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# Translocation of double-stranded DNA through membrane-adapted phi29 motor protein nanopores

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**Biological pores have been used to study the transport of DNA and other molecules, but most pores have channels that allow only the movement of small molecules and single-stranded DNA and RNA. The bacteriophage phi29 DNA-packaging motor, which allows double-stranded DNA to enter the virus during maturation and exit during an infection, contains a connector protein with a channel that is between 3.6 and 6 nm wide. Here we show that a modified version of this connector protein, when reconstituted into liposomes and inserted into planar lipid bilayers, allows the translocation of double-stranded DNA. The measured conductance of a single connector channel was 4.8 nS in 1 M KCl. This engineered and membrane-adapted phage connector is expected to have applications in microelectromechanical sensing, microreactors, gene delivery, drug loading and DNA sequencing.**

The genome of linear double-stranded DNA (dsDNA) viruses is packaged into a preformed procapsid[1–4]. This entropically unfavourable process is accomplished by an ATP-driven motor[5–8]. In bacteriophage phi29, the motor uses one ATP to package 2 base pairs[5] or 2.5 base pairs of DNA[9]. The protein hub of this motor is a truncated cone structure, termed a connector (Fig. 1a), that allows dsDNA to enter during maturation and exit during infection[10–14]. The connector has a central channel (Fig. 1b) consisting of twelve GP10 protein subunits. Although the connector proteins of viruses share little sequence homology and vary in molecular weight, there are significant underlying structural similarities[15]. By demonstrating viral DNA packaging and procapsid conversion to infectious virions, the phi29 DNA packaging motor was the first to be assembled *in vitro* in a defined system and remains one of the most studied[16]. The motor uses six packaging RNA strands[17–20] to gear the machine (Fig. 1c). Engineering such a nanomotor outside its natural environment has tremendous potential to impact biology, engineering, medicine and various other fields of nanobiotechnology.

Electrophysiological measurements of individual membrane channels have been used to study a variety of processes including the transport of DNA, RNA, pharmaceutical agents, peptides, proteins and polymers[21–25]. The transient blockade of ionic current through the $\alpha$-haemolysin ($\alpha$-HL) pore has been used to measure the length of single-stranded DNA or RNA[26]. Subsequently, DNA hairpin molecules have been used to decelerate the DNA translocation rate through the $\alpha$-HL pore to demonstrate the discrimination between single nucleotide polymorphisms[27]. Detection of base-pair stacking and strand orientation within the pore have also been investigated[28,29]. Most studies involving nucleic acid transport through nanopores have focused on $\alpha$-HL. However, the limiting lumen diameter (1.5 nm) has restricted the DNA and RNA applications to single strands[30]. A similar limitation was also reported for the MspA nanopore[31].

A few studies of channels have shown evidence of dsDNA transport[32–34], but their voltage gating properties have limited their biomedical applications. For this reason some researchers have switched to fabricating synthetic metal or silicon nanopores for potential DNA sequencing[35,36]. Conversely, synthetic nanopores are less reproducible and not as readily engineered for specific pore modification or conjugation. As a result, the search for alternate protein nanopores is still ongoing. The portal nature of the phi29 connector has inspired us to examine whether it could be used to explore membrane-based nanopore applications.

An artificial membrane architecture could allow detailed investigations into discrete motor mechanisms, as well as opening future avenues for the study of sensing, drug delivery and therapeutic dsDNA packaging. The phi29 connector is ideally suited for this endeavor because its available crystal structure allows for explicit engineering[12,13,37,38]. Furthermore, procedures for large-scale production and purification of the phi29 connector have been developed[38–41].

In this study, the connector protein was redesigned to include distinct regions of hydrophilicity. The modified connector was inserted into liposomes and a lipid bilayer. The presence of the channel across the lipid bilayer was confirmed by single channel conductance measurements and translocation of dsDNA.

## Modifying the phi29 connector

In general, membrane pores and ion channels contain a hydrophobic domain, which anchors the protein to the membrane. Analysis of the surface charge of the connector revealed that its central surface region shows slight hydrophobicity compared with the two flanking layers at the wide and narrow ends (Fig. 1a,b)[12,13]. To facilitate connector purification, a C-terminus his or strep tag was inserted just downstream of a six-glycine linker for improved affinity tag flexibility. The linker was included to provide end flexibility (Supplementary Fig. S1). After purification to homogeneity, it was found that the modified GP10 self-assembled into the dodecameric structure with similar morphology to the 12-fold symmetric wild-type connector (Fig. 1f), as observed by transmission electron microscopy (Fig. 1d,e). The existence of a native and authentic motor

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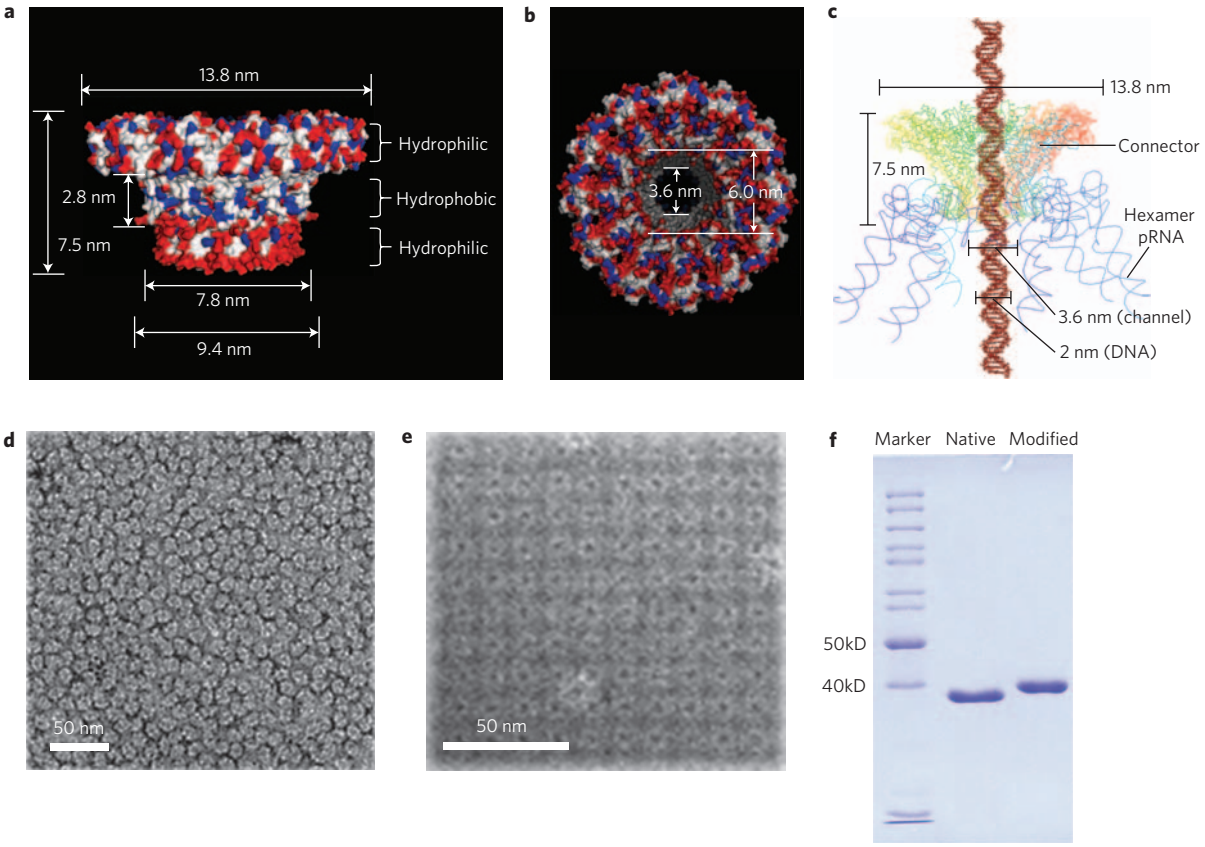


**Figure 1 | Structure of phi29 connector and DNA packaging motor.** **a**, Side view of the phi29 connector showing the acidic (red), basic (blue) and other (white) amino acids[12,13,38]. **b**, Top view of the connector showing the diameter of the narrow part and wide part of the channel. **c**, Illustration of the entire phi29 DNA packaging motor showing DNA translocation through the connector. **d**,**e**, A transmission electron microscope image of purified connectors with C-terminal modification before (**d**) and after (**e**) the array formation. **f**, Coomasie-blue stained SDS-gel showing that the modified connector is similar in size to the wild-type connector.

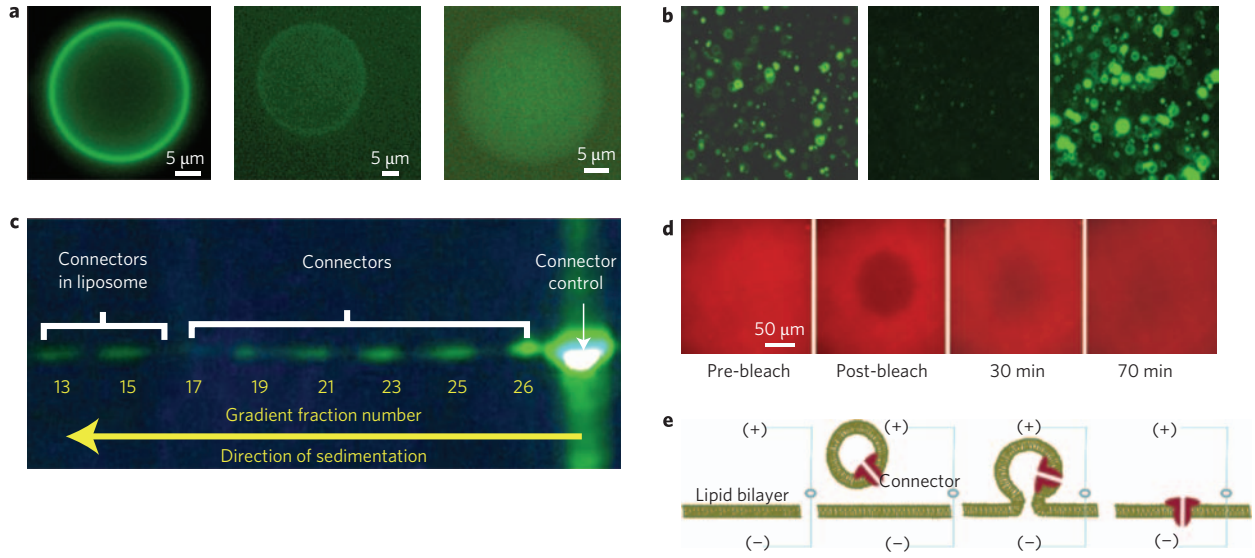


**Figure 2 | Images of a giant liposome containing the connector.** **a**, Epifluorescence images of the liposome: lipid labelled with NBD-PE without connector (left); a proteoliposome reconstituted by FITC-labelled connectors (middle); a liposome mixed non-specifically with FITC-labelled connectors (right). **b**, Membrane filtration studies. Images of before filtration (left), the filtrate (middle) and the retentate (right) demonstrate that filtration isolated most of the free connectors. **c**, Separation of liposome/FITC-connector complexes by sucrose gradient sedimentation. Free connectors appeared in the top fractions whereas proteoliposomes remained in the lower fractions. Fractions 1–12 are not shown. **d**, Fluidity of fluorescent (red) lipid bilayer demonstrated by fluorescence recovery after photobleaching showing that the fluorescence intensity of the photobleached area (black) was gradually increased over time owing to lipid diffusion. **e**, Schematic showing the insertion of the connector into a planar lipid bilayer.