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Robust Properties of Membrane-Embedded Connector Channel of Bacterial Virus Phi29 DNA Packaging Motor

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SUMMARY

Biological systems contain highly-ordered macromolecular structures with diverse functions, inspiring their utilization in nanotechnology. A motor allows linear dsDNA viruses to package their genome into a preformed procapsid. The central component of the motor is the portal connector that acts as a pathway for the translocation of dsDNA. The elegant design of the connector and its channel motivates its application as an artificial nanopore. Herein, we demonstrate the robust characteristics of the connector of the bacteriophage phi29 DNA packaging motor by single pore electrophysiological assays. The conductance of each pore is almost identical and is perfectly linear with respect to the applied voltage. Numerous transient current blockade events induced by dsDNA are consistent with the dimensions of the channel and dsDNA. Furthermore, the connector channel is stable under a wide range of experimental conditions including high salt and pH 2–12. The robust properties of the connector nanopore made it possible to develop a simple reproducible approach for pore quantifications. Such quantifications led to a reliable real time counting of DNA passing through the channel. The fingerprint of DNA translocation in this system has provided a new tool for future biophysical and physicochemical characterizations of DNA transportation, motion, and packaging.

Keywords

bacteriophage phi29; connector; ion channel; single channel conductance; membrane channel; bionanotechnology; stoichiometry quantification

INTRODUCTION

All linear double-stranded DNA or RNA viruses, including dsRNA bacteriophages^{1–3}, adenoviruses⁴, poxviruses⁵, human cytomegaloviruses (HCMV)⁶, and herpes simplex viruses (HSV)⁷, possess a common feature in that their genome is packaged into a preformed procapsid. This entropically-unfavorable process is accomplished by a DNA-packaging motor. Each motor contains a central pore, called a connector^{8–14}. In different viruses, the individual portal proteins that comprise the connector share little sequence homology and exhibit large variations in molecular weight⁸. However, the portal complexes possess a significant degree of morphological similarity accounting for the common function of DNA translocation. A propeller-shaped truncated cone architecture of the homo-

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A description for the content entry illustration: The elegant channel of bacteriophage phi29 DNA-packaging motor embedded in the lipid membrane exhibits robust nanopore properties under a wide pH range and variable salt concentrations, implying its potential application as stochastic sensor.

dodecameric connector with a central channel running along the longitudinal axis of the oligomer is shared by most bacteriophages, including T3¹⁵, T4¹⁶, SPP1¹⁷, P22¹⁸, ϵ 15¹⁹, phi29⁸ and possibly HSV²⁰. The ingenious, intricate and elegant design of the connector and its channel motivated its application in nanotechnology. However, a major concern in using protein nanopores relates to their soft nature, fluctuations in folding, dynamics in structure, and the reproducibility of the signal generated.

In phi29, the connector is a dodecameric structure composed of 12 copies of the protein subunit gp10, forming a central channel⁸ through which viral DNA is packaged into the capsid and subsequently exits during infection. The motor has been constructed in a defined *in vitro* system and was shown to be competent in DNA packaging²¹ and was determined to be the most powerful nanomotor artificially synthesized to date²². Virtually every phi29 genomic DNA gp3 molecule added can be efficiently packaged *in vitro*, and the DNA-filled capsids can be converted into infectious virions up to 10⁹ plaque forming units per milliliter with all components overproduced, purified or synthesized chemically^{23,24}. The structure of the phi29 portal protein has been determined at atomic resolution^{8,25}. The connector ring consists of a twelve α -helical subunits, with the central channel formed by three long helices of each subunit. The ring is 13.8 nm across at its wider end and 6.6 nm at the narrower end. The internal channel is 6 nm at the wider end and 3.6 nm at the narrower end. The wider end of the connector is located in the capsid while the narrower end partially protrudes out of the capsid. The mode of connector insertion and anchoring within the viral capsid is mediated via protein-protein interactions.

Nanopore analysis based on biological pores embedded in lipid membranes as well as solid state synthetic nanopores is currently an area of great interest²⁶ in cell physiology, electrical technology, and other nanotechnological applications such as single molecule sensing, molecular fingerprinting, Micro-Electro-Mechanical Systems (MEMS), microreactors, and the sequencing of DNA. Recent advances in this area have included the creation of artificial pores^{27,28} or the reengineering of biological pores²⁶, the deposition of pore channel arrays²⁹, and the incorporation of preassembled channels into preformed membranes³⁰. Such membranes inserted with reengineered protein channels potentially possess wide and diverse applications as biosensors for a range of small molecules^{31,32} such as ions³¹, nucleotides³³, enantiomers³⁴, and drugs³⁵, as well as larger polymers such as PEG³⁶, RNA/DNA^{37–39}, peptides, and proteins⁴⁰. Due to the fragility of planar lipid membranes, the practical applications of the technology are still limited. To address this issue, some techniques have been developed to improve the stability of bilayers, and these include the use of polymerized lipid membranes⁴¹, hydrogel protected lipid membranes, and tethered lipid membranes on solid support⁴². It has been experimentally demonstrated that when multiple ion channels were incorporated into a bilayer, sensing sensitivity was greatly enhanced⁴³. To build a practical and useful biosensor, an array of robust channels exhibiting uniform conductance inserted into a storable and transportable bilayer⁴⁴ is highly desirable. Herein, we demonstrate that the phi29 connector channels have great potential in this regard.

Explicit engineering of the phi29 motor nanochannel is possible due to its available crystal structure^{8,25}. Furthermore, the connector has been well characterized and procedures for large scale productions and purification have already been developed^{29,30,45–48}. Our group has previously reengineered the motor for DNA packaging⁴⁶, and demonstrated that a lipid layer possesses the ability to orient these channels into either an array pattern or rosettes^{29,49}. The connector protein was reengineered and inserted into artificial lipid bilayers³⁰. The conductance of the channels were measured and demonstrated a larger cross-sectional area than the well-studied channel protein, α -hemolysin⁵⁰, thus allowing translocation of both ds- and ss- DNA. In this manuscript, using electrophysiological

methods, we demonstrate that the connector channels are robust and stable under a wide range of experimental conditions. The channel conductance is uniform, demonstrating a perfect linear relationship with respect to the applied voltages and does not display voltage gating properties under the reported conditions. The dsDNA channel blockade events are nearly identical. The robust properties of the connector nanopore made it possible to develop a method for precise counting of the number of channels on each membrane. Taking all these factors under consideration, the phi29 connector is ideally suited for reengineering a system that can operate outside its natural environment and has tremendous potential to impact biology, engineering, medicine, and other nanotechnological fields. The work reported here represents a new system for further physical and chemical characterization of DNA translocation through the motor channel in viral DNA packaging.

EXPERIMENTAL

Materials

The phospholipids 1,2-diphytanoyl-sn glycerol-3-phosphocholine (DPhPC) and polycarbonate membrane filters were purchased from Avanti Polar Lipids (Alabaster, AL). n-Decane and chloroform were purchased from Fisher and TEDIA, respectively. Tris(hydroxymethyl)aminomethane (Tris) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were from Sigma company.

Preparation of phi29 connector channel

The construction of the plasmid for the expression of the connector protein and the assembly of the dodecameric connector has been reported previously⁴⁷. The subsequent terminal modifications of the connectors have also been described^{30,46,48,51}.

Preparation of connector reconstituted liposomes

The phi29 connector can be inserted into a bilayer membrane by vesicle fusion using phi29 connector reconstituted liposomes. To prepare the phi29 connector reconstituted lipid vesicles, 1 ml of 10 mg/ml DPhPC in chloroform solution was placed in a vial. The chloroform was evaporated under nitrogen, and the vial was further dried in a desiccator overnight. To rehydrate the lipid film, 1 ml of connector protein solution containing 200–300 mM sucrose was used to bud off vesicles into the solution. The lipid solution was then extruded through a polycarbonate membrane filter (100 nm or 400 nm) to generate unilamellar lipid vesicles. A final molar ratio of lipid vs. connector was established at 4000:1 to 16000:1.

Insertion of the connector into the planar bilayer lipid membrane

A standard Bilayer Lipid Membrane (BLM) chamber (BCH-1A from Eastern Sci LLC), was utilized to form horizontal BLMs. A thin Teflon film with an aperture of 70–120 μm (TP-01 from Eastern Sci LLC) or 180–250 μm (TP-02 from Eastern Sci LLC) in diameter was used as a partition to separate the chamber into *cis*- and *trans*- compartments. For connector insertions, 1–2 μL of liposome stock solution was further diluted by 10–20 fold and was directly added to the *cis* compartment.

Electrophysiological measurements

A pair of Ag/AgCl electrodes connected directly to the head-stage of a current amplifier were used to measure the current across the bilayer lipid membrane, and the trace was recorded using an Axopatch 200B patch clamp amplifier coupled with the Axon DigiData 1322A or Axon DigiData 1440 analog-digital converter (Axon Instruments). All the voltages reported are those of the *trans*-compartment. Data was low band-pass filtered at a

frequency of 1 kHz and acquired at 500 μ s intervals per signal, if not specified. The PClamp 9.1 software (Axon Instruments) was used to collect the data, and the software Clampfit was used for data analysis.

If not specified, all conductance measurements were determined using the slope of the current trace induced by a ramp voltage from -100 mV to 100 mV after the definite insertion of a gp10 connector occurred. Solution conductivity was measured using a Pinnacle 542 conductivity/pH meter (Corning Inc.). The cell constant of the conductivity cell used was 1 cm^{-1} . All experiments were performed at room temperature.

DNA translocation and Q-PCR analysis

In this report, three different sizes of dsDNA (20 bp, 141 bp and 2 kbp) were used for DNA translocation experiments. The current traces were recorded under -75 mV at a sampling rate of 2 kHz. For generating the histogram displaying blockade percentage of the translocation events, a 2 kbp blunt-end dsDNA was used from an EcoR V restriction fragment from a plasmid. The DNA was premixed with conducting buffer for a final concentration of 180 pM prior to connector insertion.

For quantification of the translocated DNA by Q-PCR, a 141 bp DNA was used. A higher potential of -95 mV was applied to facilitate greater translocation. The connector reconstituted liposomes were added to the *cis*-compartment (working volume 500 μ L). Prior to insertion of the first connector, the DNA was added to the *trans*-compartment with a final concentration of 25 nM after bilayer formation. Samples were collected from the *cis*-side for Q-PCR measurements at 30 min after the 1st connector insertion occurred. The number of inserted connectors represents an average number of connector channels during the entire 30 min of DNA translocation experiments.

Absolute quantification was used to determine the copy number of DNA in samples collected. Standard curves were constructed using the 141-bp DNA with 10 fold dilution of known concentration. Each dilution was assayed in triplicates. iQTM SYBR Green Supermix (Bio-Rad) was used for the Q-PCR reaction. Q-PCR was carried out in the iCycler iQTM multicolor real-time PCR detection system (Bio-Rad). The sequences for forward and reverse primers corresponding to the DNA template were 5'-TAA TAC GAC TCA CTA TTA GAA CGG CAT CAA GGT GAA CTC AAG ATT TTG TAT GTT GGG GAT TA-3' and 5'-AAG AAC GGC ATC AAG GTG AAC TTC AAG ATA ATT GAC AGC AGG CAA TCA AC-3' respectively (purchased from IDT).

RESULTS AND DISCUSSION

1. The lipid-embedded phi29 connector channels display uniform conductance

The connectors of bacteriophage phi29 (shown in Figure 1) were inserted into a lipid bilayer membrane as described previously³⁰. The occurrence of continuous insertions of single connectors is shown in the current traces of Fig. 2A–E. Each discrete step represents a current jump induced by the insertion of a single connector channel. Direct quantification of the number of connector channels inserted into the membrane was carried out by counting the steps in the current trace during the course of insertion. As demonstrated, insertion occurrence can be monitored at any voltage, either positive or negative. Extensive investigation with the phi29 connector channels revealed that the step size of the current jump per single insertion were nearly identical (Fig. 2F). For example, the insertion of one connector into the bilayer results in an increase in the current jump of approximately 65 pA (equivalent to 1.6 nS) at a potential of -40 mV in presence of 5 mM Tris (pH 7.9)/0.5 M NaCl.

2. The blockade percentage of the channel by dsDNA is nearly identical

We recently demonstrated the translocation of dsDNA using the phi29 connector channels³⁰. The principle of nucleic acid transport is based on the classic ‘coulter counter’ the— passage of non-electrolytes through the pore down an electrochemical potential gradient physically blocks the ionic current, which can then be detected. Fig 3A shows DNA translocation events in presence of multiple connectors. The current blockade percentage represents the difference between the current when the connector channel is open and the current during the DNA translocation, calculated as follows: the size of current blockade resulting from the DNA translocation through one channel divided by the step size of the current for one connector insertion. In the presence of dsDNA, the channel blockade percentage was centered at ~32%, which is determined by the dimensions of the channel (3.6 nm diameter at its narrowest end) and dsDNA (~2 nm in diameter). Furthermore, each transient current blockade event was observed to be nearly identical as demonstrated by a sharp Gaussian distribution (Inset b in Fig 3A).

It was further observed that the blockade events from the current trace were closely associated with the number of DNA molecules passing through the pores quantified by Q-PCR (Fig. 3B). The results confirm that the observed blockade events were a consequence of dsDNA translocation.

3. The phi29 connector channels display a linear response to applied voltages

A second approach to determine the conductance of the membrane/connector complex (G_m) is by calculating the slope of the current trace under a ramp voltage⁵². The data from this approach is more accurate: because the conductance equals $\Delta I/\Delta V$, the influence of electrode and contact potentials is excluded. Furthermore, since a slope represents numerous data points, the use of slope instead of a single conductance measurement greatly improves the accuracy.

The current traces from one, two and three connector insertions under a ramp voltage are shown in Fig. 4 in the presence of 5 mM Tris (pH 7.8) with 2 M NaCl. The slope calculated was 4.64 for one insertion, 9.31 for two insertions and 13.96 for three insertions, respectively. The data from these three distinct experiments indicate conductance induced by two insertions differed from that of one connector insertion by 4.67 nS, and conductance induced by three insertions differed from that of two insertions by 4.65 nS, further demonstrating the uniform size and stability of the phi29 connector channels in this model system.

A similar approach can also be used to obtain the conductance of one channel (G_c). Fig. 5 displays representative current traces obtained from more than three independent experiments. The traces were acquired under a ramp voltage after single channel insertion with varying conditions of salt type and concentration. The current-voltage curve showed an excellent linear relationship under the voltage range of -100 mV to 100 mV with each of the solutions investigated. Using the slope, we calculated the conductance in 0.5 M NaCl, 1.0 M NaCl, 1.5 M NaCl and 2.0 NaCl. Values were 1.75 ± 0.11 nS, 3.03 ± 0.02 nS, 4.07 ± 0.26 nS, and 4.60 ± 0.05 nS, respectively, demonstrating the expected increase in conductance with an increase in salt concentration. The conductance values with 0.5 M KCl, 1.0 M KCl, 1.5 M KCl and 2.0 M KCl were 2.70 ± 0.21 nS and 4.65 ± 0.11 nS, 6.23 ± 0.32 nS, and 7.83 ± 0.20 nS, respectively.

Since the phi29 connector channels were observed to be of uniform size and stable under varying salt conditions, a linear relationship between the conductance of the bilayer and the number of inserted connectors was therefore expected (Fig. 5). The linear regression

equations are included, suggesting the BLM conductance is positively proportional to the number of inserted connectors in both NaCl and KCl buffers.

4. Deriving an analytical expression for determining the number of connectors inserted in the lipid bilayer

Generally, if both the conductance of the membrane/connector sheet (G_m) and the conductance of one channel (G_c) are known, the number of connectors (N) incorporated in a membrane can be deduced by dividing the total conductance of the membrane with the conductance of one channel⁵². That is,

$$N = \frac{G_m}{G_c} \quad (1)$$

However, this method for counting the insertions in a membrane would become impractical in the situation in which connector insertions have been completed. Nevertheless, the counting of insertion steps serves as a concise reference of the number of channels per membrane for the development of the quantification procedures described in this manuscript.

The conductance of the membrane/connector complex (G_m) was determined by measuring the bulk current under a specific voltage in real time. The ratio of the measured current to the applied voltage represents the conductance value. However, the surface potentials of the Ag/AgCl electrodes, as well as membrane contact potentials, cause errors in the measurement of the voltage across the BLM. Typically, any offset potential obtained when using Ag/AgCl electrodes is compensated for before a recording as drifts of 25 μ Volts/hour have previously been reported⁵³.

For purposes of comparison, the conductance of one channel (G_c) was determined by real time direct observation of the single channel current revealed as step size of the current jump (Fig. 2). Again, this method is useful as a reference or during the course of channel insertions. Table 1 shows the results of calculating single channel conductance using the discrete current steps. As observed, standard deviation roughly increased with the number of independent experiments performed under a given set of conditions. This is attributed to electrode variation over time and the histogram in Fig. 2F further confirms the variability of the conductance obtained by these means. Another feature present in the histogram is the simultaneous insertion events of two and three connectors, although this occurs less frequently.

A formula specific to our phi29 system was derived for the calculation of single channel conductance. Solution conductivity was measured with a conductivity meter and plotted against the single channel conductance values established from the slopes of the regression equations in Fig. 6. A linear relationship was observed between these two variables as shown in Fig. 6. Therefore, using the regression equation from Fig. 7, the single phi29 channel conductance, G_c , at any NaCl or KCl concentration within the range 0.5-2.0 M can be deduced from buffer conductivity, σ_b measured by a conductivity meter.

The NaCl buffer conductivity is given by:

$$\frac{\sigma_{b,NaCl}}{(mS \cdot cm^{-1})} = \left(29.58 \times \frac{G_{c,NaCl}}{nS} \right) - 6.32 \quad (R^2=0.9994) \quad (2)$$

$$\text{Thus, } \frac{G_{c,NaCl}}{nS} = \frac{\left[\frac{\sigma_{b,NaCl}}{(mS \cdot cm^{-1})} + 6.32 \right]}{29.58}$$

Similarly, the KCl buffer conductivity can be obtained as follows:

$$\frac{\sigma_{b,KCl}}{(mS \cdot cm^{-1})} = \left(29.69 \times \frac{G_{c,KCl}}{nS} \right) - 15.61 \quad (R^2=0.9923) \quad (3)$$

$$\text{Thus, } \frac{G_{c,KCl}}{nS} = \frac{\left[\frac{\sigma_{b,KCl}}{(mS \cdot cm^{-1})} + 15.61 \right]}{25.69}$$

As noted earlier, $N = \frac{G_m}{G_c}$. Supplementary Table 1 contains the results of the application of equations 2 and 3 to the data obtained with our phi29 connector model system. For example, in the case of 0.5 M NaCl, buffer conductivity, $\sigma_{b,NaCl}$ is $44.6 \text{ mS} \cdot \text{cm}^{-1}$. Using equation 2, the single channel conductance, $G_{c,NaCl}$, is calculated to be 1.72 nS . Using the BLM conductance with one channel insertion (1.59 nS), a channel number of 0.92 is deduced. Calculated values from the derived equations were compared with those values for channel conductance calculated by means of current jump (Table 1). Again, good agreement is demonstrated for experiments conducted under the same buffer/salt conditions.

In summary, the number of channel insertions in a lipid bilayer can be calculated after two steps: measuring the bulk solution conductivity and calculating the conductance of the bilayer, given by the slope of the current/voltage plot. Using the value for the bulk solution conductivity in conjunction with Equation 2 or 3, the single connector conductance can be deduced. Then, inputting this value and the measured conductance of the bilayer into Equation 1 will deliver the number of connector insertions in the examined membrane.

5. Calculating the calibration coefficient for the KCl and NaCl buffer

We further compared the slopes from experiments with different salts (NaCl or KCl), under the same number of connector insertions. With 0.5 M, 1.0 M, 1.5 M and 2.0 M KCl and NaCl solutions, the slope ratios of KCl to NaCl were 1.50, 1.71, 1.67 and 1.70 respectively. The average was 1.65 and this value can be thus used as a calibration coefficient for translation of conductance calculated from buffers with K^+ to Na^+ . Therefore, to approximate the conductance in the same concentration of KCl, $G_{m,KCl}$, a calibration coefficient, F can be added to equation 3, i.e.;

$$G_{m,KCl} = F \times N \times G_{c,NaCl} \quad (4)$$

where $G_{c,NaCl}$ is the single channel conductance in the same concentration of NaCl; F is the calibration coefficient for the KCl and NaCl salt solutions, which was measured to be 1.65 in our experiments. For an approximation of conductance induced by multiple connector channels in the same concentration of buffer with different conducting ions, such as K^+ , equation 4 can be used only if the single channel conductance in NaCl salt solution is known.

6. The connector channel is stable under a broad range of pH values

The stability of the connector channel was further investigated under extreme pH conditions in the presence of a 20 bp DNA in both chambers. Current traces were recorded under a

constant of voltage at each of the pH conditions investigated under a constant symmetric ionic strength of 1M NaCl. The respective conductance values are 2.50 ± 0.11 nS (N=6) at pH 2, 2.73 ± 0.07 nS (N=6) at pH 7 and 2.78 ± 0.12 nS (N=6) at pH 12. Fig. 8A, B, and C shows the connector insertion step and the DNA translocation (20 bp dsDNA) at pH 2 (5 mM phosphoric acid buffer), pH 7 (5 mM Na₂HPO₄ buffer), and pH 12 (5 mM Na₂HPO₄·7H₂O buffer) respectively. The channel under the pHs were still kept open because the DNA translocation events were still found. Interestingly, when dsDNA were treated with pH 2, formation of apurinic acid occurred leading to short current blockade events (Fig. 8A). Although the structure of DNA is affected by extreme pH conditions, well-behaved uniform channel conductance were demonstrated, suggesting that the phi29 connector enables retaining its stable channel properties under the strong acidic or alkaline conditions.

DISCUSSION

The results obtained for the phage phi29 connector channels are very promising as the conductance of each pore is uniform and does not display voltage gating properties under the conditions investigated³⁰. The response of the conductance with respect to the applied voltage is linear. The blockade percentage of the channel by dsDNA is nearly identical. Finally, the channel is stable under a wide range of experimental conditions. Based on the robust conduction features, a simple analytical expression can be derived for precise counting of the number of channels in each membrane. Since its crystal structure is known, a systematic and engineering-based approach can potentially be applied to develop a system with enhanced analytical capabilities. By virtue of its channel size and well-behaved properties, it is ideally suited for biophysical studies and nanopore-based applications, such as ss- and dsDNA translocation under a wide range of experimental conditions.

Nanopore-based stochastic sensing is an emerging analytical technique that enables measurements of analytes at the single molecule level. When a molecule of interest passes through the channel, the ionic current through the pores would reflect the amplitude, the duration and the rate of the resultant blockade events. By statistical analysis, information on concentration and identity of molecular species can be derived. The DNA translocation experiments with the robust phi29 connectors have revealed its potential for nanopore analysis.

The phi29 connector channel can be ideally used as a stochastic sensor at the single molecule level. The connector has a larger diameter (~3.6 nm) at its narrowest constriction as compared to some well-established channels, such as the 1.4-nm α -haemolysin channel which can only allow translocate ss-DNA. More importantly, for the development of the phi29 connector based target-selective stochastic sensors, the larger channel of phi29 connector would provide more flexibility in the choice of bulky ligands covalently bound inside the channel.

There are many bacterial outer membrane porins with larger pores that can be potentially used as stochastic sensors. However, the gating of these channel proteins induced by voltage or solution conditions, such as protons, anions or cations, may cause transient current blockades in single channel recording that would interfere with detection of analytes. For example, OmpG porins show spontaneous gating under ± 40 mV⁵⁴. In contrast, the phi29 connector channel showed stable channel properties in the voltage range of -100 mV to 100 mV, even at extreme pH conditions. Therefore, the phi29 connector channel would be an excellent candidate for biological pore based stochastic sensors.

Viruses contain elegant and intricate structures, such as pentagons and hexagons. Viral components or capsids can self-assemble or assemble under the direction of templates into pentagonal or hexagonal arrays²⁹. The property of strong interactions involved in self-assembly in symmetrical structures, polygons or arrays has inspired the application of viral components in nanodevices. Indeed, recent applications of viruses in nanotechnology have been reported extensively^{55,56,56–62}. The phage phi29 connector channel described here can be purified to homogeneity and is capable of operating outside its natural environment. The phage connector is expected to be very robust since it is known to withstand high pressure environments. For instance, during the DNA packaging process, the motor can exert forces greater than 60 pN²² and subsequently, the DNA is compacted to near crystalline density within the capsid under an estimated pressure of 6 MPa^{22,63}. The robust properties of the phi29 connector reported here support the expectation that viral components are a new generation of nanomaterials or nanoscale building blocks. This research will inspire future studies on constructing more sophisticated sensor systems using mutant phage connector channels. The results reported here suggest that viral structural components are robust and their applications in nanotechnology are indeed feasible.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (A–C) Structure and dimensions of the phi29 connector. (D) Illustration of a lipid membrane embedded connector channels. (E) AFM electron micrograph of purified connector channels after array formation.

Figure 2. A current trace showing continuous insertions of single connectors into BLM (A) at 5 mM Tris (pH 7.8) with 1 M NaCl under -40 mV (1 insertion); (B) at 5 mM Tris (pH 7.8) with 1 M NaCl under -75 mV (2 insertions); (C) at 5 mM Tris (pH 7.8) with 2 M NaCl under 75 mV (3 insertions); (D) at 5 mM HEPES (pH 7.8) with 1 M KCl under 45 mV (4 insertions); (E) at 5 mM Tris (pH 7.8) with 0.5 M NaCl under -40 mV (5 insertions); (F) Histogram of conductance measurements for single insertion under a specific voltage -75 mV with 1 M NaCl from 40 independent experiments.

Figure 3. Analysis of DNA translocation through phi29 connector channel embedded in the membrane

(A) A current trace depicting passage of 141 bp ds-DNA through multiple phi29 connector channels. Insert a. A magnified view of a typical translocation event induced by one dsDNA molecule. Insert b. A histogram of current blockade percentage induced by linear dsDNA with a total 3264 events. (Note: The data were obtained in the presence of 1M NaCl, pH 7.8 under -75 mV holding potential). (B) A comparison of copy number of translocated dsDNA measured by Q-PCR with the relevant blockade events counted from current trace recorded in two independent experiments (Note: Only the events with 29–34% current blockades were counted). The error bars represent the standard deviation of three independent Q-PCR measurements.

Figure 4. Current traces from BLM inserted with one, two and three phi29 connector channels at 5 mM Tris (pH 7.8) with 2 M NaCl under a ramp voltage from -100 mV to 100 mV.

Figure 5. Current-Voltage traces of single connector channel
(A) Different concentrations of NaCl with 5 mM Tris, pH 7.8. (B) Different concentrations of KCl with 5 mM HEPES, pH 7.8.

Figure 6. Relationship of measured conductance with number of connectors

(A) Different concentrations of NaCl with 5 mM Tris, pH 7.8. (B) Different concentrations of KCl with 5 mM HEPES, pH 7.8. All the conductance measurements were from more than three individual experiments. Error bars represent standard deviations for the measurements. The regression equation for 0.5 M NaCl is: $G_m/nS = 1.73 \times N + 0.01$; for 1.0 M NaCl is: $G_m/nS = 2.77 \times N + 0.26$; for 1.5 M NaCl is: $G_m/nS = 3.81 \times N + 0.26$; and for 2.0 M NaCl is: $G_m/nS = 4.62 \times N - 0.00$. The regression equation for 0.5 M KCl is: $G_m/nS = 2.60 \times N + 0.11$; for 1.0 M KCl: $G_m/nS = 4.73 \times N - 0.10$; for 1.5 M KCl is: $G_m/nS = 6.35 \times N - 0.16$; and for 2.0 M KCl is: $G_m/nS = 7.86 \times N - 0.01$, respectively.

Figure 7. Relationship of buffer conductivity vs. conductance of single connector
 (A) at 5 mM Tris with 0.5–2.0 M NaCl and (B) at 5 mM HEPES with 0.5–2.0 M KCl.

Figure 8. Current traces showing connector insertion accompany by DNA translocation (20 bp dsDNA) through the channel under different pH conditions in presence of 1 M NaCl at a constant voltage of -75 mV (A) pH 2; (B) pH 7; and (C) pH 12..

Table 1

Comparison of conductance induced by single channel measured from discrete current jumps and calculated using empirical equations

No. of Independent experiments	Buffer solutions	Conductance/channel calculated by discrete steps (<i>nS</i>)	Buffer conductivity(<i>mS·cm⁻¹</i>)	Conductance/channel calculated using the empirical equations (<i>nS</i>)
2	5 mM Tris +0.5 M NaCl	1.65±0.07 (n=62)	44.6	1.72
6	5 mM Tris +1 M NaCl	3.04±0.56 (n=52)	76.3	2.79
3	5 mM Tris +2 M NaCl	4.71±0.18 (n=45)	130.9	4.64
40	TMS + 1 M NaCl	3.14±0.36 (n=500)	80.5	2.94
2	5 mM HEPES + 0.5 M KCl	2.48±0.13 (n=10)	54.4	2.73
4	5 mM HEPES + 1 M KCl	4.73±0.20 (n=9)	99.7	4.49

Note: The conductance in Table 1 was calculated using the ratio of the measured current jump induced by a discrete step to the applied voltage. “(n)” in column three represents the number of insertions.