

## **One-step aptamer selection using a non-fouling porous hydrogel**

Aptamers, commonly referred to as chemical antibodies, are used in a wide range of applications including drug delivery and biosensing. However, the process of aptamer selection poses a significant challenge, as it requires numerous cycles of enrichment and involves issues with nonspecific binding. We present a simple, fast instrument-free method for aptamer enrichment and selection based on a diffusion-binding process in a three-dimensional non-fouling porous hydrogel with immobilized target proteins. Low-affinity aptamer candidates can be rapidly released from the hydrogel, whereas high-affinity candidates are restricted due to their strong binding to the immobilized protein targets. Consequently, a one-step enriched aptamer pool can strongly bind the protein targets. This enrichment is consistent across five proteins with isoelectric points in varying ranges. With thrombin as a representative model, the anti-thrombin aptamer identified from an enriched aptamer pool has been found to have a binding affinity that is comparable to those identified over ten cycles of selection using traditional methods