Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Azobenzamide-based proteomorphous objects as a light/pH-induced photoswitchable module

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Experimental Section

Materials and Methods: All reagents used in this study were of highest purity available (sigma Aldrich) and were used without further purification. Thin-layer chromatography (TLC) was performed using silica gel (60-120 mesh size). Solvents such as CH_2Cl_2, $CHCl_3$ were dried over P_2O_5, Et_3N was dried over KOH, THF using sodium and benzophenone. DMF was vacuum distilled over anhydrous $MgSO_4$. ^1H NMR and ^{13}C NMR spectra were recorded on Jeol JNMECX 400p spectrometer at room temperature using DMSO-d_6 as solvent. All chemical shifts (δ) were recorded in ppm with reference to internal standard TMS. 4,4′-dicarboxylic azobenzene and azobenzene 4,4′-dicarbonyl chloride are synthesized and characterized according to reported procedure by Ghosh *et al*.[S1]

The optical studies were carried out on JASCO UV-Vis-NIR spectrometer (670D) using quartz cuvettes (path length = 1 cm, slit width = 10 nm, volume = 3 ml) while Varian Cary eclipse instrument was employed for all fluorescence experiments. IR spectra were characterized at Perkin-Elmer FT-IR spectrometer in range 400-4000 cm^{-1}. The pH of the solution was fixed with Metrex digital pH-meter, calibrated with buffer solution of pH 4.00 and 9.00 before each measurement. The pH of test solution was adjusted with ~10^{-3} M HCl and NaOH solution in water. The sample irradiation experiments were executed with 15 W mercury lamp equipped with a Schott WG360 filter (λ = 365 nm) for UV and incandescent lamp (Philips) with a 14 W light bulb (λ =>400 nm) for visible light.

Synthesis of (*E*)-4,4'-(diazene-1,2-diyl)bis(*N*-(pyridine-3-yl)benzamide) (1) : Triethylamine (0.150 g, 1.5 mmol) was added to 3-aminopyridine (0.235 g, 2.5 mmol) in 10.0 ml dry THF. azobenzene 4,4′-dicarbonyl chloride (0.153 g, 0.5 mmol) dissolved in 10 ml dry THF was

dropwise added to resulting solution. The reaction mixture was then stirred at room temperature for 12 h. The yellow precipitate obtained was filtered and dried. It was recrystallized by mixture of DMF/methanol. Single crystal suitable for X-ray was obtained by layering the solution of compound in DMF with methanol. IR (cm^{-1}, KBr): $\nu_{(C=O)}$ 1659 and $\nu_{(N=N)}$ 1421: ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.43 (q, 2H, pyridine), 8.09 (d, 4H, azobenzene meta hydrogen atoms), 8.22 (m, 6H, azobenzene ortho hydrogen atoms + pyridine), 8.34 (d, 2H, pyridine), 8.96 (d, 2H, pyridine,), 10.66 (s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 122.77, 123.62, 127.47, 129.25, 136.89, 142.08, 153.57, 165.06: Anal. Calcd. for $C_{24}H_{18}N_6O_2$: C, 68.24; H, 4.29; N, 19.89. Found: C, 68.04; H, 4.27; N, 19.13. UV-vis (DMF, λ_{max} (nm), ε M^{-1} cm^{-1}): 340 (10 2114).

Synthesis of (*E*)-4,4'-(diazene-1,2-diyl)bis(*N*-(pyridine-4-yl)benzamide) (2): 4-aminopyridine (0.235 g, 2.5 mmol) was dissolved in 10.0 mL dry THF and triethylamine (0.150 g, 1.5 mmol) was slowly added to it. Azobenzene 4,4′-dicarbonyl chloride (0.153 g, 0.5 mmol) dissolved in 10 mL dry THF was added drop wise to resulting solution. The reaction mixture was then stirred at room temperature for 12 h. The yellow precipitate obtained was filtered and dried. It was recrystallized by mixture of DMF/ methanol. Single crystal suitable for X-ray was obtained by layering the solution of compound in DMF with methanol. IR (cm^{-1}, KBr): $\nu_{(C=O)}$ 1668 and $\nu_{(N=N)}$ 1419; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.80 (d, 4H, pyridine), 8.0-8.22 (m, 8H, azobenzene ortho and meta hydrogen atoms), 8.50 (d, 4H, pyridine), 10.78 (s, 2H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 128.70, 129.62, 130.47, 132.42, 139.68, 140.98, 144.26, 154.27, 169.05. Anal. Calcd. for $C_{25}H_{22}N_6O_3$: C, 66.07; H, 4.88; N, 18.49. Found: C, 65.92; H, 4.77; N, 18.31. UV-vis (DMSO, λ_{max} (nm), ε M^{-1} cm^{-1}) 339 (66 391)

Table 1: Selected crystallographic data of compounds **1** and **2**

Parameters.	**1**	**2**
Formula.	$C_{24}H_{18}N_6O_2$	$C_{26}H_{26}N_6O_4$
M.	422.44	486.53
Crystal system.	Triclinic	Monoclinic
Temperature(°K)	293(2)	293(2)
Space group.	*P*-1	$P2_1/c$
a/Å	5.5110(5)	4.8327(12)
b/Å	6.6322(6)	14.850(3)
c/Å	14.0532(15)	16.252(3)
α(°)	83.397(8)	90
β(°)	81.177(8)	101.434(18)
γ(°)	72.154(8)	90
V/Å^3	481.88(8)	1213.5(4)
Z	1	2
D_c/mg.m^{-3}	1.456	1.326
Reflns.collected/Unique	3516/2155	5625/2772
Data/restraints/ Parameters.	2155/0/150	2772/1/165
R(int)	0.0232	0.0580
θ range for data collection(°)	3.24-28.94	3.65-28.96
Completeness to θ=25.00	99.8	99.8
Final R indices[I>2σ(I)]	R_1=0.0968 wR_2=0.3283	R_1=0.1807, wR_2=0.4480
R indices(all data)	R_1=0.1232, wR_2=0.3402	R_1=0.2529, wR_2=0.4864
Refinement method	Full-matrix, least-squares on F^2	
GoF	1.350	1.169

Table 2: Selected bond Lengths (Å) and bond Angles (deg) of compounds **1** and **2**.

[a] **Compound 1**			
N(1)-N(1) #1	1.242(10)	O(1)-C(7)-N(3)	123.1(6)
O(1)-C(7)	1.221(7)	O(1)-C(7)-C(1)	121.0(5)
N(3)-C(6)	1.408(3)	N(1)#1-N(1)-C(8)	113.8(6)
C(1)-C(7)	1.360(8)		
[b]**Compound 2**			
N(2)-N(2)#1	1.228(11)	O(1)-C(7)-N(1)	122.5(7)
O(1)-C(7)	1.218(9)	O(1)-C(7)-C(4)	120.2(7)
N(1)-C(7)	1.358(9)	N(2)#1-N(2)-C(1)	112.7(8)
C(1)-C(2)	1.354(11)		

[a]Symmetry transformations used to generate equivalent atoms: #1, -x+1,-y-1,-z

[b]Symmetry transformations used to generate equivalent atoms: #1, -x-2,-y-1,-z-1

Table 3. Selected parameters for weaker interactions of compounds **1** and **2**.

D-H···A	D-H(Å)	H···A(Å)	D···A(Å)	D-H...A(°)	Symmetry code
Compound 1					
C(4) --H(4) ..O(1)	0.93	2.59	3.282(9)	132	-1+x,y,z
C(9) --H(9) ..O(1)	0.93	2.37	2.905(8)	116	-
Compound 2					
N(1) --H(1) ...O(2)	1.17	1.90	3.025(11)	159	-
O(2) –H(2W) ..N(3)	1.02	1.77	2.788(12)	178	1-x,-1/2+y,-1/2-z
C(5) --H(5) ...O(2)	0.93	2.47	3.336(13)	155	-
C(9) --H(9) ...O(1)	0.93	2.24	2.827(14)	120	-
C(13)-H(13)···O(2)	0.93	2.52	3.311(13)	143	-

Photochromic test: alternative exposure to UV and Visible radiation. The solution of **1** (1×10^{-5} M in DMF, 2.5 ml, *E*-isomer) was filled in optiglass cuvette and exposed to UV-light (λ = 365 nm) in a dark-chamber for 2 min. There of, the UV-vis and fluorescence spectra were recorded immediately. Similarly, same solution was exposed to longer period of up to 15 min (for full response) with an interval of 2 min in each data collection. The resulting solution (*Z*-form) was irradiated by visible light for 5 min with an interval of 1 min till the reappearance of initial spectra (*E*-isomer).The photo-conversion was judged using ^{1}HNMR and UV-vis spectroscopic techniques. The same method was adopted for photochromic experiment for **2**.

pH dependent experiment: The pH of the solution of **1** (1×10^{-5} M in DMF, 2.5 ml) was maintained using ~10^{-3} M HCl and NaOH in water. A number of solutions of **1** were prepared having pH = 3.0, 5.0, 7.0, 9.0 and 11.0 and their UV-vis spectra were recorded. Further, each solution was exposed to UV (15 min) and visible (5 min) light before recording fluorescence spectra.

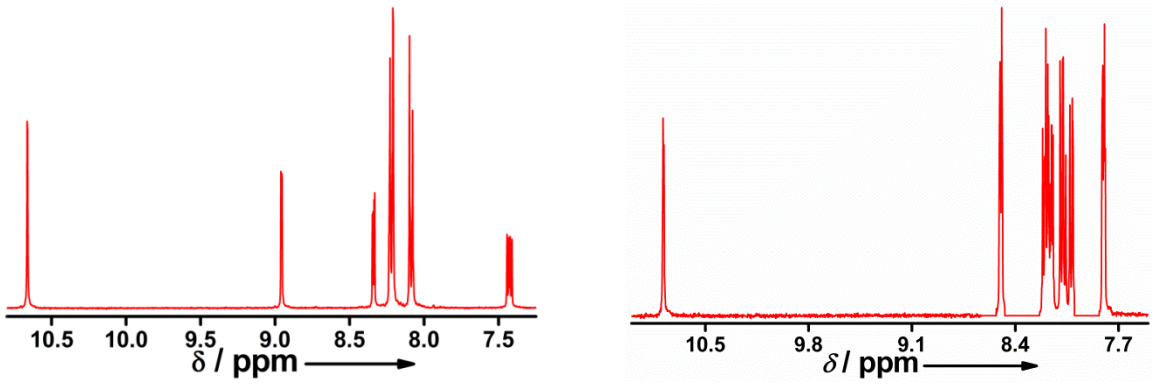

Figure S1: ^{1}H NMR spectra of **1** and **2** in DMSO-d_6 at room temperature.

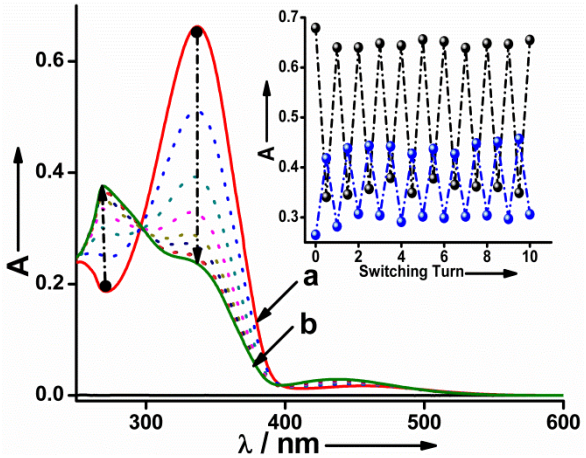

Figure S2: Absorbance intensity changes of **2** (1×10^{-5} M in DMF) upon exposing it to UV light at λ = 365 nm for 15 min (0, 2, 4, 6, 8, 10, 12, 15) (red to green line, b) and simultaneously to visible light for 5 min (green to red line, a). The dotted line serves as guide to stepwise transformation. Inset: absorption change for **2** at 337 nm (black balls) and 268 nm (blue balls) vs. the number of switching turn. Dotted lines are guide to the eyes.

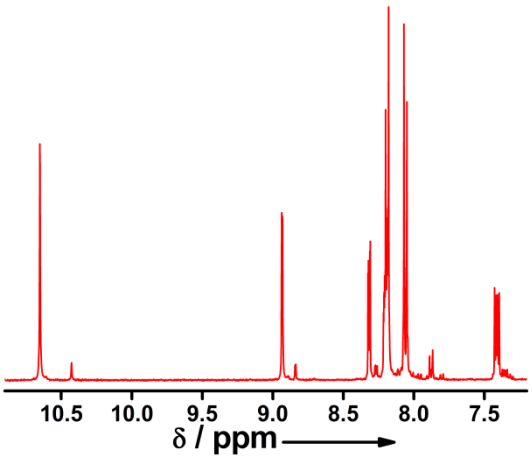

Figure S3: ^{1}H-NMR spectrum of **1** after exposure to UV-light for 15 min in DMSO-d_6.

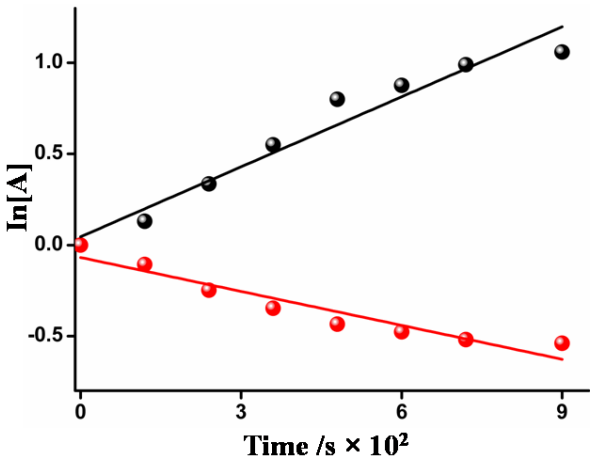

Figure S4: Absorbance intensity (In[A]) changes of **1** (1×10^{-5} M in DMF) at λ = 342 nm (black balls) and λ = 268 nm (red balls) *vs*. time.

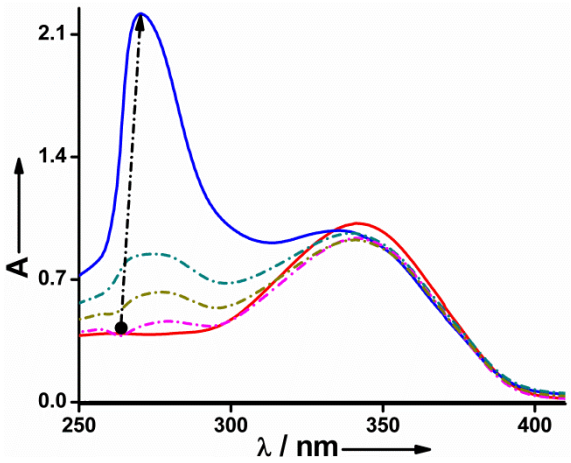

Figure S5: Absorbance changes of **1** (1×10^{-5} M in DMF) on varying the pH from 11 to 3 (11, 9, 7, 5, 3, red to blue line).

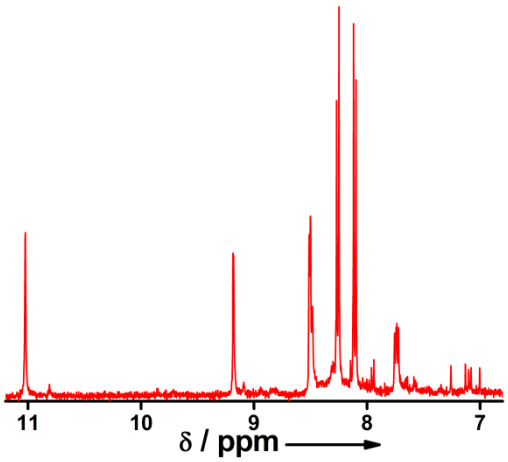

Figure S6: ^{1}H-NMR spectrum of **1** after maintaining at pH = 3 in DMSO-d_6.

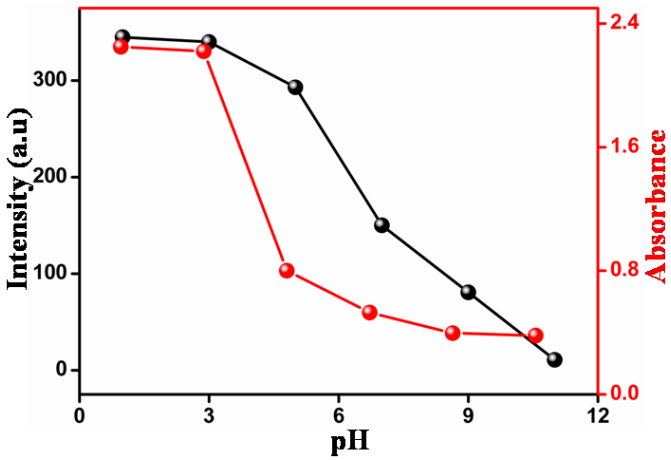

Figure S7: Monitoring the emission (at 395nm, black balls, λex = 275 nm) / absorption (at λ = 261 nm, red balls) intensity of **1** (1×10^{-5}M in DMF) as a function of pH of the solution.

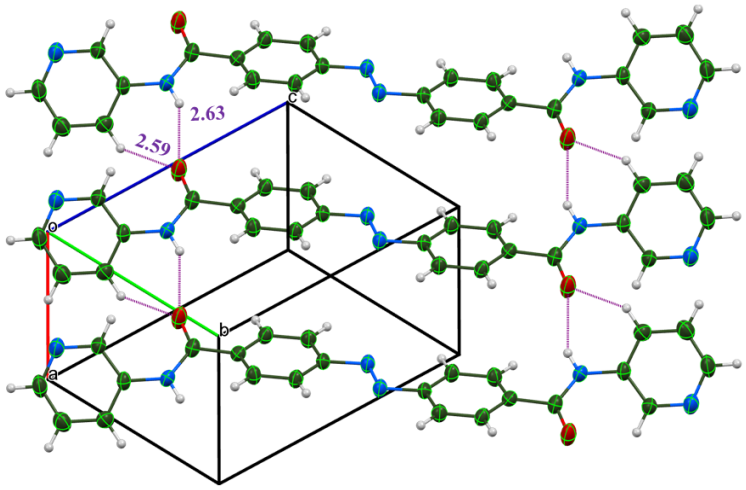

Figure S8: Crystallographic packing of **1** alike secondary *β*-sheet structure of protein.

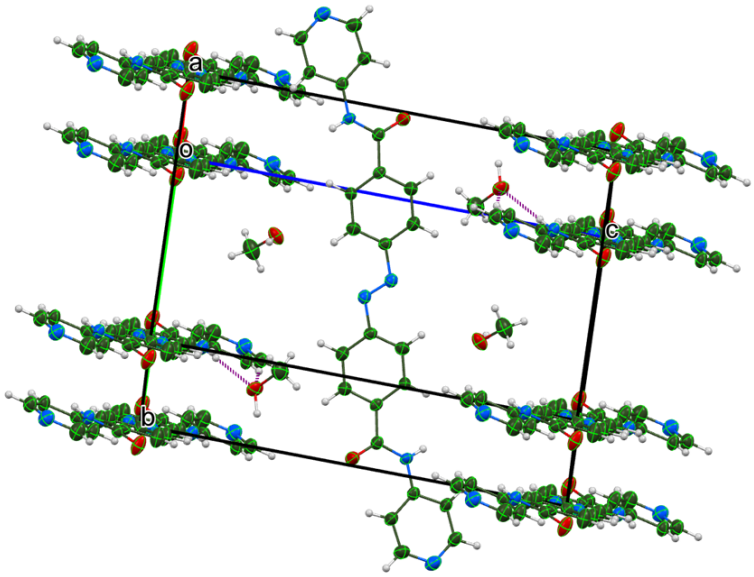

Figure S9: Crystallographic packing of **2** alike tertiary *β*-hairpin structure of protein.

Reference

S1. S. Ghosh, D. Usharani, A. Paul, S. De, E. D. Jemmis and S. Bhattacharya, *Bioconjugate Chem.* 2008, **19**, 2332–2345.