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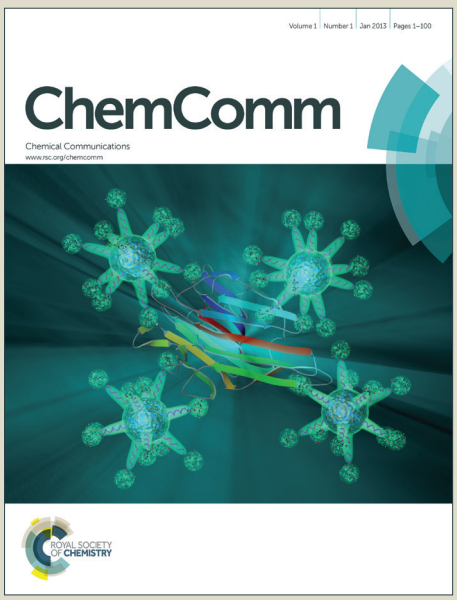

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Charge-Transfer Interactions for the Fabrication of Multifunctional Viral Nanoparticles

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A facile strategy to fabricate multifunctional viral nanoparticles was described by introducing charge-transfer interactions between pyrenyl motif with dinitrophenyl and pyridinium-contained guest molecules.

Great progress has been made in the development of multifunctional nanoparticles for their wide biomedical applications in the past decades.[1] Specifically, viral nanoparticles (VNPs) that derived from bacteria or plants have attracted considerable interests on account of their multivalent programmable and monodispersed structures, as well as their low toxicity and good biocompatibility.[2] For better using the inherent features of VNPs, a series of multifunctional VNPs has been fabricated by bioorthogonal conjugation approaches, in which various targeting catalytic units, ligands, diagnostic probes and therapeutic cargoes have been modified on the surface or inside of the internal cavity of VNPs.[3]

However, these covalent-constructed methods are irreversible, and normally need long reaction time and lengthy purification steps to introduce functional groups in the synthesis.[4] Conversely, supramolecular interactions are becoming more attractive since it is easy to realize the predicable change through attaching different stimuli-responsive groups on the basis of noncovalent synthesis, including hydrogen bonds, π-stacking, charge-transfer (CT) interactions, electrostatic interactions, and host-guest complexation.[5] In the supramolecular interactions family, CT interactions between the electron-rich and the electron-deficient species has been extensively applied to smart materials, self-assembly, drug and gene delivery due to its modularity, reversibility and stimuli-responsiveness.[6] For example, Huang and co-workers found that 2, 4, 6-Trinitrotoluene (TNT) can be encapsulated by microtubes assembled from the pillar[5]arene amphiphile using CT interactions.[7a] Guchhait and co-workers have successfully utilized the interactions of human serum albumin with CT-probe (ethyl ester of N, N-dimethylamino naphthyl acrylic acid) to study the protein micro-environment.[7b] Up to date, the reports on the application of CT-interactions modified VNPs are still rare,[8] though several works on the protein modification using supramolecular interactions have been reported.[9]

Herein, we used the pyrene (**PYR**) moiety bearing a PEG chain to link with tobacco mosaic virus, **TMV(wt)**, consequently fabricating an electron-donor based on VNPs (Figure 1a). **PYR** and its derivatives have been widely used as fluorescence probes in a large number of complex systems because of their high fluorescence quantum yields, long excited state lifetime, and the ability to form excimers.[10] **TMV(wt)** is a model VNP having rod-shape, 300 nm in length and 18 nm in diameter, consisting of 2130 identical subunit proteins arranged helically around genomic single RNA strand.[11] On the other hand, dinitrophenyl and pyridinium-contained guest molecules (Figure 1b) are chosen as the electron-acceptors to form CT complex.[12] The modified fluorescent **TMV(wt)-PYR** is able to form the supra-amphiphiles with different electron-deficient molecules through CT interactions (Figure 1c), in which a marked "switch off" of fluorescence from **PYR** motif could be observed.

As shown in Figure 1a, **PYR** was attached to the exterior surface of **TMV(wt)** by sequential diazonium-coupling and Cu^{I}-catalyzed azide-alkyne cycloaddition (CuAAC) reaction (See ESI for experimental details).[13] The formation of **TMV(wt)-PYR** was confirmed by UV-Vis and fluorescence spectra, as well as MALDI-TOF MS and SDS-PAGE analyses. As shown in Figure 2a, two new peaks at 345 and 511 nm are observed. The peak at 345 nm

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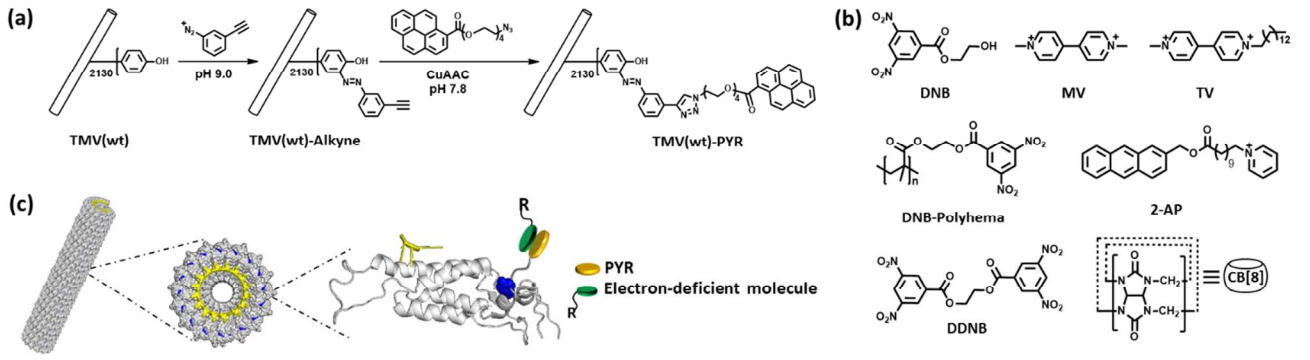

Figure 1. (a) Preparation of **TMV(wt)-PYR** using diazonium-coupling and 'CuAAC' reactions. (b) Structures of electron-deficient guest molecules and **CB[8]**. (c) Schematic demonstration of the formation of multifunctional **TMV(wt)** *via* CT interactions between **PYR** and electron-deficient molecules.

is typical for the **PYR** group, while the peak at 511 nm could be attributed to the conjugative effect between the azobenzenyl and 1, 2, 3-triazole moieties, implying a successful attachment of **PYR** moieties to the exterior surface of **TMV(wt)** by 'CuAAC' reaction. It can be further verified by fluorescence spectra, in which **TMV(wt)-PYR** shows a strong fluorescence signal at 417 nm as compared to **TMV(wt)** and **TMV(wt)-Alkyne** (Figure 2b). It should be noted that the tiny wavelength shift between **TMV(wt)-PYR** and small molecular **PYR-Azide** could be contributed to the intermolecular interactions of **PYR** groups on the virus.[14] MALDI-TOF MS result afforded the direct evidence for this complete conjugation reaction. As shown in Figure 2c, the peak of alkyne-modified **TMV(wt)** at m/z 17659 disappears completely, while a new peak at m/z 18111 is observed, indicating the full conversion of **TMV(wt)-alkyne** to **TMV(wt)-PYR** upon conjugation. It is consistent with the results from the SDS-PAGE analysis (Figure S1). In addition, the integrity of the **TMV(wt)** nanoparticles upon conjugations was confirmed by size exclusion chromatography (SEC, Figure S2), transmission electron microscopy (TEM, Figure 2d) and dynamic light scattering (DLS, Figure S3).

To test the CT interactions between **PYR** and electron-deficient molecules, six guest molecules, **DNB**, **DDNB**, **DNB-Polyhema**, **MV**, **TV**, and **2-AP** (Figure 1b), were synthesized (See ESI for experimental details). As a general protocol, **TMV(wt)-PYR** was incubated with electron-deficient molecules at different concentrations for 10 min at r.t. before the fluorescence measurement. As shown in Figure 3a, the fluorescence intensity of **TMV(wt)-PYR** is quenched dramatically upon the addition of **DNB**, revealing the CT interactions between **TMV(wt)-PYR** and **DNB**. Job's plot shows a 1:1 complex formation for **TMV(wt)-PYR**/**DNB** (Figure S4),[15] which indicates that each **TMV(wt)-PYR** subunit associates with one **DNB** molecule. Other DNB-contained

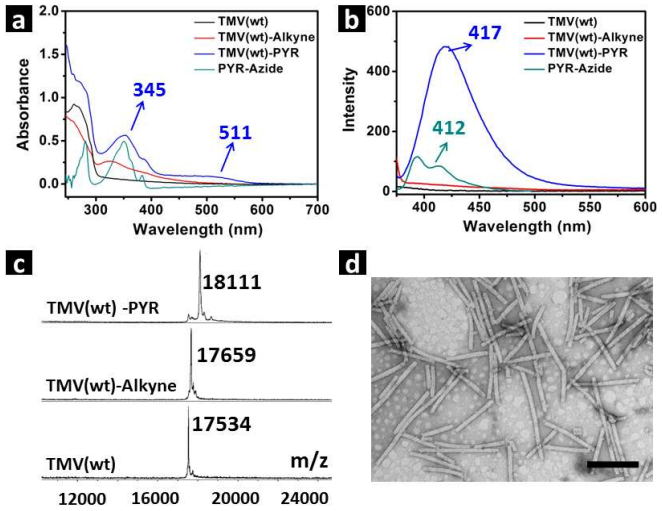

Figure 2. (a) UV-Vis and (b) Fluorescence spectra of **TMV(wt)**, **TMV(wt)-Alkyne**, **TMV(wt)-PYR**, and **PYR-Azide**. Excitation wavelength is 345 nm. (c) MALDI-TOF MS of the subunit proteins of **TMV(wt)**, **TMV(wt)-Alkyne**, and **TMV(wt)-PYR** (the calculated MS for **TMV(wt)**, **TMV(wt)-Alkyne**, and **TMV(wt)-PYR** are 17534, 17662, and 18109, respectively; the 452 m/z difference between **TMV(wt)-PYR** and **TMV(wt)-Alkyne** is consistent with the theoretical molar mass (447 m/z) of newly added **PYR-Azide** within the permitted error. (d) TEM image of uranyl acetate-stained **TMV(wt)-PYR**. Scale bar is 300 nm.

small molecule (**DDNB**) and polymer (**DNB-Polyhema**) were also tested using the above method. As shown in Figure 3b-c, **DDNB** and **DNB-Polyhema** can quench the fluorescence of **PYR** with binding modes as 1:2 and 1:1, respectively (Figure S5-6). Furthermore, there is no change in integrity of **TMV(wt)-PYR** upon the complexation as observed under TEM (Figure S7). It is apparent that the DNB-contained electron-deficient molecules can form CT-complexes with **TMV(wt)-PYR** nanoparticles. However, all attempts to measure the binding constants using

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isothermal titration calorimetry (ITC) failed, likely due to the existence of DMSO (as the co-solvent to dissolve the small molecules).

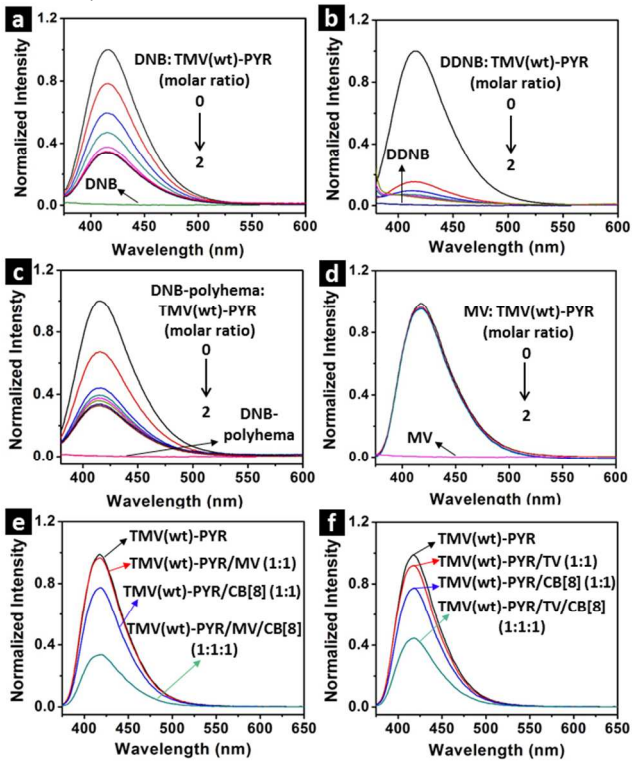

Figure 3. Fluorescence spectra of **TMV(wt)-PYR** (0.38 mg/mL in K-phosphate buffer, pH 7.8) with (a) **DNB**, (b) **DDNB**, (c) **DNB-Polyhema**, and (d) **MV** with various molar ratios. Fluorescence spectra of (e) **TMV(wt)-PYR/MV** and (f) **TMV(wt)-PYR/TV** with the addition of **CB[8]**. Excitation wavelength is 345 nm.

In contrast, when **MV** was used to form the CT-complex under the same condition as DNB-derivatives, no obvious reduction of the emission could be observed (Figure 3d). It was probably due to the electronic attractions between the positive charge of **MV** and the negative charge on **TMV(wt)**,[11] consequently leading to the absence of electron-deficient components for the formation of CT-complex. To prohibit such a binding, cucurbit[8]uril (**CB[8]**) was used as a "molecular handcuff" to bring **MV** and **PYR** moiety together.[16] It is known that **CB[8]** is a macrocyclic molecule which can form inclusive complexes with high selectivity and binding affinity in aqueous media.[17] Additionally, it was found that the size of **PYR** allowed the 1:1 complexation with **CB[8]**, and **CB[8]** is large enough to encapsulate two molecules at the same time. Therefore, we utilized **PYR** and **MV** as the guest for **CB[8]** to study their CT interactions in **CB[8]**. The emission spectra (Figure 3e) indicate the formation of supramolecular amphiphile as a significant fluorescence quenching effect could be observed from CT interactions between **PYR** and **MV** inside the **CB[8]** cavity. Even in the absence of **MV**, a slight reduction in the fluorescence intensity was still detected due to the host-guest binding.[18] Moreover, the MV-derivative **TV** (Figure 1b) that has a long alkyl chain was also used to study CT interactions with **TMV(wt)-PYR**. As shown in Figure 3f, an emission quenching could be observed similar to results from **MV**, which suggests that MV-derivatives give the similar binding behavior as **MV**. TEM image shows that there is no change of virus integrity after the complexation (Figure S7). Apparently, **TMV(wt)-PYR** could form the CT-complexes either with the neutral DNB-derivatives or positive-charged pyridinium-derivatives.

We have previously shown that **TMV** could be implanted in three-dimension porous hydrogels, and such implanted **TMV** hydrogels can enhance the cell attachment and promote the osteogenic differentiation of cultured stem cell.[19] Moreover, it exhibited the substantial decreasing immune, low toxicity, and were degradable in mice.[20] Meanwhile, it is known that **2-AP** (Figure 1b) can assemble into ultralong nanofibers bearing the electron-deficient pyridinium on their surface in K-phosphate buffer.[21] Inspired by the above results, we wonder if a supramolecular gel can form driven by CT interactions between **TMV(wt)-PYR** and the electron-deficient pyridiniums from the nanofiber surface. The results showed that a transparent supramolecular hydrogel formed with a T_g of 37 °C after mixing **TMV(wt)-PYR** and **2-AP** in K-phosphate buffer (the critical gel concentration of **TMV(wt)-PYR** and **2-AP** are 0.12 and 3.0 mg/mL, respectively, pH 7.8, Figure S8).[22] Whereas under the same condition only partial fragile hydrogel formed for **TMV(wt)**. It indicates that the CT interactions between **PYR** moieties and the pyridiniums promote the gelation. The detailed mechanism is being studied.

Conclusions

We demonstrated a facile strategy to fabricate multifunctional viral nanoparticles using CT interactions between pyrene-contained **TMV(wt)** nanoparticles with dinitrophenyl and pyridinium-based guest molecules. It is expected that the reversibility and the stimuli-responsive features of such supramolecular interactions can lead to development of novel functional biomaterials.

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Notes and references

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Electronic Supplementary Information (ESI) available: The details of instruments, reagents, and sample preparations; synthetic details, MS, and NMR spectra of **PYR-Azide**, **TMV(wt)-Alkyne**, **TMV(wt)-PYR**, **DNB**,

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DDNB, **DNB-Polyhema**, **MV**, **TV**, and **2-AP**; Data of SDS-PAGE, SEC, DLS, TEM, and Job's plot. See DOI: 10.1039/ c000000x/

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