Supplementary Material

A single-residue substitution inhibits fibrillization of Ala-based pentapeptides. A spectroscopic and molecular dynamics investigation

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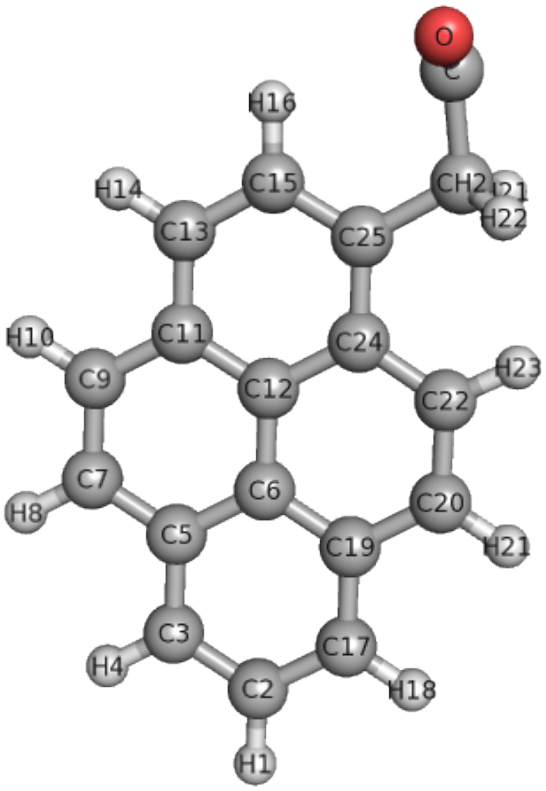
1. Peptide synthesis and characterization

Pyr-CH_2-CO-(L-Ala)$_5$-O*t*Bu (PyA5). This pentapeptide was synthesized from Pyr-CH_2-COOH and H-(L-Ala)$_5$-O*t*Bu in a 4:1 CH_2Cl_2/N,N-dimethylformamide solvent mixture in the presence of HATU and HOAt as efficient peptide coupling additives.
M. p.: 280-285 °C (with decomposition). IR (KBr): 3411, 3294, 1729, 1630, 1538 cm^{-1}. MS (ESI-ToF): $[M+H]^+_{exptl}$: 672.34; $[M+H]^+_{calcd}$: 671.33. ^1H NMR (400 MHz, DMSO, d_6): δ 8.55-7.88 [14H, 9 Pyr CH and 5 NH], 4.26-4.25 [7H, Pyr CH_2 and 5 Ala CH], 1.38 [9H, O*t*Bu CH_3], 1.27-1.11 [15 H, 5 Ala CH_3].

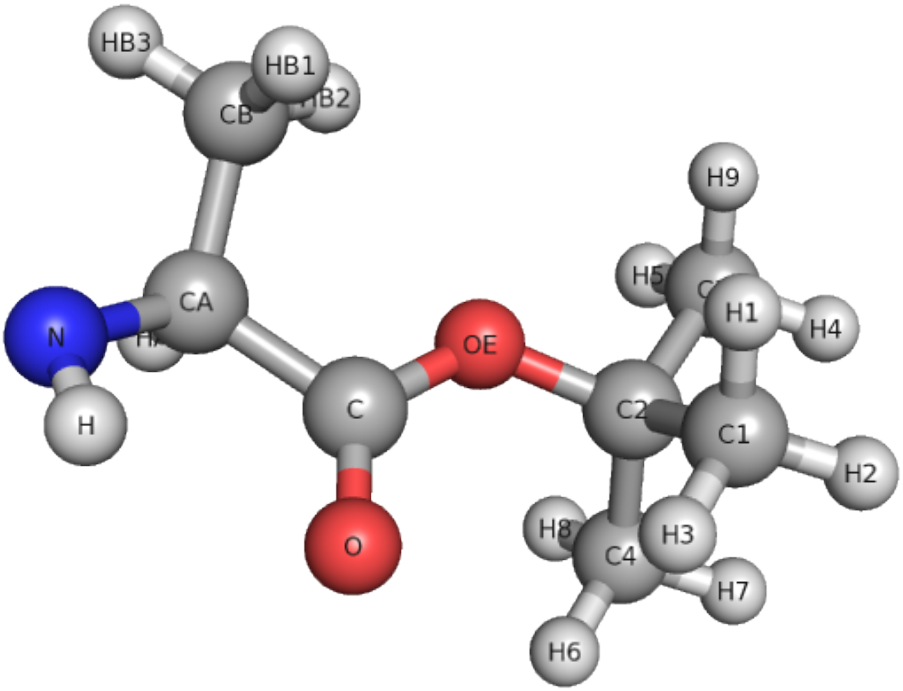
Pyr-CH_2-CO-(L-Ala)$_3$-Aib-L-Ala-O*t*Bu (PyA3UA). This pentapeptide was synthesized from Pyr-CH_2-COOH and H-(L-Ala)$_3$-Aib-L-Ala-O*t*Bu in CH_2Cl_2 solution in the presence of the coupling additives EDC and HOAt.
M. p.: 194-197 °C. IR (KBr): 3285, 1734, 1655, 1631, 1528 cm^{-1}. MS (ESI-ToF): $[M+H]^+_{exptl}$: 686.34; $[M+H]^+_{calcd}$: 685.35. ^1H NMR(400 MHz, $CDCl_3$): δ 8.24-7.95 [9H, Pyr CH], 6.94 [3H, 3 NH], 6.47 [1H, NH], 6.25 [1H, NH], 4.45-4.23 [6H, Pyr CH_2 and 4 Ala CH], 1.46 [9H, O*t*Bu CH_3], 1.35-1.29 [18H, 4 Ala and 2 Aib CH_3].

2. Partial charges of acetyl-pyrene



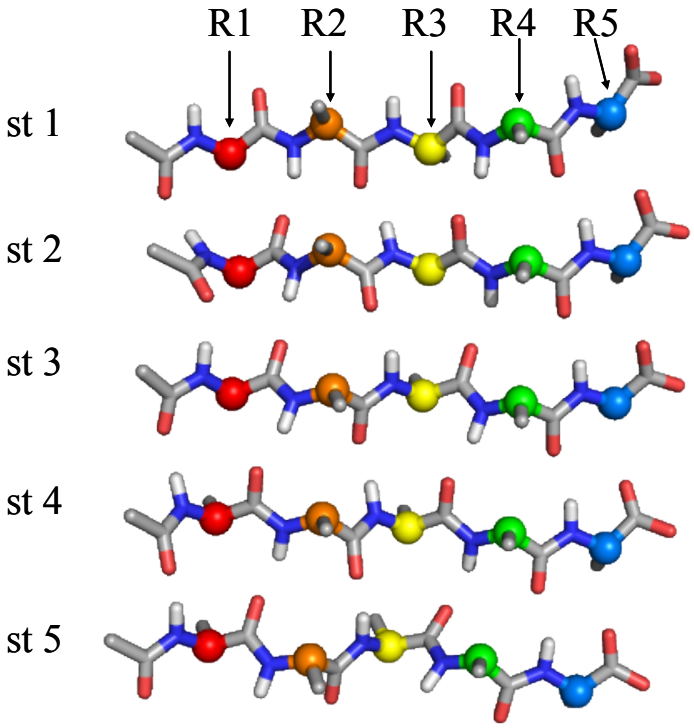
Atom name	FF Atom type	Atomic partial charges	Atom name	FF Atom type	Atomic partial charges
H1	HA	0.161000	C19	CA	0.003600
C2	CA	-0.234800	C20	CA	-0.219650
C3	CA	-0.131850	H21	HA	0.150950
H4	HA	0.142850	C22	CA	-0.185300
C5	CA	0.003600	H23	HA	0.164600
C6	CA	0.112100	C24	CA	0.036400
C7	CA	-0.219650	C25	CA	0.075900
H8	HA	0.150950	C26	CT	-0.292252
C9	CA	-0.185300	H27	HC	0.064080
H10	HA	0.164600	H28	HC	0.064080
C11	CA	0.073100	C	C	0.547987
C12	CA	0.052300	O	O	-0.506795
C13	CA	-0.169400			
H14	HA	0.136700			
C15	CA	-0.142000			
H16	HA	0.171200			
C17	CA	-0.131850			
H18	HA	0.142850			

Partial charges of *tert*-butyl ester of alanine



Atom name	FF Atom type	Atomic partial charges	Atom name	FF Atom type	Atomic partial charges
N	N	-0.404773	C2	CT	0.295404
H	H	0.294276	C1	CT	-0.335198
CA	CT	0.111037	H1	HC	0.078910
HA	H1	0.204749	H2	HC	0.078910
CB	CT	-0.229951	H3	HC	0.078910
HB1	HC	0.161375	C3	CT	-0.335198
HB2	HC	0.161375	H5	HC	0.078910
HB3	HC	0.161375	H4	HC	0.078910
C	C	0.678640	H9	HC	0.078910
O	O	-0.550940	C4	CT	-0.335198
OE	OS	-0.587163	H8	HC	0.078910
			H7	HC	0.078910
			H6	HC	0.078910

3. Schematic representation of the 5-strands system



Scheme S1 *Schematic representation of the built systems, consistent of 5 strands (st n) with 5 residues for each strand (R n)*

4. Average distances and total residence times of the H-bonds in PyA3UA and PyA5

Table S1 - Averaged distances (<*D*>) and total residence times (τ)[a] for the initial hydrogen bonds that defined the β-sheet assemblies of PyA3UA and PyA5 peptides in both water (w) and methanol (m). The position of each interaction within the assemblies is described in scheme DI: R*n* stands for the residue number in the strand st*m* (being m the strand number).

$(PyA3UA)_5^w$	τ (ns)	<D> (Å)	**$(PyA3UA)_5^w$**	***τ (ns)***	***<D> (Å)***
st2-st1_R1	11.46	2.161 ± 0.268	st2-st1_R1	14.433	2.138 ± 0.246
st3-st2_R1	7.161	2.128 ± 0.280	st3-st2_R1	13.047	2.020 ± 0.211
st4-st3_R1	4.278	2.142 ± 0.276	st4-st3_R1	2.805	2.297 ± 0.343
st5-st4_R1	0.195	2.470 ± 0.290	st5-st4_R1	7.653	2.336 ± 0.297
st2-st1_R2	13.149	2.061 ± 0.246	st2-st1_R2	5.124	2.116 ± 0.296
st3-st2_R2	8.409	2.050 ± 0.231	st3-st2_R2	3.93	2.192 ± 0.288
st4-st3_R2	5.511	2.114 ± 0.256	st4-st3_R2	8.133	2.198 ± 0.256
st5-st4_R2	0.756	2.166 ± 0.301	st5-st4_R2	14.691	1.985 ± 0.189
st2-st1_R3	7.212	2.183 ± 0.309	st2-st1_R3	4.032	2.097 ± 0.254
st3-st2_R3	1.8	2.029 ± 0.213	st3-st2_R3	2.322	2.110 ± 0.265
st4-st3_R3	6.321	2.004 ± 0.197	st4-st3_R3	7.383	2.013 ± 0.231
st5-st4_R3	5.838	1.971 ± 0.178	st5-st4_R3	11.73	2.115 ± 0.271
st2-st1_R4	——	——	st2-st1_R4	——	——
st3-st2_R4	0.12	2.502 ± 0.306	st3-st2_R4	0.282	2.317 ± 0.303
st4-st3_R4	3.183	2.418 ± 0.312	st4-st3_R4	5.184	2.085 ± 0.248
st5-st4_R4	4.266	2.160 ± 0.271	st5-st4_R4	1.464	2.500 ± 0.270
st2-st1_R5	——	——	st2-st1_R5	——	——
st3-st2_R5	——	——	st3-st2_R5	——	——
st4-st3_R5	1.257	2.538 ± 0.341	st4-st3_R5	——	——
st5-st4_R5	——	——	st5-st4_R5	——	——

$(PyA5)_5^m$	τ (ns)	<D> (Å)	**$(PyA5)_5^m$**	τ (ns)	<D> (Å)
st2-st1_R1	11.478	2.089 ± 0.226	st2-st1_R1	8.626	2.222 ± 0.299
st3-st2_R1	0.039	2.646 ± 0.346	st3-st2_R1	5.278	2.122 ± 0.254
st4-st3_R1	13.074	2.047 ± 0.226	st4-st3_R1	11.524	2.150 ± 0.234
st5-st4_R1	1.5	2.155 ± 0.284	st5-st4_R1	14.410	2.092 ± 0.232
st2-st1_R2	13.833	2.021 ± 0.201	st2-st1_R2	3.408	2.015 ± 0.233
st3-st2_R2	1.428	2.077 ± 0.237	st3-st2_R2	9.698	2.216 ± 0.303
st4-st3_R2	12.15	2.221 ± 0.289	st4-st3_R2	9.008	2.038 ± 0.225
st5-st4_R2	4.161	2.051 ± 0.224	st5-st4_R2	14.650	2.095 ± 0.239
st2-st1_R3	13.71	2.031 ± 0.231	st2-st1_R3	14.532	2.073 ± 0.248
st3-st2_R3	14.109	2.154 ± 0.261	st3-st2_R3	13.262	2.131 ± 0.309
st4-st3_R3	0.168	2.599 ± 0.307	st4-st3_R3	1.002	2.412 ± 0.334
st5-st4_R3	0.216	2.100 ± 0.246	st5-st4_R3	1.192	2.324 ± 0.328
st2-st1_R4	——	——	st2-st1_R4	——	——
st3-st2_R4	9.123	2.099 ± 0.257	st3-st2_R4	7.392	2.051 ± 0.228
st4-st3_R4	——	1.922 ± 0.000	st4-st3_R4	——	——
st5-st4_R4	0.027	2.359 ± 0.336	st5-st4_R4	——	——
st2-st1_R5	——	——	st2-st1_R5	0.006	2.842 ± 0.133
st3-st2_R5	——	——	st3-st2_R5	2.732	2.344 ± 0.363
st4-st3_R5	——	——	st4-st3_R5	0.122	1.978 ± 0.197
st5-st4_R5	0.018	2.694 ± 0.366	st5-st4_R5	——	——

[a]The presence of a hydrogen bond has been assessed by a geometrical cutoff. A hydrogen bond was computed when the distance d(N-H…O) ≤ 2.5 Å and the angle <NH…O is bigger than 120º

5. **AFM imaging of PyA3UA on a mica surface**