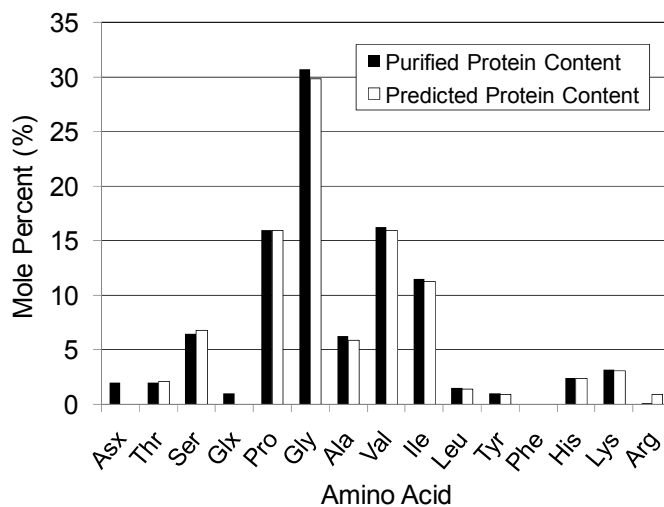
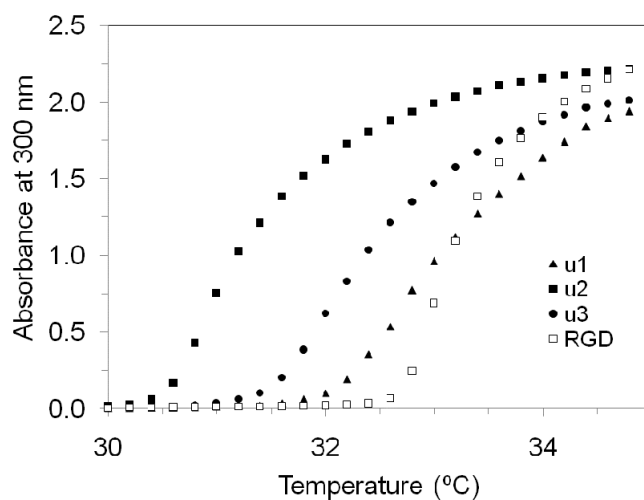


Supplementary Fig. 1 Cloning procedure for protein expression plasmids.



Supplementary Fig. 2 Amino acid analysis of purified u2 protein.

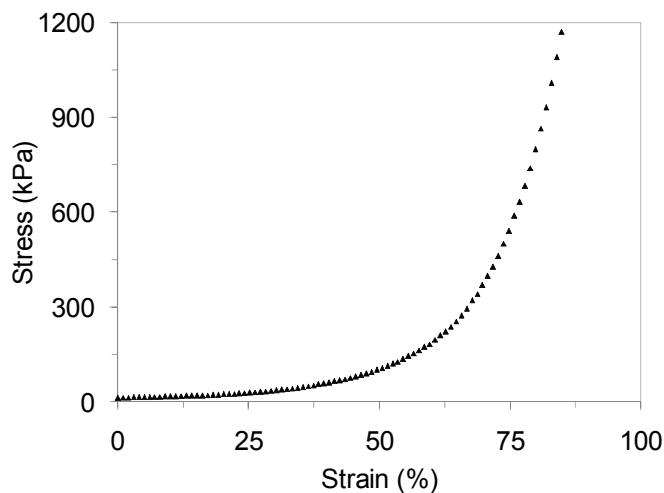
a



b

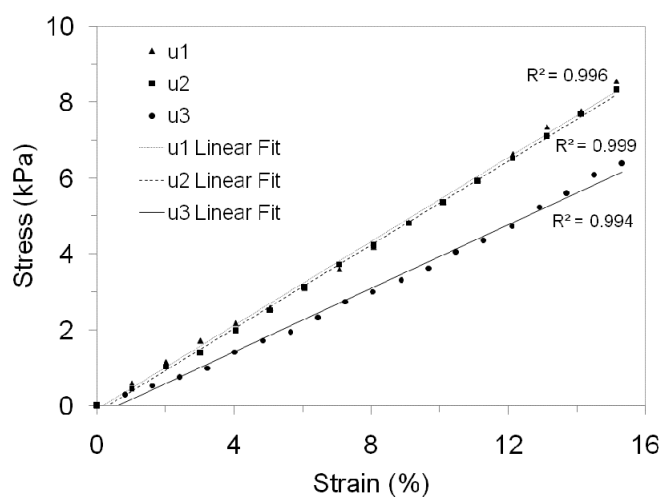
Protein	LCST (°C)
u1	33.3
u2	31.5
u3	32.6
RGD	33.3
RDG	33.3

Supplementary Fig. 3 LCST behavior of engineered proteins a) LCST curves b) calculated LCST values.



Supplementary Fig. 4 Characteristic full stress-strain curve generated from compression testing of undegraded, crosslinked u1 protein (1:1 reaction stoichiometry, 400 μm thick film).

a



b

Protein	Compressive Elastic Modulus (kPa)
u1	55.4 ± 8.6
u2	55.2 ± 6.8
u3	41.8 ± 4.0

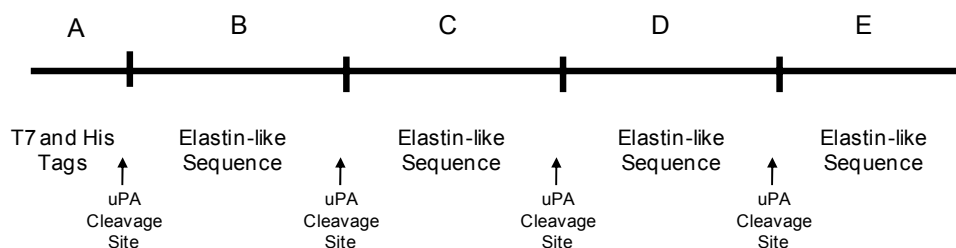
Supplementary Fig. 5 Compression testing of proteins crosslinked under similar conditions (1:1 reaction stoichiometry) a) linear portion of stress-strain curves b) compressive moduli values.

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a

T7 Tag His Tag EK Cleavage Site uPA Cleavage Site Elastin-like Sequence
MASMTGGQMG-HHHHH-DDDDK-LQ(LDAS-YAVTGGTARSASPASSA-SA((VPGIG)₂VPGKG(VPGIG)₂)₃VP)₄LE

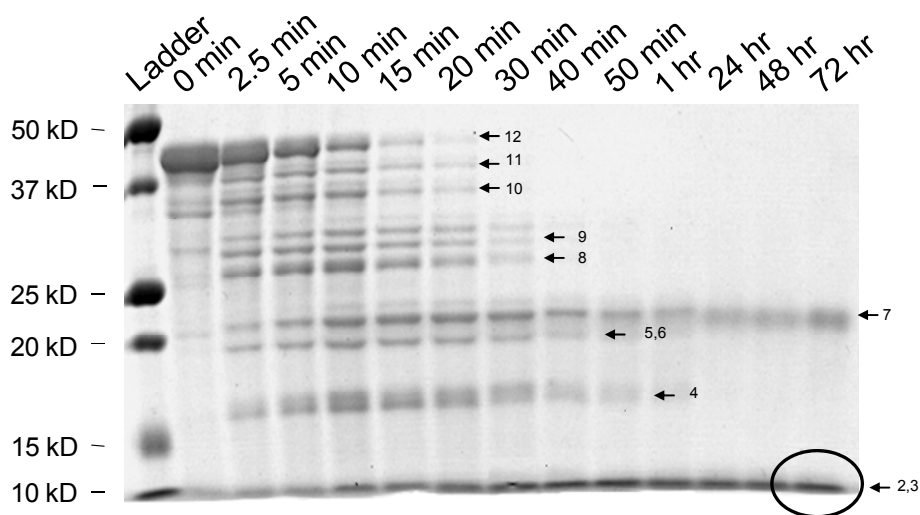
b



c

Fragment Components	Size (kDa)	Fragment Number
A	4.01	1
B, C, D	8.70	2
E	7.67	3
A+B	12.71	4
D+E	16.37	5
B+C, C+D	17.40	6
A+B+C	21.41	7
C+D+E	25.07	8
B+C+D	26.10	9
A+B+C+D	30.11	10
B+C+D+E	33.77	11
A+B+C+D+E	37.70	12

d



Supplemental Fig. 4 a) Amino acid sequence of u1 protein. b) Schematic of u1 segments between protease cut sites. c) Sizes of predicted u1 degradation fragments. d) Position of predicted u1 degradation sites on the experimental protein gel (full protein and fragments have a tendency to run slightly high on gel).

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Sample	Average cells/mm ²	Student's t-test p-values					
		BSA	Collagen	RDG	RDG:RGD	u3:RGD	u2:RGD
u1:RGD	70.4	0.015	0.052	0.026	0.210	0.685	0.578
u2:RGD	80.9	0.031	0.024	0.047	0.641	0.512	
u3:RGD	74.1	0.008	0.029	0.004	0.154		
RDG:RGD	87.9	0.022	0.033	0.006			
RDG	31.7	0.242	0.016				
Collagen	61.1	0.020					
BSA	7.6						

Supplemental Fig. 5 Student's t-test p-values of Calcein AM cell adhesion assay performed on PC-12 cells grown for 6 days (data in Fig. 7).

Sample	Percentage of alamarBlue™ Reduced (%)	Student's t-test p-values										
		PLL	Collagen	BSA	u3:RGD	u2:RDG	u1:RDG	RDG:RGD	RDG	RGD	u3:RGD	u2:RGD
u1:RGD	0.312	0.0472	0.2609	0.0026	0.0042	0.0094	0.0266	0.0657	0.0014	0.5815	0.5933	0.0280
u2:RGD	0.198	0.0211	0.0843	0.0014	0.0110	0.0282	0.0469	0.0721	0.0038	0.1413	0.8986	
u3:RGD	0.282	0.0202	0.1164	0.0717	0.0670	0.1237	0.0620	0.9466	0.1897	0.5118		
RGD	0.187	0.1122	0.6900	0.0195	0.0335	0.0552	0.0727	0.1620	0.0219			
RDG	0.287	0.0132	0.0266	0.0533	0.0745	0.3600	0.7512	0.0019				
RDG:RGD	0.158	0.0284	0.1311	0.0029	0.0126	0.0287	0.0490					
u1:RDG	0.326	0.0039	0.0004	0.2079	0.0813	0.5590						
u2:RDG	0.290	0.0130	0.0132	0.3928	0.0204							
u3:RDG	0.206	0.0072	0.0060	0.3683								
BSA	0.171	0.0042	0.0073									
Collagen	0.342	0.0226										
PLL	0.412											

Supplemental Fig. 6 Student's t-test p-values of alamarBlue™ cell adhesion assay performed on PC-12 cells differentiated for 6 days (data in Fig. 8).

Sample	Cells/mm ²	Student's t-test p-values		
		Collagen	RGD	RDG:RGD
RDG	1.0	0.00346	0.01955	0.02102
RDG:RGD	7.7	0.01875	0.11225	
RGD	10.6	0.05929		
Collagen	20.4			

Supplemental Fig. 7 Student's t-test p-values of cell adhesion data quantified from immunostained images of PC-12 cells differentiated for 6 days (data in Fig. 10a).

Sample	Cells with Neurites (%)	Student's t-test p-values		
		Collagen	RGD	RDG:RGD
RDG	4.2	0.00054	0.01091	0.00526
RDG:RGD	11.5	0.00069	0.03142	
RGD	16.7	0.00064		
Collagen	43.5			

Supplemental Fig. 8 Student's t-test p-values of percentage of cells possessing neurites greater than one cell diameter quantified from immunostained images of PC-12 cells differentiated for 6 days (data in Fig. 10b).

Sample	Cells with Long Neurites (%)	Student's t-test p-values		
		Collagen	RGD	RDG:RGD
RDG	0.3	0.00641	0.01289	0.02192
RDG:RGD	3.6	0.00538	0.00523	
RGD	6.3	0.00645		
Collagen	22.0			

Supplemental Fig. 9 Student's t-test p-values of percentage of cells possessing neurites greater than two cell diameters quantified from immunostained images of PC-12 cells differentiated for 6 days (data in Fig. 10c).