Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Amyloid Aggregates Arise from Amino Acid Condensations under Prebiotic Conditions

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Supporting Information

Materials and Methods

COS-activated amino acid polymerization
Reactions were performed in different configurations depending on the concentration of amino acid. For 10 mM and 20 mM amino acid, a balloon filled with 200-250 mg COS was attached to a 15 ml tube filled with 10 ml of an amino acid solution in 500 mM borate pH 9.2. The tube was mixed upright on an IKA Vortex 3 for 1 h by which time most of the COS had been consumed or dissolved into solution less than 50 mg left in the balloon. For the 1 mM Ala reaction, 100 ml of Ala in 50 mM borate was added to an empty ~100ml chromatography column with a stir bar in the bottom. A balloon filled with 200-250 mg COS was attached and the column outlet was opened to release 50ml of solution. The activation was allowed to proceed for 1 hour with stirring. 500 μl of the activated amino acid solution was removed for ^{1}H-NMR analysis of the thiocarbamate formation and the rest was used for the polymerization reaction. Typically, the 80-85% of the Ala and 90-95% of the Val was converted to their respective thiocarbamate. The polymerization reaction was carried out with either stepwise or continuous (dropwise) addition of the activated amino acid to a solution of potassium ferricyanate $K_3Fe(CN)_6$. In the case of the stepwise procedure 500 μl COS-treated amino acid was mixed with 60 μl 1M $K_3Fe(CN)_6$ after which an aliquot of 120 μl was removed. Every 3-5 min another 500 μl amino acid and 60 μl $K_3Fe(CN)_6$ was added to the reaction followed by the removal of another 120 μl aliquot. It is worth noting that the polymerization reactions with potassium ferricyanide also produce a precipitate of sulfur, as a result of the oxidation of hydrogen sulfide released in the hydrolysis of COS. In some cases, the sulfur precipitate gave rise to a powder pattern in the diffraction images. However, the sulfur precipitate does not interfere with the CD spectra as it is achiral and does not absorb in the far UV.

CDI-activated amino acid polymerization
Amino acid stock solutions (2M Gly, 1M Ala, 500mM sodium aspartate pH 7, 300mM ^{15}N-Val) were used to create the low-Gly and high-Gly reactions. Low-Gly reaction: 5 μl Gly, 50 μl Ala, 10 μl Asp and 75 μl ^{15}N-Val were mixed on ice and then activated with 31 mg carbonyldiimidazole (CDI, ≥97% Aldrich) for 2 min. The activated mixture was diluted to 10 ml with water (final 1 mM Gly, 5 mM Ala, 0.5 mM Asp, 2.5 mM ^{15}N-Val). High-Gly reaction: 20 μl Gly, 40 μl Ala, 10 μl Asp and 45 μl ^{15}N-Val were mixed on ice and then activated with 34 mg CDI for 2 min. The activated mixture was diluted to 5 ml with water (final 8 mM Gly, 8 mM Ala, 1 mM Asp, 3 mM ^{15}N-Val). The ^{15}N-Val was used because without the isotopic label the mass of three alanine residues is identical to 2 glycine and 1 valine residue.

Reverse-phase chromatography
Reverse-phase analyses were performed on a 3.5 μm 4.6 x 150 mm Kinetex C18 column (Phenomenex) connected to an Agilent 1200 system equipped with an auto sampler and diode array detector. The soluble reaction products were injected onto the column and resolved with a gradient from 1 % acetonitrile with 0.1 % TFA to 25 % acetonitrile with 0.1% TFA.

Mass spectrometry

The precipitate samples were washed, dried under vacuum and then solubilized with trifluoroacetic acid (TFA, Fisher HPLC grade). Acetonitrile was added to the TFA-solubilized samples to a final TFA concentration of 10-20%. The matrix (α-Cyano-4-hydroxycinnamic acid) was applied to the MALDI target (MTP SmallAnchor 384 TF, Bruker) as a 20 mg/ml solution in acetone. Approximately 300 nl of the sample in acetonitrile/TFA was applied on top of the matrix. The supernatant samples were mixed 1:1 with acetonitrile and ionized by electrospray. The FID were collected with 4M points giving an effective resolution of 300k-400k. This resolution allowed for the unambiguous identification of all possible combinations of even the most complex mixture in this study (^{14}N-Ala, ^{14}N-Asp, ^{14}N-Gly, ^{15}N-Val) as illustrated in Figure S9. The raw data were exported with CompassXport (Bruker) and the peaks were analyzed with mMass[1]. Masses were only assigned to specific peptides when the observed mass differed by less than 0.5 ppm from the calculated mass.

Circular dichroism and Fourier transform infrared spectroscopy

The precipitates from the polymerization reactions were washed with water by several rounds of centrifugation at 25k g and then resuspended in water. The CD spectra were measured on a Jasco J-815 with 1 mm path length and scanned from 260–190 nm at 50 nm/min, 1 nm band-pass, 2 second integration, averaged over 3 repetitions. The samples were diluted as necessary to maintain the PMT gain below 600 V. The same samples were applied to a diamond ATR cell on a Bruker Alpha FTIR spectrometer and air dried before measuring their spectra with 32 scans and a resolution of 2 cm^{-1}. It is important to note that the CD spectra represent primarily the material whose size does not preclude absorption. Thus very large aggregates will not give rise to absorption but rather scattering and will only be detectable via ATR-FTIR.

X-ray fiber diffraction

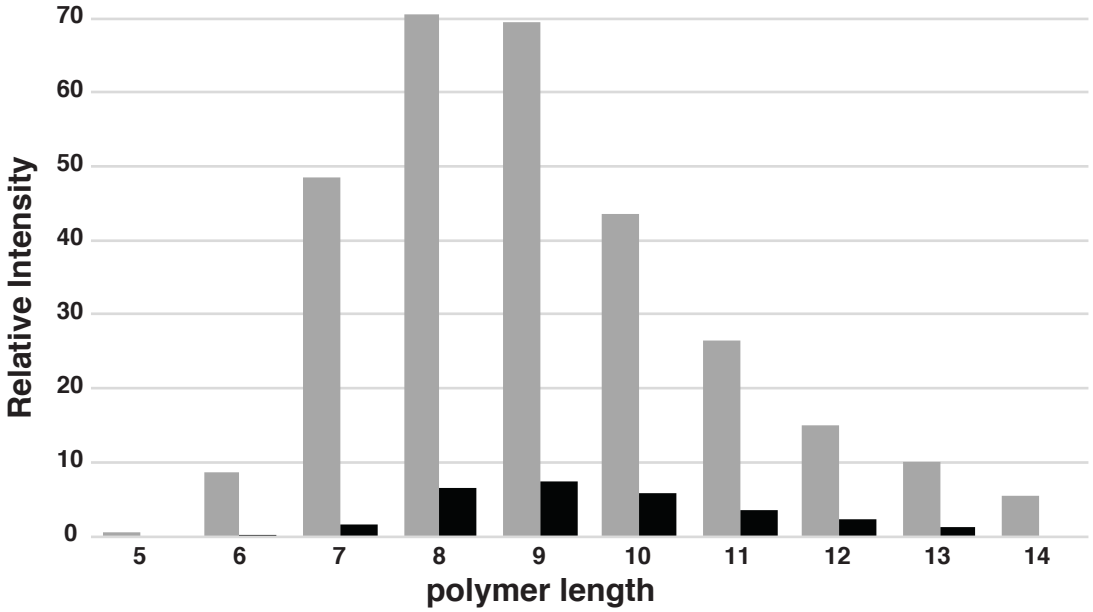
The washed precipitates were resuspended in a minimal amount of water and 4 µl of the suspension were pipetted between the melted ends of two glass capillaries, which were then left to dry overnight in a sealed petri dish. The dried material was placed in an X-ray beam (Rigaku MicroMax-007HF) at room temperature for a 60 min exposure and the diffracted x-ray intensity was measured with a Mar345 image plate detector. The beam position on the image plate was located as the center of the circles in the diffraction pattern of polypropylene using the FIT2D software [2] and the radial intensity of each ring within a 10 pixel wide band was extracted with the ADXV software.

Transmission electron microscopy (TEM)

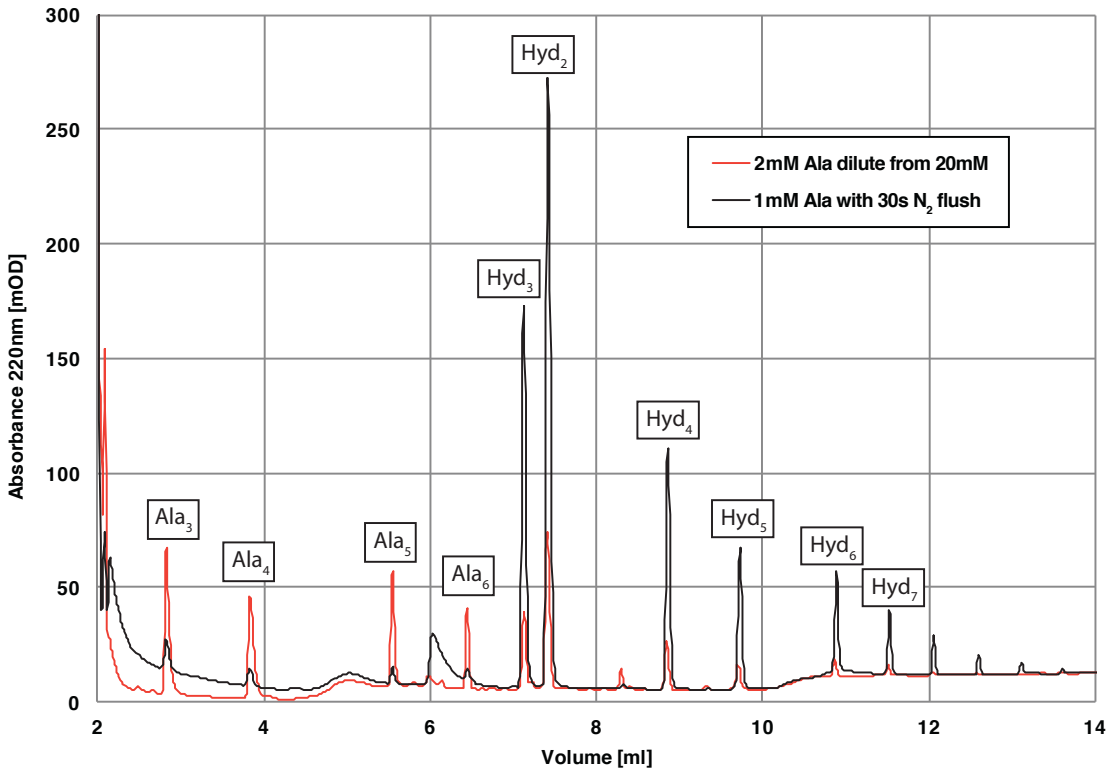
The samples prepared as for CD spectroscopy were applied directly to glow-discharged carbon-coated copper grids, washed and then stained with uranyl formate. Images were recorded on a Philips CM 12 electron microscope.

Quantitation of peptides in precipitates

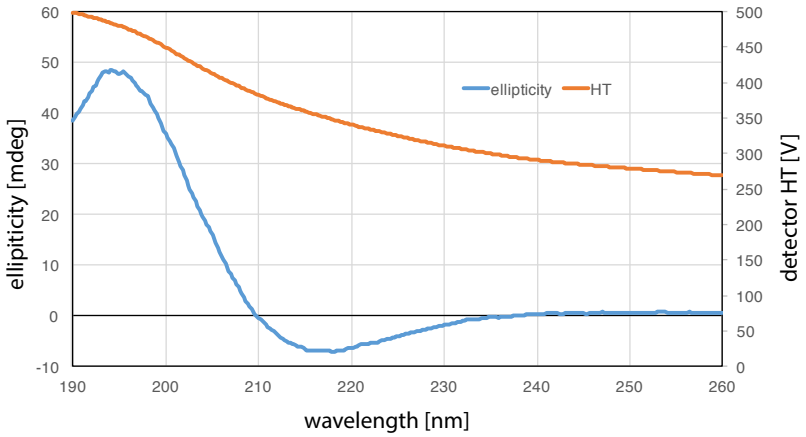
The washed precipitates were hydrolyzed in 6M HCl for 1 h at 160 °C, dried at 10 mbar and then resuspended in water. The amino acid content was measured with a ninhydrin assay preformed according to Rosen[3] and standardized with a series of Ala solutions of known concentration.



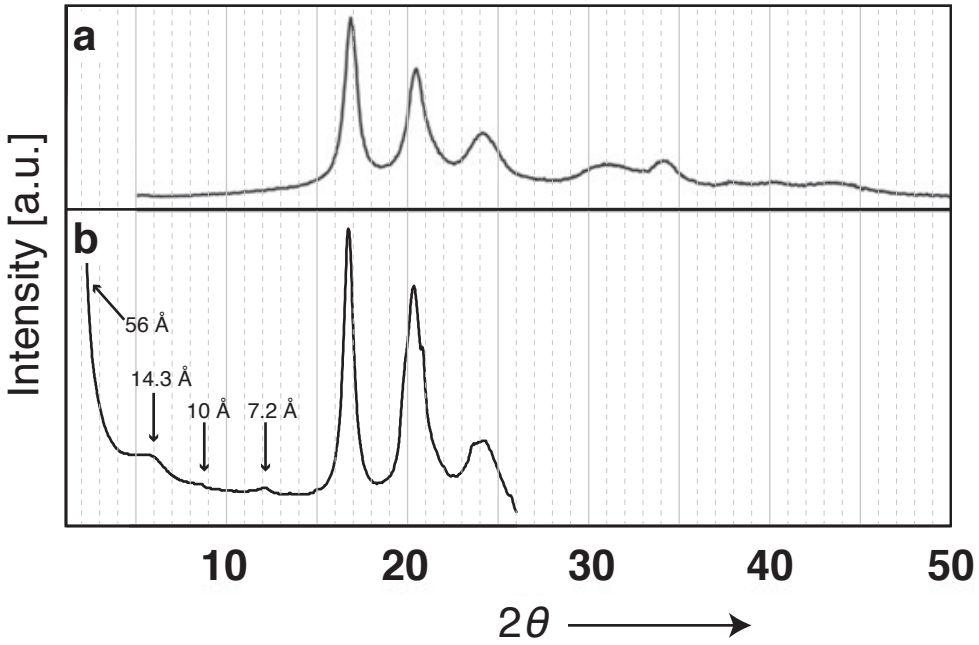
Supplemental Figure 1. Distribution of amino- and hydantoin-terminal peptides in the poly-Ala precipitate after 13 step-wise additions of 20mM Ala. The histogram depicts the signal intensity as a function of polymer length for the sodium adducts (gray : amino, black : hydantoin) from the MALDI FT-ICR mass spectrum of the final precipitate from the polymerization reaction described in Figure 1. Hydantoin peptides make up about 10% of the total signal and urea derivatives (not visible in figure) make up less than 1% of the total signal. The K^+ adducts gave a similar ratio but the H^+ adducts have only 1.6% hydantoin. The error in this estimate of the amino-terminal peptide yield based on the relative intensity of the MS signals is difficult to quantify, however the value is consistent with the trends depicted in Figure 1a-b,e. That is, as the reaction proceeds, more precipitated peptides form (Figure 1e) yet the overall yield of hydantoins decreases with reaction volume and polymer length (Figure 1b). For example, at aliquot 13 in Figure 1a-b there is half as much 3-mer amino-peptide as 3-mer hydantoin but 37% more 5-mer amino-peptide than 5-mer hydantoin. This trend indicates that for the longer peptides found in the precipitate, the ratio of amino-terminal peptides to hydantoins would be even larger.



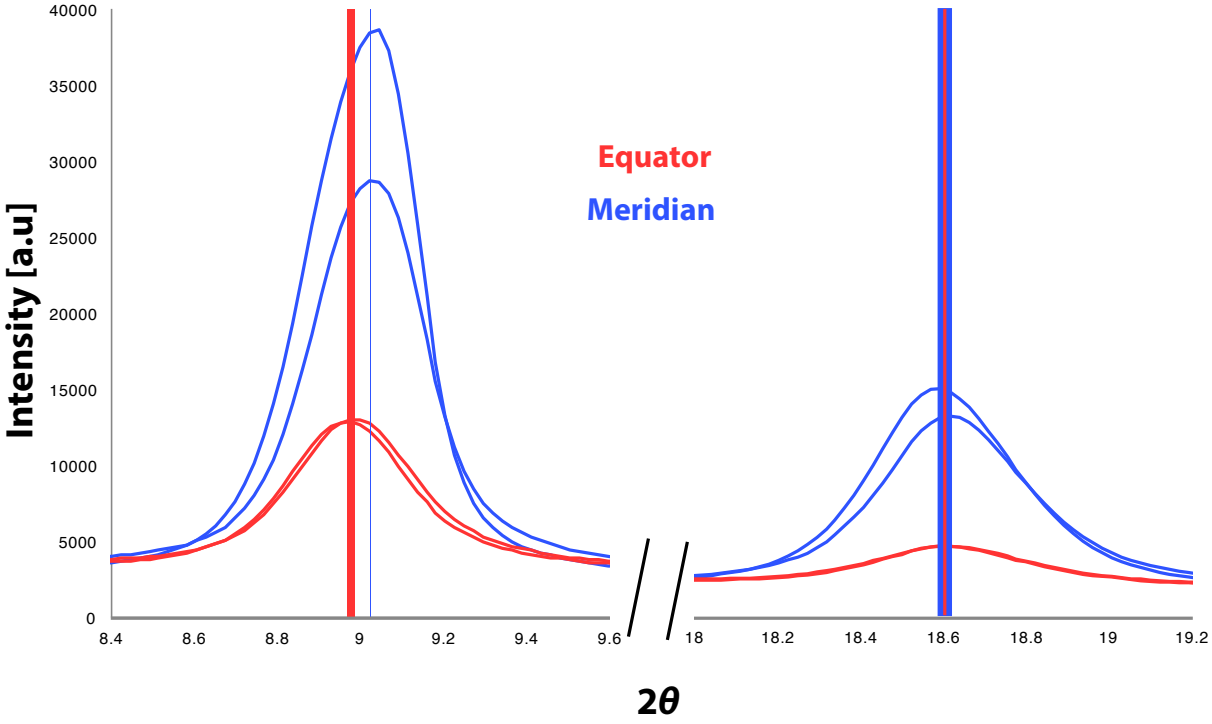
Supplemental Figure 2. HPLC chromatograms of 1 and 2 mM Ala polymerization reactions. The major peaks were identified by FT-ICR-MS and correspond to the Ala 3- to 6-mer peptide and the dimer to heptamer hydantoin peptides. The 1mM continuous flow reaction is described in the text and the 2mM reaction was performed as a routine 20mM COS-activation of Ala and then diluted with water to 2 mM. The 2 mM continuous flow reaction had a total volume of 80 ml with 120 µl additions of 1M at the start and after every 10ml increase in volume.



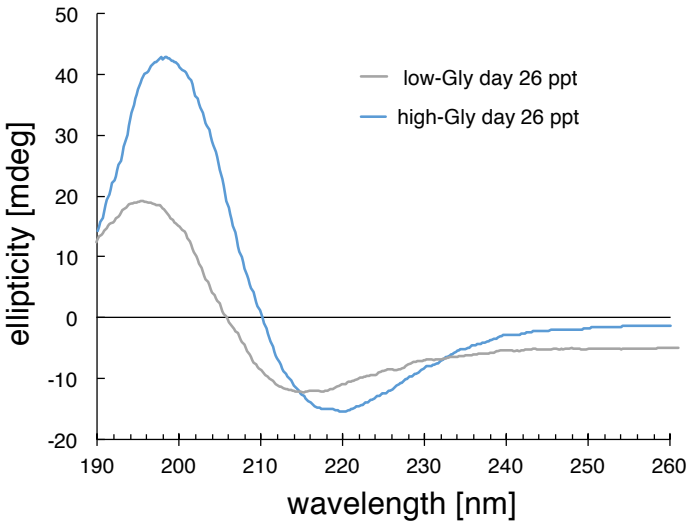
Supplemental Figure 3. The precipitate from the 1 mM COS-activated Ala reaction is β-structured. The precipitate was collected by centrifugation from a 100-fold concentrated sample of the polymerization reaction described in the main text.



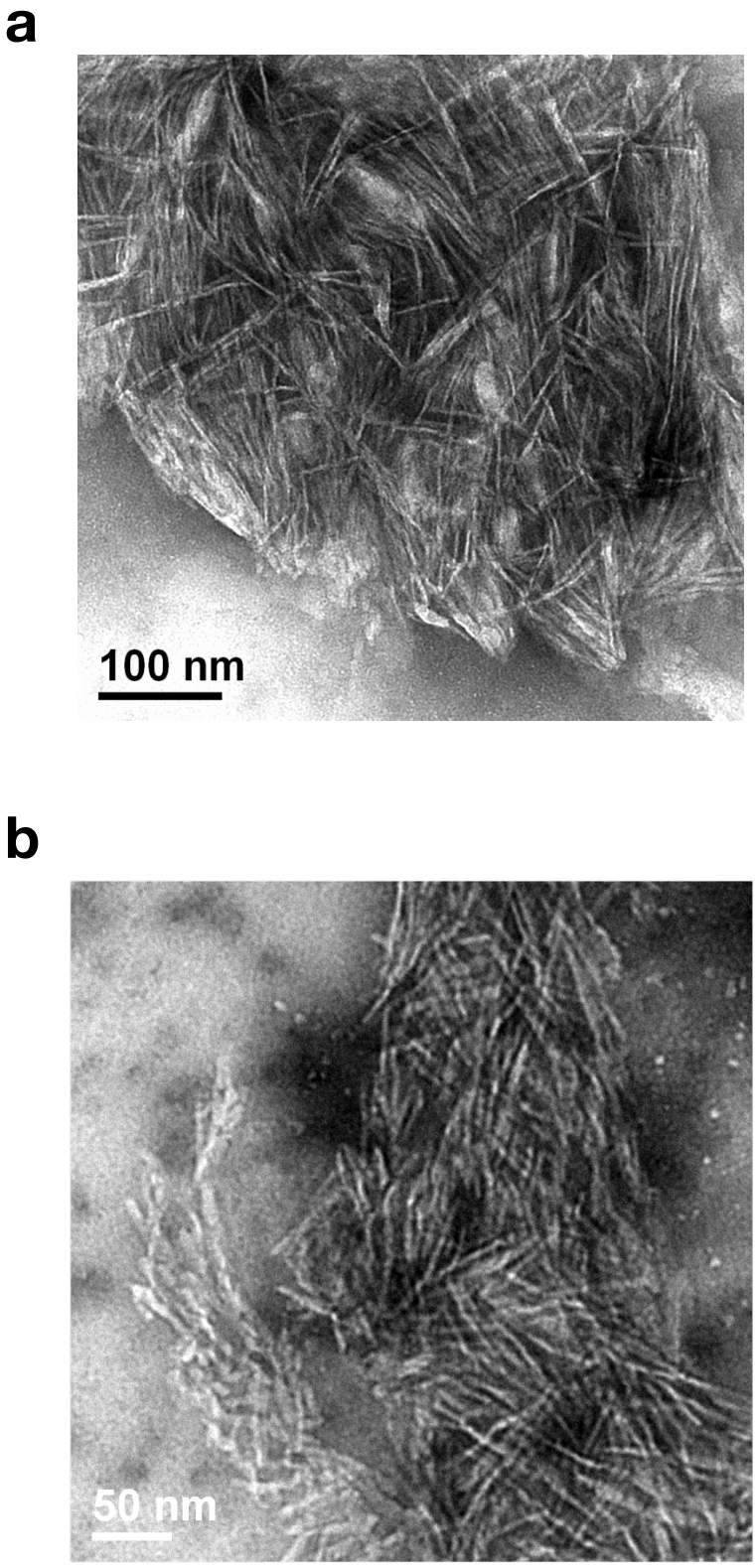
Supplemental Figure 4. Comparison of the poly-Ala diffraction reported here to that from Asakura et al. [4]. An intensity versus 2-theta plot of the data from the a) powder diffraction of an Ala heptamer[4] and b) our aligned poly-Ala sample data highlights the similarities in the higher-resolution reflections and additionally new low-resolution reflections that arise from the alignment of the fiber sample.



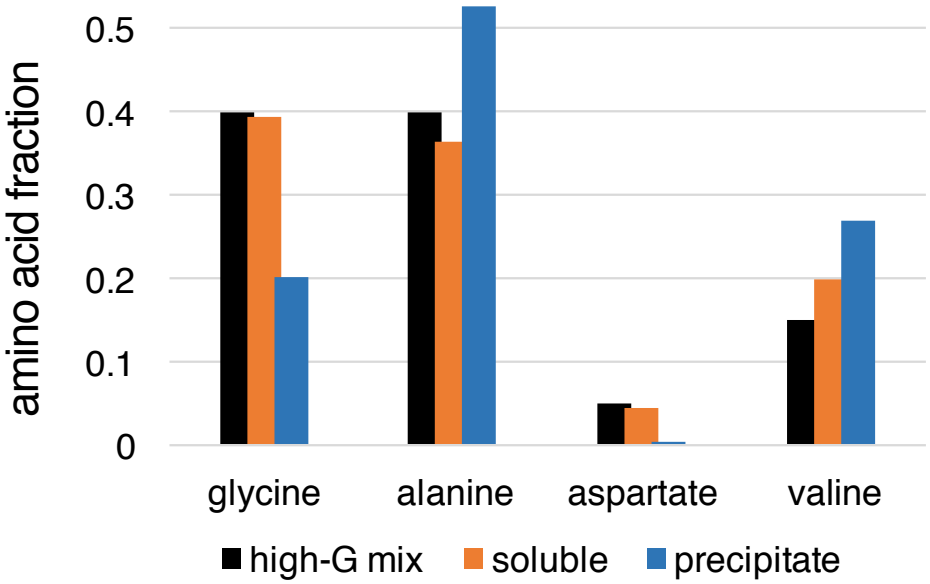
Supplemental Figure 5. An intensity versus 2-theta plot of the poly-Val sample comparing the meridional and equatorial directions. The meridian intensity as a function of 2-theta in opposite directions from the calculated beam center is in blue and the two equatorial directions are in red. The difference between the two blue or two red peak positions is indicated by the vertical line with a thickness equal to this difference. The peak centers were taken as the center of the peak at 80% intensity. The subtle difference in the equatorial and meridional peak positions for the 9.3 Å reflection (2-theta ~ 9) is indicated by the separation of the red and blue vertical lines. In contrast the peak position of the 4.63 Å peak is the same in both the meridian and equator.



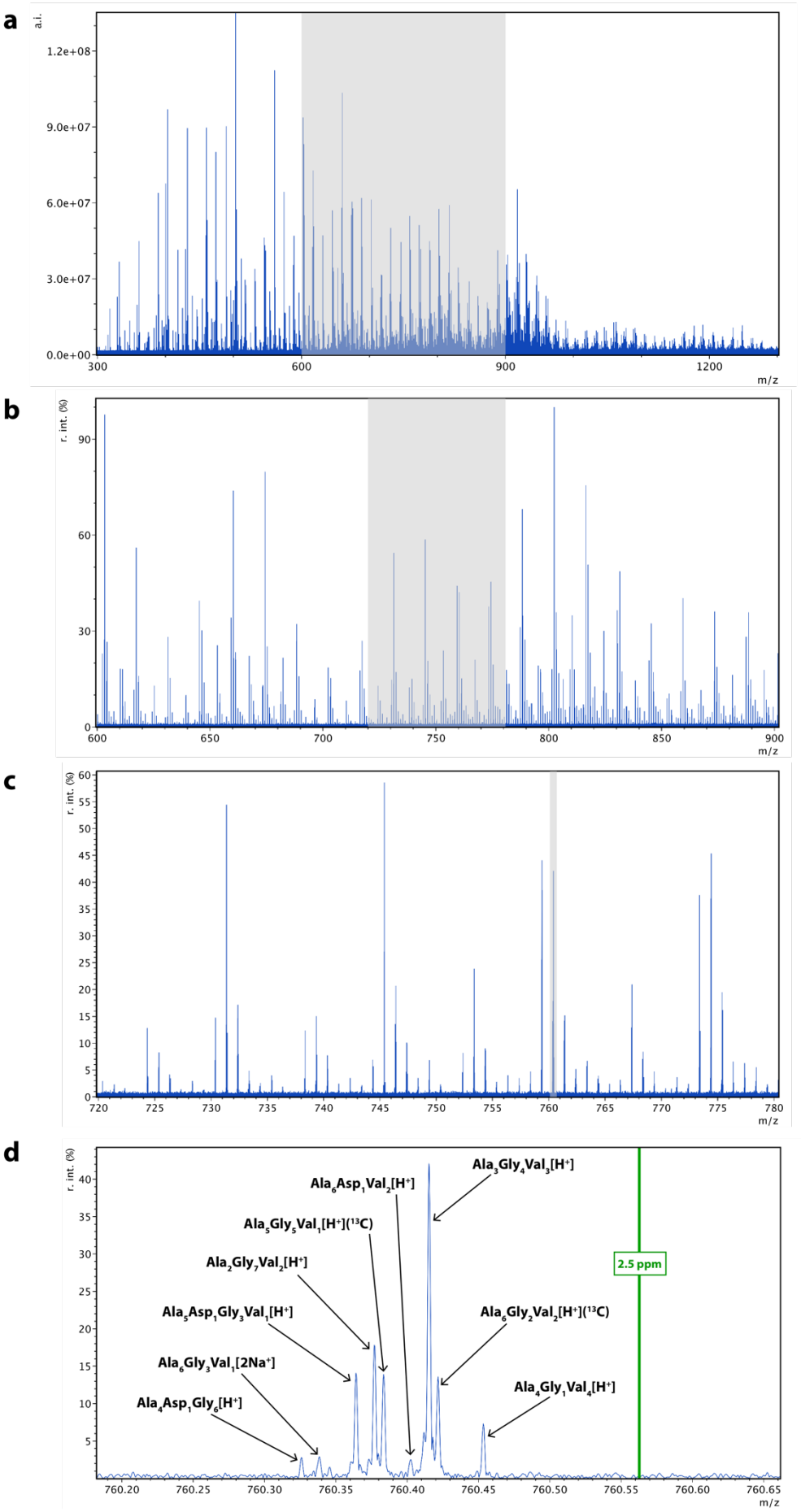
Supplemental Figure 6. The polymerization of mixtures of amino acids leads to β-structured peptides. The precipitate from the CDI-activated polymerization of the high-Gly and low-Gly mixtures was collected by centrifugation, washed with water and resuspended in an equal volume (low-Gly) or a 5-fold smaller volume (high-Gly). Their CD spectra indicate that both are highly β-structured while the low-Gly sample also exhibits a significant degree of differential scattering (negative ellipticity at 260 nm) due to some asymmetry in a structural unit that has a dimension greater than 13 nm[5].



Supplemental Figure 7. Electron micrograph of the precipitates from the polymerizations of the a) low-Gly and b) high-Gly mixtures of amino acids.



Supplemental Figure 8. Gly and Asp are selected against in the precipitate of the high-Gly mixture of amino acids. The precipitate (same sample as in Figures S6 and S7) and the soluble fraction from the high-Gly polymerization were analyzed by MALDI (precipitate) and ESI (soluble) FT-ICR-MS. A total of 231 precipitate and 503 soluble peptide compositions could be identified in the spectra within a mass accuracy of 0.5 ppm. The histogram plot shows the average amino acid composition for these identified masses compared to the starting high-Gly amino acid mixture.


a
a.i.
1.2e+08
9.0e+07
6.0e+07
3.0e+07
0.0e+00
300
600
900
1200
m/z
b
r. int. (%)
90
60
30
0
600
650
700
750
800
850
900
m/z
c
r. int. (%)
60
55
50
45
40
35
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25
20
15
10
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720
725
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735
740
745
750
755
760
765
770
775
780
m/z
d
r. int. (%)
40
35
30
25
20
15
10
5
Ala3Gly4Val3[H+]
Ala6Asp1Val2[H+]
Ala5Gly5Val1[H+](13C)
Ala2Gly7Val2[H+]
Ala5Asp1Gly3Val1[H+]
Ala6Gly3Val1[2Na+]
Ala4Asp1Gly6[H+]
Ala6Gly2Val2[H+](13C)
Ala4Gly1Val4[H+]
2.5 ppm
760.20
760.25
760.30
760.35
760.40
760.45
760.50
760.55
760.60
760.65
m/z

Supplemental Figure 9. ESI-FT-ICR-MS spectrum of the supernatant from the high-Gly polymerization. The four panels show a progressively narrower m/z range with the gray shaded region indicating the m/z range of the subsequent panel. In panel d) the assigned masses are indicated and a green vertical line with the width of 2.5 ppm is shown as a reference for the 0.5 ppm precision within which all masses were identified. The valine residues are all ^{15}N isotopes and are thus not specially designated as such. However, the single ^{13}C natural abundance isotopes are specified.

References

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