Electronic Supplementary Material (ESI) for Nanoscale.


# Electronic Supporting Information

# Sequence Dependent Proton Conduction in Self-Assembled Peptide Nanostructures

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**Synthesis of c(<u>K</u>K$_{Naph}$)$_4$**

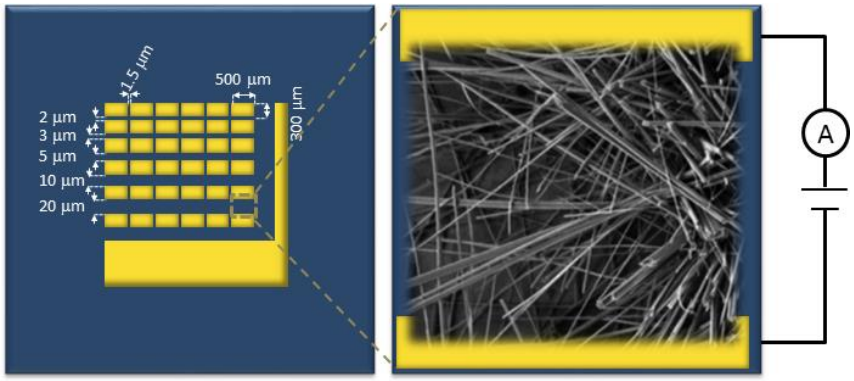
c(<u>K</u>K$_{Naph}$)$_4$ was synthesized by coupling tert-butyl acetate derivatized 1,5-dialkoxy naphthalene (DAN) to the alternating L positions of c(K<u>K(Z)</u>)$_4$ (Z denotes carboxybenzyl), followed by deprotection of the side chains. c(K<u>K(Z)</u>)$_4$ was synthesized by the methodology described in the Methods section of the manuscript. The crude material was purified by preparative RP-HPLC yielding a purity of 95%, and a mass (m/z) of 1561.9134 [M+H]+ (calculated 1561.9141), measured by LC-MS.

To prepare the Naph side chain 1 eq. 1,5-dihydroxynaphthalene (4.0 gr, 25 mmol)+ 1.2 eq. tBut-bromoacetate (5.8 gr, 30 mmol)+1.5 eq. potassium carbonate (5.2 gr, 37.5 mmol) were stirred under argon in 50 ml dry DMF overnight. The resulting solution was concentrated to dryness and the solid was dissolved in ethyl acetate. Extraction was performed with 1N HCl and then with brine (sat. aq. NaCl). The extract from the column separation was dried with $MgSO_4$ and filtered. The filtrate was concentrated and purified by flash chromatography (silica gel, hexane/ethyl acetate, 92:8%) to yield tert butyl ethoxy monosubstiuted DAN (2.0 gr, 45% purity). The obtained crud product was verified by TLC in 10% ethyl acetate in n-hexane. $^1$H NMR indicated mono substitution of the hydroxy- naphthalene. A mass of: 274.9(m/z) was measured by MALDI-TOF MS (calc. 274).

Coupling of the DAN derivative was obtained by Mitsunobu reaction.[1] In short, Triphenylphosphine ($PPh_3$) (7 mg, 0.026 mmol) was added to a solution of the monosubstituted DAN (4.27 mg, 0.0156 mmol) in THF (5 mL) and cooled to -15 ºC, After which diethyl azodicarboxylate (DEAD) was added (1µl, 0.026 mmol). After 15

minutes the cyclic peptide (2 mg, 0.0013 mmol) was added as well to yield a 20 molar access of $PPh_3$ with respect to the peptide. The reaction mixture was stirred overnight under nitrogen at room temperature. The reaction mixture was then concentrated and the crude solid was dissolved in TFA/trimethylsilyl trifluoromethanesulfonate/cresol (10:2:2, 200 mL). After 1 h, diethyl ether (2 mL) was added, and the resulting suspension was centrifuged. The supernatant was discarded, and the solid was washed twice more with diethyl ether (2 mL). The crude peptide was purified by preparative RP-HPLC to yield a solid product (0.5 mg, 25%). MS (m/z): found: 935 [M+2H]2+(calc. 935 [M+2H]2+).

Removal of the Obzl protection group of the D-Lysine amino acids was performed by hydrogenation according to a procedure described in the literature.[2] Wet Pd/C (10%/w, 0.1 g) was added to a solution of the side chain protected cyclic peptide (15.0 g, 57.4 mmol) in MeOH (15 mL). The solution was stirred under $H_2$ at room temperature overnight. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated. The crude peptide was purified by preparative RP-HPLC to yield the product solid (0.4 mg, 20%). MS (m/z: 972 [M+2H]2+ (calc. 972 [M+2H]2+).




**Scheme S1. Electrical measurements setup.** The design of the chip with the different gap sizes is shown to the left. A magnification of a single junction is shown on the right, together with the electrical circuit used for the measurements. An overlay of SEM image of c(K<u>W</u>)$_4$ assemblies is presented for illustration.

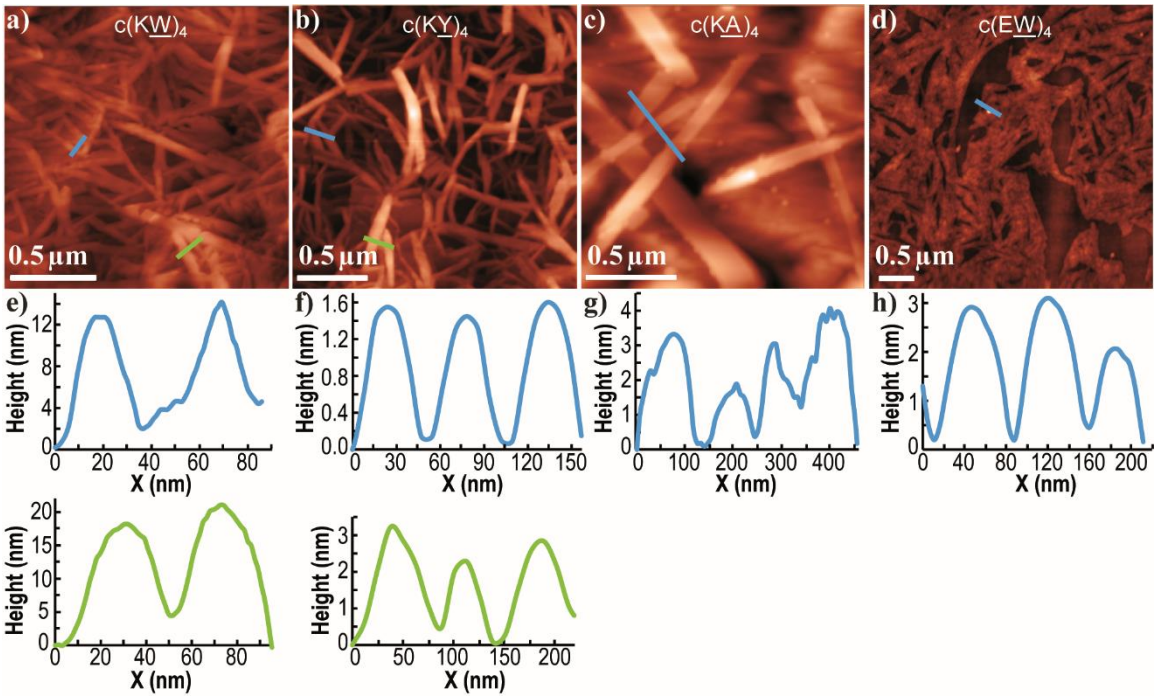



**Figure S1**: **AFM topography and cross-section images of self-assembled peptide structures.** a,b,c and d) Topography images of self-assembled peptides c(K<u>W</u>)$_4$, c(K<u>Y</u>)$_4$, c(K<u>A</u>)$_4$ and c(E<u>W</u>)$_4$ at 1mM concentration in aqueous solution (Z scales 13, 40, 50 and 10 nm, respectively). e,f,g and h) Height of the marked lines in a,b,c and d, respectively. AFM (Solver-Pro, NT-MDT, Ru) topography images were acquired in tapping mode using non-contact tips (BudgetSensorsMulti75Al-G (3 N/m, 75 kHz)). Cross section measurements and image processing, which included second order polynomial line fitting, were carried out by the NOVA AFM software. Fibers' length and height were extracted manually from the AFM data. Average values of at least 10 fibbers from at least three different samples are provided for each assembly, with the error calculated as the standard deviation.

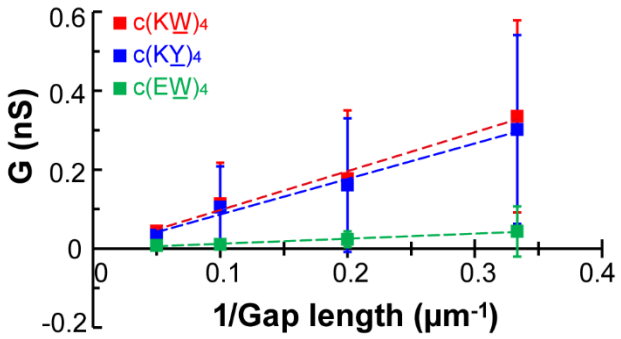



**Figure S2**: Conductance as function of electrode gap for the different peptide assemblies under vacuum.

**Extraction of electron transference number**

I-t measurement of the different peptide assemblies were obtained under vacuum conditions (P=$10^{-4}$ mbar), applying a voltage cycle of 0V – 2V – 0V with 1 h intervals for each applied voltage. Data points were colected every 5 seconds. Slow current decay during voltage onset indicated protonic contribution to the current for all the peptide assemblies (See for example Figure S3a).[3] Indeed, much lower current levels were observed in *I-V* curves immediately after the transient experiments (Figure S3b) due to exhaustion of protonic charge carriers. The original current level could be recovered by re-exposing the sample to air for a day (Figure S3b). The non-zero plateau at voltage onset was assigned to electronic contribution to the current. Hence, the data during voltage onset was fitted using eq. 1:[3]

$$I = I_{RC} e^{-t/\tau_{RC}} + I_{H^+} e^{-t/\tau_{H^+}} + I_e, \qquad (1$$

where $t$ is the time since voltage onset, $I_{RC}$ and $I_{H^+}$ are the zero time dielectric and protonic contribution to the current, respectively, with the corresponding decay times, $\tau_{RC}$ and $\tau_{H^+}$. $I_e$ is the electron contribution to the current.

Electron transference numbers $t_e\%$ were calculated as:

$$t_e\% = \frac{I_e}{I_e + I_{H^+}} \times 100 \qquad (2$$

The resulting values are tabulated in Table S1.

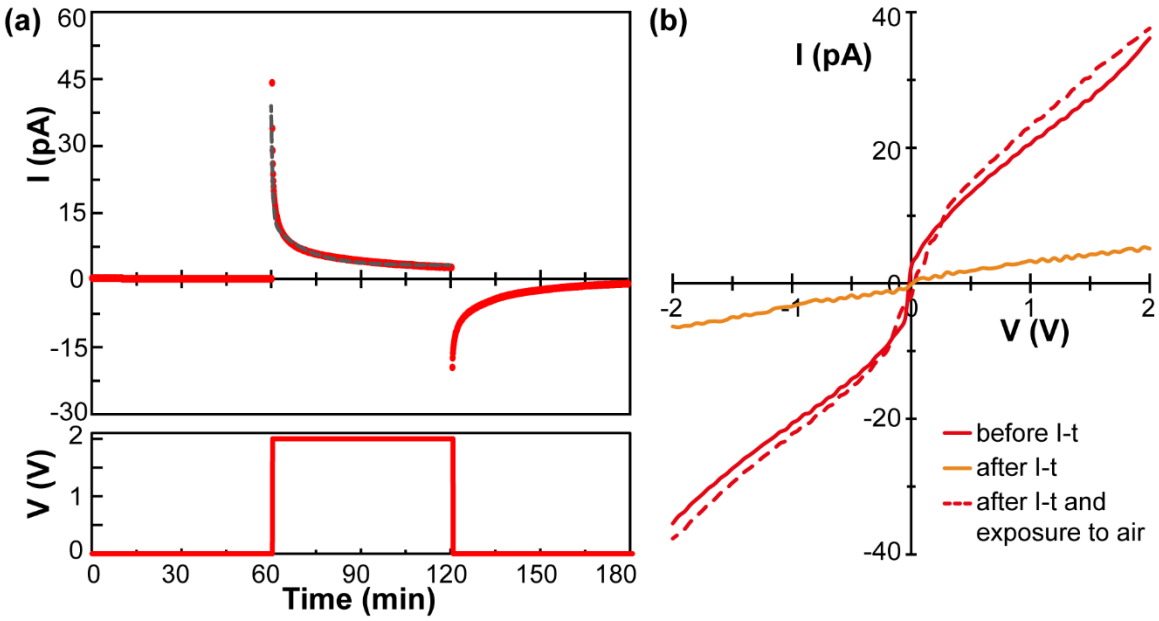



**Figure S3.** a) Transient current (I-t) measurement of c(K<u>W</u>)$_4$ under vacuum. Second order exponential decay fitting line in depicted in gray dashed line. The potential applied to the electrodes is provided below the graph. b) I-V before and after the I-t

measurement, and after exposure to air. All measurements were conducted under vacuum.

**Table S1:** Electron transference numbers of peptide assemblies.

| Peptide | c(K<u>W</u>)$_4$ | c(K<u>Y</u>)$_4$ | c(E<u>W</u>)$_4$ | c(K<u>A</u>)$_4$ | c(K<u>K</u>$_{Naph}$)$_4$ |
|---|---|---|---|---|---|
| $t_e$ % | 22 | 18 | 18 | 14 | 22 |

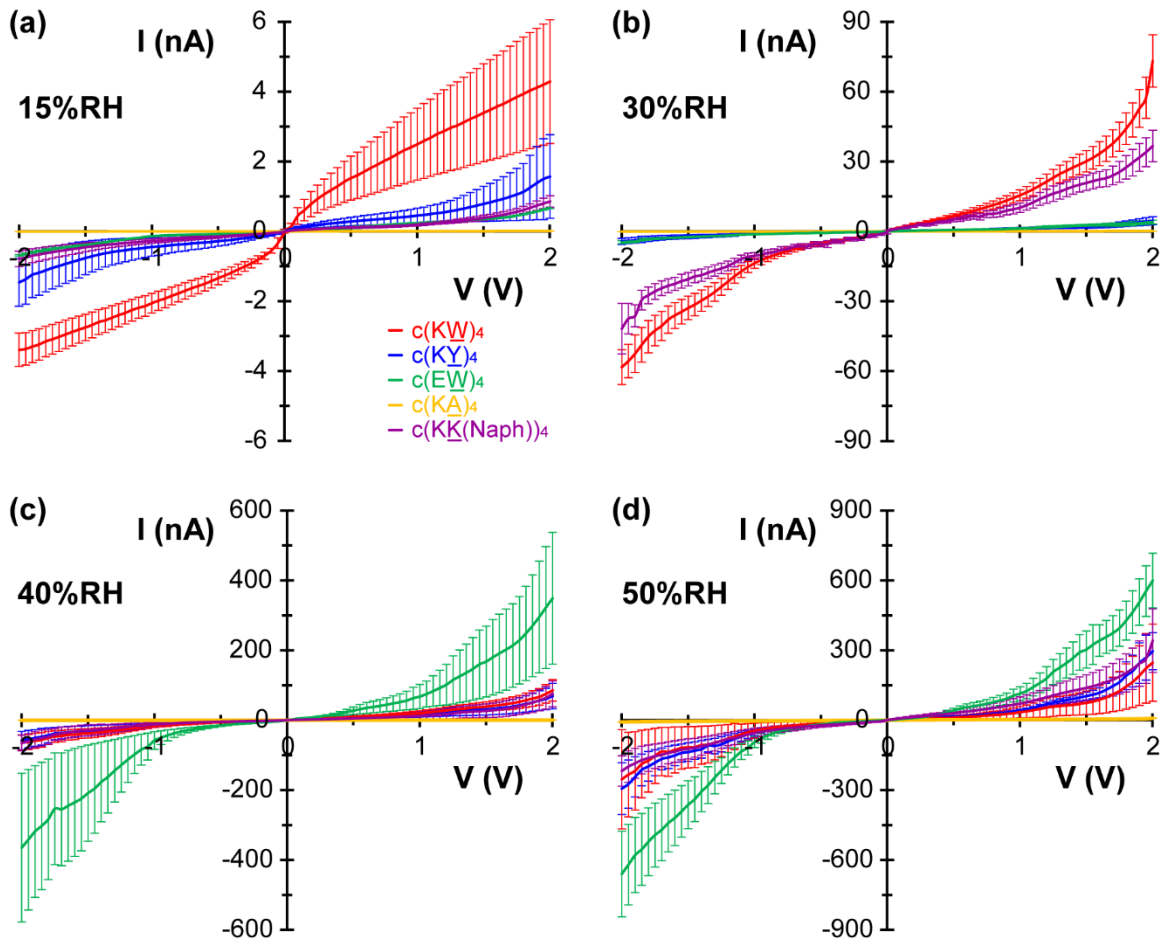



**Figure S4.** I-V of all peptides at different RH values. Legend is the same for all panels. Note the differences in y-axis scale for each panel.

**References**

1. Arya, P.; Wei, C.-Q.; Barnes, M. L.; Daroszewska, M., A Solid Phase Library Synthesis of Hydroxyindoline-Derived Tricyclic Derivatives by Mitsunobu Approach. *J. Comb. Chem.* **2004,** *6*, 65-72.

2. Xu, R.; Czarniecki, M.; Man, J. d.; Pan, J.; Qiang, L.; Root, Y.; Ying, S.; Su, J.; Sun, X.; Zhang, Y.; Yu, T.; Zhang, Y.; Hu, T.; Chen, S.-H., An Improved Synthesis of 2-Oxa-7-Azaspiro[3,5]Nonane and Analogs as Novel Reagents in Medicinal Chemistry. *Tetrahedron Lett.* **2011,** *52*, 3266-3270.

3. Amit, M.; Appel, S.; Cohen, R.; Cheng, G.; Hamley, I. W.; Ashkenasy, N., Hybrid Proton and Electron Transport in Peptide Fibrils. *Adv. Funct. Mater.* **2014,** *24*, 5873-5880.