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Single-molecule stochastic sensors for proteins using engineered **nanopores**¹ LIVIU MOVILEANU, Syracuse University — We were able to design an unusual temperature-responsive pore-based nanostructure with a single movable elastin-like-polypeptide (ELP) loop. If a voltage bias was applied, the engineered pore exhibited transient current blockades, the nature of which depended on the length and sequence of the inserted ELP. These blockades are associated with the excursions of the ELP loop into the nanopore. At low temperatures, the ELP is fully expanded and blocks the pore completely, but reversibly. At high temperatures, the ELP is dehydrated and structurally collapsed, thus enabling a substantial ionic flow. Acidic binding sites comprised of negatively-charged aspartic acid residues, engineered within the pore lumen, produced dramatic changes in the functional properties of the nanopore, catalyzing the translocation of cationic polypeptides from one side of the membrane to the other. For example, when two electrostatic binding sites were introduced, at the entry and exit of the nanopore, both the rate constants of association and dissociation increased substantially, diminishing the free energy barrier for translocation.

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