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Spotlights on Recent *JACS* Publications

DESIGNING DESIGNER DNA

Andreas Marx and co-workers use a method called X-ray crystallography to determine the 3-D structures of six different modified DNA nucleotides interacting with a DNA polymerase enzyme (DOI: 10.1021/ja3017889).

DNA—deoxyribonucleic acid, the genetic material for most life on Earth—is a biopolymer made up of nucleotide building blocks, and the enzyme called DNA polymerase is responsible for connecting each nucleotide to its neighbor. Although natural DNA is composed of only four distinct nucleotides, many biotechnology applications call for nucleotides with slightly altered structures. For example, fluorescently modified nucleotides make DNA sequencing possible.

Further advances in DNA-based technologies rely on the design and development of new modified nucleotides; however, to be useful, the modified nucleotides must still be able to interact with DNA polymerase, which evolved to accommodate unmodified nucleotides. To inform the design process, detailed structural information regarding how the modified nucleotides interact with DNA polymerase is needed. The 3-D structures described by the researchers provide insight into how the DNA polymerase works, as well as acceptable types of nucleotide modifications. These details will support the informed design of other modified nucleotides and possibly facilitate the engineering of altered DNA polymerases for further biotechnology applications. **Eva J. Gordon, Ph.D.**

NEW PROBE SELECTIVELY PICKS OUT IMPORTANT BIOTHIOL

Biological molecules containing sulfhydryl groups participate in numerous cellular processes, such as redox reactions, metabolism, and detoxification. Glutathione (GSH) is a thiol that is the most abundant sulfhydryl-containing molecule in mammalian cells. GSH can be easily oxidized into a dimer, GSSG, in response to oxidative stress. Intracellular GSH concentration is an important measure of the overall health of a cell and its ability to withstand oxidative damage.

Despite GSH's importance, there is not an imaging agent or probe to specifically and sensitively detect it. Brian K. McMahon and Thorfinnur Gunnlaugsson have now developed a new probe called 1.Tb (DOI: 10.1021/ja300887k). The probe has the lanthanide element, terbium, which luminesces under the right conditions. The terbium is embedded in a cyclical molecule connected to a maleimide group, which readily reacts with thiols.

In the absence of GSH or in the presence of GSSH, 1.Tb does not luminesce. But when free GSH is present, the maleimide group reacts with it and causes the emission from the probe to dramatically rise. The investigators demonstrated that with 1.Tb, they can monitor the conversion of GSSH to GSH by the enzyme glutathione reductase and the reducing agent NADPH in real time with high sensitivity. **Rajendrani Mukhopadhyay, Ph.D.**

ENZYME-FREE METHOD FOR SENSITIVE DNA DETECTION

Itamar Willner and colleagues have developed a new enzyme-free biocatalytic method for the amplified detection of the Tay-Sachs genetic disorder gene (DOI: 10.1021/ja3037838). The researchers use catalytic DNA, known as a DNAzyme, to detect this specific genetic sequence.

Since the introduction of DNAzymes to the chemical biology toolkit, researchers have exploited their unique capabilities for applications ranging from medical diagnostics to sensing platforms for homeland security and forensics. In an effort to develop a DNAzyme-based DNA amplification method, Willner's group looked to the Zn^{2+} ligation DNAzyme, which was originally developed by Szostak and co-workers (*Nature* **1995**, *375*, 611−614).

The readout for their assay depends on the opening of a DNA hairpin that is labeled with both a fluorophore and quencher, resulting in an increase in fluorescence signal that is correlated with the concentration of targeted DNA. The team demonstrated improved assay sensitivity by incorporating "helper" DNA strands and an enzyme, but this enzyme introduces cost and stability concerns. The researchers further modified the strategy by incorporating a second DNAzyme instead, resulting in detection of picomolar levels of DNA in an enzyme-free assay and demonstrating comparable or improved sensitivities to other DNA detection strategies. Finally, the team applied this technique to detect the Tay-Sachs mutant gene to low nanomolar levels. **Christine Herman, Ph.D.**

NOVEL THERAGNOSTIC COUPLES TARGETED CYTOTOXICITY WITH MOLECULAR IMAGING

In military press conferences, "smart bomb" strikes are often documented using video footage. Now cancer researchers can acquire similar data with their own molecular smart bombs, thanks to a novel drug-delivery system (DOI: 10.1021/ja303998y).

Chulhun Kang, Jong Seung Kim, and colleagues describe a tripartite "theragnostic" system comprising a targeting molecule (the bomb's guidance system—in this case, the cyclic Arg-Gly-Asp peptide that binds to tumor cell integrin receptors) and a fluorescent reporter (napthalimide), coupled to an anti-cancer "payload" (camptothecin) by a labile disulfide bond. In vitro, incubation with glutathione, a reducing agent prevalent in cancer cells, induces release of the molecular payload while altering the fluorescent properties of the reporter, red-shifting its emission from 473 to 535 nm.

In cultured cells, this theragnostic molecule is taken up via integrin-mediated endocytosis and works its way to the endoplasmic reticulum—events that can be monitored via confocal imaging. Release of camptothecin results in enhanced killing of targeted cells, with about half of cells expressing the integrin receptor dying within 48 h, compared to a quarter of cells that do not.

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Fluorescence-based monitoring of cellular uptake could allow for more precise control of dosage levels, as well as an improved understanding of the mechanism of action. **Jeffrey M. Perkel**

TUNING STRUCTURE–ACTIVITY RELATIONSHIPS FOR COLOR VISION

The transmembrane protein rhodopsin—which has a known crystal structure—mediates dim-light vision, while cone pigments mediate trichromate color vision. Because cone pigments all use the same retinal cofactor, their different amino acid sequences are responsible for the red, green, or blue maximum absorption (λ_{max}). No cone pigment crystal structure has yet been solved, and therefore the precise effect of amino acid substitutions can be difficult to predict.

Sivakumar Sekharan, Keiji Morokuma, and co-workers used computations to determine the spectral effect of placing a hydroxyl group at various spots in the protein active site (DOI: 10.1021/ja304820p). Starting with a single pigment amino acid sequence, the researchers found that substituting an OH-bearing amino acid at one of three positions can tune the sensitivity of the pigment from green to red. This finding shows that spectral tuning depends on specific amino acids in the active site, and not on the identity of the pigment itself.

Red-green color blindness arises from the mistuning of the red and green cone pigments, and up to 1 in 12 men have some degree of color-blindness. The authors conclude that "a critical first step toward understanding the origin of color blindness is to determine the molecular basis of variance in the λ_{max} of red and green pigments." **Sonja Krane, Ph.D.**

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