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Spotlights on Recent *JACS* Publications

ANTIFERROCENE ANTIBODY OFFERS NON-RADIOACTIVE KINASE ASSAYS

Protein kinases are enzymes that modify other proteins by phosphorylation. They are key mediators of cell signaling pathways and are implicated in development and disease. As such, they are popular drug development and research targets. Kinase assays typically require either radioactivity or phosphor-specific antibodies. Heinz-Bernhard Kraatz and colleagues describe a non-radioactive alternative that can detect phosphorylation electrochemically and via immunoassay (DOI: 10.1021/ja302586q).

In previous work, Kraatz described a novel adenosine triphosphate analogue, 5′-γ-ferrocenyl-ATP (Fc-ATP). In kinase reactions, this analogue transfers its ferrocene-coupled terminal phosphate to the enzyme's substrate, an event that can be detected electrochemically on a gold surface. In the present study, the team describes alternative detection methods using an anti-ferrocene antibody (Fc-Ab1), which, with a range of assays, was demonstrated to be specific for its ferrocene substrate in multiple protein contexts (e.g., on both phosphoserine and phosphotyrosine), matrices (i.e., crude cell extracts), and test formats. The results provide complementary information to the Fc-ATP electrochemical studies for probing phosphorylation of peptides and proteins in solution and on solid surfaces.

"As such, the Fc-Ab1 may likely be useful as a biosensor component for peptidomic, genomic, and pharmacogenomic analysis," the authors conclude. **Jeffrey M. Perkel**

THEORETICAL STUDIES REVEAL METALLOENZYME CATALYSIS OCCURS IN UNEXPECTED SITE

Inspired by metalloenzymes in nature, researchers strive to create artificial metalloenzymes composed of proteins that house a catalytic metal complex in their "womb". The goal is to achieve novel catalytic functions by combining the best of both worlds: the broad substrate scope and accessibility of metal complexes and the high efficiency and selectivity of enzymes. In addition to the numerous challenges inherent with the design of artificial metalloenzymes, researchers have found it difficult to characterize the structural details of the resulting complexes. However, a detailed understanding of the active site and reaction mechanism is important for the rational design of artificial metalloenzymes.

Researchers led by Keiji Morokuma performed theoretical studies to determine the binding site of a phenylacetylene substrate within a rhodium complex encapsulated in the protein *apo*-Ferritin (DOI: 10.1021/ja305453w). The QM/MM studies revealed that the most plausible active site is different from the sites that were suggested by the previously reported X-ray crystal structure. The results from this study highlight the important contribution of theoretical studies in the mechanistic elucidation of artificial metalloenzymes. **Christine Herman, Ph.D.**

PROTEIN FOLDING GETS DOSE OF REALITY

Unfolded proteins are floppy relative to their folded counterparts. Loss of entropy means that the shift from fluid to folded comes at an energetic cost. Calculating that cost accurately is critical to understand the balance of forces that influence protein folding, but estimates for this energy cost vary widely.

One reason for the discrepancies may be that scientists are using inaccurate models, either assuming that folded proteins are entirely immobile or that unfolded proteins have practically limitless mobility, or both. In this study, Karl Freed, Tobin Sosnick, and colleagues attempt to generate realistic virtual models of folded and unfolded proteins that may yield a more accurate assessment of the loss in entropy upon folding (DOI: 10.1021/ja3064028).

The researchers focused their efforts on ubiquitin, though their approach should be applicable to any protein. They simulated the behavior of ubiquitin in the unfolded state based, in part, on disordered regions in protein crystal structures. Their model is consistent with experimental studies and generally presents a somewhat less dynamic picture of unfolded proteins than is typical. The researchers also simulated folded ubiquitin with motions appropriate to its structure. They then calculated the difference in entropy between this and their unfolded simulation, finding that less entropy is lost upon folding than has been estimated by most previous studies. **Erika Gebel, Ph.D.**

A SWEET PERSPECTIVE ON PROTEIN GLYCOSYLATION

Immediately after their synthesis inside the cell, many proteins are decorated with carbohydrate molecules in a process called glycosylation. The carbohydrates are important for various aspects of protein activity, such as helping them adopt the proper three-dimensional structure and guiding their localization inside the cell. Notably, the misregulation of protein glycosylation has been implicated in a variety of diseases including diabetes, cancer, and neurodegenerative disorders. An enzyme called OGT is responsible for adding the carbohydrate *N*-acetylglucosamine to proteins, and a detailed understanding of the mechanism behind this process could lead to new strategies for targeting these medical conditions.

To gain insight into precisely how OGT installs *N*-acetylglucosamine onto proteins, Igor Tvaroška and co-workers employ a sophisticated computational molecular modeling approach, using quantum mechanics and molecular mechanics methods, which enables investigation of the mechanistic and energetic properties associated with OGT activity (DOI: 10.1021/ja307040m). They provide compelling evidence that the carbohydrate itself assists in the reaction, a mechanism which has not been previously observed with enzymes of this type. These findings may offer valuable clues toward the design of novel drugs targeting OGT activity. **Eva J. Gordon, Ph.D.**

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SCANNING HUMAN SOLUBLE CALCIUM-ACTIVATED NUCLEOTIDASES, COMPUTATIONALLY

Enzymes called calcium-activated nucleotidases are responsible for modulating extracellular levels of nucleotides, small biomolecules that convey important messages to cells. Originally identified in blood-sucking insects to prevent blood clotting in their prey, the human version of these enzymes—human soluble calcium-activated nucleotidases (hSCANs)—may have therapeutic potential for the treatment of blood clotting disorders.

hSCANs are unusual compared to other enzymes that perform similar biochemical transformations in that they rely on calcium to function properly, and they interact with phosphate, which is a key portion of their substrate, in an unique way. To gain a deeper understanding of the mechanism of hSCANs, Yingkai Zhang and co-workers employ cutting-edge computational methods to simulate how the enzyme functions (DOI: 10.1021/ja307267y). They identify a previously uncharacterized site in the enzyme that binds to calcium, illuminating how the metal shapes the active site to support nucleotide binding and assists the chemical reaction. Their computational analysis supports existing experimental data and offers valuable insight into how the enzyme functions, carving a path toward the design of engineered hSCAN enzymes with therapeutic applications. **Eva J. Gordon, Ph.D.**

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