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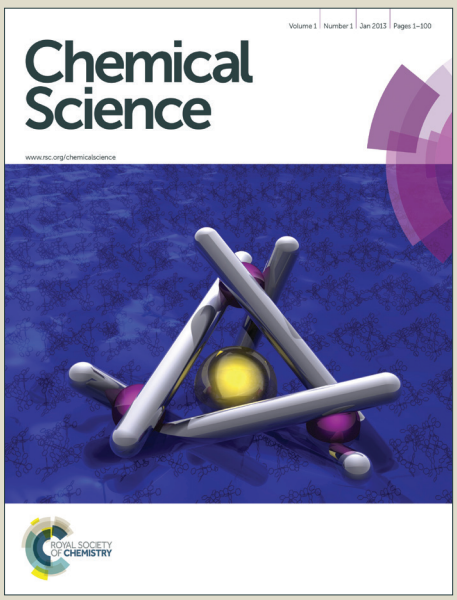

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Homotropic and Heterotropic Allosteric Regulation of Supramolecular Chirality

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Allosteric regulation, a key biological phenomenon, has been demonstrated for controlling the supramolecular handedness of an artificial self-assembled system. Supramolecular assembly of Perylene bisimide (PBI) functionalized with dipicolylethylenediamine (DPA) binding sites demonstrates bistable chiral on-off state by the non-covalent interaction of adenosine phosphate guests. Both homotropic and heterotropic allosteric control of supramolecular chirality in this otherwise helically dormant assembly could be achieved by the binding of chiral and achiral phosphate guests, respectively, hitherto unknown in a biomimetic system. Through detailed spectroscopic and morphological investigations the role of supramolecular reorganization in such an effect has been clearly established.

Introduction

Macromolecular helical assemblies have inspired chemists not only as structural mimics of biomolecules but also as model systems to understand the chiral amplification in nature.[1,2] In this respect, asymmetric synthesis of helical (supramolecular) polymers obtained via non-covalent binding of chiral guests (auxiliaries) to achiral/racemic assemblies offer a simplistic synthetic design with novel chirotechnological functions.[3] For example, this design has been used to construct metastable helical states[4] (helical memory) and chiroptical sensors[5] from conformationally stable and dynamic assemblies respectively. Another important aspect which can be envisaged from such macromolecular systems with multiple binding sites is the allosteric cooperativity, a strategy vastly employed by nature for efficient regulation of a number of biological processes.[6] Biomimetic molecular analogues of allosteric cooperative binding with non-linear response to analyte concentrations have recently gathered much attention in the design of sensors with amplified signalling.[7] However, an allosteric modulation of supramolecular chirality in helical assemblies still remains underexplored.

Allosteric communication across multiple binding sites allows the ligation at one site to influence the outcome of subsequent binding at remote place and this is generally achieved due to conformational variation of the receptors upon guest recognition. Allosteric regulation can be either homotropic or heterotropic in nature, depending on whether the subsequent binding is by same or different guest, respectively.[7] Many proteins undergo nucleotide binding induced allosteric transformation, which are very crucial for their activity.[8] In specific, adenosine triphosphate (ATP) has been well utilized as an effector molecule in various enzymatic processes, both as an inhibitor or activator. For example, glycolysis is mediated by Phosphofructokinase-1 (PFK-1) enzyme, where ATP is involved in both homotropic and heterotropic allosteric processes, leading to its autoregulation.[9] Herein we report a supramolecular polymeric analogue, which exhibits both homotropic and heterotropic allosterism in the expression of chirality, by dynamic conformational changes in response to the binding of various adenosine phosphates. Co-

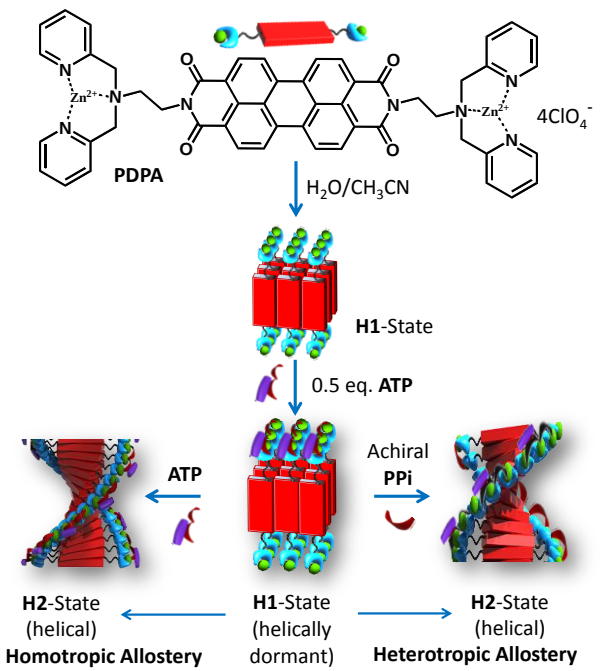

Fig. 1 Chemical structure of PDPA and schematic of the guest induced allosteric regulation of supramolecular chirality in PDPA-assembly.

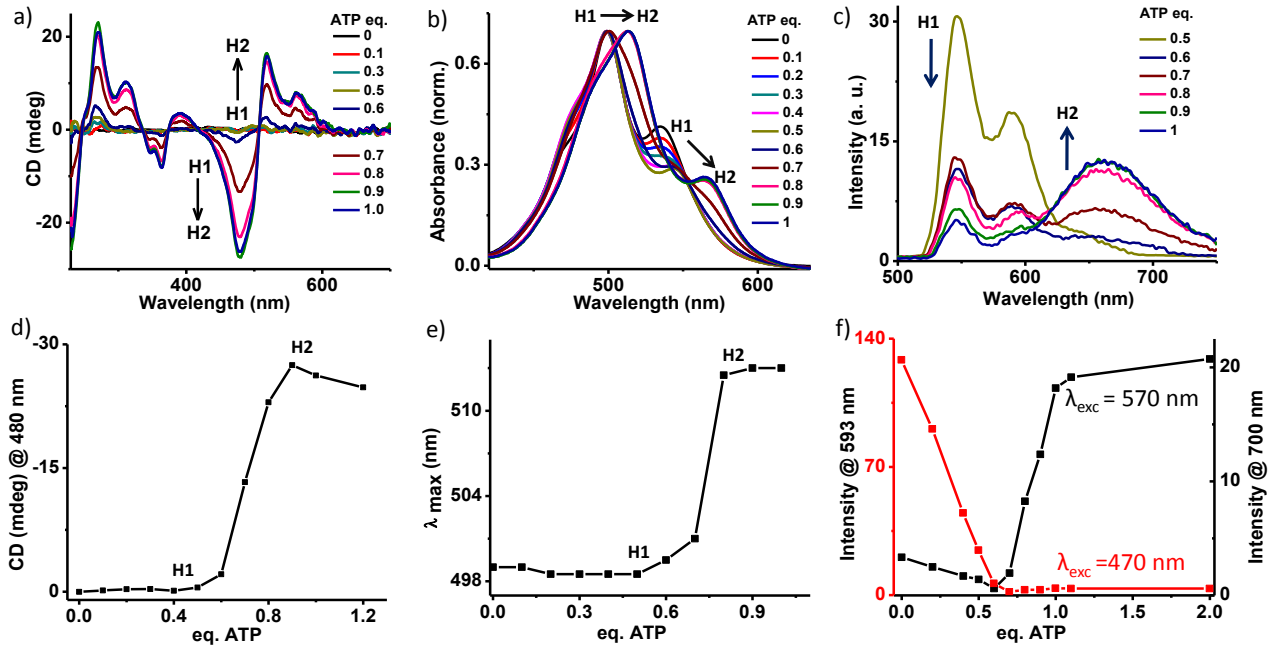

Fig. 2 Homotropic allosteric regulation of helicity: Spectroscopic changes characterizing H1 to H2 transition of PDPA-assembly upon ATP binding (legends in a-c show eq. of ATP). Evolution of a) CD signal and d) plot of CD intensity at 480 nm upon ATP titration. b) Shows the normalized absorption changes and corresponding variation in λ_{max} is plotted in e) to show two states of the assemblies on ATP binding. c) Shows decrease in monomeric emission intensity and evolution of new emission band upon ATP addition (λ_{exc}=470 nm), whereas f) shows comparative emission plot at 593 nm and 700 nm upon selective excitation at 470 nm and 570 nm respectively.[11] (CH_3CN in aq. HEPES, 1:9 v/v, c =2 x 10^{-5} M).

assembled stacks of Perylene bisimide (PBI) and ATP exist in on and off chiral states, which can further be modulated by allosteric guest binding. The helically dormant off-state could be stimulated to chiral on-state either by further addition of ATP (homotropic) or even by the addition of other ditopic guests like achiral pyrophosphate (PPi, $P_2O_7^{4-}$) or ADP (heterotropic). Through detailed investigations, a mechanistic insight into this unprecedented allosteric induction of chirality in extended chromophoric assemblies is provided.[7]

Results and Discussion

Towards the realization of this concept, we designed a PBI chromophore (**PDPA**), end functionalized with dipicolylethylenediamine-Zinc (**DPA-Zn**) receptor motifs, which is known to specifically bind to phosphates.[10] Hence, adenosine phosphates have been used as the chiral guests, which can assist in chromophoric assembly and can also bias the helical handedness of the resultant supramolecular stacks. We first constructed self-assembled stacks of **PDPA** in aq. HEPES buffer/CH_3CN solvent mixture (9/1 v/v, c = 2x10^{-5} M). **PDPA** is molecularly dissolved in CH_3CN due to the presence of two polar end groups, whereas water induces interchromophoric association due to hydrophobic and π-π stacking interactions. Spectroscopic investigations show characteristic signatures of a PBI assembly, i.e. in the absorption spectra, the sharp vibronic features of electronic transition at 460 nm, 488 nm and 521 nm in MeCN indicate monomeric state, which broadened along with quenching of the monomeric emission intensity (λ_{exc}=470 nm) with higher aq. HEPES composition (Figure S1)[11], confirming that the **PDPA** chromophores are arranged in an 'H-type' cofacial manner (**H1**-state).[2] These **H1** assembly formed 2-dimensional nanosheets (Figure S3), as visualized through transmission electron microscopy (TEM). When the **H1**-assembly was mixed with one equivalent (eq.) of ATP, a positive bisignated CD signal, i.e. positive at 518 nm followed by negative at 480 nm, in the PBI absorption region with a zero-crossing at 507 nm was observed,[2n] characteristic of excitonically coupled right-handed helical organization of PBI chromophores (Figure 2a). Such efficient chirality induction to achiral chromophoric assembly further reveals specific binding of phosphate guest molecules to the DPA-Zn sites of the receptor stacks, in congruence with the molecular design. Remarkably, CD titration experiments in which increasing eq. of ATP was added to the **H1**-stacks show that signal remains silent till 0.5 eq. ATP, beyond which sudden rise in CD signal occurs, which continues to enhance passing through an isodichroic point. Highly cooperative nature of this chiral induction process is further reflected in the plot of CD intensity at 480 nm with eq. of ATP guests, which clearly shows a non-linear sigmoidal curve with a sharp inflection point at 0.5 eq. of ATP (Figure 2d).[12]

Supramolecular Reorganization for Allosteric Effect

To gain mechanistic insights into the cooperative process in **PDPA**-assembly, we have performed detailed spectroscopic measurements, which revealed the allosteric mechanism behind the observed "all or nothing" behaviour giving rise to bistable on/off chiral assembly. Monitoring the changes in absorption spectra upon ATP titration, a continuous decrease in absorbance along with broadening of bands was noticed, without any significant shift in λ_{max} till 0.5 eq. of ATP. However, on further addition of ATP, the peak maxima takes a bathochromic jump from 499 nm to 514 nm and 535 nm to 564 nm which saturates

beyond 0.8 eq. of ATP. This two-state change is lucidly visualized in the normalized absorption spectra and the plot of λ_{max} vs eq. of ATP, consistent with the CD spectral changes (Figure 2 b, e). In addition, ATP titration resulted in a gradual decrease in the residual monomeric fluorescence (λ_{exc} = 470 nm) at 593 nm till 0.5 eq. of ATP (Figure 2c).[11] Such observation has already been assigned to H-aggregates of PBI and indicates that initial addition of ATP ensures complete aggregation of **PDPA** monomers to **H1**-assembly. Beyond 0.5 eq. of ATP, as the CD signal begins to appear, a new red-shifted emission peak at 665 nm emerged, which also saturates along with the CD signal. To probe into the genesis of this band, an excitation spectrum was recorded by monitoring the emission at 675 nm, which showed a maximum of 514 nm, which is 20 nm hypsochromically shifted compared to the monomer absorption, indicating a new kind of fluorescent H-type assembly (**H2**-assembly).[11,13] Probing the emission intensity at 593 nm (monomer) and 700 nm (**H2**-assembly) upon selective excitation at λ_{exc} = 470 nm and 560 nm respectively, revealed that the red shifted emission sets in only after 593 nm band disappears, showing their mutually exclusive nature (Figure 2f).[11]

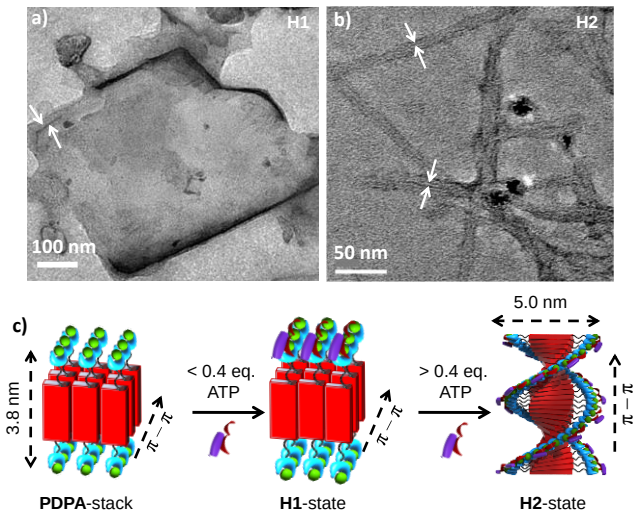

Fig. 3 Morphological evidences of H1 to H2 transition: TEM images of nanostructures obtained from $2x10^{-5}$ M solution of **PDPA** (10 % MeCN in water) with a) 0.4 eq. ATP (**H1**) and b) 1 eq. ATP (**H2**). c) Schematic depicting the morphology transition of **PDPA**-assembly upon ATP binding along with the probable molecular organization in each states.

All the above spectroscopic signatures clearly indicate a supramolecular reorganization being responsible for allosteric cooperativity. In order to obtain direct evidence of conformational transition, detailed transmission electron microscopy (TEM) imaging on various **PDPA** assemblies were performed. **PDPA** alone self assembles to form two-dimensional (2-D) sheets, where the electron density mapping of exposed edges revealed the thickness of individual layers to be around 3.8 nm, which corresponds to the molecular length of **PDPA**.[11] This suggests that the molecules are arranged in a perpendicular fashion along the thickness of the sheets via π-stacking interactions; with hydrophilic DPA-Zn binding sites exposed outside. Up to 0.4 eq. of ATP binding (**H1**-state), their 2-D morphology remains unperturbed, in agreement with their unaltered spectroscopic signatures, suggesting similar molecular organization to that of unbound stacks (Figure 3a). Upon further binding of ATP, which leads to molecular reorganization from **H1**-state to **H2**-state, a morphology transition from 2-D sheets to 1-D nanofibers was observed. TEM images show fiber bundles composed of 2-4 nanofibers with an average width of 5.0 nm, which correlates to the molecular dimension of **PDPA** molecules with ATP bound on both DPA-Zn sides.[11] The length of these fibers is in the range of 300-500 nm, where growth is the direction of π-stacking (Figure 3b).

Thus, the conformational change of **PDPA**-assembly from **H1**-state to **H2**-state is pivotal for the cooperative expression of helicity upon guest binding. The **H1**-state can be best described as a prochiral assembly, which remains in a helically dormant state despite the attachment of chiral guests to nearly half of its available binding sites. However, further binding of ATP molecules to the remaining free sites of the assembly activated its helical conformation, thus exemplifying a homotropic allosteric mechanism for the regulation of supramolecular chirality. For the present system, homotropic allostery could be achieved only in presence of ATP and failed with other phosphates like ADP/AMP/PPi.[‡]

Heterotropic Allosteric Regulation of Helicity

We envisage that, any heterotropic binding of multivalent guest molecules to prochiral **H1**-assembly, would also trigger the molecular reorganization to induce supramolecular chirality. Thus, attempts were made to demonstrate heterotropic allosteric regulation of helically dormant **PDPA**-assembly containing partially bound ATP (**H1**-state), using different achiral and chiral phosphate guests. Remarkably, the successive addition of PPi, which is an achiral ditopic phosphate, to the helically dormant **H1**-state obtained with 0.5 eq. of ATP, showed the induction of(*P*)-handed helicity similar to the **H2**-state obtained by ATP alone (Figure 4a). Thus, the system is uniquely bestowed with the ability to probe the presence of achiral guest through chiral induction, whereas amplified signalling is achieved through highly cooperative effect. Further, we have used ADP, another chiral divalent phosphate, as the heterotropic guest. ADP is shown to bias the **PDPA**-assembly to an opposite (*M*)-helicity compared to that of (*P*)-helicity induced by ATP.[11] Interestingly, addition of ADP to the prochiral **H1**-state (0.5 eq. ATP) also activates the helicity to attain the **H2**-state and more importantly with the right handed (*P*)-helicity as preferred by ATP rather than the heterotropic guest ADP (Figure 4b). Thus both PPi and ADP bind to **H1**-state to form helical **H2**-state, hence representing heterotropic allosteric regulation of supramolecular chirality even by the addition of achiral or different chiral guests. Heterotropic allosteric transformation of **H1** to **H2** was further confirmed by detailed spectroscopic and microscopic studies.[11] For example, on binding of PPi and ADP to **H1**-state, new emission bands at 665 nm were observed, a characteristic feature of **H2**-aggregates. Furthermore, addition of 0.25 eq. of PPi to **H1**-state (0.5 eq. ATP) also led to the morphology transition from 2-D sheets to 1-D nanofibers, analogous to ATP addition, thus confirming the **H2**-state.[11] Interestingly, binding of monophosphates like Pi or

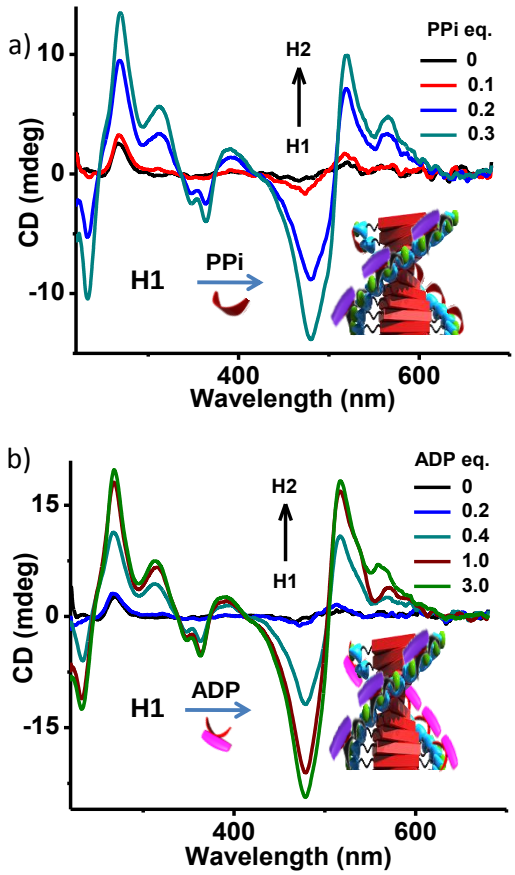

Fig. 4 Heterotropic allosteric regulation of supramolecular helicity: Variation in CD signal of PDPA-assembly in H1-state (0.5 eq. ATP) upon addition of a) achiral PPi and b) ADP (10 % MeCN in aq. HEPES solution, c = $2x10^{-5}$ M).

AMP did not show any heterotropic allosteric regulation of supramolecular handedness, indicating that multivalent guest binding is crucial for inducing the conformational change in **PDPA**-assembly. Thus the present system shows both homotropic and heterotropic allosteric effects with cooperative signalling, hitherto unknown in artificial biomimetic systems.

Detailed heterotropic allosteric experiments of prochiral **H1**-states containing varying fraction of ATP bound sites (0.1-0.5 eq.), with PPi or ADP guests, shed some light on the mechanism of allosteric regulation assisted chiral manifestation. Figures 5a and b show the results of PPi and ADP titration, respectively, with different **H1**-states, where the legends show the eq. of pre-bound ATP molecules onto the **H1**-state. It is important to note that, with the increase in pre-bound ATP (or decrease in free binding sites on **H1**-assembly), both ADP and PPi induces higher CD signal, and the maximum allosteric induction of helicity was observed, when the **H1**-assembly is pre-bound with 0.5 eq. of ATP molecules. This is further evident from the plot of ascending equivalents of pre-bound ATP versus the maximum CD intensity attained, which show a positive slope with PPi and ADP.[12] As expected, binding of monophosphate like Pi does not show induction of chirality, which reiterates the significance of multivalent guests to induce the allosteric regulation.[11,14] These observations suggest that chromophores which are bound by ATP at least on one side can only contribute to the helicity upon conversion to **H2**-state by binding with heterotropic guests like ADP and PPi. It is further evident that the handedness of **H2**-helical assemblies with bound ATP and achiral PPi guests is governed by the bound chiral ATP molecules. Similarly, in the case of ATP-ADP bound stacks ATP again controls the handedness, despite the fact that chiral ADP has a preference for opposite helicity.[15] It is clear that at lower eq. of ATP, many **PDPA** molecules would be free from ATP at both binding sites, and thus they cannot express heterotropic allosteric effects.[16] This also hints towards absence of any significant chiral amplification in these stacks. In addition, any contribution of chiral amplification in the allosteric regulation of chirality was ruled out by performing Sergeant and Soldiers like experiments with mixture of guest molecules in the **H2**-state of the assembly.[11]

Another important observation from these two graphs is the presence of lag phase, especially at lower eq. of pre-bound ATP, where the assembly remains CD silent despite the binding of ADP or PPi (Figure 5a and b).[11] For example, upon the addition of ADP, **H1**-assembly with 0.1 eq. of pre-bound ATP remains CD silent until 0.7 eq. of ADP is added, beyond which chirality induction sets in. This lag phase decreases with the increase of binding sites pre-occupied with ATP molecules and disappears completely when 0.5 eq. of ATP was added. Explanation to such a phenomenon is sought from the possibility that, initial ATP and other phosphate molecules bind preferentially to only one of the two binding sites of each chromophores in the **H1**-assembly, which does not give any CD signal as discussed above. Subsequent binding of multivalent guests to the second site is

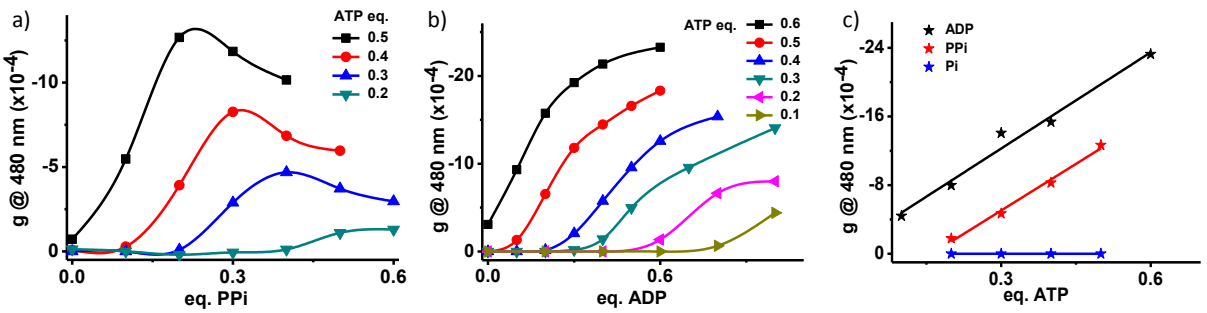

Figure 5. a-c) Heterotropic allosteric experiments of **H1**-states containing varying fraction of ATP bound sites (0.1-0.5 eq.).Variation in anisotropy value or g-value of **PDPA** in **H1**-state (0.5 eq. ATP) upon addition of a) PPi and b) ADP. c) Plot of ascending eq. of pre-bound ATP versus the maximum CD intensity attained for the **H2**-states with different heterotropic guests, which show a positive slope with PPi and ADP (10 % MeCN in aq. HEPES solution, c = 2 x 10^{-5} M). The decrease in CD signal after reaching maximum value in a) is due to competitive replacement of ATP by PPi. [12]

essential for the manifestation of **H2**-state and induction of chirality. However, the observation that chiral induction is absent till half of the binding sites in the assembly are occupied, irrespective of the equivalents of pre-bound ATP molecules, prompt us to propose a preferential binding of the guests to one side of the **H1**-assembly at the initial stages. Although, the exact reason for such a preferential facial binding of the guest molecules is not clear at this moment, we believe it could be due the morphological constraints, where multi-layered 2D assembly of **H1**-state prevents the accessibility to all binding sites. Thus, in the **H1**-assembly with 0.5 eq. of pre-bound ATP, binding sites at one face of the 2-D sheets are completely occupied. Further addition of any multivalent phosphate causes morphology transition to 1-D fibers, thereby setting in the allosteric mechanism with induced chirality. On the other hand, **H1**-assembly with 0.2 eq. of ATP, further required 0.4 eq. of ADP or PPi to completely occupy the binding sites on the exposed face of sheet, and hence they are CD silent. As expected, further addition of phosphates regulates the chirality induction, with less CD intensity. The final CD intensity of the **H2**-assembly, which reflects the induced ee in the helical stack formed during these experiments, directly correlates to the number of **PDPA** chromophores pre-bound with ATP at one site (Figure 5c).[11]

Conclusions

In conclusion, we have demonstrated a new allosteric regulation design for modulating the supramolecular chirality of helical receptor assemblies, by the homotropic and heterotropic binding of adenosine phosphate guest molecules. Through chiral guest induced helicity into the 1-D assembly of PBI, we have shown both homotropic and heterotropic allosterically responsive supramolecular chirality in a single system, which finds no precedence. Such systems with highly cooperative response to biologically relevant molecules can allow amplified signalling of both chiral as well as achiral phosphates, whereas the unique heterotropic allosteric effects could be utilized further for the autoregulation of ATP.

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Notes and references

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‡ Allosteric control of helicity was seen only with ATP and not with AMP or ADP probably due to its multivalent interactions compared to ADP and AMP. Usually only multivalent ligands are known to show such effects. Also preliminary investigations with single stranded DNA (which is a polymeric analogue of AMP) with **PDPA** also shows allosteric control over chirality, confirming the role of multivalent host guest interactions and ruling out any specific interaction with ATP being responsible for the allosterism. These results along with MD simulation data to understand the origin of opposite chirality with ADP and ATP will be published elsewhere providing detailed insights into the process.

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11 See Supporting Information.

12 ATP's three site binding should have saturated all site at 0.66 eq., however, we observe CD saturation at 0.9 eq. ATP which could be due to inability of few ATP to bind via all three sites.

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14 AMP also did not induce heterotropic allosteric effects.

15 Handedness of the stacks are dictated by strength of guest ligation.

16 This is further evident from the fact that, at very low eq. of ATP initial addition of ADP shows signal corresponding to ADP bound stacks.[11-]