Emergence of Photomodulated Protometabolism by Short Peptide-Based Assemblies

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ABSTRACT: In early earth, rudimentary enzymes must have utilized available light energy source to modulate protometabolic processes. Herein, we report the light responsive C-C bond manipulation via short peptide-based assemblies bound to photo-sensitive molecular cofactor (azo based photoswitch) where the energy of the light source regulated the binding sites which subsequently modulated the retro-aldolase activity. In the presence of a continual source of high energy photons, temporal realization of catalytically more proficient state could be achieved under non-equilibrium conditions. Further, the hydrophobic surface of peptide assemblies facilitated binding of an orthogonal molecular catalyst that showed augmented activity (promiscuous hydrolytic activity) upon binding. This latent activity was utilized for the in-situ generation of light sensitive cofactor that subsequently modulated the retro-aldolase activity; thus, creating a reaction network.

Introduction

Light played critical roles in the emergence and evolution of living matter on earth.[1] The earth's rotation about its axis leads to the periodicity of the light-dark environment changes which subsequently impacts the functioning of all extant life forms. Contemporary biochemistry features exquisite collaboration between cofactors and complex protein machineries by using such energy source to maintain the non-equilibrium biochemical oscillations required for homeostasis and metabolism.[2] For instance, photomodulation of motility in primitive organisms such archaebacterium (halobacteria) is controlled via methylation/demethylation of complex photoconvertible protein which subsequently leads to acute light responsiveness in its swimming behavior (Figure 1a).[3] In early earth, rudimentary enzymes must have utilized the diurnal cycles of light and temperature either to synthesize metabolically relevant molecules or to modulate protometabolic chemical transformations under non-equilibrium conditions.[4] Protocellular machineries gradually emerged that could exploit the available energy resources (light and chemical energy) to modulate the rudimentary yet non-equilibrium metabolic processes to be in sync with 24 h day-night cycle.[5]

Deep phylogenetic analyses have suggested that a core ensemble of rudimentary enzymes demonstrated broad catalytic activities which helped in enriching the chemical inventory.[6,7] Short peptide-based amyloids capable of accessing paracrystalline β sheet rich folds, have been shown to display catalytic activities and even in some cases promiscuous activities i.e. capabilities to catalyse more than one type of chemical transformations.[8-11] Such latent catalytic roles might have been crucial to generate an ancestral form of protometabolic activities, lending support to the argument of short peptide to be the earliest protein folds.[12] It would be intriguing to probe whether such short peptide-based promiscuous assemblies could exploit light via recruiting light sensitive cofactors to modulate its catalytic capabilities of metabolically relevant transformations (C-C bond manipulation). Temporal modulation of aldolase like activity by judiciously exploiting the environmental changes and possibilities of promiscuous activities might have been advantageous for chemical emergence of protometabolic activities (Figure 1b). Herein, we report that short peptide-based amyloid like assemblies can bind photosensitive cofactors and modulate C-C bond cleavage (aldolase-like activity), one of the important chemical transformations in the context of metabolic pathways (Figure 1a,b).[13] Solvent exposed lysines were used as catalytic units to cleave an aldol adduct (**M**) via Schiff base formation, a strategy exploited in type I aldolases (Figure 1b).[13a] Moreover, in the presence of a continual source of high energy photons, the catalytically more competent state was accessed mimicking extant biochemical processes that exploit non-equilibrium conditions. Further, the short peptide-based assemblies could bind an orthogonal molecular catalyst to demonstrate latent hydrolytic role (promiscuous) that converted a suitable chemical precursor to the light sensitive cofactor which subsequently modulated the primary catalytic activity (C-C bond breaking).

Results and Discussion

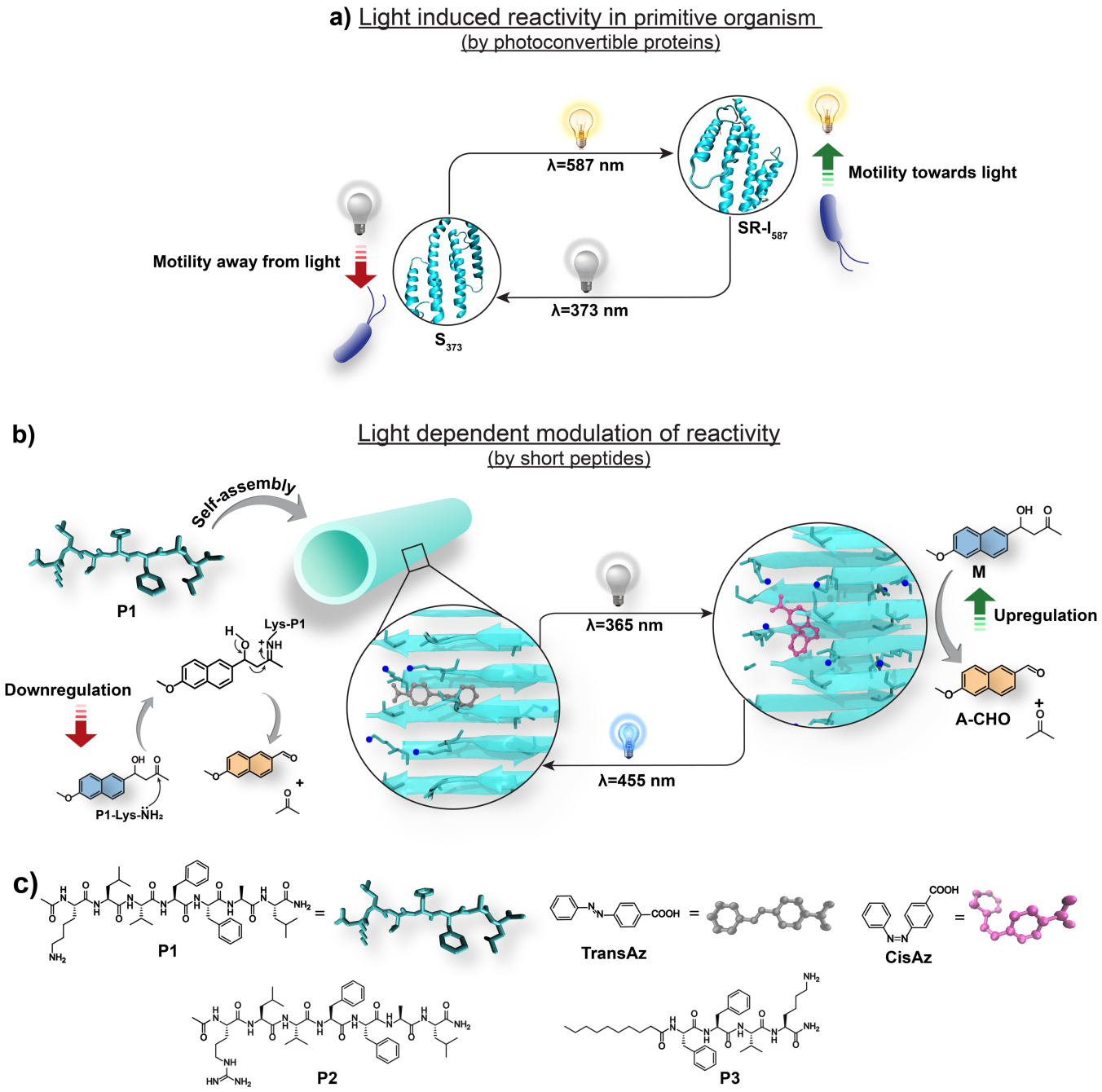

Figure 1: (a) Representative scheme showing that photosensitive proteins in halobacteria can modulate their swimming behaviour through photoactivation of sensory rhodopsin I (SR I) via methylation/demethylation of the protein. (b) UV-blue light driven photomodulation of C-C bond cleavage via cofactor bound peptide assemblies. Magnifications extracted from MD simulations where Lysine and Alanine residues shown for clarity. (c) Chemical structures of the molecules used.

Peptide sequence Ac-KLVFFAL-NH_2 (**P1**) from the nucleating core of the Aβ (1–42) was used. Circular dichroism (CD) study showed the presence of β-sheet secondary structure with nanotubular morphology (Figure S1). Each peptide strand (length of ca. 2 nm) ordered in an antiparallel out-of-register bilayer β-sheets conformation to position the N-terminal lysine residues towards the solvent. Further these sheets stack to form well-defined nanotube surfaces punctuated with lysine and leucine residues constituting collinear cross-β grooves (Figure S2).[9d,11c] Presence of cationic charged lysines over the surface of the nanotubes was confirmed by negatively charged gold nanoparticle binding study (Figure S3). This hydrophobic yet cationic surface can bind to negatively charged aromatic acids and also with hydrophobic small molecules.[11b,12c] Moreover, atomic force microscopy (AFM) images confirmed the average heights of 9.0±1.2 nm which suggested the presence of bilayers of β-strands of ~2 nm (Figure 2a). [11a]

To introduce photoresponsiveness to the **P1** assemblies, a negatively charged low molecular weight photo-sensitive cofactor 4-(phenylazo)-benzoic acid was used (**Az**, Figure 1c).[14] The aromatic backbone of the **Az** was expected to bind over the leucine rich hydrophobic surface of the **P1** assemblies.[2d] Moreover, the negatively charged carboxyl functional group of **Az** was expected to interact with positively charged arrays of lysines of **P1** assemblies. Next, trans**Az** (200 μM) was incubated with **P1** assemblies (500 μM, system termed as **P1**+trans**Az** for the trans enriched state, Figure 2b). Then, **P1**+trans**Az** was irradiated with UV light (365 nm, 50 min)

to convert to **P1**+cis**Az** (cis enriched state) and 10 min irradiation at 455 nm (blue light) to regenerate the **P1**+trans**Az** (Figure 2b). HPLC analysis of **P1**+trans**Az** state suggested the distribution of 97(trans):3(cis) while **P1**+cis**Az** showed 14(trans):86(cis) (Figure S4-S6, the ratio was similar to what observed for unbound **Az** in absence

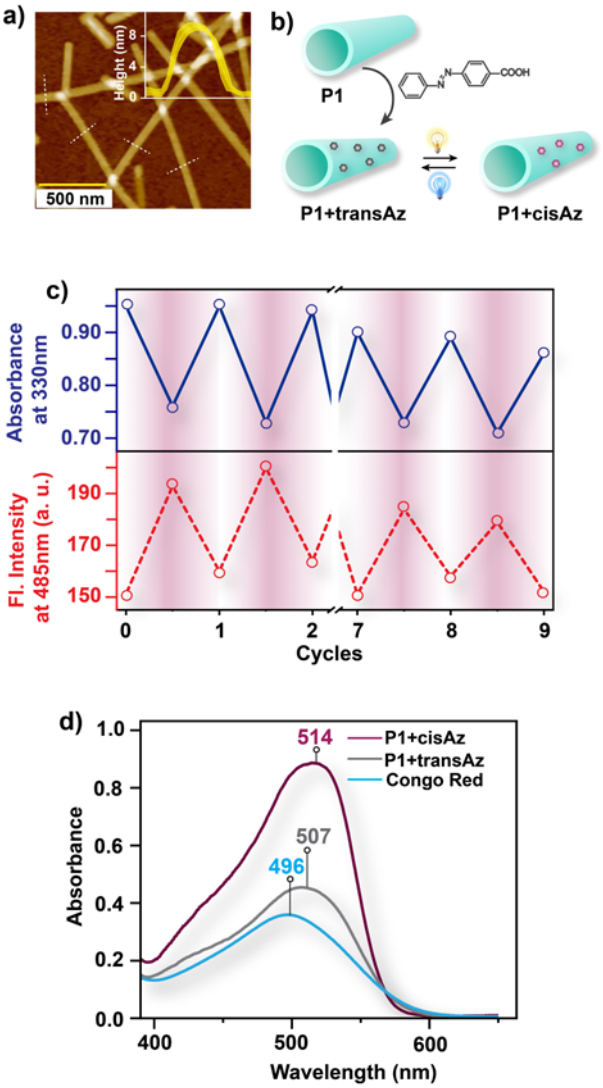

Figure 2: (a) AFM image of **P1** assemblies (inset-height profiles). (b) Schematic representation of different photostationary states. (c) UV absorbance (blue) of the trans enriched states and fluorescence intensity (red) of ANS after sequential UV/blue light irradiations. (d) UV-Vis spectra of Congo Red in different **P1**-**Az** systems.

of peptide). Next, **P1**-cofactor system was exposed to consecutive UV (50 min) and blue (10 min) light irradiation (**P1**+trans**Az**↔**P1**+cis**Az**). The reversibility of **P1**+trans**Az**↔**P1**+cis**Az** was checked by monitoring the UV absorbance at 330 nm ($\lambda_{max,\textbf{transAz}}$= 330 nm). For 9-10 consecutive cycles, the absorbance modulation was observed suggesting the photoreversibility of system (Figure 2c, blue line, top panel). TEM, AFM micrographs and HPLC analysis confirmed the structural integrity of **P1** in presence of **Az** even after multiple cycles of irradiations (see SI for details, Figure S7-S9, Temperature=25±1°C throughout the cycles). Moreover, turbidity measurements along with DLS studies showed negligible change upon irradiations for 9 cycles (Figure S10,S11). CD spectra after 9 cycles of irradiation of **P1** assemblies showed retention of secondary structure (Figure S12, CD was done in absence of aromatic cofactor to minimize the noise from high HT voltage). The reversibility of the system was further probed by using an orthogonal guest 8-anilinonaphthalene-1-sulfonic acid (ANS) which is known to exhibit an enhancement in fluorescence intensity upon binding to hydrophobic protein surfaces.[15] Indeed, the **P1** assemblies could regulate its binding capabilities towards ANS for multiple cycles of UV/blue light irradiations (Figure 2c, red line, bottom panel). The intensity of ANS was always higher for **P1**+cis**Az** suggesting the higher binding capability. Further, Congo Red, a dye known for its characteristic spectroscopic changes when bound to amyloid, registered higher bathochromic shift in presence of **P1**+cis**Az** (λ_{max}=514 nm) with increase in absorbance suggesting the greater extent of binding towards the cis enriched state (Figure 2d).[16]

To investigate further, computational studies were performed by means of atomistic molecular dynamics (MD) simulations, that have proven successful in the characterization of supramolecular systems.[17] A model of one laminate of **P1** was built and trans/cis**Az** was allowed to contact the assemblies. Analysis of the trajectories showed the decrease in hydrophobic:hydrophilic solvent accessible surface area (SASA) ratio for **P1**+trans**Az** (1.147±0.048) than the **P1**+cis**Az** (1.224±0.046, Figure 3a). The details of **P1**+**Az** interactions were obtained by means of well-tempered Metadynamics (wt-MetaD) simulations,[18] biasing contacts and distance of **Az** with the laminates of **P1**. Based on the analysis of the free-energy surfaces (FESs), it was found that the lower energy minima were located at deeper position within the laminates in trans as compared to cis-enriched state. This observation suggested that trans**Az** exhibited a greater tendency to penetrate deeper into the laminates than cis**Az**. Thus, cis**Az** demonstrated more dynamic in nature and preferred to stay closer to the surface with more exposure to solvent compared to the trans**Az** (Figure 3b,c). Indeed, loading studies of the cofactor with **P1** assemblies (varied concentration of cofactor 0-2000 µM at fixed **P1** concentration, 500 µM) suggested weaker binding of cis**Az** in comparison to trans**Az** (analysed by HPLC, see SI for details, Figure S13,S14). The loading of the cofactor per 0.2 µmole of **P1** was found to be 0.06 and 0.255 µmole for cis**Az** and trans**Az** respectively.

Early catalysts might have exploited the diurnal cycles of light and temperature for modulating catalytic functions required for protometabolic activities. To study whether the short peptide-based assemblies could manipulate C-C bonds via retro-aldolase activity, **M** was introduced to the **P1**-cofactor systems which cleaved the C-C bond to yield fluorophore **A-CHO** and acetone (Figure 1b). Retro-aldol reaction was chosen as it is a crucial step in the extant metabolic pathways where many aldolase type I enzymes assist in manipulating the C-C bond by utilizing lysines present in their active site. It would be important to mention here that the close proximity of positively charged lysine moieties at the assembled state can perturb the apparent p*K*a of lysines due to columbic charge repulsions.[12b] This might lead to augmented nucleophilicity of the lysines that subsequently could facilitate the Schiff base formation required for the C-C bond cleavage.[13c,d] Different concentrations of **M** were separately added to **P1**+cis**Az** and **P1**+trans**Az** systems and generation of **A-CHO** was monitored

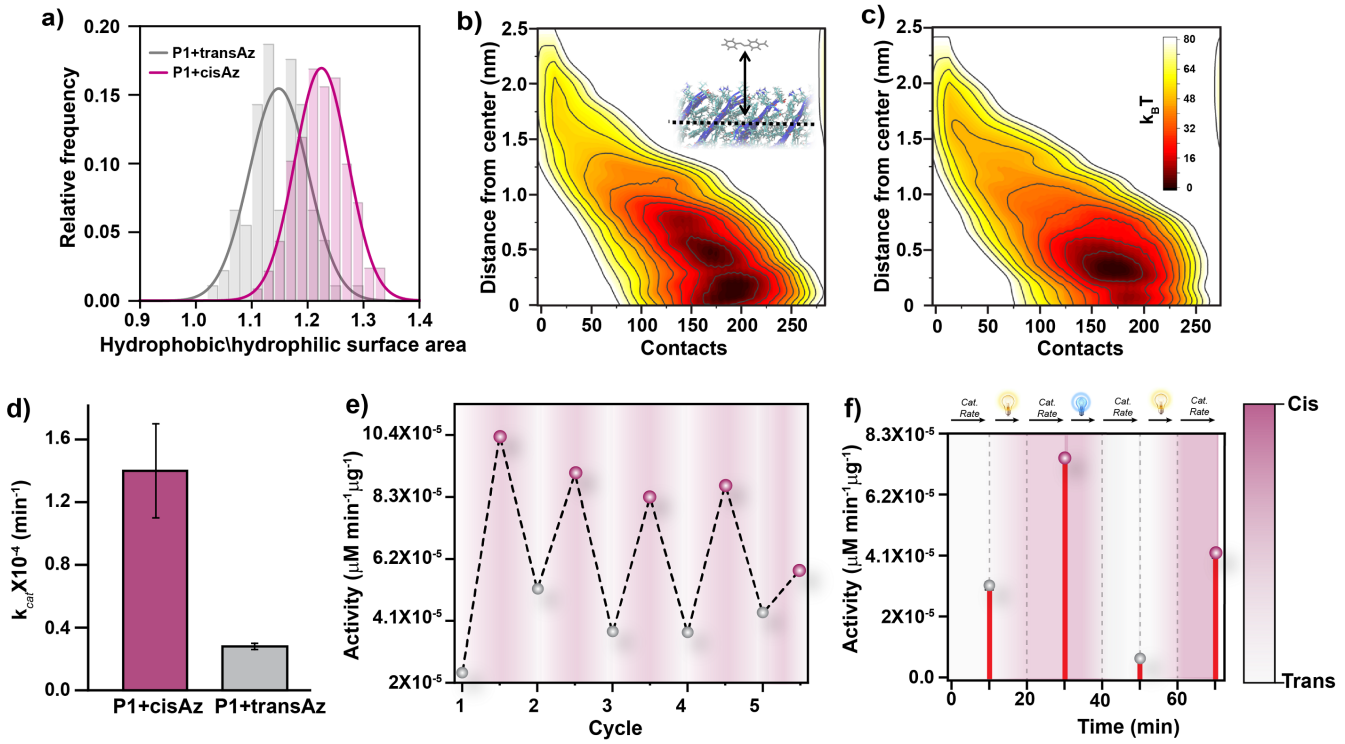

Figure 3: (a) Histograms collected along MD trajectories and Gaussian fittings of the hydrophobic:hydrophilic Solvent Accessible Surface Area (SASA). (b, c) 2D free energy surfaces of the interaction of trans (b) and cis**Az** (c) with **P1** assemblies respectively. Collective variables: number of contacts between **Az** and **P1**; distance of the center of mass of azobenzoic acid from the geometric center of the assembly (i.e. the lower the value the deeper azobenzoic acid is inserted in the assembly). (d) k_{cat} values. Impact of irradiation cycles (50/10 min UV and 10 min blue light) on activity where (e) fresh batches of **M** (200 μM) were added to **P1**+cofactor system after each cycle of irradiation and (f) when single batch of **M** (300 μM) was added from beginning.

(pH 7, HEPES, **P1**=500 μM, cofactor=200 μM). Interestingly, **P1**+cis**Az** showed 5-fold enhancement in catalytic activity compared to **P1**+trans**Az** ($k_{cat,cis}$=1.4±0.3X10^{-4} min^{-1}, $k_{cat,trans}$= 0.28±0.02X10^{-4} min^{-1}, Figure 3d,S16,S17, **P1**=500 μM, cofactor=200 μM as highest modulation was found in these cofactor concentration range, Figure S15). The population distribution in the cis enriched photostationary state was maintained during the timeframe of catalytic activity (Figure S18). The significant difference in catalytic activities can be attributed to the differential binding of the cofactor on the peptide surface. In presence of a photo insensitive control molecule which lacked the azo functional group (naphthoic acid, **B**), **P1** did not show any photomodulation of its retro-aldolase activity (Figure S19). To prove the role of primary amine of lysine to form imine bond for the retro-aldolase activity, a control sequence was chosen where the lysine was mutated with arginine to access a control sequence **P2** (accessed similar nanotubular morphologies upon assembly, Figure S20). Indeed, catalytic rate was miniscule for **P2** which implicated the role of primary amine in retro-aldolase activity (Figure S19). [13d] Catalytic activity was also monitored by soluble lysine in presence of **Az**. The modulation in catalytic activity was found to be very low with negligible rate of conversion suggesting the importance of catalytic binding surface (Figure S21). Further the catalytic modulation by soluble lysine was checked in the presence of **P2** assemblies which showed miniscule activity with very low modulation (Figure S21). In combination, these results highlighted the importance of proximity of active residue, substrate and suitable cofactor for the modulation in catalytic activity. To generalize the importance of lysine rich peptide assemblies, a different sequence C10-FFVK (**P3**) was chosen which also accessed nanotubular morphologies (Figure S22).[13d] Indeed, **P3** showed modulation of retro-aldolase activity in presence of the **Az** cofactor (Figure S23).

We further asked whether regulation of catalytic properties as a function of energies of photon could be achieved by the minimal peptide-based system (Figure 1). For this purpose, a stock solution containing **P1**+trans**Az** was taken and exposed to sequential UV (365 nm, 50 min) followed by blue (455 nm, 10 min) light irradiations for multiple times. After each irradiation, a required amount of aliquot was collected from the stock solution (**P1**+trans/cis**Az**) and **M** was added to check the activity. Remarkably, sequential up and down regulation of retro-aldolase activity could be achieved up to 4 cycles with concomitant UV and blue light irradiations (Figure 3e). Catalytic reversibility was also achieved in a closed system where the substrate **M** was added to **P1**+trans**Az** from beginning and subsequent UV and blue light irradiations were performed (Figure 3f, see SI for details). MD simulation was used to investigate the binding of **M** to the **P1**+**Az** system. The study showed competition between **M** and **Az** for the same binding sites of **P1**. The more hydrophobic trans**Az** remained buried whereas the dynamicity of the surface bound cis**Az** allowed more access and contacts between catalytic lysines with **M** (Figure 4a,b), resulting in increased catalytic activity for the **P1**+cis**Az** system (Figure 4c).

The results demonstrated that the high energy source helped in achieving a catalytically proficient state. We anticipated if both the lights were kept on, the system could temporally access a photostationary state under non-equilibrium condition.[19] For this purpose, a stock solution containing **P1**+trans**Az** was exposed to continuous blue light irradiation, with periodic on-off of high energy UV light. After switching on

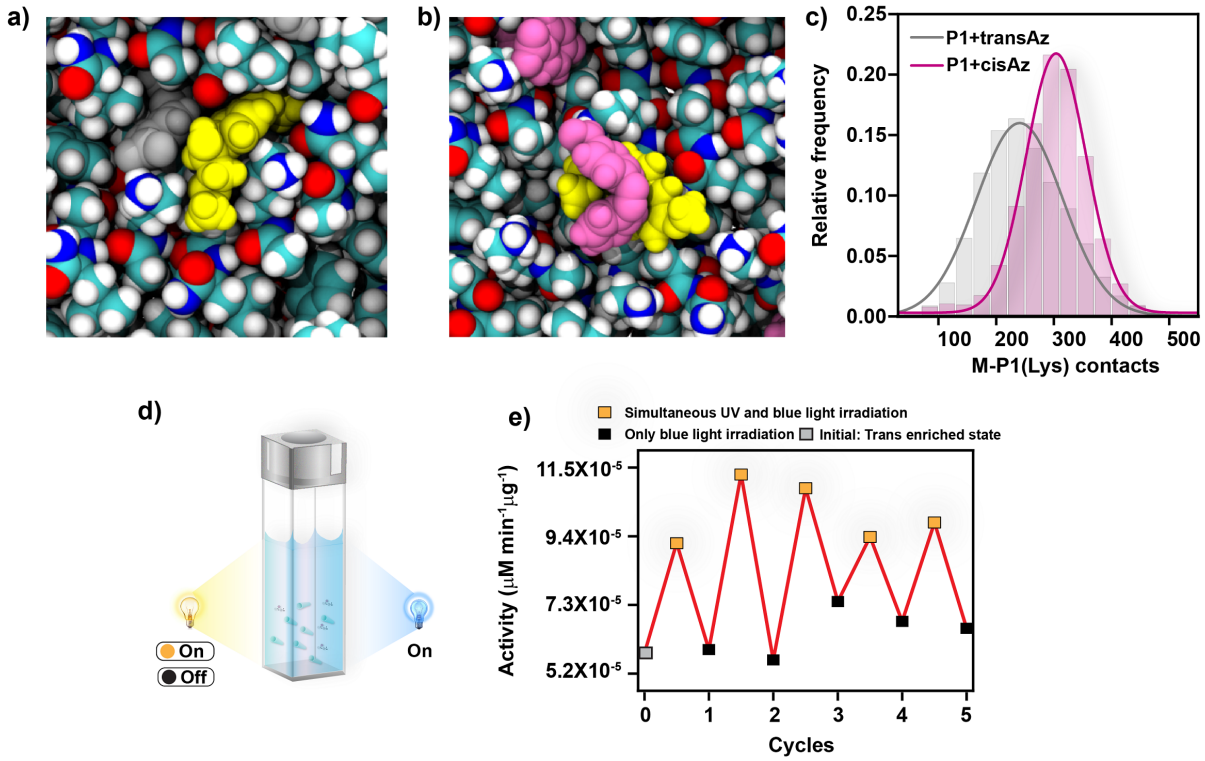

Figure 4: Snapshots of **M** (yellow) within (a) **P1**+trans**Az** (grey, trans**Az**) and (b) **P1**+cis**Az** (magenta, cis**Az**). (c) Distribution of the number of contacts between the **M** and solvent exposed lysines (d) Photoisomerization of **Az** under continuous exposure of blue light with repetitive on-off of the UV light. (e) Temporal augmentation in retro-aldolase activity in presence of high energy light source.

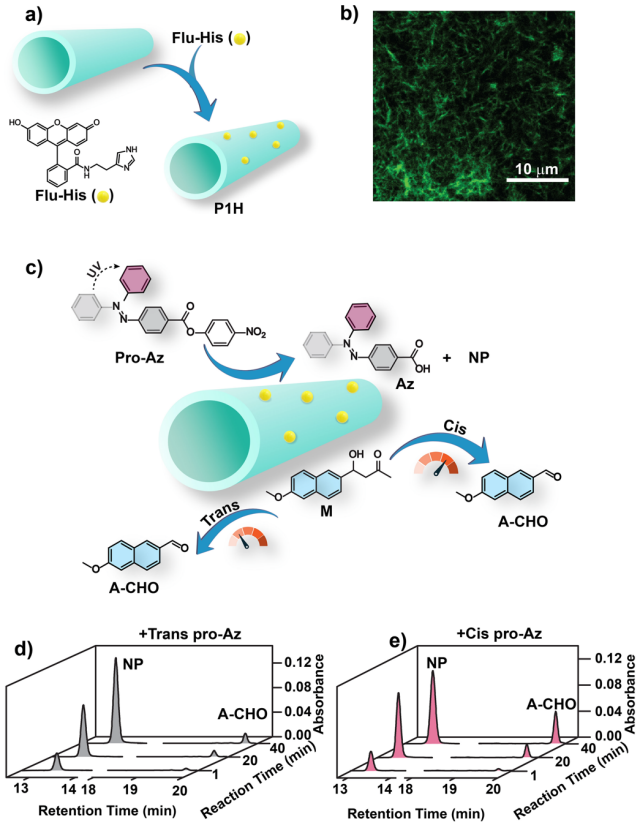

Figure 5: (a) Schematic representation of Flu-**His** bound **P1** (**P1H**). (b) Confocal micrograph of **P1H**. (c) Schematic representation of in-situ generation of **Az**. (d, e) HPLC showing generation of NP (hydrolysis) and **A-CHO** (retro-aldolase).

and off the UV light (blue light remained on), a required amount of aliquot was taken out and immediately activity measurement was done (Figure 4d,S24 see SI for details). Interestingly, catalytic activity was found to be higher during each UV irradiation (Figure 4e, see SI for details). This data suggested that light (UV) energy driven cis-enriched catalytic states could be exploited to achieve augmented catalytic activity under non-equilibrium conditions.

Earliest forms of metabolism depended on some conserved set of rudimentary yet promiscuous catalysts that facilitated latent orthogonal reactions which could be useful in the regeneration of depleted substrates/cofactors.[20] The catalytic hydrophobic surface of **P1** was utilized to explore the hydrolytic activity i.e. an additional catalytic role (promiscuity) which could possibly generate the cofactor. Hence, **P1** was exposed to Flu-**His** which was expected (due to aromatic group) to bind over the hydrophobic surface while the imidazoles could impart promiscuous hydrolytic capabilities to the assemblies (**P1H**, Figure 5a,b, binding of Flu-**His** was confirmed by confocal microscopy).[9d] Precursor esters cis-pro-**Az**+**M** and trans-pro-**Az**+**M** were incubated separately with **P1H** and both hydrolase as well as retro aldolase activity were checked (Figure 5c). Similar hydrolytic rate (**Az** formation, $rate_{cis}$=0.3 μM/min and $rate_{trans}$=0.34 μM/min, Figure 5d,e) was observed for both cis and trans enriched systems. However, the retro-aldolase activity for **P1H**+cis-pro-**Az**+**M** system was 3-fold higher than **P1H**+trans-pro-**Az**+**M** system (Figure 5d,e, only Flu-**His** or **P1** showed negligible hydrolytic activity, Figure S25). Catalytic modulation (by cis enriched versus the trans enriched states) was also checked in different control systems which showed negligible modulation (Figure S26). The data underpinned the importance of the binding surface to activate the dormant hydrolytic activity of Flu-**His** to cleave the precursor to replenish the cofactor which subsequently helped in the modulation in retro-aldolase activity.

Conclusion

In conclusion, photosensitive cofactor bound short peptide-based assemblies have been shown to modulate the activity of a

metabolically relevant biochemical transformation. Exposure to different energies of photons modulated the binding capabilities of the constructs to dynamically control its catalytic activity. Moreover, the continuous light energy source led to temporal realization of catalytically more proficient state under non-equilibrium conditions. Further, the binding to an orthogonal dormant catalytic unit resulted in the emergence of catalytic promiscuity (latent hydrolytic activity) which helped to generate the cofactor. Study of such minimal light gated catalytic systems which can show the complexities of photoresponsive proteins can help us map the emergence of primordial catalysts that can exploit the energy of light.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website. Experimental procedures, synthesis, characterizations, details of molecular models for simulations; simulation parameters (input files, etc.) are available: github.com/GMPavanLab/Azo_Peptides (will be replaced with a definitive Zenodo archive upon acceptance).

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Author Contributions

All authors contributed towards writing.

Notes

The authors declare no competing financial interest.

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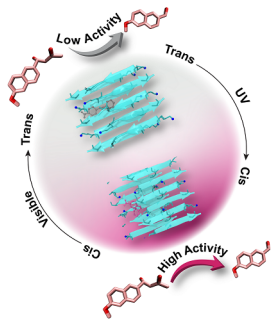

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