Directed intermixing in multi-component self-assembling biomaterials

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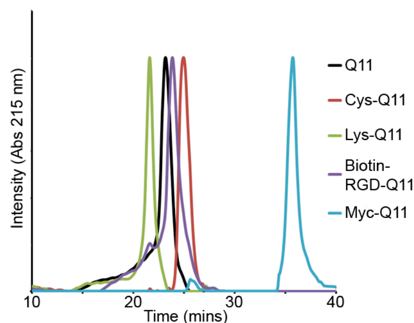
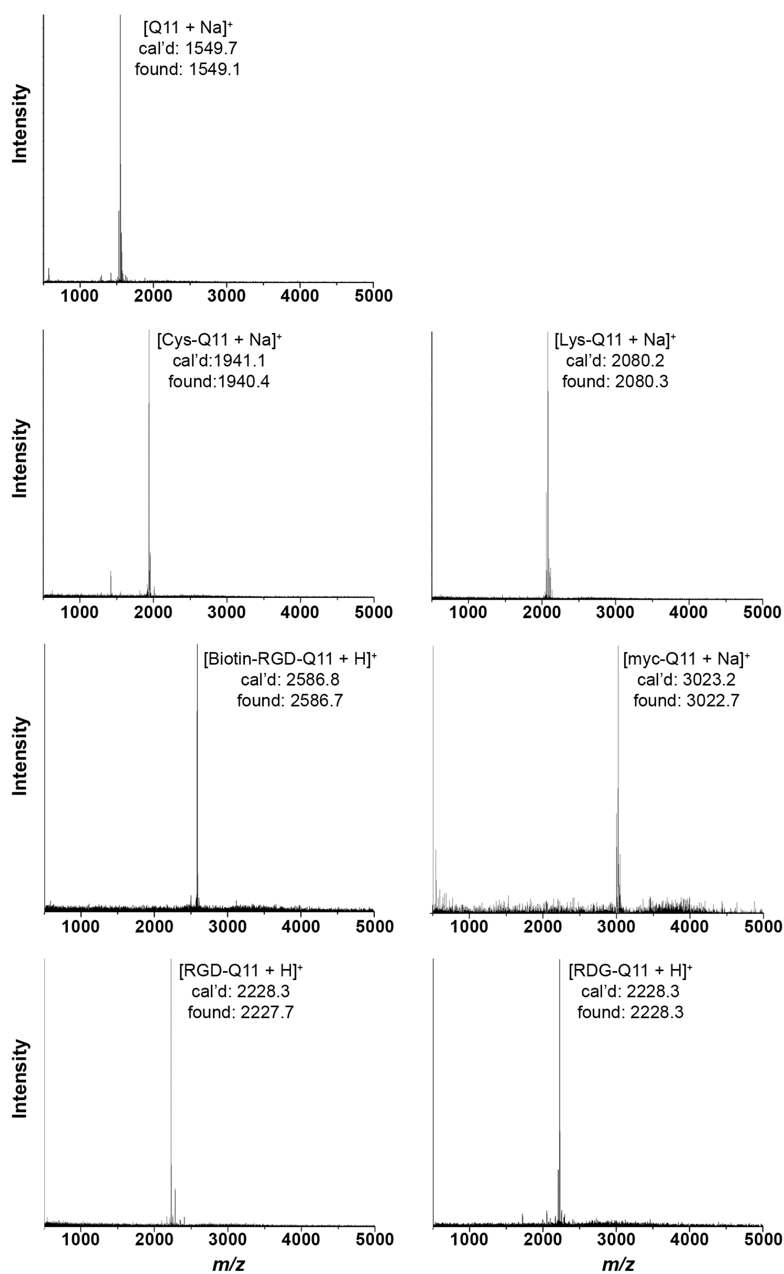
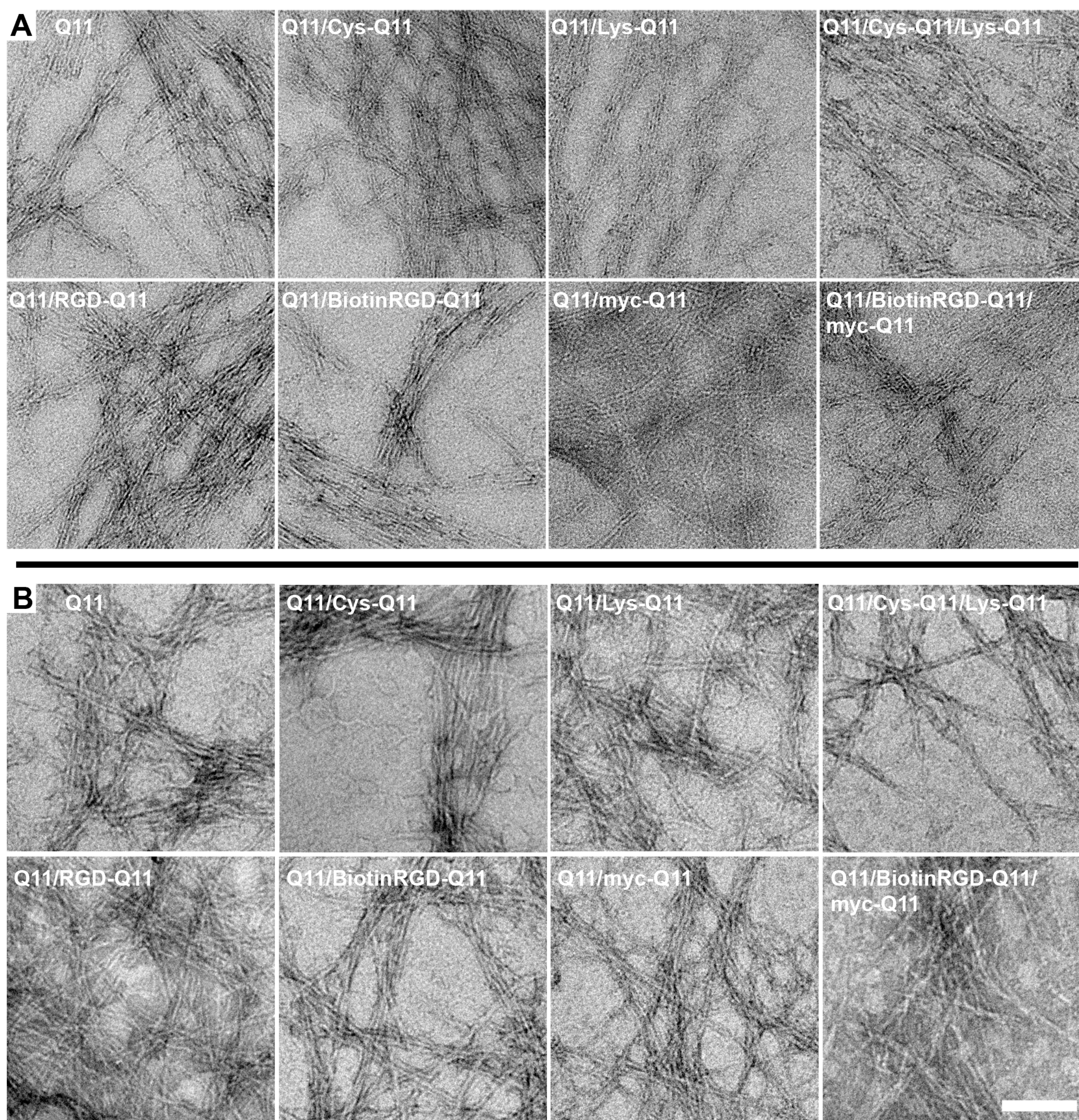
SUPPLEMENTAL INFORMATION



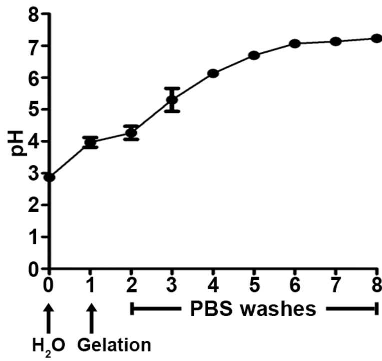
Supplemental Figure 1: Chromatograms of peptides after one round of HPLC purification on a C18 reverse phase column using a acetonitrile/water gradients.



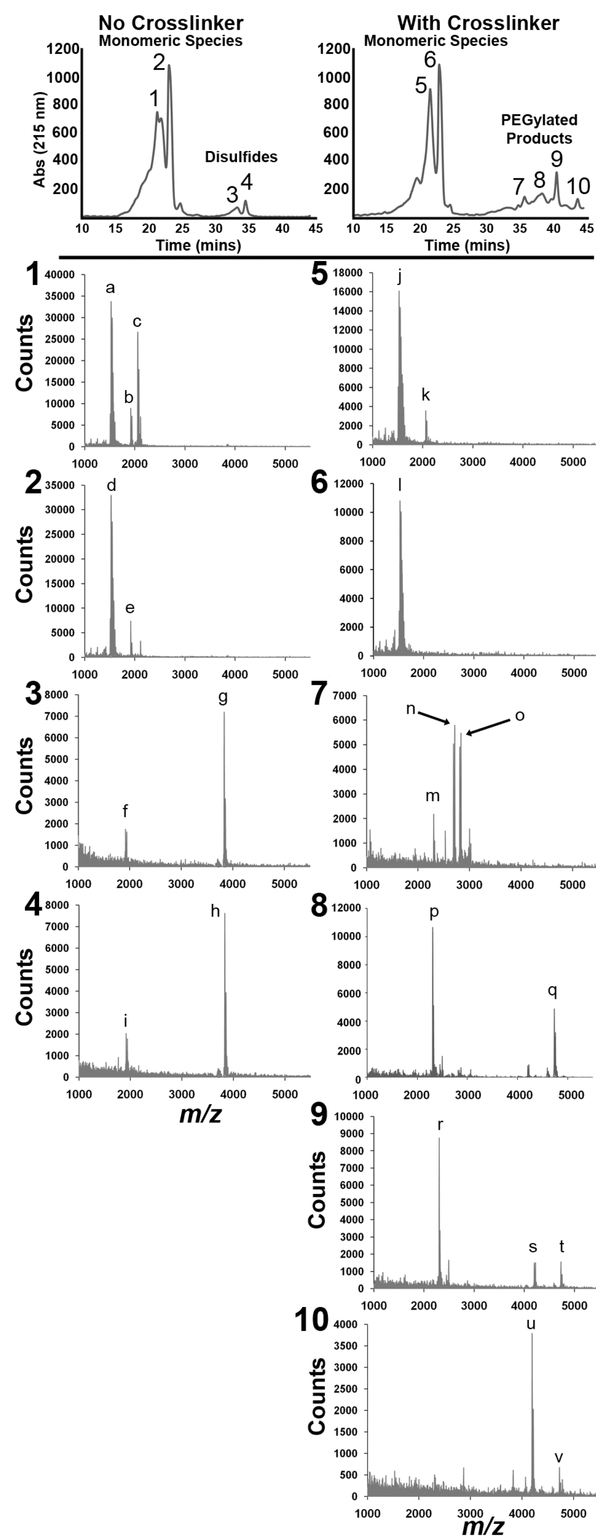
Supplemental Figure 2: MALDI-TOF mass spectra for the individual peptides used in this manuscript.



Supplemental Figure 3: TEM images of fibrillized peptides. A) Peptides formed protofibrils when incubated in water for 24 hours. B) When PBS was added to protofibrils, they became coarser and stained more heavily with uranyl acetate. For all combinations, 27 mM of Q11 was mixed with 3 mM of ligand bearing peptide (1.5 mM plus 1.5 mM when two ligand peptides were included). Scale bar = 100 nm for all.



Supplemental Figure 4: The pH of 30 mM solubilized Q11-based peptides in water was 2.8. Exposure to PBS at step 1 caused gelation, resulting in a pH of approximately 4 within the center of the hydrogel. Subsequent washes with 2 mL of PBS for 30 mins each raised the pH within the center of the gel to a physiological range.



Peaks	Species	Predicted *m/z*	Found *m/z*
a, d, l	[Q11 + H]$^+$	1527.7	1529.1, 1528.8, 1529.9
j	[Q11 + Na]$^+$	1549.7	1552.2
b, e, f, i	[Cys-Q11 + H]$^+$	1919.1	1921.6, 1921.1, 1918.1, 1918.2
c, k	[Lys-Q11 + H]$^+$	2058.2	2061.2, 2062.4
g, h	[Cys-Q11 Disulfide]$^+$	3836.2	3836.5, 3836.6
m, p, r	[Q11 + XL + Na]$^+$	2300.1	2305.3, 2306.1, 2305.4
n	[Cys-Q11 + XL + Na]$^+$	2710.1	2715.0
o	[Lys-Q11 + XL + Na]$^+$	2830.6	2836.0
u	[Q11 + XL + Cys-Q11 + H]$^+$	4196.2	4200.9
s	[Q11 + XL + Cys-Q11 + Na]$^+$	4218.2	4225.3
q, t, v	[Lys-Q11 + XL + Cys-Q11 + H]$^+$	4726.7	4731.8, 4731.4, 4731.4
Standard	Angiotensin II	1046.5	1049.2
Standard	Insulin chain B oxidized	3494.7	3500.4

Supplemental Figure 5: Chromatograms of separately assembled Cys-Q11 and Lys-Q11 gels that were either not exposed to cross-linker (top left) or were reacted with cross-linker (top right). Fractions on the chromatograms are labeled numerically, and the corresponding linear mode MALDI-TOF mass spectra are numbered accordingly, below the corresponding chromatogram. Individual mass spectra peaks are labeled alphabetically, and *m/z* values for each are provided in the table at the bottom. Although all gels were treated with TCEP prior to cross-linking, disulfide bonds are likely present because the non-cross-linked gel was exposed to PBS instead of cross-linker for 24 hours. Mass/charge ratios correspond to expected products formed by NHS/amine and maleimide/thiol cross-linking reactions. After dissolving the gels in TFA and eluting the fractions on a C18 column with an acetonitrile/water gradient, the Cys-Q11 + XL + Na^+ species demonstrate appropriate reaction with the maleimide group, but the NHS group appeared to be hydrolyzed during the process. Angiotensin II and insulin chain B standards were also tested under the same linear mode conditions and found to be +2.3 and +5.7 mass units, respectively.