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# ***FRET-based supramolecular architectures for temperature sensing and Cancer diagnosis***

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**Abstract :** Supramolecular nanostructured materials, displaying Förster resonance energy transfers (FRET) signals, have become the focus of interest for many researchers across the globe. FRET-based supramolecular systems have extended applications in areas as diverse as materials science, biochemistry, analytical chemistry, and nanomedicine. The non-covalent phenomena operating in supramolecular frameworks depends on many factors such as wide range of time scales, binding strengths, distances, and concentrations of the supramolecular components (host and guest). Herein, we focus in which FRET has been used to study non-covalent interactions having a key role of cancer diagnosis and temperature sensing in supramolecular systems. Furthermore, we have discussed FRET-based architectures with current the advancements in the field and provide a perspective on new progress for the future.

**Keywords :** SUPRAMOLECULAR ARCHITECTURES, NON-COVALENT INTERACTION, HOST-GUEST CHEMISTRY, FRET SIGNALS, DIAGNOSIS.

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## I. Introduction

Supramolecular chemistry describes chemical systems composed of a discrete number of molecules with self-organization tendency through weak and reversible non-covalent interaction. Supramolecular science heavily relies on macrocycle-based host-guest chemistry.(1,2) Typically, in a host-guest system, a large macrocyclic host recognizes a small guest molecule, binds to each other through non-covalent interactions (hydrophobic forces, electrostatic interactions, hydrogen bonding, Van der Waals forces) in a controlled manner.(3) This binding of host and guest molecules leads to the formation of host-guest complex.(1,3,4) More importantly, the guest molecules should fit in the cavity of host molecules, emphasizing on selective and spontaneous binding of host and guest, and thus realizing the molecular recognition.(5–7) Host-guest molecular recognition has brought revolutions in the development of more sophisticated supramolecular systems or materials because of their good selectivity and stimuli responsiveness. Taking advantage of weaker non-covalent host-guest interactions, the host-guest complexes are more suitable for reversibility and responsiveness towards various factors in the confined surrounding environment such as pH, light radiation, temperature, competitive factors, chemical signals, and biological interfering, etc.(3,4,6–8) The stimuli-responsive property of host-guest systems provide a favorable platform for the developing of new advanced artificial molecular machines and nanoscale smart materials.(9–11)

In the past decades, many FRET-based supramolecular system has been reported with extended applications such as optical sensor, photo-switches, artificial light harvesting system etc.(12–15). FRET got the attention of many researchers since the concept was first disclosed by Theodor Förster with a remarkable discovery of green fluorescent proteins (GFPs).(16) FRET-based approaches are considered applicable in the diagnosis of cancer, temperature sensing due to its high sensitivity, versatility and non-invasiveness.(17–19) Förster provided the basis of quantitative description for the non-radiative energy transfer between the donor and acceptor molecules. Practically, in a FRET phenomena, a donor fluorophore after absorption of light energy is excited to high energy state which in turn release the absorbed energy as emission of light, transfers its energy non-radiatively to the nearby acceptor fluorophore.(20) Moreover, the excitation energy of the donor's electron is transferred to that of the acceptor's via an induced-dipole movement interaction.(21) FRET is a more powerful tool than simple fluorescence because it is very sensitive to small changes in the ambient environment. There are many conditions that need to be met for the occurrence of FRET phenomena. Firstly, to ensure the occurrence of FRET, the process require an efficient spectral overlap of donor emission with acceptor fluorophore absorption considering molecular electronic excitation and energy release.(1,22,23) Secondly, the donor and acceptor fluorophores should be at favorable distance and needs to be less than approximately 10 nm.(24) The third and final criteria for the efficient energy transfer is the proper orientation of both the fluorophores to each other. Once all the conditions are satisfied, the FRET efficiency(25) and thus relying on the number of photons absorbed by donor fluorophores that has a key role in contribution of FRET and can provide highly sensitive, temporally specific information on molecular distance and orientation. These features broaden the scope of FRET and are well suited to study a wide-range of supramolecular phenomena with extended application in many fields. Moreover, FRET depends strongly on the distance between the donor and acceptor as given by Equation (1).

$$E = \frac{R_0^6}{R_0^6 + r^6} \quad (1)$$

where energy transfer efficiency is represented by E and r stands for the donor-acceptor distance.  $R_0$  is the Förster radius and varies with the size of donor-acceptor pairs and depends on the spectral overlap.

In general, three types of fluorescent activities have been noted in a FRET phenomenon, namely, the turn-off effect on the donor, the switch-on fluorescence of the acceptor and, sometimes the acceptor quench the emission of donor and acceptor.

Surprisingly, the functioning of FRET inside the host-guest system facilitates donor-acceptor pair association and dissociation with flexibility and controllability due to the stimuli-responsiveness and selectivity exhibited by host-guest non-covalent interactions. The combination of FRET with host-guest science possesses more advanced applications in many fields such as real-time in vivo monitoring of biomolecules, and structural manipulation,(26,27) cell imaging and drug delivery,(28,29) chemical and biological sensing(30–33) and photosynthesis mimicking.(18,34–36)

Herein, we particularly focus on FRET operating supramolecular systems used for the diagnosis of cancer, and temperature sensing. To ensure the current developments in the field, an up-to-date literature study was carried for signifying our understanding of cellular processes and help in advanced approaches applied in synthetic supramolecular systems and their in vivo applications.

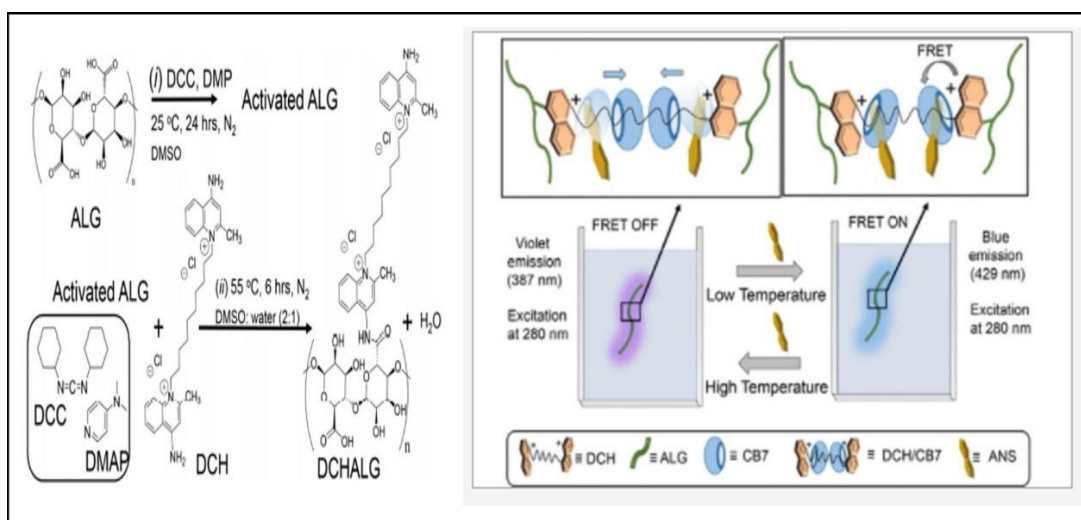
## 2. Applications of supramolecular FRET System Based on Host-Guest Chemistry

### 2.1. Temperature Sensing

Recently, Amir et al. reported the preparation of a bioactive, supramolecular carbohydrate polymer. The latter was formed by grafting cucurbit[7]uril macrocycle (CB7)-encapsulated dequalinium chloride hydrate (DCH) onto alginic acid carbohydrates (ALG) via amide linkage formation. The supramolecular assembly was shown to have the capability of light energy transfer which can be controlled by varying polymer temperature without changing the polymer.(4)

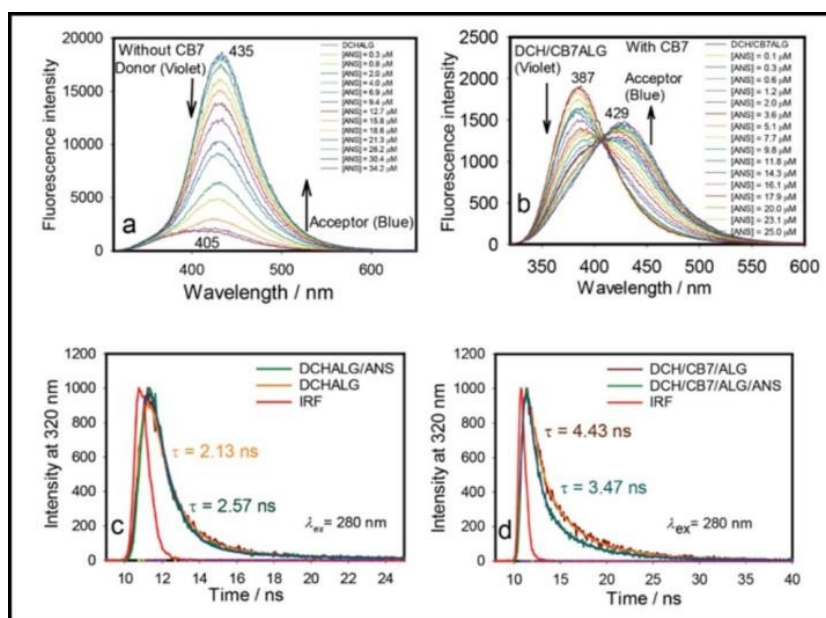
To validate energy transfer, DCHALG and DCH/CB7ALG polymer was titrated with ANS. No FRET signals were observed as no significant decrease in the excited lifetime was recorded while titrating DCHALG with ANS as shown in Figure 2a and 2c. To confirm FRET signals occurrence in DCH/CB7ALG upon titration with ANS, a substantial decrease in the excited state was recorded and thus showing the FRET signals between DCH/CB7ALG and ANS as depicted in Figure 2b and 2d.

Prior to any emission signal measurements, energy transfer was induced by introducing 0.5  $\mu\text{M}$  ANS at 298 K to guarantee the integrity of the examination. Following that, the temperature was reduced to 278 K, increased to 378 K, and then returned to 278 K. After ANS addition, the process was repeated three times with an ANS concentration rise of 2.75  $\mu\text{M}$  every cycle (up to 8.75  $\mu\text{M}$ ). The technique shown in Figure 3 was used to obtain the emission data.

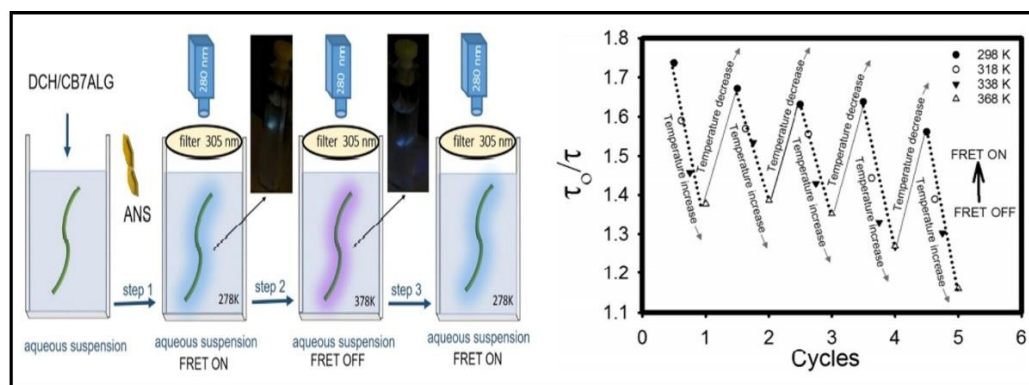


**Figure 1: Schematic representation of supramolecular carbohydrates polymer synthesis and modulation of FRET signals by altering temperature.(4)**

FRET signals were generated using DCH (donor) and 2-anilidonaphthalene-6-sulfonic acid (ANS, acceptor). The modified carbohydrate platform's stationary and time-resolved photoluminescence spectra indicated that FRET resulted in a color change from violet (387 nm) to blue (429 nm), which could be switched on and off to temperature stimuli at 298-368 K. DCH alginate polymer was prepared by covalent linkage of DCH to alginic acid (via amide linkage) with/without CB7. In the first step, alginic acid was activated in DMSO with the coupling reagent N, N'-dicyclohexylcarbodiimide (DCC), and 4-dimethylaminopyridine (DMAP) as catalyst for 24 hours under constant nitrogen purging (as depicted in Figure 1).



**Figure 2: At various concentrations, energy transfer from (a) DCHALG ( $1.5 \text{ mg L}^{-1}$  aqueous suspension) and (b) DCH/CB7ALG ( $2.75 \text{ mg mL}^{-1}$  aqueous suspension) to ANS. Corresponding 320 nm emission decays indicated by the estimated average excited-state lifetime in the absence and presence of CB7 (c) and (d).**



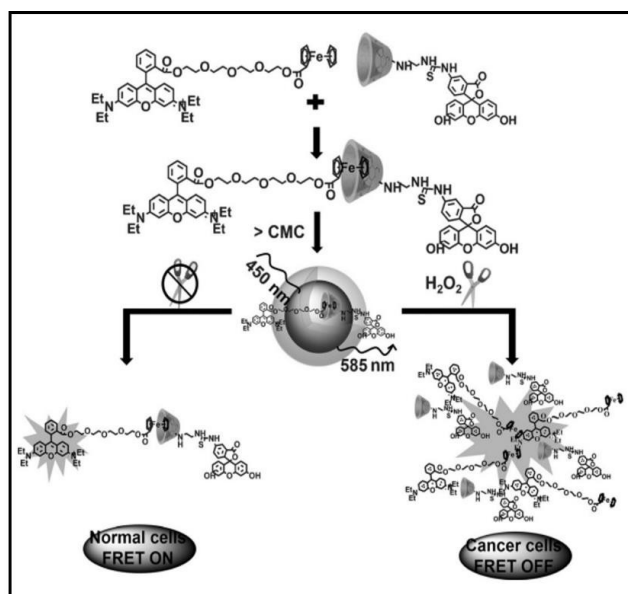
**Figure 3: Schematic representation of FRET signal regeneration in aqueous suspensions and FRET signal on/off switching between 298 and 368 K in 20 K increments was monitored by the rise in the unquenched/quenched lifespan ratio following temperature reduction (FRET ON) and increase in temperature (FRET OFF)**

The results in Figure 3 demonstrate the reversible response of DCH/CB7ALG to a temperature change from 278 to 378 K after adding an incremental quantity of ANS (7 M, from 8.75 to 33.3 M) four times to create five sensing cycles. The results validated the novel supramolecular polymers' response to temperature stimuli at 298-368 K in aqueous environments. It should be emphasized that in Figure 3, the unquenched/quenched lifespan ratio ( $\tau_0/\tau$ ) was determined in each cycle by simply changing the temperature from 298 to 368 K. Furthermore, the excited-state lifetime was assessed rather than the steady-state spectrum since the former was more suitable for validating FRET signal modulation in response to a thermal change. Furthermore, the physiological activity and toxicity of the proposed supramolecular polymers should be assessed, in view of their application to a wide range of biomedical uses in the future.

## 2.2. Cancer diagnosis

Wang and colleagues demonstrated that supramolecular fluorescent nanoparticles can be used for FRET detection of hydrogen peroxide ( $H_2O_2$ ) in cancer cells.(37) Supramolecular fluorescent nanoparticles self-assembled from fluorescein isothiocyanate modified  $\beta$ -cyclodextrin (FITC- $\beta$ -CD)/rhodamine B modified ferrocene (Fc-RB) amphiphile were employed for  $H_2O_2$  detection in cancer cells as depicted in Figure 4.

The self-assembled nanoparticles based on numerous non-covalent contacts in an aqueous medium demonstrated great sensitivity to  $H_2O_2$  while being stable under physiological conditions. The addition of  $H_2O_2$  resulted in a noticeable fluorescence shift of nanoparticles from red (RB) to green (FITC) in fluorescent tests due to the fluorescence resonance energy transfer (FRET) phenomenon. Furthermore, in vitro results clearly indicate that fluorescent nanoparticles may be effectively absorbed by cancer cells through endocytosis and then destroyed by endogenous  $H_2O_2$ , accompanied by FRET from “on” to “off.”.



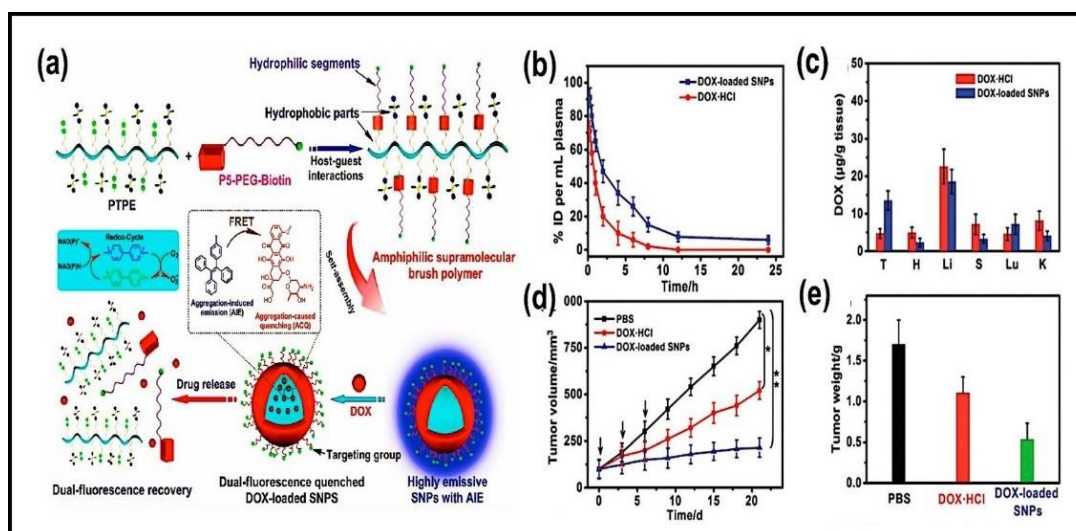
**Figure 4: The amphiphile FITC-b-CD/Fc-RB and its H<sub>2</sub>O<sub>2</sub>-activated behavior(37)**

These supramolecular fluorescent nanoparticles, which were created through a series of non-covalent interactions, are likely to have applications in the diagnosis and imaging of illnesses induced by oxidative stress, in which there is a cellular accumulation of reactive oxygen species such as hydroxyl radicals.

For many years, nanocarriers have been particularly designed for the transport and controlled release of drugs and biological molecules. However, cargo loading and release of frequently utilized carriers are difficult to monitor, particularly *in vivo*. Huang and colleagues described in their study, a [2]rotaxane complexation with pillar[5]arene (P5) to achieve mitochondria imaging and drug delivery materials.(28) In this work, aggregation-induced emission (AIE) and aggregation-caused quenching (ACQ) were introduced in a host-guest controlled FRET investigation. A [2]rotaxane with increased fluorescence was produced by attaching a tetraphenylethylene (TPE) unit, a typical AIE-active luminophore, as a stopper on one end of the axle with pillar[5]arene acting as the wheel. The anti-cancer medication Doxorubicin (DOX), as well as the ACQ agent, were covalently attached to the wheel through imine bridges and therefore positioned near to TPE, resulting in a significant failure of the fluorescence emission owing to the ACQ effect, which happened during FRET from TPE to DOX. Because of the additional negative membrane potential of mitochondria, it may be identified by the [2]rotaxane via electrostatic interactions and lighted up as a result of the recognition.

Based on the above foundation, they started to create FRET-capable SNPs for DOX delivery that were constructed using pillar[5]arene-based amphiphilic supramolecular brush copolymers (SBPs) (29) TPE and 4,4'-bipyridinium derivative (M) moieties were alternately implanted onto the polymer. The host-guest interactions formed between the M entities and PEG-Biotin (targeting group), as illustrated in Figure 5a, functionalized P5. As a result, SBPs were produced and then self-assembled into SNPs, which displayed an AIE effect caused by the aggregation of TPE units in the particles' center. The system's emission decreased when DOX was encapsulated in the SNPs because of FRET from TPE to DOX and the ACQ of the DOX units, resulting in dual-fluorescence quenching. When the guest M was reduced by the intracellular reductase NAD(P)H in an acidic environment from a bicationic entity to its radical cationic state, the binding between them and P5 was severely weakened, with the association constant dwindling by two orders of magnitude, resulting in the detachment of the host-guest pair and the dis-association of the SNPs.

DOX was therefore liberated from the confinement of the particles, and led to the restoration of fluorescence as well. Figure 5b–e demonstrates the controlled drug releasing characteristics, distributions in normal tissues, and cancer cell inhibition efficiency, indicating the good encapsulation and cancer targeting capabilities (DOX concentration was higher in tumor treated with DOX-loaded SNPs than with DOX alone in contrast to that in other organs). In this work, the proximity/separation of the FRET pair was regulated in a very circuitous, but subtle, way by modulating the assembly of SBPs and FRET at the same time, producing an exquisite collaboration between stimuli-responsiveness of host-guest chemistry and fluorescence signaling of FRET.



**Figure 5: (a) Procedure for the creation and disassembly of SNPs with DOX loading and dual fluorescence quenching; (b–e) Graphical depiction of the drug-delivering SNPs' in vivo effects: (b) blood circulation time of DOX.HCl and DOX-loaded SNPs as measured by DOX plasma concentration after injection; (d) Tumor growth inhibition curves on the HeLa tumor model treated with phosphate buffered saline (PBS), DOX.HCl, and DOX-loaded SNPs, in that order. (e) The average weight of tumors in mice carrying HeLa tumors following the three different treatments indicated above [29]. 2016. Copyright. The Royal Society of Chemistry has granted permission for this reprint**

### 3. Conclusion and future Perspectives

The innovative collaboration between FRET effects and host-guest chemistry has brought significant changes in the fabrication of a wide range of smart fluorescent materials with stimuli-responsive properties. However, in this emerging field, there are still many challenges for researchers to overcome in order to play better light tricks. These supramolecular fluorescent nanoparticles, which were created through a non-covalent interaction, are likely to have applications in the diagnosis of cancer disease and temperature sensing. Moreover, the amazing evolution of dynamically constructed FRET systems backed by supramolecular macrocyclic chemistry has resulted in the creation of a wide range of smart fluorescent materials with extended applications. Furthermore, it is believed that more advanced supramolecular frameworks will have numerous biomedical applications in the future.

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