Anion bridged nanosheet from self-assembled G-quadruplexes†

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A novel, non-covalent polymeric nanosheet has been produced through small molecule self-assembly; the structure has been characterized by solution NMR, solid-state NMR, powder XRD and AFM.

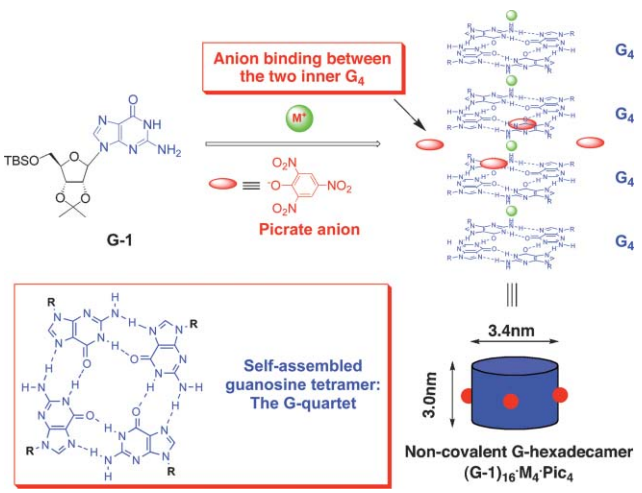
Bottom-up nanotechnology, or so-called "molecular manufacturing" or "molecular nanotechnology", has attracted increasing attention from researchers. The bottom-up approach creates larger scale architectures through the precisely controlled assembly of atoms and molecules. Self-assembly has been recognized as one of the enabling tools for the bottom-up preparation of functional molecular architectures.[1] The self-assembly approach has been applied in many areas of nanotechnology, including nanoparticle synthesis,[2] surface modification[3] and device development.[4] Herein, we report a novel, non-covalent polymeric nanosheet prepared by small molecule self-assembly, and characterized by solution NMR, solid-state NMR, AFM and powder X-ray diffraction.

One well known self-assembled structure found in nature is the guanosine quartet (G-quartet), a motif formed through self-complementary hydrogen bonding of the purine base (Scheme 1).[5] Much recent research on self-assembled G-quartets has focused on their biological functions, such as inhibition of tumor cell telomerase,[6] and the study of proteins that bind to G-quadruplexes.[7] Recently, this unique non-covalent structure has also been developed as an interesting supramolecular motif for other applications, such as ion-selective membrane channels,[8] self-assembled nanowires[9] and the construction of scaffolds for protein surface recognition.[10] In this Communication, we report the use of a bridging anion to direct the self-assembly of a G-quadruplex in the synthesis of a non-covalent polymeric nanosheet. This is a prime example of the preparation of a highly ordered nanomaterial *via* the self-assembly of small molecules.

In previous reports, we demonstrated that lipophilic guanosine **G-1** undergoes cation-templated self-assembly to form a discrete hexadecamer in the solid-state, in solution and in the gas phase.[11] The template cations, such as Na^+, K^+ and Ba^{2+}, are located along the central axis of the cylindrical complex, sandwiched between G-quartet layers.[12] Furthermore, four picrate anions were bound to the surface of the G-quadruplex through hydrogen bonds. Extensive studies have been made of this unique anion binding, and various anions, including 2,6-dinitrophenolate (DNP), SCN^- and *p*-OMe-2,6-DNP, promoted the formation of the same hexadecamer structures, albeit with different kinetic and thermodynamic properties, depending on the identity of the bound anion.[11a]

Intrigued by this unique anion binding property, we wondered whether the anion binding site could serve as a new synthetic handle to extend the supramolecular architecture of the G-quadruplex. In the crystal structure of the G-hexadecamer, picrate anions coordinate with the exocyclic amino group of the central two G-quartets through the anion's phenolate oxygen and the two nitro groups at the *ortho* positions. The *para* position, which is solvent-exposed from the G-quadruplex, provides an ideal synthetic handle for the extension of the supermolecule without disturbing the G-quartet's key non-covalent interactions. The 2,2′,6,6′-tetranitrobiphenolate (TNBP) dianion was therefore designed as a bridging anion that could be used to tether individual G-hexadecamers.

Both Na_2TNBP and **G-1** were prepared using literature procedures.[13] The formation of the polymeric G-quadruplexes was performed using two different approaches: (1) direct treatment of **G-1** with Na_2TNBP, and (2) anion exchange of preformed G-hexadecamer $(\mathbf{G\text{-}1})_{16}{\cdot}Na_4{\cdot}SCN_4$ with $(Bu_4N)_2{\cdot}$TNBP. In the first approach, the **G-1** was dissolved in CH_2Cl_2 and the Na_2TNBP was dissolved in water. Notably, the Na_2TNBP salt is not soluble in CH_2Cl_2 while **G-1** has little solubility in water.



Scheme 1

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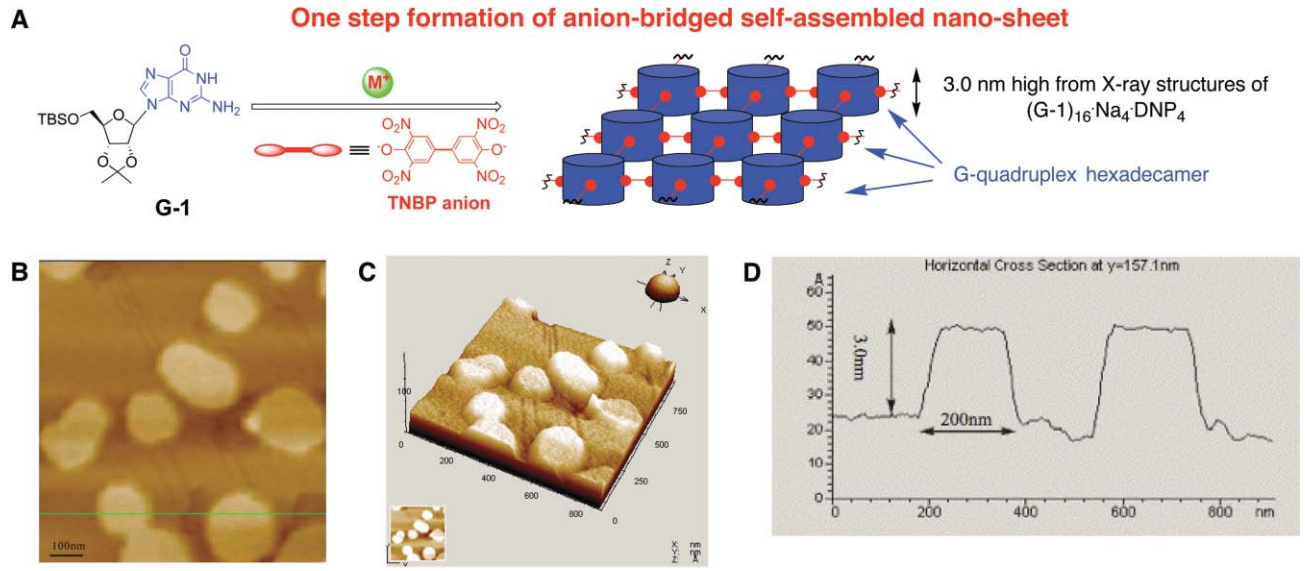

Fig. 1 (A) Schematic illustration of the nanosheet; (B) 2D topography AFM image of the $(\mathbf{G\text{-}1})_{16}{\cdot}Na^{+}{}_4{\cdot}TNBP^{2-}{}_2$ complex; (C) 3D topography AFM image of the same sampled area as in B; (D) cross-sectional profile of the $(\mathbf{G\text{-}1})_{16}Na^{+}{}_4{\cdot}TNBP^{2-}{}_2$ complex over the green line indicated in B.

Simply mixing these two clear solutions caused formation of a red solid at the interface between them.

The same polymeric complexes were also obtained through anion exchange between preformed G-hexadecamer $(\mathbf{G\text{-}1})_{16}{\cdot}Na_4{\cdot}SCN_4$ and TNBP anions. It has been demonstrated previously that sodium thiocyanate promotes the formation of G-hexadecamer by directing the self-assembly of **G-1**. Because it is a relatively poor hydrogen bond receptor, SCN^- anion binding with the G-quartets in $(\mathbf{G\text{-}1})_{16}{\cdot}Na_4{\cdot}SCN_4$ is highly dynamic.[14] Compared to SCN^-, the 2,6-DNP anion forms stronger hydrogen bonds with the G-quartets, resulting in the formation of kinetically and thermodynamically more stable guanosine complexes.[11a] The $(Bu_4N)_2{\cdot}$TNBP salt was prepared to perform this anion exchange study due to its solubility in CH_2Cl_2 and the weak binding between the Bu_4N^+ cation and the G-quartet. Upon mixing $(\mathbf{G\text{-}1})_{16}{\cdot}Na_4{\cdot}SCN_4$ with $(Bu_4N)_2{\cdot}$TNBP solutions in CH_2Cl_2, a red precipitate was again observed within 5 minutes. Because a single G-hexadecamer of formula $(\mathbf{G\text{-}1})_{16}{\cdot}Na_4{\cdot}Pic_4$ is a cylindrical structure with a height of 3.0 nm and a diameter of 3.4 nm (Scheme 1),[11] the proposed non-covalent polymers produced by the use of the bridging TNBP anion would extend along the horizontal direction without changing the vertical dimension (3.0 nm). The size and shape of the G-quadruplex polymer formed upon cross-linking with TNBP was measured by atomic force microscope (AFM), as shown in Fig. 1.

The resulting nanoparticles were about 90–200 nm in lateral size with a height of 3.0 $\pm$ 0.05 nm, which is consistent with the size of guanosine hexadecamers. This result strongly suggests that the TNBP anion bridges link the non-covalent G-quadruplexes together and produce even bigger, highly ordered supramolecular architectures.

Since the nanosheets are not soluble in water and many organic solvents, we used the polar solvent DMSO to dissociate the non-covalent interactions that hold the nanosheets together, so that we could better characterize its composition. The molecular composition of the nanocomplexes were then analyzed by ^{1}H NMR spectroscopy, as shown in Fig. 2.

As revealed by ^{1}H NMR, the nanosheet is formed from **G-1** and TNBP anions. Integration of the signals for the H8 proton of **G-1** and the TNBP protons gave a 2 : 1 ratio, indicating a 16 : 4 ratio between **G-1** and TNBP anions. This result is consistent with the proposed nanosheet structure. A ^{13}C NMR spectrum comparison of these three compounds was also performed (see the ESI†). Both ^{1}H and ^{13}C NMR confirm that the nanosheets are formed from **G-1** and TNBP through non-covalent interactions.

It is important to characterize the Na^+ cation in this non-covalent nanosheet structure. Recently, solid-state NMR techniques have been successfully developed for the determination of cation coordination within the G-quartet. Wu's group has studied the solid-state ^{23}Na, ^{39}K and ^{87}Rb NMR of guanosine complexes, and clearly demonstrated the cation-G-quartet binding in the solid-state.[14] Several ^{23}Na solid-state NMR experiments were performed on the polymeric nanosheet, and the results are shown in Fig. 3.

The Na_2(2,6-DNP) salt was examined to simulate the ion's chemical environment within the nanosheet complexes. The

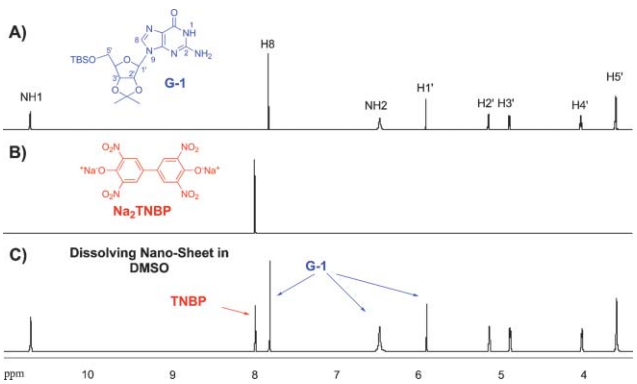

Fig. 2 ^{1}H NMR spectra of **G-1** nanosheets in d_6-DMSO (600 MHz): (A) **G-1**; (B) $Na^+{}_2TNBP^{2-}$; (C) nanosheets dissolved in DMSO. The integrated area ratio between the H8 proton of **G-1** and TNBP is 2 : 1, which is consistent with the proposed sheet structure shown in Fig. 1A.

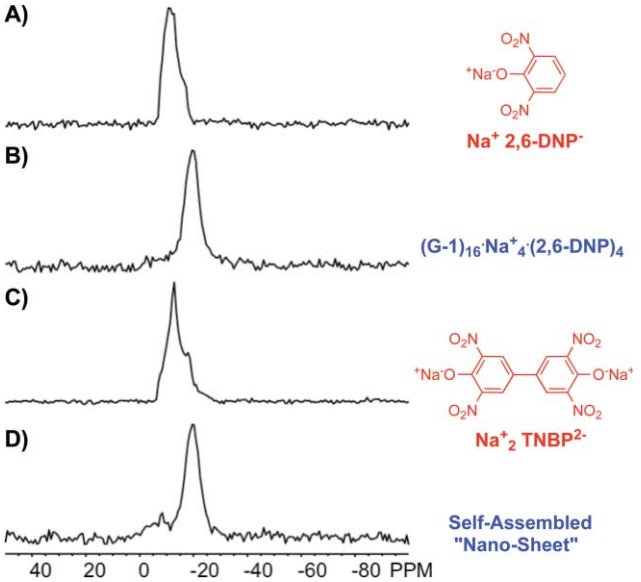

Fig. 3 ^{23}Na solid-state NMR spectra: (A) Na^+(2,6-DNP); (B) (**G-1**)$_{16}$·Na^+_4·(2,6-DNP)$_4$ G-hexadecamer, the Na^+ is bound between the G-quartet layers, as confirmed by X-ray crystallography; (C) Na^+_2(2,2,6′,6′-TNBP); (D) polymeric guanosine nanosheet.

G-hexadecamer formed from **G-1** and Na(2,6-DNP) has been previously characterized by X-ray and solution NMR studies. As shown in Fig. 3A and B, solid-state ^{23}Na NMR spectra yield different chemical shifts for "free" and G-quartet-bound Na^+. By direct comparison of these standard samples, it is reasonable to conclude that more than 90% of the Na^+ cations are bound to the G-quartets in the nanosheet complexes (Fig. 3D). Moreover, powder X-ray diffraction analysis of the nanosheet materials has been performed. The powder X-ray pattern reveals two low angle peaks observed at *d*-spacings of 47.5 and 23.75 Å, which indicates the presence of a large unit cell. As reported previously,[11a] a typical guanosine hexadecamer crystal has a unit cell of around 47 Å (for example, (**G-1**)$_{16}$·Ba_2·(*p*-OMe-2,6-DNP)$_4$ has a unit cell of a = 46.662, b = 24.259 and c = 45.111 Å). Therefore, the large unit cell revealed by the powder X-ray pattern is consistent with the guanosine hexadecamer and supports the formation of the proposed nanosheet structure (see details in the ESI†).

In conclusion, the covalently-linked dianion $TNBP^{2-}$ promotes the formation of a non-covalent polymer by cross-linking lipophilic G-quadruplexes. The AFM images, solution NMR data, solid-state ^{23}Na NMR analysis and powder X-ray measurements all strongly support the formation of a novel non-covalent network, as proposed. This novel supramolecular architecture exhibits a highly ordered structure. Notably, this novel nanoscale non-covalent complex is produced simply through the self-assembly of small molecules (**G-1** and Na_2TNBP) in one step. Since the complex construction is based on non-covalent interactions, the molecular architecture is dynamic and potentially reversible, and may provide unique properties as a novel nanomaterial. Meanwhile, the hydrophobic stacking of the G-quartet and the cation transport properties of G-quadruplexes makes this complex potentially interesting in studies of electron and ion transfer, and in nanodevice development. Screening studies of various anions and spacer linkers, and the deposition of nanosheets on metal surfaces for device development are currently under investigation in our group.

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