PRECISION EXCITATION OF FLUOROPHORES EXPANDS IMAGING PALETTE


Many researchers are accustomed to imaging cellular components with a limited palette of fluorescent dyes—typically red, green, and blue. Wei Min and colleagues have now reported the development of a refined imaging technique along with the concurrent synthesis of finely tuned dyes that brings the number of colors available for cellular imaging to an amazing 24 hues (Nature 2017, 544, 465–470).

The imaging technique begins with the traditional step of labeling target cellular components with fluorescent dyes. The cell sample is then exposed to two precisely defined laser pulses designed to stimulate the spectrally sharp vibration (which only occurs over a much narrower frequency range than that of fluorescence emission) of a specific chemical bond in one of the dyes in the sample, eliminating off-target excitation and significantly reducing background fluorescence. The dyes are excited one at a time to generate a series of images that can be overlaid to view intricate interactions between various cell structures.

Because the excitation technique precisely targets specific vibrational frequencies, which are inversely proportional to the square root of masses of each atom or functional group attached to the targeted bond, even dyes with identical molecular structure but containing different isotopes can produce distinct frequencies. Min and co-workers synthesized 14 xanthene analogs with various structures and isotopic patterns, but they predict that dozens more resolvable dyes could be developed.

Notably, the new dyes are relatively nontoxic and photo-stable, allowing the research team to perform proof-of-concept labeling experiments on live cells under normal cell culture conditions as well as in conditions designed to induce cell stress to mimic disease states. The authors expect that the technique could be further improved to allow simultaneous dye stimulation and visualization and predict that due to its sensitivity, resolution, labeling versatility, and biocompatibility, their approach will become widely applied to probing complex biological systems.

Heidi A. Dahlmann

ATP SOLUBILIZES PROTEINS UNDER PHYSIOLOGICAL CONDITIONS


Researchers investigating the physicochemical properties of adenosine triphosphate (ATP) have discovered that the biological molecule is capable of solubilizing hydrophobic proteins in aqueous solutions. Yamuna Krishnan and co-workers propose that this property is one possible reason why cells maintain a very high concentration of ATP in the cytoplasm (Science 2017, 356, 753–756).

ATP is most commonly recognized for its role in supporting protein function; hydrolysis of diphosphate (DPP) from its triphosphate side chain provides a burst of energy that fuels enzyme activity. Furthermore, ATP serves as a source of DPP for kinase proteins involved in cell signaling and is itself a building block for RNA. Thus, ATP is unquestionably one of the most critical biological molecules; however, each of these biological functions can be carried out in cells at micromolar concentrations. This begs the question, why does the cytoplasm maintain a millimolar-concentration ATP supply?

The research team knew that aqueous, high concentration protein samples tend to form aggregates, unlike in the cytoplasm, where proteins remain dissolved. So, they questioned whether ATP functions as a biological hydrotrope—a type of...
small molecule commonly used in industry to keep large quantities of insoluble molecules in solution, with a hydrophobic moiety that interacts with hydrophobic solutes and a polar side chain that interacts with polar solvent. The team demonstrated that the presence of ATP increased the water solubility of hydrophobic organic dyes such as fluorescein diacetate (FDA), then moved on to show that physiological concentrations of ATP prevented aggregation of proteins known to cause amyloids associated with Alzheimer’s disease and also dissolved previously formed protein aggregates. The authors propose that high concentrations of ATP help to keep proteins soluble in the cytoplasm, and they suggest that the decrease of ATP with age may facilitate protein aggregation and consequent age-related neurodegenerative decline.

Heidi A. Dahlmann

**SMALL MOLECULE SHUTTLES IRON ACROSS CELLULAR MEMBRANES**


Small-molecule chelators can act as shuttles to facilitate passive diffusion of iron ions across cell membranes with deficient ion-transport protein activity, as recently demonstrated by a research team led by Marianne Wessling-Resnick, Jonghan Kim, Barry H. Paw, and Martin D. Burke (*Science 2017*, 356, 608–616). The discovery may enable the development of drugs to treat certain types of anemia or to correct disorders stemming from harmful buildup of redox-reactive iron ions in susceptible tissues.

Ions are normally transported across membranes through active transport proteins that facilitate the buildup of ion concentration on one side of the membrane and passive transport proteins that release such gradients. When active ion transport proteins are functional but passive transport proteins are deficient, ions are no longer able to equilibrate across the membrane. Imbalanced iron transport can disrupt cellular functions and result in accumulation of iron ions to toxic concentrations.

To investigate whether ion-shuttling small molecules could compensate for missing or dysfunctional iron-transport proteins, the research team screened candidate compounds for their ability to restore growth to a strain of yeast lacking the iron-transporting complex FetFtr1 and identified hinokitiol, a known iron-chelating natural product isolated from the Taiwan hinoki tree. The team determined that hinokitiol also promoted iron movement across membranes of mammalian cells deficient in passive iron transporters DMT1, FPN1, and MFRN1, which are implicated in a variety of human diseases. Encouragingly, oral treatment with hinokitiol restored gut iron absorption in rats engineered to lack DMT1 and mice lacking FPN1, while hinokitiol supplementation in embryo media promoted hemoglobinization in zebrafish deficient in DMT1 or MFRN1. The authors note that their results support the pursuit of small molecules that compensate for the function of missing iron transporters for treating human diseases.

Heidi A. Dahlmann

**CHEMICAL CATALYSTS FOR CHROMATIN**


The nuclear genome is packaged into chromatin by histone proteins with access to the DNA regulated by the addition and subtraction of post-translational histone modifications. Specific histone residues are the sites of acetylation and methylation marks, and in many cases the modification status can be generally associated with transcriptional activity or repression. In the cell, histone acetyltransferases (HATs) are the catalytic engines which add acetyl groups to specific lysines using acetyl-CoA as the donor.

Amamoto et al. (*J. Am. Chem. Soc. 2017*, DOI: 10.1021/jacs.7b02138) circumvent the need for these biological catalysts by synthesizing a chemical catalyst poised for site-specific histone acylation. While HATs achieve their catalytic power by tweaking the lysine’s reactivity, the researchers instead chose a catalyst that could alter the other player in the reaction, acetyl-CoA, via thiol–thioester exchange. To deliver an acetyl group to a lysine of interest, the catalyst was coupled to one of two ligands: LANA, a DNA-binding peptide encoded by a herpesvirus, or PIP, a synthetic DNA-binding molecule with mild sequence specificity. The two resulting ligand-catalysts installed acetyl groups on specific yet distinct lysine residues. The LANA catalyst always delivered the modification to H2B K120, but the PIP catalyst modified different H3 or H4 lysines depending on the DNA sequence context. Since CoA is activated by the catalyst, other acyl groups could substitute for the acetyl moeity, making site-specific incorporation of non-natural lysine modifications simple and efficient. The researchers exploited this property to perform histone malonylation, a recently discovered *in vivo* phenomenon with unknown function. The new catalysts functioned both on recombinant nucleosomes and within intact nuclei, showcasing a new chemical modification tool to investigate the intricacies of chromatin biology.

Jason G. Underwood

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Bio-orthogonal reporters provide efficient strategies for labeling and following biomolecules within their cellular environments. However, finding the right chemistry for intracellular labeling is still difficult. Ideally, the chemistry needs to bypass unintended reactions with cysteines and other nucleophiles, and the non-natural moieties need to be small enough to be incorporated using cellular enzymes. Now, Row et al. report a new bioorthogonal strategy that incorporates cyclopropenones into proteins, which can then be labeled with phosphines (J. Am. Chem. Soc. 2017, DOI: 10.1021/jacs.7b03010).

Jennifer Prescher’s group at University of California, Irvine had previously used a strategy that employs cyclopropenones and phosphines to label biomolecules in solution. To optimize their system for use in living cells, the team first needed to tune the chemical reactivity of the components. Cyclopropenones can react with nucleophiles such as cysteine, so the team screened a variety of substituted scaffolds until they found a dialkyl combination that did not react with cysteine or glutathione at 37 °C. They then studied its reactivity with a panel of phosphine-decorated nucleophiles.

To produce a noncanonical amino acid, they appended the disubstituted cyclopropenone onto the ε-nitrogen of lysine. They then screened for the appropriate tRNA synthetase to incorporate that amino acid. They then demonstrated that they could install the modified amino acid at a single site in a modified green fluorescent protein (GFP) using an amber codon. Finally, they demonstrated the ligation of this labeled GFP to a variety of phosphines in complex mixtures, including phosphine–biotin. The reactions were quick and efficient, occurring within 1 h even in complex biological mixtures.

These results expand the chemical toolbox for biorthogonal labeling experiments. In addition, these cyclopropenone–phosphine reactions produce esters that can serve as substrates for the Staudinger reaction with azides. This traceless reaction is already recognized as an important biological labeling reaction. As a result, cyclopropenone–phosphine labeling could facilitate two-step labeling experiments within living cells.

Sarah A. Webb