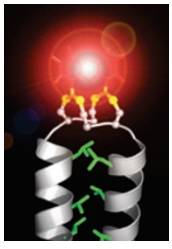
Glimpse of a single DNAzyme

Through *in vitro* selection, DNAzymes that can catalyze a wide range of reactions have been identified. However, gaining insights into the mechanism of catalysis has not been straightforward. Kim *et al.* now report the first single-molecule study of the folding and catalysis of a DNAzyme. The 8–17 DNAzyme catalyzes the metal-dependent cleavage of RNA phosphodiester bonds. Single-molecule fluorescence resonance energy transfer studies showed that with Zn^{2+} and Mg^{2+}, a folding step precedes cleavage, whereas with Pb^{2+}, the most active metal, catalysis does not require a prior folding step. These results suggest that metallo-DNAzymes, like their protein counterparts, can be preorganized to facilitate catalysis. [**Letters, p. 763; News & Views, p. 753**] *JK*

The gates of calcium

Voltage-gated calcium channels such as Ca_Vs allow the influx of Ca^{2+} and are implicated in several cardiovascular and neurological diseases. Inhibition studies of Ca_Vs generally involve the use of small molecules, which suffer from issues of specificity. Yang *et al.* now describe several genetically encoded inhibitors that allowed them to regulate specific Ca_Vs in space and time. These are based on native Rem, a Ras-like GTPase that suppresses certain Ca_Vs within the plasma membrane. The authors generated a truncated Rem fused to a membrane-targeting domain that only reaches its plasma membrane destination in the presence of a phorbol ester. The authors also developed a more tunable system that would allow them to use rapamycin analogs with different efficacies to regulate the level of Ca_V inhibition. Using these compounds, they demonstrated that the kinetics of channel inhibition are rapid, which suggests that there are no intermediate biochemical steps between Rem and the Ca_Vs. [**Articles, p. 795; News & Views, p. 754**] *MB*

A folding FlAsHlight



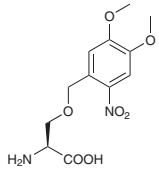
Though *in vitro* studies of protein folding have elucidated the basic principles and preferred mechanisms of folding for many proteins, *in vivo* complications such as cotranslational folding or the presence of chaperones may affect the timing and pathways of protein folding. Here Luedtke *et al.* apply the tetracysteine-targeted FlAsH and ReAsH fluorophores to monitor intra- and intermolecular protein assembly *in vitro* and *in vivo*. The authors prepared peptides and proteins containing pairs of cysteines in distal locations in the primary sequence but close in space in the folded structure, such that the fluorescent tags bind with high affinity only when the final structure is in place. Introduction of mutations at residues within helical interfaces disrupted folding, which translated to low fluorescence, thus demonstrating that folding and misfolding can be distinguished with these reagents. [Letters, p. 779] *CG*

Written by Mirella Bucci, Catherine Goodman & Joanne Kotz

Hemoglobin knows NO bounds

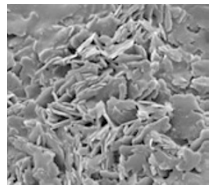
Nitrite has recently been identified as a biological signaling molecule. In one signaling pathway, the reaction of nitrite with deoxyhemoglobin generates nitric oxide (NO), which is then involved in vasodilation and other NO-mediated reactions. However, because of the reactive nature of NO, it is not clear how it can diffuse through red blood cells to reach its intended targets without any side reactions. Basu *et al.* now show that hemoglobin can catalyze the nitrite-dependent formation of N_2O_3. The authors first observed that nitrite can bind directly to methemoglobin. This species could then react rapidly with NO to form N_2O_3. Because of the more stable nature of N_2O_3 relative to NO, this previously unknown reaction of hemoglobin may underlie the physiological signaling of nitrite. [**Articles, p. 785**] *JK*

Phosphorylation inPho4rmation



Phosphorylation is a central post-translational modification, controlling protein activation and localization, among other biological functions. Correlating a biological function with a specific phosphorylation event, however, can be challenging. In the case of the transcription factor Pho4, phosphorylation of two serines (termed S2 and S3) is known to have a role in signaling nuclear export, but the relative importance of each residue has remained unknown. Lemke *et al.* now use a new genetically encoded photocleavable amino acid to probe the significance of these serine residues. Phosphorylation of S3, but not S2, was sufficient to promote export in a double mutant background, with more complicated kinetics observed for single mutants. This method should provide a general approach for investigations of this important modification. [Letters, p. 769] *CG*

Pass the electrons, please



Incorporating enzymes into traditional catalytic methods provides the chance to improve specificity or catalytic efficiency, and to broaden the types of reactions that can be performed. To generate a 'bio'-heterogenous catalyst, Vincent *et al.* attached hydrogenase to a graphite particle. Following the oxidation of hydrogen gas, electrons were transferred through the graphite particle to enable enzyme-mediated reduction of either nitrate or fumarate. This approach can now be applied to other redox-enzyme pairs for applications in biocatalysis and nanotechnology. [**Brief Communication, p. 761**] *JK*

Sulfation run amok

Heparanase serves an important role in processing heparan sulfate (HS) chains, yet a recent study showed that overexpression of this enzyme results in only a mild phenotype in mice. To understand the mechanistic basis for this observation, Galvis *et al.* examined the structure of HS generated in transfected and tumor cells containing high levels of heparanase. Under these circumstances, the authors discovered that HS sulfation increases substantially, approaching the highly modified structure of heparin. These alterations additionally led to the formation of complexes of the modified HS sequences, fibroblast growth factors and their receptors, thereby providing a new link between HS biosynthesis and tumor development. [**Letters, p. 773**] *CG*