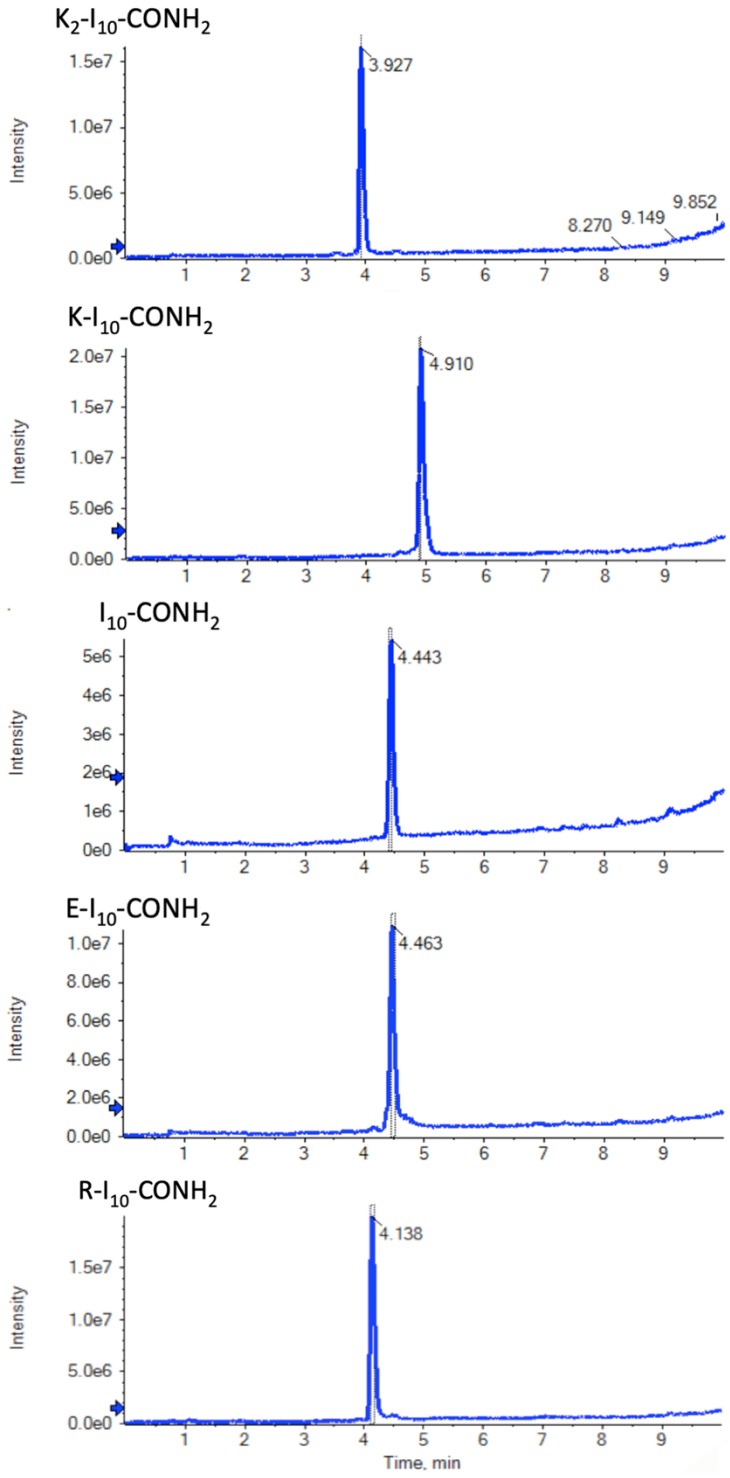
Supporting Information

Probing the molecular determinants of the activation of toll-like receptor 2/6 by amyloid nanostructures through directed peptide self-assembly

Nadjib Kihal[a,b,c], Marie-Jeanne Archambault[a,b], Margaryta Babych[a,b], Ali Nazemi[a,c], and Steve Bourgault[*a,b]
[a]*Department of Chemistry, Université du Québec à Montréal. C.P.8888, Succursale Centre-Ville, Montréal, H3C 3P8, Canada*
[b]*Quebec Network for Research on Protein Function, Engineering and Applications (PROTEO), Québec, Canada*
[c]*Quebec Centre for Advanced Materials, QCAM*, *Montreal, Canada*

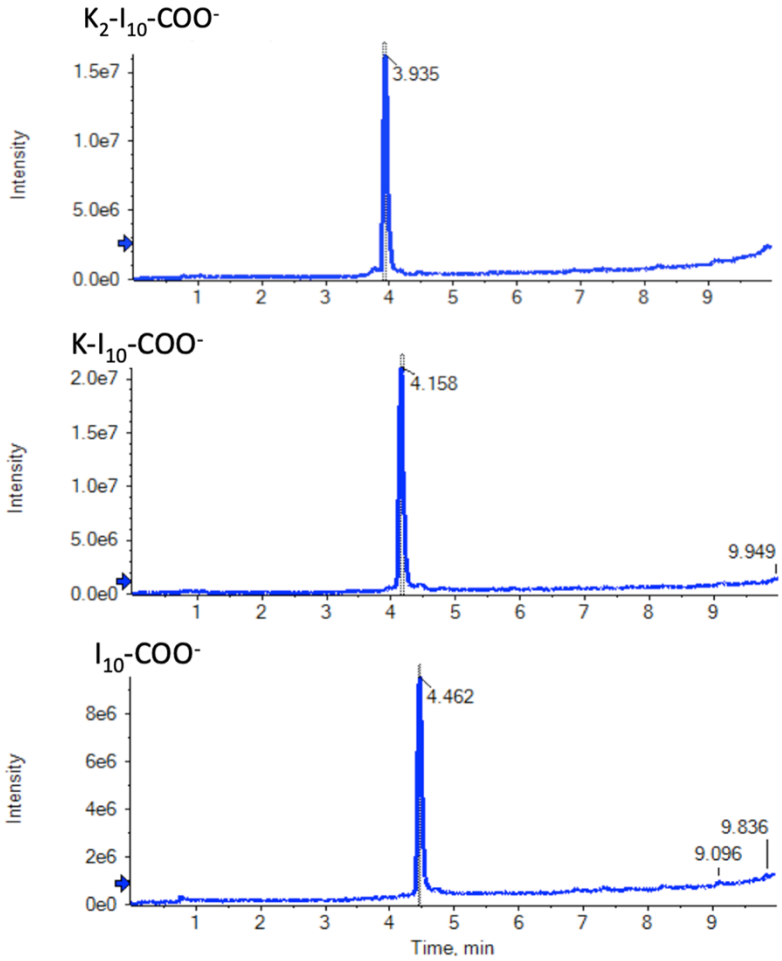
*Corresponding authors: Steve Bourgault, C.P. 8888, Succursale Centre-Ville, Montréal, H3C 3P8, Canada; e-mail address: bourgault.steve@uqam.ca

TABLE OF CONTENTS



Peptides	Formula	Exact mass	Calculated *m/z*	RT (min)	PPM Error	Observed *m/z*	Purity (%)
K_2-I_{10}-$CONH_2$	$C_{65}H_{109}N_{21}O_{23}$	1551.8005	776.9075	3.92	0.1	776.9076	>98
K-I_{10}-$CONH_2$	$C_{59}H_{97}N_{19}O_{22}$	1423.7056	712.8601	4.91	0.1	712.8602	>98
I_{10}-$CONH_2$	$C_{53}H_{85}N_{17}O_{21}$	1295.6106	648.8126	4.43	0.6	648.813	>98
R-I_{10}-$CONH_2$	$C_{59}H_{96}N_{21}O_{22}$	1451.7171	726.8631	4.13	0.3	726.8637	>98
E-I_{10}-$CONH_2$	$C_{58}H_{92}N_{18}O_{24}$	1424.6532	713.3339	4.46	0.8	713.3345	>98

Fig. S1 HPLC and MS analysis of *C*-amidated I_{10} peptides.



Peptides	Formula	Exact mass	Calculated *m/z*	RT (min)	PPM Error	Observed *m/z*	Purity (%)
K_2-I_{10}-COO$^-$	$C_{65}H_{108}N_{20}O_{24}$	1552.7845	777.3995	3.93	1.7	777.4009	>98
K-I_{10}-COO$^-$	$C_{59}H_{96}N_{18}O_{23}$	1424.6896	713.3521	4.15	0.8	713.3526	>98
I_{10}-COO$^-$	$C_{53}H_{84}N_{16}O_{22}$	1296.5946	649.3046	4.45	-1	649.304	>98

Fig. S2 HPLC and MS analysis of *C*-caboxylated I_{10} peptides.

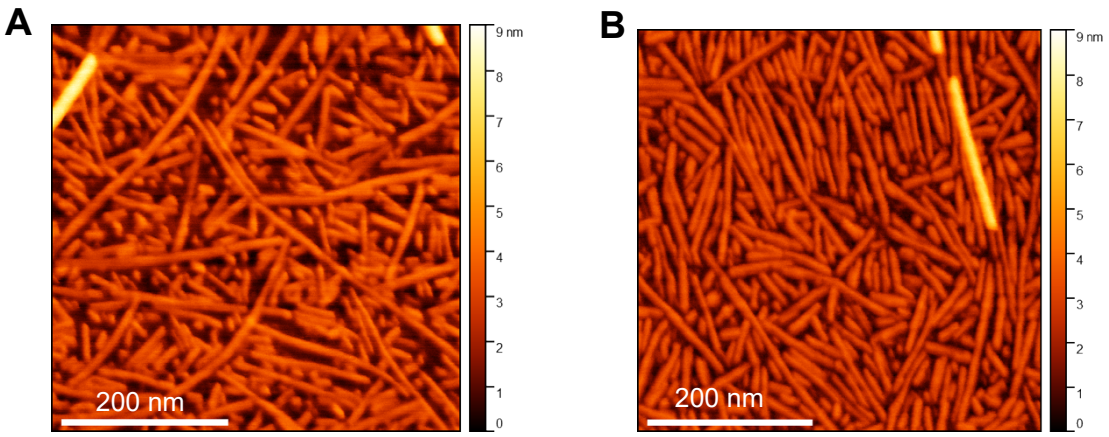


Fig. S3. Effect of adding 1% acetic acid to peptide assemblies for adsorption to AFM mica. K-I_{10}-$CONH_2$ peptide was assembled for 1 week in 20 mM Tris-HCl buffer (pH 7.4) at a peptide concentration of 1 mM and under continuous rotatory agitation at RT. Peptide assemblies were diluted to reach a final concentration of 50 μM in (A) 1% acetic acid and (B) deionized water and immediately spotted onto a freshly cleaved mica.

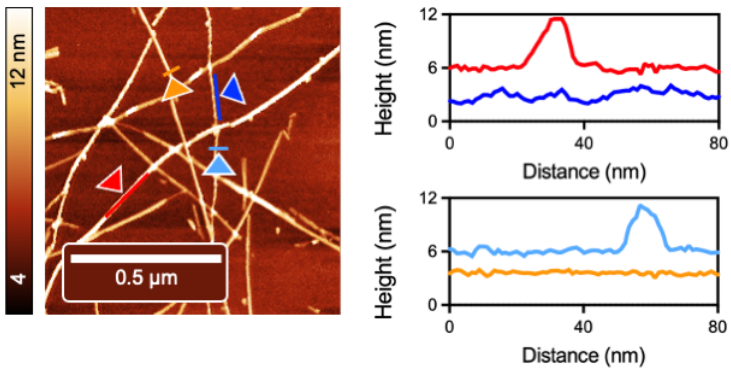


Fig. S4 AFM image and topography analysis of IAPP amyloid fibrils.

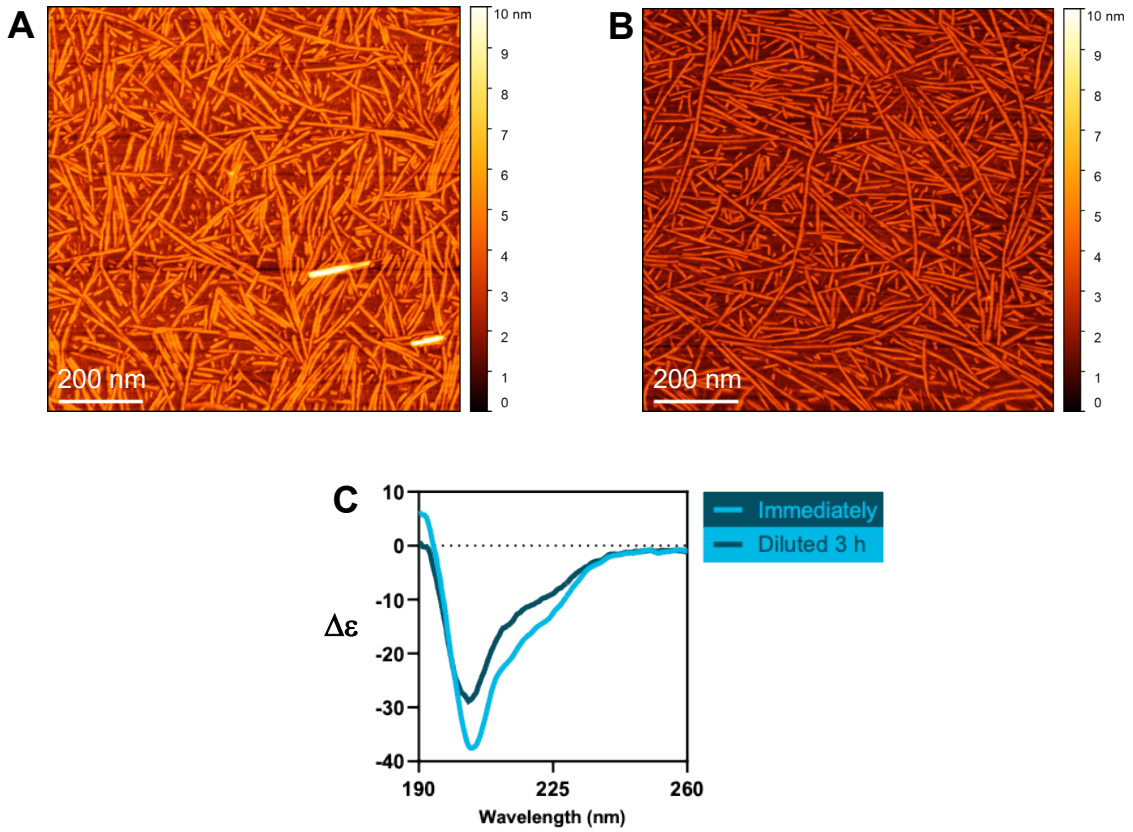


Fig. S5. Effect of diluting peptide assemblies. K-I_{10}-$CONH_2$ peptide was assembled for 1 week in 20 mM Tris-HCl buffer (pH 7.4) at a peptide concentration of 1 mM and under continuous rotatory agitation at RT. Peptide assemblies were diluted in 20 mM Tris-HCl, pH 7.4, to reach a final concentration of 50 μM. The 50 μM peptide solution was analysed immediately (A) or after 3 h incubation at RT under quiescent conditions (B) by AFM imaging (A,B) and CD spectroscopy (C).

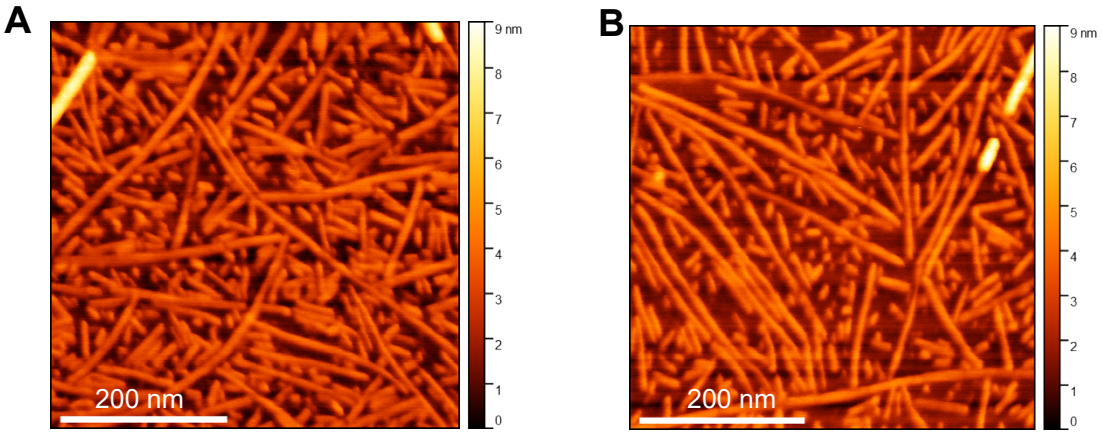


Fig. S6. Effect of lyophilisation on the stability of peptide assemblies. K-I_{10}-$CONH_2$ peptide was assembled for 1 week in 20 mM Tris-HCl buffer (pH 7.4) at a peptide concentration of 1 mM and under continuous rotatory agitation at RT. Peptide assemblies were immediately (A) analysed by AFM imaging or lyophilised (B) before being resuspended in deinozied water and analyse by AFM.

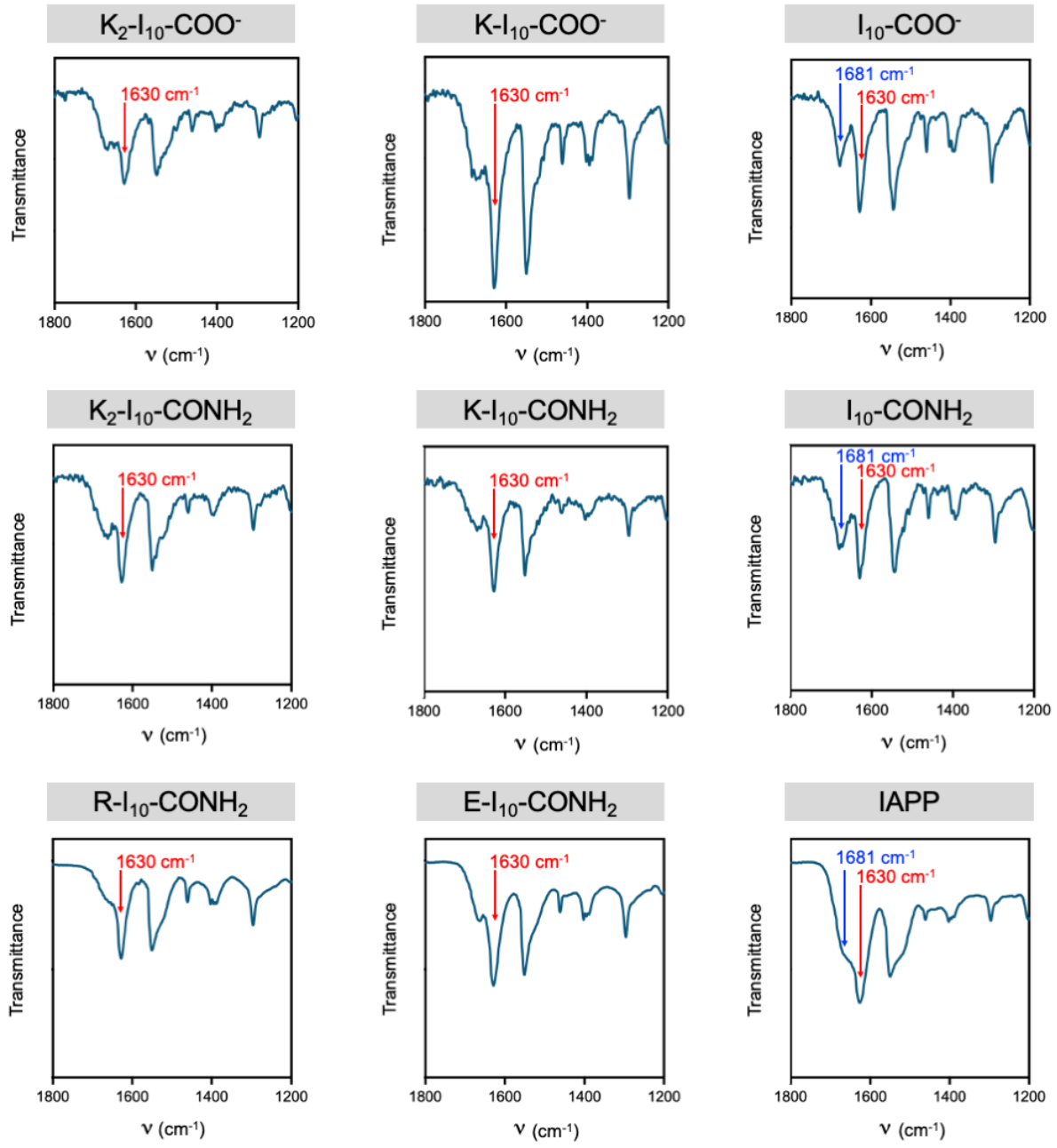


Fig. S7 ATR-FTIR spectra of *C*-carboxylated and *C*-amidated I_{10} peptides. Data were acquired after 1 week of incubation in 20 mM Tris-HCl buffer (pH 7.4) at RT.

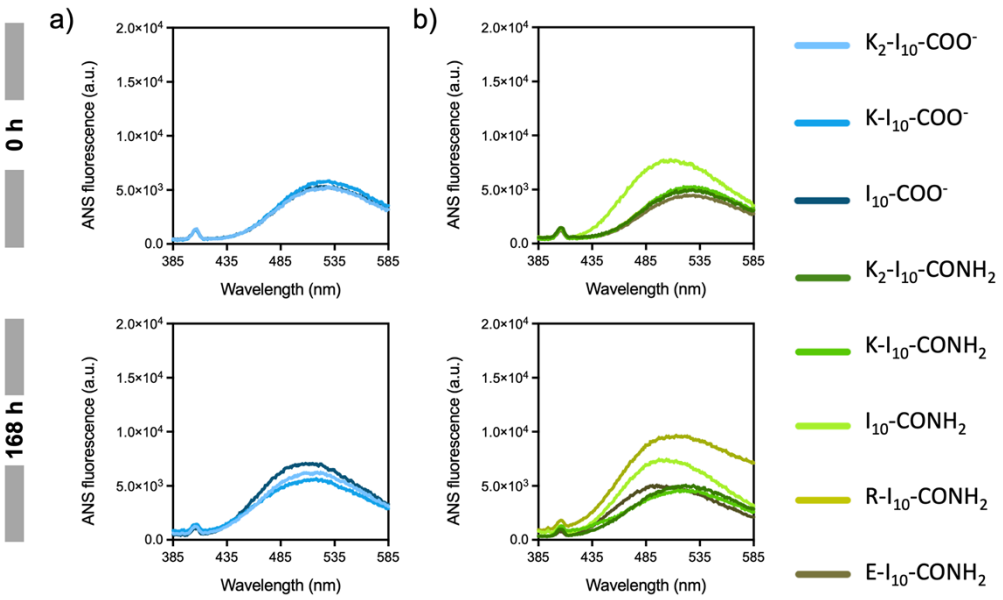


Fig. S8 ANS fluorescence spectra of I_{10} peptides. The data were acquired at 0 h and after 1 week of incubation in 20 mM Tris-HCl buffer (pH 7.4) at RT.

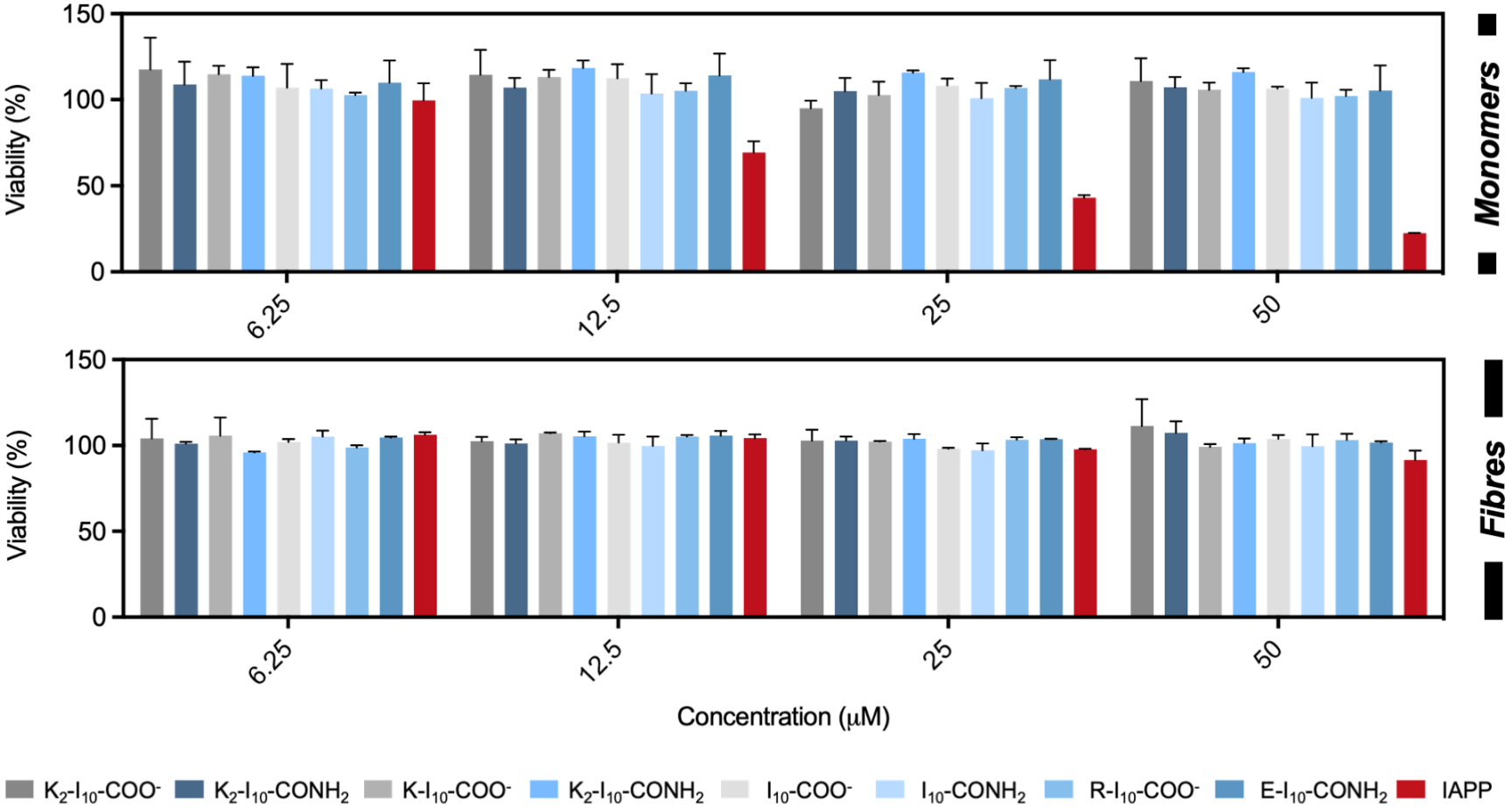


Fig. S9 Cytocompatibility of monomeric and assembled peptides. DC.2.4 cells were incubated for 24 h with increasing concentrations of monomeric (0 h pre-incubation) or assembled (168 h pre-incubation) peptides, and viability was measured by means of the resazurin-based metabolic assay. Data represent the Mean ± S.E.M. of at least three individual experiments performed in triplicate.