JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Spotlights
pubs.acs.org/JACS

Spotlights on Recent *JACS* Publications

RESEARCHERS EXTEND GENETIC ALPHABET WITH UNNATURAL BASE PAIR

Every organism on Earth, from bacteria to humans, shares the same four-letter genetic alphabet: A, C, G, T. Extending that alphabet with unnatural base pairs has synthetic biology and biotechnology applications, but getting polymerases to use those bases remains a challenge. Now Floyd Romesberg and colleagues report a novel base pair that is incorporated into DNA with near wild-type efficiency (DOI: 10.1021/ja408814g).

Romesberg previously described one unnatural base pair, d**5SICS**-d**NaM**, which forms via hydrophobic rather than hydrogen-bonding interactions. Here, the team optimizes that pair's performance by producing and testing a series of d5SICS variants. One pair, d**TPT3**-d**NaM**, can be PCR amplified "with an efficiency that is only 4-fold lower than that of DNA containing just the natural base pairs, and with a fidelity [retention of the unnatural base] in excess of 99.98%."

Fluorinated and propargyl amine variants of d**TPT3** also are efficiently incorporated and extended. The latter, d**TPT3**^{PA}, provides a chemical handle for attaching biotin or other molecules to newly synthesized DNA, which the team detects using streptavidin.

"The identification of d**TPT3**-d**NaM** represents a milestone in our effort to expand the genetic alphabet," the authors conclude.

Jeffrey M. Perkel

EFFICIENT PYROPHOSPHOPEPTIDE SYNTHESIS

Phosphorylation is a well-characterized post-translational protein modification. Less well studied is pyrophosphorylation, in which a phosphate group from inositol pyrophosphate is transferred to an existing phosphoserine residue. Now Dorothea Fiedler and colleagues describe a method to chemically synthesize pyrophosphorylated peptides with high efficiency (DOI: 10.1021/ja411737c).

The team's method couples a phosphate moiety to synthetic peptides already containing a single phosphorylated serine residue. The team tests three different phosphate donors. Both phosphoramidite and phosphoryl chloride donors work, but the phosphorimidazolide is most efficient in both aqueous and organic solvents in the presence of reactive amino acid side chains, including lysine, histidine, aspartic acid, serine, and cysteine.

The authors showcase their method to selectively pyrophosphorylate a densely functionalized 25 amino acid peptide containing five lysines, seven serines or threonines, eight acidic residues, and one phosphoserine. They envision that applications of these peptides include the development of mass spectrometry techniques and antibody production for detection of *in vivo* pyrophosphorylated proteins. The authors explain, "In all, the accessibility of pyrophosphopeptides by the reported methodology provides the necessary gateway for entering this emerging area of signal transduction research."

Jeffrey M. Perkel

INSIGHTS INTO RIBOZYMES' MECHANISMS OF CATALYSIS

Sharon Hammes-Schiffer, Philip C. Bevilacqua, and co-workers make an important contribution to the field of RNA catalysis by elucidating the mechanism of the hepatitis delta virus (HDV) ribozyme through computational and experimental studies (DOI: 10.1021/ja4104217). Ribozymes are RNA molecules that, similar to protein enzymes, are capable of catalyzing specific biochemical reactions. The HDV ribozyme is a nucleolytic ribozyme that performs site-specific cleavage in RNA using both nucleobases and metal ions. The role of the metal ions in the catalytic reactions by HDV is the focus of these studies.

Using quantum mechanical calculations combined with free energy simulations, the researchers generate a map of the free energy surface for the reaction. They observe that HDV performs its site-specific cleavage reaction in RNA with both divalent ions, such as Mg^{2+}, and monovalent ions, Na^{+}; the reaction with magnesium ion proceeds through a different mechanism that results in a faster reaction. The authors further confirm the role of the catalytic magnesium ion through kinetic and NMR measurements, thereby broadening understanding of the functional role that metal ions have in this cleavage reaction.

Dalia Yablon, Ph.D.

SINGLET OXYGEN PRODUCED AT THE FLIP OF A SWITCH

Singlet oxygen is a damage-inducing radical, which is bad news for healthy cells but good news for cancer cells—or at least for the doctors who want to kill the cancerous cells. In photodynamic therapy (PDT), light is used to create singlet oxygen radicals, which chemically oxidize—and destroy—the diseased cells. The success of PDT relies on controlling both the location and timing of singlet oxygen production.

In order to have this kind of spatiotemporal control, doctors need a reagent that allows them to turn singlet oxygen production on and off in a noninvasive way. In a new report, researchers led by Ben Feringa and Wesley Brown present a significant step toward this goal with the development of a bi-component system that controls singlet oxygen production with the flip of a switch (DOI: 10.1021/ja4122473).

In its inactive state, the colorless diarylethene-based switch does not interact with the photosensitizer or affect its ability to produce singlet oxygen. When the team shines UV light on the system, the switch converts to its active (colored) state, quenching the photosensitizer, and shutting down production of singlet oxygen. Singlet oxygen radicals can be produced again by illumination with visible light. Future work will involve developing a water-soluble version of the switch that could be compatible with PDT in living organisms, the researchers say.

Christine Herman, Ph.D.

Published: January 22, 2014

dx.doi.org/10.1021/ja5003586 | *J. Am. Chem. Soc.* 2014, 136, 821–821