conjugation of a lipophilic moiety to the protein might interfere with its conformation and render co-crystallization and solubility difficult to control, it is likely that the success of this approach will be determined on a case-by-case basis. Other sophisticated drug delivery technologies, including hydrogels, liposomes and microspheres, provide a matrix from which a drug can be released in a controlled fashion. However, these complicated formulations often reduce the efficacy of the therapeutic protein or render mass production of the drug difficult. Tweaking the drug itself, by altering the composition of the protein crystal lattice without losing efficacy, might be a simpler and more effective alternative.

Melanie Brazil

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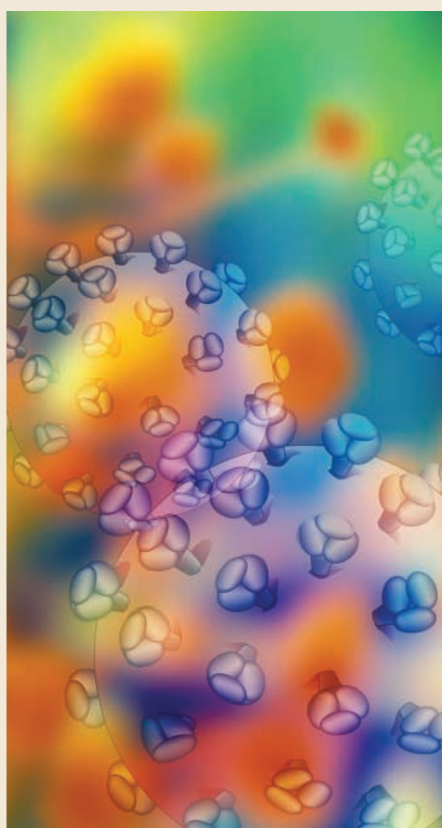

HIGH-THROUGHPUT SCREENING

Lighting up targets with reporter ribozymes

Most methods to monitor interactions between biological macromolecules, and to identify compounds that can disrupt these interactions, need one of the components to be labelled, or are not amenable to real-time detection, hindering their application in large-scale parallel screening. Now, by combining two classes of oligonucleotides — known as ribozymes and aptamers — Hartig *et al.* have developed a technology called 'reporter ribozymes', which can monitor macromolecular interactions in real time, and could potentially be compatible with the parallel screening of large compound or protein libraries.

Ribozymes are RNA molecules that can catalyse simple nucleic-acid-based reactions, such as RNA cleavage or ligation. RNA cleavage can be detected by attaching two tags to a substrate RNA oligonucleotide that is bound to the ribozyme — a fluorophore and a quencher that suppresses the fluorescence signal when close to the fluorophore. So, when the ribozyme catalyses the cleavage of the bound oligonucleotide, the fluorophore and the quencher are separated, which results in a fluorescence signal.

How can this cleavage be used to report protein interactions? This is where the aptamers — single-stranded nucleic acids that can fold into intricate globular structures capable of binding proteins with high potency and specificity — come to the fore. Linking the ribozyme to a protein-binding aptamer in such a way that the ribozyme cleavage reaction is dependent on whether the aptamer is bound to the protein or not allows the interaction between the protein and the aptamer to be monitored from the fluorescence signal due to the ribozyme activity. Other interactions of the protein — for example, with a small molecule — will influence the interaction between the protein and the aptamer, and so be detectable by a change in the fluorescence signal.

The authors explored several formats for this technology. In the first format, they coupled a naturally occuring aptamer to the HIV-1 Rev protein to a ribozyme in such a way that binding of Rev to the aptamer inactivated ribozyme cleavage, inhibiting the fluorescence signal. By contrast, in the second format, a Rev-binding aptamer that had been created previously was coupled to a ribozyme, such that binding of Rev would allow ribozyme cleavage, resulting in a

fluorescence signal. Both formats were used to screen a 96-member library of antibiotics for molecules that could disrupt the binding of Rev to its aptamer, and both identifed three compounds as hits, one of which was subsequently shown to inhibit HIV replication, indicating that the protocol can generate drug leads.

To illustrate the potential for monitoring protein–protein interactions, the authors developed a third format, using an aptamer to α-thrombin that prevents cleavage of the ribozyme unless α-thrombin is bound. The decrease in fluorescence signal on addition of a protein inhibitor to α-thrombin, which disrupts the the ribozyme–α-thrombin interaction, could report the inhibitor–α-thrombin interaction in a concentration-dependent manner.

The third format is potentially compatible with high-throughput screens for small molecules that act as superior thrombin inhibitors. Moreover, in this case, the aptamer could be swapped for other aptamers to alternative protein targets. As approaches are being developed to create such aptamers for a broad range of proteins efficiently — and importantly, with the ability to selectively target specific domains, such as the protein active site — it seems that reporter ribozymes based on aptamers are likely to become increasingly useful as tools for drug screening.

Peter Kirkpatrick

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