Electronic Supporting Information (ESI)

Cooperative, ion-sensitive co-assembly of tripeptide hydrogels

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1. Materials and Methods

a. **Reagents:** Peptides were custom synthesised from Bachem with > 98% purity. All metal ions (chloride salts) were purchased from Sigma-Aldrich.

b. **Sample preparation:** Peptides assembly and co-assembly was investigated using 30 mM of each tripeptide (unless mentioned otherwise). Peptides were dissolved in 7.4 PBS buffer at room temperature. In metal ions containing samples, a predetermined amount of the metal salt was dissolved simultaneously with other peptide components under the same conditions. Samples were vortexed and sonicated for full dissolution. Samples were left over night before performing further analysis.

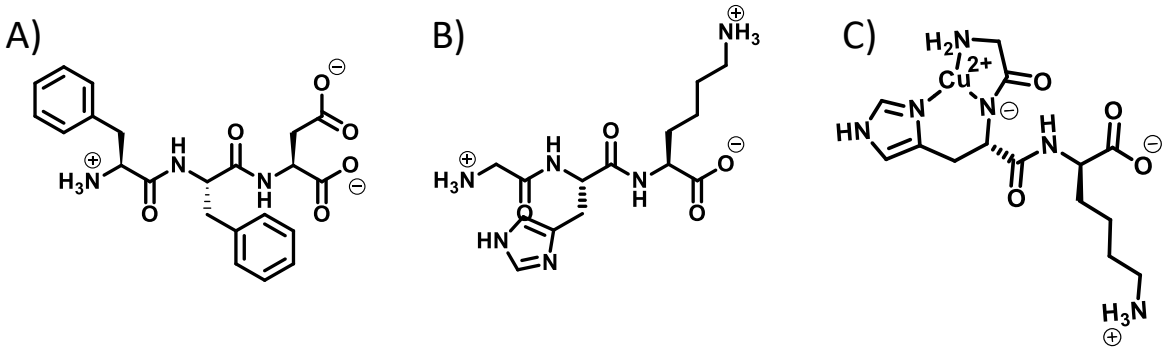
c. **Transmission Electron Microscopy (TEM):** TEM images were captured using a FEI Tecnai T20 transmission electron microscope operating at 200kV. Carbon-coated copper grids (200 mesh) were glow discharged in air for 30 s. The support film was touched onto the gel surface for 3 seconds and blotted down using filter paper. Negative stain (20 µl, 1 % aq. Methylamine vanadate obtained from Nanovan, Nanoprobes) was applied and the mixture blotted again with filter paper to remove any excess. Each sample was allowed to dry afterwards for 30 minutes in a dust-free environment prior to TEM imaging. The images were saved using Gatan Digital Micrograph software.

d. **FTIR:** Samples were prepared according to the procedure described above but using deuterated buffer. Samples were transferred to a standard IR transmission cell (Harrick Scientific) between two 2 mm CaF_2 windows, separated by a polytetrafluoroethylene (PFTE) spacer of 50 µm thickness. Spectra were recorded on a Bruker Vertex 70 spectrometer by averaging 25 scans at a spectral resolution of 1 cm^{-1}.

e. **Rheology:** Dynamic frequency sweep experiments were carried out on a strain-controlled rheometer (Kinexus rotational rheometer from Malvern) using a parallel-plate geometry (20 mm) with a 0.5 mm gap. An integrated temperature controller was used to maintain the temperature of the sample stage at 25°C. To ensure the measurements were made in the linear viscoelastic regime, an amplitude sweep was performed and the results showed no variation in storage modulus (G') and loss modulus (G") up to a strain of 0.06% (which was used for the frequency sweep). Samples were prepared according to the procedure previously described. The dynamic modulus of the gels was measured as a frequency function, where the frequency sweeps were carried out between 1 and 100 Hz. The measurements were repeated at least three times to ensure reproducibility.

f. Computational Simulation: 150 molecules of FFD, FDF or DFF was added to a 12.5 nm^3 box. To this box, a further 150 molecules of GHK was added before solvating with standard CG water giving a final peptide concentration of approximately 0.2 mol L^{-1}. The box was energy minimised for 5000 steps or until the forces on the atoms converged to below 200 pN. Each simulation was equilibrated followed by a production run for a total effective time of 9.6 µs.

2. Supporting Results



Scheme S1. Chemical Structures of (A) FFD, (B) GHK and (C) GHK-Cu^{+2} complex.

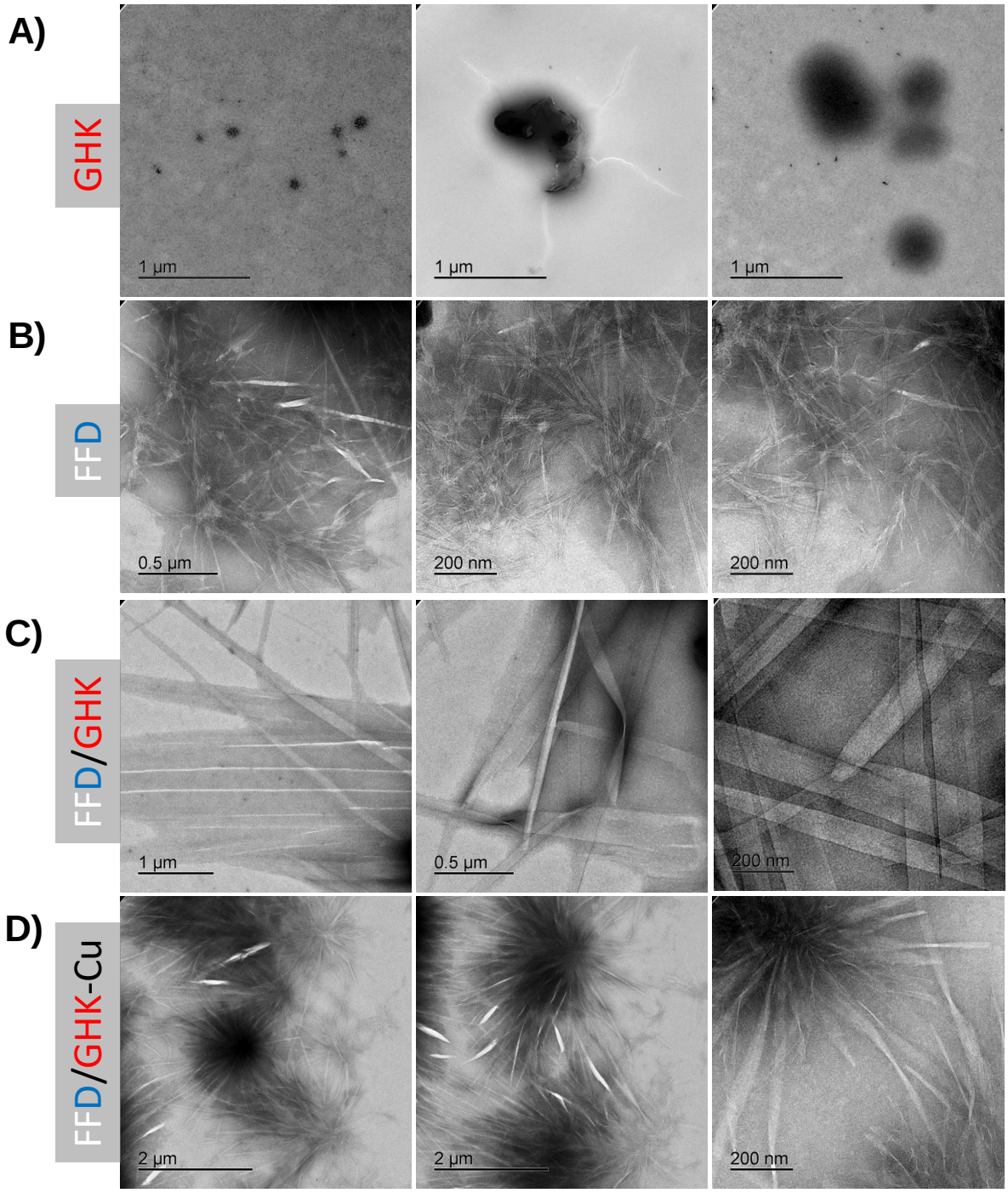


Figure S1. Addition TEM images of all tripeptides showing random aggregates of GHK (A), self-assembly of FFD nanofibers (B), nanotape formation due to the co-assembly of FFD/GHK (C) and nanofiber formation due to the co-assembly of FFD/GHK after complexation with copper ions (D).

Table S1. Gel formation ability of different tripeptide/metal ion combinations. The concentration of each component was 30 mM unless stated otherwise. All metal ions were added as Cl salts.

Sample	Metal ions	Appearance
GHK		Solution
FFD		Solution
FFD/GHK		Solution
FFD/GHK	Cu^{2+} (20 mM)	Solution
FFD/GHK	Cu^{2+} (30 mM)	Gel
FFD/GHK	Cu^{2+} (40 mM)	Gel
FFD/GHK	Cu^{2+} (50 mM)	Solution
FFD/GHK	Zn^{2+} (30 mM)	Turbid solution
FFD/GHK	Co^{2+} (30 mM)	Solution
DFF		Solution
DFF/GHK		Solution
DFF/GHK	Cu^{2+} (30 mM)	Solution
FFD	Cu^{2+} (30 mM)	Viscous solution

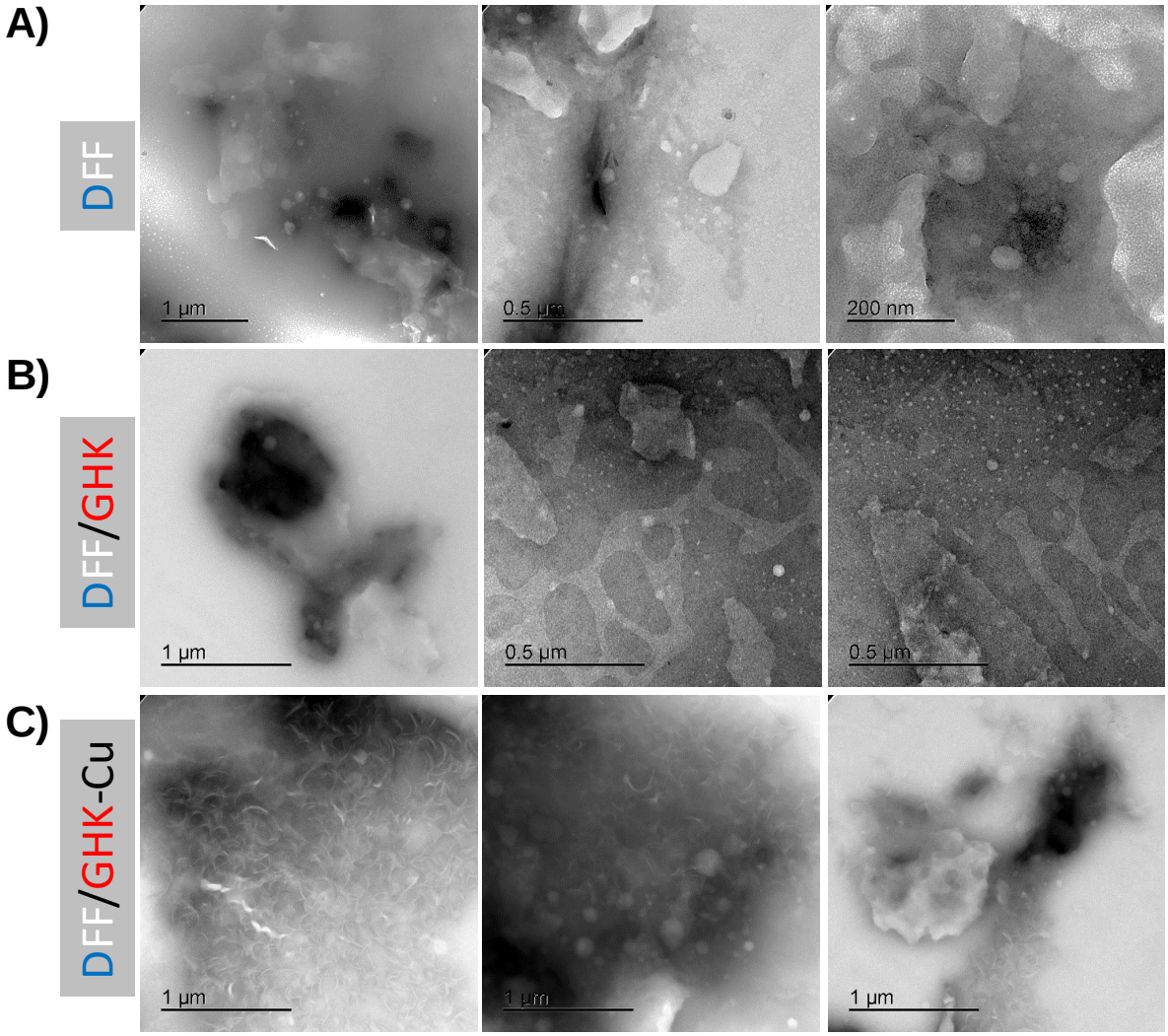


Figure S2. Additional TEM images of the control (DFF) aggregation (A), mixed with GHK (B) and co-assembled sample after complexation with copper ions (C).

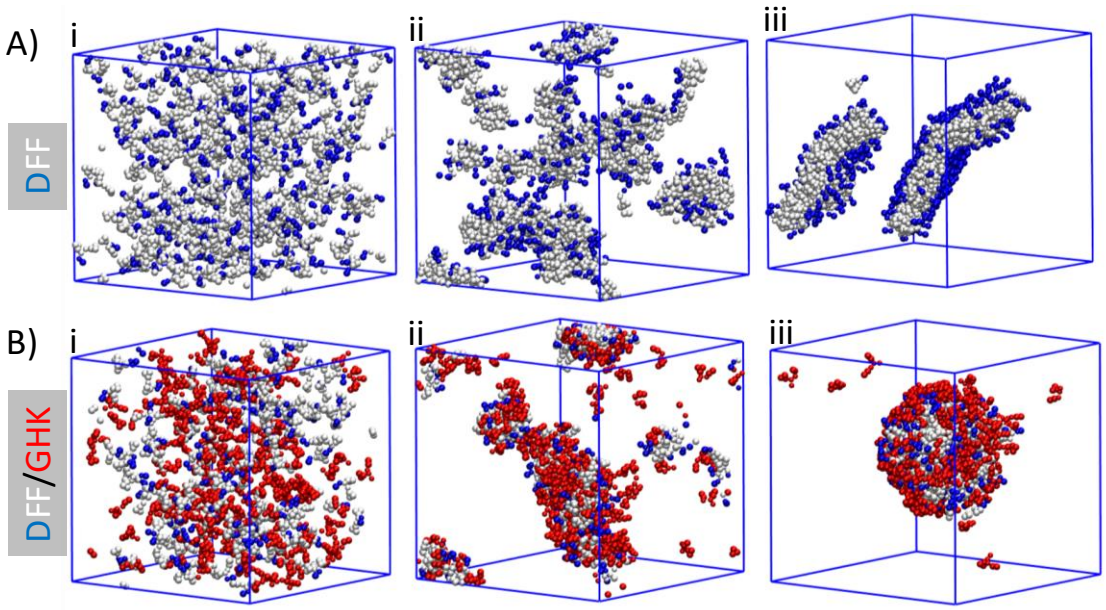


Figure S3. Computational time course for the self-assembly of the control tripeptide (DFF) in isolation (A) and co-assembled nanostructures (B). (i) Initial frame (0 μs), (ii) Mid-point (~3 μs), iii) Final frame (~9.6 μs).

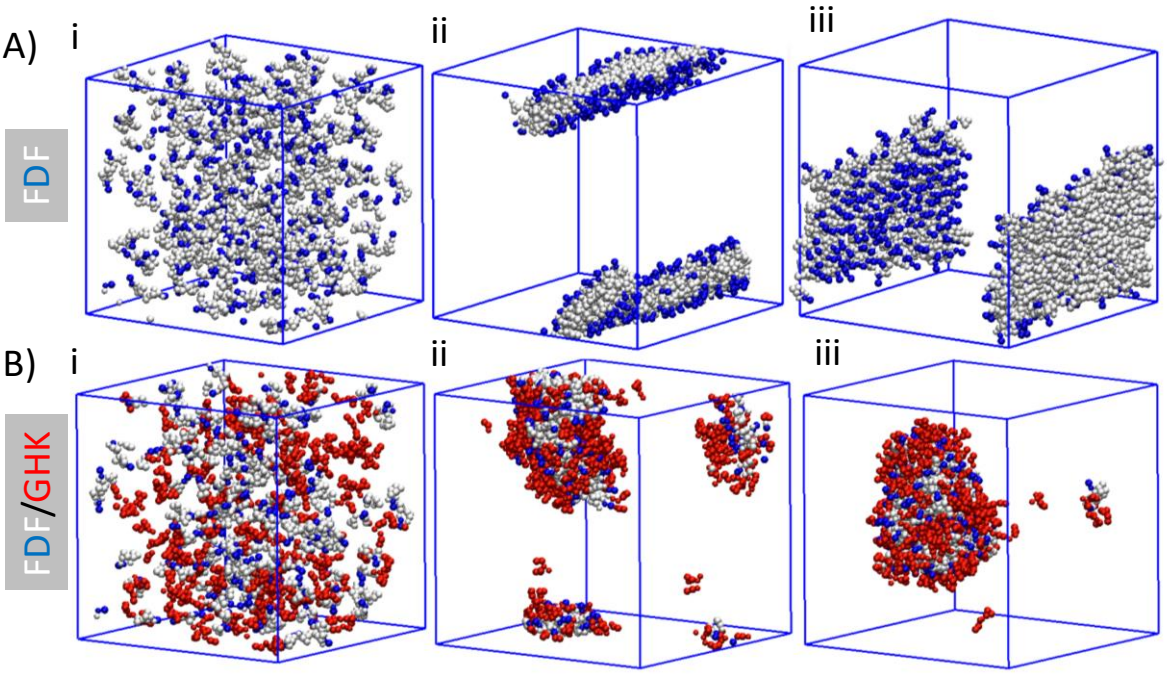


Figure S4. Computational time course for the self-assembly of the control tripeptide (FDF) in isolation (A) and co-assembled nanostructures (B). (i) Initial frame (0 μs), (ii) Mid-point (~3 μs), iii) Final frame (~9.6 μs).