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Amyloid Peptide Mixtures: Self-Assembly, Hydrogelation, Nematic Ordering, and Catalysts in Aldol Reactions

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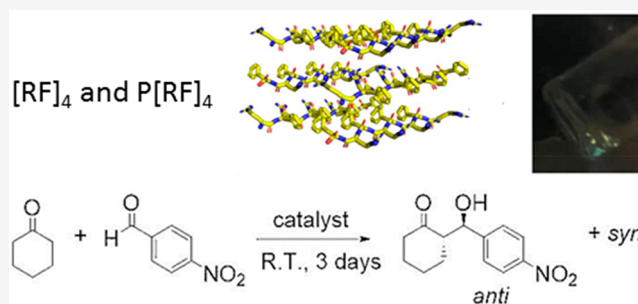


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ABSTRACT: Morphological, spectroscopic, and scattering studies of the self-assembly and aggregation of mixtures of [RF]₄ and P[RF]₄ peptides (where R = arginine; F = phenylalanine; P = proline), in solution and as hydrogels, were performed to obtain information about polymorphism. CD data confirmed a β -sheet secondary structure in aqueous solution, and TEM images revealed nanofibers with diameters of \sim 10 nm and micrometer lengths. SAXS curves were fitted using a mass fractal-component and a long cylinder shell form factor for the liquid samples, and only a long cylinder shell form factor for the gels. Increasing the P[RF]₄ content in the systems leads to a reduction in cylinder radius and core scattering density, suggesting an increase in packing of the peptide molecules; however, the opposite effect is observed for the gels, where the scattering density is higher in the shell for the systems containing higher P[RF]₄ content. These compounds show potential as catalysts in the asymmetric aldol reactions, with cyclohexanone and *p*-nitrobenzaldehyde in aqueous media. A moderate conversion (36.9%) and a good stereoselectivity (69:31) were observed for the system containing only [RF]₄. With increasing P[RF]₄ content, a considerable decrease of the conversion was observed, suggesting differences in the self-assembly and packing factor. Rheological measurements were performed to determine the shear moduli for the soft gels.



INTRODUCTION

Over the last years, self-assembled amphiphilic oligopeptides systems have attracted strong interest due to their potential for bionanotechnological applications for new materials and in the development of diagnostic and therapeutic strategies, where protein and peptide aggregation is associated with disease.^{1–4} Peptide amphiphiles (PAs) are structures based on hydrophobic blocks such as lipid chains, and bioactive peptides, which correspond to the hydrophilic part.^{5,6} Among several promising uses, we can highlight the development of sensing devices,⁷ molecular carriers,^{8,9} and bioelectronics.¹⁰ Many advantages, especially the fast synthesis, functionalization capabilities, and relatively low cost, confirm the potential of these systems.^{2,11–16}

One of the significant advantages of peptide amphiphiles as models for the aggregation process studies is their capability of self-assembly into distinct polymorphs.^{11,12,17} Thus, these systems are appropriate architectures to create biomimetic materials and establish interfaces with biological systems. The combination of amino acids with different molecular characteristics leads to amphiphilic compounds which can produce polymorphs depending on concentration and pH. Sequences based on *L*-phenylalanine (F), *L*-arginine (R), and *L*-proline (P) combine π -stacking interactions for the aromatic ring in the phenylalanine side chain,^{18,19} electrostatic interactions of the

positively charged arginine side chain,²⁰ and hydrophobic properties of the compact proline residue, which is a motif widely used in organocatalysis.²¹ Our group has observed that the concentration of these arginine/phenylalanine-based oligopeptides can influence the shape and morphology of the particles formed.¹⁷ Also, we saw that the polymorphism is fundamental for understanding the packing of these compounds, in which the presence of proline promotes more rigid and compact systems.²²

Considering the amyloid oligopeptides [RF]₄ and P[RF]₄, in our previous work,²² we found the same critical aggregation concentration (cac) in aqueous media, at 25 °C, for both systems (cac = 0.35 mmol·L⁻¹) by steady-state fluorescence spectroscopy, indicating that addition of the hydrophobic proline residue does not affect the aggregation propensity. By electron microscopy and small-angle scattering, we observed that, depending on the peptide concentrations, spherical or elongated aggregates are formed, which have radius dimen-

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sions of 9.5 and 7.5 nm for $[RF]_4$ and $P[RF]_4$, respectively. These peptides were tested as catalysts in aldol reactions involving *p*-nitrobenzaldehyde and cyclohexanone. Contrary to expectation, better conversion and stereoselectivity were observed for the systems containing $[RF]_4$. This effect was attributed to the higher packing factor of $P[RF]_4$ self-assembly, which hindered reagent attack on the substrate surface, enhancing the enamine transition state.

To examine what happens in these systems if we mix both arginine/phenylalanine peptides mainly in terms of the aldol reaction efficiency, here we investigated the self-assembly and aggregation state for mixtures of the amphiphilic amyloid peptides $[RF]_4$ and $P[RF]_4$, using spectroscopy, microscopy, and scattering techniques. The combination of these amphiphilic oligopeptides has great potential to produce novel biomaterials and enhance understanding of their self-assembly and polymorphism. It may be possible to create systems with different functional and structural properties when compared to separate peptides.^{2,3}

EXPERIMENTAL METHODS

Synthesis of Amyloid Peptides. $[RF]_4$ and $P[RF]_4$ sequences were synthesized using a solid-phase Fmoc strategy.²⁴ All chemicals were of analytical or HPLC grades. The protected amino acids, (Fmoc-F-OH), (Fmoc-R(Pbf)-OH), and (Fmoc-P-OH), 1,3-diisopropylcarbodiimide/*N*-hydroxybenzotriazole (DIC/HOBT), trifluoroacetic acid (TFA), anisole, thioanisole, dichloromethane (DCM), dimethylformamide (DMF), 1-methyl-2-pyrrolidinone (NMP), and 1,2-ethanedithiol (EDT) were purchased from Sigma-Aldrich (St. Louis, MO). Wang resin with 100–200 mesh size was purchased from Advanced Chemtech (Louisville, KY), with a substitution degree of 0.55 mmol·g⁻¹ and with the first amino acid coupled to the polymeric support. The protecting group was removed by reaction with 20% of 4-methylpiperidine in dimethylformamide for 30 min. Coupling was carried out in 5.0 fold excess of DIC/HOBT in DCM/DMF (1:1, *v:v*). The reactions were monitored using the Kaiser ninhydrin test.²⁵ The dry-protected resin was exposed to 90% trifluoroacetic acid, 5.0% thioanisole, 3.0% 1,2-ethanedithiol, and 2.0% anisole to remove all the protecting groups. After this, the material was lyophilized and analyzed on a liquid-chromatography electrospray ionization mass spectrometer, LC-ESI-MS, yielding $[RF]_4$ (MM + H) = 1232.6 (calculated = 1231.6) and $P[RF]_4$ (MM + H) = 1328.7 (calculated = 1327.7).²² The molecular structures are showed in Figure 1.

Mixing Solutions Containing the Peptides $P[RF]_4$ and $[RF]_4$. Solutions containing 0.5 wt % of each peptide were prepared and from these solutions, five systems, with different proportions of $P[RF]_4$: $[RF]_4$ (*v/v*) were made at native pH ~ 4, i.e. 0:1 (1), 3:7 (2); 5:5 (3), 7:3 (4), and 1:0 (5). All solutions were prepared with purified water from a Thermo Scientific Barnstead NANOpure system, with a resistivity of 18.2 mΩ·cm⁻¹ (at room temperature) and TOC below 10 ppb.

$P[RF]_4$ and $[RF]_4$ Hydrogels. Hydrogels of systems 1, 3, and 5 were prepared using 3 wt % of each peptide. The gelation of 3 was observed after 10 min, while heating (60 °C, 5 min) followed by cooling (-8 °C, 5 min) was performed for 1 and 5.

Spectroscopy Methods. Fourier transform infrared (FTIR) assays were recorded in the amide band regions using a PerkinElmer Spectrum 100 FTIR spectrometer. Aliquots (100 μL) of the sample (0.5 wt % peptide solution in D₂O) were sandwiched between two CaF₂ plate windows using a plastic spacer of 0.006 mm. The spectra were scanned 16 times in absorbance mode over the range 1800–1300 cm⁻¹, with 4 cm⁻¹ resolution and 1 cm⁻¹ data interval. A D₂O spectrum was used as background and subtracted from the samples' experimental data.

Circular dichroism (CD) measurements were performed using a Chirascan spectropolarimeter (Applied Photophysics, UK). Solutions containing 0.5 wt % of peptide were scanned at 25 °C in a quartz

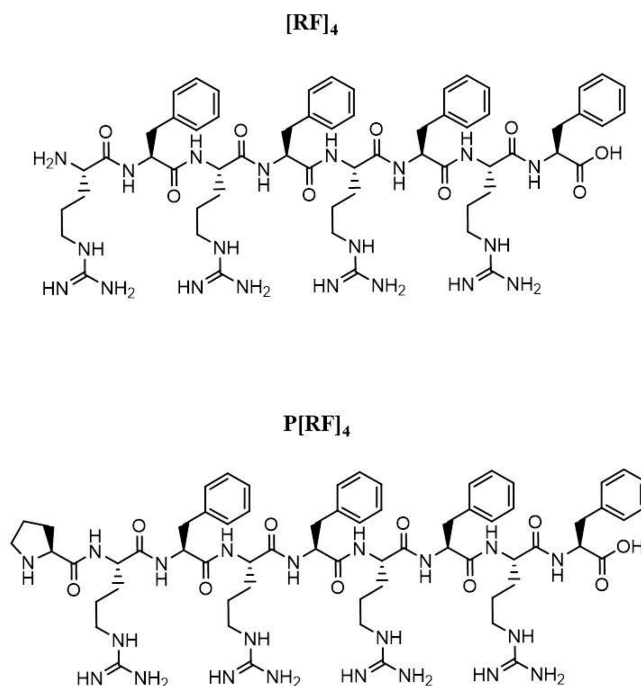


Figure 1. $[RF]_4$ and $P[RF]_4$ molecular structures.

cuvette with a thickness of 0.01 mm. The spectra were recorded with absorbance $A < 2$ at any measured point, considering 0.5 nm step, 1 nm bandwidth, and 1 s collection time per step. A water background CD signal was used to subtract the baseline from the experimental data.

TEM Imaging. Transmission electron microscopy (TEM) was performed on a JEOL 2100Plus instrument operating at 200 kV. Copper grids (Agar Scientific, UK) 5.0 mm in diameter and 10 μm thick, coated with carbon film, were used. The samples were stained with 1 wt % uranyl acetate (Sigma-Aldrich, UK) and left to dry at room temperature.

Small-Angle X-ray Scattering and X-ray Diffraction. Small-angle X-ray scattering (SAXS) was performed on beamline B21, Diamond Light Source, Didcot, UK. An EMBL BioSAXS robot containing a 96-well plate was used, where 100 μL of the 0.5 wt % peptide solutions was injected via an automated sample exchanger into a quartz capillary, with 1.8 mm internal diameter, in a vacuum chamber. For the hydrogels, 50 μL of each sample was placed in a custom-designed holder and covered with Kapton. The SAXS beamline was operated with a fixed camera length of 3.9 m and X-ray energy of 12.4 keV, respectively, and the SAXS pattern was recorded using a PILATUS 2 M detector, with 28 s per frame. Data processing was performed using ScÅtter, and fitting was performed using SASfit.²⁶

Fiber diffraction patterns were recorded using an Oxford Diffraction Gemini Ultra diffractometer with Cu Kα radiation. For this, oriented stalks were made by suspending droplets of a 5 wt % peptide solution between the ends of two wax-coated capillaries, letting them dry at room temperature. The data were collected by mounting the stalks vertically in the X-ray diffractometer and collecting diffraction patterns with a Rigaku HyPix-6000HE Hybrid Photon Counting detector, with a sample-to-detector distance of 140 mm, varying the beam angles.

Aldol Reactions. The catalytic efficiency of the peptide mixtures was monitored by the direct aldol reaction using cyclohexanone and *p*-nitrobenzaldehyde. The reactions were performed using different catalyst amounts of 5, 10, and 20 mol %. Considering the 5 mol % catalyst solutions, 20.2 μL of cyclohexanone (0.19 mmol), 1.0 mg of catalyst (0.81 μmol), 2.45 mg of *p*-nitrobenzaldehyde (1.6 μmol), and 40.4 μL of water were used. The solutions were stirred at room temperature for 3 days, and the mixtures were extracted with ethyl

acetate four times via centrifugation at 4000 rpm for 5 min. The organic phase was then removed on a rotary evaporator, and the samples were solubilized using deuterated chloroform. NMR measurements using a (^1H) Bruker Ultrashield Plus 400 instrument were performed at 400 MHz. The yield and diastereomer anti:syn ratio were calculated using the NMR spectra obtained, for which tetramethylsilane (TMS) was used as a reference.

Rheology. Dynamic shear rheometry was performed using a TA Instruments AR-2000 rheometer (TA Instruments). The shear moduli of hydrogels 1, 3, and 5 were measured using a cone-and-plate geometry (cone radius = 20 cm; cone angle = 1°). The linear regime was first determined via stress sweep experiments in the range of 0.1–500 Pa at a constant angular frequency of 6.28 rad/s and 20 $^\circ\text{C}$. Oscillatory frequency sweep experiments were then performed at constant stress within the linear regime and angular frequencies from 0.1 to 200 rad/s at 20 $^\circ\text{C}$.

RESULTS

Characterization of the Mixtures of $[\text{RF}]_4$ and $\text{P}[\text{RF}]_4$.

Following our previous study comparing $[\text{RF}]_4$ and $\text{P}[\text{RF}]_4$,²² we monitored the self-assembly of the mixtures of these peptides in aqueous solution. Considering the critical aggregation concentration (cac) of $\sim 0.35 \text{ mmol L}^{-1}$ for both $[\text{RF}]_4$ and $\text{P}[\text{RF}]_4$ at 25 $^\circ\text{C}$,²² solutions above the cac (0.5 wt %) of each peptide were prepared as stock solutions, and then the v/v mixtures of peptides at native pH (~ 6) were characterized.

FTIR and CD measurements were performed to analyze changes in the peptide secondary structure. The FTIR spectra are shown in Figure 2a. Four bands were observed for both samples. More details of the peaks are provided in Table S1. All the solutions have a sharp peak at approximately 1672 cm^{-1} (peak 1), attributed to the vibrations from TFA counterions

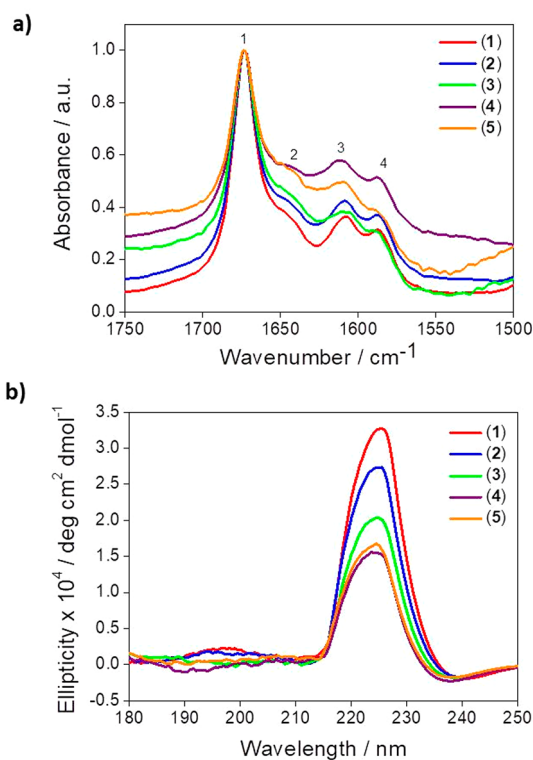


Figure 2. Spectroscopic characterization of $\text{P}[\text{RF}]_4$: $[\text{RF}]_4$ mixtures 0:1 (1), 3:7 (2), 5:5 (3), 7:3 (4), and 1:0 (5), above the cac in water, using (a) FTIR and (b) CD.

bound to peptides molecules.²⁷ Peak 2 at $1638\text{--}1646 \text{ cm}^{-1}$ is less pronounced but is correlated with unordered structures present in the system.²² The vibrational bands of the arginine side chain^{22,28,29} lead to peaks 3 and 4 (1607 and 1585 cm^{-1} , respectively). Also, peak 3 may be assigned to a contribution from β -sheet.¹⁷

CD spectra presented in Figure 2b show two positive bands, one located at $\sim 195 \text{ nm}$, correlated with antiparallel β -sheet conformation,²² and the other at 219 nm , characteristic of phenylalanine $n\text{--}\pi^*$ electronic transitions.^{30,31} Looking at the CD absorption spectra in this region (Figure S1), a hypsochromic effect and an enhancement of the absorption band intensity can be observed as the amount of proline-peptide increases in the mixtures. This effect can be correlated to a more hydrophobic environment with a higher $\text{P}[\text{RF}]_4$ content, due to the presence of the imino groups present in the proline side chain. The decrease in the intensity of the CD bands was attributed to the interacting ordered π -conjugated systems, a common feature of the β -sheet structure, with a contribution also from a β -turn-like conformation. Consequently, a slight enhancement of the CD peak width was observed for the systems containing more of the proline-containing peptide, as can be seen in Figure 2b.

Fiber X-ray diffraction was performed to obtain structural information and the influence of proline on the secondary structure. The fiber XRD patterns were reduced to one-dimensional intensity profiles, which show six peaks (Figure 3). Table 1 summarizes the associated d -spacings.

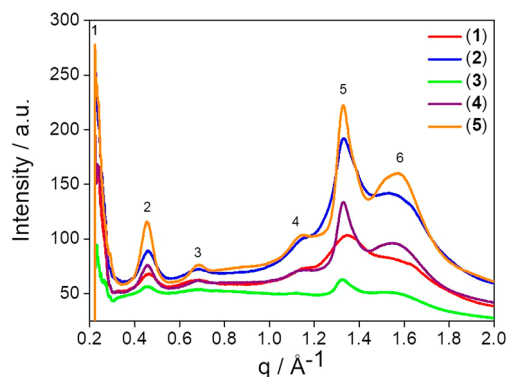


Figure 3. XRD results obtained of $\text{P}[\text{RF}]_4$: $[\text{RF}]_4$ mixtures 0:1 (1), 3:7 (2), 5:5 (3), 7:3 (4), and 1:0 (5), at native pH.

The spacing $d \sim 4.7 \text{ \AA}$ (peak 5) can be associated with the separation between adjacent peptide backbones organized into β -strands,³² and the $d \sim 9.4 \text{ \AA}$ peak corresponds to the antiparallel organization of the chains, which is characterized

Table 1. Summary of the XRD Results of $\text{P}[\text{RF}]_4$: $[\text{RF}]_4$ Mixtures 0:1 (1), 3:7 (2), 5:5 (3), 7:3 (4), and 1:0 (5), at Native pH

sample	$d \text{ (\AA)} \pm 0.1 \text{ \AA}$					
	1	2	3	4	5	6
1	28.6	13.7	9.2	5.5	4.6	3.8
2	28.6	13.7	9.2	5.6	4.7	4.0
3	27.3	13.7	9.2	5.7	4.7	4.0
4	28.6	13.9	9.2	5.5	4.7	4.0
5	28.6	13.7	9.1	5.5	4.7	4.0

by twice the β -strand separation.^{33,34} The peaks at approximately 28.6 Å (1), 13.7 Å (2), and 9.2 Å (3) suggest the presence of lamellar ordering.^{20,35} The peaks 5.5 Å (4) and 4.0 Å (6) correspond to a fraction of peptide in unordered conformation.³⁶ Thus, the peaks can be indexed to an orthorhombic unit cell, with $a = 28.6$ Å, $b \sim 9.2$ Å, and $c \sim 9.2$ Å.³⁵ The spacings in Table 1 are the same for all systems within estimated uncertainties. A representative illustration of the packing of the peptide chain in the unit cell is shown in Figure 4.

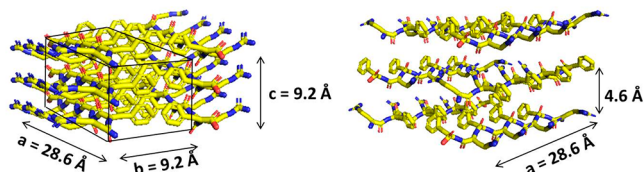


Figure 4. Schematic representation of the orthorhombic unit cell, considering the dimensions obtained by fiber XRD for [RF]₄.

TEM was used to image self-assembled structures above cac (at native pH). This revealed the presence of long fibers with lengths on the micrometer scale. Figure 5a–e shows the

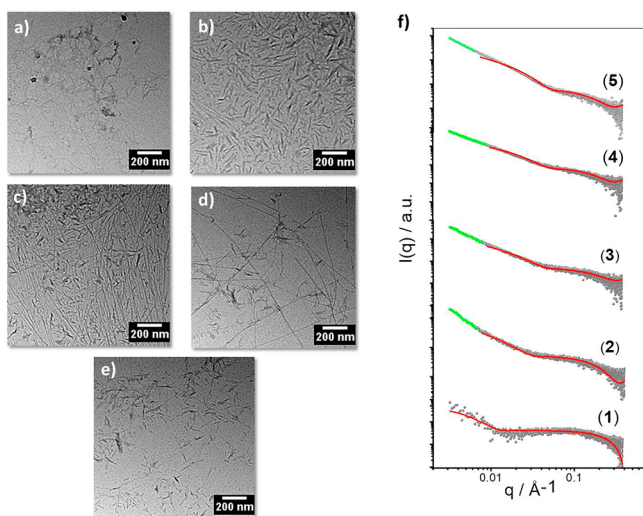


Figure 5. TEM images of 0.5 wt % peptide aqueous solutions. (a) 1, (b) 2, (c) 3, (d) 4, and (e) 5, at native pH. (f) SAXS data (gray points) of peptide solutions above the cac and at native pH. Model fits (red line) using the model described in the text and fitting excluding data (green line), characteristic of the structure factor.

influence on the orientation and size of the structures with changes in the composition of the mixtures. The system containing [RF]₄ (1) was characterized by thin fibers, with diameters <10 nm, and the coexistence of globular fractal aggregates. Adding P[RF]₄, a predominance of fibers with approximately 10 nm diameter was observed. For the mixtures, the TEM images reveal for 2 the presence of short fibers, for 3 the coexistence of short and long fibers, and for 4 the appearance of long fibers, similar to the system containing only P[RF]₄ (5).

SAXS measurements were performed to obtain more detail about the nanoscale shape and dimensions in the mixtures. A first analysis of the curves, considering the intensity decay in the Guinier regime, provides information about the form factor, as can be seen in Figure S2. Peptide P[RF]₄ and the mixtures show $I \sim q^{-n}$ with $n = 1.7$ and 1.6 to 1.8, respectively, which suggest a surface mass fractal form factor,²⁶ while the [RF]₄ samples have $n = 2.5$, characteristic of a volumetric mass fractal.²⁶ Structure factor effects at low q were excluded (as indicated by the green line regions in Figure 5f).³⁷

The SAXS data were fitted using the software SASFit.²⁶ The red curves in Figure 2f represent adjusted form factors. System 1 was modeled by applying a mass fractal form factor. The spectroscopy and microscopy results discussed above indicate that the other systems (2–5) are characterized by an extended β -sheet structure range. Consequently, a long cylindrical shell model (with fixed cylinder length, 1000 nm) was used as a second form factor, just adjusting the weighting of the two contributions. Also, for both form factor models, a Gaussian size distribution (σ_R) of the radius was included. Table 2 summarizes the fitted parameters.

The parameters in Table 2 indicate a gradual increase of the fractal cluster radius (R') with increasing P[RF]₄ content in the systems, from 0.65 nm for sample 1 to 1.0 nm for 5. However, a decrease of fractal dimension (D) was observed as the P[RF]₄ content increases, suggesting the presence of more compact clusters. Table 2 also shows a decrease in the cylinder radius (R) and the shell thickness of the cylinders (ΔR) with increasing P[RF]₄ content. This effect suggests that the proline helps molecular packing, leading to more compact structures. The model fitting also reveals variations in the (electron) scattering length density of the core (η_{core}) and the shell (η_{shell}). A considerable increase of η_{core} was observed in the samples with more P[RF]₄, which also has a lower radius. These results confirm the higher packing of the peptide molecules in the core of the fibers.

On the basis of the observed fiber XRD modeling (Figure 3) and the observed fibril structures from cryo-TEM and SAXS, we propose that the β -sheets are arranged in a typical amyloid fashion with the sheets parallel to the fiber axis and the β -

Table 2. Summary of the Model Parameters Obtained from the Fitting Procedure of SAXS Data^a

sample	mass fractal Gaussian				long cylindrical shell						fit quality	
	N_1	R' (nm)	σ_R (nm)	D (nm)	N_2	R (nm)	σ_R (nm)	ΔR (nm)	η_{core}	η_{shell}	R_v	χ^2
1	0.95	0.65	0.002	2.0	0.05	21.4	0.33	1.55	4.6×10^{-9}	2.2×10^{-9}	0.19	0.17
2	0.30	0.91	0.04	2.6	0.70	9.4	1.85	0.42	8.0×10^{-9}	4.0×10^{-8}	0.16	0.18
3	0.10	0.98	0.05	2.2	0.90	4.2	0.90	0.75	3.4×10^{-8}	1.8×10^{-7}	0.22	0.57
4	0.20	1.03	0.005	1.6	0.80	3.0	1.00	0.90	6.5×10^{-8}	2.5×10^{-7}	0.27	1.81
5	0.10	1.03	0.005	1.5	0.90	2.8	1.00	0.91	7.0×10^{-8}	2.7×10^{-7}	0.29	2.62

^aThe scattering length density of the solvent (η_{solv}) was fixed at 9.7×10^{-9} . N_1 and N_2 represent the fractional contribution of the two form factor components to the total intensity. Fit quality is assessed via the R -factor (R_v) and the reduced chi square (χ^2) value.

strands perpendicular to it. The dispersity in the cylinder radius points to differences in the number of β -sheet stacks.

In our previous work, we found that these peptides can be used as organocatalysts in direct aldol reactions, involving cyclohexanone and *p*-nitrobenzaldehyde in water. In this case, a dependence on the reaction efficiency on peptide packing was observed.²² We saw lower conversion and stereoselectivity for P[RF]₄ systems due to the formation of more elongated structures, in which the neighboring aromatic rings may be tightly packed, hindering the reactant's interactions with the active sites.^{38–42} Here, the efficiency of the P[RF]₄:[RF]₄ mixtures as catalysts was monitored considering the direct aldol reactions using cyclohexanone and *p*-nitrobenzaldehyde, examining whether it is possible to enhance the conversion in the combinations.

The reactions were performed at room temperature for 3 days, varying the catalyst amount (5 mol % and 20 mol %) in aqueous media and at native pH. The results are summarized in Table 3. The NMR results can be seen in Figure S3, and the

Table 3. Comparative Results of the Aldol Reactions between *p*-Nitrobenzaldehyde and Cyclohexanone Catalyzed by the Peptide Mixtures in the Water at Native pH^a

entry ^b	sample	catalyst (mol %)	H ₂ O ^c (equiv)	conv ^d (%)	anti:syn ^e	ee (%)
1 (s)	1	5	2	36.9	69:31	58
2 (s)	2	5	2	28.7	70:30	51
3 (s)	3	5	2	18.1	71:29	58
4 (s)	4	5	2	7.0	64:36	38
5 (s)	5	5	2	12.7	70:30	6.1
6 (g)	1	20	2	57.4	57:43	50
7 (g)	2	20	2	42.4	70:30	37
8 (g)	3	20	2	45.0	72:28	43
9 (g)	4	20	2	43.9	76:24	13
10 (g)	5	20	2	53.9	60:40	18

^aThe reactions were promoted at room temperature under vigorous stirring for 3 days, using 12 equiv of cyclohexanone, 1 equiv of *p*-nitrobenzaldehyde, 5–20 mol % of catalyst, 2 × volume of cyclohexanone for water. ^b(s) indicates molecules in solution; (g) indicates supramolecular gels. ^cExcess water relative to cyclohexanone (*v/v*). ^dConversion and diastereoselectivity were determined by ¹H NMR analysis of the crude product. ^eDiastereomeric anti:syn ratios were determined by ¹H NMR analysis of the crude product. ^fDetermined by chiral HPLC analysis of the anti-isomer.

HPLC results for the racemic aldol product are shown in Figure S4. For the lowest catalyst concentration, homogeneous solutions were observed, while with the increase of catalyst concentration to 20 mol %, supramolecular gels were formed during the reaction. Considering the results presented in Table 3, the best response is represented by entry 1, which used 5 mol % of [RF]₄ peptides which led to moderate conversion (36.9%) and a good stereoselectivity (69:31), with 58% enantiomeric excess (ee). As the amount of P[RF]₄ increases, a considerable decrease of the conversion was observed, especially for entry 4 compared to the other 5 mol % catalyzed reactions. Entry 5, containing only P[RF]₄ as catalyst, presented a low conversion, but better than the system characterized by entry 4 (70% P[RF]₄ and 30% [RF]₄), and the lowest ee. This effect can be correlated with the packing factor, because more compact systems can hinder the attack of the reagents onto the catalytic surface, increasing the energetic

barrier to the enamine transition state formation and consequently decreasing the efficiency of the aldol reactions. As observed, for example by Díaz and co-workers,⁴³ the absence of the catalyst prevents conversion for the monitored aldol reaction. Pioneering studies by Hajos and Parrish⁴⁴ and by Eder, Sauer, and Wiechert⁴⁵ described the first organocatalyzed intramolecular aldol reaction by L-proline, resulting in high yield and excellent enantioselectivity,^{45,46} proving the importance of a proline-based catalyst. The reactions mediated by proline feature high enantioselectivity, due to its ability to promote the formation of highly organized transition states, and its high nucleophilic reactivity with carbonyl compounds compared with primary amino acids.^{47,48}

The same effect was observed considering the 20 mol % catalyst reactions, but in this case, the difference between the reaction conversions and enantiomeric excess was lower, except for Entry 10, which presents an increase of three times the *ee* in comparison with the Entry 5. Also, the peptide mixtures showed similar conversion and high diastereoselectivity; Entry 9 corresponds to the best conditions with good conversion (43.9%) and stereoselectivity (76:24).

Due to the presence of self-assembled extended fibrils, we reasoned that at high concentrations, hydrogelation might be observed.^{49,50} Even without pH adjustment, hydrogel formation was observed at 3 wt % of each peptide, soft gels being obtained, as can be seen in Figure 6a. To get information about

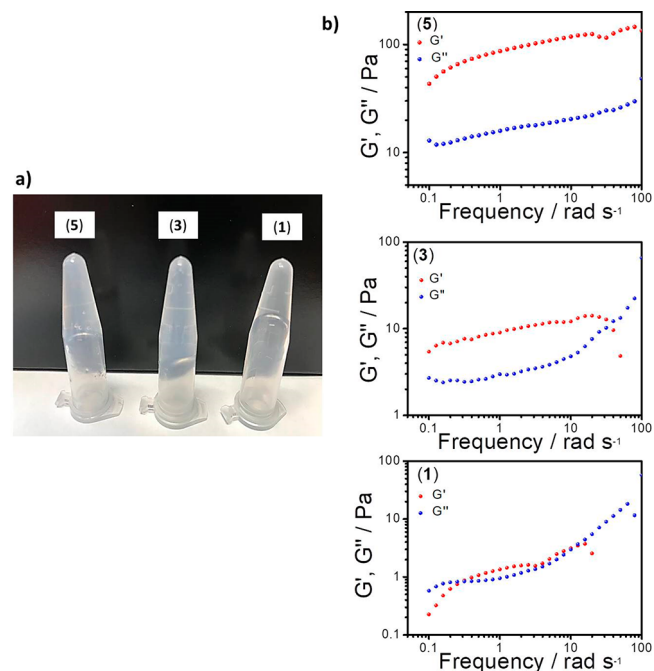


Figure 6. (a) Images of the hydrogels formed by 3 wt% peptide samples, as indicated. (b) Storage and shear modulus obtained by rheology experiments in frequency sweep mode, using $\sigma = 3$ Pa for 1, 1 Pa for 3, and 5 Pa for 5.

the viscoelastic properties of these materials, rheology experiments were performed. Initially, the stress (σ) was varied at a constant frequency ($\omega = 6.28$ rad s⁻¹) to determine the linear viscoelastic regime. Then frequency sweeps were performed at a stress value in the linear regime. Figure S5 shows the linear regime extends up to $\sigma = 5$ to 10 Pa, depending on the mixture composition.

Frequency sweep measurements were then performed. Figure 6b shows a low-frequency Newtonian plateau in the moduli with $G' > G''$, for the systems 3 and 5, consistent with a well-defined gel-like response.^{51–53} For 1, G' and G'' are similar in magnitude and have plateau values of $G' < 1$ Pa, which is characteristic of a soft gel.⁵⁴

SAXS was used to characterize the structure within the gels. The SAXS fitting curves of the 3 wt % hydrogels, shown in Figure 7a, were obtained using a long cylindrical shell form

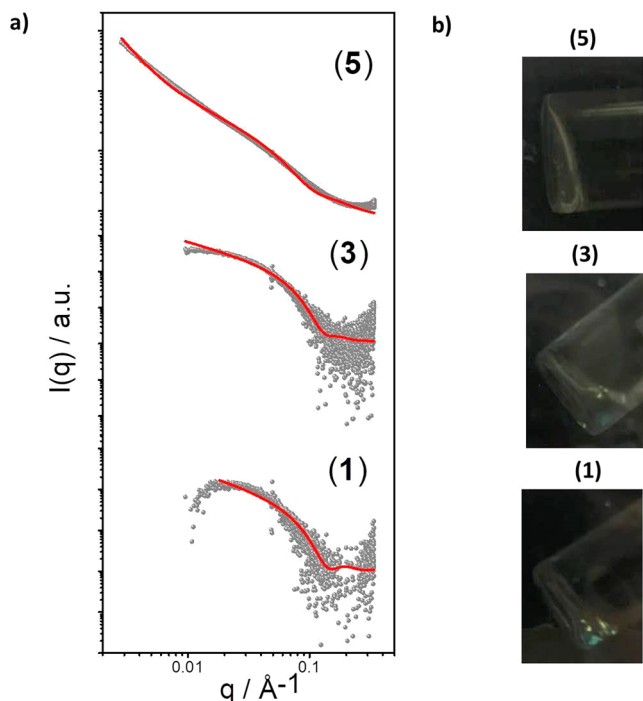


Figure 7. (a) SAXS data (gray) and fitted curves (red) using a long cylinder shell form factor model, for 3 wt % hydrogels of 1, 3, and 5. (b) Images of the birefringence between crossed polarizers for 3 wt % hydrogels of 1, 3, and 5.

factor. Systems 1 and 3 presented less information at the low q region, indicating more unordered structures. The fitted parameters are shown in Table S2, and they show a gradual decrease in fibril radius increasing the P[RF]₄ content, from 2.5 nm for sample 1 to 0.7 nm for 5. The fibril core radius and shell thickness values are lower than for the corresponding SAXS patterns for solutions (Table 2) showing that the hydrogels contain finer stranded fibril network structures. A similar shell thickness of the cylinders was observed for all systems, and considering scattering length density, samples 1 and 3 show higher core electron density. However, the scattering density value difference between the core and the shell for the system 1 is more significant than for 3. The opposite was found for the system 5, which shows a concentration of electron density in the shell.

Remarkably, the hydrogels were found to show birefringence, placing the 3 wt % hydrogels, contained in glass flasks, between crossed polarizers (Figure 7b). An increase of the anisotropy is seen for the sample containing only [RF]₄, as can be seen in Figure 7.

CONCLUSIONS

We have investigated the self-assembled morphology of the [RF]₄:P[RF]₄ mixtures, SAXS showing that the peptides self-assemble to form small fractal clusters coexisting with extended elongated fibers. The fibers comprise peptides with a β -sheet conformation, and the peptide chains pack in an orthorhombic unit cell. By studying model stereoselective aldol reactions, we showed that the concentration of the catalyst used can influence the conversion and stereoselectivity of the aldol reactions due to the polymorphism and availability of the reagents on the catalyst surface. We demonstrated that the [RF]₄ peptide, which lacks a proline residue, unexpectedly has a higher catalytic efficiency than P[RF]₄. Also, stable and soft hydrogels were observed, characterized by long cylinder structures. However, in comparison with the liquid systems, the fibrils in the hydrogels are significantly smaller in radius, especially with increasing P[RF]₄ content. Another significant change was correlated with the electron density distribution in the fibrils, with a concentration in the shell instead of the core, as was observed for the solutions. The hydrogels are birefringent, which along with SAXS data showing fibril form factor features, indicates that they possess the character of nematic hydrogels. Nematic hydrogels have rarely been reported for peptide systems. Our study provides a wealth of information on the polymorphism that can be accessed by tuning mixture composition in these model peptide systems. As the systems containing more P[RF]₄ were characterized by tight molecular packing, they do not have good aldol reaction efficiency, due to the difficulty of access of the reagents to the catalytic site of formation of the enamine transition state. Our findings show that hydrogel stiffness can also be increased by the addition of P[RF]₄ in comparison to those formed by [RF]₄ alone. Our results indicate the scope that is available to control peptide self-assembly, catalytic activity, and hydrogel formation by mixing closely related peptide homologues.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.langmuir.0c00198>.

Table of FTIR peak positions, UV absorption spectra, additional SAXS data, NMR spectra and HPLC curves for aldol reactions, stress sweep rheology data, SAXS fit parameters for hydrogels (PDF)

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Notes

The authors declare no competing financial interest.

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