Electronic Supplementary Material (ESI) for ChemComm.

Introducing Charge Transfer Functionality into Prebiotically Relevant β-Sheet Peptide Fibrils

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Supporting Information

1. Experimental section

Chemical and reagents were purchased from Aldrich or Merck and used without further purification. Amino acids, resins and coupling reagents were purchased from Novabiochem. DMF was purchased in biotech grade. Peptide **1** was synthesized on solid phase (Rink amide MBHA resin) using the standard Fmoc based synthesis. After cleavage and global deprotection the peptide was purified by HPLC and analyzed by HPLC and ESI-MS (Fig. s1). The control molecule dibutyl-NDI was synthesized as previously described,[1] and the product analyzed by ^{1}H NMR.

Analytical HPLC was performed on a Dionex 1100 using a reverse phase C18 column at a flow rate of 1.5 mL/min. Preparative HPLC was performed on a Thermo Spectra Physics instrument using a C18 reverse phase preparative column at a flow rate of 20 mL/min. Mass spectrometry analysis was performed by LCMS (LC-MS Thermo Surveyor 355). The folding and secondary structure analysis of the proteins performed using CD spectroscopy on a Jasco-815 CD spectrometer. UV-vis measurements were performed on a Jasco V-570 UV-VIS-NIR spectrometer at room temperature using a cuvette of 1 cm pass length. Fluorescence spectroscopy measurements were performed in a Fluorolog FL-3 fluorometer at room temperature using a cuvette of 1 cm pass length.

1.1 Synthesis and analysis of peptide 1^{NDI}

Synthesis of N-butylimide naphthalene 1,9-dicarboxylic acid. 1,4,5,8-Naphthalene-tetracarboxylic acid dianhydride (2.0 g, 7.46 mmol) was weighed into a 500-mL flask. Water (350 mL) was added, followed by addition of KOH (1M). In some cases, heat, sonication, and/or additional KOH were used to dissolve the starting material completely. After the solid had dissolved, the solution was acidified to pH 6.4 with H_3PO_4 (1M). n-butylamine (0.741 mL, 7.46 mmol) was added, and the solution was again acidified to pH 6.4 with H_3PO_4 (1M). The reaction vessel was fitted with a reflux condenser, and the mixture was heated to 110 ^oC, stirred overnight, allowed to cool to room temperature, and filtered. Acetic acid (5 mL) was added to the filtrate, and a white solid precipitated from the solution. The solid was collected by filtration, washed with water, and dried under high vacuum to yield the desired product (1.620 g, 64%) as an off-white solid. ^1H NMR (400 MHz, [D6]DMSO): d = 8.51 (d, J = 7 Hz, 2 H), 8.09 (d, J = 7 Hz, 2H), 4.03 (d, J = 7 Hz, 2H), 1.62 (m, 2H), 1.34 (m, 2H), 0.92 (d, J = 7 Hz, 3H).

Peptides synthesis. $\mathbf{1^{NDI}}$ was synthesized by first preparing manually on solid phase the respective sequence with orthogonally protected Dpr side chains (Dpr-Mtt). Four-fold excess of Fmoc-amino acids relative to the Rink amide MBHA resin (loading 0.74 mmol/g) was used. Coupling was performed using 1:1:20 amino acid/HBTU/DIPEA in DMF for 45 min to 2 hours. Deprotection was performed using 25% piperidine/DMF twice, for 8 min each time. DMF top washes were performed between deprotection and coupling steps. The resin bearing peptide was treated with DCM/TFA/triisopropylsilane (94/1/5) for 5 min to remove the Mtt protecting group from the Dpr β-amine, followed by washing sequentially with DCM, DMF, DMF/DIPEA (95/5), and again with DMF. Then, the resin was swelled using 1M LiCl in DMF, followed by addition of 2 mL DMF solution of N-butylimide naphthalene 1,9-dicarboxylic acid (3 eq.) and DIPEA (4 eq.) to the resin. The suspension was shaken for 30 min followed by the addition of HBTU/HOBt (0.4 M DMF solution, 3 eq). The reaction mixture was further shaken for 12 h at room temperature, then filtered through a fritted syringe, and the resin was washed thoroughly (DMF, EtOH, DCM, ether). A mixture of TFA containing the appropriate scavengers was added to the resin and stirred for 2 hours, to cleave the peptide off the resin and for side chain global deprotection. The resin was removed by filtration under reduced pressure and the TFA was evaporated out of the peptide mixture. 8-10 times volume of cold ether was added to precipitate the crude peptide. $\mathbf{1^{NDI}}$ was then purified by preparative HPLC using a C18 reverse phase column (Phenomenex Gemini-NX),

with a step gradient of solvent A (0.1 M NH_4HCO_3 in water; pH = 8) and B (acetonitrile). The peptide was analyzed by two methods: (i) Reverse phase analytical HPLC (Dionex 1100), using a C18 column and a linear water/acetonitrile gradient containing 0.1% trifluoroacetic acid, and (ii) Mass spectrometry analysis was performed by ESI-MS (LC-MS Thermo Surveyor 355), in order to verify the molecular weight.

1.2 Characterization of $\mathbf{1^{NDI}}$ fibril self-assembly

Stock solutions of peptide $\mathbf{1^{NDI}}$ were prepared by dissolving it in acetonitrile/deionized water (1/1 and up to 1/3). Prior to the self-assembly experiments, vigorous sonication (10 min) was employed in order to achieve homogeneous monomeric solutions of the peptide. Structural characterizations (CD, UV/vis, Fluorescence, AFM, cryo-TEM), seeding and reduction experiments, were each initiated at specific time after sonication (= self-assembly time) as indicated.

UV-vis spectroscopy measurements. Solutions of $\mathbf{1^{NDI}}$ (25 μM) were prepared by dissolving lyophilized sample in acetonitrile-water mixtures of different ratios and equilibration for 30 minutes, after which the absorbance spectrum of the NDI chromophore was recorded. All UV-vis measurements were performed on a Jasco V-570 UV-VIS-NIR spectrometer at room temperature using a cuvette of 1 cm pass length.

Fluorescence spectroscopy measurements. Solutions of $\mathbf{1^{NDI}}$ (20 - 120 μM), or $\mathbf{1@1^{NDI}}$ in different ratio (Fig. s4), were prepared by dissolving lyophilized samples in acetonitrile/deionized water (1/1 up to 1/3) and equilibration for 30 minutes. The fluorescence emission spectra of NDI residue were recorded after excitation at 350 or 360 nm, as specified, in a Fluorolog FL-3 fluorometer at room temperature using a cuvette of 1 cm pass length. Note that for the concentrated samples, $[\mathbf{1^{NDI}}] \geq 70$ μM, we observed a decrease in fluorescence along the entire spectrum, due probably to self-quenching.

Circular dichroism (CD) analysis. $\mathbf{1^{NDI}}$ solutions (100 - 300 μM) were prepared by weighing lyophilized peptide into Eppendorf tube and dissolving in acetonitrile/deionized water (1/1 up to 1/3). The measurements were carried out on Jasco-815 CD spectropolarimeter, at 25 ^{o}C, by using a quartz cell with 1.0 mm path length. The CD signal resulting from solvents alone was subtracted from the spectrum of each peptide solution.

Cryo-TEM imaging. Samples for direct imaging of the aqueous dispersions using cryo-transmission electron microscopy (cryo-TEM) were prepared as follows. In the controlled environment box of a vitrification robot (Vitrobot), a drop of the peptide $\mathbf{1}^{\mathbf{NDI}}$ (300 μM) solution in acetonitrile:water was deposited on a glow-discharged TEM grid (300-mesh Cu Lacey substrate; Ted Pella, Ltd.). The excess liquid was automatically blotted with a filter paper, and the specimen was rapidly plunged into liquid ethane and transferred to liquid nitrogen where it was kept until used. The samples were examined below -175 ºC using an FEI Tecnai 12 G^2 TWIN TEM operated at 120 kV in low-dose mode and with a few micrometers under focus to increase phase contrast. The images were recorded with a Gatan charge-coupled device camera (model 794) and analyzed by Digital Micrograph software, Version 3.1**.** The average mono-fibril width (e.g., Fig. 2a in the manuscript) was found by statistical analysis of more than 30 samples (e.g., Figs. 2a and s2).

Atomic force microscopy (AFM) of 1@$^{\mathbf{NDI}}$1. Peptide **1** was dissolved in acetonitrile/ water (1:9), followed by sonication for 10 minutes and equilibration for additional 20 minutes. $\mathbf{1}^{\mathbf{NDI}}$ was dissolved in solution of acetonitrile/water (1:3), followed by sonication for 10 minutes. The two solutions were then combined to mixtures of $\mathbf{1{:}1}^{\mathbf{NDI}}$ in different proportions (1:5, 1:10 and 1:20), and stirred. AFM samples after equilibration were prepared as follows. A 5 μL aliquot from the mixture solution was deposited on freshly cleaved mica surface. The mica surface with adsorbed peptide was then dried in air and imaged. Topography images were acquired on AFM machine (Solver-Pro, NT-MDT, Ru) in tapping mode using non-contact tips (BudgetSensors Multi75Al-G, 3 N/m, 75 kHz). Image processing, which included second order polynomial line fitting, was done using the NOVA AFM software.

1.3 Analyzing 1^{NDI} fibrils in reduction-oxidation experiments

Fluorescence measurements. All sample manipulations were performed in an anaerobic glove box. Peptide (50 μM) was dissolved in NH_4HCO_3 buffer 0.1 M (pH 8) followed by the addition of $Na_2S_2O_4$ in dionized water (4 mol equivalents relative to the NDI). The reduced peptide solution was mixed thoroughly, transferred to a cuvette, sealed with a teflon stopper, and measured immediately. The spectra of the compound before reduction was background corrected against NH_4HCO_3 buffer 0.1 M (pH 8), and spectra of the reduced compound were background corrected against NH_4HCO_3 buffer 0.1 M (pH 8) with $Na_2S_2O_4$ solution. Fluorescence analysis of the reoxidized sample was performed after exposure to air for up to

4 hrs. The spectrum of the original oxidized sample was obtained only after the reoxidized sample was vigorously sonicated.

UV/vis and NIR measurements. All sample manipulations were performed in an anaerobic glove box. For the UV/vis measurements, 50 μM **1**^{NDI} was dissolved in NH_4HCO_3 buffer 0.1 M (pH 8) followed by addition of an excess of $Na_2S_2O_4$ in H_2O. For NIR measurement, 300 μM **1**^{NDI} was dissolved in acetonitrile/D_2O mixture, followed by the addition of an excess of $Na_2S_2O_4$ in D_2O. D_2O was employed instead of H_2O to minimize interference from water bands in the NIR region. The reduced peptide solution was mixed thoroughly, transferred to a cuvette, sealed with a teflon stopper, and measured immediately. All spectra of the reduced compound were background corrected against a solvent with $Na_2S_2O_4$ solution. Reoxidation of the sample was done by exposure to air. The measurements were performed on a Jasco V-570 UV-VIS-NIR spectrometer at room temperature.

CD measurements. All sample manipulations were performed in an anaerobic glove box. Peptide (300 μM) was dissolved in acetonitrile:water (1:1 up to 1:3), followed by the addition of $Na_2S_2O_4$ in deionized water (4 mol equivalents relative to the NDI). The reduced peptide solution was mixed thoroughly, transferred to a cuvette, sealed with a teflon stopper, and measured immediately. The measurements were carried out on Jasco-815 CD spectropolarimeter, at 25 ^{o}C, by using a quartz cell with 1.0 mm path length. The CD signal resulting from solvents alone was subtracted from the spectrum of each peptide solution. Re-oxidation of the sample was done by exposure to air. We note that the β-sheet signal at 218 nm was only partially recovered after re-oxidation, due probably to some phase transition during this long experiment.

Cryo-TEM imaging. Reduced peptide solutions preparation was performed in an anaerobic glove box. **1**^{NDI} peptide (25-50 μM) was dissolved in 0.1 M NH_4HCO_3 buffer pH 8, followed by addition of $Na_2S_2O_4$ in deionized water (4 mol equivalents relative to the NDI). The reduced peptide solution was mixed thoroughly, transferred to a flask, sealed with a teflon stopper. Samples for direct imaging of the aqueous dispersions using cryo-transmission electron microscopy (cryo-TEM) were prepared as follows. In the controlled environment box of a vitrification robot (Vitrobot), a drop of the reduced peptide **1**^{NDI} solution was deposited on a glow-discharged TEM grid (300-mesh Cu Lacey substrate; Ted Pella, Ltd.). The excess liquid was automatically blotted with a filter paper, and the specimen was rapidly plunged into liquid ethane and transferred to liquid nitrogen where it was kept until used. The

samples were examined below -175 ºC using an FEI Tecnai 12 G^2 TWIN TEM operated at 120 kV in low-dose mode and with a few micrometers under focus to increase phase contrast. The images were recorded with a Gatan charge-coupled device camera (model 794) and analyzed by Digital Micrograph software, Version 3.1.

2. Additional Tables and Figures

Table s1: UV/Vis characterization of $\mathbf{1}^{NDI}$ in different acetonitrile-water mixtures[a,b]

ACN/H_2O ratio	λ_{max} 1	λ_{max} 2
4:1	361	381
1:1	362	383
1:4	364	385
water only	365	385

[a] λ_{max} of the two main absorption maxima, [b] $\mathbf{1}^{NDI}$ concentration = 25 μM.

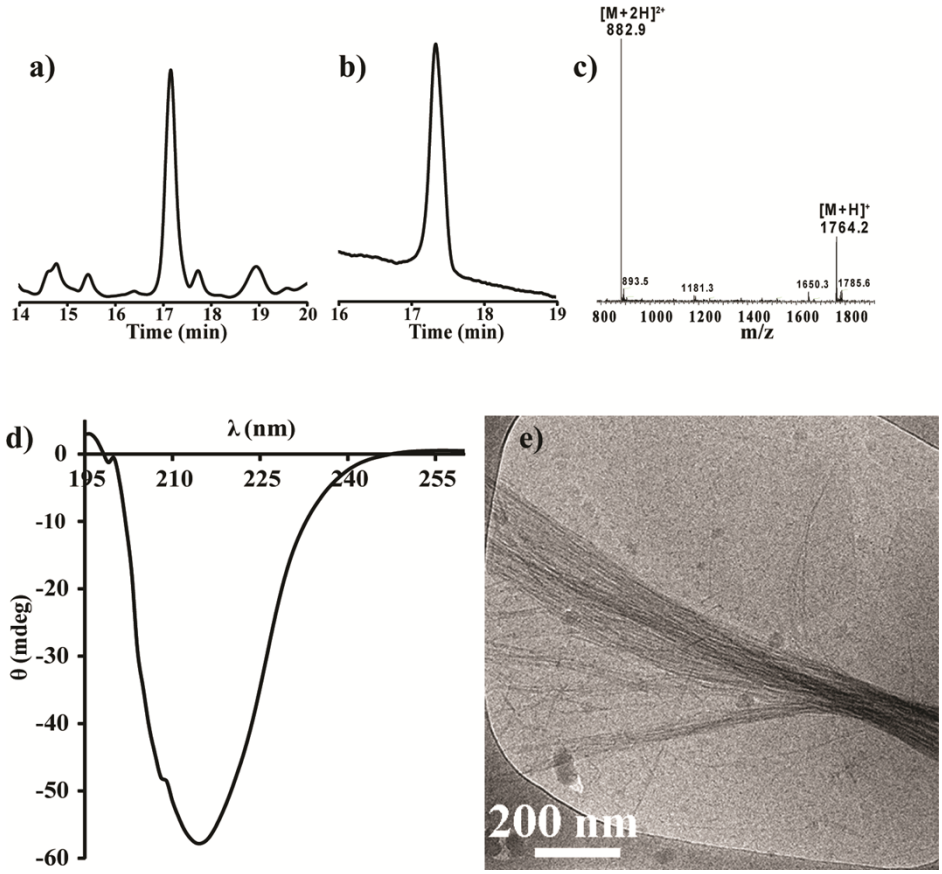

Figure s1. Peptide **1** Characterization. a) HPLC of the crude sample after synthesis, cleavage and deprotection; b, c) HPLC and ESI-MS characterization of the pure peptide; d, e) characterization of **1** self-assembly in solution by CD ([**1**] = 200 μM) and cryo-TEM ([**1**] = 1.0 mM). Additional characterization of fibrils formed by **1**, as well as by very close derivative molecules, was published by the authors recently.[2]

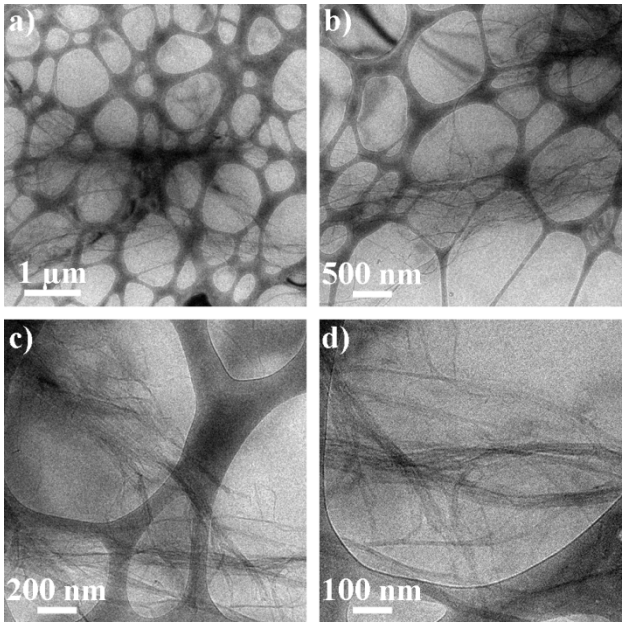

Figure s2. Additional Cryo-TEM images at different magnifications, obtained for vitrified $\mathbf{1}^{NDI}$ solutions (300 µM) in acetonitrile:water (1:1 up to 1:3, v:v).

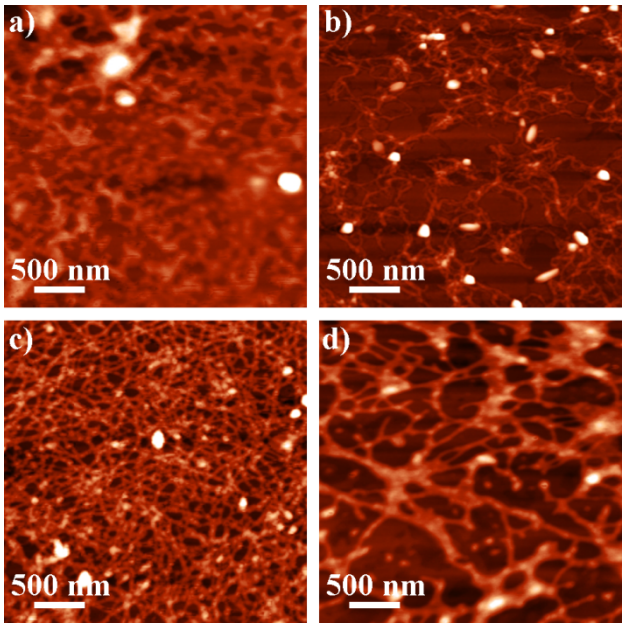

Figure s3. AFM images of fibril structures, products of the co-assembly from mixtures containing **1** and $\mathbf{1}^{NDI}$ in 1:3 v:v acetonitrile:H_2O. a, b) $\mathbf{1@1}^{NDI}$ 1:5. [$\mathbf{1:1}^{NDI}$] = 30:150 µM (a) and 5:25 µM (b). c) $\mathbf{1@1}^{NDI}$ 1:10 [$\mathbf{1:1}^{NDI}$] = 15:150 µM, and d) $\mathbf{1@1}^{NDI}$ 1:20 [$\mathbf{1:1}^{NDI}$] = 7.5:150 µM. The equilibration time before taking the images shown in panels a, c and d was 1 hour, while the image in panel b was taken after 3 days of equilibration. A typical fibril height found to be around 5 nm; z axis = 20 -25 nm.

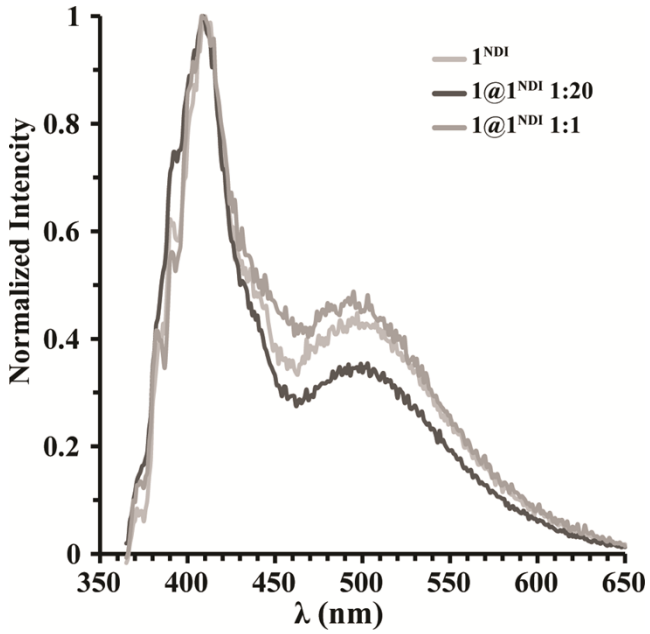

Figure s4. Fluorescence emission spectra of **1@1^{NDI}** mixtures in different ratio (in all cases [**1^{NDI}**] = 60 μM in 1:1 ACN:water). Excitation wavelength = 350 nm.

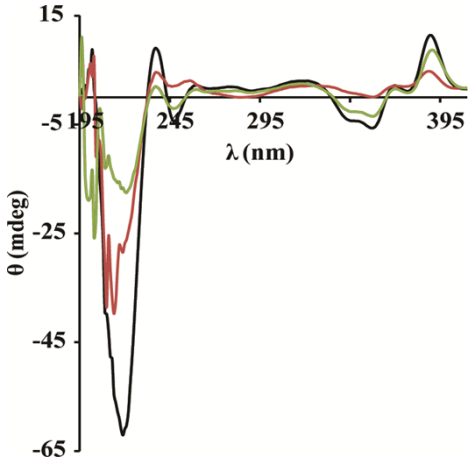

Figure s5. Active control of **1^{NDI}** fibril architecture and π-stacking interactions by reduction (with sodium dithionite) and air oxidation. CD spectra obtained for (300 μM) original (*black*), reduced (*red*), and reoxidized (*green*) samples in acetonitrile:H_2O (1:1, v:v).

References

1 W. S. Horne, N. Ashkenasy and M. R. Ghadiri, *Chem. Eur. J.* 2005, **11**, 1137.

2 a) V. Bourbo, M. Matmor, E. Shtelman, B. Rubinov, N. Ashkenasy and G. Ashkenasy, *Orig. Life Evol. Biosph.* 2011, **41**, 563; b) Y. Raz, B. Rubinov, M. Matmor, H. Rapaport, G. Ashkenasy and Y. Miller, *Chem. Commun.* 2013, **49**, 6561.