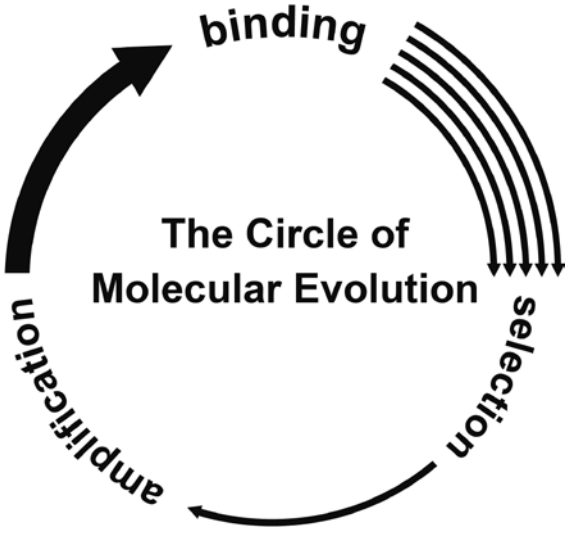
DNA Probe Specifically Binding Protein Heterodimer Rather Than Monomers

In a recent effort hunting for cancer biomarkers, researchers from the Institute of Chemistry, Chinese Academy of Science (ICCAS) identified a new DNA aptamer (termed BG2) that only binds to alkaline phosphatase (AP) heterodimer rather than its monomers. This newly-identified DNA aptamer, representing as the first molecular probe reported capable of recognizing a protein dimer, could serve as an important tool to uncover the mystery of this particular protein dimer in a biological context. This study was published in *Advanced Science* on April 9.

DNA aptamers are single-stranded DNA stands that specifically bind to targets of interest. They are functional mimics of antibodies, a molecular chain consisting of four different nucleotides instead of 20 different amino acids for antibodies. This BG2 aptamer in this report was generated using a technique called Cell-SELEX (Systematic Evolution of Ligands by EXponential enrichment).

A typical SELEX procedure for aptamer development involves three main steps – binding, selection and amplification – that form the circle of molecular evolution. Starting with a pool of random DNA sequences, experimenters could impose certain selection pressure upon the binding step to remove the nonspecific binding and weak binding DNA sequences. Only a few that survive the selection step would earn their chance to multiply into large numbers and re-enter the arena of molecular evolution for the next round. By repeating this binding-selection-amplification circle, "the winners" will gradually dominate the DNA pool and hence can be readily picked up after sequencing the enriched pool in later rounds.

By sequencing the enrich DNA pool of the fifth round, researchers identified the BG2 aptamer, which was, out of fluke, turned out to be the first reported molecular probe for the direct detection of protein dimers.

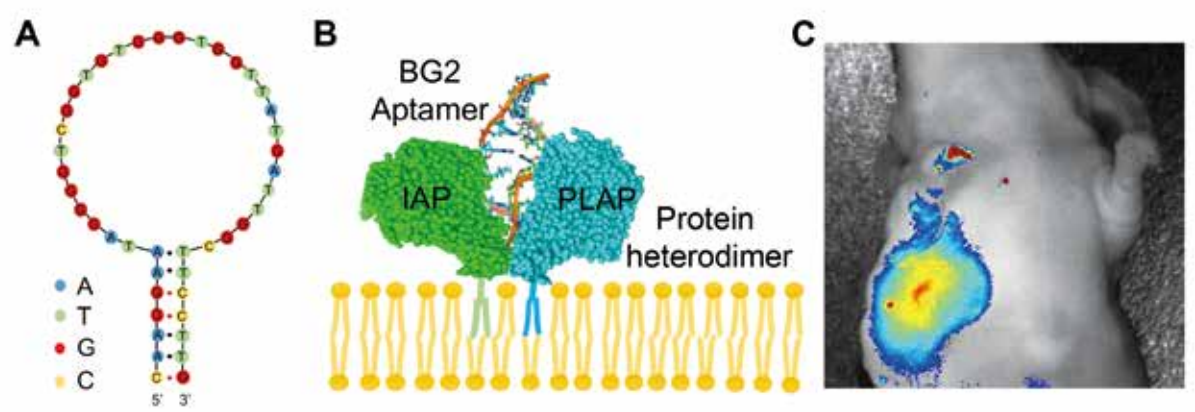


The circle of molecular evolution for aptamer development contains binding, selection and amplification. The thickness of the arrow to some extent stands for the abundance of DNA sequences (Credit: YAN Fusheng).

Protein dimerization – two proteins come together driven by the non-covalent forces such as hydrogen bonding and hydrophobic interaction – occurs frequently and plays indispensable roles in many biological events. However, no single molecular probe is available for the *in situ* detection of protein dimers on cells or tissues. This is mainly because of the great challenge in isolating complete protein dimers for probe development, which has also greatly hampered the biomedical study of protein dimers.

In this current case, the cell-SELEX strategy circumvented the need to isolate protein dimers and allowed the dimers to be present on the surface of live cells in their active state, which made the discovery of aptamers specifically binding protein dimers possible.

Probing cells with this BG2 aptamer, researchers found that AP heterodimer, as the target of BG2, was



A short DNA aptamer, termed BG2 (A), was turned out to be the first molecular probe for protein dimers, which specifically binds to AP heterodimer at the interface between two interacting monomers (B). When labeled with a fluorophore, BG2 was also demonstrated to be applicable for tumor imaging in mice (C). (Credit: Prof. SHANGGUAN Dihua, ICCAS)

upregulated on several different cancer cell lines, suggesting the potential of using AP heterodimer as a cancer biomarker. Actually, researchers chose HeLa cells as target cells for aptamer selection to ensure that the evolved DNA aptamers are likely to bind to potential cancer biomarkers. Hence, upregulated levels of AP heterodimer on cancer cells are somewhat expectable.

Then, they further demonstrated that BG2 could be used to isolate AP heterodimers from cell lysate and to illuminate the xenografted cells highly expressing AP heterodimers in mice when carrying a fluorophore. Currently, the researchers are seeking to confirm the feasibility of using AP heterodimers as a novel cancer biomarker.

This study presents a plausible way for developing molecular probes to directly detect protein dimers on cells or tissues. But it is not a guarantee for success. To make a fruitful outcome and further promote the study of protein dimerization in biological contexts, a more applicable and efficient strategy to develop such probes is still demanded.

(By YAN Fusheng)

Reference

Tao Bing *et al.*, Aptameric Probe Specifically Binding Protein Heterodimer Rather Than Monomers. *Advanced Science* 6, 1900143 (Published: April 9, 2019). doi: 10.1002/advs.201900143.