

Supporting Information

**Guanidinium-Induced Denaturation by Breaking of Salt Bridges**

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## Peptide samples

The sequences of the investigated alanine-based  $\alpha$ -helical peptides were Ac-A(AAAB)<sub>3</sub>A-NH<sub>2</sub>, Ac-A(BAAA)<sub>3</sub>A-NH<sub>2</sub>, and Ac-Ala-Glu-Ala-NH<sub>2</sub>, with Ac = acetyl, A = alanine, A = acidic residue (glutamic acid), B = basic residue (either arginine or lysine). All peptides were custom-synthesized by GL Biochem (Shanghai). Peptide purity ( $\geq 95\%$ ) was assessed by reversed-phase HPLC, and the peptides were lyophilized against a 35% HCl/H<sub>2</sub>O solution to remove residual trifluoroacetic acid. Peptide solutions of 50  $\mu$ M were prepared using a 20 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> buffer in H<sub>2</sub>O (pH = 7.0). Guanidine-HCl ( $\geq 99\%$  purity) was purchased from Sigma-Aldrich, and aliquots of a 5.0 M stock solution were added to the peptide solutions to prepare series of samples containing different Gdm<sup>+</sup> concentrations (0 – 3.7 M). After addition of guanidine-HCl, the pH of the peptide-solutions was adjusted to neutral pH level (pH = 7.0) by addition of NaOH solution (the volume added was less than 1% of the peptide solution). The Ac-Ala-Glu-Ala-NH<sub>2</sub>:Gdm<sup>+</sup> sample used for the measurements in Figs. 3g–i was prepared by acid-base reaction of the Glu COOH groups of the peptide with an equimolar solution of guanidinium hydroxide (prepared by mixing solutions Gdm<sub>2</sub>SO<sub>4</sub> and Ba(OH)<sub>2</sub> and removing the precipitated BaSO<sub>4</sub>); deuteration was achieved by evaporating the compound from excess D<sub>2</sub>O several times.

## UV-CD measurements

UV-CD spectra and thermal unfolding curves were collected on a Chirascan-plus CD Spectrometer (Applied Photophysics) using 2-mm quartz cuvettes over a UV range of 190 – 265 nm with incremental steps of 1.0 nm and averaging over 2 s of data acquisition time. Each spectrum was obtained by averaging a total of at least five scans. The ellipticity at 222 nm was collected as indicator of  $\alpha$ -helical structure, and CD band intensities were expressed in mean residue ellipticity. Temperature control was achieved using a Quantum Northwest (TC125) temperature controller in the range 268–363 K. Before each measurement, the peptide solution was allowed to equilibrate for 5 minutes at the adjusted temperature prior to data acquisition. A direct temperature probe was inserted into the peptide solution for continuously monitoring of the actual sample temperature. Global fitting of the datasets collected as function of temperature were performed using a two-state model.<sup>1</sup>

## Two-dimensional infrared measurements

The guanidinium-Ac ( $\geq 99\%$  purity), guanidine-HCl ( $\geq 99\%$  purity), and sodium acetate ( $\geq 99\%$  purity) used in the 2D-IR experiments were all purchased from Sigma-Aldrich. H/D-exchange of the carboxyl and guanidinium groups was achieved by evaporating the compounds from excess D<sub>2</sub>O, and sample solutions of 1.5 M were prepared by directly dissolving

the lyophilized solids in D<sub>2</sub>O. FTIR spectra were collected using a Bruker Vertex 70 spectrometer (2 cm<sup>-1</sup> resolution). The samples were kept at 1.5 M concentration between 2 mm thick CaF<sub>2</sub> windows separated by a 8  $\mu$ m Teflon spacer. A detailed description of the femtosecond pump-probe setup used for the 2D-IR experiments has been given elsewhere.<sup>2</sup>

## IR and 2D-IR data used for Fig. 3(a,b,c)

The data in Fig. 3(a,b,c) of the article were obtained by adding the spectra of solutions of GdmDCl and NaAc. In Figure S1 below we show the raw data used to construct these spectra. The colors in the IR spectra match those used in Fig. 3a of the main text.

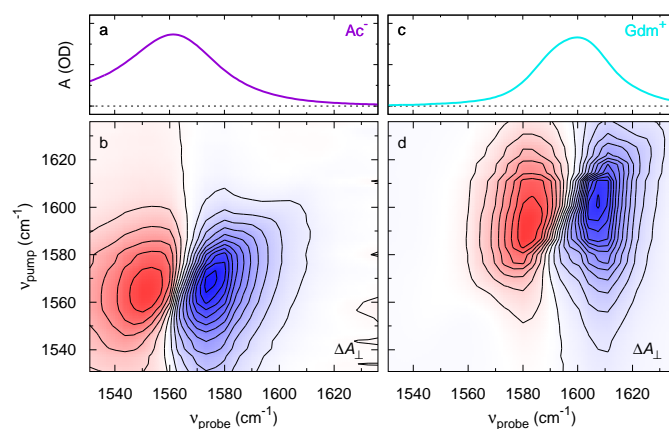


Fig. S1: (a,c) Normalized infrared absorption spectra of solutions of 1.5 M NaAc (a) and GdmDCl (c) in D<sub>2</sub>O (solvent subtracted). (b,d) 2D-IR spectra of these same solutions, recorded with perpendicular pump and probe polarizations, and with a pump-probe delay of 1 ps.

- [1] A. Fersht, *Structure and Mechanism in Protein Science*, Freeman & Co., New York, **1999**.
- [2] A. Huerta-Viga, D. J. Shaw, S. Woutersen, *J. Phys. Chem. B* **2010**, *114*, 15212–15220.