Supplementary Information

Extending the pool of compatible peptide hydrogels for protein crystallization

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Fmoc-MF characterization:

Electron Microscopy (TEM)

TEM images of xerogels were studied with a LIBRA 120 PLUS Carl Zeiss. A drop of the hydrogel was placed on a 300-mesh copper grid. The sample was dried at room temperature for 30 min.

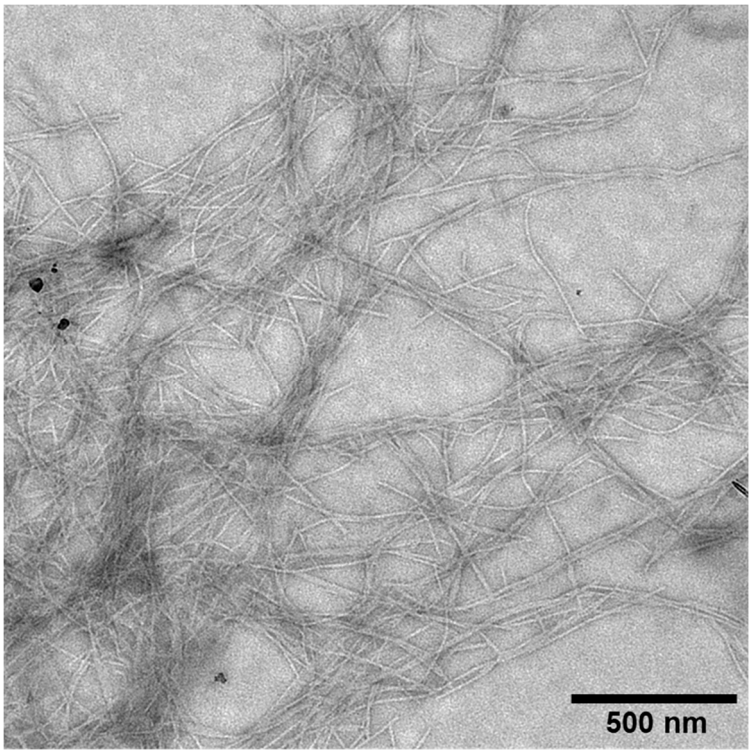

Figure S1. TEM images of dried hydrogels at 0.5% w/v.

Rheological characterization

We characterized the rheological properties of the hydrogels by using a Haake MARS III controlled-stress rheometer (Thermo Fisher Scientific, Waltham, MA, USA).We prepared the hydrogels on disposable bottom plates (made of aluminum) of the rheometer, on which we previously carved a cylindrical hole of 2 mm of depth and 35 mm of diameter to avoid spreading of the content. The hydrogels were gelled for 24 hours under water saturated atmosphere following the same protocol described above. After 24 hours we placed the plate containing the hydrogel in the rheometer and descended the upper plate (35 mm in diameter; serrated surface to avoid wall slip) until perfect contact with the hydrogel was reached, without appreciable compression. Afterwards we carried out measurements under oscillatory shear strain. First, we carried out ramps of oscillatory strains of fixed frequency (1 Hz) and increasing amplitude. Such measurements allow the identification of the linear viscoelastic region (LVR) as the low range of values of the strain amplitude for which the viscoelastic moduli (both G' and G") are approximately independent of the magnitude of the strain

amplitude. Above the LVR, G' decreases abruptly with the strain amplitude, whereas G" usually increases first and then decreases. These changes in the values of G' and G" mark the onset of the nonlinear viscoelastic region. Once we delimited the LVR, we subjected the samples to ramps of constant shear strain amplitude (we chose the value 0.002, well within the LVR) and increasing frequency in the range 0–16 Hz. From these measurements, we obtained the trends of G' and G" as a function of frequency, in the LVR.

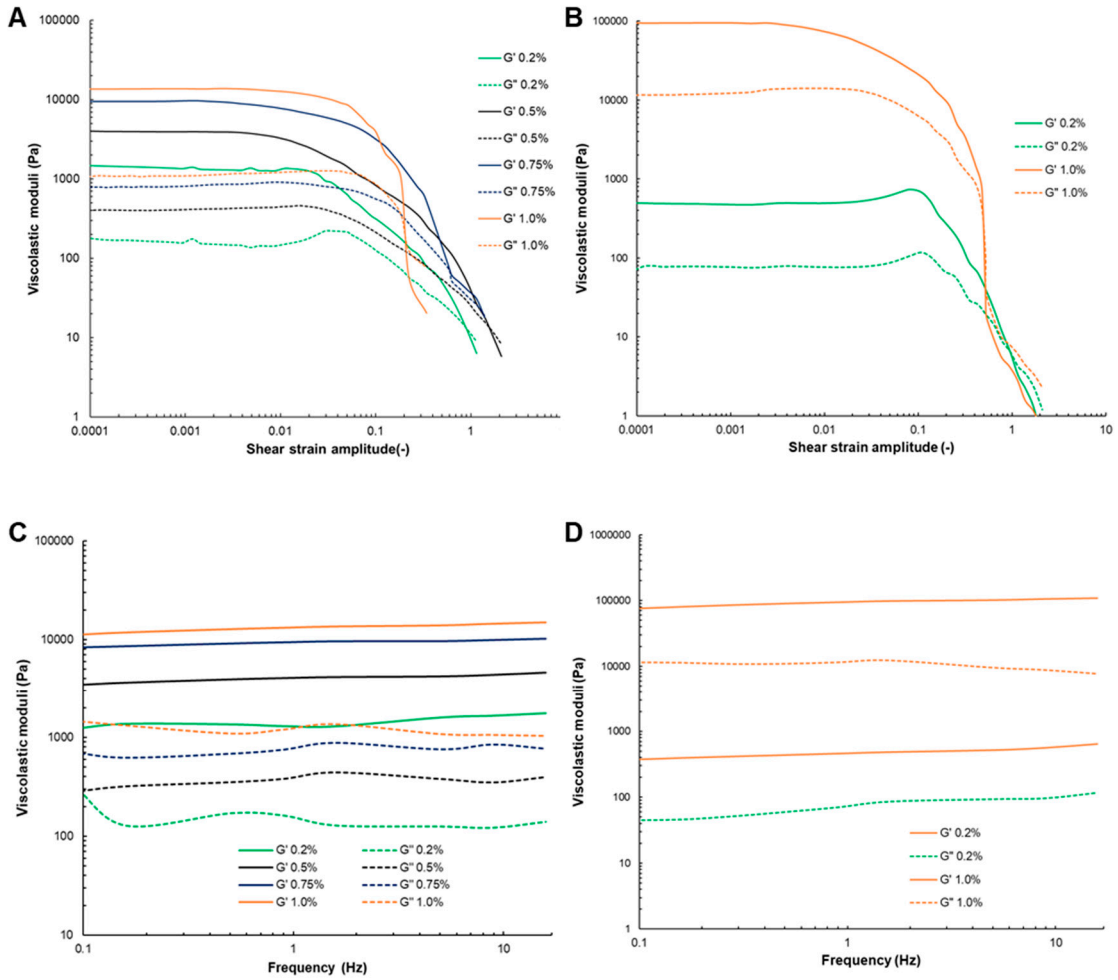

Figure S2. A) Fmoc-MF shear strain amplitude; B) Fmoc-Y shear strain amplitude; C) Fmoc-MF frequency sweeps; D) Fmoc-Y frequency sweeps.

Table S1. Summarized the data collection conditions and final statistical values of lysozyme crystals in Fmoc-MF and in agarose hydrogels (data in brackets correspond to high resolution shell). Shadowed columns correspond to those already included in the main text.

Gel type	**Agarose**		**Fmoc-MF**							
Concentration (% w/v)	0.5	0.5	0.2	0.2	0.5	0.5	0.75	0.75	1.0	1.0
Data Acquisition										
ESRF Beam-line	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2
Detector type	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS
Wavelength (Å)	0.87290	0.87290	0.87290	0.87290	0.87290	0.87290	0.87290	0.87290	0.87290	0.87290
Distance (mm)	215.97	215.97	215.97	215.97	215.97	215.97	215.97	215.97	215.97	215.97
Exposure time (s)	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Oscillation (º)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Data Statistics										
Space group	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2
Unit cell a=b, c (Å)	*79.00, 37.26*	*78.83, 37.24*	*77.33, 38.01*	*78.25, 37.58*	*77.53, 37.81*	*78.64, 37.9*	*77.43, 37.83*	*77.52, 37903*	*77.63, 37.91*	*77.56, 37.86*
Resolution (Å) (High shell)	39.50-1.20 (1.22-1.20)	39.42-1.20 (1.22-1.20)	38.66-1.30 (1.32-1.30)	39.12-1.50 (1.53-1.50)	38.77-1.20 (1.22-1.20)	39.32-1.35 (1.37-1.35)	38.71-1.30 (1.32-1.30)	38.76-1.15 (1.17-1.15)	38.81-1.40 (1.42-1.40)	38.78-1.25 (1.27-1.25)
Unique reflections	37471 (1805)	37298 (1797)	28991 (1425)	19195 (916)	36659 (1800)	26294 (1283)	28925 (1416)	41636 (2009)	26435 (1138)	32570 (1586)
R-merge (%)	5.9 (79.8)	8.5 (94.6)	10.5 (95.3)	7.7 (87.7)	4.7 (87.6)	6.3 (78.8)	7.8 (97.8)	5.2 (82.6)	5.1 (46.3)	5.1 (83.9)
I/σ(I)	20.7 (3.3)	14.8 (2.7)	11.1 (1.9)	19.3 (3.7)	27.8 (3.2)	21.9 (3.8)	18.0 (1.9)	23.6 (3.2)	29.9 (5.1)	26.7 (3.6)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	99.7 (99.4)	100.0 (100.0)	99.8 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Redundancy	13.6 (13.8)	14.0 (13.9)	13.9 (14.4)	12.9 (13.1)	13.7 (13.8)	14.1 (14.6)	13.8 (14.4)	13.7 (13.2)	12.8 (14.3)	13.7 (12.8)
B-factor ($Å^2$)	9.5	9.0	11.5	15.0	10.1	10.9	13.6	8.8	11.7	10.6
Mosaicity	0.18	0.16	0.19	0.29	0.20	0.11	0.24	0.12	0.22	0.21

Table S2. Summarized the data collection conditions and final statistical values of thaumatin obtained in Fmoc-MF and in agarose hydrogels (data in brackets correspond to high resolution shell). Shadowed columns correspond to those already included in the main text.

Gel type	**Agarose**		**Fmoc-MF**			
Concentration (% w/v)	0.5	0.5	0.5	0.5	0.75	0.75
Data Acquisition						
ESRF Beam-line	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2
Detector type	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS
Wavelength (Å)	0.87290	0.87290	0.87290	0.87290	0.87290	0.87290
Distance (mm)	215.97	215.97	215.97	215.97	215.97	215.97
Exposure time (s)	0.04	0.04	0.04	0.04	0.04	0.04
Oscillation (º)	0.1	0.1	0.1	0.1	0.1	0.1
Data Statistics						
Space group	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2
Unit cell a=b, c (Å)	*57.92, 150.43*	*57.98, 150.61*	*58.17, 151.14*	*58.17, 151.14*	*58.14, 150.63*	*58.05, 150.73*
Resolution (Å) (High shell)	45.89-1.10 (1.12-1.10)	45.94-1.05 (1.07-1.05)	46.10-1.10 (1.12-1.10)	46.10-1.20 (1.22-1.20)	46.02-1.15 (1.17-1.15)	45.99-1.15 (1.17-1.15)
Unique reflections	104237(4728)	120047 (5702)	106068 (5180)	82104 (3990)	92658 (4534)	91465 (4347)
R-merge (%)	4.4 (38.9)	5.6 (68.7)	6.0 (96.1)	5.2 (45.7)	7.2 (90.1)	6.3 (70.0)
I/σ(I)	28.4 (3.4)	24.6 (3.5)	22.6 (2.8)	28.1 (6.0)	19.6 (3.0)	22.3 (4.0)
Completeness (%)	99.6 (93.3)	99.7 (97.8)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	99.2 (96.7)
Redundancy	12.3 (4.6)	13.6 (10.4)	13.9 (13.0)	14.1 (13.8)	14.0 (13.3)	14.1 (13.8)
B-factor ($Å^2$)	6.7	5.6	6.9	7.0	7.2	7.0
Mosaicity	0.04	0.05	0.06	0.06	0.06	0.05

Table S3. Summarized the data collection conditions and final statistical values of glucose isomerase crystals in Fmoc-MF and in agarose hydrogels (data in brackets correspond to high resolution shell). Shadowed columns correspond to those already included in the main text.

Gel type	**Agarose**		**Fmoc-MF**			
Concentration (% w/v)	0.5	0.5	0.5	0.5	0.75	0.75
Data Acquisition						
ESRF Beam-line	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2	ID23-2
Detector type	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS	PILATUS
Wavelength (Å)	0.87290	0.87290	0.87290	0.87290	0.87290	0.87290
Distance (mm)	215.97	215.97	215.97	215.97	215.97	215.97
Exposure time (s)	0.04	0.04	0.04	0.04	0.04	0.04
Oscillation (º)	0.1	0.1	0.1	0.1	0.1	0.1
Data Statistics						
Space group	I222	I222	I222	I222	I222	I222
Unit cell a=b, c (Å)	*93.41,99.29, 103.09*	*93.08,98.86, 102.70*	*93.18,98.67, 102.88*	*93.48,98.86, 102.78*	*93.20,98.36, 102.91*	*93.20,98.36, 102.91*
Resolution (Å) (High shell)	46.71-1.15 (1.17-1.15)	46.54-1.15 (1.17-1.15)	49.33-1.10 (1.12-1.10)	46.74-1.50 (1.53-1.50)	46.60-1.05 (1.07-1.05)	46.60-1.10 (1.12-1.10)
Unique reflections	167421 (8287)	166929 (8172)	190602 (9407)	75999 (3732)	214576 (9993)	188000 (9105)
R-merge (%)	6.8 (77.1)	6.9 (81.2)	6.3 (73.6)	14.4 (80.1)	5.4 (83.8)	4.9 (54.8)
I/σ(I)	11.9 (1.9)	16.4 (2.3)	12.6 (1.9)	6.1 (1.7)	15.1 (1.7)	16.9 (2.7)
Completeness (%)	99.3 (99.9)	100.0 (99.9)	99.9 (100.0)	99.7 (99.8)	98.4 (93.4)	99.0 (96.8)
Redundancy	5.2 (4.8)	7.7 (7.3)	5.1 (4.7)	5.0 (4.9)	5.1 (4.4)	5.1 (4.9)
B-factor ($Å^2$)	8.2	8.1	6.3	7.4	6.0	6.0
Mosaicity	0.1	0.07	0.12	0.31	0.09	0.09

Table S4. Summarized the data collection conditions and final statistical values of lysozyme crystals obtained in Fmoc-Y and in agarose hydrogels (data in brackets correspond to high resolution shell). Shadowed columns correspond to those already included in the main text.

Gel type	**Agarose**		**Fmoc-Y**							
Concentration (% w/v)	0.5	0.5	0.2	0.2	0.5	0.5	0.75	0.75	1.0	1.0
Data Acquisition										
ALBA Beam-line	XALOC	XALOC	XALOC	XALOC	XALOC	XALOC	XALOC	XALOC	XALOC	XALOC
Detector type	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M	PILATUS 6M
Wavelength (Å)	0.980	0.980	0.979154	0.979154	0.979154	0.979154	0.979154	0.979154	0.979154	0.979154
Distance (mm)	160.35	160.35	128.0	128.0	128.0	128.0	128.0	128.0	128.0	128.0
Exposure time (s)	0.2seg	0.2seg	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Oscillation (º)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Data Statistics										
Space group	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2	P 4_3 2_1 2
Unit cell										
a=b, c (Å)	*77.27, 37.92*	*77.25, 37.9*	*78.68, 37.04*	*78.66, 37.01*	*78.57, 37.08*	*75.50, 37.16*	*78.47, 37.17*	*78.52, 37.12*	*78.56, 37.21*	*78.63, 37.22*
Resolution (Å)	38.64-1.15	38.62-1.15	39.34-1.00	39.33-1.05	39.28-1.05	39.25-1.10	39.24-1.10	39.26-1.05	39.28-1. 05	39.31-1.05
(High shell)	(1.17-1.15)	(1.17-1.15)	(1.02-1.00)	(1.07-1.05)	(1.07-1.05)	(1.12-1.10)	(1.12-1.10)	(1.07-1.05)	(1.07-1.05)	(1.07-1.05)
Unique reflections	41364 (1992)	41317 (1997)	63226 (3165)	54703 (2661)	51967 (2377)	47698 (2307)	47662 (2295)	54672 (2658)	54858 (2674)	54848 (2599)
R-merge (%)	3.2 (40.7)	8.0 (67.4)	3.9 (87.9)	4.2 (69.2)	4.4 (61.9)	4.8 (53.7)	5.6 (60.6)	4.3 (72.0)	5.1(97.5)	3.8 (95.4)
I/σ(I)	51.9 (9.0)	22.0 (4.5)	43.7 (4.2)	39.9 (4.8)	41.9 (6.1)	37.5 (6.3)	34.0 (5.6)	40.1 (4.4)	33.7 (3.5)	43.9 (4.0)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	95.3 (89.8)	100.0 (99.9)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)	99.8 (98.0)
Redundancy	24.4 (23.0)	23.7 (23.2)	24.2 (23.0)	24.3 (23.6)	25.6 (26.6)	24.5 (24.6)	24.5 (24.3)	24.3 (23.5)	24.3 (23.6)	24.5 (23.9)
B-factor ($Å^2$)	10.6	9.7	10.705	11.021	10.298	11.753	11.477	11.701	11.664	11.680
Mosaicity	0.22	0.22	0.08	0.13	0.09	0.10	0.11	0.10	0.10	0.08