Allosteric Effects in Coiled-Coil Proteins Folding and Lanthanide-ion Binding

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Supporting information

1. Experimental section

Peptide synthesis

All peptides were synthesized at 0.1-0.2 mmol scale on a Rink amide MBHA resin using standard Fmoc protocols. Fmoc deprotection was done using 25% piperidine in DMF.

Synthesis of azide-modified peptides. Triflyl azide, used for modification of amine at the n-terminus or side chain (see below), was synthesized as follows: a suspension of sodium azide (3.3 mmol) in 4 ml pyridine was cooled in ice path. Then triflic anhydride (2.78 mmol) was added to the mixture by a syringe during 5 min while stirring. After the reaction was maintained for 2 h in ice path, the TfN_3-containing solution (filtration of the salts was done when necessary) was added directly to the amine solution for subsequent diazo transfer reaction.

The peptide with free amine, on resin (100 mg of resin), was swelled in DMF for 20 min followed by the addition of 0.02 mmol of $CuSO_4$ in MeOH (1 ml), and triethylamine (0.02 mol). The mixture was cooled in ice bath for a few minutes, and then the solution of triflyl azide in pyridine was added dropwise. The reaction mixture was allowed to warm to room temperature and reacted for 5 h. The same procedure was repeated 3 times with fresh reagents. Upon completion, the reaction mixture was washed with DMF, diethyldithiocarbamic acid sodium salt in DMF (0.02 M, 3×10 min), DIPEA/DMF (99.5:0.5, 3×2 min), and finally with DMF, DCM and ether.

The azido-peptides were obtained after cleavage and global deprotection with a mixture of TFA:TIS:water 95:2.5:2.5. All the studied peptides were purified by preparative HPLC using

C18 reverse phase column (Agilent, Zorbax, 300 SB, 7 μM, 21.2×250 mm) with a step gradient of solvent A (99% water, 1% acetonitrile (ACN), 0.1% TFA) and B (90% ACN, 10% water, 0.07% TFA). The identity and purity of the peptides were analysed by analytical HPLC (Agilent, Zorbax column, 300 SB, 5 μM, 4.6×150 mm) with the same solvent system for elution and ESI-MS.

Synthesis of HPO-peptide conjugates via the 'click' reaction. The azido-peptide (1 eq.) and acetylene-HPO (0.5 eq.) were dissolved in 1 mL water/t-BuOH. A mixture of $CuSO_4$ (1% mole eq.) and TBTA (5% mole eq.) was added to the solution, degased, and then sodium ascorbate (0.5 eq.) was also added. The mixture was shaken for 24 hours at 37^0C. The progress of the reaction was monitored by analytical HPLC; upon completion the product was purified by preparative HPLC, and analyzed by HPLC and ESI-MS.

Characterization of HPO-peptides-lanthanide complexes

Stock solutions of HPO-peptides were prepared in water, and their actual concentrations were determined from the UV-absorption of the HPO moiety at 320 nm. The peptide-lanthanide complexes were prepared by mixing the respective solutions, in 200 mM MOPS buffer at pH 6.5 (in some cases up to 10% acetonitrile was added, if needed to improve solubility), at appropriate ratios, and after equilibration of at least 1 hour prior to all the experiments.

Fluorescence measurement. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer, equipped with a 96-microwellplate reader. Spectra were collected from 400 to 750 nm, with an excitation wavelength of 380 nm (excitation slit width: 12 nm), and scan speed of 100 nm min^{-1}.

Circular Dichroism (CD) measurements. CD measurements were carried on a Jasco 815 CD spectropolarimeter, at 25^0C, by using a quartz cell with 1.0 mm path length. CD spectra were obtained as the average of three scans and collecting data at 1 nm intervals from 260 to 200 nm. The CD signals resulting from buffer were subtracted from the spectrum of each peptide solution. Data was converted to molar ellipticity (Θ in deg*cm^2*$dmol^{-1}$) according to the equation: $[\Theta] = \Psi / (1000\ nlc)$, where Ψ is the CD signal in degrees, n is the number of peptide bonds, l is the path length in centimeters, and c is the concentration in decimoles per cm^3.

MALDI-TOF MS analysis. MALDI-TOF measurements were carried out on a Reflex IV mass spectrometer (Bruker, Germany) equipped with a nitrogen laser. Freshly formed complexes of

$[Ln\text{-}(HPO\text{-}1)_3]^{+3}$ were measured with DHB (2,5-dihydroxybenzoic acid) or SA (sinapinic acid) as matrix, in a mass window from 10 KDa to 100 KDa. The results obtained with accuracy of ±10 molecular units from the calculated mass.

2. Additional figures and captions

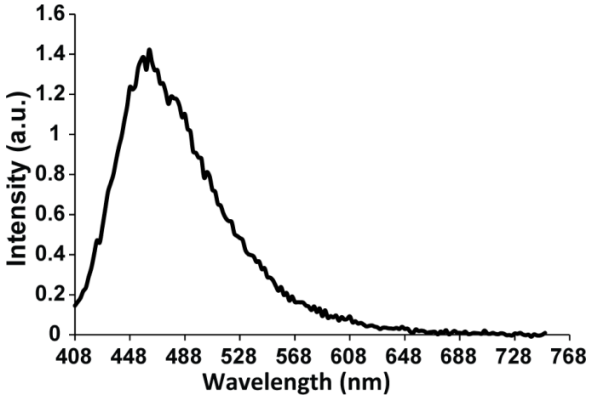

Figure s1: Emission spectrum of acetylene-HPO (1 mM) following excitation at 380 nm. The ligand was dissolved in MOPS buffer/ACN mixture (9:1) at pH 6.5.

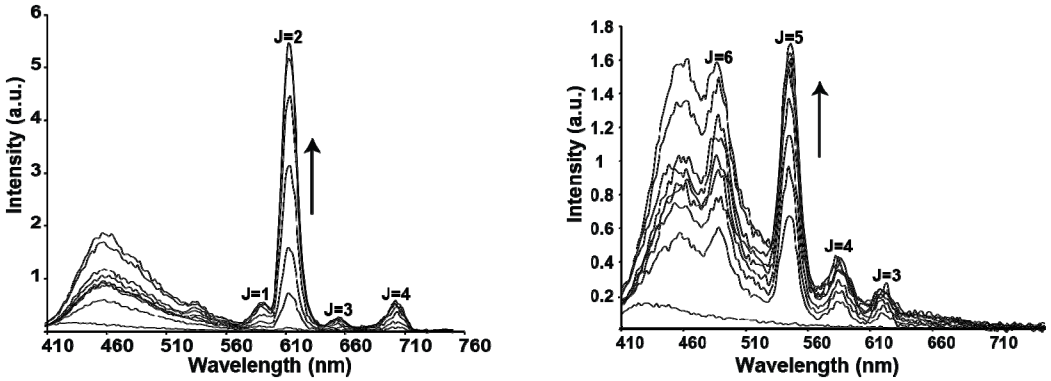

Figure s2: *left* - Spectral changes in Eu(III) (500 μM) emission upon addition of acetylene-HPO (0 → 5.0 equivalent), in MOPS buffer pH 6.5. *Right* - Spectral changes in Tb(III) (500 μM) emission upon addition of acetylene-HPO (0 → 4.0 equivalents), in MOPS buffer pH 6.5.

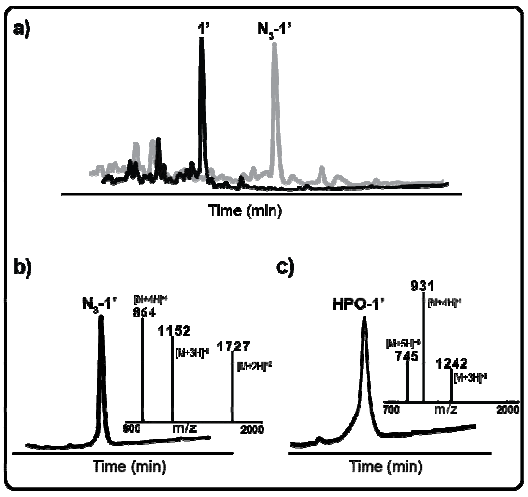

Figure s3: Characterization of 1', N_3-1' and HPO-1'. a) HPLC chromatogram of the crude peptides 1' and N_3-1' obtained after cleavage and global deprotection. b) HPLC chromatogram and ESI-MS of pure N3-1'. c) HPLC chromatogram and ESI-MS of pure HPO-1'.

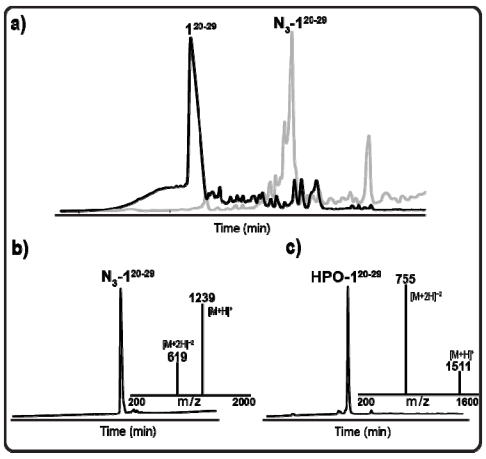

Figure s4: Characterization of $1^{20\text{-}29}$, N_3-$1^{20\text{-}29}$ and HPO-$1^{20\text{-}29}$. a) HPLC chromatogram of the crude peptides $1^{20\text{-}29}$ and N_3-$1^{20\text{-}29}$ obtained after cleavage and global deprotection. b) HPLC chromatogram and ESI-MS of pure N_3-$1^{20\text{-}29}$. c) HPLC chromatogram and ESI-MS of pure HPO-$1^{20\text{-}29}$.

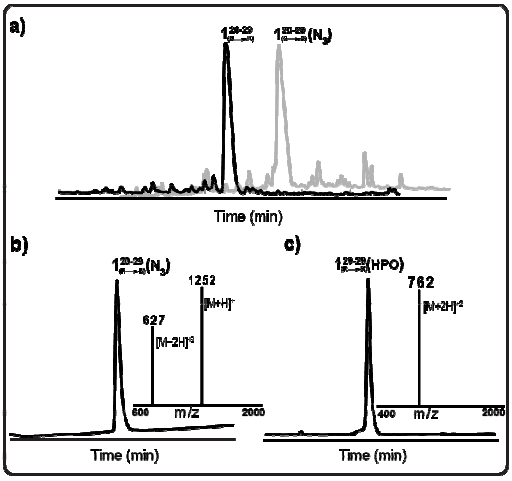

Figure s5: Characterization of $1^{20-29}_{R\rightarrow K}$, $1^{20-29}_{R\rightarrow K}(N_3)$ and $1^{20-29}_{R\rightarrow K}(HPO)$. a) HPLC chromatogram of the crude peptides $1^{20-29}_{R\rightarrow K}$ and $1^{20-29}_{R\rightarrow K}(N_3)$ obtained after cleavage and global deprotection. b) HPLC chromatogram and ESI-MS of pure $1^{20-29}_{R\rightarrow K}(N_3)$ c) HPLC chromatogram and ESI-MS of pure $1^{20-29}_{R\rightarrow K}(HPO)$.

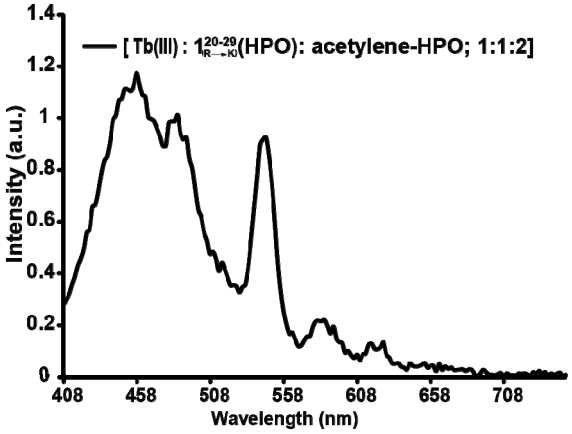

Figure s6: Emission spectrum of $Tb(III):1^{20-29}_{R\rightarrow K}(HPO):acetylene-HPO$ in ratio of 1:1:2 [500 µM: 500 µM: 1000 µM], in 200 mM MOPS buffer pH 6.5, excited at 380 nm. Characterization of this complex by MALDI-TOF MS also supported the formation of complex with 1:1:2 stoichiometry; found mass = 2254, very closely accounts to the calculated MW+Na^{+}.

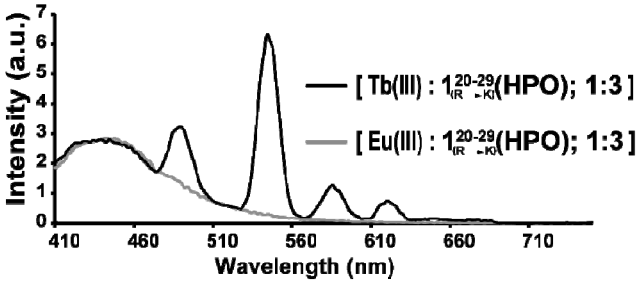

Figure s7: Emission spectra of $Tb(III):1^{20-29}_{R\rightarrow K}(HPO)$ and $Eu(III):1^{20-29}_{R\rightarrow K}(HPO)$, in a ratio of 1:3 [500 µM: 1500 µM], in 200 mM MOPS buffer pH 6.5, excited at 380 nm.