**Detecting short oligonucleotides with DNA nanopores**

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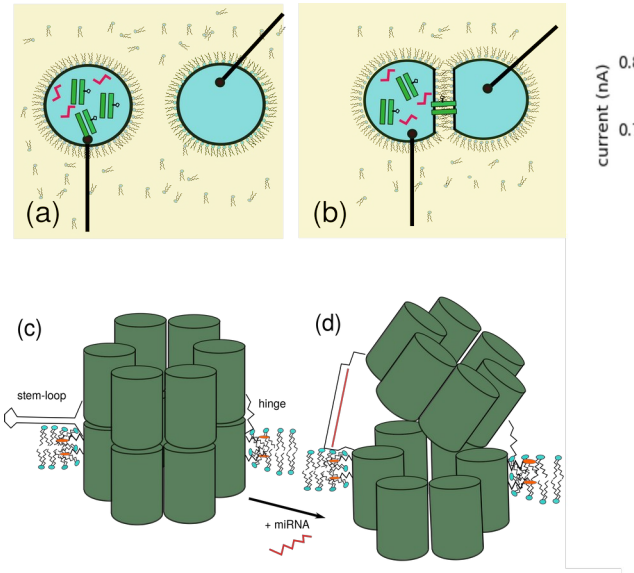
Micro-RNAs (miRNA) are short RNA sequences found in practically all aukaryotes. Despite their small size (length ~ 20nucleotides), these non-coding RNAs play an important role in the regulation of the genetic expression. The attractiveness of extracellular miRNA as cancer biomarkers relies on their stability and their dysregulation in the diseased cells. However, because of their short sequence and low concentration, miRNA detection is intrinsically difficult. In this paper, we consider a DNA based nanostructure that folds into a well defined nanopore. As shown in Figure 1(c)(d), the nanopore is actually formed by two coaxial cylinders which are linked by a flexible hinge. A locking mechanism based on a stem-loop structure controls the alignement of the two cylinders. In the closed state, the two cylinders are aligned. In the presence of a specific (signal) oligonucleotide (DNA or RNA) which can be as short as 22nucleotides, the stem-loop is disrupted and the two cylinders become unaligned.

To measure this conformational change, we modify the nanopore with cholesterol moieties so that it can insert into lipid bilayers (Figure 1(a)(b)). The conductance of a well formed lipid bilayer is usually very low. Upon insertion of a nanopore, the conductance slightly increases, as shown in Figure 1(e). The two nanopore conformations yield quite different levels of conductance. As shown in ref. [1], for 14nm long nanopores, both closed and open conformations can be monitored. For longer nanopores, only the open conformation yields a measurable signal. Counting the number of open nanopore insertions provides with an accurate measure of signal concentration (detection at the single molecule level). In this paper, we discuss the experimental conditions required to obtain (I) stable current recordings (ii) efficient nanopore insertion, significantly improving results in ref. [1].



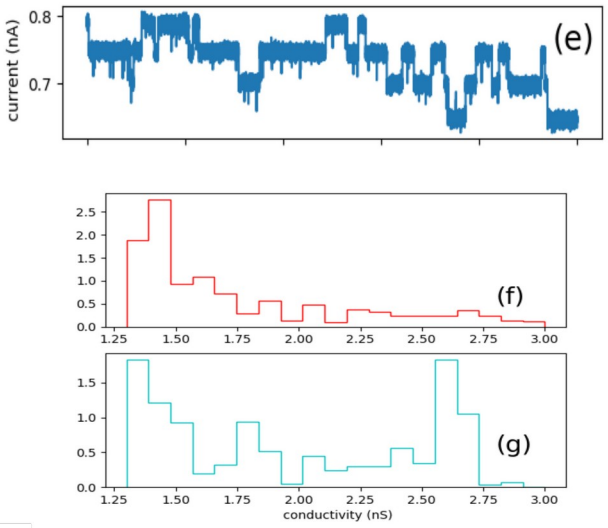




Figure 1: (a) and (b) show the formation of a well controlled lipid bilayer between two aqueous droplets immersed in an oil bath (yellow) containing lipids. Nanopores in the left droplet may insert into the bilayer. (c) and (d) illustrate the opening mechanism of the nanopore: each green cylinder corresponds to a double helix. (e) current recording showing insertions and desorption (respectively positive and negative current jumps) of single nanopores. (f) and (g) are histograms of nanopore conductance in the presence (g) or the absence (f) of a signal nucleotide, 22nt long, DNA analog of the miR-21 miRNA.

[1] L. Yang, C. Cullin and J. Elezgaray, Chem. Phys. Chem. (2022) http://dx.doi.org/10.1002/cphc.202200021.