Electronic Supplementary Information

Structural Characterisation of Amyloid-like Fibrils Formed by an Amyloidogenic Peptide Segment of β-Lactoglobulin

Vasantha Gowda [a], Michal Biler [b], Andrei Filippov [cd], Malisa V. Mantonico [a], Eirini Ornithopoulou [a], Mathieu Linares [bef], Oleg N. Antzutkin [cg], Christofer Lendel *[a]

[a] *Dept. of Chemistry, KTH Royal Institute of Technology, Stockholm, Sweden.*

[b] *Dept. of Theoretical Chemistry, KTH Royal Institute of Technology, Stockholm, Sweden.*

[c] *Chemistry of Interfaces, Luleå University of Technology, Sweden.*

[d] *Dept. Medical and Biological Physics, Kazan State Medical University, 420012, Kazan, Russia.*

[e] *Laboratory of Organic Electronics and Group of Scientific visualization, ITN, Linköping University, 60174 Norrköping, Sweden*

[f] *Swedish e-Science Research Centre (SeRC), Linköping University, 60174 Norrköping, Sweden*

[g] *Dept. of Physics, University of Warwick, Coventry, UK.*

* *Correspondence to C.L.: lendel@kth.se*

Table S1. ^{1}H {H_2O+D_2O (10%) solution} Chemical shift table for the β-LG_{11-20} peptide are compared with random coil chemical shifts[49].

	H^N	H^{α}	$H^{\beta1}$	$H^{\beta2}$	$H^{\gamma1}$	$H^{\gamma2}$	H^{δ}	$H^{\varepsilon1}$	$H^{\varepsilon2}$
D	8.27	4.62	2.7	2.57					8.27
I	8.08	4.06	1.78		1.38	1.1	0.8		8.08
Q	8.34	4.21	1.96	1.88	2.25			7.17/7.08	8.34
K	8.21	4.22	1.64	1.55	1.25				8.21
V	8.05	4.03	1.97	-	0.88	0.81			8.05
A	8.4	4.23	1.31						8.4
G	8.26	3.86							8.26
T	7.84	4.2	3.97		0.96				7.84
W	8.08	4.54	3.12						8.08
Y	7.7	4.31	2.78	2.66					7.7

Table S2. Backbone torsion angles ϕ and ψ in β-LG_{11-20} fibrils obtained using TALOS and MD simulations.

	Predicted TALOS ϕ angle in fibrils	Predicted TALOS ψ angle in fibrils	MD simulated β-sheet structure (AB4)		MD simulated β-sheet structure (AA3)	
			ϕ angle	ψ angle	ϕ angle	ψ angle
D	-	-	-80 ± 8	125 ± 12	-90 ± 14	121 ± 13
I	-122.218	123.814	-113 ± 12	126 ± 16	-118 ± 14	127 ± 12
Q	-112.957	137.24	-121 ± 16	130 ± 11	-113 ± 13	130 ± 10
K	-120.806	132.618	-133 ± 10	130 ± 10	-124 ± 11	121 ± 16
V	-117.161	125.93	-127 ± 13	135 ± 10	-128 ± 9	133 ± 6
A	-112.417	126.779	-144 ± 13	141 ± 22	-137 ± 19	134 ± 27
G	-119.366	135.807	-111 ± 23	113 ± 24	-94 ± 28	118 ± 25
T	-127.937	136.017	-137 ± 14	137 ± 19	-125 ± 16	120 ± 18
W	-107.677	134.448	-124 ± 14	129 ± 19	-117 ± 16	122 ± 35
Y	-	-	-113 ± 14	102 ± 26	-94 ± 24	112 ± 17

* Data are extracted from the last 5 ns of relevant MD simulations with 500 points collected (10 ps/point) per each amino acid.

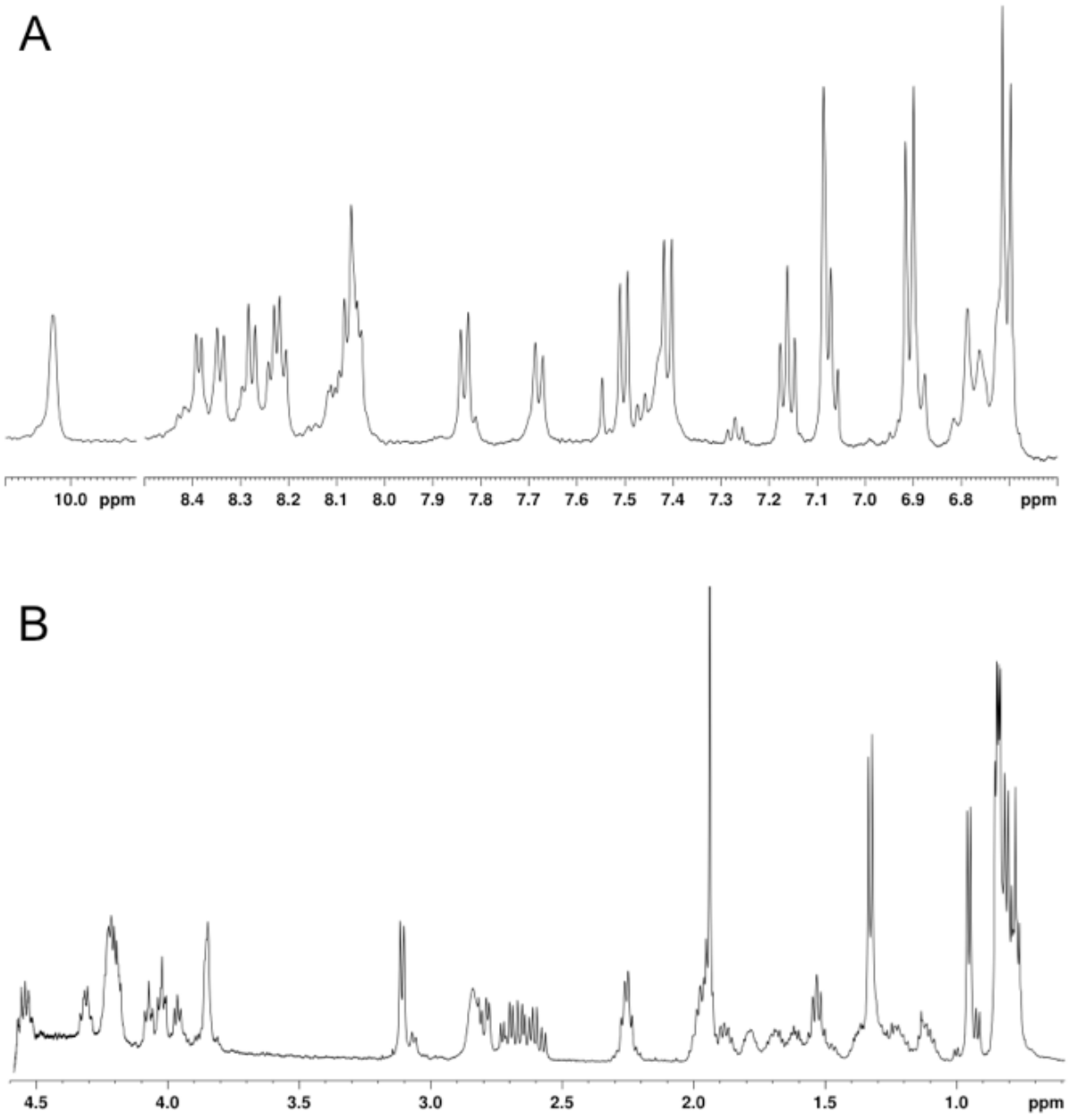

Figure S1. ^{1}H NMR spectrum of the β-LG_{11-20} peptide in H_2O+10% D_2O solution, expanded regions: A) 6.6-10.1 ppm B) 0.5- 4.6 ppm.

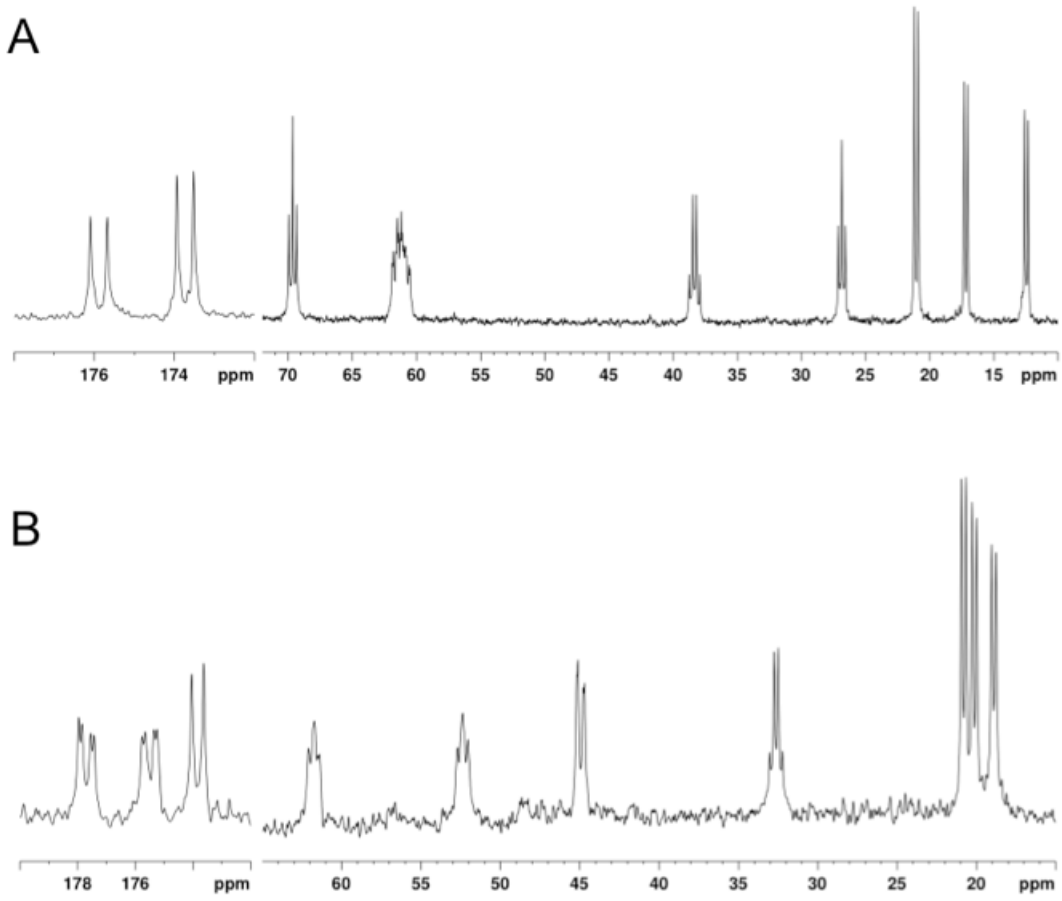

Figure S2. **A**) ^{13}C (^{15}N coupled) NMR spectrum of the uniformly $^{13}C/^{15}N$ labelled (I and T residues) β-LG_{11-20} peptide in H_2O+10% D_2O solution. **B)** ^{13}C (^{15}N coupled) NMR spectrum of the uniformly $^{13}C/^{15}N$ labelled (V, A, and G residues) β-LG_{11-20} peptide in H_2O+10% D_2O solution.

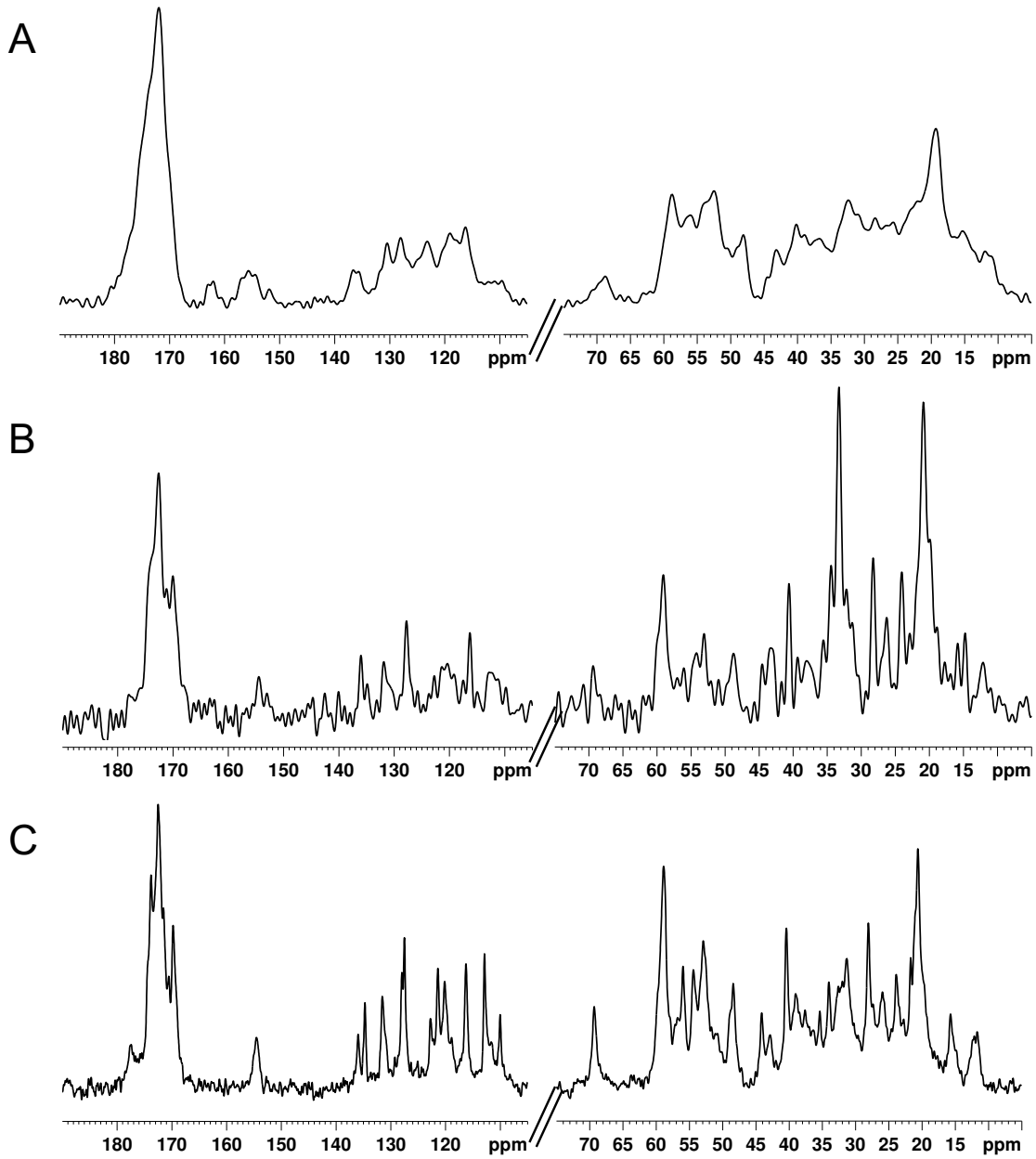

Figure S3. **A)** ^{13}C CP-MAS NMR spectrum of monomeric unlabelled β-$LG_{11\text{-}20}$ peptide (20480 acquisitions, 2.5 s relaxation delay, and 1.5 ms contact time). **B)** ^{13}C CP-MAS NMR spectrum of lyophilised unlabelled β-$LG_{11\text{-}20}$ fibrils (40960 acquisitions, 2.5 s relaxation delay, and 1.5 ms contact time). **C)** ^{13}C CP-MAS NMR spectrum of fully hydrated unlabelled β-$LG_{11\text{-}20}$ fibrils (61440 acquisitions, 2.5 s relaxation delay, and 1.5 ms contact time).

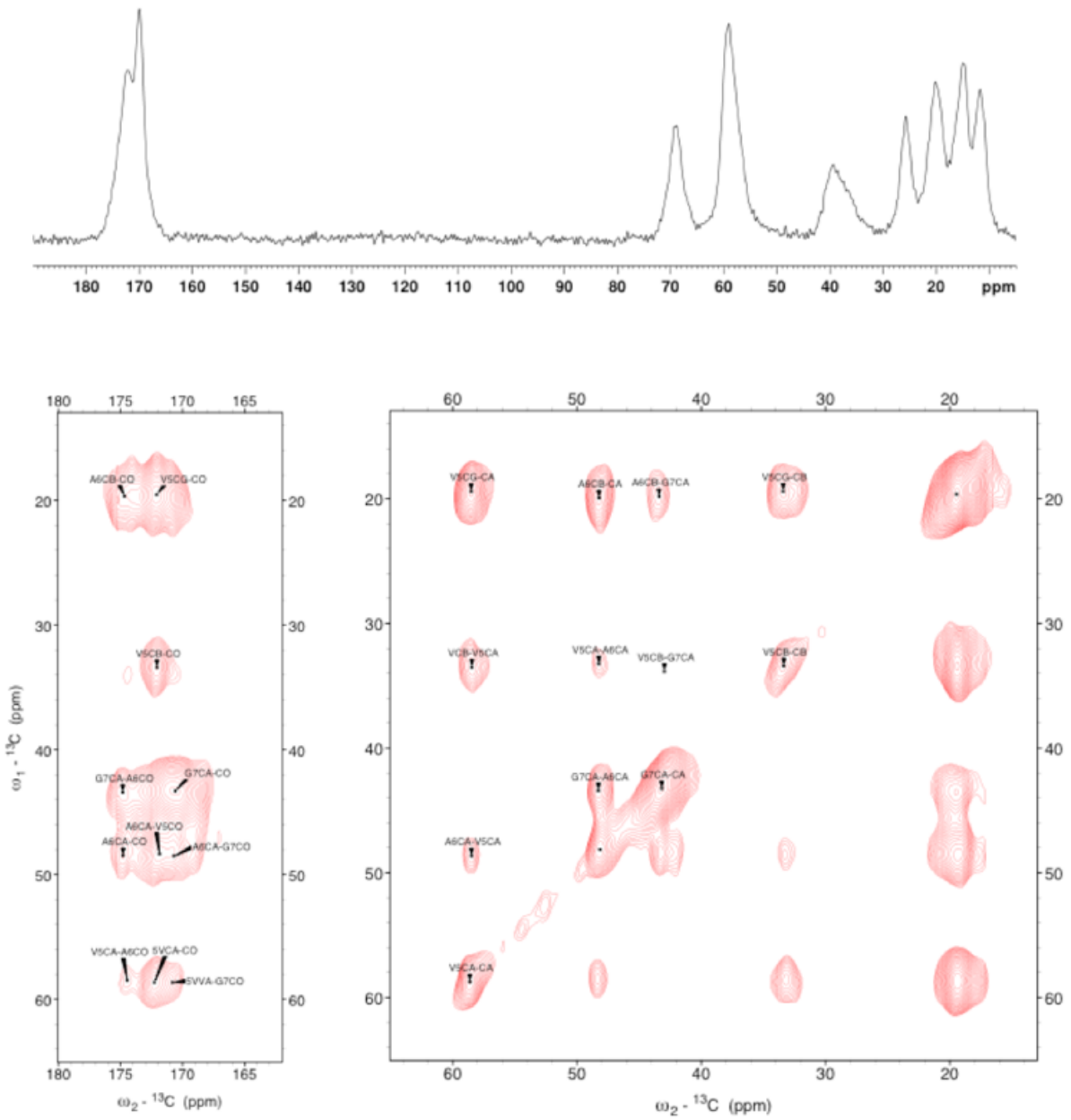

Figure S4 A) ^{13}C CP-MAS NMR spectrum of the uniformly $^{13}C/^{15}N$ labelled (V, A, and G residues) β-$LG_{11\text{-}20}$ peptide (1024 acquisitions, 2.5 s relaxation delay, and 1.5 ms contact time). **B)** ^{13}C-^{13}C solid-state PDSD NMR spectra of non-fibrillar peptide-VAG (DIQK**<u>VAG</u>**TWY)

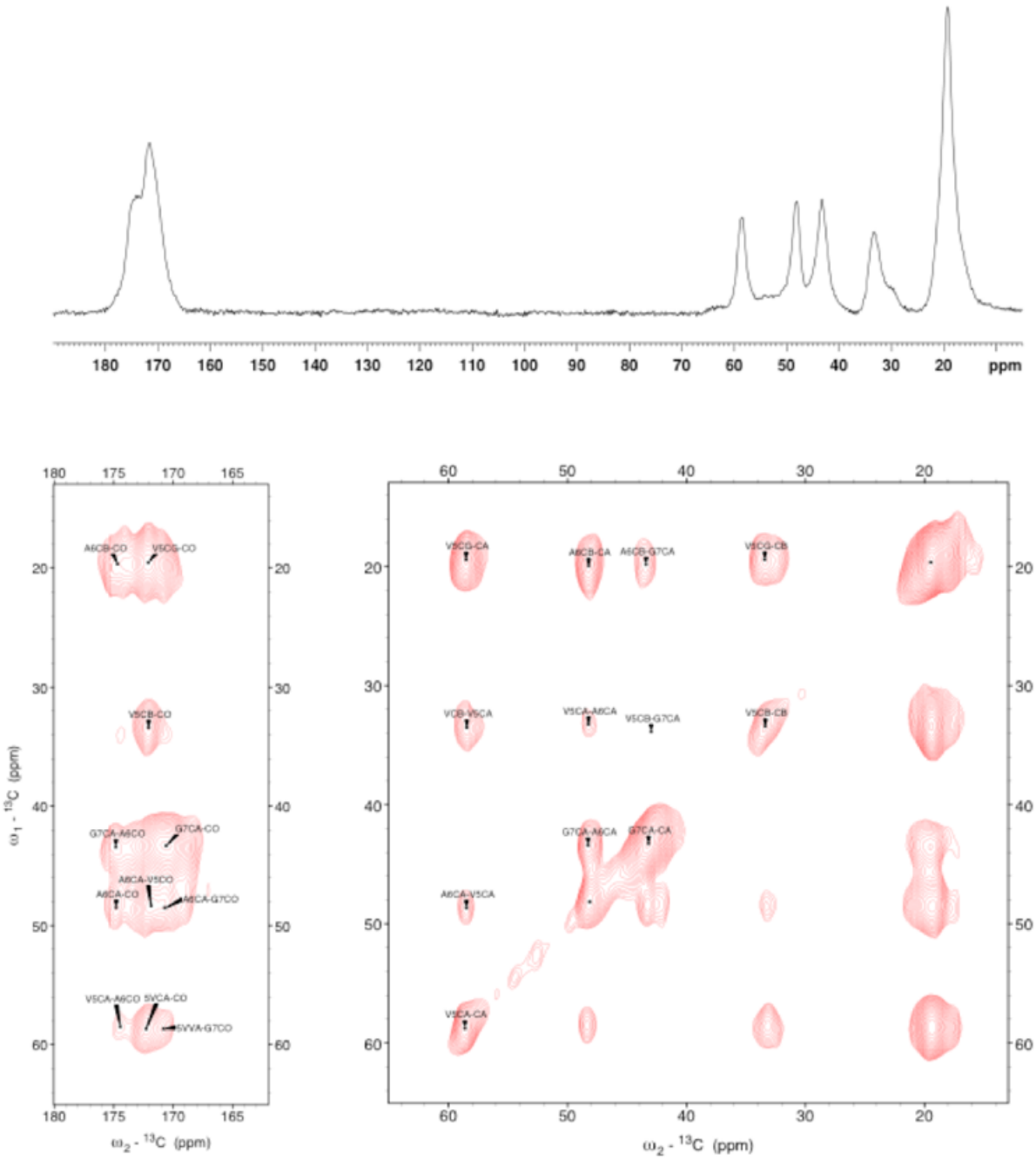

Figure S5. **A)** ^{13}C CP-MAS NMR spectrum of the uniformly $^{13}C/^{15}N$ labelled (I and T residues) β-LG_{11-20} peptide (1024 acquisitions, 2.5 s relaxation delay, and 1.5 ms contact time). **B)** ^{13}C-^{13}C solid-state PDSD NMR spectra of non-fibrillar peptide-IT(D**I**QKVAG**T**WY)

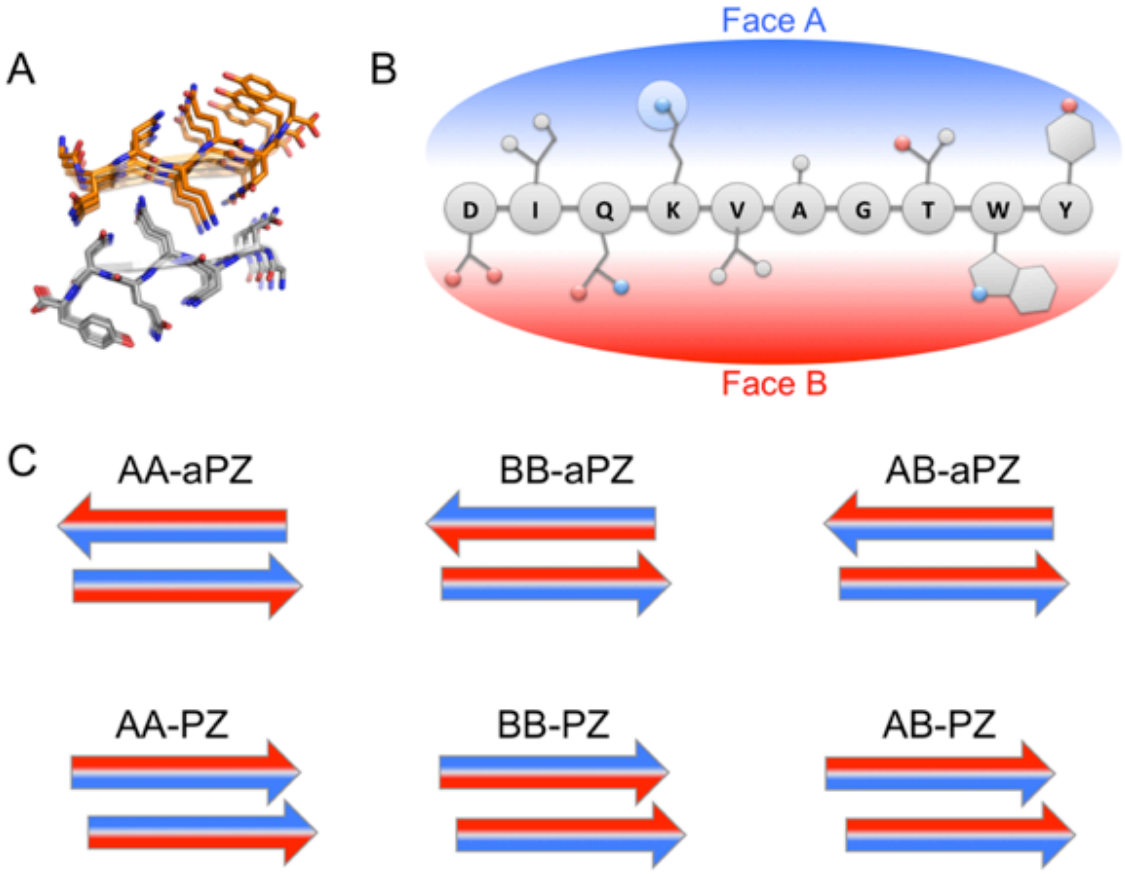

Figure S6. A) Illustration of a steric zipper structure (from PDB ID 1YJP). **B)** The two faces of the β-$LG_{11\text{-}20}$ peptide in extended strand conformation. **C)** Potential packing of anti-parallel (top row) and parallel (bottom row) β-sheet in "steric zippers". The colours indicate the faces as shown in panel B.

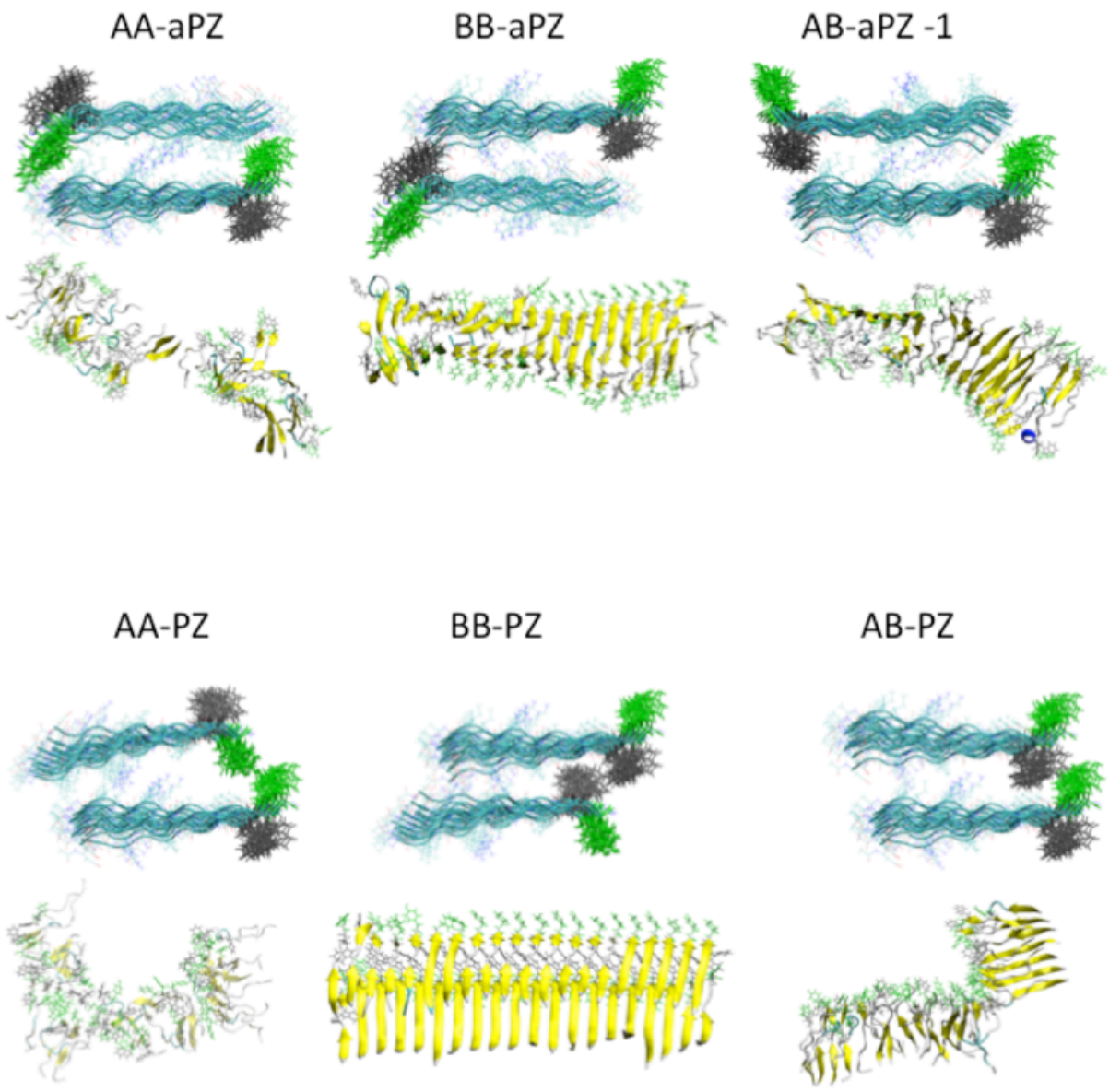

Figure S7. Six different arrangements of 40 peptides, considered in our 30 ns long MD simulations, in the *anti-parallel* A-A, B-B and A-B, and the *parallel* A-A, B-B and A-B zipper mode packing. Tyrosine and tryptophan moieties are shown in green and gray, respectively, and the beta sheet structures are highlighted in yellow. The most stable arrangements, BB-aPZ and BB-PZ, have *ca* 50 more H-bonding interactions and keep stable configuration during the simulation time with respect to the π-π stacking interactions.