Electrostatically tuned rate of peptide self-assembly resolved by multiple particle tracking
Electronic supplementary information

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Molecular model of peptide KFE8

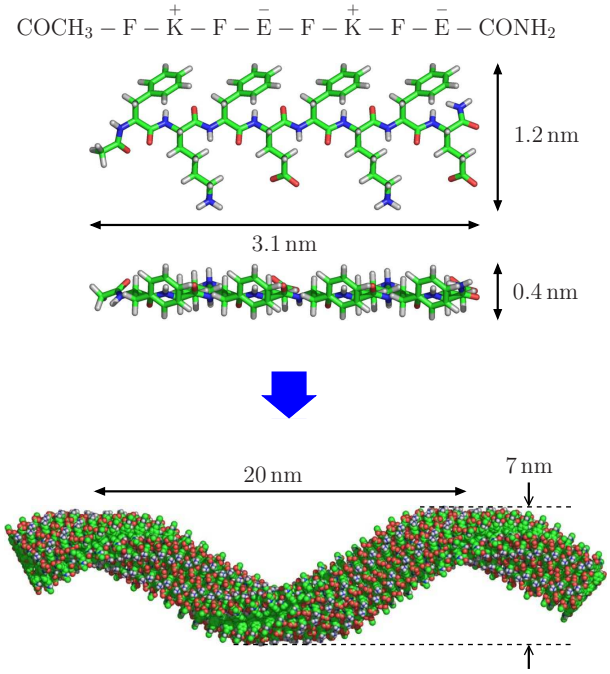

Fig. S1 Molecular model of KFE8. Lysine (K) and glutamic acid (E) are polar hydrophilic and phenylalanine (F) is hydrophobic. The N- and C-termini are respectively acetylated and amidated. KFE8 self-assembles into left-handed helical ribbons formed by a bilayer β-sheet, where hydrophobic side chains are buried between the two helices (courtesy of W. Hwang).

Peptide solution titration

Titration was performed on a sample of KFE8 solution prepared the same way as described in the main text. However, after reducing the concentration of powder from 3 mg ml^{-1} to 1 mg ml^{-1}, small volumes of NaOH were added to

perform the titration, such that the final concentration of powder is not modified by more than 2%. Moreover, we observed that the pH is independent of the time of measurement during the self-assembly. Also, we verified that the addition of beads does not affect the titration result, whether their surface chemistry is amine or carboxylate.

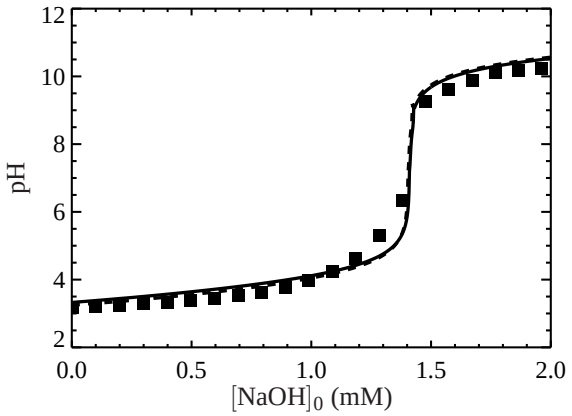

Fig. S2 Titration curve of the KFE8 solution at 1 mg ml^{-1} of powder. The squares are experimental results, whereas the lines are from the model described by eqn (S3). The solid line is the best match with the experimental jump in pH, assuming the powder contains only KFE8 and TFA complexes (that is the purity in peptide is exactly given by $1162.5/(1162.5+k\times 114)$, see text). We find $k = 1.95$ and $p = 84\%$. The dotted line is obtained for 70% purity in peptide, and the corresponding number of TFA per peptide is $k = 2.3$.

The solid squares in Fig. S2 give the result of the titration of the powder. We observe that the pH of the solution with no addition of NaOH is very low at 3.2, consistent with other reported values [1, 2]. This low pH is due to the presence of residual trifluoroacetic acid (TFA) from peptide synthesis. The pH variation of the solution as the concentration of NaOH resembles a classic titration curve with a sharp jump of pH at a concentration $[\mathrm{NaOH}] = 1.4\,\mathrm{mM}$.

We can use a simple titration model to evaluate the concentration of TFA in the original powder. The dissociation constants for the glutamic acid EH and for the Lysine KH^+ present in the peptide are written

$$K_\mathrm{E} = \frac{[\mathrm{E}^-][\mathrm{H}^+]}{[\mathrm{EH}]} = 10^{-\mathrm{pK_E}} \quad \text{and} \quad K_\mathrm{K} = \frac{[\mathrm{K}][\mathrm{H}^+]}{[\mathrm{KH}^+]} = 10^{-\mathrm{pK_K}} \tag{S1}$$

where $\mathrm{pK_E}$ and $\mathrm{pK_K}$ are the pK values of EH and KH^+ respectively. The standard value for these constant, $\mathrm{pK_E} = 4.3$ and $\mathrm{pK_K} = 10.8$ [3], are modified by the surrounding peptide chain and the electrostatic interaction, and we use the "apparent" pK values, $\mathrm{pK_E} = 3.6$ and $\mathrm{pK_K} = 11.2$ for the individual titratable groups [4, 5]. The same way, for the trifluoroacetic acid TH we write

$$K_\mathrm{T} = \frac{[\mathrm{T}^-][\mathrm{H}^+]}{[\mathrm{TH}]} = 10^{-\mathrm{pK_T}} \tag{S2}$$

with $\mathrm{pK_T} = 0.52$. If we call $[\mathrm{KFE8}]_0$ and $[\mathrm{T}]_0$ the initial concentration of peptides and TFA in the powder solution, we write the conservation of species by $[\mathrm{E}^-]+[\mathrm{EH}] = [\mathrm{K}]+[\mathrm{KH}^+] = 2[\mathrm{KFE8}]_0$ and $[\mathrm{T}^-]+[\mathrm{TH}] = [\mathrm{T}]_0$. The neutrality of the solution gives $[\mathrm{Na}^+]+[\mathrm{KH}^+]+[\mathrm{H}^+] = [\mathrm{E}^-]+[\mathrm{T}^-]+[\mathrm{OH}^-]$ so that finally, the pH variations are described by

$$[\mathrm{NaOH}]_0 + \frac{2[\mathrm{KFE8}]_0}{1+10^{\mathrm{pH}-\mathrm{pK_K}}} + 10^{-\mathrm{pH}} = \frac{2[\mathrm{KFE8}]_0}{1+10^{\mathrm{pK_E}-\mathrm{pH}}} + \frac{[\mathrm{T}]_0}{1+10^{\mathrm{pK_T}-\mathrm{pH}}} + 10^{\mathrm{pH}-14} \tag{S3}$$

which can be numerically solved for the pH at each value of $[\mathrm{NaOH}]_0$ reached in the titration, and knowing the initial concentrations $[\mathrm{KFE8}]_0$ and $[\mathrm{T}]_0$. The molecular weight of the peptide molecule is 1162.5 g mol^{-1} and the one of TFA

is 114 g mol^{-1}. Assuming both species, peptide and TFA, form a complex during the synthesis, this complex has a mass $1162.5 + k \times 114$ g mol^{-1} where k is the number of TFA molecule per molecule of peptide. When mixing 1 mg of powder in 1 ml of water, we obtain the concentrations $[\mathrm{KFE8}]_0 = p/1162.5\,\mathrm{M}$ and $[\mathrm{T}]_0 = pk/1162.5\,\mathrm{M}$, where p is a purity level of peptide in the powder which is less than $1162.5/(1162.5 + k \times 114)$. We plot in Fig. S2 the pH variation from this model by assuming that the non-purity of the powder comes only from the residual TFA. The best match for the pH jump is obtained when 1.95 molecules of TFA are attached to each peptide molecule (this leads to a purity of 84%, meaning that 0.84 mg of actual peptide is found in 1 mg of powder). However, the peptides were ordered crude and purity in that case are usually advertised between 60% and 80% by the fabricant. In addition to TFA traces, the crude powder is likely to contain solvents, other counter ions and salts from the synthesis. In Fig. S2, we also reported the titration curve from our model that best matches the experimental data with a purity of 70%. This leads to a value of $k = 2.3$. In both case we see an excellent agreement between the model and the experimental data. This suggests that the eventual impurities are inert for the titration, as assumed in our model. The value of $k \approx 2$ is also in agreement with the intuition that there is initially one TFA anion on each of the two lysines in the peptide [2]. The corresponding concentration of TFA is then $[\mathrm{T}]_0 \approx [\mathrm{T}^-] \approx 1$ mM, in agreement with what was used by Hwang et al. in [4] to develop their model. In the experiments presented in the article, we used concentration $[\mathrm{NaOH}]_0$ between 0.65 and 1 mM to obtain pH between 3.5 and 4.

DLVO interaction in the crossed cylinders geometry

In this section we calculate the interaction driving the self-assembly of the peptide KFE8 assuming that the elementary block interacting are infinitely long cylinder with radius R. This geometry presumably approximates the early helical ribbons depicted in Fig. S1. For this geometry, eqn (6) in the main article describing the chemical equilibrium at the surface of the cylinder is unchanged. However, because of the helical shape (a pitch, constituted of 100 molecules of KFE8, has a radius 3.5 nm and a length 20 nm), we find that $\sigma^{\max}$ is reduced to $0.26 \times 100 \times 0.4 \times 3.1/(\pi \times 20 \times 3.5) = 0.15\,\mathrm{C\,m^{-2}}$. Eqn (7) in the main text must be modified to account the new geometry of the surface. Ohshima [6] found

$$\tilde{\sigma} = 2\sinh(\tilde{\psi}_\mathrm{s}/2)\left[1 + \frac{K_1^2(\kappa R)/K_0^2(\kappa R) - 1}{\cosh^2(\tilde{\psi}_\mathrm{s}/4)}\right]^{1/2}, \tag{S4}$$

where K_n is the modified Bessel function of the second kind of order n, and the other notations are the same as the ones used in the article. Note that eqn (S4) (as well as eqn (7) of the article) is *not* obtained from a linearized Poisson-Boltzmann (obtained for $\tilde{\psi} \ll 1$) description of the double-layer. Although eqn (S4) is strictly valid for $\kappa R \gg 1$, it has been shown that it returns precise results even when $\kappa R \sim 1$ [6]. Notably, we see that these remarks are important for the model used here, as we find that both $\tilde{\psi} > 1$ and $\kappa R \sim 1$ at the pH investigated. Eqn (S4) above and (6) in the article can then be used to calculate σ and ψ_s at all pH for the cylindrical geometry, and the results are reported in the inset of Fig. S3. We see that both σ and ψ_s follow the same trends and magnitudes than in the planar geometry presented in the article.

The expression of the DLVO potential is also modified in the cylindrical geometry. Between two crossed cylinders with perpendicular axis, the interaction is written [6]

$$\frac{U_\mathrm{DLVO}(d)}{k_\mathrm{B}T} = \frac{4\pi^2 c e^{-2\kappa R}}{\kappa^3 K_0^2(\kappa R)}\Psi_\mathrm{s}^2 e^{-\kappa d} - \frac{AR}{6d} \tag{S5}$$

with the following expression for the effective potential at the surface:

$$\Psi_\mathrm{s} = \frac{8\tanh(\tilde{\psi}_\mathrm{s}/4)}{1 + \left[1 - (1 - K_0^2(\kappa R)/K_1^2(\kappa R))\tanh^2(\tilde{\psi}_\mathrm{s}/4)\right]^{1/2}}. \tag{S6}$$

The potential $U_\mathrm{DLVO}(d)$ is shown on Fig. S3. We see that the magnitude of the potential barrier is significantly higher and occurs at a shorter range than the one corresponding to a planar description (shown in Fig. 5 of the main article) at identical values of pH. Consequently the drop in reaction time $\propto e^{E/(k_\mathrm{B}T)}$ (E being the height of the potential barrier)

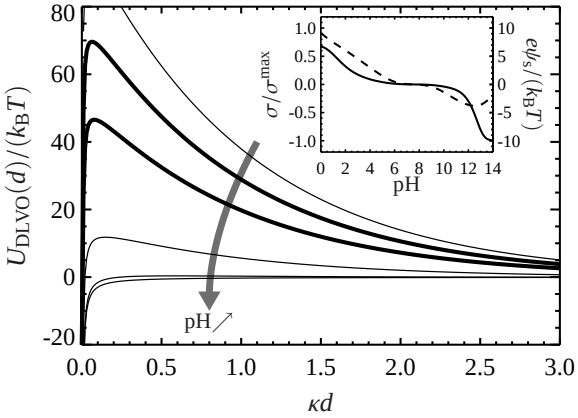

Fig. S3 DLVO interaction potential for pH=3.5 and pH=4 (thick lines) for a cylinder with radius $R = 3.5\,\mathrm{nm}$ and maximum surface charge density $\sigma^{\max} = 0.15\,\mathrm{C\ m^{-2}}$ (see text). The thin lines are for pH=3, 5, 6 and 7. The inset gives the surface charge density and electrostatic potential of the peptides for $[\mathrm{T}]_0 = 10^{-3}\mathrm{M}$ as a function of the pH of the bath solution. The solid line represents the surface charge density $\sigma/\sigma^{\max}$ and the dashed line is for the surface potential $\tilde{\psi}_{\mathrm{s}} = e\psi_{\mathrm{s}}/(k_{\mathrm{B}}T)$.

when increasing pH from 3.5 to 4 will be much bigger than the one observed experimentally and reported in the article. In this regard, the planar geometry exposed in the article provides more realistic magnitudes. A reason why the cylinder geometry fails to describe the elementary building block of self-assembly could come from neglecting the helical shape whose pitch (20 nm) is comparable to the Debye length (10 nm).

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