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Review

Nanopores formed by DNA origami: A review

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ABSTRACT

Nanopores have emerged over the past two decades to become an important technique in single molecule experimental physics and biomolecule sensing. Recently DNA nanotechnology, in particular DNA origami, has been used for the formation of nanopores in insulating materials. DNA origami is a very attractive technique for the formation of nanopores since it enables the construction of 3D shapes with precise control over geometry and surface functionality. DNA origami has been applied to nanopore research by forming hybrid architectures with solid state nanopores and by direct insertion into lipid bilayers. This review discusses recent experimental work in this area and provides an outlook for future avenues and challenges.

1. Introduction

Nanopores are a relatively new technique for detecting single molecules in solution and are garnering great interest due to their applications in areas such as DNA sequencing [1,2]. The basic idea for single molecule sensing is to apply a voltage across a single nanoscale pore separating two electrolyte solutions and measure the resulting ionic current. Individual molecules are detected as they translocate the pore since they reduce the flow of ions and therefore the measured current. Seminal research in the 1990s first showed that single molecules such as nucleic acids could be detected in this way [3–5]. In the past two decades nanopores have been applied to the detection of a wide range of biomolecules such as DNA, RNA and proteins – see Wanunu [6] for a recent review of biomolecule detection with nanopores. Nanopores have also uncovered a wealth of interesting phenomena covering diverse research topics such as nanoscale electrokinetics [7], polymer physics [8] and surface chemistry [9].

Nanopore research has traditionally been split into two disciplines: solid state nanopores and biological nanopores. Solid state nanopores are entirely synthetic and can be fabricated by techniques such as ion beam drilling of thin insulating membranes [10] or laser assisted pulling of glass capillaries [11–14]. Biological nanopores are made by reconstitution of a membrane spanning protein into a lipid bilayer [4,15]. A crucial aspect of solid state and biological nanopore sensing is the ability to control both the geometry and surface functionality of the nanopore. Nanopore geometry determines the signal of measurements and enables selectivity based on physical size. Surface functionality allows for chemical specificity in nanopore experiments.

First we consider aspects of nanopore geometry. Advances in fabrication techniques have recently enabled the production of solid state nanopores with similar dimensions to biological nanopores such as alpha-hemolysin [16] and atomically thin nanopores can be made in graphene [17]. However atomic reproducibility of solid state nanopore 3D profiles is still not achievable and small (below 10 nm) diameters also require time consuming transmission electron microscopy (TEM) techniques. Biological nanopores are atomically reproducible, however they are mostly limited to studying single stranded DNA or smaller biomolecules there being only a few examples of engineered non-gating pores with diameters wider than 2 nm that show DNA translocation [18,19]. The most widely used biological nanopore, alpha-hemolysin, is 1.4 nm at its narrowest constriction [20]. In contrast, solid state nanopores can be tuned to arbitrarily large diameters with nanometre accuracy and therefore are useful for testing larger biomolecules such as double stranded DNA and proteins [21].

The second aspect of control for nanopore sensing is surface functionality. Biological nanopores significantly outperform their solid state counterparts when it comes to the ability to engineer surface functionality. Genetic engineering of biological nanopores

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allows for the precise positioning of chemical groups which bind to analytes. In the first protein nanopore experiments of the 1990s molecules simply passed through the nanopore driven under an electric field [3]. In later experiments, the introduction of engineered binding sites in a protein nanopore was used to create stochastic blockades as individual molecules bind on and off thereby modulating the ionic current [9]. This has enabled the specific detection of analytes such as ions [22], small organic molecules [23] and DNA bases [24]. Solid state nanopores can be chemically modified with monolayers [25,26] however the precise positioning of single binding sites remains extremely challenging.

Having considered these aspects of nanopore geometry and surface functionality it is apt to ask what other nano-engineering techniques are available for fabricating isolated three-dimensional structures at the scale of a few to tens of nanometres? A method that has recently gathered significant momentum has been the design of nanoscale objects self-assembled from DNA. DNA follows simple rules of base pairing and synthetic DNA strands can be designed to form nanoscale structures. This field of DNA nanotechnology has been pioneered by Ned Seeman who first realised its potential in the 1980s [27]. A significant leap forward in the sophistication of designed DNA nanostructures was so-called "DNA origami" first described in 2006 by Paul Rothemund [28]. The DNA origami method is to take a long single "scaffold" strand and fold it into a desired shape using hundreds of short "staple" strands. The scaffold strand is often the genome of the m13mp18 virus and the staple strands are made by chemical synthesis. The base sequences of the synthetic strands hybridise at specific positions on the scaffold strand and thereby fold it into the designed nanostructure with a typical molecular weight of ~5 MDa. DNA origami immediately opened up new avenues for DNA nanotechnology due to its simplicity and high yield. Previous work on DNA nanostructures had nearly always used short synthetic oligonucleotides as the only building block and very tight controls of stoichiometry and purification were needed to get reasonable yields. DNA origami overcomes issues of matched stoichiometry since the staple strands can be used in large excess to the scaffold. This also allows the full length staples to preferentially bind at their designed positions and remove potential secondary structure [28]. Modifications on the synthetic single strands can be used for site specific attachments on DNA origami for instance with gold nanoparticles [29,30], proteins [31] and fluorophores [32]. Readers are referred to several reviews for further information on the DNA origami method [33–36].

DNA origami represents an attractive method for forming nanopores since it can be used to fabricate 3D shapes with nanoscale level accuracy in geometry and surface properties. DNA origami can be used to form structures such as plates [28], cubes [37], curved beams [38] and shapes with complex curvatures [39]. Functional groups can be reliably positioned in a design with an accuracy of a few nanometres [40]. Recently a molecular model of a DNA origami design was made by cryo-EM imaging and this shows that the field can progress towards even greater positional accuracy [41]. Billions of nearly identical copies are created in parallel since the method relies on self-assembly. Finally we also add that the design of DNA origami structures is simple using freely available software such as caDNAno [42] and annealing protocols for synthesising DNA origami are typically less than a day.

DNA origami has been applied to nanopore research in two distinct ways – (1) by trapping a DNA origami structure at the mouth of a solid state nanopore and (2) by insertion of a DNA origami structure, coated with hydrophobic moieties, into a lipid bilayer. Recent work in these two branches is now discussed.

2. Hybrid nanopores formed by trapping DNA origami onto a solid state nanopore

The combination of DNA origami and solid state nanopores was first described in 2012 [43,44]. The method was to make a hybrid nanopore by inserting a single engineered DNA origami into a solid state nanopore using the electrophoretic force. This idea is similar to that used by Hall et al. [45] in 2010 for guiding a protein nanopore, alpha-hemolysin, into a solid state nanopore and thereby forming a hybrid architecture. This system combines the advantages of atomically engineered nano-constructs and a substrate that is significantly more robust than a lipid bilayer.

Our work [43] used a funnel shaped DNA origami designed with a narrowest constriction of 3×3 double helix widths (7.5 nm $\times$ 7.5 nm) and a double stranded DNA tail 2.3 kilobasepair (kbp) long to aid trapping in the correct orientation (Fig. 1a and b). These DNA origami structures were added in solution and a voltage applied with a polarity to attract the negatively charged DNA origami towards the solid state nanopore. A characteristic decrease in ionic current was observed after a few seconds of applying the voltage (Fig. 1c). This corresponds to a single DNA origami being trapped into the solid state nanopore and thereby reducing the flow of ions. The trapping process was found to be highly controllable and reversible. The DNA origami could be simply expelled from the solid state nanopore by switching the polarity of the potential and therefore the direction of the electrophoretic force (Fig. 1d). Another DNA origami could then be assembled by again reversing the potential. This process could be performed many times without significant clogging of the bare solid state nanopore. The waiting time between trapping events can be easily tuned by adjusting the applied voltage or the DNA origami concentration. This means there are several parameters that can be adjusted in order to quickly obtain a single insertion.

Wei et al. [44] reported experiments on DNA origami nanoplates with a thickness of 6 nm and varying pore diameters (Fig. 1e). A hybrid architecture was also formed by electrophoretic guiding of the DNA origami into a single solid state nanopore. They found that the relative conductance of the nanoplate decreased as the pore size was reduced giving evidence that the nanoplate was trapped in the correct orientation (Fig. 1f). They also showed ionic current traces that suggest that the DNA origami could be used as a size selective filter since proteins larger than the aperture of the nanoplate did not translocate through.

Several challenges are immediately apparent from the first two papers of Bell et al. and Wei et al. Simple geometrical models of the systems showed that the ionic flow was substantially higher than what would be expected if the DNA origami was completely insulating. This is somewhat surprising given the dense packing of helices suggested by TEM images (Fig. 1b and e) but shows that there is substantial leakage current through the structure and its seal. Therefore the signals for translocating molecules through the hybrid nanopore are not as high as might be hoped. Also there can be a significant increase in ionic current noise when the hybrid nanopore is formed compared to the bare solid state nanopore. This fluctuating current is also highly variable for different DNA origami trappings (on the same solid state nanopore). This is clearly undesirable for single molecule sensing however it can be ameliorated to some degree by exchanging DNA origami structures until a low noise hybrid nanopore is achieved. The increase in current noise upon hybrid nanopore formation may be explained by the fluctuations of the DNA origami. The source of the wide variability in noise levels is still uncertain but one can speculate that this is due to different conformations in which the DNA origami is trapped or small differences between individual DNA origami structures such as missing staple strands or slightly misfolded