Abstract

Synthetic nanotubes that have the ability to broadly mimic the function of biological ion channels have extraordinary potential for various applications, from ultrasensitive biosensors to efficient water purification devices. As a result of their immense potential, the design and fabrication of such synthetic nanotubes is rapidly gaining momentum. We briefly review recent theoretical and experimental studies on nanoscale cylindrical hollow tubes constructed from carbon, boron, and nitrogen atoms that are able to selectively transport water molecules, cations (positively charged ions), or anions (negatively charged ions) similar to various biological ion channels.

From the Clinical Editor: This review discusses the current status of synthetic nanotube research, including recent theoretical and experimental studies on nanoscale cylindrical hollow tubes constructed from carbon, boron, and nitrogen atoms that are able to selectively transport water molecules, cations or anions similar to biological ion channels.

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Nanotubes (NTs) are cylindrical hollow tubes similar in diameter to biological ion channels (a few nanometers). They can be fabricated from a range of materials, from the more commonly studied carbon to boron nitride (BN) and silicon carbide. In recent years there have been rapid advances in technologies for synthesizing NTs made from various materials and modifying their surface chemistry. An excellent summary of current techniques of fabricating NTs is given by Harris.1

NT length can be precisely controlled2,3 so as to target cells with a specific membrane thickness. For example, certain gram-negative bacterial cell membranes are thinner than human cell membranes and therefore may be a suitable target for tailored NTs. The surface chemistry of NTs can also be modified4,5 to make the tubes soluble6 or to target specific cells.6 During fabrication carbon nanotubes (CNTs) will acquire hydrophilic ends (such as -OH, -COOH, -H) and, if desired, they can be further modified such that other functional groups are covalently attached to the partially charged termini. Moreover, the sidewalls of NTs can be modified either by covalently bonding functional groups7 or by wrapping a molecule around the surface.8,9 A recent review by Karousis et al10 outlines various approaches used to modify CNTs by covalently bonding functional groups on their external surface or otherwise.

There is a general consensus that pristine CNTs, or NTs with no functional groups attached at their ends or to their surface, are toxic to cells,11 whereas BN NTs are considered nontoxic.12 The cytotoxicity of pristine CNTs is largely due to their insolubility in aqueous solutions, hydropathy, and tendency to aggregate. The cytotoxicity of NTs, however, can be reduced13 by rendering them soluble by modifying their surface chemistry.5

It may be possible that synthetic NTs can be rendered selectively permeable to specific ionic species, thus mimicking one of the key features of biological membrane ion channels. Current theoretical and experimental findings suggest that there is a real possibility of assembling ion-selective NT-based pores in novel ways, creating practical nanodevices. These engineered nanoscale pores, once successfully designed and fabricated, could lead to a host of pharmaceutical products, such as antimicrobial agents and drugs to combat certain human diseases, in addition to having potential applications as ultrasensitive biosensors and water purification devices. The feasibility of utilizing CNTs as efficient water purification devices14,15 or single-ion detectors16 has already been demonstrated experimentally. These biology-inspired NTs will lead to a new generation of products that are more efficient than their current counterparts. For example, NT-based water channels are shown to conduct water an order of magnitude faster than commercially available reverse-osmosis membranes.17,18 Studies on CNTs that sieve water molecules from seawater have been summarized in a

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number of excellent review articles. Moreover, Hilder and Chung have designed a NT that can mimic the antibiotic gramicidin but with potassium conductance six times larger. This suggests that a NT-based drug, more potent in destroying microorganisms than the naturally occurring one, could be engineered. NTs have also been shown to embed into a cell membrane and conduct ions much faster than biological channels. As a result of their immense potential, artificial ion channels constructed from synthetic NTs is a unique field of research that has recently begun to gain momentum. For a number of years synthetic ion channels based on molecular structures other than NTs, such as cucubitiril macrocycles, have been investigated. For further details on synthetic channels made from organic molecules, the reader is referred to a review by Sakai et al.

Here, we briefly review some of the recent studies carried out on cylindrical NTs, which broadly replicate the dynamics of ion permeation across membrane ion channels. Many of these investigations aim to theoretically demonstrate the feasibility of creating synthetic ion-selective carbon or BN NTs. As the technology of fabricating nanodevices advances in the near future, these NTs conceived at the proof-of-principle level may be eventually manufactured and brought into practical use.

The article is organized as follows. We first survey the computational studies on the interaction between engineered NTs and cell membranes. For NTs to be useful in a biological setting, they must remain stable in a cell membrane, which is composed of two hydrophilic outer layers separated by the inner hydrophobic core. We next highlight some of the groundbreaking experimental and computational work on water-conducting, ion-rejecting pores. These devices have obvious uses for water purification and desalination, as well as potential biological applications. Finally, we review some of the recent investigations on nanopores that are selectively permeable to either positively or negatively charged particles. It is of theoretical as well as practical interest to explore how a simple nanodevice made from one or two atomic species can mimic some of the functions of complex biological ion channels made of bulky membrane proteins.

### Stability of synthetic NTs in lipid bilayer

Potential biological applications of engineered tubes as nanosyringes or artificial ionic channels mandate that they be stable in the lipid bilayer. Therefore, it is important to determine whether or not synthetic NTs will incorporate into a cell membrane, align vertically across it, and remain stable, similar to the polypeptide gramicidin and other membrane proteins, including ion channels. There are several computational studies that examine the interaction between NTs and lipid bilayers using coarse-grained or steered molecular dynamics. The results of these calculations reveal that CNTs with hydrophilic ends are potentially stable in a lipid bilayer.

Lopez et al. first demonstrated, using coarse-grained molecular dynamics, that small-diameter CNTs ~1.3 nm in diameter, 3 nm in length, and functionalized with hydrophilic termini, can spontaneously insert into and align across a lipid bilayer. Their nanopores are terminated at each end with generic hydrophilic units constructed from coarse-grain water sites. The hydrophilic ends are crucial for stability, because close matching to the hydrophobic and hydrophilic domains of the bilayer is necessary to avoid pore blocking. Insertion of a purely hydrophobic NT results in the NT remaining in the hydrophobic core of the bilayer, parallel to the bilayer plane. Lopez et al observe a lipid-assisted mechanism for the passive insertion of these hydrophilic-terminated CNTs, as shown in Figure 1. An animation of the lipid-assisted mechanism is available as supplementary information in Lopez et al.

Subsequently, Shi et al. investigated single-walled CNTs with diameters from 1 to 1.5 nm and lengths of 6 nm. Their NTs are purely hydrophobic, because they are not terminated with hydrophilic functional groups. They found that small-diameter tubes enter the membrane via a piercing mechanism, and for larger diameter NTs the bilayer changes shape to wrap around the tube.

According to a coarse-grained molecular dynamics study by Nielsen et al. close matching to the hydrophobic and hydrophilic domains is crucial for stability in the bilayer. The bilayer thickness thins as it approaches contact with a NT, and as the ratio of NT radius to length decreases, tilting of the tube in the bilayer becomes more pronounced. These findings are in accord with those reported previously by Lopez et al.

Wallace and Sansom, on the other hand, used steered coarse-grained molecular dynamics to investigate bilayer penetration by a hydrophobic NT. They simulated NTs with diameters from 1.4 to 6.1 nm and lengths of 5 and 10 nm. Their simulation results are in broad agreement with a recent experimental study, in which the force required to push
large-diameter NTs into a living cell is measured using atomic force microscopy. As expected, the force required to penetrate the bilayer is shown to monotonically increase with an increase in NT diameter. Moreover, they found that there is no apparent effect on bilayer integrity following NT penetration.

Similar to Wallace and Sansom, Hilder et al used steered molecular dynamics, this time using an all-atom force field. They investigated the stability of a CNT 0.9 nm in diameter and with a length of 3.4 nm in a lipid bilayer. Because all atoms were explicitly represented, rather than a generic hydrophilic terminus, their NTs were terminated with carbonyl groups (C = O). In agreement with Lopez et al., they determined a deep energy well of 250–300 kcal mol\(^{-1}\), suggesting that the NT is likely to be confined to the center of the lipid bilayer.

Studies on water flow across NTs

The flow of water through synthetic NTs has been extensively investigated using computer simulations. These studies have illustrated that synthetic NTs are capable of conducting water molecules exceptionally fast, while rejecting charged particles. The results of these theoretical studies indicate that membranes embedded with NTs may one day be used for water purification or desalination. Given the exciting possibilities presented by these NT-based membranes, researchers are beginning to focus on their fabrication and testing. By functionalizing their ends or external surface, a synthetic NT can be embedded in various membranes. Recent techniques have enabled the arrangement of aligned CNTs within membranes such as polymers and silicon nitride (Si\(_3\)N\(_4\)). Figure 2 illustrates a schematic of a CNT-Si\(_3\)N\(_4\) membrane. Such a membrane exhibits an enhanced mechanical strength, thus enabling it to withstand large applied pressures. However, these experimental studies have predominantly focused on the flow of water molecules only. In agreement with theoretical studies, experiments have demonstrated exceptionally fast water flow rates, which have been attributed to the near-frictionless surface of the NT wall. Recently, because of the increasing interest in these NT-based membranes for desalination, Fornasiero et al investigated their ion exclusion capability. Their <2-nm-diameter CNTs embedded in Si\(_3\)N\(_4\) could achieve ion exclusion as high as 98%, but at salt concentrations of 10 mM ions were no longer excluded. In more recent work, Fornasiero et al demonstrate experimentally that the ion permeability and exclusion through these NTs can be controlled by adjusting the solution pH, which affects the ionized carboxylic groups at the NT opening.

Water flow across NTs has been extensively investigated theoretically. In their molecular dynamics simulations, Hummer et al observe spontaneous and continuous filling of a CNT 0.8 nm in diameter with a single-file chain of water molecules, illustrated in Figure 3. In the absence of an applied pressure, and with no ions present, water is found to flow in both directions at a rate of 17 water molecules per nanosecond. Moreover, the filling or emptying of these NTs occurs by sequential adding or removing of water molecules to or from the single-file hydrogen-bonded chain, and this is shown to occur rapidly. As a comparison, aquaporin-1 conducts at a rate of 3 water molecules per nanosecond. This initial finding, that a CNT is populated with a single-file chain of water molecules, has been confirmed in ab initio simulations. Mann and Halls observe a single-file chain of water molecules inside a CNT 0.8 nm in diameter. In contrast to the biological water channel aquaporin-1, proton conduction can occur across CNTs 0.8 nm in diameter under the influence of an applied electric field. Similar results have been obtained by Garate et al. Aquaporin-1 allows the rapid flow of water with no proton transport, which is postulated to result from the transient reversal of the water chain due to residues lining the pore.

Kalra et al studied, using molecular dynamics simulations, the osmotically driven transport of water through a hexagonally packed array of 0.8-nm-diameter CNTs, as illustrated in Figure 4. They, too, obtained a single-file water chain with a flow rate of
5.8 water molecules per nanosecond, twice that of aquaporin-1. Their simulations reveal that the water flow rate is independent of NT length, suggesting that permeant water molecules encounter virtually no friction. The rate-limiting step appears to be the barrier presented to the water at the entry and exit of the pore. Similarly, the transport of water through arrays of CNTs ranging in diameter from 0.6 to 1.1 nm was investigated by Corry. He found that ions face a large energy barrier and do not enter 0.68- and 0.8-nm-diameter CNTs. However, as the diameter increases above 0.95 nm, ions can pass through. In contrast, water molecules conduct through all NTs studied, with the conductance increasing with increasing diameter. Similar to Kalra et al., the conduction was independent of NT length. This work suggests that 0.95-nm-diameter CNTs could obtain 95% salt rejection with a water flow rate much faster than existing semipermeable membranes used in desalination.

In recent work Corry examined water and ion transport through CNT-based membranes. This work aimed to investigate whether larger diameter (1.1 nm) NTs could be made to reject ions by attaching various charged and polar functional groups to the NT ends. Although the addition of charges is shown to prevent the passage of ions, it also reduces the flow of water through the membrane. Despite this the performance of these membranes was shown to be many times better than existing desalination membranes.

Alternatively, double-walled CNTs have been proposed as artificial water channels by Liu et al. This would enable functionalization to be included along the length of the inner NT while avoiding hydrophobic mismatch of the hydrophobic and hydrophilic domains of the lipid bilayer. The structure outside the NT has been shown to greatly affect water permeation across the channel. Thus, use of a double-walled CNT enables protection of the inner tube from the disturbance of the membrane environment.

It is possible to fabricate NTs from a range of materials, such as from boron and nitrogen atoms to form BN NTs. As yet there are no experimental studies that investigate the flow of water through BN NTs. However, theoretical studies have shown BN NTs to have superior water permeation properties when compared to CNTs. Water molecules encounter a lower energy barrier at the pore entrance of a BN NT as a result of the stronger van der Waals interaction between the BN NT and water. For example, a BN NT with a diameter of 0.69 nm can conduct water, whereas a similar-sized CNT has only intermittent filling of water. Similarly, Suk et al. finds that a higher water flux exists through a BN NT as compared to a CNT, and both NTs had significantly higher flux than that of a polymethyl methacrylate pore.

Water conduction and salt rejection through BN NTs embedded in a Si₃N₄ membrane was investigated by Hilder et al. They find that a BN NT 0.69 nm in diameter embedded in a Si₃N₄ membrane can, in principle, obtain 100% salt rejection at concentrations of seawater (500 mM) while allowing water to flow at rates as high as 10.7 water molecules per nanosecond. Moreover, ions continue to be rejected at hydrostatic pressures as high as 612 MPa. When the NT diameter is increased above 0.83 nm, ions can enter the tube. By assuming a pore density of 2.5 × 10¹¹ cm⁻² and at an operating pressure of 5.5 MPa, Hilder et al predict water flow rates approximately four times that of a commercially available reverse-osmosis element.

Most computational studies on these synthetic NTs are conducted using a nonpolarizable force field. This has been shown to be a reasonable approximation, in that the effect of water on a polarizable NT is negligible and never accounts for more than 8% of the total interaction energy. In contrast, in the biological channel gramicidin the polarization of water is important in calculating the interaction energy.

**Mimicking ion-selective biological ion channels**

Although most research has focused on the use of synthetic NTs as water channels, increasing attention has been given to the design of ion-selective synthetic NT channels that could potentially be useful in unique devices such as ultrasensitive biosensors, and may help to gain insight into complex biological ion channel processes. As a result of the relatively recent interest in ion-selective NTs and the degree of difficulty in fabricating such tubes, there are very few related experimental studies. Therefore, initial theoretical studies may prove to be useful to facilitate further experimental studies. In this section we first highlight recent studies that have demonstrated the potential of NTs to nonselectively exclude or conduct ions. Following this, we review research studies that have achieved exclusive selectivity to positively charged (cations) and negatively charged (anions) ions.
Peter and Hummer\textsuperscript{54} illustrate using molecular dynamics simulations that nonpolar pores less than 0.5 nm in diameter can successfully block ions, but increasing the pore diameter to 1 nm allows the passage of ions. They observe a drastic increase in the diffusion coefficient of ions once the tube diameter is above this critical value, and this critical diameter can also be linked to changes in the water structure within the NT. This critical diameter has also been noted in a study by Beu,\textsuperscript{55} which included a polarizable model for water and solute ions. Beu found that polarizability has a negligible influence on the ionic currents through CNTs 6 nm in length with diameters ranging from 1 to 1.6 nm. Ions are thought to move through the tube almost frictionlessly, but as ions enter the NT they encounter an energy barrier that increases as the pore radius decreases.\textsuperscript{55} As a result, ion currents show a strong dependence on pore radius. In recent experiments, Yu et al\textsuperscript{56} report on the gated ionic diffusion through densely packed CNT membranes with both 3-nm NT and 3-nm interstitial pore diameters. At a temperature of 298 K, ions become trapped in the membrane by discontinuous water clusters. However, ion diffusion rates were shown to increase upon increasing the temperature or exposing the membrane to ultrasound. Although the membrane is able to gate the flow of ions, it is not selective to a particular ion type.

It is possible to increase ion occupancy within a CNT by placing a rim of partial charges at the NT ends.\textsuperscript{57} In molecular dynamics simulations Joseph et al\textsuperscript{57} demonstrated a fourfold increase in ion occupancy in a CNT 2.2 nm in diameter and 1.3 nm long, using a partial charge of ±0.38 e placed at the NT rim.

\begin{figure}[h]
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\caption{Schematic of ion separation using a Y-junction carbon nanotube (CNT). Reproduced in part with permission from Joseph et al.\textsuperscript{57} Copyright 2006. IOP Publishing Ltd.}
\end{figure}

Atoms. Fornasiero et al\textsuperscript{37} were able to control ion transport through fabricated CNT pores 0.8 to 2.6 nm in diameter embedded in a Si$_3$N$_4$ matrix. Following Joseph et al,\textsuperscript{57} their NT ends were terminated with negatively charged carboxylic groups. By varying the solution pH they were able to modulate the NT tip charges so that the membrane would either reject ions or permeate ions. In addition, they found that an increase in the number of multivalent ions in the solution decreased the rejection capability toward monovalent ions.

The above research illustrates the potential of NTs to exclude or conduct ions depending on diameter and environmental factors, but these studies are not selective to a particular ion type. In other words, cations will flow in one direction and anions in the opposite direction across these tubes under the influence of an applied field. In the following sections we outline research in which exclusive cation and anion selectivity is achieved. For example, Park et al\textsuperscript{58} designed a novel ion separator using a Y-junction CNT, illustrated in Figure 5. They demonstrated, using molecular dynamics, the ability to separate potassium and chloride ions from KCl solution by applying a negative and positive surface charge density to the two branches, respectively. Park et al suggest that a Y-junction CNT could be used as a fluidic component in complex fluid networks and nanoscale lab-on-chip applications.

\textbf{Cation-selective channels}

Majumder et al\textsuperscript{59} fabricate membranes constructed from vertically aligned CNTs ~7 nm in diameter embedded in a polystyrene matrix and investigate the effect of anionically charged functional groups at the NT open ends. They show that these charged groups increase the flux of cationic species approximately fourfold compared to their unmodified CNT membrane. The effects of an applied voltage across the NT-embedded membrane are investigated in subsequent work.\textsuperscript{60} Their membranes exhibit voltage-gated control of ionic transport through the cores of the NTs. For example, at a positive bias the tethered tips are drawn into the NT, occluding the pore. They demonstrate the effectiveness of such a device using a relatively large cation, ruthenium bi-pyridine (Ru(bipy)$_3$$^{2+}$). For example, increasing the voltage from 0 mV to 50 mV reduces the flux of Ru(bipy)$_3$$^{2+}$ to ~25%.

In a molecular dynamics computational study by Yang and Garde,\textsuperscript{61} a negative charge density of −$n$ e is uniformly distributed to all atoms of a CNT 0.67 nm in diameter and 1.1 nm in length (comprising 100 carbon atoms), so that each atom carries a charge of −$n$/100 e. When the surface charge is −5 e the NT interior becomes favorable to all three investigated cations, namely potassium, cesium, and sodium. In contrast, when the surface charge is reduced to −2 e no cations enter the tube. At a charge of −3 e, sodium ions take 25 times longer to enter the NT than potassium and cesium ions. Studies such as these may aid better design of high-energy storage devices.

Again using molecular dynamics, Liu et al\textsuperscript{62} demonstrate that a hexagonal array of noncharged CNTs, each with an effective diameter of 0.44 nm, is able to discriminately select potassium ions over sodium in the presence of an applied pressure of 5 MPa. They suggest that the key to this ion selectivity is the...
higher binding energy of water molecules in the first hydration shell of sodium, which renders it more stable, and therefore sodium has a higher desolvation energy, making it more difficult to permeate through a narrow NT. The flexibility of the hydration shell of a potassium ion enables it to maintain better coordination within the NT.\textsuperscript{62-64} Similarly, Song and Corry\textsuperscript{65} showed that narrow CNTs with no functionalization have intrinsic ion selectivity. Dehydration energy plays a dominant role in determining selectivity in narrow pores, because in narrow pores all ions have to be largely dehydrated; for example, because sodium has a larger dehydration energy, it faces a steeper barrier to enter the tube. Although the work of Liu et al\textsuperscript{62} presents interesting possibilities, the simulation times of only 0.35 ns are too small to have confidence in the result. In similar work, they find that positive or negative ions will exit the NT only when one or two other ions of the same charge are present in the tube, suggesting that the permeation mechanism involves the presence of multiple ions and Coulomb repulsion.\textsuperscript{66}

A single-walled CNT of 1.2 nm diameter and 1.34 nm length is shown to discriminate Na\textsuperscript{+} over K\textsuperscript{+} or vice versa, depending on the patterns of carbonyl oxygens placed on its interior surface.\textsuperscript{67} The positions of the carbonyl oxygens in the molecular dynamics study by Gong et al\textsuperscript{67} are chosen such that they mimic the arrangement of amino acids in the selectivity filter of the biological potassium channel. Unfortunately, the investigated NTs are not exclusively selective, so that although preference will result for one ion type, the other will also traverse the NT.

Recently, Lee et al\textsuperscript{16} fabricated single-walled CNT ion channels with negatively charged ends ~1.5 nm in diameter that conduct protons and are blocked by cations present in the surrounding electrolyte solution. Moreover, they observe complete rejection of ions for voltages <100 mV. Once the electric field is large enough the larger cations move across the channel, and this blocks the flow of protons. These synthetic nanopores are therefore capable of single-ion detection.

For the first time, it has been demonstrated theoretically that a functionalized CNT embedded in a lipid bilayer can selectively allow cations to move across under the influence of an applied electric field.\textsuperscript{25} The NT, 0.91 nm in diameter and 3.6 nm in length, is functionalized with hydrogen at the ends and in two thin circular rings near the entrances of the NT, as illustrated in Figure 6. This NT is selectively permeable to monovalent cations, binds divalent cations, and rejects anions. The characteristics of this channel resemble the antibiotic gramicidin A, one of the first antibiotics used clinically, but the potassium current is six times larger. Moreover, Hilder et al\textsuperscript{68} designed a CNT 1 nm in diameter and 3.6 nm in length that is functionalized with carboxylic acid at the ends. In the presence of an electric field the NT exhibits the ability to selectively conduct cations by an ion chaperoning mechanism, whereby a chloride ion ferries a sodium ion across the channel.

Anion-selective channels

A number of studies have demonstrated the ability of synthetic NTs to be selective to cations. In comparison, few have demonstrated anion selectivity. Combining density functional theory and molecular dynamics simulations, Won and Aluru\textsuperscript{69} designed a single-walled BN NT with a diameter of 1.36 nm that demonstrates selectivity of chloride over potassium ions. They found that although both ions can enter the NT, chloride ions are 10 times more likely to be observed inside the NT. In contrast, they find that a similar-sized CNT selectively transports potassium ions. Water within the BN NT was shown to orient such that there exists a net positive charge at the center of the NT, facilitating the attraction for negatively charged chloride ions.

In recent studies that combine molecular and stochastic dynamics, Hilder et al\textsuperscript{28} designed CNTs with an effective...
diameter of 0.9 nm and terminated with carbonyl functional groups that were shown to be exclusively selective to chloride ions. Figure 7 illustrates schematically the carbonyl-terminated CNT embedded in a lipid bilayer. Moreover, these NTs broadly mimic the functions of biological chloride channels but with a chloride conductance four and two times larger than through the CLC-1 and GABA\textsubscript{A} biological chloride channels, respectively.

Future perspectives

Synthetic NTs show enormous potential as artificial ion channels in both theoretical and experimental work. It is now possible to fabricate and design synthetic NTs that are selectively permeable to water molecules, cations, or anions. Although research into mimicking biological ion channels has recently gained momentum, it is still very much in its infancy and much work needs to be done before practical applications will be realized. With the work of Fornasiero et al., the development of desalination membranes incorporating NTs is closer to being achieved than any other application. For the application of these synthetic ion channels in a biological setting to be realized, it is vital that there be more studies confirming theoretical work and confirming their lack of toxicity. It is hoped that this review will facilitate interest and expedite progress in this unique field of research.

References