Supplementary Information

The bionic water channel of ultra-short and high-affinity carbon nanotubes with high water permeability and proton selectivity

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Materials and Methods

Experimental Materials

In this study, ultrapure water with a resistivity of 18.2 MΩ·cm was prepared through an ultrapure water system. NaCl, KCl, $MgCl_2$, NaOH, HEPES buffer solution(100 mM NaCl, 10 mM HEPES, pH 7.0), chloroform agarose, sucrose, glycerol TBE buffer, tryptone, TB (Terrific Broth) medium, antibiotics, absolute ethanol, methanol, polycarbonate with a purity of more than 99% Membrane, dodecyl-β-D-maltoside, Triton®X-100, and dimethyl sulfoxide were all purchased from Aladdin. The chemicals used in AQPs expression and purification involve isopropyl-β-D-1-thiogalactoside, chloramphenicol, ampicillin, lysozyme, Tris, honey Pinease, Ni-NTA resin, β-mercaptoethanol, which purchased from Sigma-Aldrich. The n-octyl-β-D-glucopyranoside (OG, purchased from Aladdin) used as a detergent during the preparation of proteoliposomes.1,2-dioleoyl-sn-glycero-3-phosphocholine(DOPC), cholesterol, E. coli extract lipid, E. coli sensory Cell strain C41-pLysS, monolauryl phosphate, lauryl ether phosphate, valinomycin, imidazole, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) were from Shanghai Sangon Biotech. CNTs with a diameter of 1.5 nm were purchased from Shenzhen Nano Harbor.

Preparation of Homogeneous Liposomes

The liposomes of 10mM DOPC supplemented with 10% cholesterol were dissolved in chloroform to a concentration of 5 mg/mL [29]. In order to build a uniform lipid film on the inner wall of the flask, evaporating 1ml of DOPC solution containing chloroform in a 5ml round-bottomed flask. This process must be carried out under N2 blowing. The remaining liposomes were placed in a vacuum desiccator and evaporated for 2 hours to dry and remove the chloroform that was not exhausted. Then, the lipid membrane was hydrated at 40°C for 2 hours with the buffer solution HEPES (2 mL, 100 mM NaCl, 10 mM HEPES, pH 7.0) to obtain a milky suspension. Then the sample was frozen in liquid nitrogen (-196°C) for 3 minutes, and quickly transferring it to a 50°C water bath for 3 minutes. Reciprocating 10 times to make the obtained liposomes more stable. Subsequently, the solution was sonicated by using a sophisticated sonicator at 8 W for 5 minutes until the solution began to become transparent, forming liposomes and it was purified by centrifugation at 1500rpm for 5 minutes. A DOPC vesicle solution with a concentration of 10mM was obtained finally. The solution was kept at -80°C.

Morphological Observation of Liposomes

Using a pipette to absorb 1 microliter of the prepared liposome solution and dropping it on a carbon-coated Cu400TEM grid used for glow discharge to form a carrier and it was dyed with 2% uranyl methyl formate solution containing 25 mM NaOH. The transmission electron microscope was operated at 100kV for imaging. An AMT 4×4pixel CCD camera was used to acquire the image. Using the 2D catalase crystal lattice constant as the length reference to calibrate the microscopic image scale. Browsing images at 11500× magnification and imaging most images at 28500× magnification.

Reconstituted Liposomes Inserted into Water Channels

The expression and purification of AQPs refer to the previous method [4]. In this study, detergent-mediated remodeling was used to insert AQPs into lipid membranes. During the membrane hydration step, 5μL of the AQPs stock solution was added to the liposome-detergent (5 mg/mL, 9 mM dodecyl-β-D-maltoside, DDM) mixture and incubated at room temperature for 45 minutes. Thoroughly wash through polystyrene gel (Bio-beads S-X3), including 2 washes with methanol (100%) and 3 washes with ultrapure water to ensure complete removal of detergent to form proteoliposomes, and then in HEPES Equilibrate in buffer solution (1.5mL, pH 7.8, 10 mM HEPES, 100 mM NaCl). Adding biological beads (160 mg/mL) to the lipid-AQPs-detergent mixture and stirring gently at room temperature for 6 hours. During this period, transferring the solution to a new batch of Biobeads every 1 hour, and then at 4°C Gentle mixing with the new batch of Bio-beads finally prepared liposome polymer bodies incorporating AQPs. UV-Vis spectroscopy was used to ensure the successful incorporation of AQPs into liposomes. The sample was passed through a SEC column, eluted with 10 mM HEPES buffer pH 7.8, and the fractions containing liposomes were combined. Before the water permeability measurement, the proteoliposome was diluted 3 times. Finally, the protein-containing reconstituted liposome solution was extruded through a polycarbonate membrane with a diameter of 400 nm and stored at -4°C.

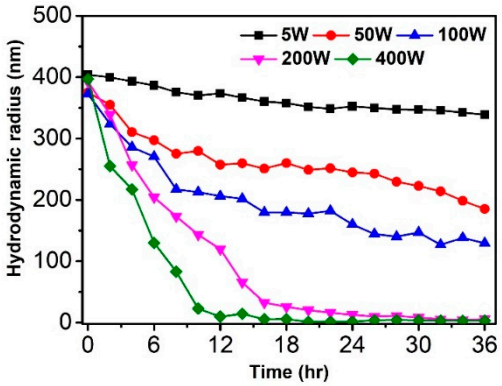
Reconstituted liposomes containing CNTCs: 10 mM DOPC lipid supplemented with 10% cholesterol were dissolved in chloroform to a concentration of 5 mg/mL. A rotary evaporator was used to evaporate 1ml of DOPC solution in a 5 mL round bottom flask to build a lipid membrane. Storing the DOPC membrane in a vacuum desiccator for at least 2 hours to evaporate the remaining chloroform. In order to incorporate CNTCs into liposomes, 2mL of CNTCs solution was firstly dried overnight in a vacuum dryer to get rid of the solvent. The dried CNTs membrane was mixed with HEPES buffer (1ml) and sonicated for 30 seconds to ensure that the membrane was completely dissolved and separated from the glass bottle. The lipid membrane was hydrated at 40°C for 2 hours with the buffer solution HEPES, and the CNTCs solution was added during this process to obtain a milky suspension. The sample was frozen in liquid nitrogen (-196°C) for 3 minutes, and then put it in a water bath for 3 minutes (50°C). Reciprocating this process for 10 times. The obtained sample was subjected to 21 times extruder filtration with a 400nm polycarbonate filter membrane to obtain a liposome solution with a low polydispersity index (PDI). Size exclusion chromatography (SEC) was performed with a column involving Sepharose CL-6B agarose to separate the unincorporated free CNTCs from the reconstituted liposome mixed solution and collect fractions in 96-well plates. At the same time, 10mM HEPES, pH7.8 buffer was used as the eluent. Finally, the reconstituted liposomes were kept at -4°C.

Ion Selectivity Test of CNTCs

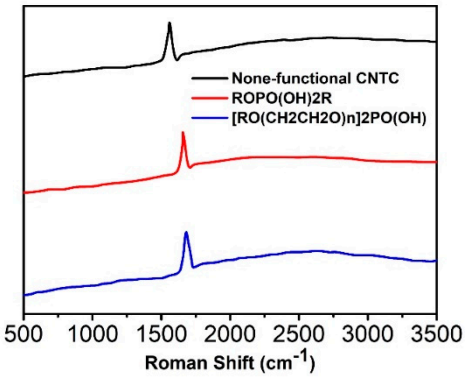
Preparation of liposomes containing fluorescent dyes (HPTS): The mixture of 225μl EYPC chloroform solution (100mg/ml, 30μmol) and 60μl EYPG chloroform solution (20mg/ml, 1.5μmol) were evaporated in a 5 ml flask with a rotary evaporator, and then dried in high vacuum for 3 hours. Using HEPES buffer solution containing HPTS (0.1 mM) hydrated at 40°C for 2 hours to obtain a milky suspension. The sample was put in liquid nitrogen (-196°C) and frozen for 3 minutes, then transferring it to a 50°C water bath for 3 minutes. Reciprocating this process for 10 times. Uniform liposomes are obtained by ultrasonic treatment. Size exclusion

chromatography was used to separate the liposomes suspension from the extravesicular dye. Using UV-Vis spectroscopy to verify whether the dye is exhausted. Afterwards, the liposomes were purified by centrifugation at 1500rpm for 5 minutes. 10 mM vesicle suspension was obtained by diluting with HEPES buffer eventually.

The vesicular suspension (104 μL) was placed in a fluorometric cell and diluted to 3040μl with a suitable buffer solution (20 mM HEPES, 100 mM NaC1, pH 7.0). The total liposomes concentration in the fluorescence measurement solution was 0.17mM. Then an aliquot of the DMSO solution of the CNTCs were added to the liposomes suspension and incubated at 25°C for 30 minutes. After incubation, the time course of fluorescence was recorded for 200 seconds, and then 50μL of 0.5M NaOH was quickly added to the vesicular suspension, and recording the fluorescence emission for 1200 seconds. The maximum change in dye emission is gained by finally lysing liposomes with detergent (5% Triton®X-100, 40μL).

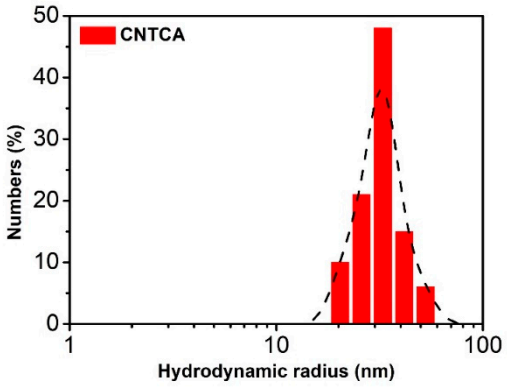


Supplementary Fig.1. The relationship between the length of CNTC and time under different ultrasonic power. It can be seen from the figure that under 200W power, the length of CNTC has a linear decreasing relationship with time.

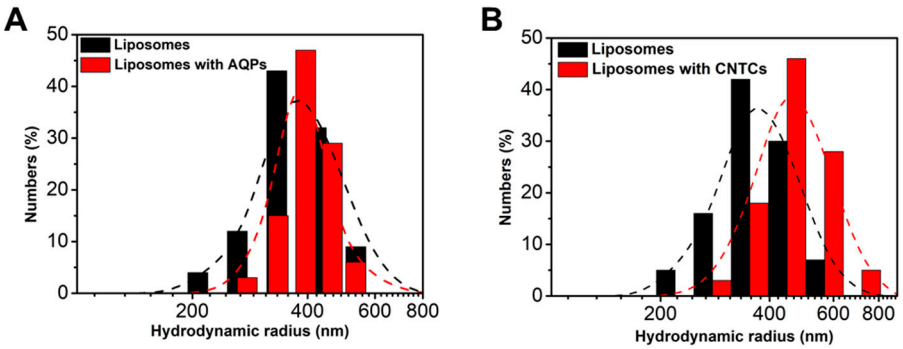


Supplementary Fig.2. Raman spectrum (black curve) of the unsuccessfully functionalized CNTC. Raman spectrum (red curve) of the sample after functionalization of CNTC with monolauryl phosphate as the active agent. Raman spectrum (blue curve) of the sample after functionalized CNTC with alcohol ether

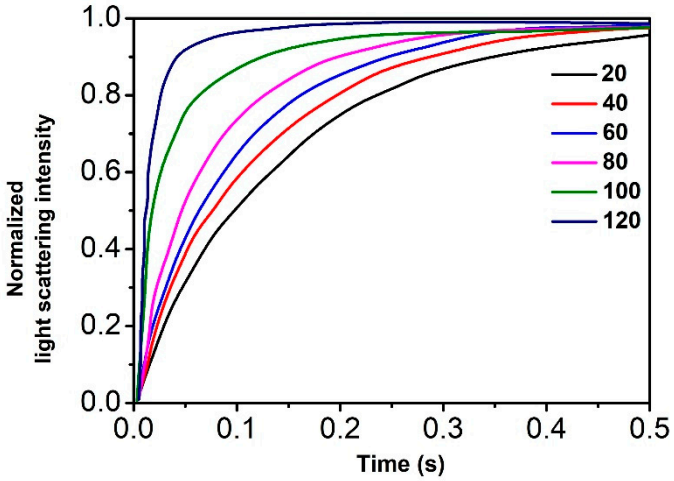
phosphate as the active agent.



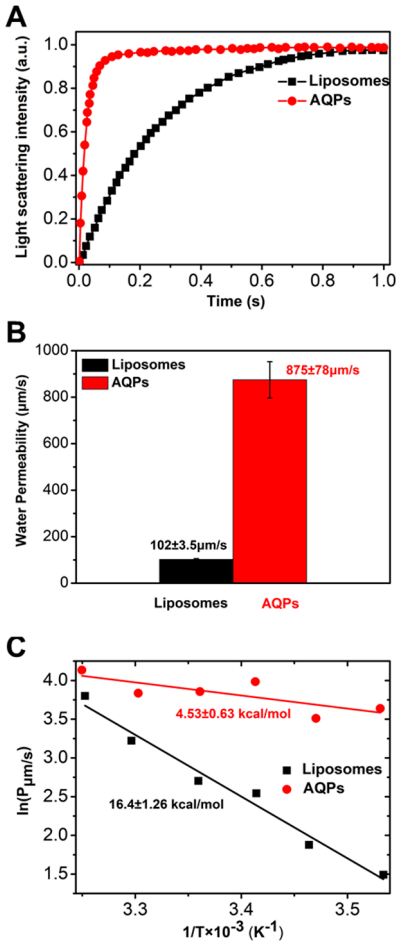
Supplementary Fig.3. Kinetic size of CNTCA sample.



Supplementary Fig.4. The change in particle size of liposomes after insertion into the water channel. The change in the particle size of liposomes after insertion of AQPs (Fig.7A); the change in the size of liposomes after insertion of CNTC (Fig.7B). It can be seen from the figure that the particle size distribution of the vesicles is almost unchanged after being inserted into the water channel, that is, the insertion of the water channel has no obvious effect on the vesicles.

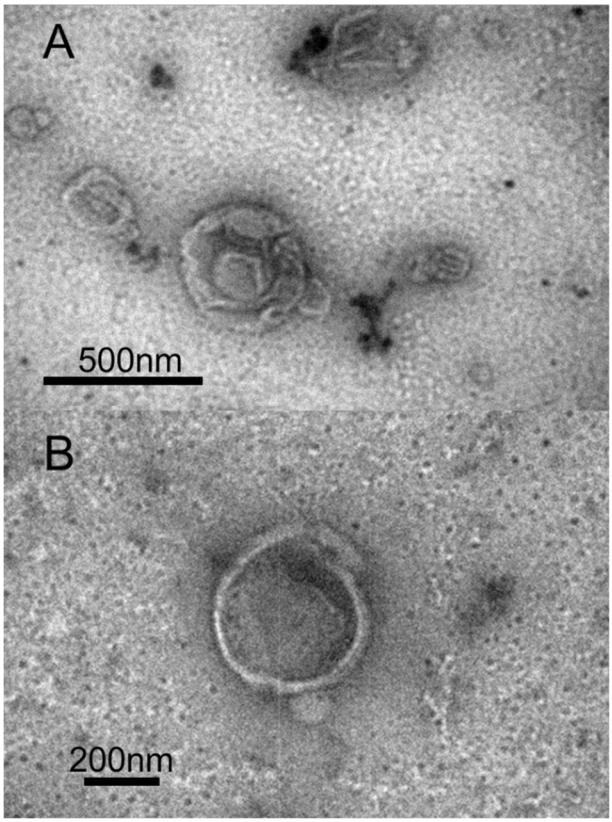


Supplementary Fig.5. Light scattering curve of CNTC under different osmotic pressure gradient.

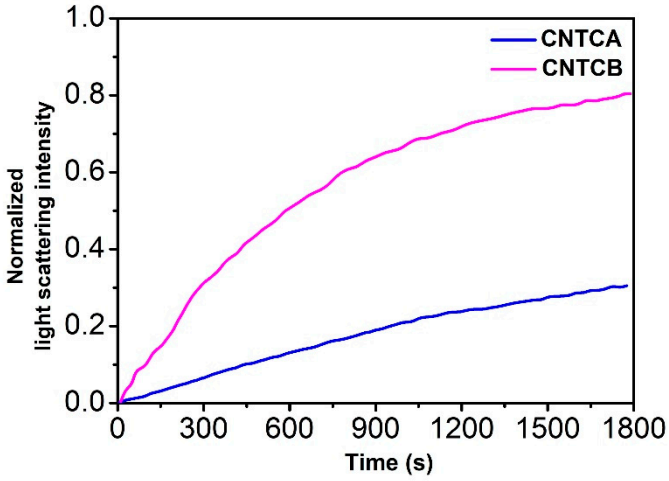


Supplementary Fig.6. Light scattering curves of DOPC and AQPs (Figure A); water permeability of DOPC and AQPs calculated based on light scattering curves (Figure B); Arrhenius diagram showing the water permeability of DOPC liposomes and

AQPs channels Relationship with temperature. All values are measured under neutral conditions, and the activation energy determined according to the slope of the linear regression of the data is consistent with the previously reported values of DOPC liposomes and AQPs channel.



Supplementary Fig.7. Liposomes were stained with phosphotungstic acid (2%) and morphologically characterized by TEM.As shown in the figure, the liposomes are approximately round with a particle size distribution around 400 nm.



Supplementary Fig.8. Fluorescence curve of proton selectivity of CNTCA and

CNTCB samples.