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Supporting Information

Biocatalytic amide condensation and gelation controlled by light

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Materials and Methods

Materials:

All commercial reagents were used as purchased. All solvents were used as supplied (analytical or HPLC grade) without further purification. All chemical reactions were performed in oven-dried glassware and magnetically stirred. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates. All compounds were visualized either by UV light source (254 nm) or by dipping in basic permanganate solution. Column chromatography was carried out by using silica gel 60 (230-400 mesh). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV300 spectrometer in the deuterated solvents. All chemical shifts (δ) are quoted in ppm and coupling constants (J) given in Hz. Residual signals from the solvents were used as an internal reference.

Methods:

Biocatalytic hydrogelation

The precursors **Azo-Y** (20 mM) and each **X-NH$_2$** (80 mM, X= F, L, V, Bachem, Germany) were weighed (at 1:4 ratio) in a glass vial individually. The mixture was solubilised in 1.0 mL of 100 mM phosphate buffer (pH 8) followed by addition of thermolysin (1 mg) powder (*bacillus thermoproteolyticus rokko,* Sigma-Aldrich, UK, Mol.wt 34.6 kDa). The mixture was mixed by vortex for 20 s and sonicated for 30-60 s depending on the amino acid sequence for complete dissolution. Samples were left at room temperature without disturbing for 24 h before analysis.

UV-visible absorption spectroscopy

UV-Vis absorption spectra were recorded in Jasco V-660 spectrophotometer. Samples for the measurement were prepared in PMMA cuvettes (Fisher Scientific).

Fluorescence emission spectroscopy

Fluorescence emission spectra were measured on a Jasco FP-6500 spectrofluorometer with light measured orthogonally to the excitation light, at a scanning speed of 200 nm min^{-1}. The excitation wavelength was 343 nm and emission data were recorded in the range between 360 and 700 nm. The spectra were measured with a bandwidth of 3 nm (or 5 nm) with a low (or medium) response and a 1 nm data pitch. Samples were prepared in PMMA cuvettes (Fisher Scientific) and the time-dependent spectra were recorded immediately.

Circular dichroism (CD)

Circular dichroism (CD) spectra were measured on a Jasco J600 spectropolarimeter in a 0.1 mm path length cylindrical cell, with 1 s integration, step resolution of 1 mm, response of 0.5 s with a bandwidth of 1 nm and slit width of 1 mm. The freshly prepared samples were directly added to the cell (200 µL) using a pipette and the spectra were recorded after 24 h. The High Tension (HT) voltage value reaches maximum below 225 nm and CD couldn't be measured.

High performance liquid chromatography (HPLC)

Dionex P680 HPLC system was used to quantify the percentage conversion of the enzymatic reaction. A 50 µL sample was injected onto a Macherey–Nagel C18 column of 250 mm length with an internal diameter of 4.6 mm and 5 mm fused silica particles at a flow rate of 1 mL min-1 (eluting solvent system: linear gradient of 20% (v/v) acetonitrile in water for 4 min, gradually rising to 80% (v/v) acetonitrile in water at 35 min. This concentration was kept constant until 40 min when the gradient was decreased to 20% (v/v) acetonitrile in water at 42 min.) Sample preparation involved mixing 20 µL of the sample with 1 mL acetonitrile-water (50:50 mixture) containing 0.1% trifluoroacetic acid. The intensity of each identified

peak was determined by UV detection at 280 nm. The experimental data was acquired in triplicate and the average data was shown. The samples were vortexed before collecting the aliquots for HPLC and the percentage yields are calculated from HPLC integrated peak areas.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) images were captured using a LEO 912 energy filtering transmission electron microscope operating at 120kV fitted with 14 bit/2 K Proscan CCD camera. Carbon-coated copper grids (200 mesh) were glow discharged in air for 30 seconds. The support film was touched onto the gel surface for 3 seconds and blotted down using filter paper. Each sample was allowed to dry afterwards for 2-3 minutes in a dust-free environment prior to TEM imaging. Negative stain (20 μL, 1 % aqueous methylamine vanadate (Nanovan), Nanoprobes) was applied and the mixture blotted again using filter paper to remove excess. The dried grids with the samples were then imaged using the microscope.

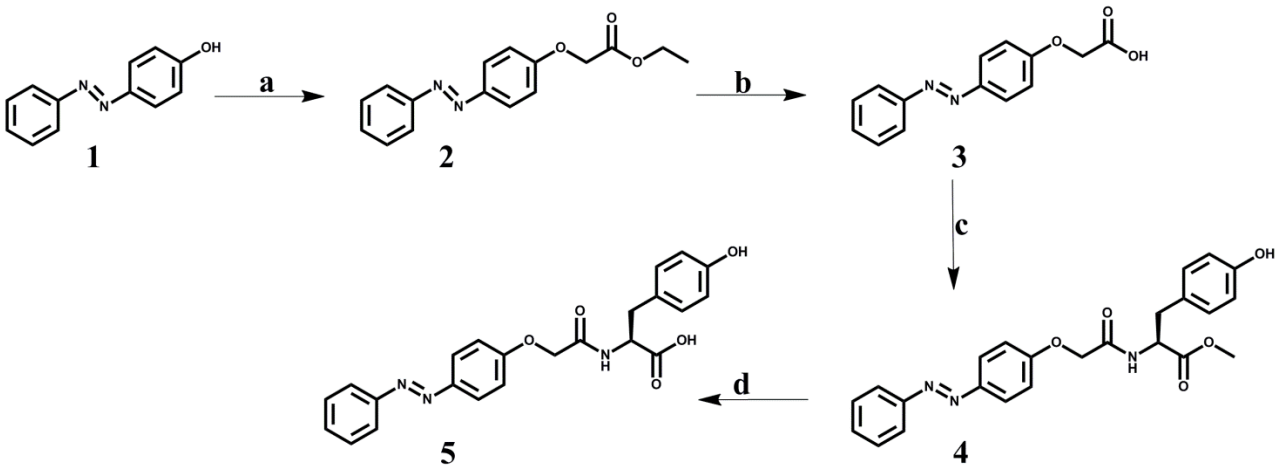
Rheology

Rheometric characterisation was performed in order to confirm the gel-like behaviour and mechanical properties of the resulting hydrogels. Dynamic frequency sweep experiments were performed on strain-controlled rheometer (Kinexus Pro rheometer) using 20 mmparallel-plate geometry with a gap of 0.5 mm. The experiments were performed at 25 °C. Viscometry measurements were carried out by monitoring the viscosity and shear stress over controlled shear rates from 0.1-100 s^{-1}. The dynamic modulus of the hydrogel was measured as a frequency function, where the frequency sweeps were carried out in the range of 0.1-100 Hz with constant strain value. In order to ensure the measurement were done in linear viscoelastic region, amplitude sweeps were performed at constant frequency (1 Hz) from shear strain 0.01-100% where no variation in G' and G'' was observed. The gels were made in

small fractions in wide-mouth vials from which they were transferred with a spatula for rheological measurements.

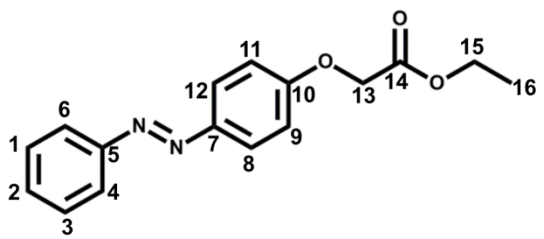
Synthesis:

Azo-Y was synthesized by 4-step procedure as shown in **scheme S1**.



Scheme S1. Reagents and conditions: (a) Ethyl bromoacetate, K_2CO_3, dry acetone, room temperature (b) NaOH (1N), methanol, room temperature (c) HBTU, DIPEA, L-Tyrosine methyl ester, room temperature (d) LiOH, THF, 55 °C, room temperature.

Synthesis of (E)-2-(4-(phenyldiazenyl) phenoxy) Ethyl acetate (2):



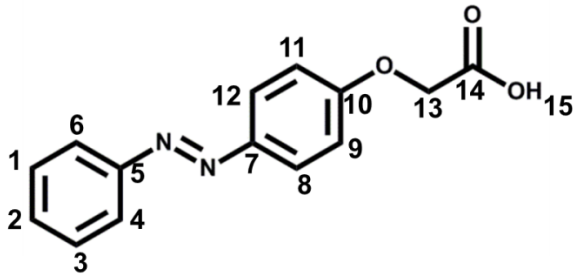
To a solution of 4-phenyl azophenol (5 g: 25 mM) in dry acetone (100 mL), 13.55 g of ethyl bromoacetate and 12.51 g of K_2CO_3 was added and refluxed overnight. After the reaction got over, it was allowed to cool to room temperature and solvent was reduced *in vacuo*. The

remaining compound was dissolved in DCM and was washed in HPLC water and brine. The organic fraction was dried in $MgSO_4$ (dry) and was evapourated *in vacuo*. The residue was then purified by silica gel column chromatography (DCM:MeOH 95:5) to obtain the desired product.

^{1}H NMR (400MHz, $CDCl_3$): 7.97 – 7.84 (4 H, m), 7.55 – 7.38 (3 H, m), 7.07 – 6.96 (2 H, m), 4.69 (2 H, s), 4.33 – 4.18 (2 H, m), 1.30 (3 H, t, *J* 7.2 Hz).

^{13}C NMR (100MHz, $CDCl_3$): 169.79, 160.21, 152.80, 147.73, 130.63, 129.13, 124.82, 122.72, 115.02, 68.32, 65.61, 61.62, 14.27.

Synthesis of (E)-2-(4-(phenyldiazenyl) phenoxy) acetic acid (3):

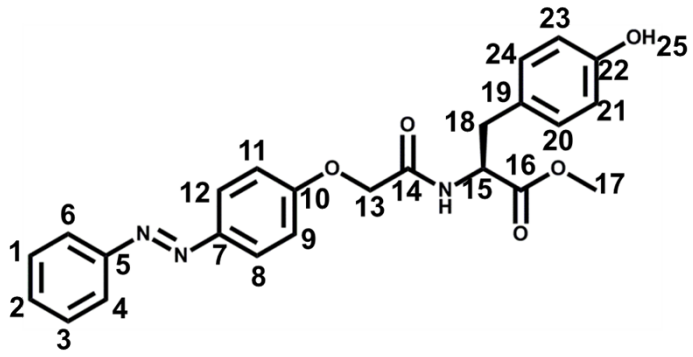


To a solution of compound **2** (2.21 g; 7.77 mM) in 120 mL of methanol, 20 mL of NaOH (1N) was added and stirred at room temperature for 2h. After the reaction got over, methanol was removed in a rotary evaporator and the remaining solution was mixed with 50mL of ethyl acetate. The aqueous layer was acidified to pH 2 with 1N HCl. The aqueous phase was separated and the organic layer was further washed with brine, dried in $MgSO_4$ (anhydrous) and concentrated by evaporation *in vacuo* to get the desired product. The obtained compound was characterised by ^{1}H NMR and ^{13}C NMR spectroscopy.

^{1}H NMR (400MHz, DMSO): 13.10 (1 H, s), 7.93 – 7.81 (4 H, m), 7.61 – 7.48 (3 H, m), 7.16 – 7.08 (2 H, m), 4.82 (2 H, s).

^{13}C NMR (100MHz, DMSO): 169.76, 160.45, 151.99, 146.45, 130.85, 129.34, 124.43, 122.25, 115.14, 64.75.

Synthesis of 4:

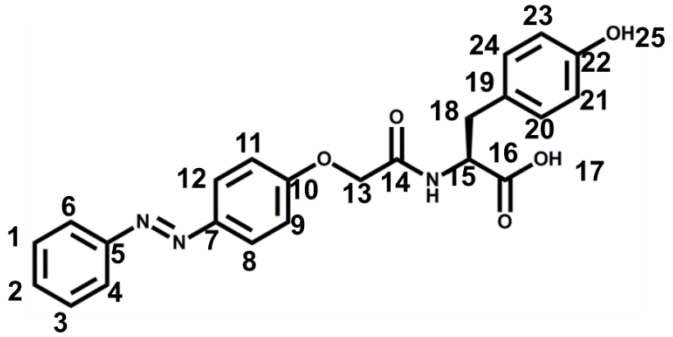


For the synthesis of **4,** compound **3** (1.4 g; 5.46 mM), L-tyrosine methyl ester (1.526 g; 6.58 mM), HBTU (2.49 g; 6.58 mM), diisopropyl ethyl amine (DIPEA) (2.39 mL, 13.15 mM) was mixed in 30 mL of anhydrous DMF and stirred over night at room temperature. After the reaction got over, 40 mL of HPLC grade water was added to the mixture and was separated in 100 mL of ethyl acetate. The compound in organic fraction was further washed with sodium bicarbonate ($NaHCO_3$; 60 mL), Brine (60 mL) and HCl (1N) and brine (60 mL) respectively. The organic fraction was collected and dried in anhydrous $MgSO_4$ followed by evaporating the solvent *in vacuo*. The crude product was finally purified by using column chromatography (DCM as eluent) to obtain 1.4 g of desired product. The compound was characterised by ^{1}H NMR and ^{13}C spectroscopy.

^{1}H NMR (400MHz, DMSO): 9.23 (1 H, d, *J* 3.6), 8.47 (1 H, d, *J* 8.0), 7.93 – 7.81 (4 H, m), 7.64 – 7.49 (3 H, m), 7.10 – 6.94 (4 H, m), 6.70 – 6.62 (2 H, m), 4.62 (2 H, s), 4.56 – 4.44 (1 H, m), 3.63 (3 H, s), 3.04 – 2.81 (2 H, m).

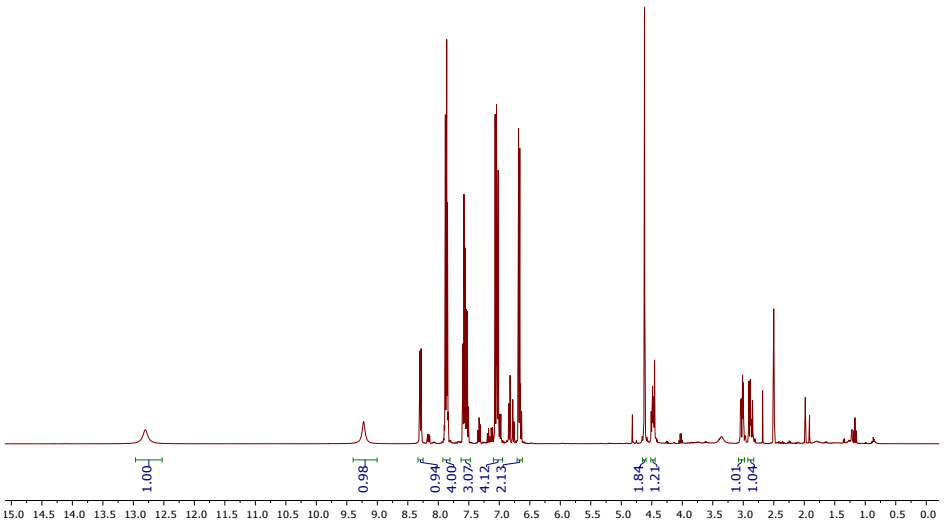
^{13}C NMR (100MHz, DMSO): 171.72, 167.25, 160.35, 156.02, 151.97, 146.51, 130.87, 130.01, 129.34, 129.04, 126.99, 124.36, 122.73, 122.24, 119.27, 115.30, 115.06, 114.58, 66.67, 53.46, 51.91, 35.72.

Synthesis of 5:



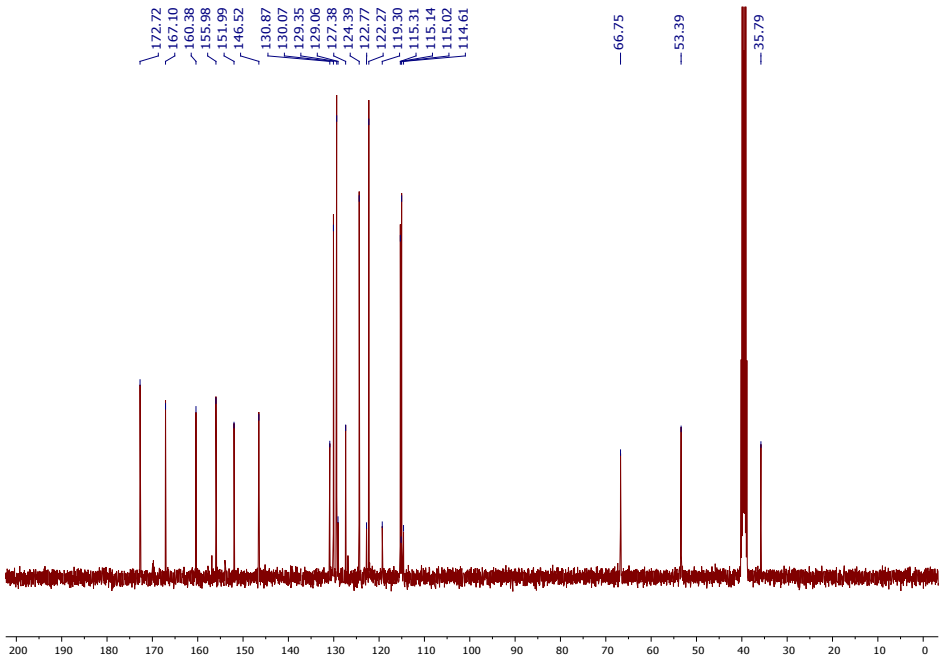
For the synthesis of compound 5, compound 4 was added to 1:1 mixture of THF:LiOH (1M in H_2O) in a round bottom flask and stirred at 55 °C for 1h. After the reaction got over, the THF was evaporated *in vacuo* to get the aqueous layer. The aqueous layer was acidified to pH 3 by adding HCl (1 M) drop wise. The acidified solution was then extracted in ethyl acetate (5X), dried in anhydrous $MgSO_4$ and evaporated *in vacuo* to get **Azo-Y** (solid, mango yellow in colour).

^{1}H NMR (400MHz, DMSO): 12.80 (1 H, s), 9.22 (1 H, s), 8.29 (1 H, d, *J* 8.2), 7.94 – 7.80 (4 H, m), 7.64 – 7.47 (3 H, m), 7.10 – 6.94 (4 H, m), 6.72 – 6.61 (2 H, m), 4.62 (2 H, s), 4.54 – 4.41 (1 H, m), 3.02 (1 H, m), 2.95 – 2.79 (1 H, m).



^{1}H NMR spectra of Azo-Y-OH.

^{13}C NMR (100MHz, DMSO): 172.72, 167.10, 160.38, 155.98, 152.00, 146.52, 130.87, 130.07, 129.35, 129.06, 127.38, 124.39, 122.77, 122.27, 119.30, 115.32, 115.14, 115.03, 114.61, 66.75, 53.39, 35.79.



^{13}C NMR spectra of Azo-Y-OH.

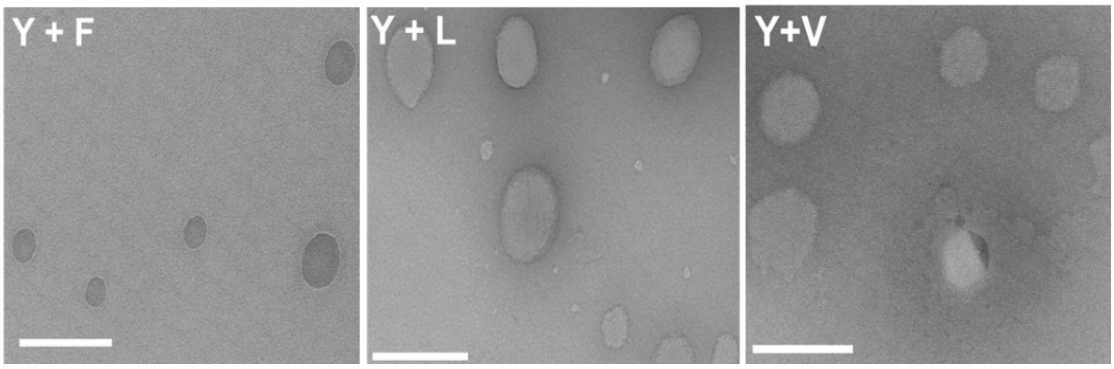


Figure S1: TEM images before addition of thermolysin; Scale bar: 200 nm.

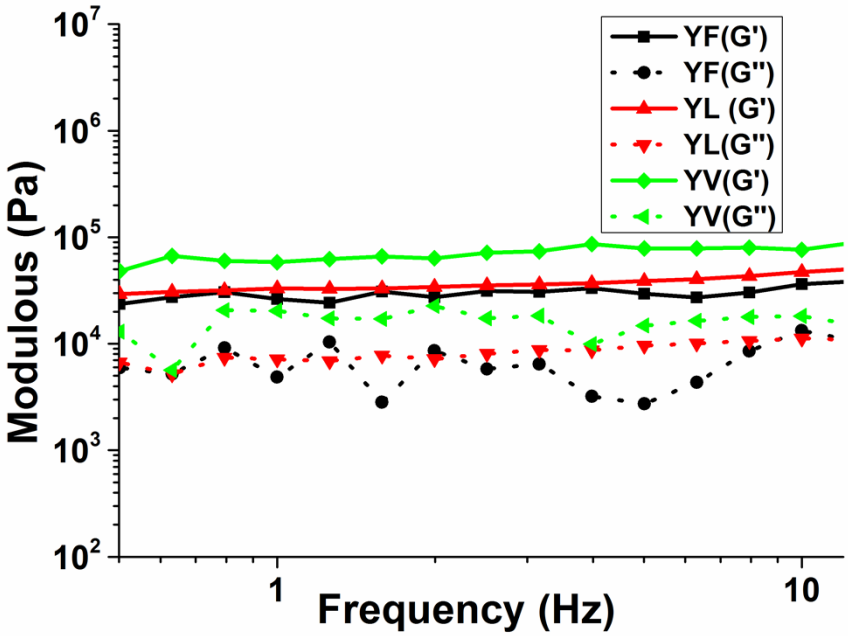


Figure S2: Rheological data of different dipeptide derivatives at 20 mM concentration.

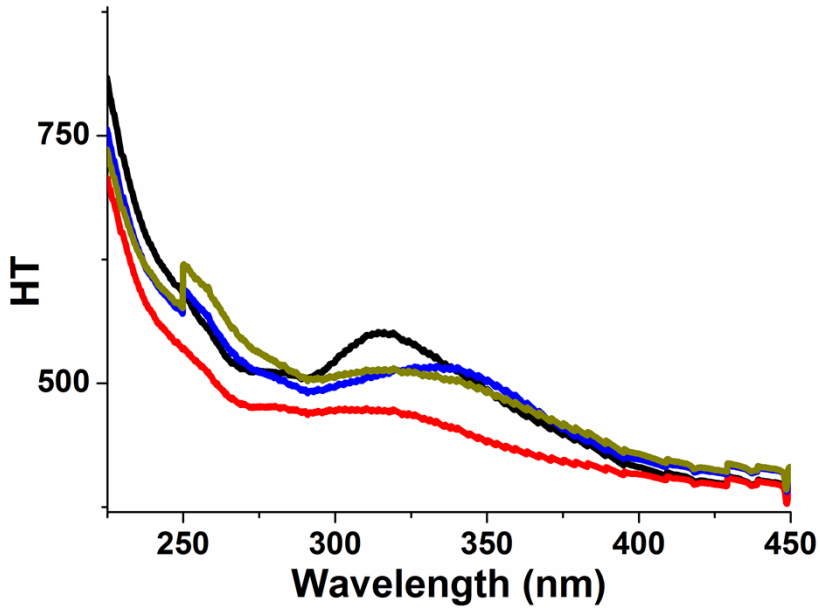


Figure S3: High Tension (HT) voltage.

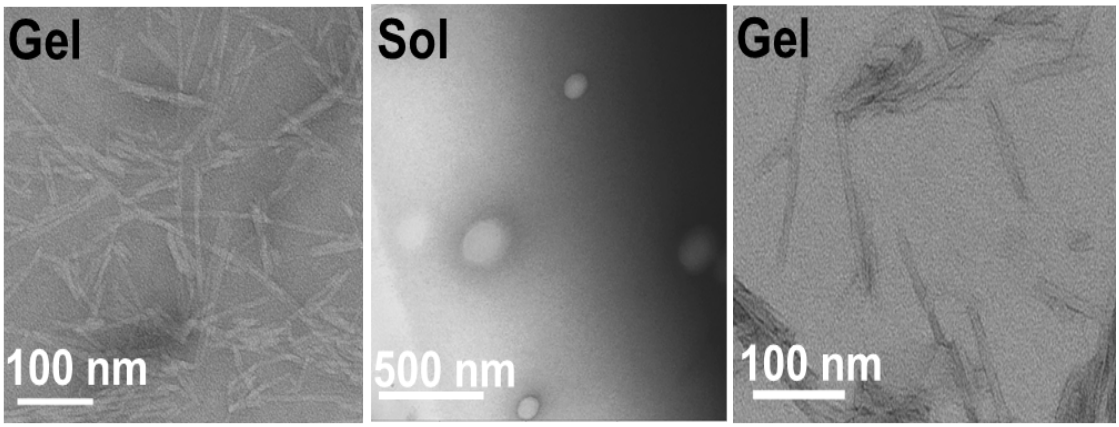
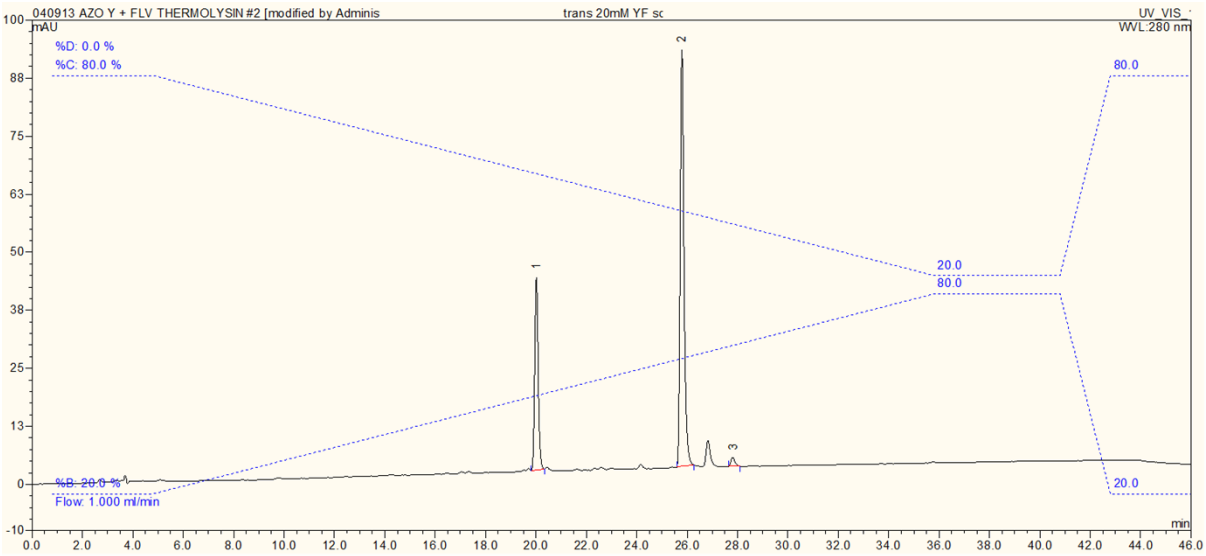


Figure S4: TEM images of different stage of light induced morphological change (gel-sol-gel).



		min	mAU*min			mAU	%	
1	n.a.	20.017	6.5672	n.a.	BMB	41.54	29.1	22.85
2	n.a.	25.794	15.6687	n.a.	BMB*	89.599	69.43	7.57
3	n.a.	27.824	0.3327	n.a.	BMB*	1.851	1.47	n.a.
Total:			22.5685		0	132.99	100	

Figure S5: HPLC trace showing the presence of *cis*-**azo Y** and *trans*-**azo Y** after UV light irradiation on *trans*-**azo YF-NH$_2$** (λ=280 nm).

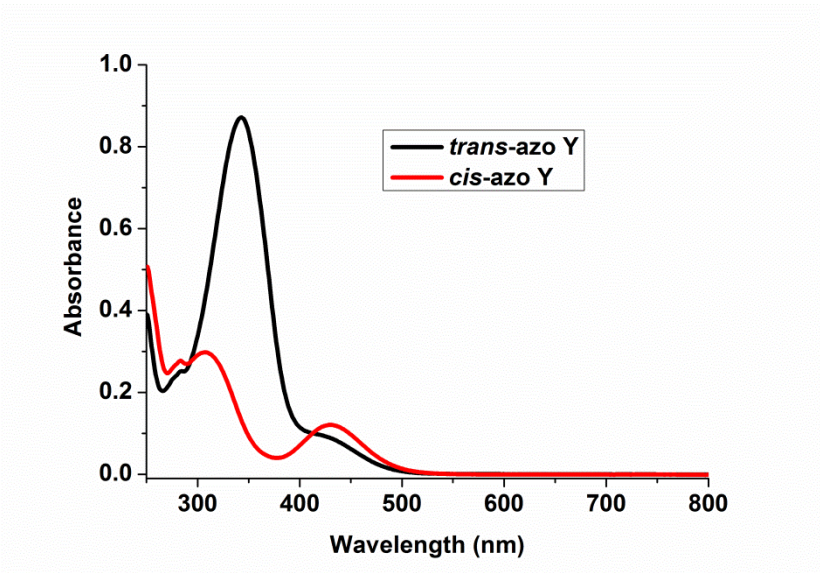
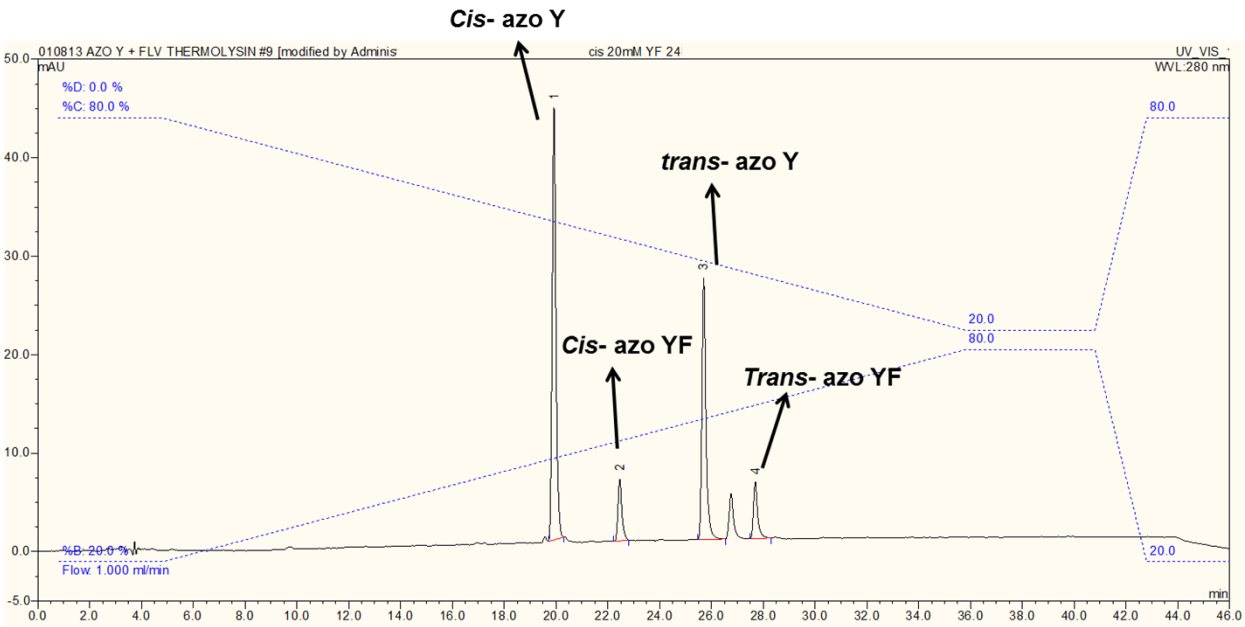


Figure S6: UV-Vis spectra of **Azo Y** (2.5 x 10 $^{-5}$ M) in phosphate buffer (100 mM) at pH 8 before (black line; *trans*-**Azo Y)** and after UV light irradiation (100 W, λ = 365 nm, 5 minutes) (red line; *cis*-**Azo Y**).



No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
		min	mAU*min			mAU	%	
1	n.a.	19.924	7.3146	n.a.	BMB	43.85	51.08	10.03
2	n.a.	22.464	1.0943	n.a.	BMB	6.292	7.64	12.55
3	n.a.	25.703	4.8282	n.a.	BMB*	26.56	33.72	7.57
4	n.a.	27.696	1.0824	n.a.	BMB	5.755	7.56	n.a.
Total:			14.3195	0		82.457	100	

Figure S7: HPLC trace showing the percentage peptide conversion of cis-**azo YF-NH_2** after 24 h (λ=280 nm).