

## STRUCTURE OF A VIRAL DNA-UNWINDING PROTEIN

The three-dimensional structure of a virus protein that plays a key role in DNA replication has been solved by a group of researchers from the University of Colorado Health Science Center, School of Medicine; Argonne National Laboratory; and the Dana-Farber Cancer Institute at Vanderbilt University. Simian virus 40 produces a protein called large tumor antigen (LTag), which disrupts host cells' anti-tumor machinery and begins the viral DNA replication process. Studying LTag's structure could help elucidate the mechanism behind DNA replication in human and other cells. The research helps explain how LTag forms a six-membered complex and contacts host anti-tumor proteins and suggests that it uses an "iris" effect to separate DNA.

During viral DNA replication, LTag forms a double six-membered complex around DNA, opens gaps in the two DNA strands, and unwinds them so the replication machinery can copy them. A protein in archaeal cells has the same function and also seems to form a double hexamer, so the two proteins may use a similar mechanism. Now, this group of experimenters have isolated the smallest LTag fragment that acts as a DNA-unwinder (helicase) and determined its structure from the x-ray diffraction pattern. They collected structural data at the SBC-CAT beamline 19-ID at the APS.

Viewed from the side, six LTag proteins combine to form two stacked tiers, a smaller one on top of a larger one, with a central channel running from top to bottom. From the top the hexamer looks like a six-pointed gear or star (see Fig. 1). In the top tier, each monomer reaches over counterclockwise to touch its neighbor. A cleft between each pair of monomers in the bottom tier might provide room for an energy-dependent conformational change to drive DNA remodeling.

Each monomer consists of three domains, D1-D3. A zinc atom holds the five helices of domain D1 in place. D2 folds into a sheet sandwiched by helices. D3 consists of two interlocked rings composed of three and four helices, respectively. Hydrophobic interactions between D1 domains and hydrogen and ionic bonds between D2 domains cause adjacent monomers to stick together. D3 domains form the triangular points radiating outward in the bottom tier.

The position of the zinc-binding region suggested it might be important for hexamerization. To test the idea, the group

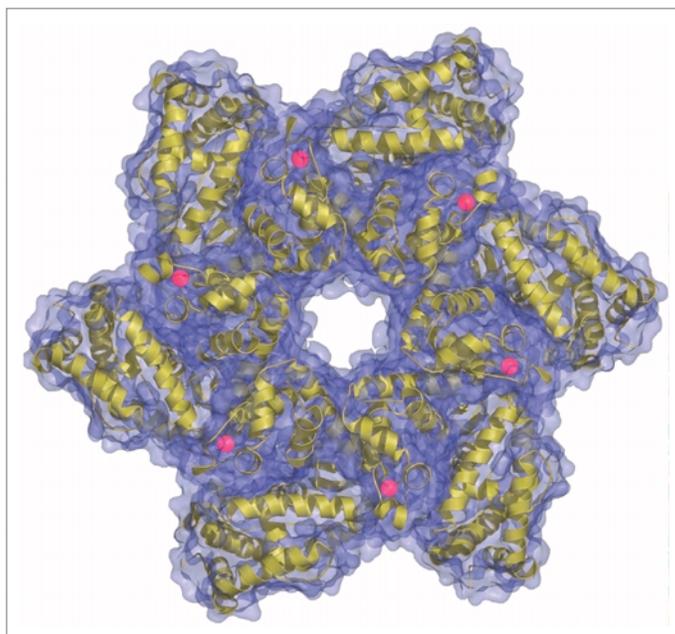


Fig. 1. Top view of the LTag hexamer showing the central channel. Each pointed arm in the star-like shape represents one monomer. Zinc atoms are in red.

deleted part of the region and passed solutions of the complete fragment and deletion mutant through a gel chromatograph. The complete fragment formed mostly or all hexamers, whereas the deletion mutant seemed to form only monomers, supporting the hypothesis.

Researchers had previously identified several mutations that disrupt LTag's ability to bind the anti-tumor protein p53. Four of the amino acids altered in these mutations form a patch on the outer surface of the hexamer, suggesting they contact p53 or lie where LTag and p53 meet.

The central channel of Hexameric LTag is strongly positively charged and has six similarly charged, smaller channels radiating out from it, perfect for manipulating negatively charged DNA. The opening in the larger

tier initially appears wide enough for just single-stranded (ss) DNA. But the same opening in LTag crystals formed at different pH or salt concentrations varies in width, suggesting the opening can open and close. The researchers suggest the hexamer operates like the iris (diaphragm) of a camera by flexing the helices that connect the two tiers.

They propose two linked hexamers bind DNA along the inner channel surface and use the iris effect to pull apart the twin strands of DNA at two locations. LTag repeats the iris motion again and again, extruding the strands as ssDNA loops through side channels on opposite sides of the protein complex. The hypothesis is consistent with electron microscope images showing DNA "rabbit ears" connected to the complex. ○

**See:** D. Li<sup>1</sup>, R. Zhao<sup>1</sup>, W. Lilyestrom<sup>1</sup>, D. Gai<sup>1</sup>, R. Zhang<sup>2</sup>, J.A. DeCaprio<sup>3</sup>, E. Fanning<sup>4</sup>, A. Jochimiak<sup>2</sup>, G. Szakonyi<sup>1</sup>, and X.S. Chen<sup>1</sup>, "Structure of the replicative helicase of the oncoprotein SV40 large tumor antigen," *Nature* **423**, 512-518 (29 May 2003).

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## CAPTURING AN ENZYME'S UNSTABLE INTERMEDIATE

Researchers from the Boston University School of Medicine and the University of New Mexico have determined the structure of the first high-energy intermediate of an enzymatic reaction. Enzymes sometimes accelerate chemical reactions by stabilizing such states, allowing the reaction to proceed with less energy input. The group crystallized a mixture of an enzyme and its substrates, capturing the enzyme in the act of catalysis. The result provides direct evidence of a key enzymatic strategy.

Without an enzyme around, reactions happen by chance. Substrates must bump into each other at just the right angle and with enough energy to form a short-lived structure, called a transition state, which resolves itself into the products. Enzymes carefully orchestrate reactions to place substrates automatically in the optimal positions. To lower the energy barrier, they stabilize the transition state, sometimes proceeding via a high-energy intermediate that is unique to the enzyme-catalyzed reaction. Structural biologists perform various experimental tricks to observe or infer the structure of intermediates and transition states. They introduce enzyme inhibitors believed to mimic the transition state, alter the enzyme to make the intermediate build up, or, in rare cases, perform time-resolved crystallography on a cold sample to extend the intermediate's lifespan. Until March 2003, however, nobody had determined the structure of a high-energy intermediate in its natural environment.

The group chose the enzyme  $\beta$ -phosphoglucomutase ( $\beta$ -PGM) to fill this gap because it crystallizes in a closed form that traps its high-energy intermediate in place.  $\beta$ -PGM can switch the location of a phosphate group back and forth between two positions on a glucose molecule. In the process, it forms an intermediate state in which a phosphorous atom is bound to five oxygen molecules—one from the enzyme, one from glucose, and three of its own—which are arranged as the points of a "trigonal-bipyramidal" structure (Fig. 1), reports the group. They combined the enzyme with either of its substrates and allowed the mixture to come to equilibrium, in which each enzyme molecule is in either the substrate-bound ground state or the intermediate state. By crystallizing the mixture and analyzing its x-ray diffraction pattern, they were able to reconstruct the elusive intermediate. High-intensity x-rays from the BioCARS beamline 14-BM at the APS allowed them to resolve the intermediate structure. (Additional work was done at the HHMI beamline X4A at Brookhaven National Laboratory.)

The research indicates that  $\beta$ -PGM stabilizes the intermediate in two ways. It anchors the substrate by forcing it to bond precisely with a magnesium ion, water molecules, and amino

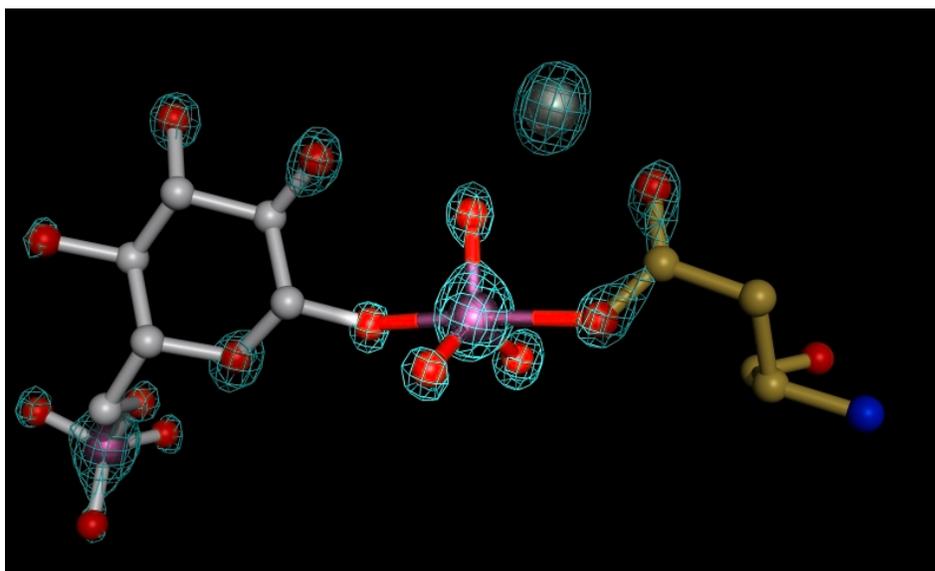


Fig. 1. The high-energy intermediate formed by the enzyme  $\beta$ -PGM as it rearranges a phosphate group on a glucose molecule. Five oxygen atoms (red) are oriented around a phosphorous atom (purple) in a so-called trigonal bipyramid. The two outer atoms can be viewed as the apices of two triangular pyramids, which share a common base in the three middle oxygen atoms.

acids. The anchoring prevents a large loss of entropy, which would make the reaction unfavorable in solution. The enzyme also forms strong bonds with the oxygen atoms at either end of the trigonal bipyramid; otherwise they would be unstable and likely to break away. Both kinds of interaction make the intermediate more thermodynamically stable, giving the reaction a better chance of proceeding. Researchers had suspected that similar structures are involved in several phosphorous transfer reactions that occur with or without enzyme catalysis but had never observed such an intermediate.  $\odot$

**See:** S.D. Lahiri<sup>1</sup>, G. Zhang<sup>2</sup>, D. Dunaway-Mariano<sup>2</sup>, and K.N. Allen<sup>1</sup>, "The Pentacovalent Phosphorus Intermediate of a Phosphoryl Transfer Reaction," *Science* **299**, 2067-2071 (28 March 2003).

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## HOW AN IMMUNE SYSTEM PROTEIN BINDS ITS RECEPTORS

**M**ice expressing too much of the protein TALL-1 experience symptoms that mimic the human autoimmune disease systemic lupus erythematosus. TALL-1 seems to bind receptors in a novel way. Studying that interaction may increase our understanding of autoimmune disease and aid in designing drugs to counteract it. Researchers have now determined the crystal structure of the functional section of TALL-1 bound to the key sections of two of its receptors. Their research, carried out at the SBC-CAT beamline 19-ID at the APS reveals why one receptor binds TALL-1 instead of a closely related protein.

Mice deficient in TALL-1 (called a ligand) or its receptor BAFF-R cannot grow mature B cells, which produce antibodies. At low pH, functional TALL-1 fragments form threesomes and fold into the same shape as other proteins in the same family: a “jellyroll,” which looks like a bent paper clip. At high pH, the fragments cluster together and acquire their biological function (Fig. 1). The binding regions of BAFF-R and the related receptor BCMA have many fewer cysteines (sulphur-containing amino acids) than proteins of the same family do. Such amino acids often form bonds within proteins to hold the three-dimensional (3-D) structure in shape. The receptors still bind TALL-1 strongly, however, suggesting a unique binding mechanism. To test the hypothesis, researchers from the National Jewish Medical and Research Center, Howard Hughes Medical Institute, The University of Colorado Health Science Center, Peking University, and Argonne National Laboratory diffused fragments of either BCMA or BAFF-R into crystallized TALL-1 and reconstructed their 3-D shapes from x-ray diffraction patterns.

The overall structure resembles a sunflower, with receptors as the petals and 60 TALL-1 ligands assembled into the seedbed. Each receptor fragment consists of two domains and folds into a saddle-like shape. BCMA has a helical section positioned like a “rider” on the saddle. BAFF-R has a lone extended coil in place of the helix. Both structures are unique among proteins of the same family.

Each saddle sits slightly askew on a “horseback”-like surface formed by four loops of a TALL-1 fragment. In related proteins, one elongated receptor binds the cleft formed by two ligands. Both receptors bind TALL-1 through a combination of ionic bonds and two so-called hydrophobic cores, in which non-polar amino acid side chains cluster together. Nonpolar or hydrophobic side chains create disorder among water molecules. Proteins become more energetically stable by burying hydrophobic side chains away from water. The major difference between the two fragments is in BAFF-R, which distinguishes it from typical proteins of the same family. The coil makes several additional ionic and hydrogen bonds with TALL-1.

Mice lacking April, the protein that most resembles TALL-1, die as embryos. The researchers have speculated previously that April may compete with excess TALL-1, offsetting the latter's ability to cause symptoms of autoimmune disease symptoms. BCMA binds April as strongly as it does TALL-1, but the

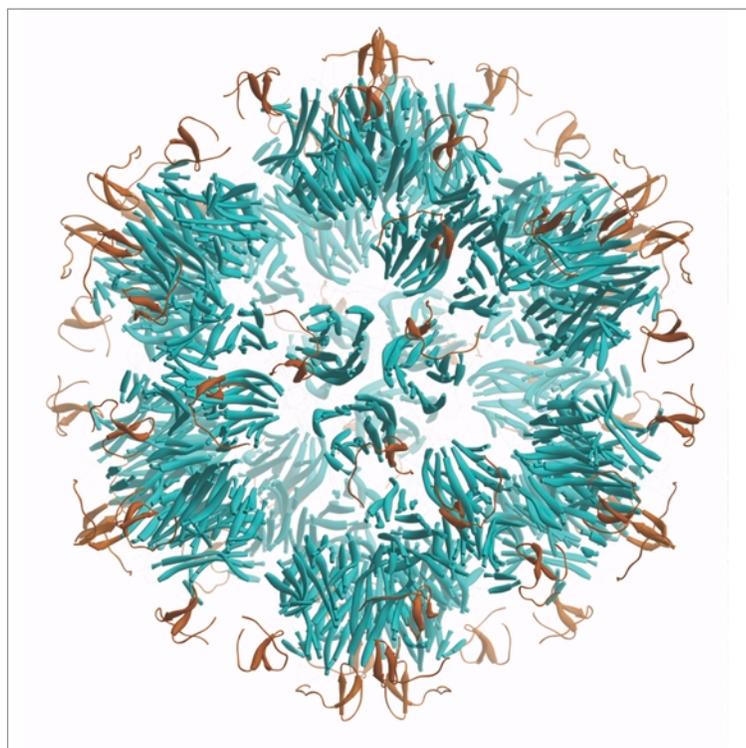


Fig. 1. Crystalline structure of 60 monomers of TALL-1 (blue) complexed with 60 monomers of eBAFF-R (orange).

structurally similar BAFF-R does not. To figure out why, the researchers mutated BAFF-R in several ways, removing or altering different sections. When they mutated either of two cysteines in the middle of BAFF-R, the protein could suddenly bind April strongly. The researchers conclude that one of BAFF-R's two natural cysteine-cysteine bonds makes the protein rigid and unable to conform to April's shape. ○

**See:** Y. Liu<sup>1</sup>, X. Hong<sup>1</sup>, J. Kappler<sup>1,2,3</sup>, L. Jiang<sup>1</sup>, R. Zhang<sup>5</sup>, L. Xu<sup>1</sup>, C.-H. Pan<sup>1</sup>, W. E. Martin<sup>1</sup>, R. C. Murphy<sup>1</sup>, H.-B. Shu<sup>1,4</sup>, S. Dai<sup>1,2</sup>, and G. Zhang<sup>1,3</sup>, “Ligand–receptor binding revealed by the TNF family member TALL-1,” *Nature* **423**, 49-56 (1 May 2003).

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## IN SEARCH OF NEW ANTIBIOTICS

The antibiotic vancomycin is often the last resort for life-threatening infections of the wounds or blood of hospital patients, but bacteria are becoming increasingly resistant to the drug. In an early step toward developing new antibiotics on the same model, researchers have solved the structure of an enzyme that synthesizes a derivative of vancomycin. Changing the enzyme's structure on the basis of such information could lead to new antibiotics.

Vancomycin consists of a short series of amino acids, which form the "peptide core," with sugars projecting from it. Researchers have found that changing the sugars on the core can overcome bacterial resistance, but such derivatives are complicated to synthesize, which has prevented drug companies from manufacturing them. If researchers could reprogram enzymes that naturally produce vancomycin and related antibiotics to form new products, companies could use the enzymes in developing or producing new drugs. Researchers would need detailed structural information about how the enzymes recognize different substrates to enact such a scheme, however.

Add an extra sugar to a specific spot on vancomycin and the antibiotic chloroeremomycin results. Researchers from Michigan State University, Harvard University, and DND-CAT have solved the structure of the bacterial enzyme that adds the extra sugar, called GtfA. The enzyme, which swaps a sugar from a nucleotide anchor to a vancomycin precursor, snaps from an open to closed form when the nucleotide-sugar molecule binds to it. To determine the structure, they crystallized mixtures of the enzyme and vancomycin or its precursor DVV, then analyzed the x-ray diffraction pattern, collected at the SBC-CAT beamline 19-ID and the DND-CAT beamline 5-ID at the APS.

GtfA, like related enzymes, is made of two domains, each of which recognizes and binds a different substrate. The reaction joining the two substrates takes place in a deep cleft between the two domains (Fig. 1). A shallow pocket on the outer surface of one domain holds the cup-shaped vancomycin or DVV molecule, accommodating their different sugar attachments by slight shifts in structure. The nucleotide-sugar molecule would bind to the other domain along the cleft. Although the sugar to be swapped was not included in the structure,

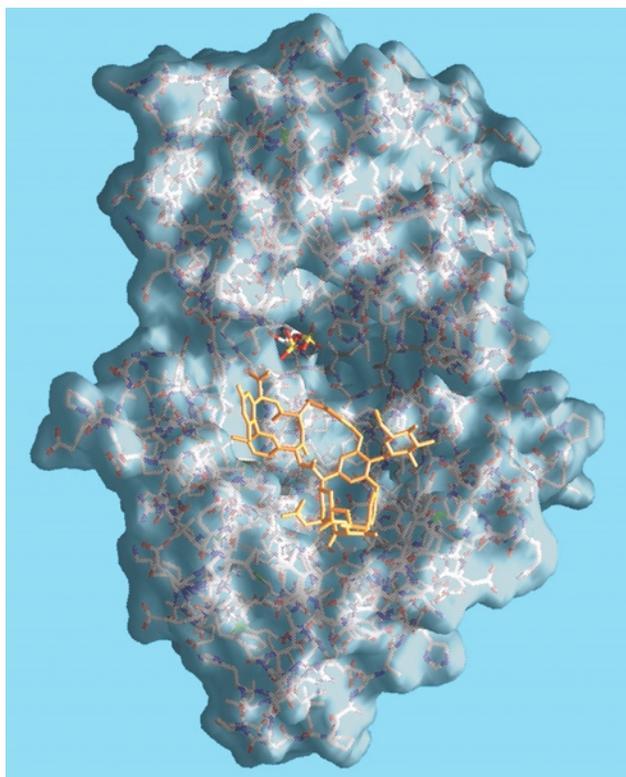


Fig. 1. The molecular surface (and underlying atoms) of the GtfA enzyme, with the antibiotic precursor DVV (gold) bound to the so-called active site, and TDP (red and yellow) buried between the two protein domains.

comparison with other enzymes suggests it would project further into the cleft. The structure revealed two forms of the enzyme: an open form in which the nucleotide anchor is absent, and a closed form with a smaller cleft when the nucleotide anchor is present. The result indicates that binding of the nucleotide triggers the cleft to close, allowing the molecule to interact with both domains. A small rotation of the two domains about a flexible link is responsible for the closure.

The result marks the first direct observation of a sugar acceptor (DVV or vancomycin) bound to a member of this family of enzymes and provides hints at how different members accomplish their specific reactions. GtfA seems to prefer DVV because vancomycin's extra sugar would interfere with cleft closure. A structurally similar enzyme transfers the same sugar to a different spot on DVV, and the authors suggest that variability in the antibiotic binding site makes the difference. How such differences

reprogram the enzyme is unclear, but swapping domains between different enzymes could be one way to generate novel antibiotics. ○

**See:** A.M. Mulichak<sup>1</sup>, H.C. Losey<sup>2</sup>, W. Lu<sup>2</sup>, Z. Wawrzak<sup>3</sup>, C.T. Walsh<sup>2</sup>, and R.M. Garavito<sup>1</sup>, "Structure of the TDP-epivancomosaminyltransferase GtfA from the chloroeremomycin biosynthetic pathway," *Proc. Natl. Acad. Sci. USA* **100**(16), 9238–9243 (5 August 2003).

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## A FIRST LOOK AT THE STRUCTURE OF CFTR NBD1

Cystic fibrosis (CF) is a life-threatening, autosomal-recessive genetic disease, most common among Caucasians, affecting tens of thousands of people worldwide. Defects in the cystic fibrosis transmembrane conductance regulator (CFTR) protein are the root cause of CF. Cystic fibrosis transmembrane conductance regulator is a member of the ABC transporter family of proteins that typically conduct various ions and small molecules through the cell membrane and consist primarily of two membrane-spanning domains (MSD1 and MSD2) and two nucleotide binding domains (NBD1 and NBD2). CFTR acts as a chloride channel and is important for maintaining proper mucus viscosity in the lungs and other vital organs. Most frequently, CF results from a genetically inherited deletion of a single amino acid in NBD1: phenylalanine at position 508 in the protein chain referred to as  $\Delta F508$ .

SGX (Structural GenomiX, Inc.) and Cystic Fibrosis Foundation Therapeutics (CFFT), the nonprofit drug discovery and development affiliate of the Cystic Fibrosis Foundation, are collaborating to use x-ray crystallography to determine the structure of CFTR with the goals of explaining disease etiology and supporting drug discovery efforts. At SGX, an intense effort to determine the structure of the most important globular domain of CFTR, NBD1, has spanned two years and involved molecular biology efforts with cDNAs from 10 different organisms. After characterizing more than 500 different versions of NBD1, a truncated form of mouse CFTR containing NBD1 was crystallized. SGX, San Diego, determined the structure of NBD1 using diffraction data collected at the SGX-CAT beamline 31-ID at the APS (Fig. 1).

It was expected that NBD1 of CFTR would possess strong similarities to the known structures of NBDs from other ABC transporters. While overall similar in fold to the bacterial NBD structures, there are significant differences that distinguish mammalian NBDs from their bacterial counterparts. These structural differences include an unexpected domain definition where both the N- and C-termini of the domain are more extended than would be predicted from sequence alone. In addition, the protein was crystallized and analyzed in several different liganded states including adenosine triphosphate

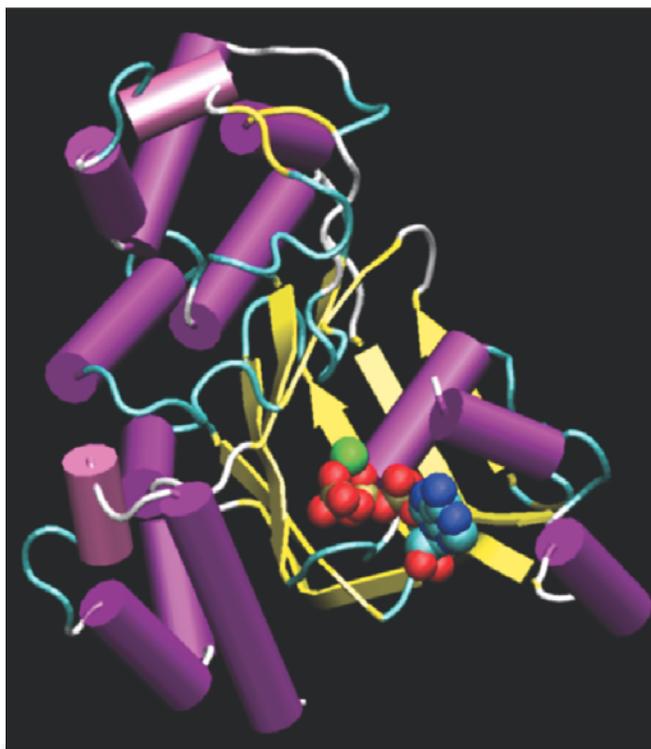


Fig. 1. X-ray structure of the NBD1 domain of mouse CFTR. The protein is drawn in cartoon style, where purple cylinders represent alpha helices and yellow arrows represent beta strands. Atoms of the ATP molecule, with coordinated magnesium ion, are shown as spheres (lower right).

(ATP) bound, adenosine diphosphate (ADP) bound, and with no ligand present (apo) and surprisingly showed no conformation change. ATP was found bound to the domain, as expected, but no hydrolysis of the nucleotide was observed, either in the structure or in accompanying biochemical tests, in contrast to the known ATPase functions of NBDs in other ABC transporters. Residue Phe508 falls in a surface-exposed short loop or turn region of the NBD1 fold. It is not apparent from the structure how its deletion would affect CFTR function. Further studies with this important mutation will be required.

In summary, the structure of NBD1 from mouse CFTR has raised many new questions relating to CFTR function and the role of  $\Delta F508$  in the development of CF. Ongoing and future projects to help answer these questions include the determination of a structure or structures of forms of NBD1 bearing the  $\Delta F508$  mutation; the determination of the structure of human NBD1 structure to support drug discovery efforts the crystallization and structure determination of the other nucleotide-binding domain in CFTR, NBD2; and ultimately, the co-crystallization of NBD1 with NBD2 to provide a direct look at how these two important domains may interact. ○

**See:** H.A. Lewis<sup>1</sup>, S.G. Buchanan<sup>1</sup>, S.K. Burley<sup>1</sup>, K. Connors<sup>1</sup>, M. Dickey<sup>1</sup>, M. Dorwart<sup>2</sup>, R. Fowler<sup>1</sup>, X. Gao<sup>1</sup>, W.B. Guggino<sup>3</sup>, W.A. Hendrickson<sup>4</sup>, J.F. Hunt<sup>5</sup>, M.C. Kearins<sup>1</sup>, D. Lorimer<sup>1</sup>, P.C. Maloney<sup>3</sup>, K.W. Post<sup>1</sup>, K.R. Rajashankar<sup>1</sup>, M.E. Rutter<sup>1</sup>, J.M. Sauder<sup>1</sup>, S. Shriver<sup>1</sup>, P.H. Thibodeau<sup>2</sup>, P.J. Thomas<sup>2</sup>, M. Zhang<sup>1</sup>, X. Zhao<sup>1</sup>, and S. Emtage<sup>1</sup>, "Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator," *EMBO J.* **23**(2), 282-293 (2004).

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## AN APPETITE FOR APATITE

The three-dimensional (3-D) structure of the second most abundant protein in bone after collagen has been solved by a group of researchers from the Illinois Institute of Technology, Microstar Biotech, Inc., Mount Sinai Hospital, and the University of Toronto, led by experimenters from McMaster University. Called osteocalcin, the protein binds to the calcium-containing mineral hydroxyapatite and thereby helps regulate the amount of calcium in bone. The group crystallized the protein and used x-rays to determine its structure. The result shows that osteocalcin's binding site complements the crystal structure of the mineral.

The body constantly builds up bone and tears it down. The net balance of these two processes determines the amount of calcium in bone and thus the overall health of the skeletal system. Because osteocalcin plays an important role in regulating bone growth, its blood level can serve as a marker for skeletal health. Researchers would like to better understand how the protein interacts with hydroxyapatite and other molecules to aid in designing drugs to combat osteoporosis, for example. Previous structural studies had found that it has a globular structure made of many helices packed together, but researchers had not ascertained its precise mechanism of action on the molecular scale.

To determine how the protein recognizes hydroxyapatite, the group crystallized pig osteocalcin (all vertebrates share a very similar form of the protein) and analyzed the crystal's x-ray diffraction pattern, measured at the IMCA-CAT beamline 17-ID at the APS. Osteocalcin has a novel, roughly pyramidal shape built from three helices. One helix contains an elongated negatively charged region, including three amino acids that researchers had already implicated in hydroxyapatite binding. The negatively charged areas on two adjacent osteocalcin molecules are capable of holding five positively charged calcium ions in place through a network of ionic bonds (Fig. 1). The researchers were surprised to observe that the calcium ions have a periodic arrangement reminiscent of a crystal lattice, so they performed a computer search to identify corresponding calcium arrangements in hydroxyapatite. The best match was found in the most common surface presented by natural and synthetic versions of the mineral. Evidence for the structure of hydroxyapatite in bone is inconclusive but consistent with the best match surface.

The group, which includes members from Microstar Biotech, Inc., the Samuel Lunenfeld Research Institute of Mount Sinai Hospital, the University of Toronto, and the Illinois Institute of Technology, speculates that osteocalcin binds hydroxyapatite in a way that is analogous to the way antifreeze proteins recognize ice crystals and inhibit their growth. Antifreeze proteins have different shapes that complement, and thus bind specifically to, different planes of ice crystals, allowing the protein to modify their structure. The high complementarity between osteocalcin and hydroxyap-

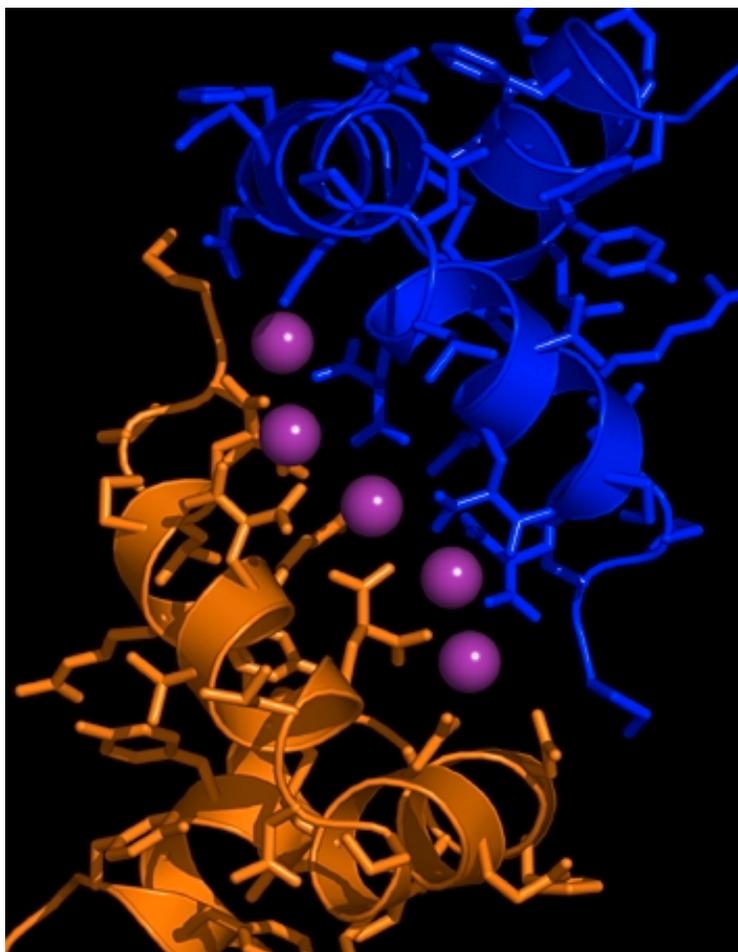


Fig. 1. Crystal structure of two osteocalcin molecules bound to five calcium ions (purple).

atite suggests the protein may likewise directly modify crystal growth and structure. The remaining portions of the protein would then be well positioned to transmit signals to and from the surrounding cellular environment. ○

**See:** Q.Q. Hoang<sup>1,2</sup>, F. Sicheri<sup>3,4</sup>, A. J. Howard<sup>5</sup>, and D.S.C. Yang<sup>1</sup>, "Bone recognition mechanism of porcine osteocalcin from crystal structure," *Nature* **425**(30), 977-980 (October 2003).

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## COLLECTING PROTEIN STRUCTURAL INFORMATION WITHOUT CRYSTALS

The most common method of determining protein structure is to crystallize a protein and collect its x-ray diffraction pattern, but researchers have been able to crystallize only a few percent of all proteins. Some have therefore turned to another method: measuring x-ray scattering from proteins in solution—without crystallizing them. The intense power of third-generation synchrotron sources, such as the APS, should allow researchers to obtain high-quality structural information from scattering experiments, but one of their key concerns has been the effect of radiation damage on the observed scattering. The results reported here indicate that such protein damage is measurable and avoidable.

Scattering data provide less-detailed structural information than crystallography because individual protein molecules are oriented randomly in solution instead of locked into the fixed, repetitive arrangement of a crystal. But the technique complements crystallography by providing information about proteins that either do not crystallize or undergo large structural changes that would be confined in a crystalline lattice. Researchers flash-freeze crystallized proteins to liquid nitrogen temperatures; otherwise, x-rays can disrupt their structure and chemistry by splitting important structural bonds, cleaving chemical groups from amino acids, and even distorting the crystal geometry. But flash-freezing causes additional experimental problems for scattering experiments, so a group of researchers from Argonne National Laboratory and the Illinois Institute of Technology scattered increasing doses of x-rays off proteins in solution to observe and quantify radiation damage at room temperature.

Working at the Bio-CAT beamline 18-ID at the APS, the group is studying the protein fold-dependence of the wide-angle x-ray scattering (WAXS) pattern (Fig. 1). Specific peaks within the pattern correlate with the secondary and tertiary features formed by the amino acid residues of protein molecules such as helices and sheets. To assess radiation damage, the group went on to resolve medium-scale features of hemoglobin and myoglobin, two oxygen-carrying proteins found in blood, and cytochrome C, a metabolic enzyme. They examined each protein by using three protocols with different exposure times. First, samples were made to flow through a quartz capillary such that no part of the sample was exposed longer than 100 ms, which is a typical exposure time for WAXS experiments. Secondly, samples were oscillated slowly in a capillary during an 8-s exposure. Third, samples remained stationary during a 0.7-s exposure. The group found that scattering from the stationary hemoglobin showed signs of protein degradation compared to that of the flowing samples. Hemoglobin consists of four protein chains, and the scattering experiments demon-

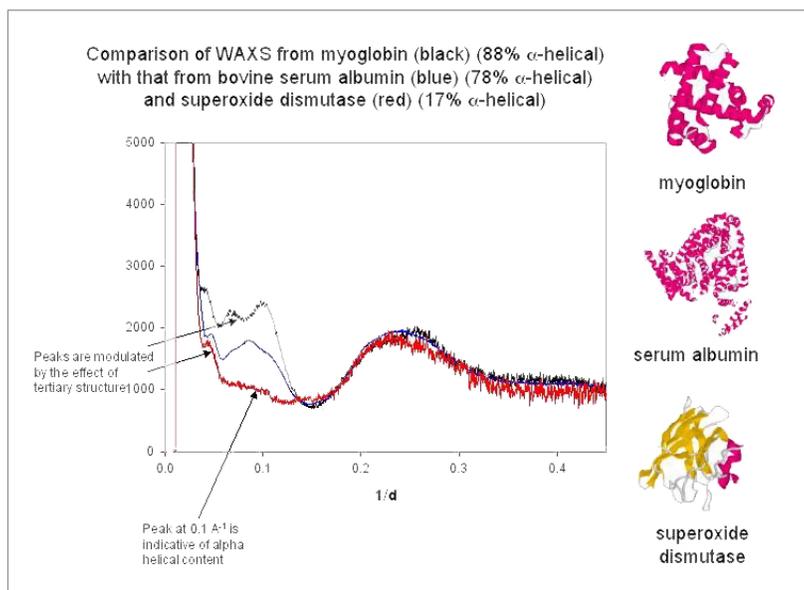


Fig. 1. Wide-angle x-ray scattering data from three proteins with decreasing helical content show progressive flattening of the scattering peak at  $0.1 \text{ \AA}^{-1}$ . Myoglobin (88% helical) is in black; serum albumin (78%) in blue; superoxide dismutase (17%) in red.

strated that these chains fall apart after repeated stationary exposures. Myoglobin and cytochrome C showed much less sign of breakdown, even in the stationary samples.

To demonstrate the potential of WAXS to detect structural changes in proteins, scattering experiments were also performed on hemoglobin exposed to increasing concentrations of a denaturing salt — a salt that is known to unfold proteins. The resulting scattering patterns showed a progressive loss of structural features as the concentration of the salt increased. The group concluded that WAXS experiments can (1) provide accurate and informative data about the structure of a protein in solution; (2) can observe changes in protein structure; and (3) can be carried out in a way to minimize radiation damage to the protein. ○

**See:** R.F. Fischetti<sup>1,2</sup>, D.J. Rodi<sup>1</sup>, A. Mirza<sup>3</sup>, T.C. Irving<sup>3,4</sup>, E. Kondrashkina<sup>4</sup>, and L. Makowski<sup>1</sup>, "High-resolution wide-angle x-ray scattering of protein solutions: effect of beam dose on protein integrity," *J. Synchrotron Rad.* **10**, 398–404 (2003).

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