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The influence of the localised charge of C- and N-termini on peptide self-assembly

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Supporting Information

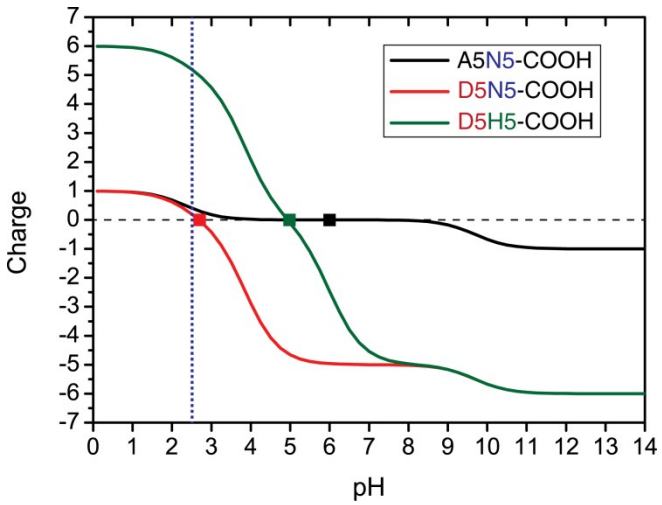

Fig. S1 Charge curves for all 3 sequences. Isoelectric points are indicated with squares: A5N5 = 6.0, D5N5 = 2.8 and D5H5 = 5.0. A green dashed line indicates the pH value at which all peptides were incubated in (pH = 2.5).

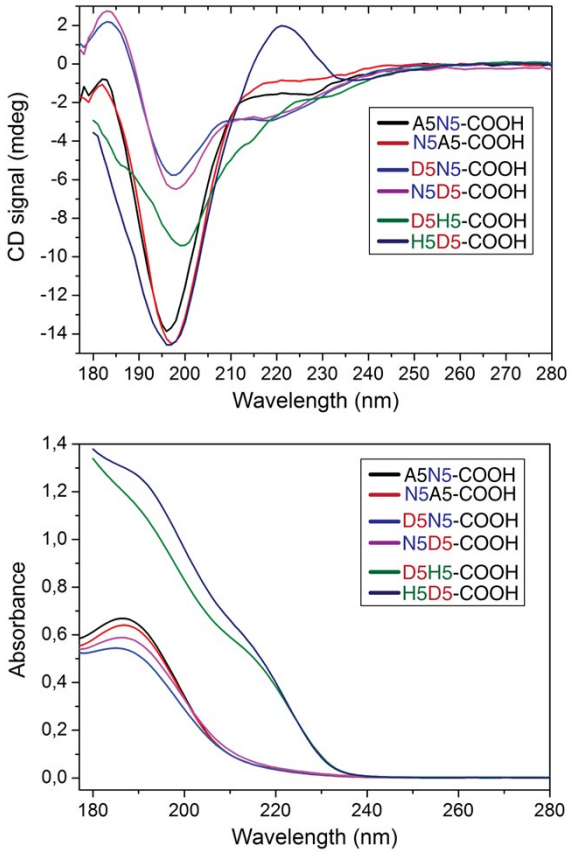

Fig. S2 SRCD and Absorbance spectra of all peptides analyzed in this work.

Figure S2 shows the SRCD spectra (upper panel) and the corresponding absorbance spectra (lower panel) for all peptides measured with a concentration of 1 mg/ml for each peptide . The absorbance of histidine rich sequences is much higher than the others, most likely due to the presence of aromatic rings.

	α–helices ordered	α–helices distorted	β–strands ordered	β–strands distorted	Turns	random coil	α–helices TOTAL	β–strands TOTAL
A5N5-COOH	0.02	0.02	0.15	0.12	0.17	0.51	4%	27%
N5A5-COOH	0.00	0.00	0.16	0.12	0.16	0.53	0%	28%
D5N5-COOH	0.02	0.06	0.23	0.11	0.15	0.43	8%	34%
N5D5-COOH	0.01	0.06	0.22	0.11	0.15	0.44	7%	33%
D5H5-COOH	0.17	0.08	0.12	0.08	0.15	0.39	25%	20%
H5D5-COOH	0.03	0.02	0.13	0.13	0.18	0.53	5%	26%

Table S1 Synchrotron radiation circular dichroism (SRCD) secondary structure contents of peptides predicted using Dichroweb[1, 2].

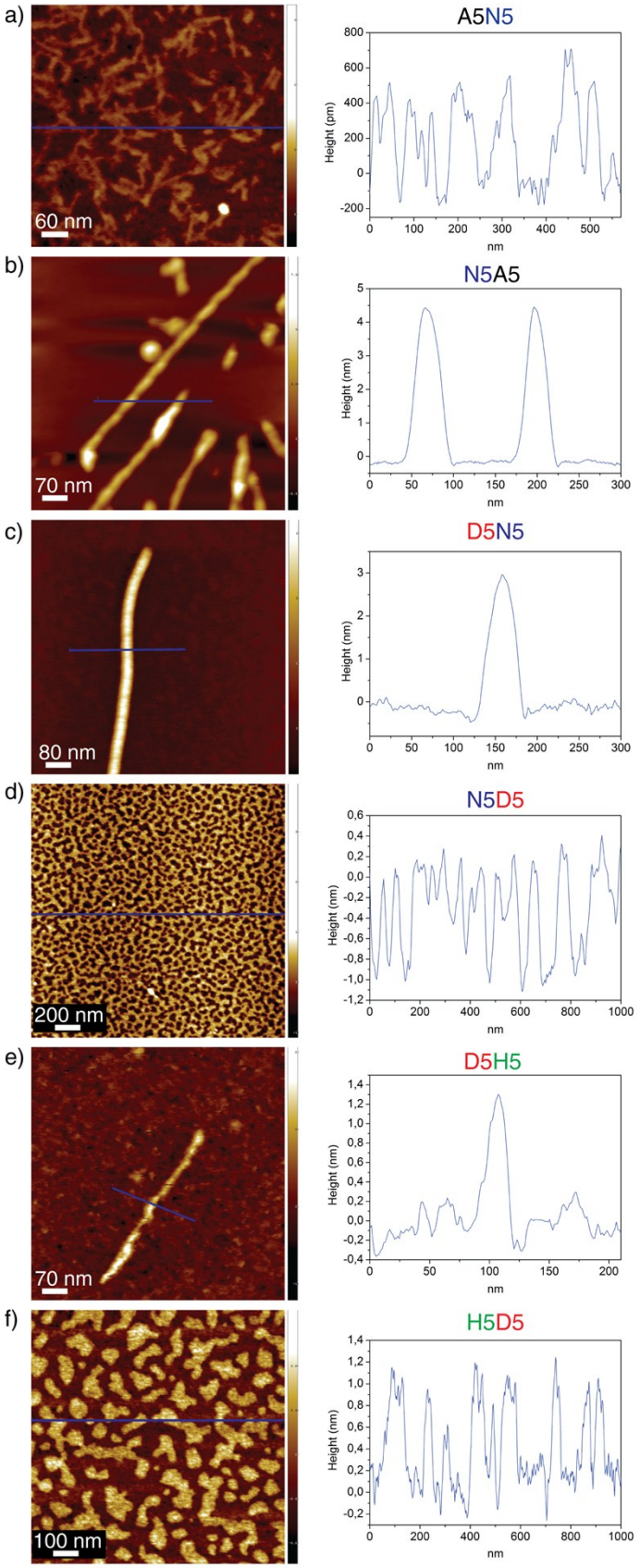

Fig. S3 AFM images and line profiles of peptide structures: a) **A5N5**; b) **N5A5**; c) **D5N5**; d) **N5D5**; e) **D5H5**; f) **H5D5**.

Experimental

Atomic Force Microscopy (AFM)

AFM experiments were performed in air at room temperature (21 ± 1°C, humidity 22%) using a Multimode SPM system with a Nanoscope VIII controller (Veeco Instruments Inc., Santa Barbara, CA). All the recorded AFM images consist of 512 x 512 pixels and several images were obtained at separate locations across the mica surfaces to ensure a high degree of reproducibility of the recorded molecular nanostructures. Samples are prepared by pouring 10 µl of the peptide solution on a freshly cleaved mica surface, rinsed with distilled water and dried with pressurised air.

Peptides incubation

Monomers were incubated in an aqueous solution (in which the pH was adjusted to 2.5 by HCl titration) at room temperature (or at 35°C when specified) and continuously shaken at 500 rpm. Monomer concentration was 2 mg/mL.

Peptide charge charts and ISO determination

Peptide charge charts (including the ISO point) were computed by using the following equation:

$$Z = \sum_i N_i \frac{10^{pKa_i}}{10^{pH} + 10^{pKa_i}} - \sum_j N_j \frac{10^{pH}}{10^{pH} + 10^{pKa_j}}$$

where Z is the peptide net charge, pKai and Ni are the pKa values and number of the N-terminus and the side chains of Arginine, Lysine and Histidine, whereas pKaj and Nj are the pKa values and number of the C-terminus and the Aspartic Acid, Glutamic Acid, Cysteine, Tyrosine amino acids (see, for example: D. S. Moore, Amino Acid and Peptide Net Charges: A Simple Calculational Procedure, in Biochemical Education, Volume 13, Issue 1, Wiley, 1985, from which this formula can be easily derived).

Data Analysis

All the AFM images were analysed by means of the commercial Scanning Probe Image Processor (SPIP™) software. All force curves were analysed with offline software NanoScope Analysis (Bruker, Santa Barbara, CA).
SR-CD spectra were analysed by using Dichroweb; in particular, we employed the CDSSTR programme with reference set SP175 (Optimised for 175-240 nm) and no scaling factor (i.e. scaling factor = 1.0).
http://dichroweb.cryst.bbk.ac.uk/html/userguide.shtml#Analysis%20Programme

Synchrotron Radiation Circular Dichroism (SR-CD)

Synchrotron Radiation Circular Dichroism (SR-CD) spectra were collected on the AU-UV beam line on the ASTRID2 storage ring (ISA, Aarhus University, Denmark). Similar to the CD experiment previously described on the CD1 beam line on ASTRID[3, 4], light from the AU-UV beam line was polarized with a MgF_2 Rochon polarizer (B-Halle GmbH, Berlin) and a photo elastic modulator (Hinds, USA) produced alternating left and right handed circular polarized light. The light was then passed though the sample, with concentrations of 1 mg/mL, and was detected by a photo multiplier tube (Type 9406B, ETL, UK). Spectra of water were recorded for baseline subtraction. Samples were measured in a 0.1 mm path-length Suprasil cell (Hellma GmbH). Sample and baseline spectra (1 nm steps size and 2 second dwell time) were each collected in triplicates, averaged and slightly smoothed with a Savitzky-Golay filter using a purpose made Excel template.

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