diseases. Other areas of key importance to the DOE include the clean up of its facilities, the development of treatments for genetic damage by ionizing radiation, and the supply of technology for the energy industry. A fundamental understanding of the structure and dynamics of proteins, nucleic acids, and their interactions will help in finding better and cheaper approaches to these problems. Technology transfer potential in the area of biomolecular structure, dynamics and function is also far reaching. Industries to target include the multi-billion dollar drug industry, the environmental remediation technology industry destined to grow rapidly, and the energy industry as we develop new tools and novel materials of utility there.

Scientific Approach and Accomplishments

In order to strengthen Los Alamos' core competency in *Bioscience and Biotechnology* we formed a group of co-investigators from CST, LS, P and T Divisions to formulate a plan. This plan includes long range goals, as well as staged short term goals prioritized to optimize the return for investment. Our approach has been to:

i) Select "Structure, Function, and Dynamics of Biomolecules" as a central theme. Within this topic area, we selected a small number of specific technological goals for

intense work.

ii) Strengthen bioscience and biotechnology efforts where Los Alamos has serious deficiencies by supporting specific technical projects in the areas identified and prioritized.

iii) Form an intellectual center for intense and frequent discussions and collaboration, facilitate meetings among staff members, host long-term or short-term visitors and speakers, and interact closely with users and visitors to LANSCE.

iv) Arrange for a steady stream of visitors and lecturers to publicize Los Alamos efforts and build stronger external networks that would benefit our user facilities (both present and future).

v) Organize two series of lectures: one tutorial ("Biology for Physicists") and one oriented toward the selected research topic area.

Accomplishments in Team Work and Integration (iii-v above)

- Our lecture series "Issues in Modern Biology," attracted about two dozen distinguished lecturers, more than two thirds of whom were members of the National Academy of Sciences and/or Nobel Prize winners. This series helped raise awareness of the greatest challenges and opportunities in bioscience and biotechnology.
- We helped to form and then continued to support the multidisciplinary *Science Based Bioremediation*, and *Integrated Structural Biology Resource* teams at Los Alamos, both of which competed successfully for competency development thrust initiatives.
- We successfully worked with a multidisciplinary team to put forward the *Genome and Beyond* as a Laboratory tactical goal and we have provided input to this goal in its first year (FY1997).

Accomplishments in Technological Goals (i-ii above)

Small-Angle Neutron-Scattering Instrument Design for Structural Biology: This project supported small-angle neutron-scattering instrument development at LANSCE's Manuel Lujan Neutron Scattering Center (MNLSC) focusing on the needs of the structural biology community. Work was completed to design a better optimization of the existing Low-Q Diffractometer (LQD) at MLNSC (Figure 1), as well as on the development of design parameters for small-angle neutron-scattering instrumentation on a 1-MW, long-pulse spallation source (LPSS) neutron source, and LANSCE's upgraded short-pulse spallation source (SPSS) neutron source (1,2,3,4). These design projects required upgrading a Monte Carlo-based computer code (MCLIB) to test feasibility and parameter optimization for different designs (3,4). Instruments planned for future LPSS and SPSS

facilities could make DOE neutron facilities a major center for structural biology focusing on complex assemblies and adaptive behavior in biomolecules during communication and signaling.

The upgrades for the existing LQD have been implemented and have yielded significant gains in intensity. These gains have increased the utility of the LQD to the structural biology community. The Los Alamos small-angle scattering capabilities have contributed to structural studies of protein/DNA complexes such as the gene 5 protein/DNA complex from fd phage (5), the muscle proteins complex troponin C/troponin I (6), structural studies of Ca^{2+} induced conformational changes in blood coagulation factor X (7), and of calmodulin complexed with myosin light chain kinase (8). The studies of troponin C/troponin I also led to new modeling algorithms for small-angle scattering data that have wide application (6).

Studies of Receptor/Ligand Interactions: The predominant pathway for uptake of iron by most vertebrate cells is achieved by receptor-mediated endocytosis of transferrins. These proteins consist of a bilobal structure in which each lobe is made up of two domains containing a single high-affinity iron binding site in the interdomain cleft. Despite structural similarities, different transferrins have different physiological roles. Lactotransferrin (in milk) and ovotransferrin (in egg) provide a means for depriving the extracellular environment of iron, hence negating the damaging effects of iron-catalyzed free radical cascades and exerting bacteriostatic effects through the denial of iron needed for bacterial growth. On the other hand, the role of serum transferrin (sTf) is to transport iron to all sites of utilization or storage in the circulation. Because of the indispensable role of transferrin in the regulation of iron metabolism and in iron transport, considerable attention has been directed to understanding mechanisms entailed in uptake and release of iron by the transferrins.

We have completed small-angle scattering experiments on sTf in solution in order to elucidate the tertiary changes associated with iron release from sTf activated by acidification (pH 7.4 -> pH 5.6) that occurs in the endocytosis process. By making a simple comparison of scattering and absorption data as a function of pH, we were able to track the protein shape changes associated with iron release. The radius of gyration, R_g, which is determined using scattering for the di-ferric protein at pH 7.8, is 31.2 ± 0.2 Å while that of the apo protein at pH 5.7 is 33.9 ± 0.4 Å. The absorption data reveal that the N-lobe iron release is nearly complete by pH 7.0, while the C-lobe remains primarily iron-laden. In contrast, the R_g values for sTf remain constant between pH 7.8 and 7.1, and begin to change only for pH values < 7.0. An empirical pH of 6.6 was determined for the change in R_g as a function of pH for values less than 7.0. While R_g is effectively constant in the

pH range associated with iron release from the N-lobe, the radius of gyration of cross section, R_c, increases from 16.9 ± 0.2 Å to 17.6 ± 0.2 Å.

Using the crystal structure of di-ferric lactoferrin as a scaffold for model simulations, the scattering data from sTf with decreasing pH < 7.0 can be understood in terms of a domain opening involving a rotation/twist motion that we have modeled using the crystal structure of apo-ferritin as a scaffold. These tansferrin studies are published in the Journal of Molecular Biology (9).

Protein/DNA Complexes, Human Flap Endonuclease: Human flap endonuclease (FEN-1) is a member of the structure-specific endonuclease family, and is essential in DNA replication and may be involved in DNA repair. FEN-1 has high endonuclease activity with regards to repairing nicked double-stranded DNA substrates with the 5'-end of the nick expanded into a single-stranded tail. FEN-1 has been shown to be highly homologous to the structure-specific nucleases encoded by the RAD2 gene family: human XP-G, yeast *S. cerevisiae* RAD2 and *S. cerevisiae* YKL510. As with FEN-1, these homologous structure-specific nucleases bind and cleave DNA 5'-flap and pseudo "Y" structures.

As the role of FEN-1 in mammalian DNA replication and repair is becoming more clear, it is important to structurally characterize this enzyme in order to better understand how it functions as an endonuclease. At present, no crystal structure has been reported for FEN-1. In order to examine the structure-function relationship of FEN-1 in its endonuclease capacity, we have studied the affect of magnesium on its conformation in aqueous solution. Magnesium is an important cofactor for enzymatic activity. In addition, we have performed these same studies on a D181A mutant of FEN-1, in which Asp 181 is substituted for Ala. This D181A mutant still selectively binds to the 5'flap DNA structure but it has lost its ability to cleave the single strand in the presence of Mg^{2+}. We showed that no measurable structural change was evident in either the FEN-1 or the D181A mutant due to the presence of Mg^{2+}. On the other hand, Mg^{2+} induces self-association of the DNA.

Measurements of the FEN-1 and D181A mutant were also performed with a 34mer DNA fragment which was constructed so that it readily adopts a 5'-flap structure. Mg^{2+}-dependent activation of the FEN-1/34mer DNA complex was observed and the scattering profiles are consistent with the protein remaining bound to the double-stranded fragment after cleavage of the DNA flap. Cleavage was not observed for the D181A/34mer DNA complex. In addition, in the absence of Mg^{2+} the FEN-1/34mer DNA complex is considerably more compact compared to the D181A/34mer DNA complex as observed by the R_g values (35 cf 41 Å) suggesting that Asp 181 is crucial for proper binding of the protein to the DNA substrate.

Our studies of FEN-1 and its complexes with DNA, including the effects of Mg^{2+} and the D181A mutation, are being prepared for publication (10). In fiscal year 1997, we also completed a series of neutron scattering experiments from the FEN-1/34mer DNA complex at LANSCE (using LQD) and at the National Institute of Standards and Technology (NIST). These experiments were successful and will provide additional structural information on the FEN-1 while complexed with its DNA substrate.

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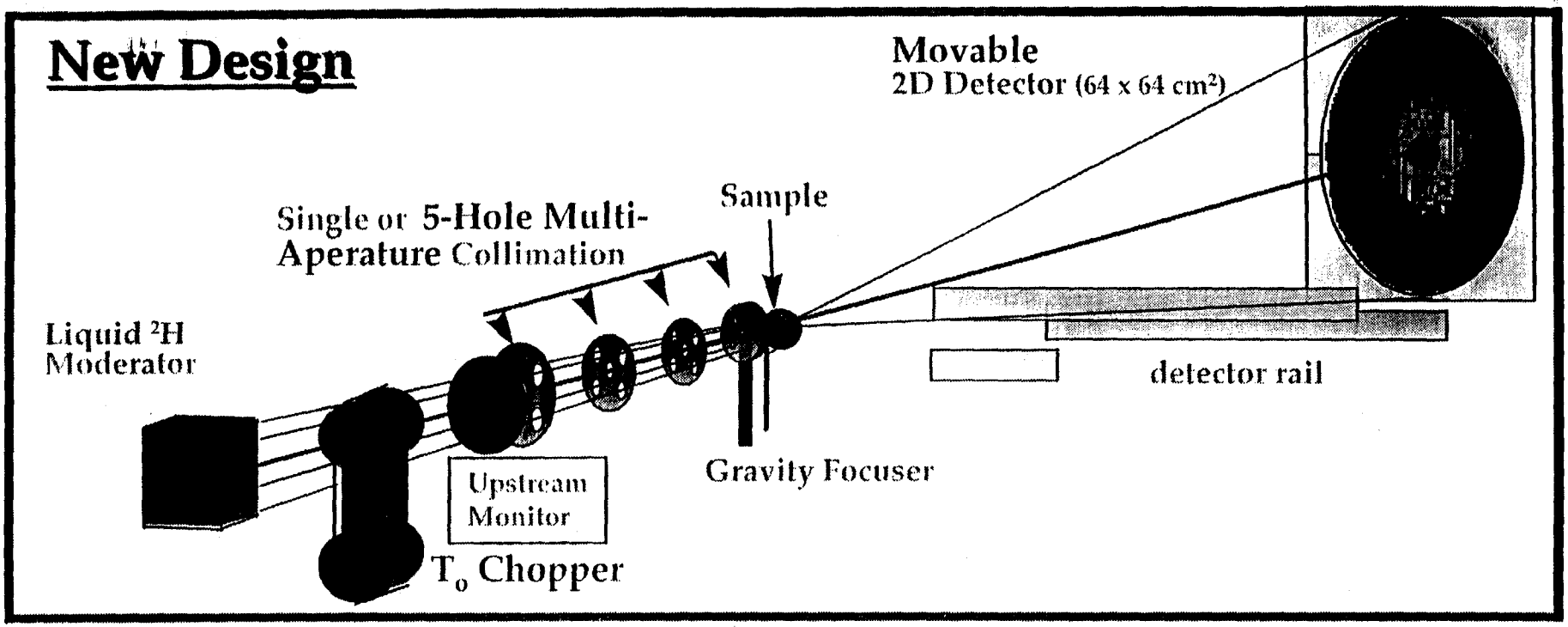
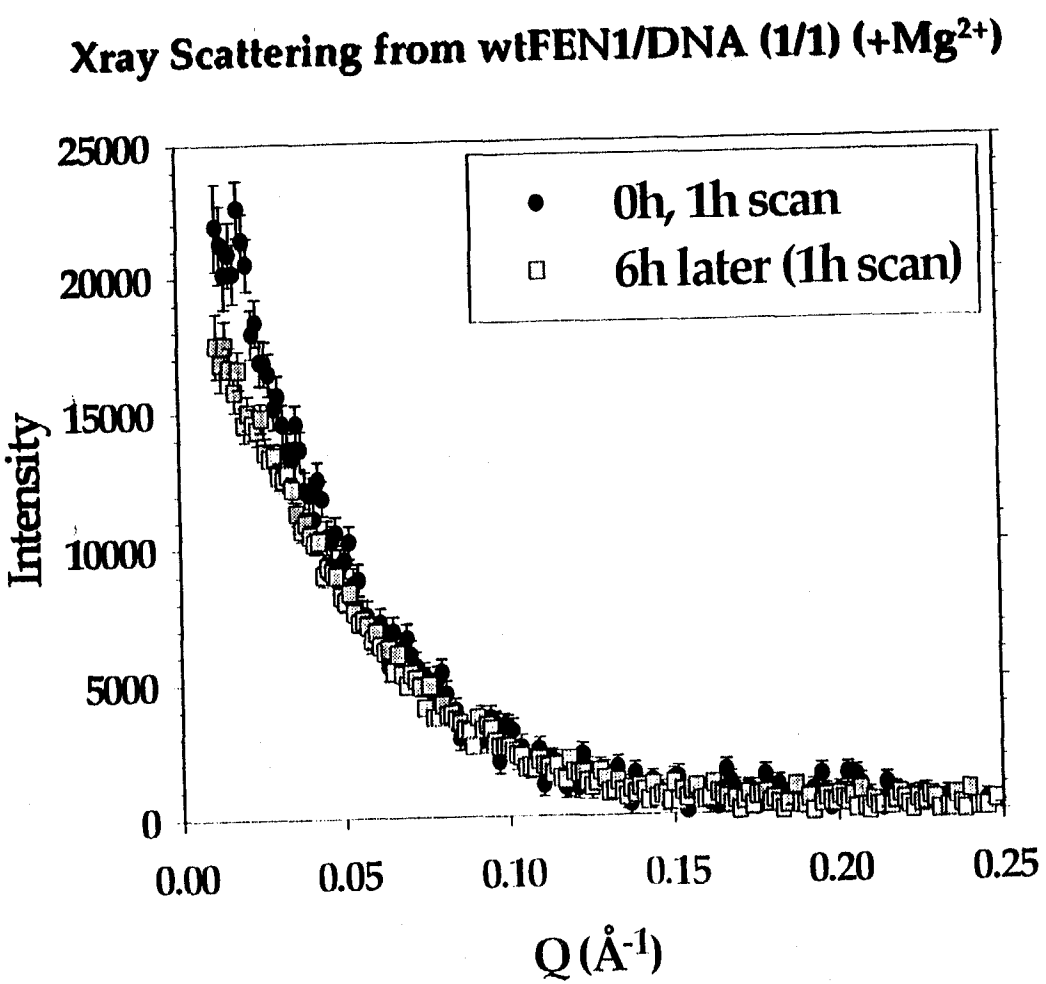
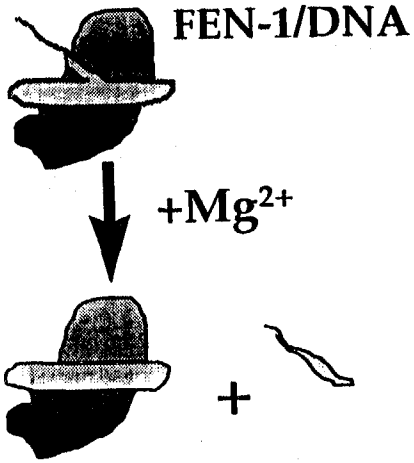


Figure 1. Schematic showing the designed upgrades to the Low-Q neutron diffractometer (LQD) at LANSCE. Structural biology applications using LQD have been limited by the neutron flux from this instrument. In order to increase the flux of neutrons on a sample at high resolution, a number of changes were designed and implemented. The 5-hole multiaperture collimator allows the sample to "see" a larger area of the neutron source moderator and hence capture more neutrons. The T_0 chopper minimizes unwanted background and the variable detector position allows for better optimization of resolution and neutron flux for individual experiments.





◆DNA bound to FEN-1is needed to see Mg^{2+} activation affect.

◆Decrease in scattering amplitude is consistent with wtFEN1 remaining bound to double stranded DNA after cleavage.

Figure 2. Results of an x-ray scattering experiment on FEN-1, a protein that binds to double-stranded DNA that has been "nicked" to produce an expanded single-strand flap. FEN-1 binds to the DNA and cleaves the flap. This process requires a number of additional agents, including Mg^{2+}. X-ray scattering from the FEN-1/DNA complex has shown that when Mg^{2+} is added to the complex, the DNA flap is cleaved and FEN-1 remains bound to the DNA.