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Journal:	<i>ACS Applied Materials & Interfaces</i>
Manuscript ID	am-2020-05191u.R1
Manuscript Type:	Article
Date Submitted by the Author:	10-Apr-2020
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Magnetic field-induced alignment of nanofibrous supramolecular membranes: a molecular design approach to create tissue-like biomaterials

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KEYWORDS aromatic cationic peptides, self-assembly, nanofibers, magnetic field, alignment

ABSTRACT: A molecular design approach to fabricate nanofibrous membranes by self-assembly of aromatic cationic peptides with hyaluronic acid (HA) and nanofiber alignment under a magnetic field is reported. Peptides are designed to contain a block composed of four phenylalanine residues at the C-terminus, to drive their self-assembly by hydrophobic association and aromatic stacking, and a positively charged domain of lysine residues for electrostatic interaction with HA. These two blocks are connected by a linker with a variable number of amino acids and ability to adopt distinct conformations. Zeta potential measurements and circular dichroism confirm their positive charge and variable conformation (random coil, β -sheet or α -helix), which depend on the pH and sequence. Their self-assembly, examined by fluorescence spectroscopy, small-angle X-ray scattering and transmission electron microscopy, show the formation of fiber-like nanostructures in the micromolar range. When the peptides are combined with HA, hydrogels or flat membranes are formed. The molecular structure tunes the mechanical behavior of the membranes and the nanofibers align in the direction of magnetic field due to the high diamagnetic anisotropy of phenylalanine residues. Mesenchymal stem cells cultured on magnetically-aligned membranes elongate in direction of the nanofibers supporting their application for soft tissue engineering.

INTRODUCTION

Soft materials are pliable materials that easily deform as a result of the relatively weak non-covalent interactions that hold their individual building blocks together. Additional properties arising from these weak interactions are stimuli-responsive behavior and non-linear response to external mechanical forces (viscoelastic behavior).¹ Soft matter encompasses biological (living tissues) and a vast number of synthetic materials (gels, emulsions, foams, polymers, colloids). Biology uses multiscale self-assembly, the spontaneous formation of ordered mesoscopic structures from small building blocks, to create complex structural materials with hierarchical organization like collagen. Inspired by biology, supramolecular chemistry uses

non-covalent forces to create molecular structures and materials mimicking biological counterparts. Mastering self-assembly and organization across the nano and microscales have been achieved in supramolecular peptide materials through molecular design and extrinsic forces,² but hierarchically oriented organization over large length scales (tissue size) is still challenging.

Manipulation of soft materials by external forces (heat, light, pressure, chemicals, electric, acoustic, gravitational and magnetic fields) is an attractive possibility to reshape their geometries/architectures and obtain new functions beyond their original properties.³ The use of magnetic fields to actuate soft materials is promising to create orientational order in

1 biomaterials by controlling the magnitude and direction of the
2 field.⁴ Many molecular materials are endowed with
3 diamagnetism,⁵ a very weak form of magnetism that is induced
4 by a change in the orbital motion of electrons due to an applied
5 magnetic field. However, the force generated from the
6 movement of the electrons is very small on the molecular level
7 and its effects under normal magnetic fields is negligible due to
8 the much stronger effect of Brownian motion. The magnetic
9 response of soft materials can be enhanced by incorporating
10 ferro- or para-magnetic materials into their structure. On the
11 other hand, diamagnetism effect can exceed the effect of
12 Brownian motion when large magnetic fields are applied (1-20
13 T). For example, aromatic rings have a high magnetic
14 susceptibility due to the free movement of electrons around the
15 ring. The magnetic susceptibility of a molecule is highly
16 dependent on its volume and structure.⁵ The stacking of
17 aromatic rings (π - π interactions) in assembled molecules
18 amplifies their magnetic susceptibility. Taking advantage of
19 diamagnetic anisotropy of peptide bonds and aromatic rings,
20 magnetic fields have been used to align peptide,⁶⁻⁹ protein
21 (collagen,¹⁰ fibrin and outer membrane protein (Omp)¹¹) fibers
22 in the absence of ferro- or para-magnetic materials.

23 Our group has been developing soft membranes obtained by
24 co-assembly of rationally designed peptides (peptide
25 amphiphiles and multidomain peptides not containing aromatic
26 residues) with hyaluronic acid (HA) without any external
27 intervention.¹²⁻¹⁴ Here we combine supramolecular and
28 magnetic forces to fabricate self-assembling peptide/HA
29 membranes and induce nanofiber alignment over the millimeter
30 scale. We designed novel cationic aromatic peptides to self-
31 assemble with HA and leverage the magnetic anisotropy of the
32 peptides to control the nanofiber orientation by a magnetic field.
33 These soft self-assembled membranes with aligned nanofibers
34 can be used as scaffolds for engineering soft tissues and in
35 mechanobiology studies.

EXPERIMENTAL SECTION

36 **Peptide synthesis.** All peptides were synthesized in an
37 automated peptide synthesizer (Liberty Blue, CEM, UK) using
38 standard 9-fluorenylmethoxycarbonyl (Fmoc) based solid
39 phase chemistry. 4-methylbenzhydrylamine (MBHA) rink
40 amide resin (100-200 mesh) was used as a solid support and
41 Fmoc-protected amino acids were coupled using (4:4:4)
42 equivalents of amino acids, 1-hydroxybenzotriazole hydrate
43 (HOBt) and N,N'-diisopropylcarbodiimide (DIC). Fmoc groups
44 were deprotected using 20% (v/v) piperidine in
45 dimethylformamide (DMF). Before cleaving the peptide from
46 the resin, the N-terminal of all peptides was capped with acetic
47 anhydride (10% in DMF). The removal of the protecting groups
48 of amino acid side-chains and peptide cleavage from the resin
49 was achieved by incubating the peptide-bound resin with a
50 mixture of trifluoroacetic acid (TFA)/trisopropylsilane (TIS)/
51 water (95%/2.5%/2.5%). The cleaved peptide was collected and
52 the resin washed with dichloromethane (DCM). The solutions
53 were combined and reduced down in a rotary evaporator to a
54 viscous solution. Cold diethyl ether was used to precipitate the
55 peptide. After centrifuging, the supernatant was discarded and
56 the solid allowed to dry overnight. The peptide mass was
57 confirmed by electrospray ionization mass spectrometry (ESI-
58 MS) using a single quadrupole mass detector (SQ Detector 2,
59 Waters, UK). The crude peptide was purified using a
60

preparative reverse-phase C18 column (X-Bridge prep OBD, 5 μ m, 30 \times 150 mm column, Waters, UK) in a Waters 2545 Binary Gradient high-performance liquid chromatography (HPLC) system and using a gradient of ultrapure water/acetonitrile (0.1% TFA) as mobile phase. Fraction collection was carried out by mass detection and acetonitrile was removed from the collected fractions by rotary evaporator and then lyophilized. The purity of the peptides were confirmed by analytical HPLC (Alliance HPLC system coupled with 2489 UV/Vis detector, Waters, UK). Peptide samples (1 mg/ml) were injected into an analytical reverse-phase C18 column (XBridge analytic 3.5 μ m, 4.6 x 150 mm, Waters, UK) and eluted through a gradient of water/acetonitrile (0.1% TFA) at 1 ml/min and detected at 220 nm.

Calculation of the diamagnetic susceptibility of the peptides. The diamagnetic anisotropy (DA) of the peptides was calculated based on the magnetic susceptibility of the amino acids and their spatial position with respect to one another using a recently developed computational method.¹⁵ Briefly, the magnetic susceptibility tensor for amino acids was transformed to a single global coordinate followed by summing the tensors to build the net magnetic susceptibility tensor for the entire peptide structure, and the diamagnetic anisotropy was calculated for the peptides based on the eigenvectors and eigenvalues of the total magnetic susceptibility tensor for the peptide structure.¹⁵ The molar and volumetric (dimensionless) diamagnetic anisotropy for the peptides are presented in **Table S1**.

Zeta-potential Measurements. Peptide solutions at different pHs (7, 10 and 11) were analyzed in a Nano-ZS Zetasizer (Malvern Instruments) for measuring their zeta potential. Solutions were prepared at 0.1 wt% in Milli-Q water and the pH adjusted with 0.1 M HCl and 0.1 M NH₄OH.

Circular Dichroism (CD) spectroscopy. Secondary structure of pure peptides was examined by CD spectroscopy using a PiStar-180 spectrometer from Applied Photophysics (Surrey, UK), under a constant flow of nitrogen (8 L min⁻¹) at a constant pressure value of 0.7 MPa. The measurements were recorded at 25 °C from 190 to 300 nm far-UV spectra. Peptide solutions were prepared in de-ionized water with a final concentration of 0.04 mM and the pH was adjusted to 3, 7, 10 and 11 with 0.1 M HCl and 0.1 M NH₄OH. Then, they were analyzed in a quartz cuvette with 1 mm path-length and all scans were performed in the steady state with a bandwidth of 1. The solvent spectrum was subtracted at each measurement and mean residue ellipticity obtained.

Fourier Transform Infrared (FTIR) Spectroscopy. To further investigate the secondary structure of the peptides, peptide solutions were prepared at concentration of 20 mg.ml⁻¹ in D₂O (pH 7) and ND₄OD (pH 11). Then, the IR spectra of solutions were analyzed using Bruker Tensor 27 FTIR spectrometer using an attenuated total reflection (ATR) diamond crystal accessory (Bruker, Germany). The absorbance spectrum was measured in the range of 4000–400 cm⁻¹ by averaging individual scans per sample at a resolution of 4 cm⁻¹.

Fluorescence Spectroscopy. The π - π interactions of the phenylalanine residues were analyzed on a LS55 fluorescence spectrometer (PerkinElmer, UK). Peptide solutions were prepared in the concentration range of 0.02 - 800 μ M. The pH of peptide solutions was adjusted to 7 and 11 and then excited

1 at 210 nm and the fluorescence emission measured in the range
2 of 280 - 400 nm¹⁶.

3 **Critical Aggregation Concentration (CAC).** The CAC of the
4 peptides was obtained by using the hydrophobic
5 solvatochromic dye Nile Red¹⁷. Aqueous solutions of peptides
6 with various concentrations, ranging from 0.02 to 82 μ M, were
7 prepared and added to the tubes containing dried Nile Red
8 giving a final concentration of 1 μ M of the dye. The mixture of
9 peptides and Nile Red were left overnight to equilibrate.
10 Fluorescence emission spectrum of each sample was recorded
11 on a LS55 fluorescence spectrometer (PerkinElmer, UK)
12 ranging from 580 to 720 nm with an excitation wavelength at
13 550 nm. The maximum intensity and the corresponding
14 wavelength of each spectrum were plotted as a function of
15 logarithm of peptide concentration.

16 **Transmission Electron Microscopy (TEM).** Peptide
17 assemblies were imaged by TEM to analyze their morphology.
18 Peptide aqueous solutions (0.05 wt%) were prepared and then
19 10 μ L casted onto a carbon film-coated copper grid (400 mesh,
20 Agar Scientific) followed by negative staining with 10 μ L of
21 uranyl acetate solution (2%, v/v) for 30 seconds. The excess
22 staining solution was removed with a piece of filter paper and
23 the grid allowed to dry at room temperature for at least 3 hours.
24 Bright field TEM imaging was performed on a JEOL-1230
25 TEM operated at an acceleration voltage of 80 kV and the TEM
26 images were recorded by a SIS Megaview III wide angle CCD
27 camera.

28 **Small Angle X-ray Scattering (SAXS).** SAXS experiments
29 were performed at the Diamond Synchrotron (Harwell, UK) on
30 beamline B21. Solutions containing 0.5 wt% peptides in PCR
31 tubes were loaded into the 96 well plate of an EMBL BioSAXS
32 robot, and then injected via an automated sample exchanger into
33 a quartz capillary (1.8 mm internal diameter) in the X-ray beam.
34 The quartz capillary was enclosed in a vacuum chamber, in
35 order to avoid air scattering. Data was collected using a Dectris
36 EIGER 4M detector at a fixed camera length of 3.9 m with a
37 wavelength $\lambda = 1 \text{ \AA}$. Data are presented as a function of $q = 4\pi$
38 $\sin \theta / \lambda$, where 2θ is the scattering angle. Membranes were
39 mounted in a custom-designed enclosed gel cell holder¹⁸ for
40 gels and pastes. All measurements were performed at 20 °C.

41 **Membrane Assembly.** The self-assembly of peptides with HA
42 was tested by casting HA solutions on the bottom of a well-plate
43 and peptide solutions were added on top at equal volume ratio.
44 HA with different molecular weights (20, 60, 200 and 700 kDa)
45 were tested and prepared at 2 wt% concentration except for 1.5
46 MDa HA where 1 wt% was used. The peptide solutions were
47 prepared at 2 wt%. Peptides were left to incubate with HA at 37
48 °C overnight.

49 **Scanning Electron Microscopy (SEM).** The microstructure of
50 the surface and cross-section of the obtained self-assembled
51 materials (membrane or hydrogel) was examined by SEM.
52 Samples were fixed with 2% glutaraldehyde in PBS. After
53 cycles of washing with PBS, samples were progressively
54 dehydrated using graded ethanol concentrations. Ethanol
55 removal was performed using a critical point dryer (EMS 850,
56 Electron Microscopy Sciences, USA). All samples were first
57 coated with a gold layer (5-30 nm) using an Emitech SC7620
58 sputter coater (Quorum Technologies, UK) then imaged in a
59 field emission gun scanning electron microscope (FEG SEM,
60 Inspect F50, FEI, Netherlands). Analysis of aligned fibers in the
membranes were performed by fast Fourier transform (FFT)

61 using ImageJ. Simply, the SEM images were used in ImageJ
62 followed by processing the FFT function, giving the FFT image
63 as output.

64 **Mechanical Testing.** Mechanical properties of all membranes
65 were measured in a tensile testing machine. Membranes were
66 formed in a mold with dimensions (5 mm \times 15 mm) customized
67 for this test allowing them to be clamped between tensile testing
68 grips. The final dimensions of each sample were measured with
69 a digital Calliper. To obtain the membrane thickness, each
70 membrane was sandwiched between two coverslips and their
71 thickness imaged using high resolution camera. The tensile test
72 was performed in an Instron 3342 (UK) with a load cell of 10 N
73 at the rate of 0.1 mm/s. Each test was repeated three times. After
74 plotting the stress-strain curves, the elastic modulus was
75 obtained by calculating the slope of the linear region of the
76 curve. Statistical analysis was performed by one-way ANOVA
77 using Bonferroni test for comparing membranes properties. (p-
78 value: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***),
79 <0.0001 (****)). The mechanical behavior of obtained
80 hydrogels was analyzed by rheological measurements using a
81 Discovery Hybrid Rheometer (DHR-3, TA Instruments, USA)
82 fitted with a 8 mm diameter plate geometry (advanced Peltier
83 plate, APP). Strain and frequency sweeps were examined at 1
84 Hz frequency and 0.1% strain, respectively. All experiments
85 were carried out at 25 °C with a loading gap height set to 0.5
86 mm. Amplitude and frequency sweeps were performed on three
87 repeats of samples.

88 **Membrane Assembly under Magnetic Field.** The effect of
89 magnetic field on membrane fiber alignment was performed at
90 25 °C using a cryofree superconducting magnet (10 cm inner
91 bore diameter, Cryogenic, UK) at fields of 12, 6 and 1 T. The
92 alignment of fibers were confirmed using a graphite marker
93 (lead pencil). Briefly, HA solutions were cast in the bottom of
94 vials (or 96 well-plate for cell adhesion studies). Then, the
95 peptide solution was added on top and the vial immediately
96 inserted in the superconducting magnet at a set field. The
97 membrane was allowed to form under the field for different
98 periods of time. The formed membranes were removed after
99 different time periods and inspected by SEM analysis as
100 described above. Membranes were imaged with light
101 microscopy (Olympus SC100 digital camera) with polarizing
102 filters to assess their birefringence properties.

103 **Cell Culture on the Membranes.** Human-derived
104 mesenchymal stem cells (hMSCs, passage number 6,
105 PromoCell GmbH, Germany) were used for the cell adhesion
106 studies. Cells were cultured in 75 cm² flask with cell density of
107 3 \times 10³ cells cm⁻² and maintained in culture medium (DMEM,
108 low glucose, GlutaMAXTM) supplemented with 10% FBS, 1%
109 Pen/Strep and 0.1% bFGF (FGF-Basic (AA 10-155)
110 recombinant human protein, Thermo Fisher Scientific, UK).
111 Flasks were incubated at 37 °C in an incubator supplied with
112 5% CO₂ flow. Medium of the cells was refreshed twice a week
113 and cells were trypsinized when 80% confluence was reached.

114 All membranes were prepared using 1.5 MDa HA for the cell
115 adhesion studies. RGDS-K₂A₅F₄/1.5 MDa HA membranes were
116 also prepared with peptide solution containing 10% RGDS-
117 K₂A₅F₄ and 90% of K₂A₅F₄ (**Figure S1**). All membranes were
118 formed under sterile conditions except the ones prepared under
119 magnetic field. Before MSC culture, the membranes were
120 exposed to UV irradiation for 20 minutes. MSCs (30,000 cells
121 per well (1.1 cm²)) were suspended in a culture medium (serum-
122 free DMEM) and added to the membranes.

1 containing condition: DMEM supplemented with 10% FBS and
2 1% Pen/Strep; serum-free condition: DMEM supplemented
3 with 1% Pen/Strep), seeded onto the membranes and incubated
4 at 37 °C in an incubator supplied with 5% CO₂. They were left
5 for attachment for 24 hours. The membranes were then washed
6 with PBS and incubated in a permeabilization solution (5% v/v
7 Triton X-100 (Sigma, USA), 0.14 wt% NaCl, 0.01 wt% MgCl₂
8 and 5 wt% sucrose) for 40 minutes at room temperature, washed
9 twice with PBS and fixed using 4% paraformaldehyde (PFA,
10 Sigma-Aldrich, Germany) for 15 minutes at room temperature.
11 Cells cultured on the membranes were stained with 4,6-
12 diamidino-2-phenylindole-dilactate (DAPI, nucleus staining,
13 Invitrogen) and phalloidintetramethylrhodamine B
14 isothiocyanate dyes (Phalloidin, cytoskeleton staining, Alexa
15 Fluor™ 488 Phalloidin, Invitrogen). Briefly, for each staining,
16 samples were incubated with 10 µl phalloidin in 1 ml PBS for
17 40 minutes following a three-cycle of PBS washing. Then DAPI
18 staining was performed with 1 µl of dye in 1 ml of PBS with a
19 15 minutes incubation time. Cell monolayers cultured on the
20 well-plate were used as a controls. Cell density and aspect ratio
21 were both measured using the Fiji Image J software. The
22 number of cells per mm² on the membranes was calculated by
23 counting the nuclei (DAPI-stained) of the cells in a square of
24 0.5×0.5 mm dimensions and the aspect ratio was measured
25 applying an ellipse fit to single cells and the ratio of the ellipse's
26 dimensions acquired. Three replicates of membranes were
27 imaged with epifluorescence microscopy (Leica DMI8
28 Epifluorescence). Statistical differences were obtained with
29 GraphPad Prism 7 software (USA) using a two-way analysis of
30 variance (ANOVA) with a Bonferroni's multiple comparison
31 test (p-value: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***),
32 <0.0001 (****)).

33 MSCs-cultured membranes were fixed and prepared for SEM
34 as described above. Samples were coated with gold (Emitech
35 SC7620 sputter coater, Quorum Technologies, UK) and imaged
36 with a field emission gun scanning electron microscope (FEG
37 SEM, Inspect F50, FEI, The Netherlands).

38 RESULTS AND DISCUSSION

39 **Design and characterization of aromatic cationic peptides.** The peptides designed in this work follow the K₂X_nF₄ template,
40 with X serving as a linker segment between a positively charged
41 lysine (K) domain and an aromatic block of four phenylalanine
42 (F) residues. Phenylalanines were selected to establish π-π
43 interactions between aromatic rings while the charged domain
44 provides water solubility and imparts responsiveness to pH and
45 (poly)electrolyte addition. The variable linker (X_n) is
46 incorporated to control the degree of hydrogen bonding hence
47 the secondary structure of the peptides. The number of alanine
48 residues (K₂A₃F₄, K₂A₃F₄ and K₂A₇F₄) was investigated as well
49 as different amino acids (serine and glycine: K₂S₅F₄ and
50 K₂G₇F₄) (Table 1). Alanine residues were selected considering
51 their α-helix propensity¹⁹, while serine provides hydroxyl
52 groups, through its side chain, for additional hydrogen
53 bonding.²⁰ Glycine is achiral with a single hydrogen atom as its
54 side chain.²¹

55 Peptides were successfully synthesized and purified (Figure
56 S1) and their charge measured through zeta-potential (ζ-
57 potential) analysis. All of the peptides showed positive zeta
58 potential at pH 7, 10 and 11 (Table 1), as expected, but the zeta
59 potential decreased with increasing pH as lysines become de-

60 protonated (Figure S2). However, at pH 11 (over the pKa value
of lysine side chains), the peptides showed a rather sharp drop
in zeta potential compared to pH 10.

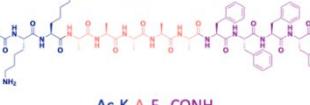
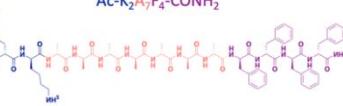
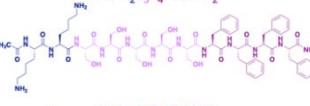
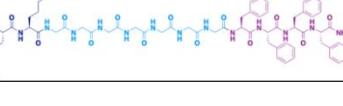
61 Aromatic dipeptides (e.g. diphenylalanine) or single aromatic
62 amino acids (e.g. phenylalanine) are known to self-assemble
63 into fibril nanostructures through well-ordered stacking of
64 aromatic rings by π-π interactions.^{22,23} These studies
65 demonstrate the power of aromatic rings in triggering self-
66 assembly and providing order and directionality of the
67 assembly. Conveniently, the aromatic ring of phenylalanine
68 also imparts high diamagnetic anisotropy, or susceptibility to
69 directional alignment in a magnetic field.¹⁵ This prior work
70 suggests that the secondary structure of the peptide has a
71 significant effect on its diamagnetic anisotropy. While a protein
72 with randomly oriented phenylalanine amino acids would not
73 exhibit directional alignment, self-assembly of these residues
74 into secondary and tertiary structures can translate into
75 substantial diamagnetic anisotropies at the peptide or fibril
76 level. We calculated the molar and volumetric diamagnetic
77 anisotropy values for a series of peptides with different residue
78 compositions Table S1. Generally, the volumetric diamagnetic
79 anisotropy increases as the number of phenylalanine in the
80 sequence increases.

81 **Self-assembly behavior of aromatic cationic peptides.** The
82 secondary structure of designed peptides was investigated in
83 acidic, neutral and basic environments by circular dichroism
84 (CD) spectroscopy. All of the peptides exhibited extended 3₁-
85 helix or polyproline type II helix (with negative peak at 194 nm
86 and a positive peak at 220 nm)²⁴ at acidic and neutral pHs, at
87 which the lysine residues are charged (Figure S2). However, at
88 pH 10, the side chain of lysine becomes less charged (Figure
89 S2) and peptides with alanine linker adopt α-helical
90 conformation, with a maximum at 180 nm and two negative
91 minima at 200 and 212 nm. Alanine-rich peptides have been
92 shown propensity to adopt helical conformation.^{25,26} Increasing
93 the number of alanine residues, which extends the linker length,
94 resulted in a decrease in the intensity of the maximum peak at
95 180 nm, where this is more significant for K₂A₇F₄ peptide. The
96 peptide containing serine residues in the linker region (K₂S₅F₄)
97 showed a spectra similar to α-helix conformation with a slight
98 shift in the second minima peak (210 → 227 nm), which may be
99 due to the contribution of aromatic side chains of
100 phenylalanines²⁷ and hydrogen bonding between hydroxyl side
101 chains of serine. For the peptide with glycine-containing linker
102 (K₂G₇F₄), a β-sheet structure was observed at pH 10. Being
103 achiral, glycine does not contribute significantly to the
104 peptide's CD signal compared to the chiral amino acids at the
105 peptide termini (K₂ and F₄).

106 The secondary structure of K₂A₅F₄ and K₂A₃F₄ peptides
107 changed from being α-helix at pH 10 to β-sheet structures at pH
108 11 (Figure 1-A). At this pH, the peptides are mostly neutral
109 (Figure S2) allowing the stacking of the phenylalanine
110 aromatic rings as well as hydrogen bonding between the peptide
111 backbones. FT-IR analysis of peptide solutions at pH 11
112 revealed mostly parallel β-sheet structures with a more intense
113 peak at 1630 cm⁻¹ compared to anti-parallel β-sheet structures
114 at 1690 cm⁻¹ (Figure S3, Table S3). Combined parallel and
115 anti-parallel β-sheet structures were also observed by FT-IR for
116 peptides with similar design (H-F₂ALGLAGK₂-OH).²⁸ The
117 contribution of phenylalanine side chains in shifting the CD
118 spectra is also visible for the K₂A₇F₄ and K₂S₅F₄ peptides. These

1 results are in agreement with the self-assembly of noncharged
 2 diphenylalanine peptides into β -sheet structures.²⁹
 3
 4
 5
 6

7 **Table 1. Chemical structure of designed aromatic cationic peptides and their properties.**

Peptide sequence	Critical Aggregation Concentration, CAC (μM)	Volumetric Diamagnetic Anisotropy (DA)* 10^{-6}	Zeta potential (mV)	
			pH 7	pH 11
Ac-K ₂ A ₃ F ₄ -CONH ₂ 	6.16	—	32.26 \pm 0.87	2.15 \pm 0.27
Ac-K ₂ A ₅ F ₄ -CONH ₂ 	4.26	0.956	35.5 \pm 3.87	4.90 \pm 2.82
Ac-K ₂ A ₇ F ₄ -CONH ₂ 	2.50	1.98	34.13 \pm 0.40	2.64 \pm 0.29
Ac-K ₂ S ₅ F ₄ -CONH ₂ 	7.40	3.23	50.53 \pm 2.45	-7.06 \pm 0.51
Ac-K ₂ G ₇ F ₄ -CONH ₂ 	4.40	4.51	45.06 \pm 1.53	11.8 \pm 0.1

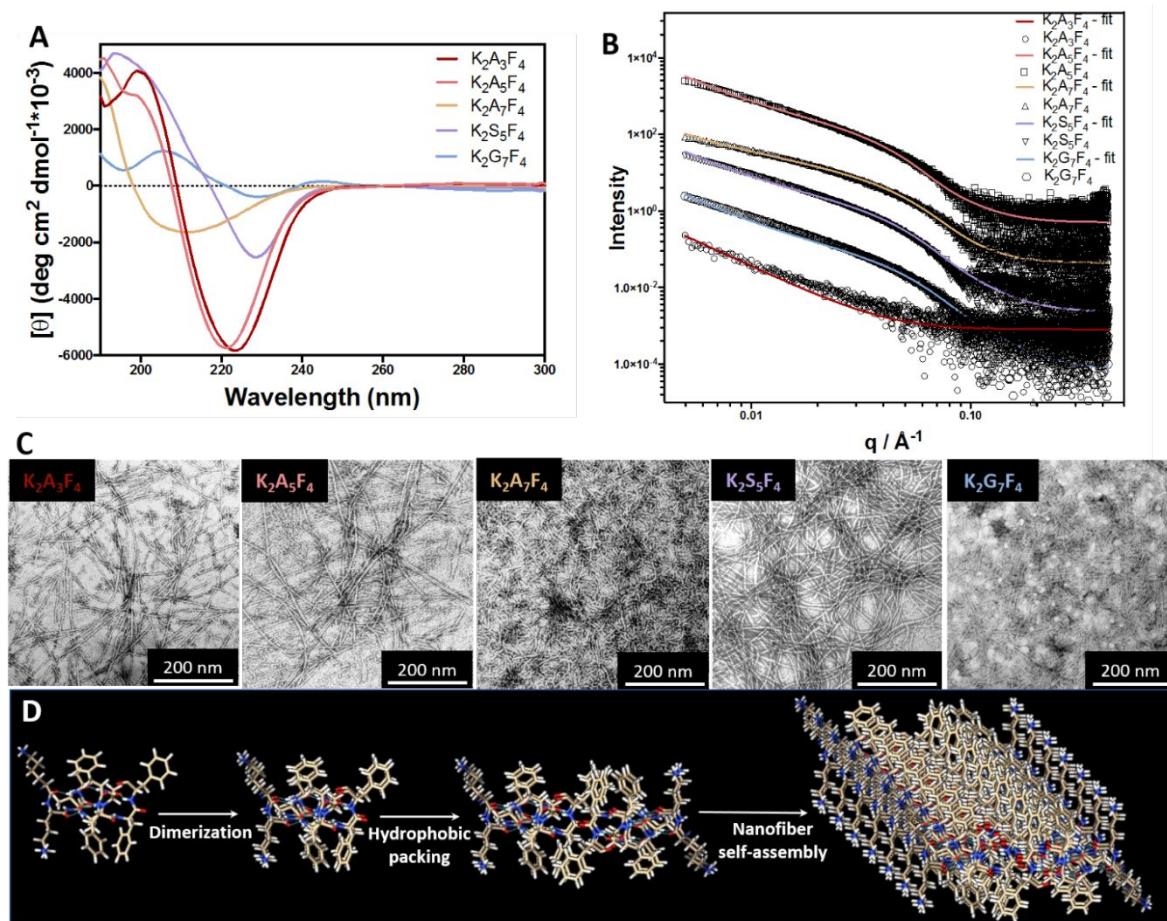


Figure 1. Self-assembly behavior of cationic aromatic peptides at pH 11. (A) CD spectra; (B) SAXS patterns measured for 0.5 wt% solutions (open symbols) along with model form factor fits (solid lines). For ease of visualization, the data for $K_2S_5F_4$ has been multiplied $\times 10$, that for $K_2A_7F_4 \times 100$ and that for $K_2A_5F_4$ by $\times 1000$. Also for clarity, only every 5th data point is plotted; (C) TEM images of 0.05 wt% of peptide solutions. (D) Proposed model for self-assembly for $K_2A_5F_4$ peptide.

The morphology of the assemblies formed by the cationic aromatic peptides are shown through TEM images taken from solutions at pH 11 (Figure 1-C). No defined structures were detected for $K_2A_5F_4$ at pH 7, but intertwined long fibers were observed at basic pH (pH=11). Therefore, TEM imaging was done on the remaining peptide solutions at pH 11. TEM images show nanofibrillar structures for all of the peptides but their length and density varies depending on the peptide sequence. Differences seen in the CD spectra and TEM images highlight the influence of the linker segment on the conformation and morphology of formed structures. As the alanine linker gets longer, dense nanofibers are formed but the length of the fibers gets shorter (denser fibers for $K_2A_7F_4$). In contrast, the serine linker ($K_2S_5F_4$) promotes the formation of long and well-defined nanofibers. Peptides with glycine linker ($K_2G_7F_4$) also formed long fibers, in addition to grape-like structures. SAXS analysis on the peptide solutions was also performed to further elucidate the morphology of the assemblies at pH 11. The results are presented in Figure 1-B along with fitted form factors based on the form factor of cylindrical nanofibrils (Table S2). All of the samples, except $K_2A_3F_4$, form well-defined nanofibrils with radii in the range 2.0 – 3.1 nm. This is an agreement with the TEM images, apart from $K_2A_3F_4$ which may form fibrils upon drying as part of the TEM sample preparation.

To further investigate the π -interactions of fluorenyl moieties of the peptides, fluorescence spectroscopy was used. Aromatic

amino acids are intrinsically fluorescent and are mostly responsible for inherent fluorescence of proteins, with changes in their environment resulting in a fluorescence shift. Peptides solutions where excited at 210 nm¹⁶ and a redshift (from 290 to 310 nm) in the peak of maximum fluorescence was observed at pH 11 (Figure S4), confirming the formation of π - π -interactions upon screening of amine groups of lysine side chains. However, this was not observed for $K_2S_5F_4$ peptide. At pH 11, only a decrease in the shoulder peak at 290 nm was detected, inferring the repulsion of deprotonated hydroxyl moieties in cancelling the π -interactions of aromatic groups.

The peptides designed in this work have an amphiphilic structure with a hydrophilic charged portion at the N-terminus and a hydrophobic aromatic segment at the C-terminus. Based on this amphiphilic structure and on the obtained experimental data, their self-assembly was proposed taken $K_2A_5F_4$ peptide as model (Figure 1-D). SAXS on solutions of $K_2A_5F_4$ peptide at pH 11 estimated a cylinder radius of 3.1 nm (6.2 nm in diameter). Considering a distance of 3.4 Å per residue and a linear peptide of 11 residues, the expected molecular length of a single peptide molecule is 37.4 Å. Upon charge screening (basic pH or addition of oppositely charged molecules or ions), peptides can dimerize via hydrophobic association and π - π interactions. Antiparallel association of dimers via hydrophobic effects (Figure 1-D, hydrophobic packing) results in the growth into extended nanofibers, stabilized by hydrogen bonding between peptide

1 backbones, with estimated diameter of ≈ 7.5 nm. This value is in
 2 accordance with the diameter obtained by SAXS.

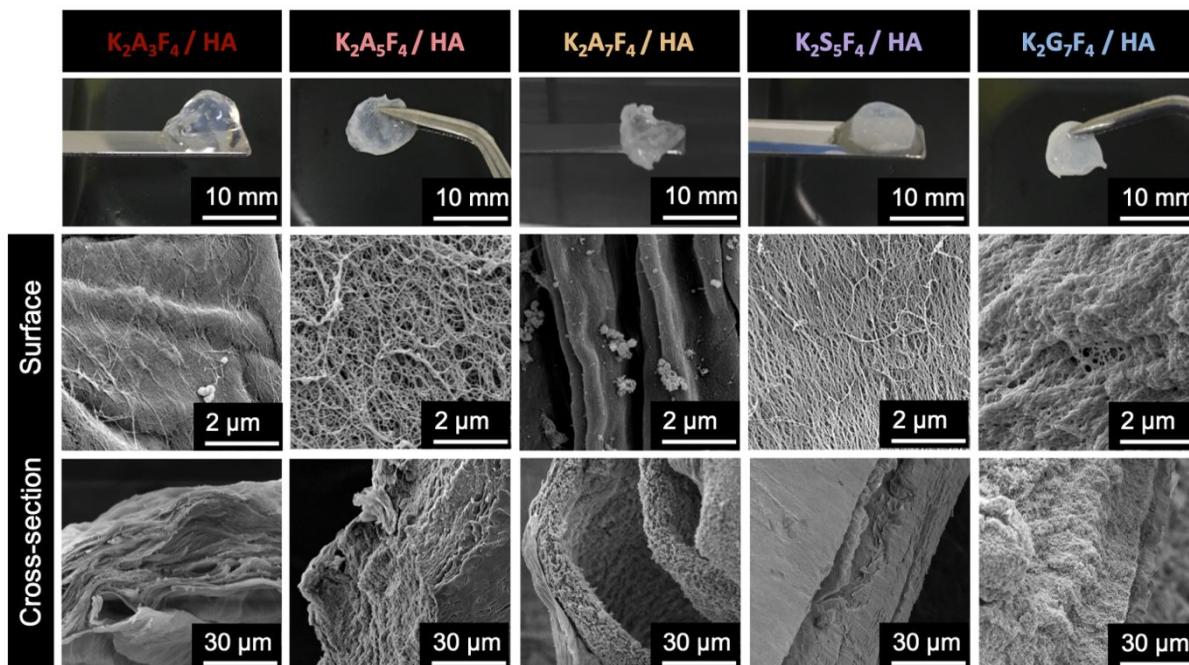
3 The critical concentration for self-assembly for each peptide
 4 was also determined using the solvatochromic fluorescent dye,
 5 Nile Red¹⁷. Nile Red is poorly soluble in water, hence has a great
 6 tendency towards hydrophobic binding sites of molecules present
 7 in solution.³⁰ Therefore, the critical aggregation concentration
 8 (CAC) of peptides can be determined with Nile Red through
 9 plotting the shift in maximum wavelength or with changing in
 10 fluorescent intensity as function of the logarithm of concentration
 11 (**Figure S5**). CAC results (**Table 1**) showed that all sequences
 12 aggregate at low concentration, in the micro-molar (μ M) range,
 13 and the linker segment has a slight effect on the CAC being more
 14 evident in the peptides with an alanine-based linker. As the
 15 alanine length is longer, the aromatic stacking took place at lower
 16 concentration (2.5 μ M for $K_2A_7F_4$) and this increases for $K_2A_3F_4$
 17 being 6.16 μ M. Overall, the CAC of the peptides are shown to be
 18 between 2–7 μ M which is lower than the CAC determined for
 19 similar peptides²⁸ containing only two phenylalanine residues. A
 20 higher number of phenylalanine residues enhances the
 21 hydrophobic effects and favors the self-assembly of these cationic
 22 aromatic peptides at lower concentrations.

23 **Self-assembly of cationic aromatic peptides with hyaluronic
 24 acid (HA).** Lysine residues were incorporated in the peptide
 25 design to establish electrostatic interactions with (poly)electrolytes of opposite charge. Here, peptides were self-
 26 assembled with HA in aqueous solutions, first casting HA
 27 solution on the bottom of a well plate and the peptide solution
 28 was then added on top of the HA. Different concentrations of
 29 peptides and HA were tested, but only robust membranes were
 30 formed when using 2 wt% of peptide with 1 wt% of 1.5 MDa HA

31 and 2 wt% of HA of lower molecular weights. Self-assembly
 32 occurred immediately and the formation of an opaque layer at the
 33 interface was observed as reported in previous studies using HA
 34 and other self-assembling peptides.^{12–14,31} However, the
 35 membranes were left to grow overnight at 37 °C.

36 All sequences, except $K_2A_3F_4$, formed membranes at the
 37 interface of the two liquids, whereas the peptide with shorter
 38 alanine linker formed a hydrogel, acting as kind of hydrogelator.
 39 Self-assembly with higher molecular weight HA (1.5 MDa)
 40 resulted in a transparent hydrogel (**Figure 2-top**). All aromatic
 41 cationic peptides were tested with 1.5 MDa HA, as well as $K_2A_5F_4$
 42 sequence self-assembled with different concentrations of 700
 43 kDa and 1.5 MDa HA. As a result, membranes were formed with
 44 different thicknesses (**Figure S6**), which ranges from 18 μ m for
 45 $K_2A_5F_4$ (2 wt%)/1.5 MDa HA (0.5 wt%) up to 71 μ m for $K_2S_5F_4$
 46 (2 wt%)/1.5 MDa HA (1 wt%). The higher thickness of the
 47 $K_2S_5F_4$ /HA membrane is believed to be due to the presence of
 48 polar hydroxyl groups on the side chains of serine, which retain
 49 more water content compared to other membranes.

50 **Figure 2-bottom** shows a highly compact fibrillar structure for
 51 $K_2A_3F_4$ /HA hydrogel. Membrane microstructure varies in density
 52 of fibers depending on the peptide sequence and the length of HA
 53 chain. The self-assembly of $K_2A_7F_4$ with HA resulted in
 54 membranes with rough surface for 1.5 MDa. The self-assembly
 55 of HA with a peptide amphiphile (PA)^{14,31} and multi-domain
 56 peptides (MDPs)^{12,13} into membranes showed two distinct faces,
 57 one rich in HA and the other in the peptide component. However,
 58 these membranes showed no distinct structure for both surfaces
 59 (peptide and HA sides) with randomly distributed nanofibers on
 60 both surfaces.



51 **Figure 2.** Photographs of hydrogels and membranes formed by self-assembly between designed cationic aromatic peptides and
 52 HA and their nanofibrous structure revealed by SEM images.

53 Analysis of the cross-section also shows nanofibrous structure
 54 and no evident layer showing the interface between the two mixed

55 components (**Figure 2-bottom**). SAXS was also used to
 56 investigate the nanostructure organization of the membranes.

SAXS data for dried membranes is shown in **Figure 3-A**. $K_2A_5F_4$ and $K_2S_5F_4$ membranes show well-developed Bragg peaks at $q = 0.087 \text{ \AA}^{-1}$ and $q = 0.099 \text{ \AA}^{-1}$ respectively, corresponding to average mesh sizes within the membranes of $d = 73 \text{ \AA}$ and $d = 63 \text{ \AA}$ respectively. SAXS from $K_2A_7F_4$ and $K_2G_7F_4$ also shows Bragg peaks, but they are broader than for the other two samples corresponding to spacings of $d = 56 \text{ \AA}$ (the peak for $K_2A_7F_4$ is asymmetric, indicating a population of structures with longer spacings in addition). SAXS on hydrated as well as rehydrated membranes was also performed (**Figure S7**). The data for $K_2A_7F_4$ and $K_2G_7F_4$ shows very distinct structure in hydrated and dried states, with reversibility on hydration (the SAXS profiles show the same shape as the initial profile for the hydrated sample, with only a loss of intensity). The initial SAXS profiles for hydrated samples show fibril form factor features, however for the dried membranes, clearly defined Bragg peaks are observed at $q^* = 0.115 \text{ \AA}^{-1}$ and $q = 0.23 \text{ \AA}^{-1}$ (close to $2q^*$) for $K_2A_7F_4$ (**Figure S7**) which indicates a lamellar structure with spacing of $d = 54.6 \text{ \AA}$. Upon rehydration, the SAXS profile reverts to the same initial form factor shape. Similarly, the first SAXS profile for dried $K_2G_7F_4$ (**Figure S7**) shows Bragg peaks at $q = 0.103 \text{ \AA}^{-1}$ and $q = 0.21 \text{ \AA}^{-1}$, corresponding to a lamellar structure with $d = 61.0 \text{ \AA}$. Considering a peptide layer of around 40 \AA thick, if highly interdigitated, and the total lamellar spacing, a 20 \AA thick HA layer is estimated, and corresponding to four to six HA chains. Some order in this pattern is lost subsequently, but the intensity profile in the hydrated state is very similar after multiple rehydrations as shown in **Figure S7**. In contrast to $K_2A_7F_4$, the SAXS profile in the hydrated state retains a Bragg peak, i.e. a structure factor peak with $q^* = 0.077 \text{ \AA}^{-1}$ corresponding to a domain spacing of $d = 82 \text{ \AA}$. There is a weak second order peak near $\sqrt{3} q^*$, which suggests remarkable hexagonal order of fibrils in the hydrated state for the $K_2G_7F_4/\text{HA}$ membrane. SAXS on

membranes obtained by self-assembly of HA and a positively charged peptide amphiphile revealed Bragg peaks in the scattering profile indicative of a cubic phase.³²

Mechanical testing of cationic aromatic peptide/HA biomaterials. The mechanical characterization of $K_2X_nF_4/\text{HA}$ membranes was performed by tensile testing (**Figure 3-C-E**). In a tensile test, the uniaxial forces are used to stretch the clamped sample with known dimensions and measures the elongation versus applied load. The stress-strain curves show an evident dependence on the linker region used in the peptide design, illustrating either highly elastic soft membranes ($K_2S_5F_4/\text{HA}$ stretched up to 3 times its initial length) or stronger but brittle membranes ($K_2A_5F_4/\text{HA}$).

Among the membranes made from peptides with different linkers, $K_2A_5F_4/\text{HA}$ presents the highest elastic modulus (0.02 MPa) (**Figure 3-D**), which is lower compared to the PA/HA membranes with tensile strength of 0.9 MPa in hydrated state³¹. This can be due to the lack of hierarchical organization observed for the cationic aromatic peptide/HA membranes. However, these membranes can sustain stresses of around 1.2 MPa until failure or stretch up to twice their length. Therefore, it is possible to obtain membranes with tunable mechanical properties through the design of the peptide component as well as through the changes in the concentration of the components.

The membranes were imaged after tensile testing to check their microstructure at the breaking point. The microstructure was affected by the stretching of the membranes, showing fibers are more aligned in the pulling direction at the breaking region compared to the mid-region of the membrane (**Figure S8**). The fiber alignment post stretching is seen for all membranes, but for the $K_2S_5F_4/\text{HA}$ membrane the stretching-induced alignment resulted in the formation of fiber bundles as this is the most stretchable among the tested membranes.

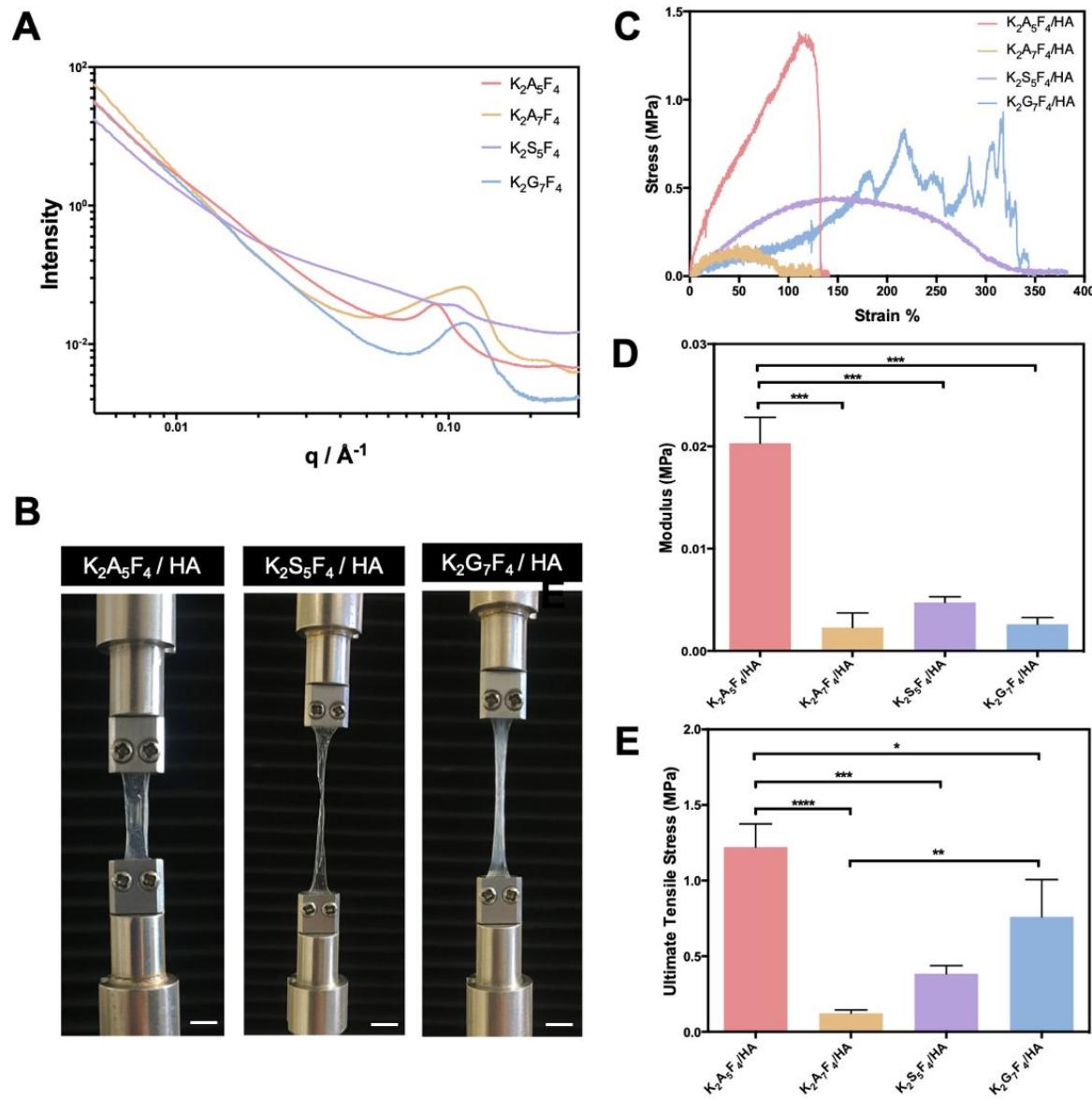


Figure 3. Structural and mechanical analysis of the peptide/HA membranes: (A) SAXS measurements on dried membranes; (B) Images showing membranes clamped between two grips allowing uniaxial stretching (scale bar: 4.5 mm); (C) Stress-strain curves of tensile tested membranes, (D) Tensile modulus and (E) Ultimate tensile stress.

The mechanical properties of the $\text{K}_2\text{A}_3\text{F}_4/\text{HA}$ hydrogels were obtained in a oscillatory rheometer using HA of two molecular weights (700 kDa and 1.5 MDa) to assess the effect of HA length on the stiffness of the hydrogels. The linear viscoelastic region (LVER) was obtained from amplitude sweeps for each group of hydrogels. Both $\text{K}_2\text{A}_3\text{F}_4/700$ kDa HA and $\text{K}_2\text{A}_3\text{F}_4/1.5$ MDa HA maintained their linearity up to 5% strain at frequency of 1 Hz. In order to assess the stiffness of the hydrogels, frequency sweeps were measured at 0.1% strain (in the LVER) with changing in frequency. In both graphs (Figure S9), the storage modulus exceeds the loss modulus showing typical behavior of a gel system. The effect of HA Mw on the stiffness of the hydrogels showed higher elastic modulus for $\text{K}_2\text{A}_3\text{F}_4/1.5$ MDa HA gel (1.8 kPa) compared to $\text{K}_2\text{A}_3\text{F}_4/700$ kDa HA (0.6 kPa), indicating the versatility of the system to obtain hydrogels with different stiffness.

Magnetic alignment of peptide nanofibers during membrane assembly. External fields (electric, magnetic) have been used to direct peptide self-assembly.¹⁹ For example, the effect of electric field on the self-assembly of oppositely charged molecules (anionic HA and cationic peptide amphiphile) was investigated.³³ Electrical field was applied to promote or prevent HA diffusion and shown to affect the kinetics of membrane formation and their final properties (nanostructure organization, thickness, and mechanical properties).

Considering the diamagnetic anisotropy of the aromatic rings of the phenylalanine residues, and based on the previous work showing the alignment of diphenylalanine nanotubes under magnetic field,⁶ we exploited the application of magnetic field during formation of the membrane. As the peptide is expected to play a major role in the magnetic alignment of the fibers, the secondary structure of peptides were first analyzed at pH 7 and 11 after being exposed to a 1 T magnetic field for 24 hours

(Figure S10). The magnetic field had the most effect on peptide conformation at pH 11, where the π - π stacking is stronger. The β -sheet structure of the peptides at pH 11 is affected after exposure to a 1 T field suggesting the orientation of aromatic rings with the

field disturbed the β -sheet configuration. At pH 7, a slight shift and decrease in the minimum peak at 190 nm is evident. Overall, CD results showed that the magnetic field affected the secondary structures of the peptides.

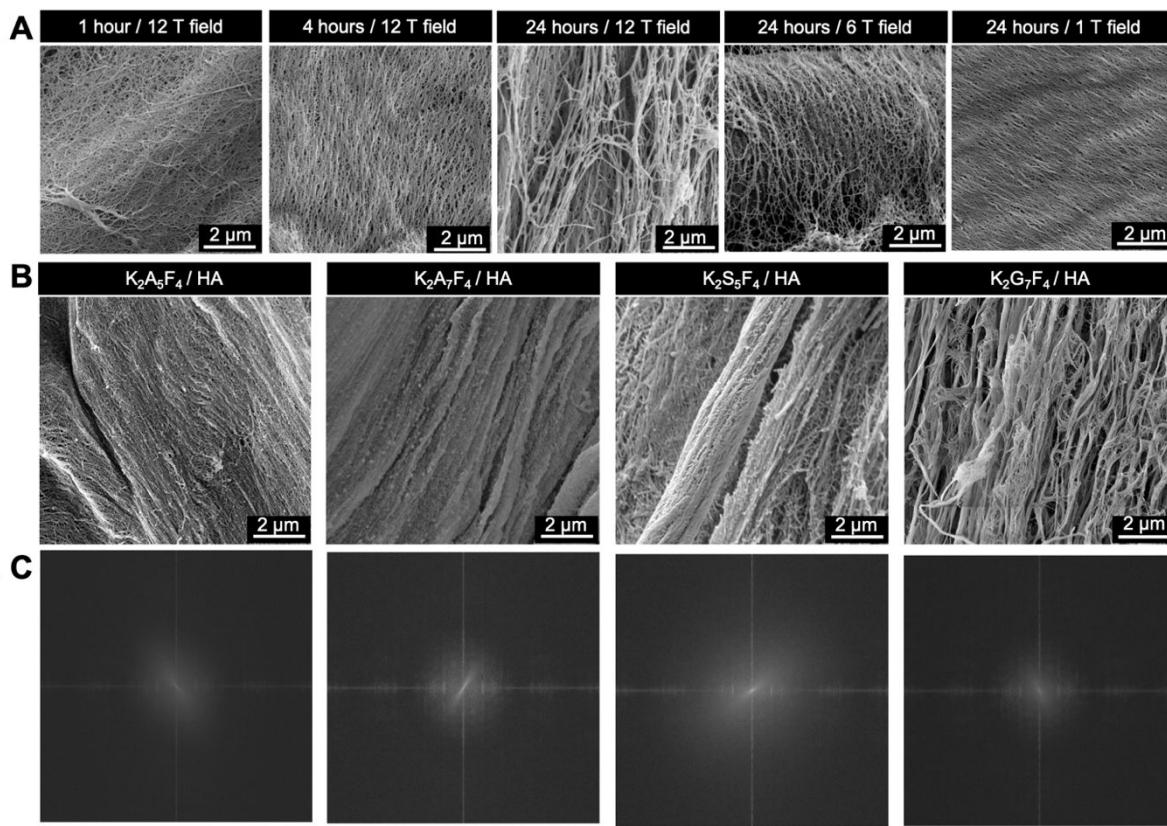


Figure 4. Effect of magnetic field during the membrane assembly on nanofiber alignment: (A) SEM images showing the surface of K₂A₅F₄/HA membranes formed under a magnetic field for different periods of time and of intensity and (B) membranes assembled with the designed peptides in the presence of 1 T magnetic field for 24 h ; (C) FFT analysis of aligned fibers membranes.

The effect of magnetic field intensity and exposure time was investigated on the K₂A₅F₄/1.5 MDa HA system at 1 and 6 T for 24 hours and 12 T for 1, 4 and 24 hours (Figure 4-A). Exposure time affected the alignment and only 1 hour exposing time is not enough to obtain uniform fiber alignment on the membrane. Fibers can be aligned at lower magnetic field, such as 1 T, if exposure time is longer (24 hours). The nanofiber alignment was confirmed by microstructure analysis (SEM) for all membranes at 1 T magnetic field (Figure 4-B, C). A peptide amphiphile (PA, C₁₆V₃A₃K₃) previously used to form membranes with HA, but not containing aromatic residues, was used as a control peptide. The PA/HA membranes were also formed under a 12 T magnetic field for 24 hours. Randomly distributed fibers of PA/HA were observed confirming the role of aromatic groups in the nanofiber alignment (Figure S11-A). The aligned fibers were maintained after incubating in water for one month, indicating their stability (Figure S11-B). The nanofiber organization of the membranes was also analyzed by polarized light microscopy. Due to their fibrillar characteristics, they showed structural birefringent properties.^{34,35} Figure S11-C shows the alignment of fibers enhanced the birefringence of the K₂A₅F₄/HA membrane in one direction compared to the membrane without magnetic field exposure. To further analyze the direction of the fibers, a graphite rod was used as a probe to confirm that fibers are aligned in the

direction of the magnetic field. Figure S12 shows the SEM images with the aligned fibers in the direction of the graphite pencil lead. Having the graphite on the peptide surface, the HA and peptide sides were identified.

Attachment of MSCs cultured on aligned peptide/HA membranes. In order to maintain proper function and morphology, cells need to attach to their surrounding environment. This attachment can be either binding to the ECM or to other cells.³⁶ Specific peptide segments in ECM proteins (e.g. RGDS in fibronectin) are known to act as anchoring points for binding to cell surface receptors (integrins) leading to the formation of focal adhesions.^{37,38} Here, we have investigated the effect of nanofiber alignment on the attachment of mesenchymal stem cells (MSCs) onto the various membranes.

The effect of nanofiber alignment on cell attachment was first studied on membranes formed under 1 T magnetic field and using serum-containing medium. Fluorescent microscopy images show the spreading and morphology of MSCs (Figure 5-A) on aligned and non-aligned membranes. Compared to cells on non-aligned membranes, MSCs elongated on the membranes in the direction of the aligned nanofibers, and this elongation is more evident for K₂G₇F₄/HA membranes (K₂G₇F₄ with highest diamagnetic anisotropy, Table S1). Cell attachment was also assessed in

1 serum-free conditions using $K_2A_5F_4/HA$ membranes and also on
2 membranes formed with 90% of $K_2A_5F_4$ and 10% of RGDS-
3 $K_2A_5F_4$ peptide. **Figure 5-B** compares the density and aspect ratio
4 of cells cultured on $K_2A_5F_4/HA$ membranes in presence or
5 absence of serum, and on RGDS-containing membranes in
6 serum-free medium. It is clear that the presence of FBS has a
7 significant role on cell adhesion and the fiber alignment tends to
8 favor cell attachment compared to non-aligned membranes, in
9 particular when membranes contain the RGDS cell-adhesive
10 sequence. We postulate that the magnetic alignment of the
11 nanofibers promoted the presentation of the RGDS epitopes on
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the surface, hence cell anchorage increased compared to non-aligned membranes.

Further investigation on the interaction of MSCs with these membranes by SEM analysis revealed the spreading of cells along the nanofibrous network of the membranes. Cells showed lamellipodia and filopodia, which are more extended in the direction of aligned fibers (**Figure 5-C**). Furthermore, cells are able to pull the nanofibers and seem to remodel these soft supramolecular membranes. Through the incorporation of RGDS sequence, membranes were able to promote the adhesion of MSCs under serum-free conditions and nanofiber alignment directed their spreading.

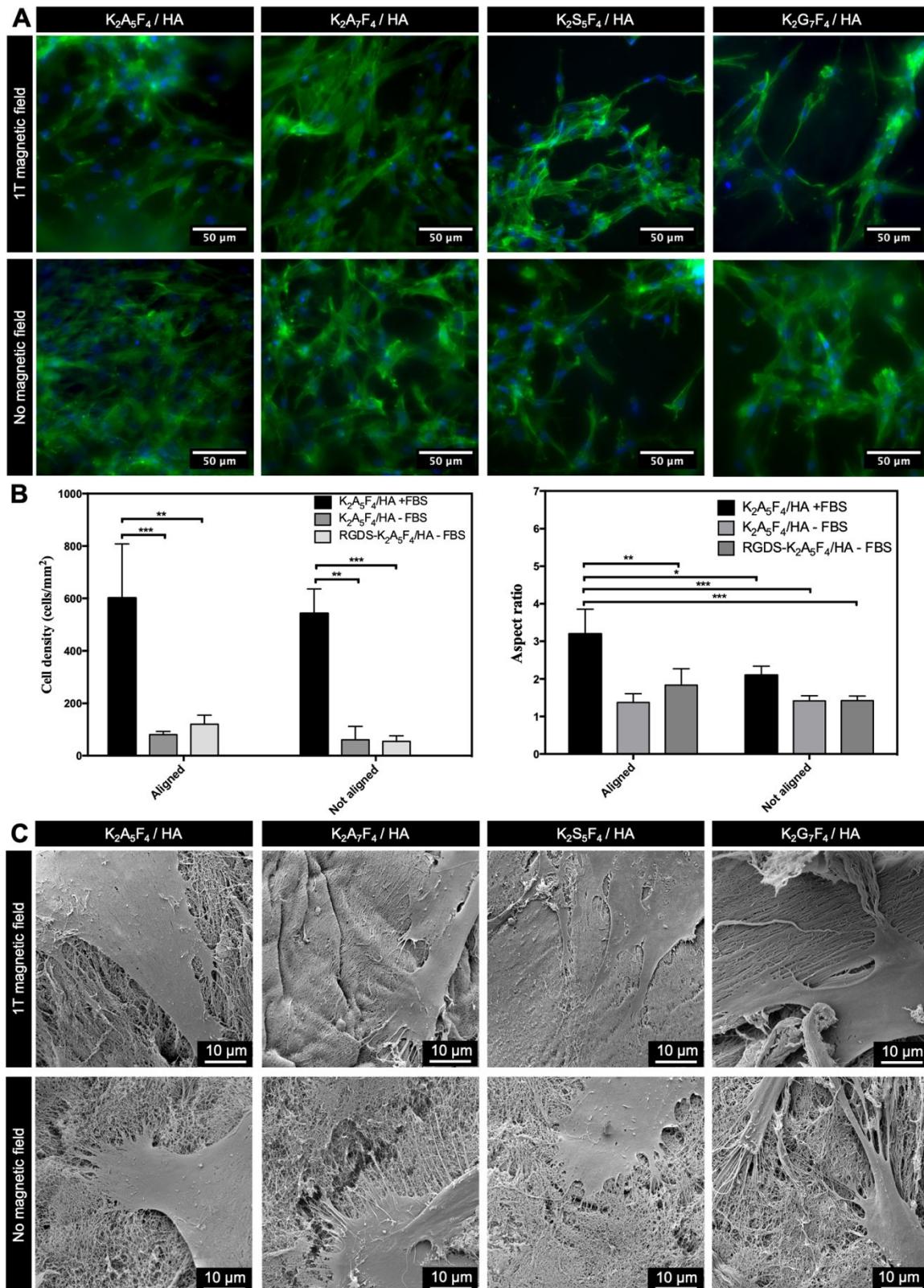


Figure 5. Cell behavior on the various peptide/HA membranes: (A) Fluorescence microscopy images of hMSCs cultured on aligned and non-aligned membranes (serum-containing cell media) for 24 hours; (B) Graphs displaying cell density (left) and cell aspect ratio (right) comparing culturing conditions (in presence of serum and serum-free) as well as the effect of RGDS on cell attachment on $K_2A_5F_4 / HA$ membranes; (C) SEM micrographs showing the cell interaction with the membrane nanofibrous surface (24 hrs) in the presence of serum.

CONCLUSION

Novel aromatic cationic peptides have been designed for supramolecular assembly with hyaluronic acid into hydrogels or membranes. The designed peptides can adopt different secondary structures depending on the pH and their self-assembly is driven by electrostatic screening followed by π - π stacking of phenylalanine residues. The linker segment in the peptide sequence was shown to affect the mechanical properties of the obtained membranes. The modulus of the resulting materials (hydrogels or membranes) can be tuned through the HA molecular size and concentration of components. Owing to the diamagnetic anisotropy of phenylalanine, the peptide nanofibers in the membranes were able to align while forming in the presence of magnetic field. The alignment of fibers enhanced the attachment of mesenchymal stem cells. These findings suggest the potential of these self-assembled membranes as cell culture substrates to study the effect of biochemical and physical (nanofiber organization) signaling on stem cell behavior.

ASSOCIATED CONTENT**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website.

Peptide characterization (HPLC, ESI-MS, CD, fluorescence and FTIR spectra; zeta potential, DA calculation, SAXS, CAC determination); Membrane characterization (thickness, SAXS data, SEM and polarized microscopy images) (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

Any funds used to support the research of the manuscript should be placed here (per journal style).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

H. S. A. thanks the financial support from the European Union (CIG Grant "SuprHApolymers," PCIG14-GA-2013-631871) and The Royal Society (Research Grant RSG\R1\180005). I. W. H. thanks EPSRC for the award of a Platform Grant (EP/L020599/1) and Diamond Light Source for the award of beamtime (SM18523-2 and SM21470-1) and Nathan Cowieson and Nikul Khunti for assistance during SAXS measurements.

X. L. and M. M. gratefully acknowledge the support by the U.S. National Science Foundation under Award No. 1710143. We also acknowledge Mr Maurizio Leo for his technical assistance with magnetic alignment experiments. We would also like to acknowledge the support EPSRC who funded the superconducting magnet to set up the MagMat facility (EP/M022714/1).

ABBREVIATIONS

CAC, critical aggregation concentration; CD, circular dichroism; DA, diamagnetic anisotropy; FTIR, Fourier Transform Infrared; HA, hyaluronic acid; HPLC, high performance liquid chromatography; hMSCs, human-derived mesenchymal stem cells; SAXS, small angle X-ray scattering; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

REFERENCES

(1) van der Gucht, J. Grand Challenges in Soft Matter Physics. *Front. Phys.* **2018**, *6*, 87.

(2) Yuan, C.; Ji, W.; Xing, R.; Li, J.; Gazit, E.; Yan, X. Hierarchically Oriented Organization in Supramolecular Peptide Crystals. *Nat. Rev. Chem.* **2019**, *1*–22.

(3) Lum, G. Z.; Ye, Z.; Dong, X.; Marvi, H.; Erin, O.; Hu, W.; Sitti, M. Shape-Programmable Magnetic Soft Matter. *Proc. Natl. Acad. Sci.* **2016**, *113* (41), E6007–E6015.

(4) Erb, R. M.; Martin, J. J.; Soheilian, R.; Pan, C.; Barber, J. R. Actuating Soft Matter with Magnetic Torque. *Adv. Funct. Mater.* **2016**, *26* (22), 3859–3880.

(5) Smal, N.; Löwik, D. W. P. M. Magnetic Fields to Align Natural and Synthetic Fibers. In *Self-assembling Biomaterials*; Elsevier, 2018; pp 321–340.

(6) A. Hill, R. J.; Sedman, V. L.; Allen, S.; Williams, P.; Paoli, M.; Adler-Abramovich, L.; Gazit, E.; Eaves, L.; Tendler, S. J. B. Alignment of Aromatic Peptide Tubes in Strong Magnetic Fields. *Adv. Mater.* **2007**, *19* (24), 4474–4479.

(7) Löwik, D. W. P. M.; Shklyarevskiy, I. O.; Ruizendaal, L.; Christianen, P. C. M.; Maan, J. C.; van Hest, J. C. M. A Highly Ordered Material from Magnetically Aligned Peptide Amphiphile Nanofiber Assemblies. *Adv. Mater.* **2007**, *19* (9), 1191–1195.

(8) Kwon, S.; Kim, B. J.; Lim, H.-K.; Kang, K.; Yoo, S. H.; Gong, J.; Yoon, E.; Lee, J.; Choi, I. S.; Kim, H. Magnetotactic Molecular Architectures from Self-Assembly of β -Peptide Foldamers. *Nat. Commun.* **2015**, *6*, 8747.

(9) Wallace, M.; Cardoso, A. Z.; Frith, W. J.; Iggo, J. A.; Adams, D. J. Magnetically Aligned Supramolecular Hydrogels. *Chem. Eur. J.* **2014**, *20* (50), 16484–16487.

(10) Torbet, J.; Malbouyres, M.; Builles, N.; Justin, V.; Roulet, M.; Damour, O.; Oldberg, Å.; Ruggiero, F.; Hulmes, D. J. S. Orthogonal Scaffold of Magnetically Aligned Collagen Lamellae for Corneal Stroma Reconstruction. *Biomaterials* **2007**, *28* (29), 4268–4276.

(11) Klara, S. S.; Saboe, P. O.; Sines, I. T.; Babaei, M.; Chiu, P.-L.; DeZorzi, R.; Dayal, K.; Walz, T.; Kumar, M.; Mauter, M. S. Magnetically Directed Two-Dimensional Crystallization of OmpF Membrane Proteins in Block Copolymers. *J. Am. Chem. Soc.* **2016**, *138* (1), 28–31.

(12) Mendes, A. C.; Smith, K. H.; Tejeda-Montes, E.; Engel, E.; Reis, R. L.; Azevedo, H. S.; Mata, A. Co-Assembled and Microfabricated Bioactive Membranes. *Adv. Funct. Mater.* **2013**, *23* (4), 430–438.

(13) Ribeiro, S.; Radvar, E.; Shi, Y.; Borges, J.; Pirraco, R. P.; Leonor, I. B.; Mano, J. F.; Reis, R. L.; Mata, Á.; Azevedo, H. S. Nanostructured Interfacial Self-Assembled Peptide-Polymer Membranes for Enhanced Mineralization and Cell Adhesion. *Nanoscale* **2017**, *9* (36), 13670–13682.

(14) Ferreira, D. S.; Marques, A. P.; Reis, R. L.; Azevedo, H. S. Hyaluronan and Self-Assembling Peptides as Building Blocks to Reconstruct the Extracellular Environment in Skin Tissue. *Biomater. Sci.* **2013**, *1* (9), 952–964.

(15) Babaei, M.; Jones, I. C.; Dayal, K.; Mauter, M. S. Computing the Diamagnetic Susceptibility and Diamagnetic Anisotropy of Membrane Proteins from Structural Subunits. *J. Chem. Theory Comput.* **2017**, *13* (6), 2945–2953.

(16) Yu, Z.; Tantakitti, F.; Palmer, L. C.; Stupp, S. I. Asymmetric Peptide Nanoribbons. *Nano Lett.* **2016**, *16* (11), 6967–6974.

(17) Shi, Y.; Hu, Y.; Ochbaum, G.; Lin, R.; Bitton, R.; Cui, H.; Azevedo, H. S. Enzymatic Activation of Cell-Penetrating Peptides in Self-Assembled Nanostructures Triggers Fibre-to-Micelle Morphological Transition. *Chem. Commun.* **2017**, *53* (52), 7037–7040.

(18) Hutchinson, J. A.; Hamley, I. W.; Edwards-Gayle, C. J. C.; Castelletto, V.; Piras, C.; Cramer, R.; Kowalczyk, R.; Seitsonen, J.; Ruokolainen, J.; Rambo, R. P. Melanin Production by Tyrosinase Activity on a Tyrosine-Rich Peptide Fragment and PH-Dependent Self-Assembly of Its Lipidated Analogue. *Org. Biomol. Chem.* **2019**, *17* (18), 4543–4553.

(19) Mendes, A. C.; Baran, E. T.; Reis, R. L.; Azevedo, H. S. Self-assembly in Nature: Using the Principles of Nature to Create Complex Nanobiomaterials. *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology* **2013**, *5* (6), 582–612.

(20) Aulisa, L.; Dong, H.; Hartgerink, J. D. Self-Assembly of Multidomain Peptides: Sequence Variation Allows Control over Cross-Linking and Viscoelasticity. *Biomacromolecules* **2009**, *10* (9), 2694–2698.

(21) Paramonov, S. E.; Jun, H.-W.; Hartgerink, J. D. Self-Assembly of Peptide–Amphiphile Nanofibers: The Roles of Hydrogen Bonding and Amphiphilic Packing. *J. Am. Chem. Soc.* **2006**, *128* (22), 7291–7298.

(22) Reches, M.; Gazit, E. Casting Metal Nanowires within Discrete Self-Assembled Peptide Nanotubes. *Science* **(80-.)** **2003**, *300* (5619), 625–627.

(23) Adler-Abramovich, L.; Vaks, L.; Carny, O.; Trudler, D.; Magno, A.; Caflisch, A.; Frenkel, D.; Gazit, E. Phenylalanine Assembly into Toxic Fibrils Suggests Amyloid Etiology in Phenylketonuria. *Nat. Chem. Biol.* **2012**, *8* (8), 701–706.

(24) Kelly, S. M.; Jess, T. J.; Price, N. C. How to Study Proteins by Circular Dichroism. *Biochim. Biophys. Acta (BBA)-Proteins Proteomics* **2005**, *1751* (2), 119–139.

(25) Smith, S. J.; Du, K.; Radford, R. J.; Tezcan, F. A. Functional, Metal-Based Crosslinkers for α -Helix Induction in Short Peptides. *Chem. Sci.* **2013**, *4* (9), 3740–3747.

(26) Shi, Y.; Ferreira, D. S.; Banerjee, J.; Pickford, A. R.; Azevedo, H. S. Tuning the Matrix Metalloproteinase-1 Degradability of Peptide Amphiphile Nanofibers through Supramolecular Engineering. *Biomater. Sci.* **2019**, *7* (12), 5132–5142.

(27) Krittanai, C.; Johnson Jr, W. C. Correcting the Circular Dichroism Spectra of Peptides for Contributions of Absorbing Side Chains. *Anal. Biochem.* **1997**, *253* (1), 57–64.

(28) Son, J.; Kalafatovic, D.; Kumar, M.; Yoo, B.; Cornejo, M. A.; Contel, M.; Ulijn, R. V. Customizing Morphology, Size, and Response Kinetics of Matrix Metalloproteinase-Responsive Nanostructures by Systematic Peptide Design. *ACS Nano* **2019**, *13* (2), 1555–1562.

(29) Mahler, A.; Reches, M.; Rechter, M.; Cohen, S.; Gazit, E. Rigid, Self-assembled Hydrogel Composed of a Modified Aromatic Dipeptide. *Adv. Mater.* **2006**, *18* (11), 1365–1370.

(30) Stuart, M. C. A.; van de Pas, J. C.; Engberts, J. B. F. N. The Use of Nile Red to Monitor the Aggregation Behavior in Ternary Surfactant–Water–Organic Solvent Systems. *J. Phys. Org. Chem.* **2005**, *18* (9), 929–934.

(31) Capito, R. M.; Azevedo, H. S.; Velichko, Y. S.; Mata, A.; Stupp, S. I. Self-Assembly of Large and Small Molecules into Hierarchically Ordered Sacs and Membranes. *Science* **(80-.)** **2008**, *319* (5871), 1812–1816.

(32) Bitton, R.; Chow, L. W.; Zha, R. H.; Velichko, Y. S.; Pashuck, E. T.; Stupp, S. I. Electrostatic Control of Structure in Self-Assembled Membranes. *Small* **2014**, *10*

1 (33) (3), 500–505.
2 Velichko, Y. S.; Mantei, J. R.; Bitton, R.; Carvajal, D.;
3 Shull, K. R.; Stupp, S. I. Electric Field Controlled
4 Self-Assembly of Hierarchically Ordered Membranes.
5 *Adv. Funct. Mater.* **2012**, *22* (2), 369–377.

6 (34) (37) Zhang, S.; Greenfield, M. A.; Mata, A.; Palmer, L. C.;
7 Bitton, R.; Mantei, J. R.; Aparicio, C.; De La Cruz, M. O.;
8 Stupp, S. I. A Self-Assembly Pathway to Aligned
9 Monodomain Gels. *Nat. Mater.* **2010**, *9* (7), 594–601.

10 (35) (38) Murphy, D. B.; Spring, K. R.; Fellers, T. J.; Davidson, M. W. Principles of Birefringence
11 https://www.microscopyu.com/techniques/polarized-light/principles-of-birefringence.

12 (36) Ruoslahti, E. RGD and Other Recognition Sequences for
13 Integrins. *Annu. Rev. Cell Dev. Biol.* **1996**, *12* (1), 697–715.

14 Storrie, H.; Guler, M. O.; Abu-Amara, S. N.; Volberg, T.;
15 Rao, M.; Geiger, B.; Stupp, S. I. Supramolecular Crafting
16 of Cell Adhesion. *Biomaterials* **2007**, *28* (31), 4608–4618.

17 Hirano, Y.; Okuno, M.; Hayashi, T.; Goto, K.; Nakajima,
18 A. Cell-Attachment Activities of Surface Immobilized
19 Oligopeptides RGD, RGDS, RGDV, RGDT, and YIGSR
20 toward Five Cell Lines. *J. Biomater. Sci. Polym. Ed.* **1993**,
21 4 (3), 235–243.

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