

## SINGLE MOLECULE

## Touch-and-go sensing

A protein nanopore sensor detects transient protein–protein interactions at single-molecule resolution.

Isothermal calorimetry, surface plasmon resonance, and biolayer interferometry are routinely used methods for the analysis of protein–protein interactions (PPIs). However, these techniques yield only average values for the affinity and kinetic parameters and are not suitable for heterogeneous samples.

Avinash K. Thakur and Liviu Movileanu from Syracuse University engineered a protein nanopore sensor that allows the real-time detection of transient PPIs at single-molecule resolution, even in complex biological fluids. The nanopore sensor consists of a  $\beta$ -barrel pore scaffold linked to a protein receptor. PPIs occur outside the pore in the aqueous phase and are detected as conductance changes in single-channel electrical recordings using planar lipid bilayers.

The authors used catalytically inactive RNase barnase (Bn) as a protein receptor and probed the binding to its inhibitor barstar (Bs). Bn was fused to the N terminus of a truncated ferric hydroxamate uptake component A (t-FhuA) construct. A 12-residue-long adaptor was added to the N terminus of Bn. The peptide adaptor is likely to interact with the t-FhuA pore entrance, which decreases the conductance of the pore, and when Bs binds to Bn this obstruction is released. When the authors added Bs to the chamber, they observed reversible current transitions between a higher-current-amplitude open substrate ( $O_{\text{off}}$ ), reflecting Bs–Bn binding events, and a lower-current-amplitude open substrate ( $O_{\text{on}}$ ), corresponding to a release of Bs by Bn. The association and dissociation rate constants can be derived from the duration of the  $O_{\text{off}}$

and  $O_{\text{on}}$  states. The dissociation constant  $K_d$  for Bs–Bn binding is 64 nM. The D39A Bs mutant has a  $K_d$  of 146  $\mu$ M. Importantly, binding of wild-type and mutant Bs can be measured simultaneously, and measurements can also be done in mammalian serum.

Protein nanopore sensors could be incorporated into microfluidic devices and might be useful for diagnostic applications and high-throughput compound screening.

Karin Kuehnel

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Research papers

Thakur, A. K. & Movileanu, L. Real-time measurement of protein–protein interactions at single-molecule resolution using a biological nanopore. *Nat. Biotechnol.* **37**, 96–101 (2019).



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