EXPERIMENTAL PROCEDURES

Recombinant Protein Expression and Purification

All chemicals were obtained from Sigma unless specified otherwise. The expression plasmid (pET23b), provided by Philippa Talmud (University College London Medical School, London, UK) (1), was transformed into BL21(DE3) pLysS competent cells (Invitrogen). This construct contains a C-terminal hexa-histidine tag following LE as two spacer residues. Cells were grown at pH 7.0 and 37 °C with 25% dissolved oxygen using a 10-L BioFlo 110 fermentor (New Brunswick Scientific) supplemented with 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, 14.2 g/L Na₂HPO₄, 3.5 g/L glucose, 0.5 g/L MgSO₄·7H₂O, and 100 mg/L ampicillin. Cells were induced upon reaching an O.D. ~3.0 at 600 nm with 0.5 mM isopropyl β-D-1-thiogalactopyranoside. Glucose also was added (4 g/L) at induction and whenever dissolved oxygen levels increased. Cells were harvested after 2 h using a continuous centrifuge (Sharples) at a flow rate of 250 mL/min and stored at −80 °C until use.

Cell pellets (∼80 g) were resuspended in 300 mL of 100 mM sodium phosphate (NaPi) pH 7.4 buffer containing 100 mM NaCl, 8 M urea, and 6 protease inhibitor cocktail tablets (Roche 04693159001) for 90 min at RT, homogenized mechanically, and then passed twice through a microfluidizer (M-110PS, Microfluidics) at 18,000 psi. Cell debris was removed by centrifugation at 14,000 g for 90 min and the resulting supernatant was shaken overnight (160 rpm) at 4 °C with 30 ml of Ni-NTA resin (Qiagen) in 100 mM NaPi, 100 mM NaCl, 8 M urea, pH 7.4 buffer. The resin was then washed with 200 mL of 100 mM NaPi, 100 mM NaCl, 8 M urea, pH 7.4 buffer. Protein was eluted with 80 mL of the same buffer containing 300 mM imidazole and concentrated to a final volume of ∼3 mL using Amicon Ultracel MWCO 3 kD filters. Further purification was achieved by gel filtration chromatography (Hi Load 16/60 Superdex 75, GE Healthcare) equilibrated in 10 mM NaPi, 0.8 M urea, pH 7.4, followed by anionic exchange chromatography (Mono Q HR 16/10, Pharmacia) using a linear salt gradient of 50–150 mM NaCl in 10 mM NaPi, 0.8 M urea, pH 7.4. Fractions containing ApoCIII were pooled, concentrated to ∼100 µM, and stored at −80°C after freezing in liquid nitrogen. Protein
concentration was determined using an extinction coefficient $\varepsilon_{280\text{nm}} = 19,480 \text{ M}^{-1} \text{ cm}^{-1}$, estimated based on amino acid content (ProtParam). SDS-PAGE analysis confirmed sample homogeneity and mass spectrometry indicated two primary components corresponding to ApoCIII without (9829.6, 82% based on ion counts) and with an N-terminal methionine (9960.8, 18%), respectively.

**Protein Preparation**

Frozen protein stocks were thawed and buffer exchanged into 10 mM NaPi, 100 mM NaCl, 1 mM EDTA pH 7.4 buffer using a PD-10 desalting column (GE Healthcare). To ensure consistency, 500 µL of protein (~100 µM) were loaded onto the column each time, followed by 2 mL of buffer, and then collection of 2 mL from the column. The typical resulting concentration is ~24 µM which was then diluted in NaPi buffer to the specified concentration. The inclusion of EDTA is to ensure any trace metals would be chelated and not bind to the C-terminal His-tag.

**Monitoring Aggregation Kinetics**

ApoCIII was agitated for ~3 days in 96-well flat bottom, untreated plates (Costar) sealed with silicone adhesive film (VWR) using a Tecan Infinite M200 Pro microplate reader at 37 °C, with 1-mm orbital shaking (ca. 88 rpm), in NaPi buffer (10 mM NaPi, 100 mM NaCl, pH 7.4, 1 mM EDTA), inclusion of one 2-mm glass bead per well, and a total volume of 180 µL per well. Thioflavin T (ThT, 10 µM) was included for some experiments and results were similar in the presence or absence of ThT. ThT (excitation at 415 nm, emission at 480 nm, 25 flashes, gain 100) and Trp fluorescence (excitation at 280 nm, emission at 350 nm, 25 flashes, gain 80) were measured from the top at 45 min intervals until completion.

**Congo Red Binding Assay**

A Congo Red (CR) stock solution was prepared by mixing 1 mL of CR solution (0.2% Congo Red, Sigma Aldrich HT60-3) with 10 µL of 0.25 M NaOH, filtered (0.22 µm), and used immediately. The CR stock (10 µL) was then added to 900 µL of buffer (pH 7.4, 100 mM NaCl, 10 mM NaPi), followed by 100 µL of 20 µM protein sample, and then incubated at RT for ~45 min. Absorption spectrum was measured in a Cary 300 Bio UV-Visible spectrophotometer using a 1-cm quartz cuvette.
**Circular Dichroism Spectroscopy**

Circular dichroism spectra were measured on a Jasco J-715 spectropolarimeter (200–260 nm, 1 nm steps, 50 nm/min, 1 nm bandwidth, 0.5-s integration time) at 22 °C. Background signal for the sample buffer was independently measured and subtracted.

**Transmission Electron Microscopy**

TEM was conducted with a JEOL JEM 1200EX electron microscope (JEOL USA) equipped with an AMT XR-60 6 megapixel digital camera (Advance Microscopy Techniques). 400 mesh Formvar/carbon grid (Electron Microscopy Sciences) were glow discharged for 1 min. Subsequently, 3 µL of sample was applied to the grid for 10 s and then five drops of 1 % w/v uranyl acetate were applied at approximately 1 drop per second. The stain was slowly wicked off using a filter paper and then air dried for at least one minute before imaging.

**Atomic Force Microscopy**

20 µM aggregated sample (stored at 4 °C) was diluted 100 fold (10 mM NaPi, 100 mM NaCl, pH 7.4) and then 10 µl was applied to freshly cleaved mica, excess liquid was wicked off, and then the sample was air dried for approximately 10 minutes before imaging using a Multimode 8 atomic force microscope (Bruker) and TESP7 tips (Veeco). Height maps were analyzed using Nanoscope Analysis 1.40 software (Bruker).

**Electron Diffraction**

Samples were exchanged into pure water to eliminate salt contributions to the electron diffraction pattern. Aggregated ApoCIII (100 µL of 20 µM) was diluted to 500 µL in Ultra Pure Water (Quality Biological Inc., Molecular Biology Grade) and then concentrated to ~70 µL in an Amicon 100 kD microcon at 2000 g for 5 min. This procedure was repeated for a total of five times and then 5 µL of sample was immediately applied to a recently glow-discharged 400 mesh Formvar/carbon grid (Electron Microscopy Sciences) and allowed to air dry at RT. Diffraction images were acquired on a Philip CM120 electron microscope (FEI, Hillsboro, OR) operating at 120 kV and recorded on photographic films (SO-163, Kodak). Negatives were digitized by a Super CoolScan 9000 ED scanner (Nikon, Melville, NY). Diffraction patterns were taken from areas of 6 µm diameter with doses of 1–4 e⁻/Å². Lattice spacings were standardized using thallium chloride crystals (Electron Microscopy Sciences).
Proteinase K Digestion

Monomeric and aggregated ApoCIII (20 µM protein, 10 mM NaPi, 100 mM NaCl, pH 7.4, 1 mM EDTA) were mixed with 100 ng/mL Proteinase K (PK, Invitrogen) and incubated at 37 °C for 5 min and 60 min. Samples were subsequently heated at 100 °C for 3 min to deactivate PK and then stored at 4 °C for SDS-PAGE analysis and liquid chromatography mass spectrometry (LC-MS). For gel analysis, samples were mixed 1:1 with 8 M urea, 100 mM NaCl, 100 mM NaPi, pH 7.4. NuPage Bis-Tris 10% gels (Invitrogen) were used with NuPage MES SDS running buffer. 10 µL 4x NuPage LDS sample buffer (Invitrogen) was mixed with 30 µL of sample followed by heating for 90 s at 100 °C. 30 µL of the mixture was loaded on the gel followed by staining with SimplyBlue Safe Stain (Invitrogen).

Mass Spectrometry

Sample (7 µL) after digestion by PK was injected onto a reverse phase HPLC (Agilent 1100 series HPLC, Agilent Technologies) with a C18 column (Vydac 218TP5205, 2.1 x 50 mm) and introduced into the mass spectrometer as described (2). Positive-ion electrospray ionization (ESI) mass spectra were obtained with an Agilent G1946D mass selective detector (MSD) equipped with an ESI interface (Agilent Technologies). Data were analyzed using LC/MSD ChemStation software (Rev. A.10.02, Agilent Technologies). FindPept (3) was used to map molecular weights to specific amino acid sequences corresponding to full length ApoCIII.
Table S1. Mass Spectrometry Results

| Sequence residues          | Measured Mass (daltons) | Calculated Mass (daltons) | ΔMass (daltons) | Ion Count (%) |  |
|----------------------------|-------------------------|---------------------------|---------------|---------------|---|
| ApoCIII Monomer            | 9829.56                 | 9829.787                  | 0.227         | 1634148       | 1–87 |
|                           | 9960.82                 | 9960.980                  | 0.159         | 362996        | Met1–87 |
| ApoCIII Amyloid Digested by Proteinase K | 8364.71 | 8365.219                  | 0.508         | 75831         | 1–74 |
|                           | 8365.219                | 8365.270                  | 0.508         | 75831         | 2–75 |
|                           | 8365.219                | 8365.270                  | 0.560         | 75831         | 10–83 |
|                           | 8495.64                 | 8496.411                  | 0.771         | 16255         | Met1–74 |

1^FindPept (3) was used to identify masses and corresponding peptide sequences. A mass tolerance of ±1 dalton for average and non-ionized mass was used. Full-length ApoCIII sequence with an N-terminal methionine was used:

M^1SEAEASLLLSFMQGYMKHATKDALSSVQESQVAQQARGWVTDFSSLKDYWSTVKDFSEFWDLPERVEPRTSAVAA^79LEHHHHHH^87
Figure S1. Width of ApoCIII loops determined by TEM. TEM image of ApoCIII loops (A) was analyzed using ImageJ. We selected several cross sections marked in yellow (B) and obtained an average of ~13.5 nm (C). Resolution of the image is 0.35 nm/pixel.
Figure S2. Periodicity of ApoCIII loops determined by TEM. TEM image of ApoCIII loops (A) was analyzed using ImageJ. We selected segments which showed two clear and consecutive left-handed helical repeats (B) and obtained an average of ~77 nm (C). The surface interaction probably distorts the helicity and it is not possible to define this procedure rigorously.
Figure S3. Height of ApoCIII loops determined by AFM. Three sections of each AFM image (top row) were selected as red, green, and blue. The corresponding height map (bottom row) gives values of ~2-3 nm.
Figure S4. Periodicity of ApoCIII loops determined by AFM. AFM images of ApoCIII loops were prepared using Nanoscope Analysis (Bruker) (A). Using ImageJ, segments were selected which appeared to be consecutive helical repeats and marked in blue (B) to obtain an average of ~35 nm (C).

References

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2. (a) Apffel, A., Fischer, S., Goldberg, G., Goodley, P. C., and Kuhlmann, F. E. (1995), J. Chromatogr. A 712, 177-190; (b) Taggart, C., Cervantes-Laurean, D., Kim, G., McElvaney, N. G., Wehr, N., Moss, J., and Levine, R. L. (2000), J. Biol. Chem. 275, 27258-27265.
3. Gattiker, A., Bienvenut, W. V., Bairoch, A., and Gasteiger, E. (2002), Proteomics 2, 1435-1444.