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Molecular Recognition Controlled Stereomutation Cycle in a Dynamic Helical Assembly

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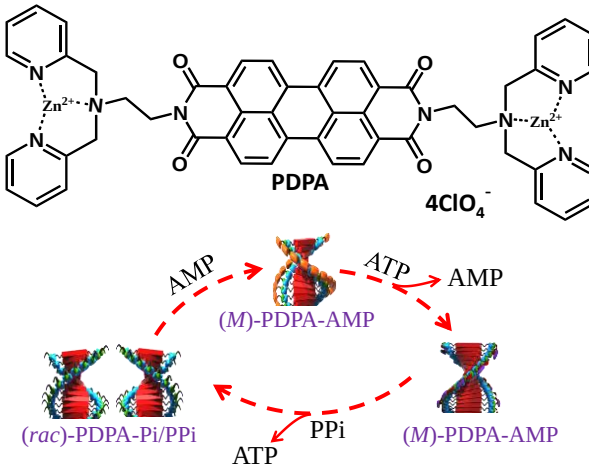
Perylenebisimide functionalized with phosphate recognition unit assembles into left-handed, right-handed or racemic helical assembly on binding with AMP, ATP and inorganic phosphates, respectively. Thus, competitive binding among these multivalent guests were utilized for completing an unprecedented helix mutation cycle in a dynamic supramolecular assembly.

Natural helical macromolecules display an elegant control over their helix handedness through preferred configuration of homochiral building blocks.[1] In artificial helical polymers[2] and supramolecular assemblies,[3] handedness has been controlled by employing monomers of opposite enantiomers.. Such an approach involves additional synthetic challenge of obtaining both enantiomeric monomers. However, homochiral guest (chiral auxiliaries) induced helicity into the assembly of achiral molecules present a smart design strategy, whose handedness can be easily controlled by configuration of the easily accessible guest molecules.[4] Very recently, this design has been further utilized for the construction of metastable states, enantioselective sensing and other chiroptical applications.[5] An evolved level of control over the helical assembly would demand a dynamic switching of main chain chirality without affecting the configuration of stereocenters. Such a rational design of precise control over the helix handedness of one-dimensional (1-D) supramolecular polymers has not been reported.

Biomacromolecules like polynucleotides represent a class of polymers which is shown to display stimuli responsive reversible stereomutation.[6] A biomimetic approach of stereomutation in synthetic helical polymers has been demonstrated by controlling parameters like temperature, solvent, pH, light, redox states, guest molecules, metastable assemblies etc.[7] However, a rational design for dynamic switching of helical states leading to complete control over helix mutation cycle in a synthetic supramolecular polymer remains unprecedented. Here we report, an efficient strategy of competitive guest binding among multivalent guests for dynamic switching of helical states of a receptor functionalized supramolecular polymer, from racemic to left-handed followed by right-handed helix before converting them back to racemic stacks, thus completing a helix mutation cycle. Our recent report of dipicolylethylenediamine–zinc (DPA–Zn) functionalized perylenediimide derivative (**PDPA**) show opposite helical assembly of **PDPA** molecules upon interaction with AMP (left-handed, *M*-helix) and ATP (right-handed, *P*-helix).[8] Taking advantage of the competitive guest binding among multivalent guests, AMP can be replaced by ATP leading to dynamic helix reversal from *P*-helix to *M*-helix. Using this strategy, we could replace bound AMP by ATP, which can further be replaced by achiral pyrophosphate (PPi, $P_2O_7^{4-}$). This results in a helix transformation from racemic to *M*-helix followed by *P*-helix and finally converting them back to racemic assembly, completing one helix cycle (Scheme 1). Thus, we demonstrate a tandem guest exchange for step-wise control of the helix handedness of a synthetic assembly, resulting in one complete helix mutation cycle.



Scheme 1 Chemical structure of **PDPA** along with schematic representation of step-wise change in helix handedness by competitive replacement of phosphate guests.

Towards this goal, homochiral stacks with preferred handedness were constructed by binding of chiral adenosine phosphates to **PDPA** racemic assemblies. Addition of AMP to **(*rac*)-PDPA**

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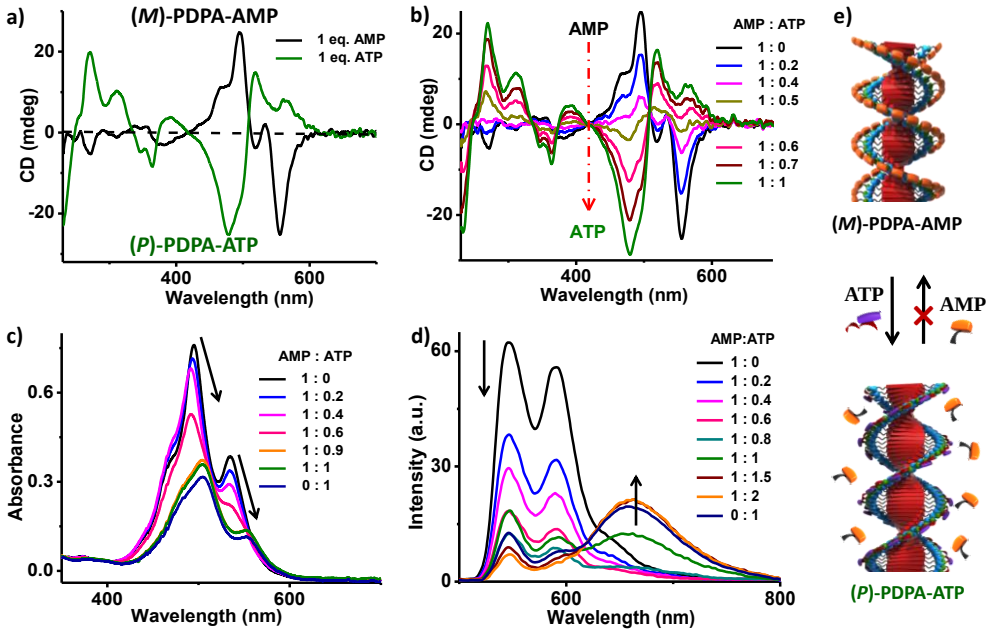

Fig. 1 a) CD spectra of (***M***)**-PDPA-AMP** and (***P***)**-PDPA-ATP** assemblies showing their mirror image relation. Variation in b) CD signal, c) absorption spectra and d) emission spectra (λ_{ex}= 470 nm) upon addition of ATP to (***M***)**-PDPA-AMP** solution (90% aq. HEPES in MeCN, c = 2 x 10^{-5} M). e) Schematic representation of dynamic helix reversal through competitive replacement of AMP by ATP.

stacks (90% HEPES in MeCN, 2 x 10^{-5} M) resulted in negative bisignated circular dichroism (CD) signal (negative and positive maxima at 557 nm, 496 nm, respectively, Fig. 1a, Fig. S1). This indicates a left-handed (*M*-helix type) organization of PBI chromophores i.e. (***M***)**-PDPA-AMP**. On the other hand, addition of adenosine triphosphate (ATP) to **(*rac*)-PDPA** stacks induced a positive bisignated CD signal (positive and negative maxima at 518 nm, 480, nm respectively, Fig. 1a). This is a clear signature of right-handed (*P*-helix type) organization i.e. **(*P*)-PDPA-ATP**, which is opposite in handedness compared to **(*M*)-PDPA-AMP** assemblies as shown in Fig. 1a. The origin of opposite helicity upon AMP and ATP binding to DPA derivative was previously attributed to the differences in their mode of binding and the differential positioning of adenosine moiety with respect to the stacks.[9]

Another important aspect of these multivalent guests is their strength of interactions which is easily tuneable based on the number of available binding groups. For example, AMP being mono phosphate can be competitively replaced by ATP with three phosphate binding groups, due to significant differences in association constant (Ka). The highest value for Ka (ATP) was 1.2 x 10^6 M^{-1}, whereas Ka (AMP) was 8.8 x 10^4 M^{-1} (Fig. S2-4). These data were obtained by fitting the titration curves using "GraphPad PRISM" software. We notice that indeed ATP binding is much stronger than AMP, thus AMP can be replaced easily by ATP. This strategy was recently employed for demonstrating helix reversal in naphthalenediimide (NDI) derivatives.[10] We envisaged that such an approach could be used to impose a well-defined control over the helix handedness of stacks. Thus, aliquots of ATP were added to **(*M*)-PDPA-AMP** solution while monitoring the CD spectra. We notice that the CD spectra inverts gradually from a negative bisignated signal to a positive bisignated signal, passing through an isodichroic point at zero crossing of 418 nm (Fig. 1b). These changes were very fast, where the addition of an aliquot of ATP to **(*M*)-PDPA-AMP** led to sharp jump in the CD signal (Fig. S5). Moreover, the final CD signal obtained after replacement of AMP by ATP was same in intensity as compared to the **(*P*)-PDPA-ATP**, confirming complete substitution of guest molecules. Apart from the opposite CD signal, the replacement of AMP by ATP was further confirmed by bathochromic shift of the band maxima from 499 nm to 514 nm and 535 nm to 564 nm on formation of **PDPA**-ATP stacks (Fig. 1c). Fluorescence spectra also show evolution of a new red shifted band at 665 nm upon addition of ATP (Fig. 1d). These spectral features were shown to be the characteristic of **(*P*)-PDPA-ATP**, confirming the complete replacement of AMP from the PDPA stacks (Fig. S6).

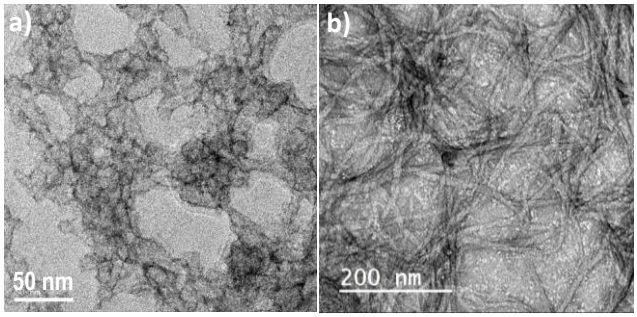

Fig. 2 TEM micrographs of **PDPA** assembly in presence of a) 1 eq. AMP, b) 4 eq. AMP followed by 1 eq. ATP showing morphology transition from ill-defined aggregates to 1-D nanofibers confirming replacement of bound AMP by ATP (90% water in MeCN, 2 x 10^{-5} M). Helical structures could not be visualized in their microscopic images probably due to bundling of aggregates.

To gain further insight into the supramolecular organization replacement of **PDPA** bound AMP by ATP, microscopic investigations were undertaken. Transmission electron micrograph of **(*M*)-PDPA-AMP** shows formation of irregular nanostructures (Fig. 2a). However, upon competitive replacement of AMP by ATP, we observe a morphology transition to well-defined one-dimensional

(1-D) nanofibers (Fig. 2b). The widths of these fibers were nearly 5 nm, which is close to the length of **PDPA**-ATP complex (ATP bound on both side of **PDPA**). Moreover, these fibers closely resemble the nanostructure obtained by **(*P*)-PDPA-ATP** (Fig. S7). This unambiguously confirms the transformation from **(*M*)-PDPA-AMP** to **(*P*)-PDPA-ATP**, presenting a very simple strategy for the dynamic reversal of supramolecular helicity.

Having shown the dynamic reversal of supramolecular helicity in these self-assembled stacks, a method to convert homochiral to racemic stacks is essential in gaining a complete control over their helical states. In this regard, we utilized an inorganic achiral phosphate, PPi [$(P_2O_7)^{4-}$], which can replace AMP as well as ATP due to their high charge density. Addition of PPi to either **(*M*)-PDPA-AMP** or **(*P*)-PDPA-ATP** led to complete loss of negative bisignated CD signal (Fig. S8) due to formation of **(*rac*)-PDPA-PPi** stacks. With these experiments, we have shown switching of helicity from a left-handed to right-handed assembly as well as dynamically convert them into their racemic form.

With the demonstration of control over various single step transitions like inversion of helicity, racemic to homochiral and vice-versa, the next challenge was to perform them in a sequential manner in one pot (Fig. 3). This was attempted by tandem addition of different phosphates, in order to complete one helix cycle from racemic to left-handed followed by right-handed helix before converting them to racemic stacks again. Thus, we started with **PDPA** stacks pre-bound with achiral inorganic mono-phosphate, Pi [$(PO_4)^{3-}$], to give racemic assembly **(*rac*)-PDPA-Pi**. To this solution aliquots of AMP were added, which can replace Pi, thereby converting them into a homochiral left-handed assembly. This could be easily monitored by the evolution of negative bisignated CD signal, confirming the formation of **(*M*)-PDPA-AMP** stacks (Fig. 3a). Subsequent addition of ATP to the above solution resulted in reversal of CD signal from negative to positive bisignated CD signal, passing through an isodichroic point with zero crossing at 423 nm (Fig. 3b). This clearly indicates transition between two states, i.e. from left-handed **(*M*)-PDPA-AMP** to right-handed **(*P*)-PDPA-ATP** through dynamic helix reversal. Next step in this sequential process was the addition of achiral diphosphate, PPi, to the above obtained **(*P*)-PDPA-ATP** stacks. CD spectra show continuous decrease in signal intensity, which finally resulted in CD silent state at higher eq. of PPi (Fig. 3c). This confirms the transformation from ATP bound right-handed helix to **(*rac*)-PDPA-PPi** stacks. Absorption spectra of **(*rac*)-PDPA-Pi** and **(*rac*)-PDPA-PPi** confirmed that the molecules are not in their monomeric state, but are assembled as racemic stacks. A better picture of the whole process could be obtained by the plot of maximum CD intensity (near 495 nm) against sequential addition of various phosphates. Thus, CD silent feature of **(*rac*)-PDPA-Pi**, show increase in signal upon AMP addition (left-handed helix),

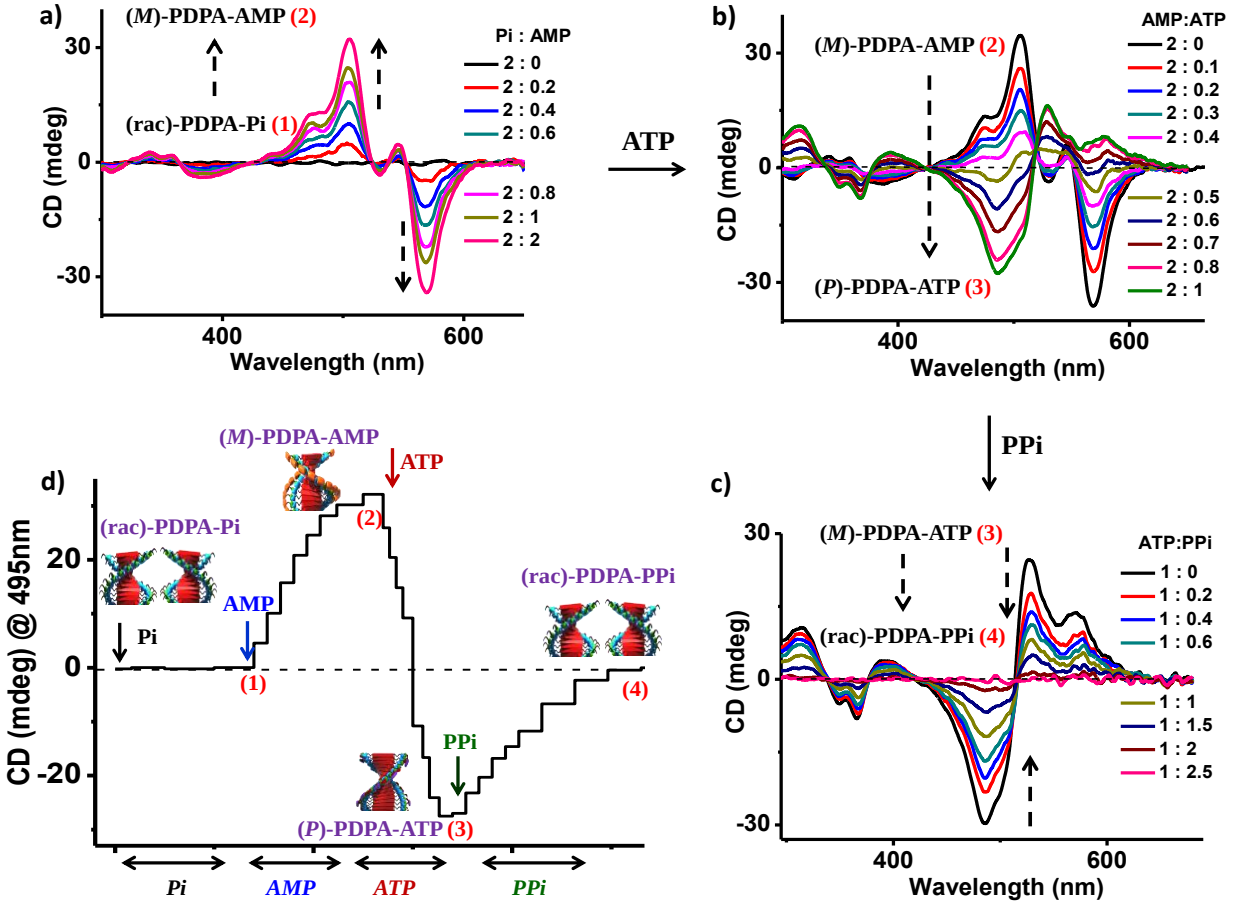

Fig. 3 Variation in CD signal upon sequential addition of a) AMP to **(*rac*)-PDPA-Pi** followed by b) ATP and subsequently c) PPi (90% aq. HEPES in MeCN, c = 2 x 10^{-5} M). d) The plot of CD maxima near 495 nm upon sequential addition of various phosphates, whereas the schematic represents the respective helical states obtained. All spectra in a, b and c were obtained from the same solution with subsequent addition of different phosphates each time.

followed by its reversal in presence of ATP (right-handed helix), before further decreasing to zero CD signal in presence of PPi (racemic) (Fig. 3d). Thus, we have shown a sequential change of helical states from racemic **(*rac*)-PDPA-Pi** to left-handed **(*M*)-PDPA-AMP**, followed by right-handed **(*P*)-PDPA-ATP** before converting them back to racemic **PDPA** stacks **(*rac*)-PDPA-PPi** (Fig. 3d), completing one helix cycle. These transitions were also confirmed by changes in the absorption spectra, which show characteristic features of respective states (Fig. S9).

In conclusion, we have shown a molecular recognition driven helical assembly of **PDPA**, whose handedness can be easily tuned based on the type of bound chiral auxiliary like AMP, ADP or ATP. Binding with AMP produced *M*-helix, whereas ATP binding resulted in the formation of opposite handed *P*-helix. Interestingly, AMP bound *M*-form could be switched to its mirror imaged *P*-form by adding ATP, which competitively replaces AMP due to multivalent interactions. Thus we demonstrate a rational and simple competitive binding strategy for dynamic helix reversal of **PDPA** assemblies. Such a design strategy was employed to gain step-wise control over the helix mutation cycle from racemic assemblies to left-helix followed by right-helix before converting them into racemic stacks again. Such a rational design for unprecedented control over the helix mutation cycle holds great promise as material for switchable enantioselective and other chirotechnological applications.

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