Supporting Information

Unzipping the Role of Chirality in Nanoscale Self-Assembly of Tripeptide Hydrogels

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I – Analytical Characterization of Peptides

a. L-Leu-L-Phe-L-Phe

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\begin{align*}
\text{H-NMR} & \quad (400 \text{ MHz, DMSO, TMS}): \delta 8.65 (d, J = 8 \text{ Hz}, 1\text{H, NH}), 8.42 (d, J = 8 \text{ Hz}, 1\text{H, NH}), 8.03 (s (br), 3\text{H, NH})^3, \\
& \quad 7.23-7.14 (m, 10\text{H, Ar}), 4.55 (ddd, J = 4 \text{ Hz}, 8 \text{ Hz}, 8 \text{ Hz}, 1\text{H, } \alpha \text{CH}), 4.42 (ddd, J = 4 \text{ Hz}, 8 \text{ Hz}, 8 \text{ Hz}, 1\text{H, } \alpha \text{CH}), \quad 3.69 (m, 1\text{H, } \alpha \text{CH}), 3.04 (dd, J = 8 \text{ Hz, J}^\text{gem} = -14 \text{ Hz, 1H, } \beta \text{CH}_2), 2.95 (dd, J = 8 \text{ Hz, J}^\text{gem} = -14 \text{ Hz, 1H, } \beta \text{CH}_2), 2.88 (dd, J = 10 \text{ Hz, J}^\text{gem} = -14 \text{ Hz, 1H, } \beta \text{CH}_2), 2.76 (dd, J = 10 \text{ Hz, J}^\text{gem} = -14 \text{ Hz, 1H, } \beta \text{CH}_2), 1.57 (m, 1\text{H, } \gamma \text{CH}), 1.45 (m, 2\text{H, } \beta \text{CH}_2), \\
& \quad 0.83 (d, J = 4 \text{ Hz, 3H, CH}_3), 0.81 (d, J = 4 \text{ Hz, 3H, CH}_3). ^{13}\text{C-NMR} (100\text{MHz, DMSO, TMS}): \delta (\text{ppm}) 173.1, 171.1, 169.4 (3 \text{ x CO}); 137.8 (1\text{C}), 129.6 (2\text{C}), 129.5 (2\text{C}), 128.6 (2\text{C}), 128.5 (2\text{C}), 126.9 (1\text{C}), 126.8 (1\text{C}), (10 \text{x Ar}); 54.6, 53.9, 51.1 (3 \text{ x } \alpha \text{C}); 40.6 (1 \text{ x CH}); 39.2, 37.1 (2 \text{ x } \beta \text{CH}_2); 23.8, 23.3, 21.9 (\gamma \text{CH}, 2 \text{ x CH}_3). \quad \text{ESI-MS: } m/z 426.1 (\text{M+H})^+ \text{ C}_{24}\text{H}_{31}\text{N}_3\text{O}_4 \text{ requires 426.2.}
\end{align*}
\]

HPLC

ESI-MS
**H-NMR of LFF**

**C-NMR of LFF**
b. D-Leu-L-Phe-L-Phe

\[
\begin{align*}
\text{H-NMR (400 MHz, DMSO, TMS):} & \quad \delta \text{ (ppm)} 8.69 (d, J = 8 \text{ Hz, 1H, NH}), 8.56 (d, J = 8 \text{ Hz, 1H, NH}) 7.95 (s \text{ (br), 3H, NH}_3^+), 7.27-7.12(m, 10H, Ar), 4.66 (m, 1H, \alpha\text{CH}), 4.42 (m, 1H, \alpha\text{CH}), 3.59 (m, 1H, \alpha\text{CH}), 3.08-3.03 (m, 2H, \beta\text{CH}_2), \\
& \quad 2.90 (dd, J = 8 \text{ Hz, } J_{gem} = -12 \text{ Hz, 1H, } \beta\text{CH}_2), 2.60 (dd, J_{gem} = -12 \text{ Hz, 2H, } \beta\text{CH}_2), 1.13-0.99 (m, 3H, \gamma\text{CH, } \beta\text{CH}_2), 0.63 (d, J = 6 \text{ Hz, 3H, CH}_3), 0.62 (d, J = 6 \text{ Hz, 3H, CH}_3). \\
\text{C-NMR (100MHz, DMSO, TMS):} & \quad \delta \text{ (ppm)} 173.2, 171.6, 169.1 (3 \times \text{ CO}), 138.0 (1C), 129.8 (2C), 129.6 (2C), 128.7 (2C), 128.4 (2C), 127.0 (1C) (10 \times \text{ Ar}), 54.2, 54.1, 51.1 (3 \times \alpha\text{C}); 40.7 (1 \times \text{ CH}); 38.6, 37.0 (2 \times \beta\text{CH}_2); 23.6, 22.9, 22.2 (\gamma\text{CH, 2 x CH}_3). \\
\text{ESI-MS:} & \quad m/z 426.1 (M+H)^+ \text{ C}_{24}\text{H}_{31}\text{N}_{3}\text{O}_4 \text{ requires 426.2.}
\end{align*}
\]

HPLC

[Graph of HPLC analysis showing a sharp peak at around 10 minutes]

ESI-MS

[Graph of ESI-MS analysis showing a peak at m/z 426.1]
$^1$H-NMR of $^5$LFF

$^{13}$C-NMR of $^5$LFF
II- TEM images with negative staining displaying D_LFF short fibers originating from globular structures.

Scale bar = 200 nm.

Cryo-TEM images of D_LFF displaying how a globule responds to laser radiation damage just before disappearance.

Scale bar = 500 nm.

III- Cryo-TEM images showing how globular nuclei of LFF respond to laser radiation damage before disappearance. Scale bar = 500 nm.
IV- Confocal images for Thioflavin T-stained samples of LFF after 7 days. Scale bar = 50 microns.

V- Confocal images for Thioflavin T-stained samples of LFF showing crystal needles aligning into plates. Scale bar = 50 microns.
VI - TEM image with negative staining for LFF on fresh samples. Scale bar = 200 nm.

VII - Cryo-TEM image detail showing globules for LFF superimposed on a crystal plate. Scale bar = 200 nm.
VIII- \( d \) spacings from XRD diffraction analysis

<table>
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<th>( d ) spacings (Å)</th>
<th>D LFF</th>
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IX - Theoretical average distances from molecular modelling

**D LFF**

- molecular length \( \sim 17.4 \text{Å} \)
- central Phe \( \pi-\pi \) stack distance \( \sim 4.2 \text{ Å} \)
- beta-strand distance \( \sim 4.9 \text{ Å} \)
- antiparallel distance \( \sim 10.3-10.5 \text{ Å} \)

**LFF**

- molecular length \( \sim 13.5 \text{Å} \)
- central Phe \( \pi-\pi \) stack distance \( \sim 4.3 \text{ Å} \)
- beta-strand distance \( \sim 4.2 \text{ Å} \)
- antiparallel distance \( \sim 8.8 \text{ Å} \)