

Communication

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A DNA-Inspired Synthetic Ion Channel Based on G-C Base Pairing

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Supporting Information Placeholder

ABSTRACT: A dinucleoside containing guanosine and cytidine at the end groups has been prepared using a modular one-pot azide-alkyne cycloaddition. Single channel analysis showed that this dinucleoside predominantly forms large channels with 2.9 nS conductance for the transport of potassium ions across a phospholipid bilayer. Transmission electron microscopy (TEM), atomic force microscopy (AFM) and circular dichroism (CD) spectroscopy studies reveal that this dinucleoside can spontaneously associate through Watson-Crick canonical H-bonding and π - π stacking to form stable supramolecular nanostructures. Most importantly, the ion channel activity of this G-C dinucleoside can be inhibited using the nucleobase cytosine.

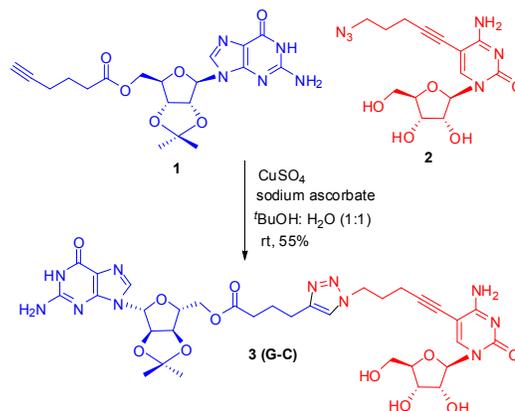
Natural ion channels are essential for life processes.¹ They are high-molecular weight pore forming proteins that promote transport of ions across biological membranes. Inspired by nature, there is an increased interest in developing artificial ion channels which can mimic the fundamental functions of natural ion channels.²⁻⁵ The creation of artificial ion channels may improve our understanding of natural ion channels and open up possible applications in materials and biological sciences. Therefore it is important to devise easily synthesizable, cost-effective and bio-compatible transmembrane channels with tailored physiological properties.

While peptide based synthetic ion channels have been widely studied,^{2,3,5} only a few examples of synthetic ion channels based on nucleobases have been reported.⁶⁻⁸ Mostly guanine derivatives have been demonstrated to form synthetic ion channels in the membrane.^{7,8} In these examples, guanine can self-assemble in the presence of cations to form macrocyclic G-quartets which further stack to form G-quadruplexes.^{7,8} Recently, Langecker *et al* reported that self-assembled DNA nanostructures can form ion channels in the lipid bilayer.⁹

We intended to utilize self-complementary guanosine (G) and cytidine (C) nucleobases to program biomimetic supramolecular structures in the membrane, which can facili-

tate the transport of ions. Guanosine has ionophoric properties and in the presence of potassium ions guanosine can assemble to form G-quadruplexes,¹⁰⁻¹² while guanosine and cytidine can be stabilized by Watson-Crick hydrogen bonds.¹³ Synthetic analogues of the natural G-C hydrogen bonding motifs have been used to assemble supramolecular structures.¹³ Additionally, cytidine diphosphate diacylglycerol is the most common natural nucleolipid, which plays an important role in the biosynthesis of membrane phospholipids.¹⁴

In an attempt to prove the principle, herein we delineate a modular access to the synthesis of a G-C dinucleoside via a triazole linkage that can spontaneously assemble into stable supramolecular nanostructures with the potential to form ion channels in the membrane and whose conductance can be selectively inhibited. For the synthesis, we incorporated an acetylene unit in guanosine and an azide unit in cytidine (Scheme 1, Supporting Information, S.I., Scheme S1). The guanosine alkyne **1** underwent smooth Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition¹⁵ with the azido cytidine **2** using Na-ascorbate and CuSO₄·5H₂O in *t*-BuOH/H₂O (1:1) as solvent to give the triazole linked guanosine-cytidine dinucleoside **3** (G-C) in 55% yield.



Scheme 1. Modular synthesis of a G-C dinucleoside.

To test its ability to transport the physiologically important cation K⁺ across membranes, voltage-clamp experiments were employed on planar solvent-free bilayers in 1 M KCl, phosphate (2 mM) buffer at pH 7.4. Planar solvent-free bilayers were produced by spreading giant unilamellar vesi-

cles (GUVs) consisting of 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) and cholesterol (9:1) on a small aperture in glass.¹⁶ Current traces were recorded after insertion of G-C into the membrane. Similar to the natural ion channels, synthetic ion channels formed by the G-C dinucleoside exhibit conductance values that appeared and disappeared for several minutes (Figure 1, Figure S1-a). The histogram of conductance values showed existence of two distinct conductance levels, one around 0.64 nS and one around 2.9 nS. It is noteworthy that large-conductance channels were present in a majority of experiments (80%) with conductance values of 1-8 nS with a mean value of 2.9 nS. G-C also formed channels (~20%) with conductance values of 0.1-1 nS with a mean value of 0.64 nS. The presence of distinct channel types indicates the formation of two different species in the membrane. The lifetime of G-C channels spans a wide range from <10 ms to 5 s (Figure S1-b). Control experiments showed no measurable conductance in the absence of the G-C dinucleoside. No current changes in the presence of G-C were observed using BaCl₂, which suggests an influence of the electrophoretic mobility of the cation or the inability of the G-C channels to adapt the cation.¹⁷

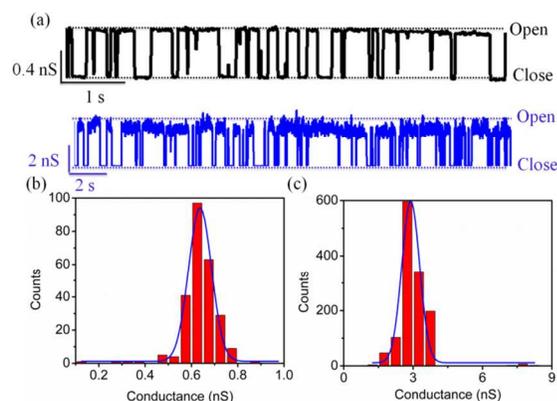


Figure 1. (a) Traces of single-channel recordings obtained by the insertion of G-C dinucleoside (20 μ M) to the *cis* side of the chamber after the planar bilayer was formed. Distribution of the two distinct conductance states (1557 events) of G-C dinucleoside: (b) 0.1-1 nS (mean conductance 0.64 ± 0.05 nS) for the conductance trace shown in the top panel (a); (c) 1-8 nS (mean conductance 2.9 ± 0.4 nS) for the conductance trace shown in the bottom panel (a).

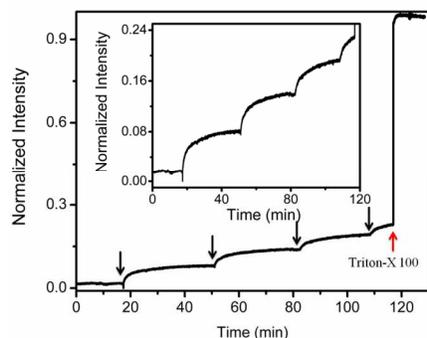


Figure 2. Plot of percentage of CF release from liposomes (DPhPC /cholesterol = 9:1) induced by the G-C dinucleoside (4 X 25 μ M) as a function of time. The black arrows indicate the addition of G-C. Complete release of CF was determined after destroying the liposomes with Triton X-100 (red arrow).

Next, we investigated if G-C dinucleoside can induce release of carboxyfluorescein (CF) from vesicles.¹⁹ G-C dinucleoside (4 X 25 μ M in DMSO) was added to the CF encapsulating vesicles and the fluorescence intensity was monitored over time at 520 nm (excitation at 492 nm). A release of the dye from the vesicles would increase its fluorescence intensity and indicate pore formation. Figure 2 shows the fluorescence time course of CF-containing LUVs over a time period of 130 min. No release of CF was observed in the absence of G-C dinucleoside or using DMSO as a control. In the presence of G-C dinucleoside, a slow increase in fluorescence intensity was observed indicating the formation of pores. The value after disruption of the vesicles using Triton-X corresponds to 100% release of CF. G-C dinucleoside induced CF release was concentration dependent and approximately 23% of the entrapped carboxyfluorescein was released at 100 μ M concentration of G-C after 2 h.

Transmission electron microscopy (TEM) and atomic force microscopy (AFM) images were taken to investigate possible structure formation due to the self-assembly of GC dinucleoside. TEM imaging revealed coiled helical filamentous nanostructures in addition to linear nano-fibers of width 4.8 nm and ranging up to a few micrometers (Figure S3). The primary nanofibrils were even interconnected to form thick bundles up to 14.9 nm in width. The formation of the higher order structures is a result of the H-bonded head-to-tail polymeric ability of the dinucleoside as it contains guanosine and cytosine at its two ends (Figure 5). The formation of helical nano-filaments and subsequent bundle formation in TEM may be explained based on iterative intermolecular H-bonding between complementary nucleobases in combinations with π - π stacking and hydrophobic interactions (Figure 5), where the triazole ring may contribute to enhanced π - π interactions between two adjacent stacks. Further AFM analysis supported formation of a network of cross-linked fibrils of the G-C with an average height of 5.5 nm (Figure 3 and Figure S4). PXRD patterns for G-C exhibited a broad peak at $2\theta = 28.9$ ($d = 0.33$ nm), which could be related to guanosine-cytidine stacking between two adjacent vertical G-C stacks (Figure S5). Dynamic light scattering (DLS) also confirmed the aggregation behavior of the dinucleoside in solution with an average diameter of ~28 nm (Figure S6).

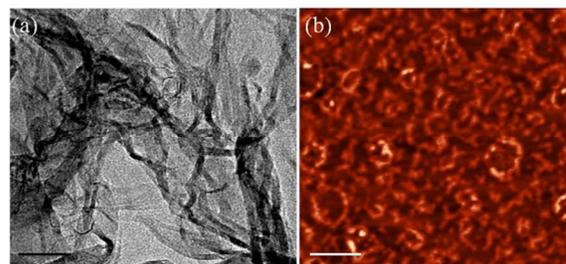


Figure 3. (a) TEM image; scale bar: 50 nm and (b) AFM image; scale bar: 200 nm.

Spectroscopic studies were performed in order to get insights into the supramolecular assemblies of G-C in the absence or presence of LUV suspensions (Figure 4). The UV-Vis spectrum of G-C dinucleoside showed peaks at 247 nm, 278 nm corresponding to the π - π stacking of the nucleobases. Observation of a shoulder at 306 nm was attributed to the π - π stacking of the triazole ring. The addition of LUV suspensions to a solution of G-C dinucleoside resulted in a marginal hypochromicity of the absorption peak at 247 nm

along with a blue shift of ca. 5 nm, suggesting its interaction with the membrane. Circular dichroism (CD) spectroscopy revealed a negative peak at 235 nm, a positive peak at 250 nm and a broad positive peak at 288 nm in accordance with the absorption peaks in the corresponding UV spectra, suggesting presence of supramolecularly oriented chirality of the G-C irrespective of the LUVs (Figure 4b). Moreover, the UV and CD prediction spectra are quite comparable with the experimental UV and CD spectra (Figure S10).

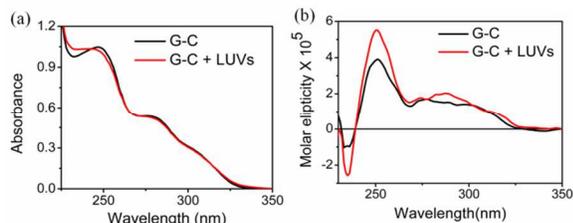


Figure 4. (a) UV and (b) CD spectra of G-C dinucleoside (20 μ M) in the absence and in the presence of vesicles in PBS (2.7 mM KCl, 136.9 mM NaCl, 1.5 mM KH_2PO_4 and 8.1 mM Na_2HPO_4 , pH 7.4).

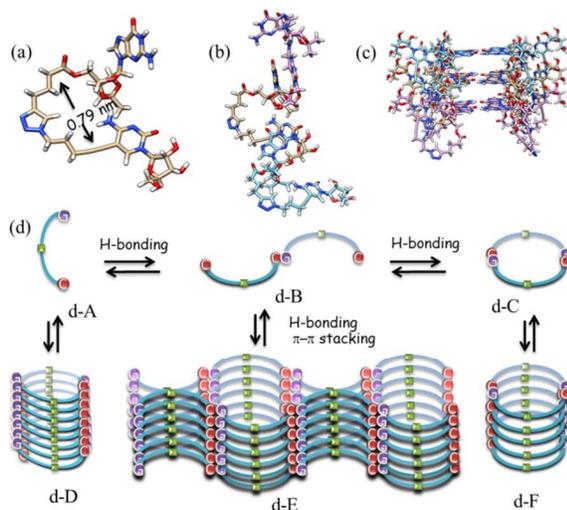


Figure 5. (a) Energy minimized structure of G-C monomer obtained using B3LYP/6-31G level (Gaussian03); proposed model the G-C ion channels: (b) oligomer based on G-C base pairing and (c) G-quartet; (d) schematic representation of ion channel formation.

Density functional theory (DFT) structural analysis of the G-C dinucleoside reveals that the triazole containing linker between the two end nucleosides is flexible to make a peripheral pore of 0.79 nm diameter (Figure 5a, d-A, S8). The end-to-end distance of the lowest energy conformer of the G-C dinucleoside is determined to be 1-1.5 nm (Figure S8). The larger TEM diameter compared to the molecular length of G-C indicates lateral association of the vertically stacked G-C on top of each other. We propose the presence of a flexible linker between G and C end groups may facilitate 'barrel-stave' type structures with nano-pore channels of \sim 0.79-1.6 nm (Figure 5d-E), where the triazole rings and the nucleobases facilitate self-assembly of linear chains to fibrous structures by π - π stacking. However, we certainly cannot rule out the possibilities, where a linear chain of G-C dinucleoside can form helical pores (Figure 5d-D, Figure S9, S.I.). Despite the flexibility of the small linker, the self-

assembly of the G-C monomer may just form cyclic dimers (Figure 5d-C). The cyclic dimers can further self-assemble side-by-side to form aggregated pores (Figure 5d-F, Figure S9).

As the guanosine (G) and cytidine (C) motifs are involved in H-bonding, the triazole ring and the ester carbonyl may weakly coordinate to the potassium ion and contribute to the K^+ transport. The slow release of CF can be rationalized as follows: i) the size of CF (\sim 1 nm) is close to that of these channels and ii) the negative charge of CF may lead to repulsion due to the presence of the partially negative charged carboxyl group in the G-C dinucleoside. The opening and closing of the channels at an applied potential may be due to the dynamic conformational change in the self-assembly of G-C in the membrane. The observed smaller conductance values (0.1-0.5 nS) may be explained based on the formation of a 'barrel-rosette' model using G-quartets as the basic building blocks (Figure 5c).

Molecular disassembly was investigated primarily by two approaches; (i) competition with guanosine and (ii) inhibition with cytosine. Ion channel blockers/inhibitors are compounds which bind to the pores or break the self-assembly and inhibit the activity of a channel in the membrane. When a solution of guanosine (5 mM) was added to the G-C ion channels in the lipid bilayer, the G-C ion channels conductance of 2.9 nS disappeared and much smaller conductance values were monitored with a mean conductance value of 0.24 nS and a mean open life time of 0.71 s (Figure S2). This smaller conductance can be attributed to the formation of competitive G-quadruplex type ion channels by guanosine.^{7,8} However, a complete disappearance of the G-C ion channel conductance was observed when excess cytosine (5 mM) was added to the lipid bilayers (Figure 6b). CD spectroscopy was used to investigate the structural changes of the G-C channels upon addition of guanosine and cytosine (Figure S7). As the concentration of guanosine was increased, a reduction in the molar ellipticity of all the peaks was observed in a dose dependent manner till the pores are populated with G-quadruplex scaffold in the presence of K^+ .^{8,11} Upon titration with cytosine, several peaks were observed with the disappearance of the negative peak at 235 nm and a positive peak at 250 nm. This suggests that cytosine can induce disassembly of the supramolecular organization of G-C in the membrane.

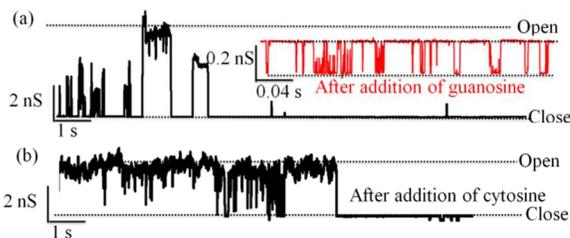


Figure 6. Inhibition of ion channel formed from G-C dinucleoside using (a) guanosine and (b) cytosine.

In summary, we created the first biomimetic artificial transmembrane ion channel construct using a self-complementary G-C dinucleoside. The biomimetic ion channel shows a half-life in the range of seconds and exhibits two distinct conductance levels for potassium ions. A competitive assay with guanosine and channel inhibition with cytosine confirms the role of a GC hydrogen bonded framework for channel formation. In contrary to the natural ion channel systems, this synthetic ion channel possesses

tunability with flexibilities in choosing functionality and length of the linker.

ASSOCIATED CONTENT

Supporting Information

Experimental details, synthetic procedures, ^1H NMR and ^{13}C NMR spectra, voltage-clamp experiment traces, AFM, TEM analysis and CD spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interests.

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