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## Tracking host-guest recognition in cells by a BODIPY•CB[7] complex

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Interested in host•guest binding events in cellular environments, intracellular recognition between fluorescent BODIPY<sup>+</sup> and cucurbit[7]uril was explored for lysosome tracking in living cancer cells. Sequential deaggregation from spontaneously dimerized BODIPY<sup>+</sup> was unusually discovered upon complexation with CB[7].

Intracellular construction of synthetic materials has been an intriguing topic for advanced biological applications in recent years.<sup>1–3</sup> As one of crucial process in living systems and attractive strategies in material science, supramolecular chemistry has been interestingly applied for in-situ construction of functional materials in cells via self aggregation. By contrast, supramolecular association in cells between synthetic host and guest has started to be investigated and it is a new promising field of research. Given potential of cucurbiturils (CB[n]s) in biological applications<sup>4</sup>, in this communication, we were interested in investigating possible intracellular complexation process of CB[n]s with guests. Cucurbit[7]uril (CB[7], Fig. 1) was chosen as host due to possible ultra-strong binding affinity, low toxicity profile and sufficient solubility in water.<sup>5</sup>

As one of popular fluorophores for dyes designing, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) derivatives are widely applied in laser technology, optical imaging, photosynthesis, sensing, and theranostics.<sup>6</sup> These compounds are featured by structural diversities, low cytotoxicity and visible to near-infrared absorption and emission with high molar extinction coefficients, quantum yields and photostability.<sup>7–9</sup> The planar and electron-deficient skeleton renders BODIPY as fantastic building blocks for supramolecular assemblies (*H*- and *J*-aggregates) and donor-acceptor (D-A) conjugates.<sup>10–14</sup>

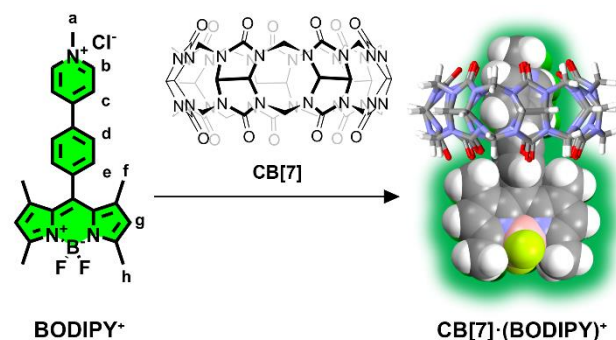


Fig. 1 Structures and host-guest complexation of CB[7] and BODIPY<sup>+</sup>.

However, applications in optical devices and biology are limited by aggregation-caused quenching (ACQ) and poor aqueous solubility of BODIPY derivatives<sup>15</sup>. Strategies have been developed to overcome these issues by integrating aggregation-induced emission gen (AIEgens), bulky cycles, spiral structures, and dendrimers on pyrrolic, meso- and BF<sub>2</sub> positions.<sup>7, 9, 16</sup> In a supramolecular approach, Kuang et al.<sup>17</sup> used a 1:1 complex of pillar[5]arene and a meso-aromatized BODIPY for lysosome-targeted imaging of MCF-7 cells. While several groups showed that BODIPY complexation by CB[7] significantly improved their fluorescence in water<sup>18,19</sup>, the recognition was limited to unstable external binding between CB[7] and BODIPY derivatives. Conjugation with aromatic rings can be an excellent strategy to enhance binding between CB[7] and BODIPY<sup>20–22</sup>. Therefore, in combination with our previous researches on BODIPY<sup>23–25</sup>, we attempt to graft a 4-phenylpyridinium group to

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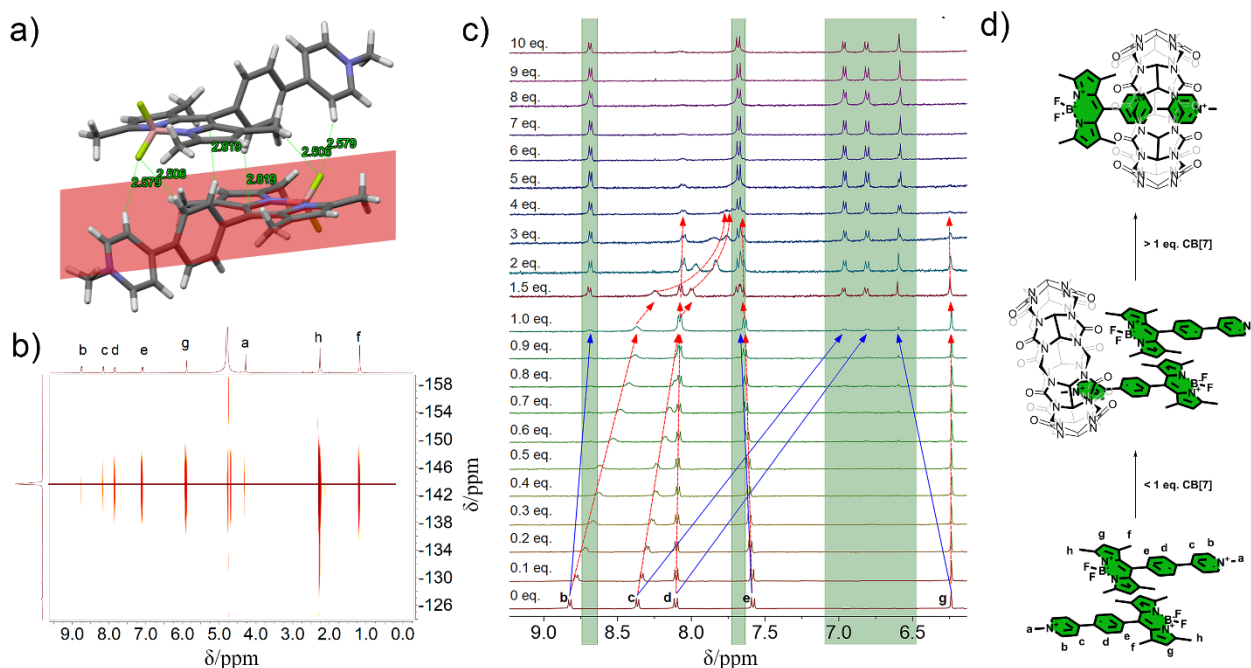


Fig. 2 a) Multiple hydrogen-bonding and intermolecular stacking in BODIPY<sup>+</sup> single crystal; b) <sup>1</sup>H-<sup>19</sup>F HOESY spectrum (400 MHz, D<sub>2</sub>O, 298.8 K, mixing time = 0.8 s) of BODIPY<sup>+</sup> (30 mM); c) Partial <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O, 299.6 K) of 1.0 mM BODIPY upon adding CB[7]; d) Schematic illustration of proposed BODIPY<sup>+</sup> dimer and complexation with CB[7].

improve binding with CB[7] in water<sup>22</sup> by integrating charge-assisted hydrogen bonds and hydrophobic interaction (Fig. 1).

The BODIPY<sup>+</sup> was synthesized as Cl<sup>-</sup> salt and characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR, ESI-MS, COSY and ROESY (Fig. S1-S9). Concentration-dependent <sup>1</sup>H NMR titration unraveled BODIPY<sup>+</sup> aggregation in water (Fig. S10). UV-Vis (Fig. S11a) and fluorescence (Fig. S11b) spectroscopies respectively showed S<sub>0</sub>-S<sub>1</sub> π-π\* transitions at 500 nm and emission quenching of BODIPY<sup>+</sup> at high concentration, consistent with aggregation. Single crystal X-ray diffraction (Fig. 2a and Fig. S12) showed the presence of BODIPY<sup>+</sup> supramolecular dimers. Indeed, multiple C-H...F hydrogen bonds and C-H...π interactions were found to synergistically stabilize BODIPY<sup>+</sup> dimer with phenyl rings perpendicular to adjacent BODIPY<sup>+</sup> cores (Fig. 2a). B-F...π interactions and C-H...π stacking (Fig. S12) were also observed. Finally, <sup>1</sup>H-<sup>19</sup>F HOESY spectrum (Fig. 2b) was recorded in D<sub>2</sub>O and showed intramolecular correlations of H<sub>f</sub>-H<sub>h</sub> and intermolecular correlations of H<sub>a-e</sub> with fluorine, in line with anti-parallel dimerization of BODIPY<sup>+</sup> in water. Fit of variation of <sup>1</sup>H NMR chemical shifts at H<sub>e</sub> considering a dimerization model<sup>26</sup> afforded a  $K_{\text{dim}} = 49 \text{ M}^{-1}$ .

Host-guest interaction between CB[7] and BODIPY<sup>+</sup> was then studied via <sup>1</sup>H NMR, UV-Vis absorption and fluorescence spectroscopy. As shown in Fig. 2c and Fig. S13, two independent sets of proton chemical shifts of BODIPY<sup>+</sup> were observed upon CB[7] addition. In the presence of less than 1 eq. of CB[7], the majority of guest proton signals shifted upfield (red arrows). Early in the titration, chemical shift changes were observed to be generally significant but weak (< 0.45 ppm) suggesting a rather moderate complexation. The up-field shift of signals of protons H<sub>a-c</sub> are in line with partial encapsulation of the methyl pyridinium moiety into CB[7], while down-field shift of other

protons (H<sub>d-g</sub>) rather suggests a proximity of the host carbonyl rims toward the phenyl ring and the BODIPY core. After the threshold over 1 eq., new sharp signals (blue arrows in Fig. 2c) appeared with slow-exchange kinetics on NMR time scale in the 6.5-7.0 ppm region corresponding to protons H<sub>b</sub>, H<sub>c</sub> and H<sub>d</sub>, deeply engulfed in the cavity of CB[7] ( $\Delta\delta$  up to -1.41 ppm). At the same time, signals of protons H<sub>a</sub>, H<sub>g</sub> and H<sub>f</sub> were significantly down-field shifted, indicating strong H-bonding between CB[7] rim and H<sub>f</sub> of BODIPY and deeper penetration of the phenylpyridinium moiety into the cavity of CB[7]. COSY and ROESY spectra (Fig. S14-S15) of BODIPY<sup>+</sup> in the presence of 10 eq. CB[7] supported the proposed assignment. Remote correlation between H<sub>e</sub> and H<sub>f</sub> disappeared due to restricted rotation of phenyl group upon inclusion complexation.

For stoichiometry, ESI-mass spectrometry (Fig. S16) showed formation of a CB[7]-(BODIPY<sup>+</sup>) adduct with a peak at  $m/z$  765.77930 corresponding to [H-G-BF<sub>2</sub>+H]<sup>2+</sup> (calculated  $m/z$  765.78146). UV-Vis and fluorescence spectroscopies were then recorded to confirm BODIPY<sup>+</sup> binding by CB[7]. The gradual addition of CB[7] to an aqueous solution of BODIPY<sup>+</sup> induced a bathochromic shift of the absorption at 250-350 nm with an isosbestic point at 315 nm and a hypsochromic shift of the absorption at 500 nm, consistent with formation of a supramolecular complex (Fig. S17a). Fluorescence titration showed enhanced emission of BODIPY<sup>+</sup> in 500-600 nm upon complexation with CB[7] (Fig. S17b). Job plots (Fig. S17c-S17e) by UV-Vis at 295 nm and emission at 540 nm are in line with a 1:1 host:guest stoichiometry. Non-linear fitting based on 1:1 complexation by absorption at 500 nm and emission at 540 nm gave binding constants of  $1.4 \times 10^4 \pm 470 \text{ M}^{-1}$  and  $1.2 \times 10^4 \pm 965 \text{ M}^{-1}$ , respectively. The affinity is much stronger than those

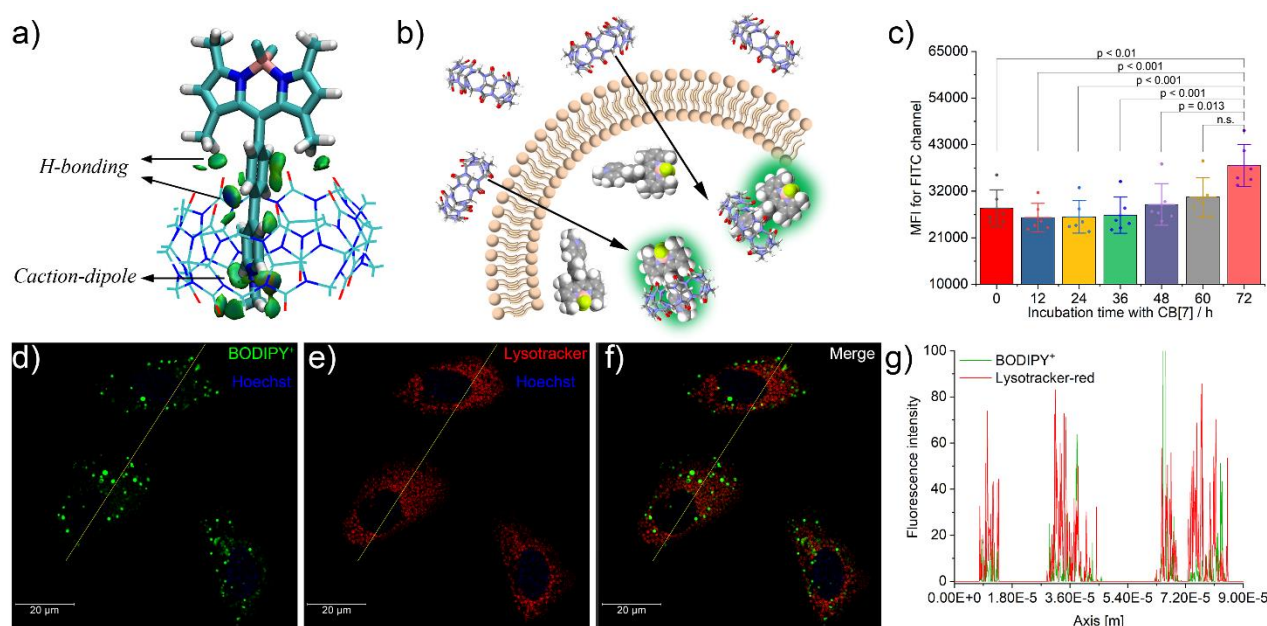


Fig. 3 a) DFT simulation and IGM analysis of CB[7]•(BODIPY<sup>+</sup>) complex; b) Scheme illustration of uptake tracking of CB[7] by cells; c) Flow cytometry of 5 μM BODIPY<sup>+</sup> in A549 cells pre-incubated with 500 μM CB[7]; d-f) LCFM and g) colocation analysis of BODIPY<sup>+</sup> in A549 cells pre-incubated with 500 μM CB[7] for 3 days. Cellular nucleus was stained with Hoechst33342 (blue), BODIPY<sup>+</sup> was imaged under FITC channel (green), lysosomes were stained by lysotracker red and imaged under Ex/Em=647/668 channel (red).

corresponding to external binding reported previously.<sup>18</sup> The inclusion complexation improved fluorescence lifetime and quantum yield (Table S1) of BODIPY<sup>+</sup> benefited from restricted rotation by CB[7]. Isothermal titration calorimetry (ITC, Fig. S18) revealed 1:1 complexation between CB[7] and BODIPY<sup>+</sup> with a binding constant of  $(4.35 \pm 0.29) \times 10^4 \text{ M}^{-1}$ , which is on the same order of magnitude to the above UV-Vis and fluorescence titration results.

At the beginning, the dual binding behavior suggested by the <sup>1</sup>H NMR titration puzzled us. However, consideration of BODIPY<sup>+</sup> dimerization in crystal and 1:1 stoichiometry of the CB[7]•(BODIPY<sup>+</sup>) complex put us on the track of sequential deaggregation of the BODIPY<sup>+</sup> dimer upon complexation with CB[7] (Fig. 2d). These results were confirmed by further performing concentration-dependence <sup>1</sup>H NMR titrations increasing CB[7] in BODIPY<sup>+</sup> solution of different concentrations. As shown in Fig. S19, less CB[7] was needed to transform BODIPY<sup>+</sup> from dimer to CB[7]•(BODIPY<sup>+</sup>) complex.

Density functional theory (DFT) calculations were conducted to afford a structure of the inclusion complex. Energy minimized conformation (Fig. 3a) for the complex showed threading of phenylpyridinium in CB[7] cavity. Independent gradient model (IGM) analysis<sup>27-29</sup> using Multiwfn<sup>30, 31</sup> supported significant hydrogen bonding between CB[7] rims and BODIPY<sup>+</sup>.

Supramolecular complexes of CB[n]s and dyes have been widely applied for biological applications.<sup>32</sup> As exemplified in Fig. S20a, CB[7] remains high viability to A549 cells with concentration up to 500 μM. However, BODIPY<sup>+</sup> shows viability less than 50% at concentration over 125 μM. Complexation with

CB[7] dramatically alleviated toxicity of BODIPY<sup>+</sup>. At low concentration of 5 μM for BODIPY<sup>+</sup>, no dimer is expected to be formed. Excess CB[7] up to 500 μM was used to guarantee complexation of BODIPY<sup>+</sup> with CB[7] at this concentration. Flow cytometry (Fig. S20b) and inverted fluorescent microscopy (Fig. S20c) verified enhanced green fluorescence of CB[7]•(BODIPY<sup>+</sup>) complex. Laser confocal fluorescence microscopy (LCFM) (Fig. S21) demonstrated colocation of BODIPY<sup>+</sup> and CB[7]•(BODIPY<sup>+</sup>) complex with lysosomes in A549 cells.

In contrast to cellular imaging using in-vitro prepared host-guest complexes, visualization of host-guest recognition processes in biological media remains a challenging task due to competitive binding. Interested in in-situ construction of synthetic material in living cells,<sup>33-35</sup> we attempted to visualize intracellular host-guest complexation by incubating cells with CB[7] and BODIPY<sup>+</sup>, separately (Fig. 3b). Flow cytometry (Fig. 3c) showed that BODIPY<sup>+</sup> could be internalized by cells in few minutes, while CB[7] was slowly taken up by A549 cells in a timescale of days without compromising cell viability (Fig. S22). LCFM and co-localization analyses (Fig. 3d-3g) revealed no influence of CB[7] in the lysosome targeting capability of BODIPY<sup>+</sup>. Even if the small fluorescence difference between free and complexed BODIPY<sup>+</sup> impeded further development, preliminary results support the use of BODIPY<sup>+</sup> as a lysotracker probe. Internalization inhibition experiments proved the clathrin-mediated endocytosis of CB[7] by A549 cells (Fig. S23).

In conclusion, BODIPY<sup>+</sup> was found to be an interesting fluorescent dye for lysosome tracking in A549 cells. In the course of this work, an interesting cascade complexation with CB[7] was discovered. This macrocyclic host significantly

alleviated the dye toxicity enabling for its use at a higher concentration. Intracellular host-guest recognition between CB[7] and BODIPY<sup>+</sup> was tracked and showed uptake of CB[7] for more than two days. These results support further exploration of intracellular host-guest chemistry. However, the fluorescence enhancement is limited due to the emission quenching of BODIPY by methylation. Further work to improve the emission could be performed by structural modifications such chlorine substitution at H<sub>g</sub>.<sup>36</sup>

Liu was responsible for the whole investigation and manuscript writing. Gao and Xiao supervised researches on biological experiments. Bardelang and Siri contributed to DFT investigation. This work was funded by the National Natural Science Foundation of China (22475230), Shenzhen Medical Research Fund (D2403001) and National Key Research and Development Program of China (2024YFF0507803). The final version of this paper was reviewed by all authors.

### Data availability

The data supporting this article have been included as part of the Supplementary Information. Crystallographic data for BODIPY<sup>+</sup> has been deposited at the CCDC [accession number 2414976] and can be obtained from [https://www.ccdc.cam.ac.uk/?locale=zh\\_CN/](https://www.ccdc.cam.ac.uk/?locale=zh_CN/).

### Conflicts of interest

There are no conflicts to declare.

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