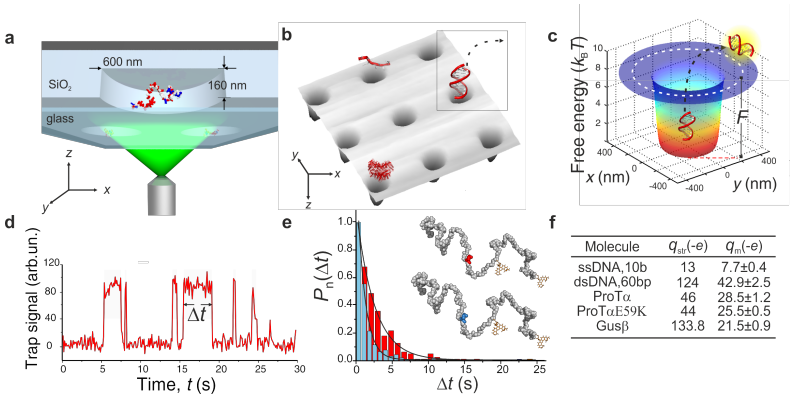
Single-molecule electrometry

F. Ruggeri[1], F. Zosel[1], B. Schuler[1], M. Krishnan[1]

[1]University of Zurich

Electrical charge is a fundamental property of biomolecules, strongly influencing their function [1] and stability. We demonstrate for the first time a high-precision (<1*e*), measurement of the electrical charge of biomolecules in solution. The method is based on parallel, external field-free trapping [2] at an unprecedentedly low estimated sensitivity of yoctomoles (1-10 molecules).
Our single molecule trap is created in a fluid-filled gap between two charged walls. Nanoscale patterning of one of the surfaces leads to a modulation of the local electrostatic potential, creating a deep thermodynamic potential well for a like-charged entity (Fig.a-b). In our new method "Escape Time Electrometry" (ET*e*) [3] we show for the first time stable trapping of biomolecules in solution, for tunable timescales ranging from hours to milliseconds. The depth of the well, F, experienced by a charged molecule is linearly proportional to its effective charge q_m (Fig.c). A molecule undergoing Brownian motion will reside in a trap for a time (t_{esc}) given by Kramer's analytical expression, $t_{esc}=t_r\exp(F/k_BT)$, where t_r is a relaxation time that depends on the diffusion coefficient of the molecule. When occupied by a fluorescently-labelled molecule the optical intensity of the trap region is high (Fig.d). The duration of the intensity bursts (Dt) follows an exponentially decaying probability distribution $P_n(Dt)$, which is fitted to extract t_{esc}, yielding information on the well depth and thus directly giving the effective charge q_m. Finally, q_m can be theoretically modelled and related to the molecule's known structural charge (q_{str}); the table in Fig.f summarizes a few representative results.



The measured charge of DNA molecules is in remarkable agreement with existing theoretical predictions [4] and suggest that ET*e* can serve to readout the inter-nucleotides spacing of a nucleic acid molecule or polyelectrolyte. The study of the enzyme Gusb suggests substantial regulation of the structural charge in a globular molecule [5], while our measurements on ProTa, a disordered one-dimensional polypeptide, provides unique insight into the charge renormalizing behavior of short, strongly charged segments within the molecule. Crucially, the exponential dependence of t_{esc} on the charge of the molecule permits us to distinguish between two ProTa variants that differ by a mutation of a single amino acid (E59K, 4% of the structural charge) (Fig.e). The Electrometry measurement can also be performed on a single molecule in real time, with the potential of detecting charge fluctuations, making ET*e* a new tool for ultrasensitive, rapid structural studies on biological macromolecules in the fluid phase.

[1] Perutz, *Science,* **1978,** 201:1187-1191. [2] Krishnan et al., *Nature,* **2010,** 467:692-695. [3] Ruggeri et al., 2017; *Nature Nanotechnology*, **2017,** 12:488-495 [4] Manning, *Journal of Chemical Physics,* **1969,** 51:924-& 492. [5] Ninham, Parsegian, *Journal of Theoretical Biology,* **1971,** 31:405-428