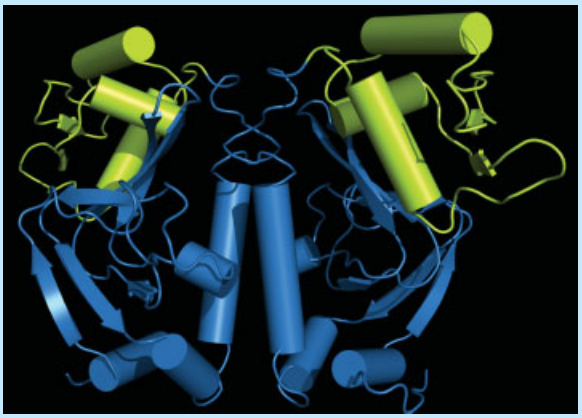
What Turns CAP On?

The catabolite activator protein (CAP) from *E. coli* holds an esteemed role in biochemistry history. In addition to being described in countless textbooks as a canonical example of allosteric regulation and a component of the much-studied *lac* operon, CAP was also the first transcriptional regulator to have its three-dimensional structure solved. Some mysteries remain, however. CAP operates as a homodimer that exists in two states: an 'on' conformation in which it tightly binds DNA regulatory sequences when bound to cyclic AMP (cAMP), and an unbound 'off' conformation in which it only associates weakly with DNA. The mechanism of the off-on transition is unclear, however, because no structural data is available for the unbound complex.

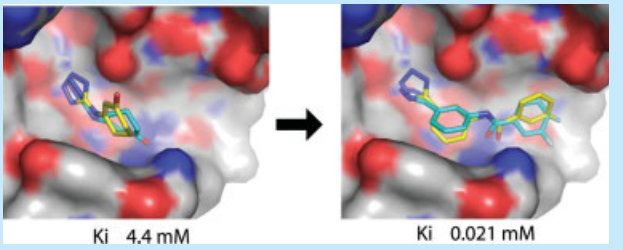


New nuclear magnetic resonance structural data from Popovych et al. provide the missing piece to this puzzle. As described recently in the *Proceedings of the National Academy of Sciences, USA*, the major difference between the two states lies in the positioning of CAP's DNA recognition helices, which can only interact with the major groove of DNA when cAMP is bound. The movement of these helices is mechanically mediated by partial unwinding of the coiled-coil domain that connects the DNA-binding domain to the cAMP-binding domain, and the researchers identified several mechanisms by which cAMP binding might bring about this transition. These findings enable them to demonstrate why inappropriate substrates such as cGMP fail to turn CAP 'on', and explain the constitutive DNA binding activity of certain CAP mutants. Most importantly, the authors suggest that these revelations may offer valuable insights about hundreds of other proteins that make use of similar allosteric regulatory mechanisms.—*Michael Eisenstein*

Popovych, N. et al. *Proc. Natl. Acad. Sci., USA*, published online 9 April 2009, doi: 10.1073/pnas.0900595106.

Starting Small

For researchers seeking the perfect inhibitor for a given enzyme, 'fragment-based' drug discovery—screening performed with extra-small compounds that occupy only a portion of the active site—represents a powerful tool. The hits obtained by this approach may be relatively low-affinity by themselves, but they can provide valuable starting points for constructing highly effective therapeutics. Unfortunately, even though more than 300,000 fragments are now available to the scientific community, current experimental methods don't provide the throughput needed to exploit the full diversity of this stockpile. Computational docking strategies could help boost throughput, but serious questions remain about how accurately such algorithms can model fragment-active site interactions. Chen and Shoichet tackle this issue in a new article in *Nature Chemical Biology*, using a molecular docking program to search a library of 67,489 fragments from their ZINC compound database for candidate inhibitors of CTX-M, a bacterial β-lactamase involved in antibiotic resistance. Their docking program proved remarkably accurate in its interaction predictions, as confirmed by crystallographic analysis of several hits from their screen. Among the strongest inhibitors identified were several tetrazole derivatives, and these were used to search for analogues in a second library of larger 'lead-like' compounds.



This enabled them to retrieve several promising compounds that would have been overlooked if docking had instead been used to screen the lead-like library, including one that provides micromolar inhibition of CTX-M with relatively strong specificity. The authors conclude that molecular docking should offer an effective general strategy for initial screening of large numbers of fragments, and a means for achieving greater diversity in the hunt for drug candidates.—*Michael Eisenstein*

Chen, Y. & Shoichet, B. K. *Nat. Chem. Biol.*, published online 22 March 2009, doi: 10.1038/nchembio.155.

Structure of a Glycoprotein

The availability of good three-dimensional structural data for glycoproteins is diminished by difficulties in crystallization as well as by the absence of convenient isotope enrichment methods for NMR studies. In a recent report in the *Journal of the American Chemical Society*, Slynko et al. describe a new scheme for selective isotope enrichment of the glycan and peptide portions of a glycoprotein along with isotope-filtered NMR methods that greatly facilitate acquisition, assignment and interpretation of NMR data for glycoproteins. Their method involves importation of the *Campylobacter pgl* gene cluster into *E. coli*. The single protein oligosaccharyl transferase, PglB, much simpler than its eukaryotic counterpart, is active in an *in vitro* system for transfer of the glycan from a lipid-linked oligosaccharide to a natively folded protein acceptor. This convergent synthesis joins a natural abundance glycan to an isotope-enriched protein facilitating their separate observation in ^{13}C- and ^{15}N-filtered NOE experiments. Although the glycan is linked to asparagine in this bacterial system, its structure differs substantially from that of its eukaryotic cousins, having GalNAc-α-1-4-GalNAc linkages. The proposed glycoprotein model, based on longer range NOE data than commonly observed in free glycans, shows a protein conformation largely unchanged by the glycosylated asparagine located in a flexible peptide loop and a relatively well defined rod-like glycan. This conformation, which contrasts with the more commonly held picture of carbohydrate chains as relatively flexible, could result from its unusual axial-to-axial glycosidic linkages. With so few known structures in the protein data bank, it is quite difficult to generalize about glycoproteins but the model proposed by Slynko et al. is certainly a most intriguing addition to a very small collection.—*C. Allen Bush*

Slynko, V. et al., *J. Amer. Chem Soc.* 131, 1274–1281, 2009.