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Novel dual-functional regulation of a chair-like antiparallel G-quadruplex inducing assembly–disassembly of a cyanine dye†

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Biomolecules are promising templates that play important roles in controlling the supramolecular assembly process more flexibly and precisely due to their unique characteristic structures. Here we first present a G-quadruplex with a chair-like antiparallel motif as a novel dual-functional regulation template in the aggregation of a cyanine dye.

Chiral supramolecules have been a topic of keen interest in the scientific community for decades because of their novel properties and wide applications.[1,2] Constructing a chiral assembly and regulating its arrangement is always an essential issue in supramolecular chemistry to develop novel functional materials.[3]

Recently, many researchers have focused on constructing and regulating chiral supramolecules of cyanine dyes templated by biomolecules,[4–6] owing to their unique 3D structures and biological activities. It has been well studied that duplex DNA with proper grooves is able to facilitate the self-assembly of a cyanine dye,[7,8] where two dye molecules could first assemble into dimers then a few dimers could further assemble orderly in a head-to-end adjacent manner along the minor groove of the duplex DNA template. However, the duplex DNA templates reported previously mainly play a single role in regulating cyanine dye assembly, different from protein templates,[9,10] which cannot control the assembly process reversibly.

Besides linear double helix, DNA can also fold into some other secondary motifs, such as G-quadruplex and i-motif, which have distinctive steric and chemical environments,[11–13] and might play unique functional roles in regulating the aggregation of cyanine dyes. So far, attempts to utilize these kinds of unconventional DNA motifs as supramolecular assembly templates have been rarely reported.

A G-quadruplex is a special DNA secondary structure which is stabilized by Hoogsteen hydrogen bonding among four guanines arranged in a square planar configuration.[14–16] Benefiting from this distinctive structure, on one hand, a G-quadruplex has various grooves and loops which might interact with dye molecules and act as chiral templates inducing the assembly of dyes,[17] as duplex DNA and proteins do. On the other hand, the terminal G-quartet of a G-quadruplex can strongly bind to planar cyanine dyes in the form of a monomer due to the π–π stacking interaction and thus disassemble the dye aggregates.[18–20] Therefore, it might be rationally inferred that specific G-quadruplex could probably regulate the assembly process of a cyanine dye in bi-directional ways, which may play important roles in controlling the supramolecular assembly process more flexibly and precisely.

In this paper, we firstly report the dual-functional regulation of a chair-like antiparallel G-quadruplex, d(GGTTGGTGTGGTTGG) (termed **TBA**), in the aggregation of cyanine dye 2,2′-diethyl-9-methyl-selenacarbocyanine bromide (termed **DMSB**, shown in Chart 1). The whole assembly process of **DMSB** on **TBA** was studied in detail and consequently it was found that the unique steric conformation and electrostatic potential of **TBA** are two key factors that influence the arrangement of **DMSB** molecules.

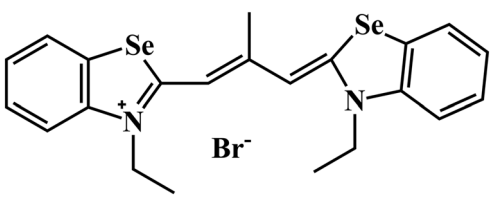

Chart 1 The molecular formula of DMSB.

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Chair-like antiparallel G-quadruplex has dual-functional regulation ability in DMSB assembly

In methanol, **DMSB** exhibits only one monomer band (M-band) at about 554 nm.[21] While in phosphate buffer solution (PBS, 20 mM K_2HPO_4/KH_2PO_4, 100 mM KCl, pH 7.4), it exhibits a primary monomer band at 554 nm, as well as a smaller shoulder peak at around 512 nm (D-band)[22,23] (Fig. S5, ESI†) according to the exciton model[24] and two reported analogues, DTC[25] and Cy3.[26] The result indicates that the monomers and dimers of **DMSB** co-exist in PBS without any higher-order aggregate formation. However, in the presence of the chair-like antiparallel G-quadruplex **TBA**, **DMSB** can be induced to assemble into J-aggregates (J-band) (Fig. S5, ESI†), whose absorption band is located at about 633 nm.

More than that, a further systematic study indicates that **TBA** can regulate **DMSB** bi-directionally: assembling into chiral J-aggregates and disassembling in reverse, as shown in Fig. S6 (ESI†) and Fig. 1(b). Firstly, an increase in [**TBA**] from 0.05 μM to 1 μM in 12 μM **DMSB** leads to a gradual decrease in the D-band of **DMSB** in UV-vis spectra accompanied by the appearance and gradual increase in the J-band, while the M-band almost remains invariable. It indicates that **DMSB** begins to assemble into J-aggregates from the dimers templated by a very tiny amount of **TBA**, and the **DMSB** monomer might not be involved in this process. Further increase in [**TBA**] from 1 μM to 8 μM leads to opposite UV-vis spectral changes: the J-band falls sharply and eventually disappears, whereas the D-band and the M-band increase simultaneously, implying that **DMSB** J-aggregates are totally disassembled into dimers or monomers. Finally, excess **TBA** (more than 8 μM) leads to further disassembly of the **DMSB** dimer into monomers.

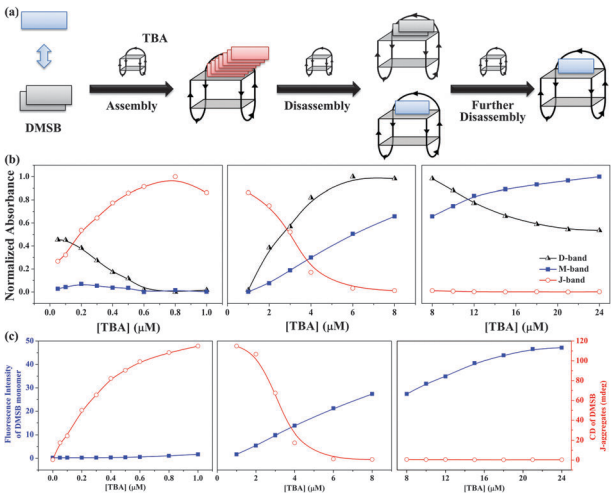

Fig. 1 (a) The schematic illustration of the assembling state changes of **DMSB** induced by **TBA**. (b) The changes in the M-band (blue), D-band (black) and J-band (red) of 12 μM **DMSB** against increasing concentrations of **TBA**. (c) The changes in monomer fluorescence intensity (blue) and the J-aggregates CD signal (red) of 12 μM **DMSB** against increasing concentrations of **TBA**.

Furthermore, the chirality of induced **DMSB** J-aggregates was also studied. As shown in Fig. S7 (ESI†) and Fig. 1(c), in the presence of **TBA**, **DMSB** exhibits a positive CD band at 645 nm assigned to J-aggregates. And the change in the CD signal is in good agreement with that in the J-band absorbance, indicating that the chiral **DMSB** J-aggregates are indeed induced by the **TBA** template.

The results show that **TBA** is able to bi-directionally induce either the assembly of **DMSB** at a high [**DMSB**]:[**TBA**] (more than 12:1) ratio, or the disassembly of J-aggregates into dimers and monomers, and eventually into monomers at a low [**DMSB**]:[**TBA**] ratio (less than 12:1) [Fig. 1(a)]. Besides **TBA**, another DNA sample d(TAGGTTAGGTTAGGTTAGG) (termed **BM**), which presents a similar chair-like antiparallel G-quadruplex motif to **TBA** in PBS,[27] also exhibits a similar bi-directional function in regulating the assembly of **DMSB** (Fig. S8, ESI†). This implies that the unique structural features of the chair-like antiparallel G-quadruplex is the crucial factor of this kind of dual-functional template.

Reasons for inducing the disassembly of DMSB

Owing to the methine bridge, free **DMSB** can facilely rotate and the radiationless transition results in quenching of molecular fluorescence.[28] When **DMSB** is bound, the rotation is hindered and radiationless transition is inhibited a lot, resulting in the enhancement of fluorescence intensity. As shown in Fig. S9 (ESI†) and Fig. 1(c), in the **DMSB** disassembly stage, the fluorescence intensity of the **DMSB** monomer is dramatically enhanced with the increase in the M-band; while in the **DMSB** assembly stage, the fluorescence intensity of the **DMSB** monomer is rarely observed. The results indicate that the disassembly of **DMSB** J-aggregates and dimers is supposed to result from the binding of the **DMSB** monomer to **TBA**.

To further investigate the binding site, a ^{1}H-NMR titration experiment at a relatively low [**DMSB**]:[**TBA**] ratio (<7:1) was performed (Fig. S10, ESI†). As shown in Fig. 2(a), the chemical shifts of aromatic protons on G6, T7, G8, T9 and G10 (patterned orange columns) and those of methyl protons on T7 and T9 (solid orange columns) exhibit more remarkable changes ($\Delta\delta$) than those of other protons (patterned and solid black ones), suggesting that the **DMSB** monomer end-stacks on the G1–G6–G10–G15 G-quartet of **TBA** and has a strong interaction with the T7–G8–T9 loop [Fig. 2(c)]. This binding mode of combining end-stacking and loop interaction is in accordance with our previous study on the interaction between a cyanine dye and a specific G-quadruplex.[18–20] It is believed that owing to the stronger binding affinity (including end-stacking and loop interaction) between **DMSB** and **TBA** than that among **DMSB** molecules in an aggregate, J-aggregates are disassembled into monomers when **TBA** is excess.

Reasons for inducing the aggregation of DMSB

It is well-known that the self-aggregation ability of a cyanine dye depends on its hydrophobic nature and charge distribution.[22,23] In the case of **DMSB**, it has an extended planar π-electron conjugated system which is a critical feature for the formation

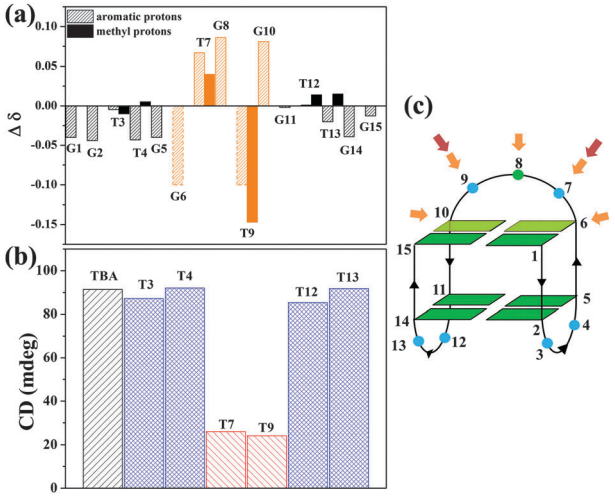

Fig. 2 (a) The changes in proton chemical shifts ($\Delta\delta$) derived from difference in ^{1}H-NMR titration spectra of 0.5 mM **TBA** without and with 3.5 mM **DMSB**. The $\Delta\delta$ of the aromatic protons on G6 and T9 are in dashed boxes, which means their values are imprecise since the two proton signals almost disappeared under the experimental conditions due to strong interaction. (b) The CD signal intensity of 12 μM **DMSB** J-aggregates in the presence of 0.5 μM **TBA** (black) and 6 mutation sequences (blue and red), respectively. (c) The NMR-based folding topology of **TBA**. The orange arrows show the key bases involved in the interaction between **TBA** and the **DMSB** monomer, while the red ones show bases involved in the interaction between **TBA** and **DMSB** J-aggregates.

of stable π-stacked aggregates. But the one net positive charge on **DMSB** in solutions would repulse molecules from each other which consequently hinders the formation of higher-order aggregates. Therefore, although **DMSB** has the potential to self-assemble, there is no higher aggregate formed in PBS.

A previous study[29] showed that extrinsic templates with opposite charge can reduce the electrostatic repulsion among cyanine dye molecules and "activate" their assembly ability. In order to explore the reason of the **DMSB** assembly induced by the G-quadruplex template, the properties of the surface electrostatic potential of **DMSB** [Fig. 3(a)] and that of the bases on **TBA** around the binding site (G1–G6–G10–G15 G-quartet and T7–G8–T9 loop) [Fig. 3(b)] were evaluated. It is clear that the former is positive while the latter, especially the binding cavity, is primarily negative. The positively-charged **DMSB** would be neutralized when bound on **TBA** by the negative atmosphere in its binding cavity. In addition, it is reported that the thymine (T) bases are more electronegative than the adenine (A)[30] because of the presence of two oxygen atoms. Thus, the one-by-one mutation of T to A changes the local electrostatic environment of **TBA** and leads to less electronegativity in the mutated position [Fig. 3(d)], but is proved to not change the conformation of **TBA** (Fig. S11, ESI†). Consequently, the mutation around the binding sites of **TBA** (T7 and T9 to A7 and A9) dramatically weakens the formation of **DMSB** J-aggregates [Fig. 2(b)]. Therefore, both the calculation and experimental results support that the negatively-charged environment on **TBA** is of importance to "activate" the assembly ability of **DMSB**.

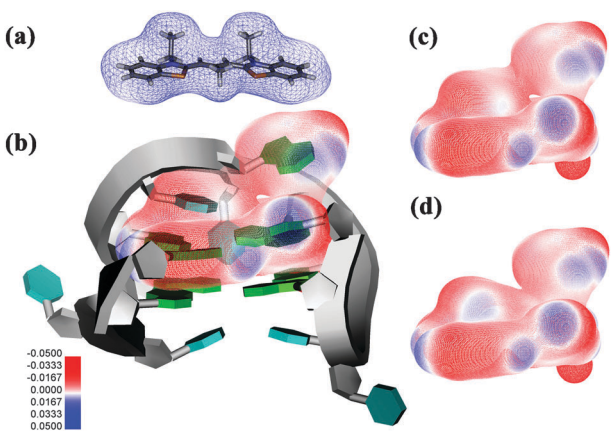

Fig. 3 The calculated electrostatic surface according to partial charge distribution for (a) **DMSB** and (b) bases on **TBA** around the binding site (G1–G6–G10–G15 G-quartet and T7–G8–T9 loop). (c) The calculated surface of the binding site with the **TBA** backbone removed. (d) The calculated surface of the binding site with the **TBA-T9** base mutated to **A9**.

Furthermore, our present work shows that only a chair-like antiparallel G-quadruplex can "activate" the assembly ability of **DMSB** while other G-quadruplex motifs (including parallel and hybrid ones) cannot.[31] It implies that besides the electrostatic factor, the unique motif of the template is probably another key factor in "activating" the assembly ability of **DMSB**. However, the detailed "activation" mechanism still needs further investigation.

In summary, this work firstly reports the chair-like antiparallel G-quadruplex as a novel dual-functional template in regulating assembly and disassembly of cyanine dye **DMSB**. This kind of motif can bind a **DMSB** monomer and "activate" its ability to assemble into aggregates. Therefore, when the G-quadruplex is in excess, **DMSB** exists in the form of a bound, "activated" monomer; while when **DMSB** is excess, free **DMSB** molecules can further stack on the "activated" ones and assemble into chiral J-aggregates. The results can help in the design of bi-directional function templates, which might play important roles in controlling the supramolecular assembly process more flexibly and precisely.

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