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Visualization of Stereoselective Supramolecular Polymers via Chirality Controlled Energy Transfer

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Abstract: Chirality driven self-sorting is envisaged to efficiently control functional properties in supramolecular materials. However, it faces challenge due to lack of analytical methods to directly monitor the enantioselectivity of resulting supramolecular assemblies. In this context, we present two fluorescent core-substituted naphthalene diimide based donor and acceptor molecules with minimal structural mismatch and comprising of strong self-recognizing chiral motif, for solely chirality to determine the self-sorting process. As a consequence, stereoselective supramolecular polymerization with an unprecedented chirality control over energy transfer has been achieved. This chirality controlled energy transfer has been further exploited as an efficient probe to visualize microscopically the chirality driven self-sorting.

Naturally occurring systems have an extraordinary ability to competently discriminate between self and non-self from complex mixtures of similar structural motifs. Moreover, biological systems such as enzymes and proteins can chirally recognize between the two enantiomers via supramolecular interactions to facilitate biological processes.^[1] Inspired by this, structural mismatch^[2] and pH variation^[3] driven self-sorting process has been investigated in synthetic supramolecular assemblies. This has been exploited further to control organization in functional supramolecular assemblies to modulate their resultant properties.^[2] For example, in chromophoric assemblies, the self-sorting process is utilized to modulate their opto-electronic properties^[2] and its spectroscopic properties are often used to probe these assemblies.^[4] More recently, high resolution microscopic techniques were used to even visualize the self-sorted supramolecular polymerization.^[5]

However, bio-inspired chirality driven self-sorting systems have been rarely investigated.^[6] In this context, chirality driven self-sorting of enantiomeric monomers has been exploited to achieve stereoselective supramolecular polymerization.^[7] Our group has also shown the chirality driven self-sorting of donor (D) and acceptor (A) supramolecular polymers to control the chargetransfer properties between the chromophores.^[8] However, the scarceness of chirality based self-sorted supramolecular assemblies can be attributed to the dynamicity of these assemblies, which results in low level of chiral differentiation and lack of analytical methods to characterize them. In this communication, we present a chirality driven control on the selfsorting characteristics of supramolecular fibers to modulate their energy transfer properties and thereby the resulting optical properties. Furthermore, we have utilized the chirality controlled energy transfer as an efficient probe to spectroscopically investigate and to visualize the resulting co-assembled and self-

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E-mail: <u>george@jncasr.ac.in;</u> Web: <u>www.jncasr.ac.in/george</u> Supporting information (SI) for this article is given via a link given at the end of this document sorted supramolecular polymers. Although, various molecular designs have been reported to modulate the energy transfer process in supramolecular assemblies,^[9,10] a chirality based approach to control energy transfer is seldom reported.^[11,4b]



Figure 1. a) Molecular structures of donor SS-diOEt and acceptors RR/SS-OEtiPa. b) Schematic representation of chirality controlled co-assembly and self-sorting and resultant chirality controlled energy transfer process.

Our group has previously reported the stereoselective supramolecular polymerization of D-A charge-transfer assemblies, using trans-1,2-bis(amido)cyclohexane (BAC) as strong chiral self-recognizing motif.^[12] In the present study, we have modified the D and A chromophoric components into a Förster resonance energy transfer (FRET) pair for a chirality controlled energy transfer, which we further employ for the visualization of stereoselective supramolecular polymerization. The two key requirements for an efficient chiral recognition driven energy transfer are: i) a minimum structural mismatch between the chromophores for chirality to control the self-sorting process and, ii) well-separated optical properties of D and A. To fulfill the requirements, we chose core-substituted naphthalene diimide (NDI) derivatives as fluorescent chromophores. For this, naphthalene diimides were appropriately core substituted, with both side -OEt (ethoxy) as a green emissive donor chromophore and with unsymmetrical core-substitution with -OEt and -iPa (Nisopropyl amine) groups for the red-emissive acceptor chromophore (Fig. 1a). The donor was tethered in (S,S)-chiral BAC core (SS-NDI-OEt), whereas the acceptor NDI derivative was tethered in both (R,R) and (S,S)-chiral cores of BAC (RR/SS-NDI-OEtiPa) to investigate the chiral self-sorting process (Fig. 1b). The donor and acceptor NDI derivatives were synthesized and characterized (Scheme S1 to S4) by NMR and mass spectrometry.

Although, core-substituted naphthalene diimides (cNDIs) are well studied for their tunable optical and electron transfer properties,^[13] their self-assembly has not been well investigated

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Fig. 2. Self-assembly of SS-diOEt (top panel) **and SS-OEtiPa** (bottom panel): Composition dependent a), e) Normalised absorption spectra and b), f) Normalised emission spectra ($\lambda_{ex} = 415$ nm for **SS-diOEt** and 430 nm for **SS-OEtiPa**), c), g) CD spectra in molecularly dissolved (TCE) and self-assembled states (TCE/MCH). c) also shows the LD spectrum of **SS-diOEt** and g) also shows the mirror image CD spectra of both enantiomer **SS-OEtiPa** and **RR-OEtiPa** and d), h) TEM images showing 1-D nanofibers in aggregated 25/75 and 1/99 (*v*/*v*) TCE/MCH solutions respectively. Insets of b) and f) show the photographs of the corresponding molecularly dissolved (left) and self-assembled (right) solutions under UV-light (365 nm irradiation) ([**SS-diOEt**] = [**SS-OEtiPa**] = **RR-OEtiPa** = 2.5x10⁻⁵ M).

so far. Thus, we first investigated the self-assembly of the individual D and A molecules. SS-diOEt and SS-OEtiPa exists in molecularly dissolved state in polar solvents such as 1,1,2,2tetrachloroethane (TCE) which is signified by sharp vibronic features at 347 nm and 367 nm in the absorption spectrum (Fig. 2a,e, S1, S4). Upon introducing non-polar solvents like methylcyclohexane (MCH), their self-assembly is induced which is depicted by the broadening of absorption spectra and reversal in intensity of vibronic features of the π - π * band at 347 nm and 367 nm (Fig. 2a,e, S1-S6). The assembled state (25/75 (v/v) TCE/MCH) SS-diOEt emission spectra shows a new red-shifted emission band at 516 nm in comparison to 502 nm band in molecularly dissolved state (TCE) (Fig. 2b, S2-S3). A similar red shift from 591 nm to 641 nm is observed in SS-OEtiPa assembly (Fig. 2f, S5-S6). Lifetime decay profiles of SS-diOEt assembly (6.89 ns) shows increased lifetime than that of the corresponding lifetime of monomer in TCE (1.78 ns) (Fig. S7a, Table S1), which along with the excitation spectra (Fig. S2) suggests the formation of fluorescent J-aggregates. However, lifetime decay profile of the SS-OEtiPa assembly showed a sharp decay component (1.89 ns) in comparison to monomeric state (10.96 ns) suggesting formation of J-aggregates with an efficient excitonic migration in the stacks (Fig. S7b, Table S2).[14,9a]

Although, **SS-diOEt** exhibited a positive bisignated CD signal in the monomeric state (in TCE), characteristic of intramolecular excitonic coupling in bischromophoric assemblies, the CD signal of the corresponding self-assembly is highly contaminated with the contribution of linear dichroism (LD) suggesting the presence of long aligned supramolecular fibers (Fig. 2c). However, CD spectra of **RR-** and **SS-OEtiPa** show mirror image bisignated CD signal in both molecularly dissolved and self-assembled state (Fig. 2g), suggesting the presence of both intramolecular and intermolecular excitonic coupling.

The morphological investigations into D and A assemblies show that both of them form 1-D fibers as visualized by TEM and

FE-SEM (Fig. 2d,h, S8, S9). The fluorescent nature of these assemblies were further confirmed by confocal microscopy that shows green and red fluorescent 1-D nanofibers of **SS-diOEt** and **SS-OEtiPa**, respectively (Fig. S8, S9).

Spectoscopic investigations revealed sufficient spectral overlap of self-assembled **SS-diOEt** emission and **RR/SS-OEtiPa** absorption spectra which is necessary to obtain an efficient energy transfer (Fig. S10a). Moreover, our previous study on BAC based D-A system suggest that due to strong hydrogen bonding between the chiral BAC motif, there is significant energy difference between homochiral and heterochiral stacks. Hence, we envisage to study the effect of chirality to determine the self-sorting behavior of present D and A system and to use FRET as a probe to spectroscopically and microscopically discriminate between chirality driven self-sorted and co-assembled D and A assemblies.

First we investigated the spectroscopic properties of the homochiral mixture of D and A molecules. To avoid any kinetic traps, all the samples were annealed by heating and cooling prior to measurements. Upon excitation of donor at 415 nm in a solution of **SS-diOEt** (c = 2.5x10⁻⁵ M, 25/75 (v/v) TCE/MCH) and 0.2 % SS-OEtiPa shows a significant quenching of donor fluorescence at 516 nm and buildup of acceptor emission at 591 nm (Fig. 3a). Increase in acceptor percentage beyond 0.2% does not result in further quenching of donor emission, suggesting saturation of energy transfer process with mere 0.2 % of acceptor. This indicates an efficient co-assembly of D and A molecules and a very efficient energy transfer in these homochiral stacks, which is also confirmed by the excitation spectra (Fig. S10e). The indirect excitation of the acceptor at 415 nm in the homochiral mixture showed emission at 591 nm corresponding to its monomeric emission, suggesting that the acceptors is distributed in the stack as isolated molecules. Moreover, the direct excitation of the A at 550 nm shows a much lesser intensity than that by indirect excitation at 415 nm indicating an amplified emission via

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Figure 3. Energy-transfer probing of chiral self-sorting: Top panel corresponds to solution of SS-diOEt and SS-OEtiPa whereas bottom panel corresponds to the solution of SS-diOEt and RR-OEtiPa a), e) Emission spectra and corresponding confocal microscopy images collected by b), f) Channel I (480-560 nm), c), g) Channel II (570-620 nm) and d), h) Merged Channel I and II. a) Co-assembly of SS-diOEt and SS-OEtiPa depicted by quenching in donor and amplified acceptor emission that show FRET. b-d) Confocal microscopy images with 0.2 % SS-OEtiPa. b) Shows SS-diOEt green fluorescent fibers, c) shows energy transfer to incorporated SS-OEtiPa acceptor in donor stacks and d) merged image depicting spatial overlap of fibers emitting green and red emission confirming co-assembly and energy transfer. e) Self-sorted assemblies of SS-diOEt and RR-OEtiPa depicted by negligible changes in donor and acceptor emission. f-h) Confocal microscopy images with 1 % RR-OEtiPa. f) Shows SS-diOEt green fibers, g) shows absence of red emitting species and absence of energy transfer and h) merged image depicting presence of solely SS-diOEt green emitting fibers. Note: Insets of a) & e) shows the corresponding photographs of the donor and acceptor mixture under UV-light (365 nm irradiation), ([SS-diOEt] = 2.5x10⁵ M, 25/75 (v/v) TCE/MCH))

energy transfer (Fig. 3a).

We next investigated the D and A mixture of opposing chirality, SS-diOEt and RR-OEtiPa, to see the effect of chirality on assembly characteristics. A solution of SS-diOEt (c = 2.5x10⁻⁵ M, 25/75 (v/v) TCE/MCH) with upto 30 % RR-OEtiPa, on excitation at 415 nm, did not result in any significant quenching of donor emission suggesting the absence of energy transfer process, which also suggests a chirality driven self-sorting of D and A assemblies (Fig. 3e). The energy transfer process was further validated by time-resolved fluorescence measurements. Homochiral, co-assembled solutions of SS-diOEt and SS-OEtiPa show decrement in lifetime of donor and enhancement in lifetime of acceptor (Fig. S10b,c, Table S3-S4) whereas chiral self-sorted assemblies of SS-diOEt and RR-OEtiPa do not show any change in donor and acceptor lifetime, compared to the individual homochiral stacks of D and A molecules (Fig. S10d, Table S5-S6). The enatioselectivity of SS-diOEt towards SS-OEtiPa over RR-OEtiPa was calculated to be 98.6% (Fig. S11).

Next we visualized this chirality driven co-assembled and self-sorted supramolecular polymers microscopically using energy transfer as a probe. The confocal images of various solutions dropcasted on glass slide were imaged. The donor was excited at 458 nm and the emission was collected in two channels, channel I for 480 to 560 nm and channel II for 570 to 610 nm emissions, corresponding to green and orange/red emission respectively. The solution of **SS-diOEt** (c = 2.5×10^{-5} M, 25/75 (*v*/*v*) TCE/MCH) and 0.2 mol% **SS-OEtiPa** showed fibers of green and

orange emission, when viewed though channels I and II (Fig. 3b,c, S12). On merging them, we observe a perfect overlay of the two colours which confirms that the emission originates from the same fibers confirming co-assembled fibers of D and A molecules, via stereoselective supramolecular polymerization (Fig. 3b-d, S12). A similar observation was concluded in fluorescence microscopic images (Fig. S13).



Figure 4. Visualization of self-sorted fibers of 1:1 mixture of SS-diOEt and RR-OEtiPa. Fluorescence microscopy images obtained by a) Excitation at the donor absorption using GFP (BP 470/40) filter and collection at donor emission showing green fluorescent domains, b) excitation at acceptor absorption using TX2 (BP 560/40) filter and collection at acceptor emission showing red fluorescent domains and c) merged image showing separate domains with no overlap of green and red domains. ([SS-diOEt] = 2.5×10^{-5} M, [RR-OEtiPa] = 2.5×10^{-5} M, 1/99 (v/v) TCE/MCH.)

In contrast, the solution of **SS-diOEt** (c = 2.5×10^{-5} M, 25/75 (*v*/*v*) TCE/MCH) and 1 mol% **RR-OEtiPa** showed only presence of green fibers on excitation of donor band at 458 nm (Fig. 3f-h, S12). No orange/red emissive species were obtained due to

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absence of any energy transfer process suggesting that no incorporation of A happened in the D stacks. To visually distinguish the chiral self-sorting process, a 1:1 solution of **SS-diOEt:RR-OEtiPa** (c = 2.5×10^{-5} M, 1/99 (*v*/*v*) TCE/MCH) were seen under fluorescence microscope (Fig. 4, S14). Interestingly, distinguished regions of green and red emission were obtained, which show domain formation and hence we were able to successfully distinguish both donor and acceptor supramolecular fibers in the self-sorted assembly **SS-diOEt** and **RR-OEtiPa**.

In conclusion, we have reported an unprecedented chirality driven energy transfer and self-sorting of fluorescent supramolecular fibers assembled from core-substituted NDI derivatives. We have shown a very efficient chiral recognition and resulting energy transfer with maximum efficiency with just 0.2 mol% acceptor in the co-assembled solution. We have successfully distinguished the co-assembly and self-sorting of the molecules spectroscopically and visually by our design strategy of structurally similar and chiral novel FRET pairs. We believe that, the present study will unveil the applications of chirality driven self-sorting process in opto-electronic materials and for the understanding of self-sorting events. It should also open the paths for the emerging kinetically controlled stereoselective supramolecular polymerization processes to be visualized.

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Visual discrimination of self-sorted and co-assembled stereoselective supramolecular polymers is presented using chirality-controlled energy transfer.



Aritra Sarkar, Shikha Dhiman, Aditya Chalishazar and Subi J. George*

Page No. – Page No.

Visualization of Stereoselective Supramolecular Polymers via Chirality Controlled Energy Transfer