PROBE DYNAMICS IN AN ALPHA-HEMOLYSIN-BASED SENSOR

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We have developed an alpha-Hemolysin nanopore-based sensor for single-molecule spectroscopy experiments or specific biomolecule detection. Briefly, a single alpha-Hemolysin nanopore is allowed to assemble in a lipid bilayer separating two chambers. A molecule of interest is placed in the bottom (*trans*) chamber, while a DNA probe molecule, with either a hairpin loop or a biotin-Avidin complex at the 5' end is added to the top (*cis*) chamber (see figure 1). The sensor is assembled by electrophoretic insertion of the probe into the *cis*-side of the pore; the hairpin or Avidin at the 5' end prevents translocation through the pore. Hybridization of the probe to an analyte molecule on the *trans*-side of the pore traps the probe in place, and increases the time constant for exit on subsequent voltage reversal. Probe insertion and exit are observable as changes in electrical impedance of the pore. This design retains the high signal-to-noise ratio observed when the pore is used as a simple DNA detector; however, by appropriately designing the 3' end of the probe molecule, we are able to make the sensor specific for a given analyte.

We have previously demonstrated that the sensor is capable of distinguishing between 14-mer oligonucleotides differing by as little as a single base from one another [Ref 1]. More recently, we have focused on experiments to better understand both the limitations of the sensor, and the factors governing interaction between the probe molecule, the analyte and the pore and bilayer. A basic characteristic of the sensor is the relationship between detection speed and sensitivity; in 1M KCl, we have found that the sensor retains a signal-to-noise ratio greater than 25, even when sampled at speeds up to 10kHz.

To investigate interactions between the probe and the alpha-Hemolysin pore, we have performed experiments on probes of various designs (varying both the 5' end of the molecule and the nucleotide sequence that extends into the pore), and have been able to make estimates of a number of different physical properties of the sensor , including electric field geometry in the pore, the effective charge per nucleotide for single-stranded DNA in the pore, diffusion constant for probes held in the pore, and the contributions of the DNA and protein portions of the probe to observed probe/pore interactions. Based on these results, we present a refined probe design.

References

[1] Nakane J, Wiggin M, Marziali A. A nanosensor for transmembrane capture and identification of single nucleic acid molecules. *Biophys J* 87:615-621 (2004).

Figures

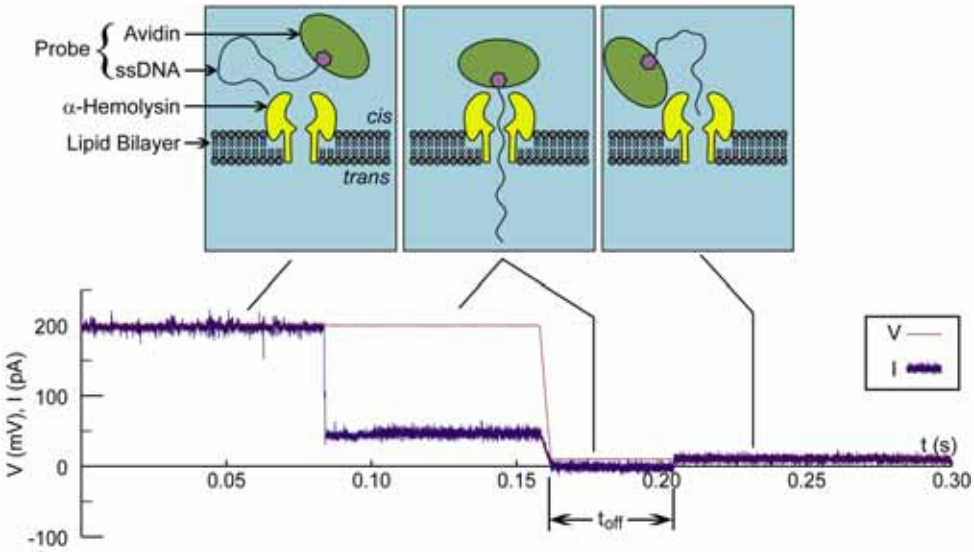

Figure 1: Animation with voltage and current traces for a probe-pore interaction experiment. A 200mV potential across the lipid bilayer captures a probe molecule in the pore, observed as a reduction in the current. The potential is then lowered to a level insufficient to hold the probe in the pore (in this case, 10mV) against diffusion. Probe exit causes an increase in current; the time required for exit is recorded as t_{off}. By observing many events, it is possible to determine the time-constant governing probe escape.