

TOGGLING A NUCLEAR RECEPTOR

Science is beginning to understand how chemicals in cells spur on and rein in a major hormone receptor. The human glucocorticoid receptor is biologists' primary source of basic knowledge about the function and mechanism of action of nuclear receptors, which turn genes on and the off. Now, researchers from Karo Bio company in Sweden and Abbott Laboratories has solved the three-dimensional (3-D) structure of the protein bound to drugs that stimulate and suppress its activity. By comparing the two, they found that the position of a short stretch of protein determines whether the protein will be highly active or sluggish.

Human glucocorticoid receptor (GR) waits in the cell for the arrival of steroid hormones such as cortisol, a stress hormone, or progesterin, a reproductive hormone. The receptor latches onto them and burrows into the cell nucleus, where it activates some genes and deactivates others. As a result, the body may begin accumulating fat or (in women) ovulating, depending on the hormone. Drugs and other chemicals in the body can make it easier or harder for GR and similar receptors to do their jobs. Although researchers began studying GR before most other nuclear receptors, whose 3-D structures are now known, they only recently determined the structure of one of its key pieces. The so-called ligand-binding domain (LBD) is where the receptor grabs hormones and recognizes other chemical signals.

The researchers decided to compare the structure of the LBD when bound to the agonist, or stimulator, dexamethasone and the antagonist, or suppressor, mifepristone (RU-486), which counteracts the effects of progesterin in a related receptor. They crystallized two different forms of the complete mifepristone-bound protein. Mifepristone deactivates GR by nudging a stretch of protein called helix 12 over a cavity the protein uses to bind a so-called co-activator protein (Fig. 1), the group reported in the June 20, 2003 issue of the *Journal of Biological Chemistry*, based on x-ray diffraction patterns recorded at the IMCA-CAT beamline 17-ID at the APS, and beamline ID14-4 at the European Synchrotron Radiation Facility in Grenoble, France. In the presence of dexamethasone, helix 12 bends away from the cavity (Fig. 1), giving the co-activator access to GR. Other nuclear receptors have a similar mechanism of inhibition.

The second form of GR crystallizes in units made of symmetric pairs of receptors, as opposed to asymmetric groups. In this crystal form, each protein helps antagonize the other. Helix 12 of one member of the pair extends out and covers the activating cavity of the other. The researchers say this structure is unusual and may be an artifact of crystallization. If not, it indicates that GR is quite flexible and open to antagonism in more than one way. The group says the structural comparisons reveal how a single protein can exert different biological func-

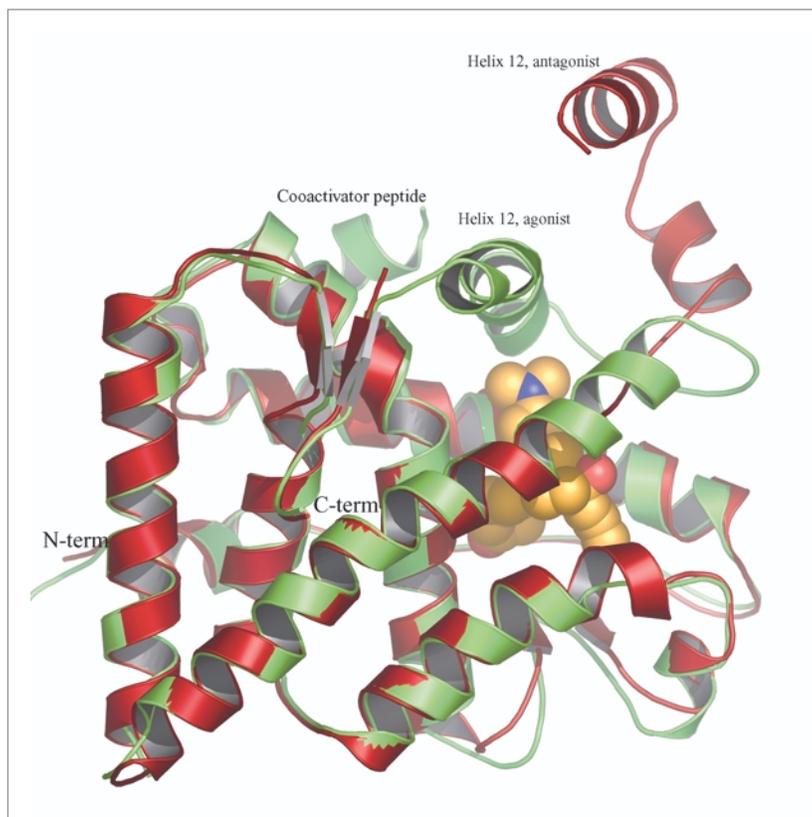


Fig. 1. Comparison of GR with bound agonist (green) or antagonist (red). A chemical group on the side of mifepristone (RU-486) displaces helix 12.

tions based on its contacts with stimulating and suppressing factors. Researchers can now begin interpreting biochemical data from an assortment of GR mutants based on these structures, they add. ○

See: B. Kauppi¹, C. Jakob², M. Färnegårdh¹, J. Yang², H. Ahola¹, M. Alarcon¹, K. Calles¹, O. Engström¹, J. Harlan², S. Muchmore², A.-K. Ramqvist¹, S. Thorell¹, L. Öhman¹, J. Greer², J.-Å. Gustafsson³, J. Carlstedt-Duke³, and M. Carlquist¹, "The Three-dimensional Structures of Antagonistic and Agonistic Forms of the Glucocorticoid Receptor Ligand-binding Domain," *J. Biol. Chem.* **278**(25), 22748-22754 (2003).

Author affiliations: ¹Karo Bio AB, ²Abbott Laboratories, ³Karolinska Institute

The facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team at the Advanced Photon Source are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology (IIT), executed through the IIT Center for Synchrotron Radiation Research and Instrumentation. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

NEW CRYSTAL STRUCTURE FOR NICKEL-RESPONSIVE TRANSCRIPTION FACTOR

Many bacteria require nickel for anaerobic metabolism. The transcription factor NikR regulates nickel uptake in such a way that sufficient amounts, but not too much, of the potentially toxic metal are obtained. NikR, a member of the ribbon-helix-helix family of transcription factors, requires nickel to bind DNA, and there is some evidence to suggest that the binding of DNA changes the geometry of NikR. Using an array of data sets, including some collected at the NE-CAT 8-BM beamline at the APS, investigators from the Massachusetts Institute of Technology determined the crystal structures of two forms of *E. coli* NikR (the full-length apo-repressor at a resolution of 2.3 Å, and the nickel-bound C-terminal regulatory domain at a resolution of 1.4 Å) that establish a better understanding of the mechanism of nickel binding.

Overexpressed full-length NikR and the C-terminal regulatory domain were each purified by nickel-affinity followed by size-exclusion chromatography. Cells grown in a medium supplemented with selenomethionine (SeMet) were used to produce full length SeMet NikR. Crystals were obtained by hanging-drop vapor diffusion and flash-cooled in gaseous N₂ at 100K, the temperature at which all data were collected.

X-ray wavelengths near the N, K-edge and far from the edge were used to determine the structure of the nickel-bound C-domain, using multiwavelength anomalous diffraction (MAD) techniques. Several rounds of iterative manual building and refining by using SHELXL resulted in a model of the nickel-bound C-terminal regulatory domain of NikR. Single-wavelength anomalous dispersion was used to calculate experimental electron density maps for apo-NikR. The C-domain tetramer and two DNA-binding domains were then built into this map. A high-resolution native data set was used to refine the model, which was compared to electron density maps calculated in CNS for manual adjustment.

The basic structure of NikR proves to be a homotrimer: two ribbon-helix-helix DNA binding domains attached at each end of the tetrameric C-terminal regulatory domain (Fig. 1). Two NikR monomers intertwine at their N-termini to each form a two-fold symmetric ribbon-helix-helix domain. A core dimer is formed by the C-terminal regulatory domains of two NikR monomers. Together, the dimers form a tetramer with approximate D₂ symmetry, stabilized by a hydrophobic cluster and hydrogen bonding. At the high-affinity nickel-binding sites at the tetramer interface, the nickel ligands show a square-planar coordination, which may account for the specificity of binding nickel at this site rather than other metal ions that do not take on this geometry.

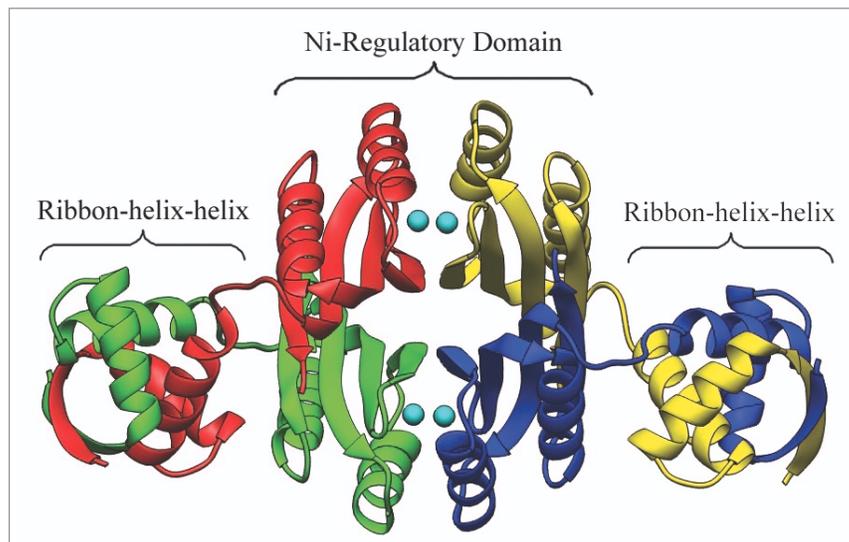


Fig. 1. Model of Ni-NikR, highlighting the modular domain organization. This model was produced from structures of Apo-NikR and the isolated Ni-bound regulatory domain. The NikR tetramer is displayed as a ribbon diagram, colored by the NikR monomer. Nickel ions are displayed as cyan spheres.

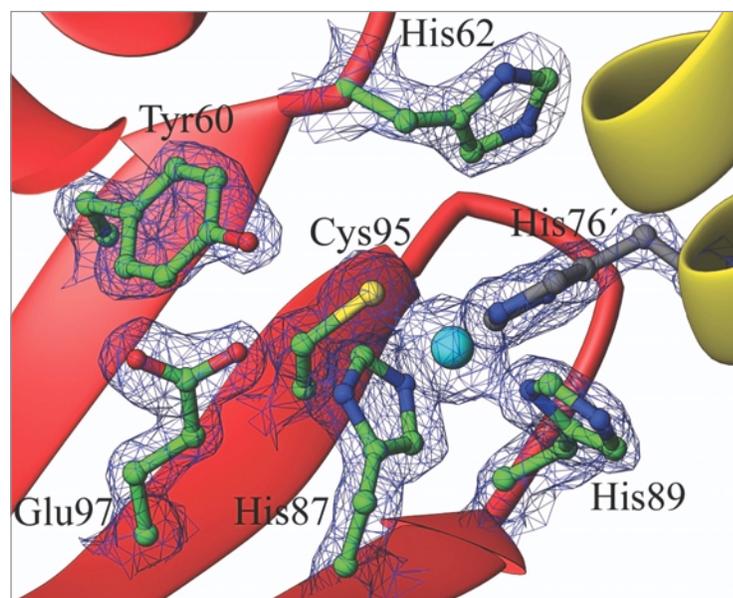


Fig. 2. The high-affinity nickel-binding site in NikR. Strictly conserved amino-acid sidechains are shown with a ribbon diagram of the protein backbone. Note the square-planar coordination of a nickel ion and the contribution to nickel binding from two NikR monomers. The electron density map around the amino-acid sidechains is a $2F_o - F_c$ map contoured at 1.2 sigma.

The C-domain structures are similar either with and without nickel, though some differences are related to the movement of nickel ligands and adjacent residues (Fig. 2). NikR exhibits a C-domain most similar to the regulatory domains of enzymes such as phosphoglycerate dehydrogenase (PGDH), ATP phosphoribosyltransferase (ATP-PRTase) and phenylalanine hydroxylase (PheOH), suggesting a common mechanism for propagating ligand-binding signals to adjacent protein domains. Though NikR has a dimeric structure similar to other proteins in the ribbon-helix-helix family, such as Mnt, Arc, and MetJ, it is the only such transcriptional repressor known to be metal-responsive.

Several models for nickel-binding regulation of NikR can be inferred from the established structures. Nickel could be used to induce a conformational change in the protein to make it capable of binding DNA; it could affect transcription by its role in formation or stabilization of the NikR tetramer; or it could make an interaction with DNA electrostatically favorable by decreasing the net negative

charge of the regulatory C-domain tetramer. All three mechanisms may be operating in activating NikR for operator binding. ○

See: E.R. Schreiter, M.D. Sintchak, Y. Guo, P.T. Chivers, R.T. Sauer, and C.L. Drennan, "Crystal structure of the nickel-responsive transcription factor NikR," *Nature Struct. Biol.* **10**(10), 794-799 (October 2003).

Author affiliation: Massachusetts Institute of Technology

This research supported in part by the U.S. National Institutes of Health (NIH), Searle Scholars Program, Cecil and Ida Green Career Development Fund, Lester Wolfe Predoctoral Fellowship, and the Gray Fund for Undergraduate Research. Data were collected at the Advanced Photon Source, National Synchrotron Light Source, Advanced Light Source, and Stanford Synchrotron Radiation Laboratory synchrotrons. Synchrotron facilities are funded by the U.S. DOE, NIH National Center of Research Resources, and the U.S. National Institute of General Medical Sciences. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

PICKING A PICKY INHIBITOR

Why do some potential drugs specifically target an enzyme that plays a key role in type II diabetes and obesity?

A team of researchers from the Merck Frosst Center for Therapeutic Research and the Merck Research Laboratory has solved the three-dimensional structure of an enzyme bound to several compounds that inhibit its activity as much or more than a similar enzyme. The results may provide a framework for designing potent and selective drugs against diabetes and obesity.

Chemical signals circulating in the blood tell cells when to grow, divide, etc. When a signal reaches an individual cell, the cell often responds by turning on enzymes that add or remove phosphorous groups from proteins inside it, which turns those proteins on or off. Mice lacking a specific phosphorous-removing enzyme, called protein tyrosine phosphatase 1B (PTP1B), become resistant to weight gain and more sensitive to insulin, a hormone that tells cells to burn fuel. In humans, researchers have observed that obese people gradually lose sensitivity to insulin and develop adult onset or type II diabetes. Pharmaceutical companies would like to develop drugs that turn off PTP1B in hopes of counteracting those diseases.

The group synthesized a set of compounds that inhibit PTP1B but ran into a common problem: the compounds also inhibit a T-cell phosphatase that is required for a healthy immune system. With the help of existing 3D structures for the two phos-

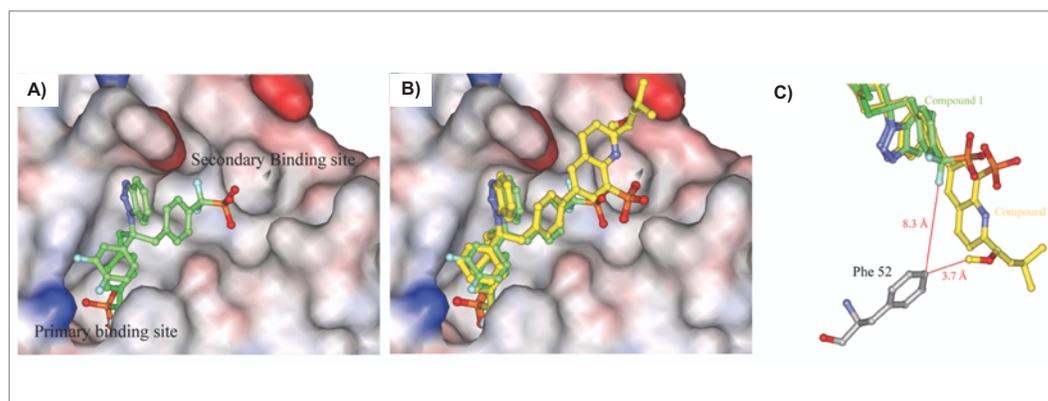


Fig. 1. A) Surface of the substrate binding site of PTP1B. A first-generation, non-selective inhibitor (compound 1) binds in the primary binding site, but does not reach into the secondary binding site. B) A second-generation, more selective inhibitor (compound 2) has been overlaid onto compound 1: compound 2 reaches into and interacts with the secondary binding site. C) The moderate selectivity observed for compound 2 is consistent with its proximity (3.7 Å) to the amino acid phenylalanine (Phe 52).

phatases, the group designed a second set of inhibitors to take advantage of the slightly different shape of the secondary site in each enzyme, and indeed some of the resulting compounds prefer PTP1B moderately over the T-cell phosphatase. To confirm the validity of their approach, the group solved the structure of the enzyme bound to members of both sets of inhibitors. They crystallized a mix of enzyme and inhibitor and determined the resulting structure by analyzing the crystal's x-ray diffraction pattern, measured at the IMCA-CAT beamline 17-ID at the APS.

Each phosphatase has a shallow, elongated crevice on its surface for binding substrates (Fig. 1A,B). Inhibitors block that crevice, which is divided into a primary and secondary site. The more an inhibitor occupies the secondary binding site, the more specific it is for PTP1B. The only difference between the two sec-

ondary binding sites is that two of the amino acids lining PTP1B's pocket—phenylalanine and alanine—are replaced by bulkier amino acids in the T-cell phosphatase. Compounds from the earlier, non-specific set of inhibitors fill the primary binding site, which gives them their potency, but don't extend far enough into the second to make contact with the amino acids that distinguish the two phosphatases (Fig. 1A). With no way to differentiate the two enzymes, the inhibitors bind them both with essentially equal strength.

The group looked at three second-generation inhibitors. The crystal structures confirm that the least specific inhibitor, containing the smallest arm projecting into the secondary binding site, does not make contact with the key amino acids. The other two inhibitors have arms that extend further across the binding site and are both selective for PTP1B. The inhibitor that gets closer to the crucial phenylalanine is also the more selective one (Fig. 1B,C).

The compounds take advantage of most of the available binding sites for potency and selectivity, the researchers con-

clude. They suggest that possible ways to improve compound design would be to simplify the molecules while trying to retain the interactions with the primary binding site, which determine potency, or anchor them better in the secondary binding site. ○

See: G. Scapin¹, S. B. Patel¹, J.W. Becker¹, Q. Wang², C. Desponts², D. Waddleton², K. Skorey², W. Cromlish², C. Bayly², M. Therien², J.Y. Gauthier², C.S. Li², C.K. Lau², C. Ramachandran², B.P. Kennedy², and E. Asante-Appiah² "The Structural Basis for the Selectivity of Benzotriazole Inhibitors of PTP1B," *Biochem.* **42**, 11451-11459 (2003).

Author affiliations: ¹Merck Research Laboratory, ²Merck Frosst Center for Therapeutic Research

The facilities at IMCA-CAT are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology (IIT), executed through IIT's Center for Synchrotron Radiation Research and Instrumentation. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

PLANT VIRUS DEFENDS ITSELF WITH TINY CALIPER

In the long arms race against viruses and other parasitic bits of genetic material, plants have evolved a mechanism for chopping up foreign genes called RNA silencing. Some viruses have retaliated with proteins that negate RNA silencing, and researchers have now discovered how one of these suppressor proteins recognizes and interacts with the silencing signal. They solved the three-dimensional structure of the plant virus protein and found that it acts like a tiny caliper, measuring lengths of nucleic acid and binding preferentially to nucleic acid of the right length. The result may give researchers a new tool for studying RNA silencing in other organisms.

Certain viruses and other genetic "freeloaders," seeking only to reproduce or insert themselves into a host's genome, spend part of their lives as a double-stranded molecule of RNA (a variant of DNA). The host responds by chopping up these RNA double helices into short pieces. Specialized proteins each nab a small fragment of the diced RNA and use the fragments to recognize and destroy (or silence) RNAs from molecular parasites. Some plant viruses produce a protein called p19, which researchers believe interferes with RNA silencing by scouring up all the small RNA fragments of a specific length. To understand how p19 recognizes those molecules, researchers from the National Institutes of Health and the Agricultural Biotechnology Center of Hungary, crystallized and solved the structure of p19 from Carnation Italian Ringspot Virus (CIRV) bound to an RNA fragment.

Based on x-ray diffraction studies performed at the SER-CAT beamline 22-ID at the APS and additional biochemical studies, the group concluded that p19 protein can select the

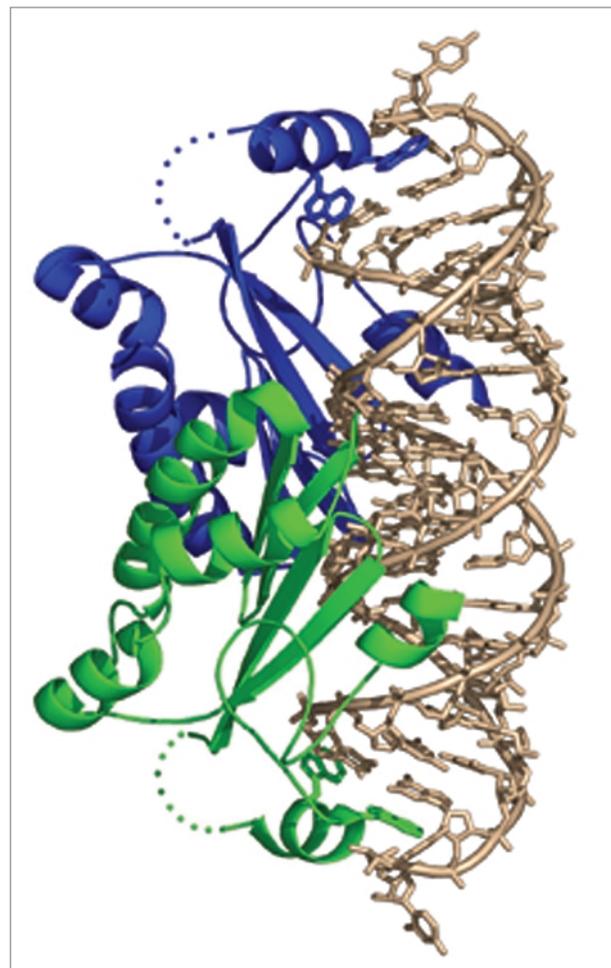


Fig. 1. Two viral p19 protein molecules (green, blue) join to form a long groove for binding RNA fragments that would destroy the virus if left alone.

size class of small RNA fragment that it prefers. Two p19 molecules join together to form an elongated concave surface, and the double-stranded RNA molecule rests in this groove (Fig. 1). The protein binds to atoms along the edge of the RNA that are the same for every RNA molecule but does not bind to the bases in the core of the helix, so the protein can't recognize the specific sequence of those bases. Instead, two amino acids at either end of the protein stick to the bases at either end of the RNA. Replacing one or both amino acids with different ones made CIRV less potent in plant cells, indicating that they are needed for p19 to function properly. One could imagine that the distance between the two amino acids determines the size of the RNA molecule the protein will recognize, namely 21 bases. Shorter segments wouldn't be anchored at the ends and longer ones would kink, missing out on some of the generic bonding along their edges. Consistent with this possibility, the group measured the binding strength of RNAs ranging from 19 to 26 bases long and found that binding strength decreased for each base added to or subtracted from 21.

It is known that p19 suppresses fruit fly RNA silencing and binds short, artificial RNAs in test tubes, so the protein may be useful as a tool in studying the silencing process in animals as well as plants. Animals exploit a version of it to turn their own genes off. ○

See: J.M. Vargason¹, G. Szittyá², J. Burgyán², and T.M. Tanaka Hall¹, "Size Selective Recognition of siRNA by an RNA Silencing Suppressor," *Cell* **115**, 799–811 (26 December 2003).

Author affiliations: ¹National Institutes of Health, ²Agricultural Biotechnology Center

This research supported by grants from the Hungarian Ministry of Education (NKFP 4/023/2001) and "VIS" EU project (QLG2-CT-2002-01673) and the intramural program of the National Institute of Environmental Health Sciences. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

STRUCTURE OF UNUSUAL PROTEIN PROVIDES INSIGHT INTO DEVELOPMENT OF NEURONS

The semaphorins are extracellular proteins that play important roles in growing neurons. These molecules provide chemical signals that guide axonal growth cones. The process begins with the initial stages of axon outgrowth and ends with the formation of a functioning synapse. Many parts of these signaling events, however, are not well understood. Experimentalists from the Memorial Sloan-Kettering Cancer Center, Brookhaven National Laboratory, Cornell University, and Westfälische Wilhelms Universität used data collected at the NE-CAT 8-BM beamline at the APS and the Cornell High Energy Synchrotron Source F2 beamline to determine the crystal structure of one form of a semaphorin, identified the site at which it binds one of its receptors, and may have found the site where it binds a coreceptor. They developed a model describing how the signaling starts and suggested that this model could also explain how signaling is initiated by other molecules with similar structures.

Semaphorin 3A (Sema3A) is particularly interesting because it is a potent repulsive molecule inhibiting or repelling neurite outgrowth. It may also be important for growing blood supplies, which makes it of interest to cancer researchers looking for ways to block the growth of blood vessels around tumors. Ultimately, the researchers want to understand the molecular mechanisms of semaphorin-mediated signaling.

As a small part of that goal, they need to understand how Sema3A interacts with its two coreceptors. Sema3A is unusual in that it needs two receptors (Plexin-A1 and Neuropilin-1) in order to function. Plexin-A1 acts as the signal-transducing unit, but Sema3A cannot bind directly to it. Another receptor, Neuropilin-1 (Nrp-1), brings the two together.

In order to investigate the structure of the protein, they used the techniques of x-ray crystallography to study a func-

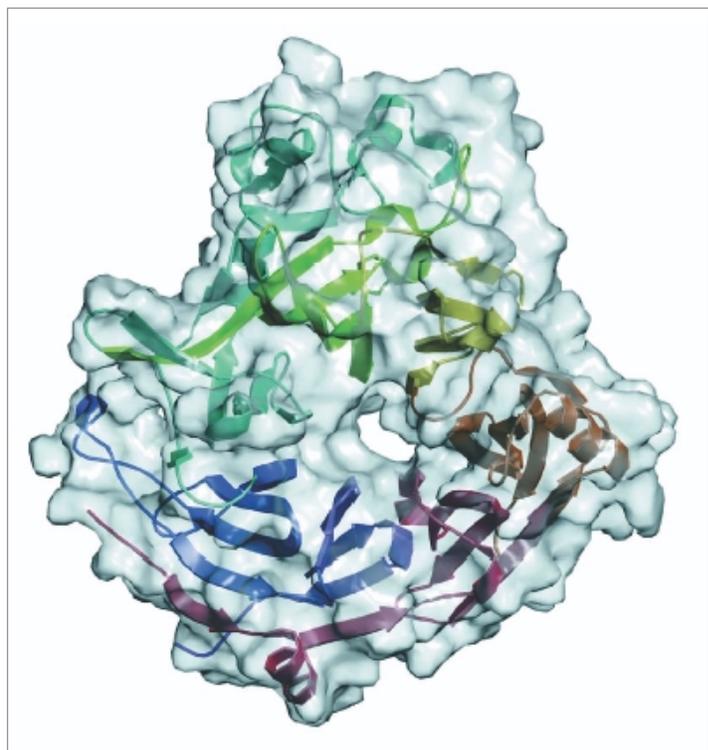


Fig 1. Structure of semaphorin Sema3A-65K contains seven propeller blades. Starting at the N-terminal end of the protein, each of the seven blades is assigned a color (red, orange, yellow, green, cyan, blue, and magenta) before reaching the C-terminal end of the molecule.

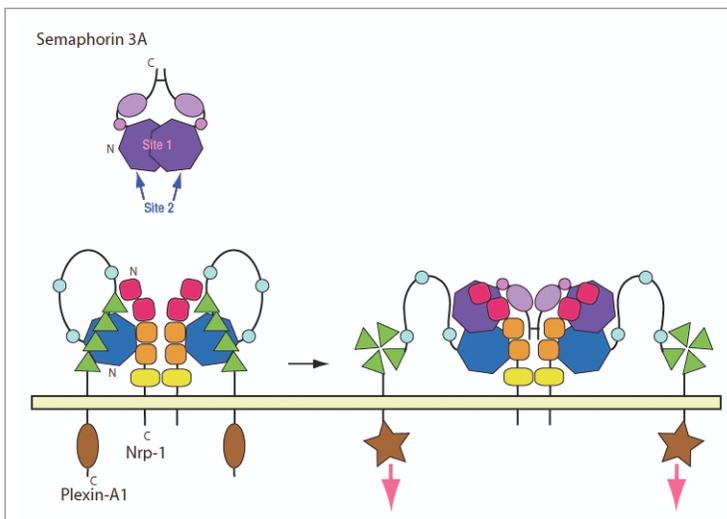


Fig. 2. A model for semaphorin-initiated signaling. The authors suggest that (left) in the absence of Semaphorin 3A, the Nrp-1/Plexin-A1 complex is both stable and inactive. When Semaphorin 3A binds to the two receptors, it releases a part of the plexin molecule that was previously bound by Nrp-1, allowing it to adopt an active conformation. Plexin is shown in dark blue, light blue, green, and brown. NRP-1 is shown in red, orange, and yellow. Semaphorin is shown in purple, lavender, and pink.

tional 65-kDa Semaphorin 3A form (Semaphorin 3A-65K). It contains the entire semaphorin domain (the roughly 500 amino acids that define semaphorins). Nikolov's group used two-wavelength multiple anomalous diffraction (MAD) phasing, a method that requires changing the wavelength—a requirement that is beyond the capability of many x-ray sources but possible using the APS. The high-intensity source also aided the researchers by providing a good signal from weakly diffracting crystals. The data obtained at APS allowed them to build a semaphorin model, which they refined at 2.8-Å resolution.

They found that the protein is shaped like an elongated disc and measures about $60 \times 70 \times 45$ Å. To their surprise, the group also found that the protein folds in a propeller-like fashion, a variation of the so-called beta propeller topology, in which seven blades are arranged radially around a central axis (Fig. 1). Interestingly, most of the blades contain a larger than usual number of amino acids. Some of the extra secondary structure elements are likely to have important biological functions, including the four protruding loops that form the proposed neuropilin-binding site.

The researchers were able to compile clues about the way neuropilins and semaphorins interact with each other to identify their potential binding sites. Because neuropilin binding competes with semaphorin dimer formation, they surmised that the binding spot is probably near the dimerization interface. Also, since some semaphorins do not bind neuropilin-1, they must lack some binding site features that are present in Semaphorin 3A. As an additional indication that they'd found the right site, analysis of the electrostatic potential at the molecular surface showed that the binding sites are oppositely charged and therefore attractive. To test their theory, they created two Semaphorin 3A-65K variants with alterations in the proposed binding site: neither would bind to Nrp-1.

Given the structural information and previously published data, the researchers propose a series of molecular events that occur at the beginning of semaphorin signaling (see Fig. 2). In the absence of ligands, the plexins remain in an inactive (autoinhibited) state. Nrp-1 interacts with the extracellular region of Plexin-A1, stabilizing this inactive form of the molecule. When Semaphorin 3A binds to the Nrp-1/Plexin-A1 complex, however, the shape of Plexin-A1 changes both outside and inside the cell. This process releases the plexin from inaction, allowing it to begin signaling.

This signal-initiation sequence could be similar to that of other cell surface receptors (such as integrins and the LDL receptor) with the same beta propeller topology.

See: A. Antipenko¹, J.-P. Himanen¹, K. van Leyen¹, V. Nardi-Dei¹, J. Lesniak¹, W. A. Barton¹, K.R. Rajashankar², M. Lu³, C. Hoemme⁴, A. W. Püschel⁴, and D.B. Nikolov¹, "Structure of the Semaphorin-3A Receptor Binding Module," *Neuron* **39**, 589–598 (14 August 2003).

Author affiliations: ¹Memorial Sloan-Kettering Cancer Center, ²Brookhaven National Laboratory, ³Weill Medical College of Cornell University, ⁴Westfälische Wilhelms Universität

This work was supported by the New York State Spinal Cord Injury Research Program (D.B.N) and by DFG and Fonds der Chemischen Industrie (A.W.P.). D.B.N. is a Bressler Scholar. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.



THE MORE, THE MERRIER: MULTIPLE PRECIPITANTS ARE BETTER THAN ONE

X-ray crystallography studies to determine a protein's structure are notoriously limited by many proteins' stubborn refusal to crystallize. Experimentalists from the National Institutes of Health and Columbia University have found that combining several types of precipitants, or chemicals that nudge molecules out of solution, can work better than adding just a single type. They screened 64 precipitant mixtures and reported that mixtures increased the chance of crystallization and the quality of crystals formed.

Although researchers have dramatically increased the number of protein structures determined by X-ray diffraction over the last decade, the crystallization process is still somewhat mysterious and finicky. Precipitating agents, used to force proteins out of solution and possibly into crystal form, have become key ingredients. Researchers often resort to screening a range of solution conditions when trying to crystallize a protein, but typically treat the precipitant as a single variable, even though precipitants come in a number of distinct flavors: Salts make water repel oil more strongly, forcing the greasy parts of proteins together; organic solvents prevent water from shielding charged parts of proteins, allowing complementary charges to stick; and high molecular weight polymers crowd the dissolved molecules, forcing them to cluster together. Previous researchers had discovered that fortuitous mixtures of precipitants sometimes give crystals, but no one had systematically tested the effect of precipitant mixtures on crystallization.

The group devised a 64-member screen varying along five dimensions: pH, the presence of an additive (positive or negative ion, detergent, etc), and the choice of three dissimilar precipitants. Many combinations didn't mix, but those that did often lowered the concentration of protein required for precipitation. Compared to a commercially available screen, the so-called precipitant synergy (PS) screen covered a wider and denser range of possible precipitation conditions. They tested the two screens on ten HIV proteins whose long flexible loops make them extremely difficult to crystallize. Although both screens produced the same number of initial crystals, the PS produced three times as many crystals large and ordered enough for structural analysis--six in total--based on x-ray studies on the SER-CAT 22-ID beamline at the APS. (Several of the structures were recently published.)* The experimental screen also crystallized three proteins that the commercial screen couldn't, but failed to crystallize two proteins the commercial screen could. Many of the commer-

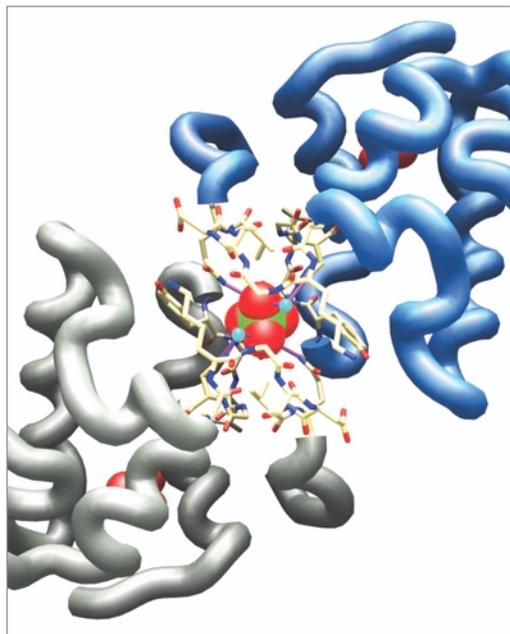


Fig. 1. A never-before-seen type of egg white lysozyme—the first new crystal lattice of lysozyme to be reported since 1967. Pairs of lysozyme molecules (silver, blue) are held together by sulfate ions (red, green).

cial screen's successes were from mixtures of precipitants, though, and when the initial crystals were optimized to find the best crystallization conditions, all formed the best crystals in precipitant mixtures.

The group also tested the PS screen on hen egg white lysozyme, a protein studied for over 50 years and commonly used to evaluate screens. 29 out of 64 combinations supported crystal growth, and one combination even produced a novel crystal structure that hadn't been reported previously. The crystal was built out of symmetric pairs of protein molecules, held together electrostatically by sulfate ions (Fig. 1) with adjacent pairs joined by hydrophobic (water-excluding, non-electrostatic) interactions.

The researchers speculate that the interactions holding the crystal together reflect the mixture of precipitants responsible for crystallization. Researchers believe crystal formation may begin from a small number of contacts. Precipitants stabilize different types of protein interactions, which may enhance the probability of forming specific types of contacts.

The results suggest that existing robotic technology should be able to search through significant regions of "crystallization space," the sum of all possible precipitant combinations. Pharmaceutical and biotech companies would like to assemble as complete a library of structures of proteins in the human genome as possible, the researchers point out, and even doubling the average probability of crystallization would have a big effect. ○

*Huang et al. 2004 PNAS 101:2706-2711

See: S. Majeed^{1,2}, G. Ofek¹, A. Belachew², C.-c. Huang¹, T. Zhou¹, and P.D. Kwong^{1,2}, "Enhancing Protein Crystallization through Precipitant Synergy," *Structure* **11**, 1061-1070 (September 2003).

Author affiliations: ¹National Institutes of Health, ²Columbia University

These studies were supported by the National Institutes of Health. P.D.K. was a recipient of a Burroughs Wellcome Career Development award. Beamline X4A at the National Synchrotron Light Source, a Department of Energy facility was supported by the Howard Hughes Medical Institute. Use of SER-CAT at the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.