Supporting Information

An ion transport switch based on light-responsive conformation-dependent G-quadruplex transmembrane channels

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Experimental Procedures

S1. Materials and reagents

The azobenzene-incorporated DNA oligonucleotides were purchased from Sangon Biotech. DNA synthesis was performed by solid-phase phosphonamidite method, purified by high-performance liquid chromatography and analysed by mass spectrometry (MS). 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, 99%), 8-hydroxyindole-1,3,6-trisulphonic acid trisodium (HPTS, 87%) and were obtained from Sigma Aldrich. All solutions were prepared in MilliQ water (18.2 MΩ).

S2. Instrumentation and characterisation

Absorption measurements were conducted using a Shimadzu UV-vis 1601 spectrophotometer. Fluorescence emission spectra were performed using a Hitachi F-7000 fluorescence spectrophotometer. Circular dichroism (CD) spectra were generated using a MOS-500 spectropolarimeter. The DDSJ-318F conductivity instrument was used to measure conductivity with a conductivity constant k = 10, stability constant α = 0.05. Light source is 15 W xenon lamp (PLS-SXE300, Trusttech Ltd Co., Beijing).

S3. UV/Vis-light mediated G-quadruplex formation

The gradual introduction of K^+ ions (0–100 mM) into the AzoG4 ssDNA sequences (2.0 μM), UV- or Vis-light alternative irradiation of the sample for 30 min. UV irradiation (λmax = 365 nm) of the sample was performed in a custom-built irradiation setup with 15 W power each (Nichia NCCU033(T)). A light source from Müller Elektronik Optik was used with a xenon arc lamp for Vis-light irradiation.

S4. Fluorescent imaging of giant unilamellar vesicles (GUVs)

GUVs were prepared according to our previous report.[31,32] FAM-labelled AzoG4 (FAM-AzoG4, 4.34 μmol) was added to 10 mL of GUV suspensions, and the solutions were incubated for 5 h at room temperature. The fluorescence staining of GUVs was visualised using confocal fluorescence microscopy. The FAM dye was excited using a laser at 488 nm, and the emission bands were set at 500–550 nm. The obtained images were processed using ImageJ.

S5. 8-Hydroxypyrene-1,3,6-trisulfonic acid (HPTS) assay in large unilamellar vesicles (LUVs)

LUVs were prepared using a lipid film hydration method in a Tris-HCl buffer.[31,32] The HPTS-filled LUV stock solutions were diluted with 200 μL Tris-HCl buffer containing 2.25 mM DPPC in cuvettes. Appropriate volumes of the UV or Vis-light irradiation AzoG4 with G-quadruplex ion channels (5, 10 μM) were subsequently added to the cuvettes, and the solutions were stirred for 1–2 min. The pH was subsequently raised by 1.0 unit using an aqueous KOH solution (3 μL, 1 M), and the fluorescence intensity of the HPTS dye was monitored in real-time at 520 nm. Finally, the maximum changes in the dye emission were obtained at the end of each experiment by lysing the vesicles using a detergent (5 μL of 5% aqueous Triton X-100). The final transport trace was obtained as a ratio of the emission intensities monitored at 460 nm and normalised to 100 % of ion transport.

S6. Conductivity recording in U-type cells

A lightning DDSJ-318F conductivity metre was used to measure the ionic conductivity. The track-etched polyethylene terephthalate membrane (PET, Hostaphan RN12 Hoechst, 5 μm thick, with single ion track in the centre) was mounted between the two half-cells of the feed and permeation half-cell. A chloroform solution of DPPC/EYPC was dried under N_2 and then re-suspended in decane (25 mg/mL) before this process. The lipid solution was used to precoat and form a planar lipid bilayer membrane on the PET membrane. Then, the AzoG4 carrier was used to coat on the lipid bilayer membrane and embedded in a membrane.

Furthermore, feed half-cell was filled with KCl standard electrolyte solution, and permeation half-cell was filled with similar volume MilliQ water. Black glass electrodes were used to apply a transmembrane potential across the film. The main conductivity of permeation half-cell used in this work was measured by lightning DDSJ-318F conductivity. The process and conditions of all the measurements mentioned in this communication were the same unless otherwise stated, and each test was repeated three times to obtain the average current value.

Figures

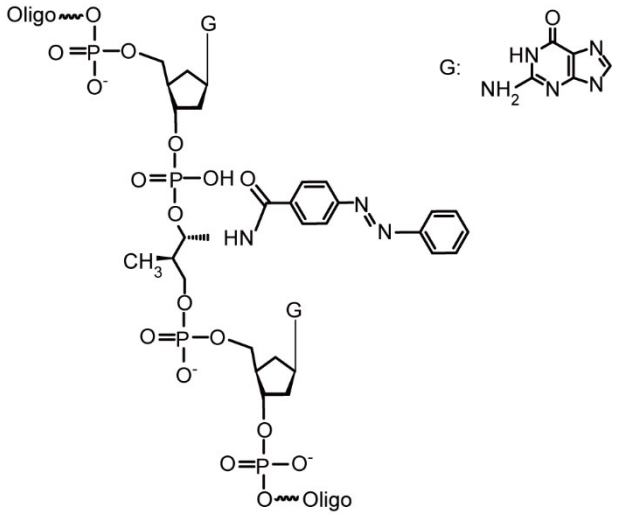

Figure S1. Scheme of azobenzene- conjugated phosphoric acid skeleton of AzoG4.

A

Oligonucleotide sequences used in this work.

Entry	Sequence (from 5' to 3')
AzoG4	GGG-Azo-GGG-Chol
FAM-AzoG4	FAM-GGG-Azo-GGG-Chol

Azo, Chol and FAM indicated the Azobenzene, Cholsterol and FAM fluorescent dyes that conjugated to the sequences through solid phase phosphonamidite method.

B

AzoG4

Calculated MW	Measured MW	Relative error (ppm)
2288.61	2288.7	0.01%

FAM-AzoG4

Calculated MW	Measured MW	Relative error (ppm)
3452.21	3450.4	0.05%

C

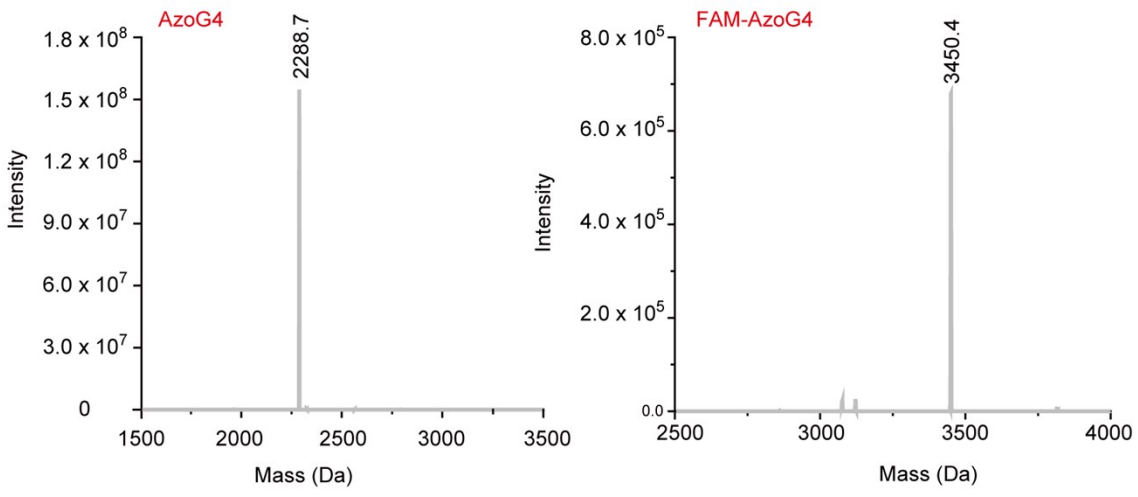

Figure S2. Characterization of oligonucleotides. (A) Oligonucleotide sequences used in this work: AzoG4, FAM-AzoG4. Azobenzene and FAM are conjugated to the nucleotides during DNA solid phase synthesis based on the phosphoramidite method. (B) List of molecule weight of different DNA sequences. (C) ESI-MS for different DNA sequences. The molecular weights determined from ESI-MS were all consistent with the calculated values. Experimental conditions: DNA is dissolved in isopropanol solution, single electron, 10-2000 amu, Negative ion multi-charge mode. AzoG4/FAM-AzoG4: 1 μM.

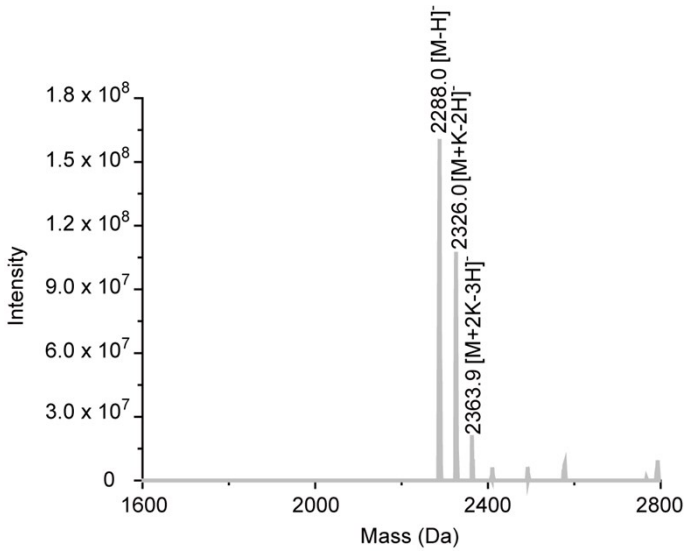

Figure S3. ESI-MS of 1 μM AzoG4 in the presence of 50 mM K^+; ESI-MS experiments in the negative-ion mode, three distinct peaks at m/z = 2288.0, 2326.0 and 2363.9 were determined for K^+ stabilized AzoG4. The former corresponded to $[M-H]^-$ and the latter corresponded to $[M+K-2H]^-$ and $[M+2K-3H]^-$, which indicated the presence of one or two K^+ ions bound with AzoG4.

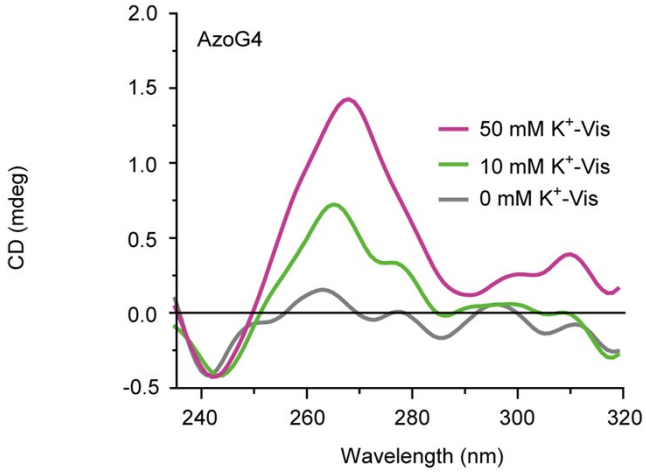

Figure S4. Circular dichroism (CD) spectra of K^+ stabilized AzoG4 G-quadruplex complex after addition of different concentrations of K^+ ions in homogeneous solution. The total sample volume is 200 μL, K^+: 0, 10, 100 mM. AzoG4: 2 μM. An instrument scanning speed of 100 nm/min, a 1 nm pitch, and a 1 nm bandwidth, with a response time of 0.05 s, over a wavelength range of 235-320 nm, Experimental conditions: 20 mM Tris-HCl (pH 7.14), 298 K.

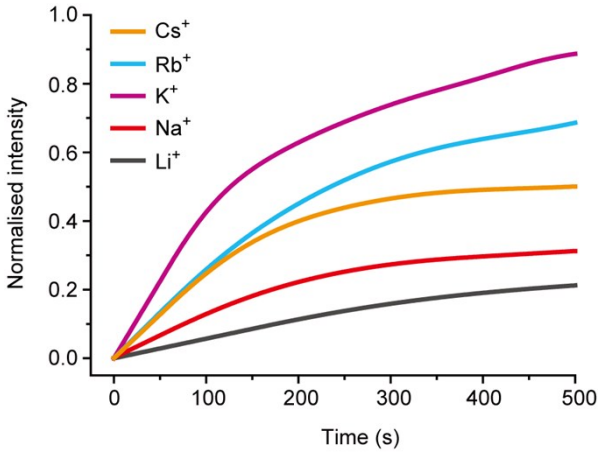

Figure S5. Representative fluorescence traces of cation selectivity test of AzoG4 using HPTS assay. The LUVs contained 100 mM KCl buffered at pH 7.14 with 20 mM Tris-HCl buffer and pH sensitive dye HPTS (0.5 mM). Outside of the vesicles was 20 mM Tris-HCl solution buffered at pH 8.14 containing 100 mM MCl (M= Li^+, Na^+, Rb^+ or Cs^+); AzoG4: 5 μM.

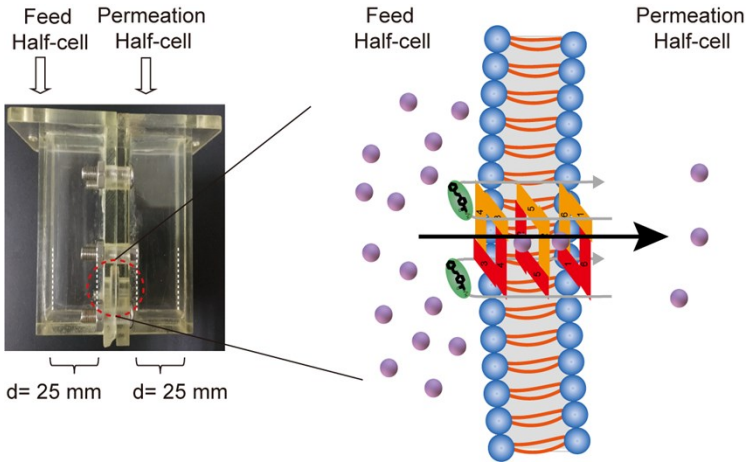

Figure S6. Schematic representation of the U-tube setup for light modulated ions transport experiments.

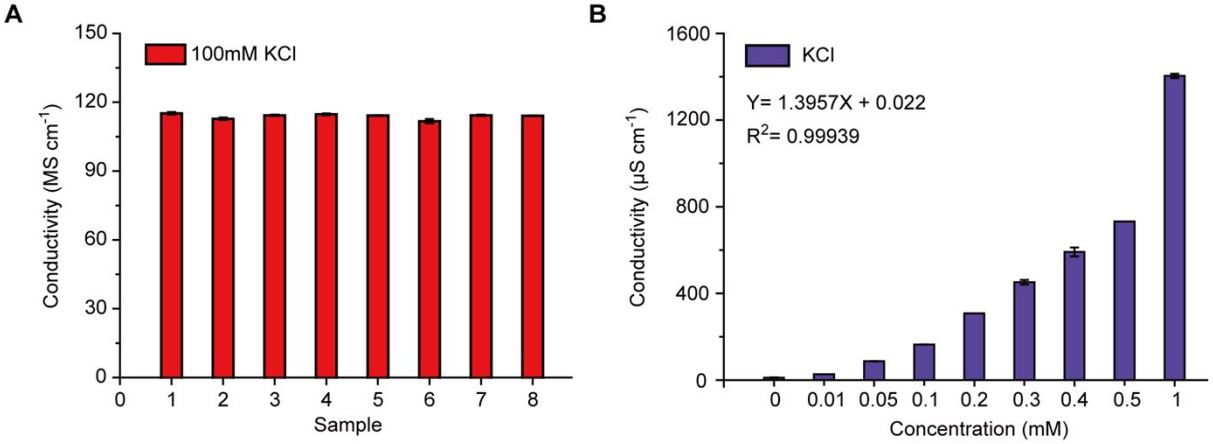

Figure S7. (A) Conductance standard curve at different concentrations of KCl. (B) Correlation curve of a serious of KCl standard solution.