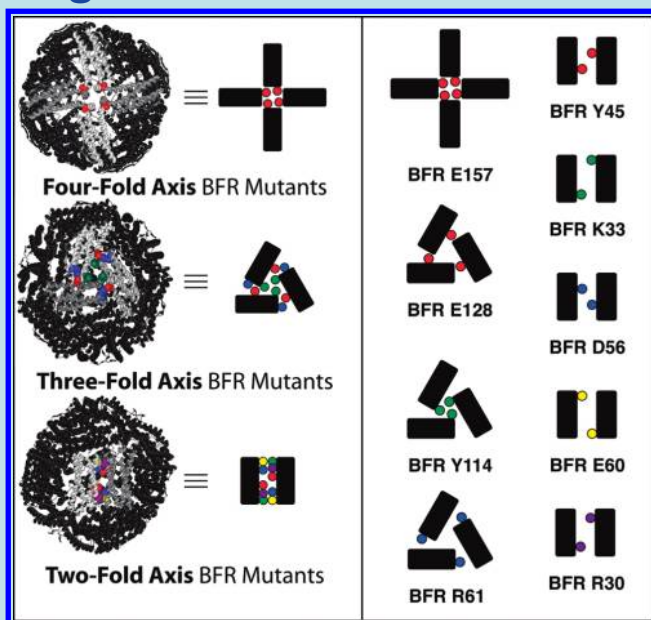


Spotlight

Oligomerization Switches



Zhang, Y., *et al.*, *J. Biol. Chem.*, 2010, 285, 12078–12086. Copyright 2010 The American Society for Biochemistry and Molecular Biology.

sion chromatography, dynamic light scattering, native polyacrylamide gel electrophoresis, and circular dichroism. Although all of the mutants were capable of adopting proper secondary and even tertiary structures, they exhibited dramatically different macromolecular properties. For example, four of the mutants failed entirely to form the 24-mer in solution, forming dimers instead. The mutated residues in these proteins thus appear to function as oligomerization switch residues, serving to guide the self-assembly process. In addition, two of the mutations resulted in proteins with increased thermodynamic stability relative to that of the native protein, pointing to the complex interplay between structure and stability that likely influences ferritin function. These studies demonstrate the power of combining computational, biochemical, and structural methods for the investigation of macromolecular self-assembly processes and help point the way toward the rational design of novel nanostructures. Eva J. Gordon, Ph.D.

The self-assembly of proteins into macromolecular structures is a key part of the protein folding process and proper protein function; improper assembly has been implicated in a variety of human diseases including Alzheimer's disease and type II diabetes. Ferritins, proteins involved in iron storage in cells, form 24 subunit-containing macromolecules called nanocages that are convenient model systems for exploring the nature of the self-assembly process. To this end, Zhang *et al.* (*J. Biol. Chem.* 2010, 285, 12078–12086) perform an alanine-shaving mutagenesis study to investigate the roles of amino acid residues situated at the protein–protein interfaces within the nanocage structure.

Structural analysis and virtual alanine scanning guided the design of nine mutants in which residues suspected of engaging in important interactions at either the 2-fold, 3-fold, or 4-fold axis of symmetry within the 24-mer were replaced with alanine. The mutant proteins were recombinantly expressed and purified, and then examined using numerous structural analytical techniques, including transmission electron microscopy, size exclu-

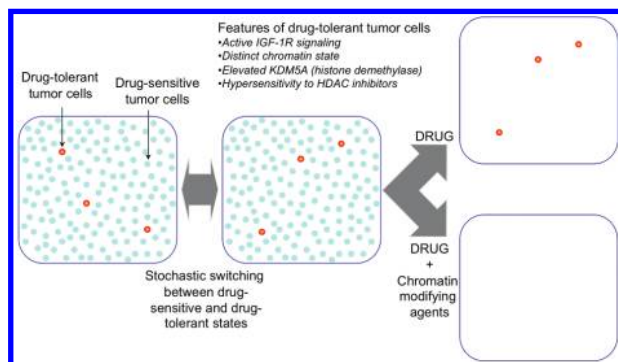
Targeting Tenacious Tumors

Promising cancer treatments are often thwarted when tumor populations acquire resistance to chemotherapy. Although many mechanisms for drug resistance depend on rare genetic mutations, recent research suggests that epigenetic changes could also orchestrate reversible drug resistance pathways. Even drug-sensitive human cancer cell lines maintain a subpopulation of reversibly tolerant cells when exposed to the stress of a lethal drug. Now Sharma *et al.* (*Cell*, 2010, 141, 69–80) have demonstrated how modifications to chromatin can lead to reversible drug resistance and a possible clinical treatment.

After several days of treatment with lethal drugs, a small subpopulation of cells (up to 0.5%) from the drug-sensitive lines persist, usually as quiescent cells, called “drug-tolerant persisters” (DTPs). A portion of these cells may then resume normal proliferation as “drug-tolerant enhanced persisters” (DTEPs). Using drug-free passaging, drug sensitivity can be restored over time in proliferating DTEPs. Global gene expression analysis of normal cells, DTPs, and DTEPs pointed to alterations in the global chromatin in drug-tolerant cells. In DTPs and DTEPs, expression of KDM5A, an enzyme that modifies chromatin, was elevated, stabilizing the reversible drug tolerance.

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Reprinted from *Cell*, 141, Sharma, S.V., et al., A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations, 69–80, Copyright 2010, with permission from Elsevier.

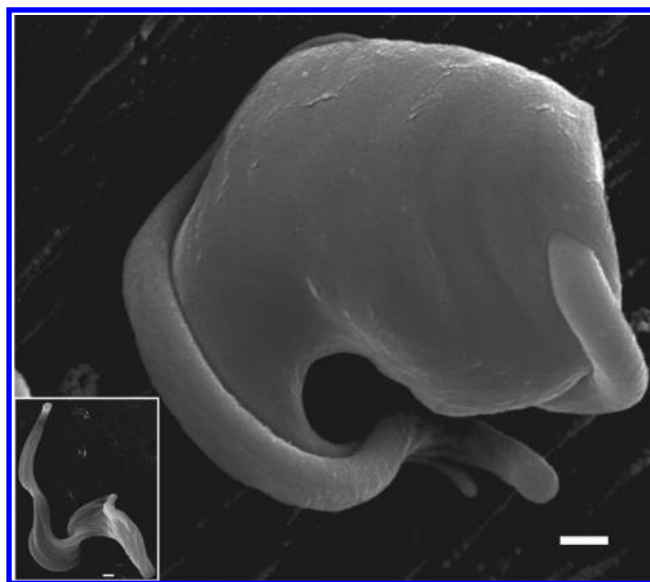
No drug inhibitors of KDM5A have been developed, but KDM5A associates with histone deacetylases (HDACs). Therefore, the researchers looked to HDAC inhibitors as a way to counteract drug tolerance. Treatment with HDAC inhibitors successfully killed DTPs and DTEPs but not drug-susceptible cancer cells. Simultaneous treatment of cancer cell lines with the targeted drug therapy and HDAC inhibitors prevented the formation of drug-tolerant subpopulation.

A clinical study of non-small-cell lung cancer patients combining the drug erlotinib and a chromatin-modifying agent is underway with promising initial results among patients whose tumors had shown signs of drug resistance. These survival strategies that Sharma *et al.* observed within tumor cell populations are strikingly similar to those used by microbial populations to tolerate antibiotics. This drug-tolerance mechanism could be occurring in parallel with drug-resistant genetic mutations. In addition, these chromatin modifications could facilitate stress-induced mutagenesis within cancer cells. **Sarah A. Webb, Ph.D.**

A Wake-Up Call for Sleeping Sickness

Trypanosomiasis, or African sleeping sickness, is a disease caused by parasites of the *Trypanosoma brucei* species. Transmitted to humans through the bite of a parasite-infected tsetse fly, the disease is responsible for the death of tens of thousands of people each year, and few effective treatments exist. Now, Frearson *et al.* (*Nature*, 2010, 464, 728–732) report the identification of *N*-myristoyl-transferase (TbNMT) as a new target for African sleeping sickness, along with small molecule inhibitors that represent exciting new drug leads to treat this complex and destructive disease.

TbNMT is responsible for the addition of myristic acid to proteins, a post-translational modification that directs membrane targeting and is involved in the signal transduction activity of many proteins. Essential for parasite growth, TbNMT is an intriguing drug target, as it participates in the myristoylation of over 60 proteins in *T. brucei*. Screening of more than 60,000 small molecules led to the identification of a se-



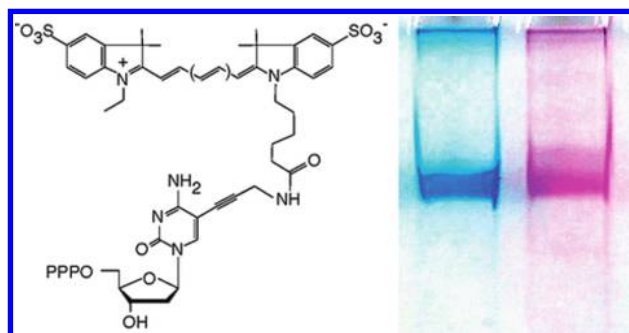
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ries of pyrazole sulfonamide-containing compounds capable of potent inhibition of TbNMT. When probed for their activity in biological systems, the inhibitors were shown to prevent *T. brucei* growth *in vitro* and, importantly, cured trypanosomiasis in mouse models of the disease. Fluorescence-activated cell sorting analysis demonstrated that *T. brucei* exposed to the inhibitors resulted in trypanosome death, and radiolabeling and protein expression experiments offered compelling evidence that the TbNMT protein indeed was the target of the compounds. Further, though the structure of TbNMT has yet to be solved, a protein–ligand complex with *leishmania* NMT and enzyme kinetic studies confirm that the inhibitors bind in the peptide substrate binding site of the enzyme. This structural insight will facilitate further optimization of this series of promising compounds as new trypanosomiasis drugs. **Eva J. Gordon, Ph.D.**

DNA Takes the Cyanine Pill

As sensitive detection techniques such as microarrays emerged, the demand for clever ways to make more intense fluorescent complementary RNA or DNA probes arrived in concert. For highest fluorescence, many standard methods revolve around enzymatically incorporating a reactive nucleoside into the DNA or RNA strand and then performing a secondary reaction to attach a cyanine fluorophore to the reactive base. This two-part scheme is usually necessary because polymerases are, by nature, highly selective enzymes that cannot readily incorporate bases with a foreign, and bulky cyanine dye hanging off of them. To address this issue, a new study by Ramsay *et al.* (*J. Am. Chem. Soc.* 2010, 132, 5096–5104) took advantage of an *in vitro* evolution procedure to hunt for polymerase mutants that could directly incorporate Cy3 or Cy5-labeled dCTP.

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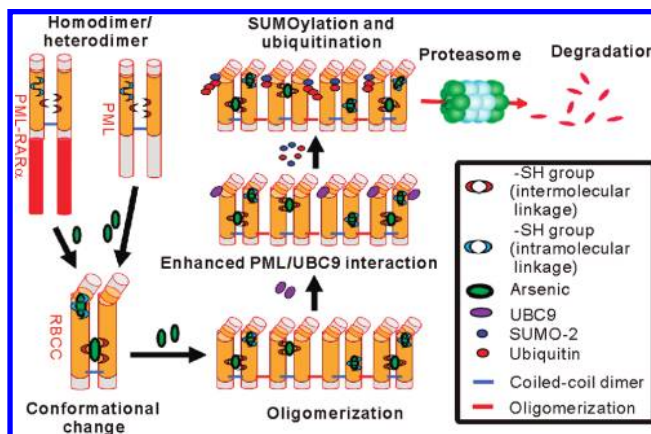


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Starting with the thermostable polB polymerase from *Pyrococcus furiosus* (Pfu), the authors made random mutations near the active site of the enzyme, reasoning that such mutations might reduce steric clashes with the bulky cyanine dyes. A library of mutant enzymes were individually isolated into microdroplets dispersed in oil emulsion serving as tiny bioreactors and given the task of replicating a 460 nucleotide DNA corresponding to a patch of the mutant polymerase gene itself. They dubbed this method short-patch compartmentalized self-replication or spCSR. Given only cyanine-dCTP as a source of C, only mutants capable of replicating their own gene using the fluorescent base survived. After just two rounds of selection, a mutant designated E10 emerged as the best candidate because it could readily incorporate Cy3 or Cy5-labeled dCTP with high efficiency. E10 showed excellent fidelity with the other dNTPs and only a mild loss of fidelity when dCTP was replaced with Cy-dCTP. The authors went on to study what they term, CyDNA, or DNA where every C replaced with a Cy-labeled-C. Due to the large number of cyanine dyes, CyDNA not only displayed bright fluorescence but a number of novel properties not normally seen in nucleic acids such as vivid color and organic phase partitioning. CyDNA appeared to have specific hybridization properties and gave improved signals on microarrays. In all, this study shows both the impressive utility of the spCSR technique and the potential for CyDNA as a new weapon in the war on sensitivity. **Jason G. Underwood, Ph.D.**

A Poison for Cancer

Notorious for both its poisonous and medicinal properties, arsenic has recently found distinction as an anticancer agent targeting acute promyelocytic leukemia (APL). Specifically, arsenic trioxide (As₂O₃) promotes the destruction of PML-RAR α , an oncogenic fusion protein that is present in nearly all cases of APL and is critical for the pathogenesis of the disease. Despite the success of As₂O₃ in the clinic, the mechanism by which it facilitates the degradation of PML-RAR α has remained elusive. Using an impressive suite of molecular, biochemical, and cellular methods, Zhang *et al.* (*Science* 2010, 328, 240–243) elucidate with remarkable detail just what happens when As₂O₃ interacts with PML-RAR α .



From Zhang, X.-W., et al., *Science*, 2010, 328, 240. Reprinted with permission from AAAS.

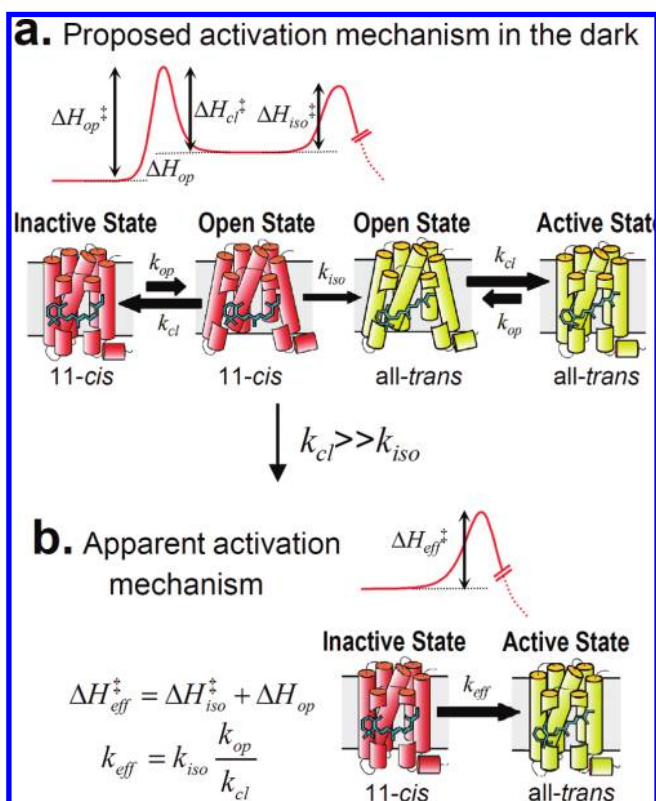
It was known that As₂O₃ promotes PML-RAR α degradation by facilitating the addition of the ubiquitin-like protein SUMO to PML, which subsequently directs the protein to its demise in the proteasome. Protein localization experiments, As₂O₃ derivatives, and PML deletion mutants were used to explore the specific role of As₂O₃ in PML degradation, and it was determined that arsenic directly binds to PML through a cysteine-rich, zinc-finger-containing domain. Structural characterization of the interaction using a combination of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry, X-ray absorption spectroscopy, circular dichroism, and gel filtration experiments indicated that arsenic likely replaces zinc in the protein and induces its conformational change and oligomerization. Moreover, *in vitro* SUMOylation experiments and mammalian two-hybrid assays revealed that arsenic binding in PML enhances its interaction with an enzyme required for conjugation of SUMO to PML. Together, the data suggest a model in which arsenic binding to cysteines in PML causes a conformational change that enhances SUMOylation of PML, resulting in its degradation. The insights into how As₂O₃ exudes its anticancer activity offered by this study will facilitate further progress into the use of arsenic for medicinal purposes. **Eva J. Gordon, Ph.D.**

A Window Into Dark Events

Vision cells in vertebrates are amazingly sensitive: protein complexes within rods can reliably process single photons into electrical signals, but in extremely low light, vertebrates still have practical vision limits. Those same proteins also experience “dark events,” random electrical signals that occur in the rods without exposure to light. A critical photochemical reaction, the conversion of 11-*cis*-retinal to the all-*trans* isomer, governs the conversion of light into electrical signals. Scientists have proposed that a thermal isomerization might explain the “dark events,” but the low activation enthalpy for dark events, just half that for the photochemical

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process, has seemed inconsistent with this mechanism. Dark events are also remarkably infrequent, occurring just 20 times per hour within a cell that contains more than 100 million photon receptor molecules.



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Now, Lórenz-Fonfria *et al.* (*J. Am. Chem. Soc.* 2010, 132, 5693–5703) offer a clever solution to this long-time conundrum: a two-step mechanism involving a transient opening of the retinal binding pocket of the photon receptor, rhodopsin, followed by a thermal *cis*–*trans* isomerization. First, they measured the kinetics and thermodynamics of the thermal *cis*–*trans* isomerization of a free retinal analogue. The activation enthalpy (94 kJ mol^{-1}) matches the range observed for dark events ($80\text{--}110 \text{ kJ mol}^{-1}$), which suggests that the isomerization likely occurs in a solvent-like environment. But the rate of dark events is one-million-fold slower within the rhodopsin (Rho) protein than the rate of these reactions in solution. This much slower reaction rate suggested a transient opening of the retinal binding pocket, so the researchers looked for experimental evidence of an opening. Through hydrogen–deuterium exchange experiments, they demonstrated that a critical threonine residue in the retinal binding pocket showed exchange of its OH-hydrogen. The researchers then analyzed the hydrogen–deuterium exchange at this residue using global kinetic analysis to find that the rate of this opening over a specific time, temperature, and pH range was consistent with the observed rate of dark events.

These results also provide tantalizing clues to related questions about visual processing. For example, cones have dark events up to 10,000 times more frequently than rods. These results further bolster the hypothesis that the retinal binding pocket is less stable in cones than in rods. In addition to probing “dark events” these investigations provide hints that other important biological processes may be controlled by high-energy, short-lived protein conformations not observed by conventional structural methods. **Sarah A. Webb, Ph.D.**