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Self-assembly of a Guanosine Derivative to Form Nanostructures and Transmembrane Channels

Rabindra Nath Das,[a] Y. Pavan Kumar,[a] S. Arun Kumar,[a] Ole Mathis Schütte,[b] Claudia Steinem,[b] Jyotirmayee Dash[a]*

Abstract: We herein report the self-assembly of a lipophilic bromoguanosine derivative (**G1**) in homogeneous solution, in the solid state, and in planar bilayer membrane. The self-assembly of **G1**, driven by H-bonding and π-π stacking interactions can form different nano-structures depending on incubation time. The **G1** nanostructure is able to bind a bioactive dye like Rose Bengal. In crystal state, it shows ribbon type H-bonding pattern and exhibits birefringence in polarized light. And further, the self-assembled nanostructure of **G1** can form discrete transmembrane ion channels in the biological membrane, enabling transportation of potassium ions.

Guanosine derivatives, owing to their self-assembling properties, have been used as building blocks for fabricating well-defined nanostructures, hydrogels and synthetic ion channels.[1] Guanosine derivatives can self-assemble using Hoogsteen type hydrogen bonding and π-π stacking interactions to generate tunable three-dimensional highly ordered structures.[1] Lipophilic guanosine derivatives, depending on the substituents and the experimental conditions, are able to form supramolecular architectures like ribbons, sheets and helices.[2] In the presence of metal ions, guanosine derivatives self-assemble to form macrocyclic G-quartet,[1a] which is a prime building block of biologically relevant four stranded DNA and RNA secondary structures.[3] The G-quartet like assembly has been demonstrated to incorporate aromatic dyes and drug molecules; useful for industrial and biomedicinal applications.[1] It has been reported that 8-substituted guanosine derivatives form stable self-assembled structures[4,5] as C-8 modification causes the nucleobase to preferentially adopt the *syn*-conformation and enables Hoogsteen type H-bonding. In our previous report, we have used a combination of guanosine **1** and 8-bromo guanosine **2** to prepare stable hydrogels.[5] We herein report the self-assembling and ion transportation properties of a 8-bromosubstituted lipophilic guanosine derivative **G1**.

The lipophilic *tert*-butyldimethylsilyl (TBDMS) protected bromo guanosine derivative **G1** was prepared from guanosine nucleoside **1** (Figure 1, Scheme S1, see Supporting Information, S.I.). The self-assembling property of **G1** on solid surfaces was studied using AFM and TEM imaging.

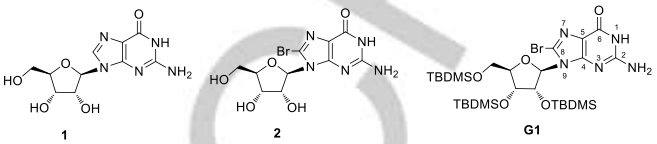

Figure 1. Guanosine and its derivatives.

The images showed that the TBDMS protected bromoguanosine, **G1** formed circular honeycomb type supramolecular structures after 1 h incubation (Figure 2 a, b).[6] The average height of the ring was determined to be 30 nm and the average width was 125 nm. The images also showed that some of these rings were fused together forming dumbbell shaped loops. Interestingly, incubating a solution of **G1** for 4 days, a network of G-wires interconnected at few nodes was observed (Figure 2 c, d). AFM and TEM images highlight the dependence of self-assembly of **G1** on incubation time. These results indicate that the ring or dumbbell shaped self-assembled structures of **G1** open up and join end-to-end to form longer and thinner G-wire architectures with time.

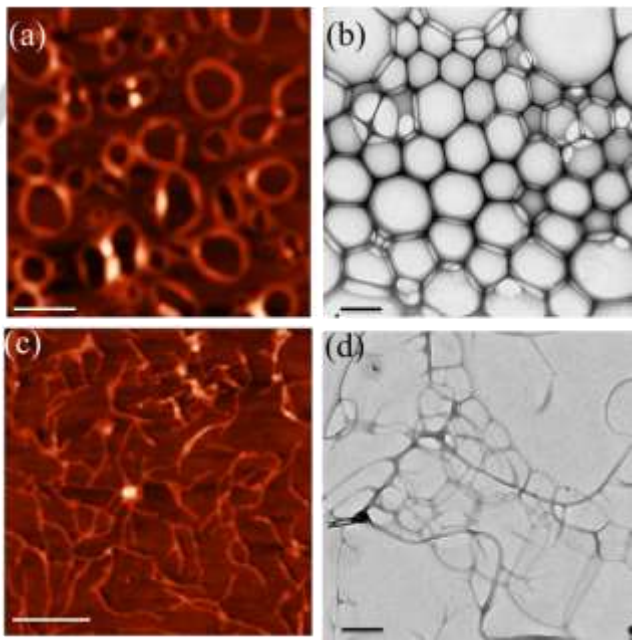

Figure 2. AFM and TEM images of **G1** after 1 h (a, b); 4 days (c, d) incubation (scale bar 400 nm).

The solid-state conformation of **G1** was studied using single crystal X-ray analysis.[7] The structure analysis reveals that it forms H-bonded 1D chain like structures in which each guanosine is connected through four Hoogsteen type H-bonding

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with four-neighbouring guanosine motifs (Figure 3). In these extended H-bonded layers, the amino (N2) hydrogen of a guanosine motif (A) H-bonds with N7 of another guanosine motif (B); the imino hydrogen of A is further H-bonded with the O6 of guanosine motif (C). In a similar fashion, the O6 and N7 of A are H-bonded with imino and amino hydrogens of guanosine motifs D and E, respectively leading to the formation of a ribbon type nanowire.[2a,e] The extended H-bonded networks of **G1** further held together through stacking interactions of the aromatic rings.

It has been reported that guanine crystals are responsible for amazing color in animals e.g. fish skin, widow spider etc. due to their extremely high refractive index.[8] We observed that **G1** crystals exhibited strong birefringence upon exposure to polarized light (Figure 3c).[5] This result indicates that **G1** crystal is anisotropic in nature due to the difference in the refractive indices (n) along two different parallel H-bonded crystallographic directions. The densely stacked H-bonded layers present in the crystal structure exhibiting birefringence would be of great interest in different applications like data storage, development of optical devices and bio-imaging.[9]

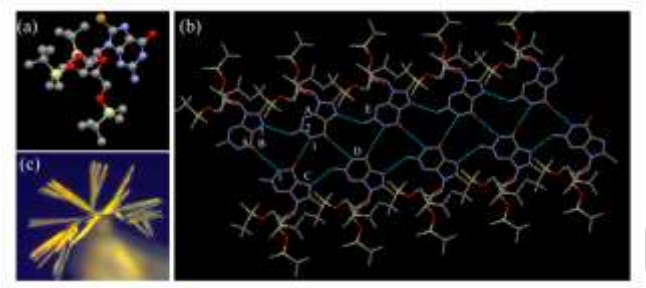

Figure 3. (a) Crystal structure of **G1**, (b) H-bonding pattern in **G1**, (c) **G1** crystals exhibiting birefringence.

The self-assembly of **G1** was then studied in solution state ($CDCl_3$) by using variable temperature (VT) NMR (Figure 4). ^1H NMR spectra of **G1** were recorded at various temperatures (-40 to 25 °C) to probe the intermolecular H-bonding (N1-O6 and N2-N7). At room temperature (25 °C), **G1** showed a strong broad peak at 6.52 ppm for the $–N(2)H_2$ protons, due to fast exchange of protons. By decreasing the temperature (-40 °C), two broad peaks at 8.82 and 4.95 ppm were observed for $–N(2)H_2$ protons. At low temperature, the exchange rate of the H-bonded protons became slow enabling discrimination of $–N(2)H_2$ protons. In addition, a relatively sharp peak at 12.12 ppm for –N(1)H was also observed by lowering the temperature. These results indicate the formation of a H-bonded network of **G1** in solution state. The self-assembly of **G1** in the presence of K^+ was also studied by using NMR spectroscopy.[10] ^1H NMR spectra of **G1** in the presence of K-picrate showed a peak at 12.0 ppm indicating the involvement of imino–N(1)H proton in the H-bonding to form G-quartet like arrangement (Figure S1).

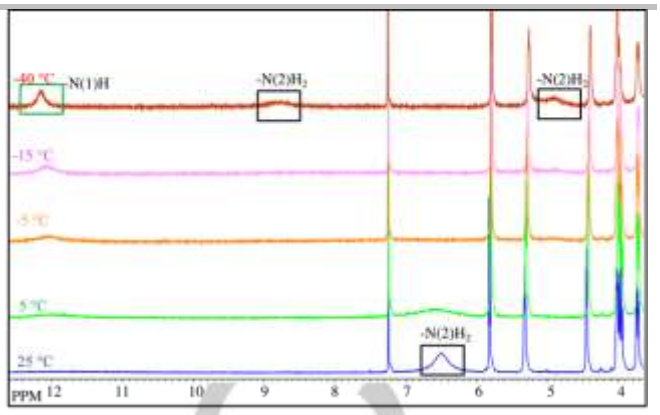

Figure 4. 1H- NMR spectra of **G1** in $CDCl_3$ at variable temperatures (-40 °C-25 °C).

It has been demonstrated that guanosine derived hydrogels can find useful applications in drug-delivery as well as optical and organic electronic systems as the supramolecular assembly can bind bioactive aromatic dyes using π-π stacking and electrostatic interactions.[5,11] To gain insights into the binding property of the **G1** assembled nano-construct, we studied the emission and steady state anisotropy of a bioactive dye rose bengal in the presence of **G1**. The fluorescence intensity of rose bengal was significantly decreased suggesting its incorporation into the **G1** nano-construct (Figure 5a). We also observed a remarkable enhancement in the fluorescence anisotropy of rose bengal in the presence of **G1** and that further confirms the binding of the dye molecules into **G1** network (Figure S2). AFM imaging was performed to investigate the morphological changes of **G1** assembled nanostructures after the encapsulation of rose bengal. The AFM images showed the accumulation of dyes in **G1** network (Figure 5b).

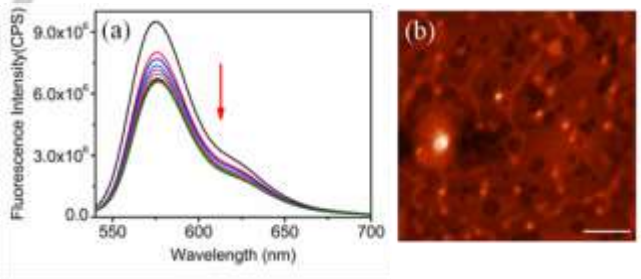

Figure 5. (a) Fluorescence spectra of rose bengal (10 M) with addition of **G1**, (b) AFM image of **G1** after the encapsulation of rose bengal (scale bar 400 nm)

Guanosine derivatives have also been used as building blocks for synthetic ion channels.[1f, 12-15] The Davis group reported that a lipophilic guanosine derivatives can assemble into a unimolecular G-quadruplex structure that can transport Na^+ ions across a phospholipid bilayer.[12] Matile and Kato groups reported that a folic acid derivative containing similar H-bonding motif as in guanine can self-assemble to form ion channels.[13] Subsequently, diguanosine derivatives containing different covalent linkers have been demonstrated to form membrane spanning channels by self-assembly.[14,15] In recent years, there is an emerging interest in the development of

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synthetic ion channels that can mimic the structural and functional aspects of natural ion channels.[16] Synthetic channels can be useful for a diversity of applications like therapeutics[17] and sensors.[18] Small molecules, peptides, proteins and DNA structures have been reported to form synthetic ion channels.[19] Since only a few guanosine derivatives have been evaluated for transporting metal ions across the biological membrane, we have studied the ability of lipophilic 8-bromoguanosine, **G1** to form ion channels. The voltage-clamp experiments were carried out on planar solvent-free bilayers in 1 M KCl, phosphate (2 mM) buffer at pH 7.4. First, giant unilamellar vesicles (GUVs) were prepared from 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) and cholesterol (9:1). Then planar solvent-free bilayers were generated by spreading GUVs on a small aperture in glass.[20] Guanosine derivative **G1** (20 µM) was added to the cis-side of the chamber and current traces were recorded at an applied voltage of -100 mV.

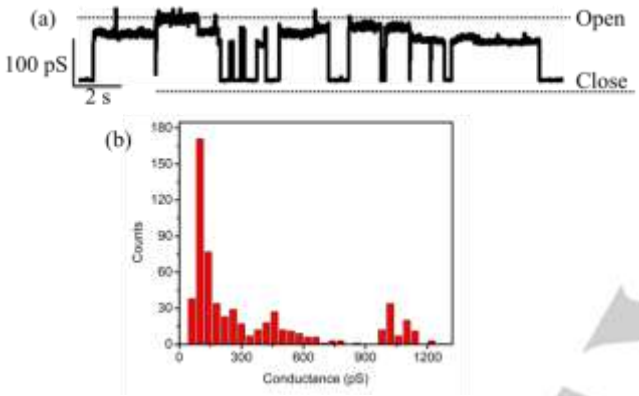

Figure 6. Traces of single-channel recordings obtained by the insertion of (a) **G1** (20 µM) to the cis side of the chamber after the planar bilayer was formed. The experiment was performed at -100 mV in 1 M KCl, phosphate (2 mM) buffer at pH 7.4. Statistical distribution of the observed conductance states for (b) **G1** (592 events).

The characteristic current steps were observed upon insertion of **G1** into the lipid membrane. These results suggest the formation of distinct channels (Figure 6a, Table S1) that can transport K^+ ions across the bilayer. The opening and closing of these ion channels indicate a dynamic self-association process of guanosine derivatives. The histogram analysis of the current traces showed that **G1** formed pores with a maximum conductance value of 1.2 nS (Figure 6b) and it also frequently formed channels with conductance values of 0.1-0.5 nS (60.1 %) (Table S1). Owing to weak lipophilicity and low solubility, simple guanosine **1** and 8-bromoguanosine **2** (Figure 1) failed to form ion channels, whereas in case of **G1**, the lipophilic TBDMS groups enabled the insertion of **G1** nanostructures into the lipid bilayer resulting in significant conductance values. The most frequently observed channels with conductance values (0.1-0.5 nS) indicate the formation of G-quartet like ion channels in the lipid bilayer (Figure 7).[15a,c] The CD spectrum of **G1**, embedded in the membrane showed a positive peak at 253 nm and negative peak at 275 nm, suggesting the formation of G-quartet like structures in the membrane (Figure S3, S.I.). The G-quartets could stack into columns in the lipid bilayer enabling transport of K^+ ions through the G-quartet pores (Figure 7).

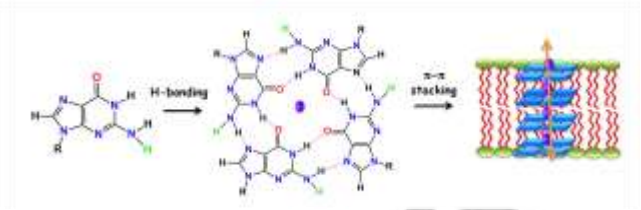

Figure 7. Self-assembly of guanosine derivatives to form ion-channels in biological membrane.

In summary, we have demonstrated that guanosine derivative **G1** self-assemble to form distinct nanoarchitectures. The lipophilic TBDMS protected bromoguanosine derivative forms different nanostructures with time, exhibits birefringence in its crystal state and binds dye molecules. It can also transport ions through the membrane. This study reveals that simple monoguanosine derivatives can be used to build functional nanoarchitectures as well as membrane spanning channels for ion transportation.

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Keywords: • biomimetic ion channel • birefringence • dye binding • lipophilic guanosine • self-assembly• phospholipid bilayer

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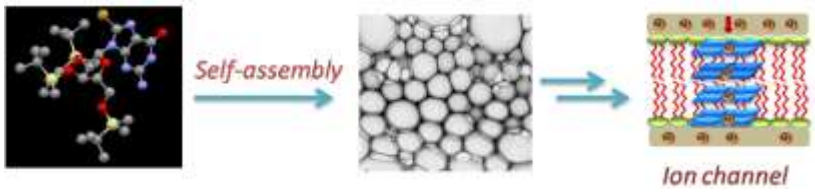

Nucleoside nanostructure and ion channel: A simple lipophilic nucleoside analogue forms self-assembled structures in solution, solid state and in lipid bilayer. Interestingly, the self-assembled structure of this nucleoside exhibits birefringence, incorporates a bioactive dye and forms transmembrane ion channels across a phospholipid bilayer.

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