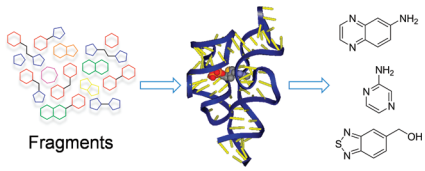
In this ISSUE

Making the Switch to RNA

Methods to find small molecules that bind diverse drug targets such as enzymes and RNA are a key part of the drug discovery process, and also provide molecular tools for exploring biological processes. Fragment-based screening methods, which involve the screening of low molecular weight structural motifs, have found considerable success in identifying molecular fragments that bind to proteins. This approach, however, is just beginning to be applied to finding small molecule partners for RNA. To this end, Chen *et al.* (DOI: 10.1021/cb9003139) now describe a fragment-based approach for identifying small molecules that bind to the *Escherichia coli* riboswitch *thiM*.

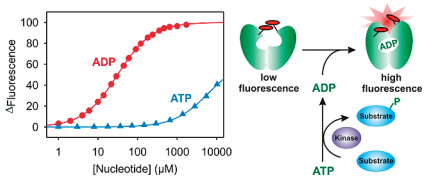
Integral to their approach was the integration of several techniques for characterizing fragment-riboswitch binding events. To initiate the search, equilibrium dialysis was used to screen approximately 1300 compounds for binding to *thiM*. Hits were subsequently confirmed using NMR spectroscopy methods, further characterized using isothermal calorimetry, and finally screened in another round of equilibrium dialysis to eliminate generic RNA binders. Several leads for development into *thiM* modulators were identified using this process.



Quenching the Thirst for ADP Detection

Adenosine diphosphate (ADP) is a product of two vitally important enzymatic reactions, protein phosphorylation and the hydrolysis of adenosine triphosphate (ATP). Thus, methods to detect ADP have general applications in the investigation of these processes, as well as in the search for drugs that target their corresponding enzymes, kinases and ATPases. Inspired by a strategy used to monitor cleavage of small peptides by proteases, Kunzelmann and Webb (DOI: 10.1021/cb9003173) now report creation of an ADP biosensor comprised of the bacterial actin homologue ParM labeled with two molecules of tetramethylrhodamine.

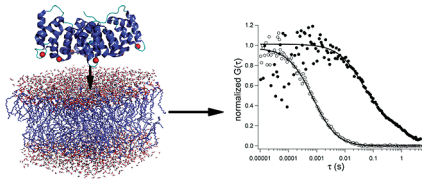
The approach exploits the large difference in rhodamine fluorescence depending on whether the fluorophore is monomeric and highly fluorescent, which occurs when ADP is bound, *versus* when the fluorophore is in a stacked state and undergoes quenching, which is the case in the absence of ADP. The sensor is highly sensitive to ADP binding, can be used at low concentrations relative to ADP, and enables detection of ADP even in the presence of high concentrations of ATP. The utility of the sensor was demonstrated by monitoring ADP generation in two distinct phosphorylation reactions.



Probing the Protein—Lipid Relationship

Cell membranes are the gatekeepers of the cell, controlling what goes in and what comes out, which has profound influences on numerous cellular processes. Made up primarily of lipids and proteins, the organization and relative composition of cell membranes is dynamic and depends on a variety of factors, both chemical and structural in nature. Using the peripheral intracellular protein annexin a5 and biomimetic membranes, Vats *et al.* (DOI: 10.1021/cb900303s) investigate the structural and functional nature of the protein—lipid relationship in membranes.

Fluorescence imaging and correlation spectroscopy were used to characterize the binding of annexin a5 to phospholipid analogues. It was found that binding of annexin to membranes leads to formation of annexin assemblies, as well as a dispersed population of the protein. In turn, annexin binding affects the lateral movement of lipids within the membrane, the extent of which is dependent on lipid structure. The findings suggest that protein binding to membranes purposefully influences lipid structure and organization, which potentially facilitates distinct aspects of protein function.



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