Single-particle resolution of copper-associated annular α-Synuclein oligomers reveals potential therapeutic targets of neurodegeneration

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Contents

Fig. S1. Quantification of the embryonic and lag phase of α-Syn aggregation at gold-water interface.
Fig. S2. Quantification of the saturation phase of α-Syn.
Fig. S3. Raman spectroscopic analysis.
Fig. S4. Background subtraction analysis.
Fig. S5. AFM analysis of oligomeric and fibrillar aggregates of α-Syn when incubated with Cu²⁺ for time period of 1-8 days.
Fig. S6. AFM analysis of fibrillar aggregates of α-Syn when incubated with Cu²⁺ for time period of 9-10 days.
Fig. S7. Designated domains of α-Syn, atomic model of Au(111), and starting models of monomers and oligomers on gold-water interface for MD simulations with denoted position of copper(II) coordination.
Fig. S8. Representative models of α-Syn monomers and oligomers on gold-water interface during 200 ns dynamics and stretch of residues considered in calculating mean monomeric and oligomeric lengths.
Fig. S9. Simulation timelines of size and shape parameters of α-Syn monomers and oligomers.
Fig. S10. Model computed α-Syn intra-domain interaction energies of monomers and oligomers.
Fig. S11. Model computed α-Syn domain-wise interaction energies of monomers and oligomers with Au(111) sheet.
Fig. S12. Model computed α-Syn domain-wise interaction energies of monomers and oligomers with Cu²⁺ ion.
Fig. S13. α-Syn E46–K80 salt bridge stabilities of monomers and oligomers, their percentage of residues in β-sheet conformation, and their timelines of conformational energies.
Fig. S14. Timelines of intra-monomer and inter-monomer hydrogen bonds (H-bonds) number of α-Syn monomers and oligomers.
Fig. S1. Quantification of the embryonic and lag phase of α-Syn aggregation at gold-water interface. (a) and (b) Large-area AFM topographic and phase-contrast image of α-Syn peptides in the form of monomers and small oligomers adsorbed on the terraces of ultra-flat gold (surface roughness of bare gold regions: ~0.2 nm). The AFM data shown in panels a, f, k, m was recorded after incubating the α-synuclein solution for 1, 2, 3 and 4 days at 37° C under mechanical agitation, respectively, as labelled D1-D4. (c-e) Individual cross-sectional profiles measured across the particles visible from the AFM topography (panel a) and indicated by the respective coloured lines in panel b. The sectional profile analysis highlights the differences in the distribution of particle sizes ranging from ~0.5 to ~5.5 nm. (f) and (g) AFM height and phase-contrast image of major α-synuclein oligomer populations on Au(111). (h) and (j) Cross-sectional profiles measured along the colour-coded lines across the oligomeric aggregates as marked in panel g.
Fig. S2. **Quantification of the saturation phase of α-Syn**. High-resolution AFM topographs revealing the evolution in spatial organization of fibrils from a sparse to dense network on gold after α-Syn solution was incubated for 6 (a), 7 (b), 8 (c), 9 (d) and 10 (e) days. The AFM data shown in panel a-g were recorded in clean water environment. The phase-contrast images (f-g) reveal the close-packed and layered arrangement of α-synuclein mature fibrils on gold recorded on day 10 of the aggregation pathway of α-synuclein. (h-k) Height profiles extracted along the respective color-coded lines indicated in panels a, b, c and d showing the differences in fibril height with respect to the underlying gold surface. Despite rinsing the buffer solution with clean water and imaging in water medium, a residual buffer salt layer is present in certain regions of the gold surface (bottom inset image panel c) on which the fibrillar network is adsorbed. The height profiles are then calculated by measuring the difference in height between the maximum peak position and underlying gold rather than calculating the difference in height between the buffer salt layer and fibril maximum height.
**Fig S3. Raman spectroscopic analysis.** Averaged spectral signature of aggregates adsorbed on gold recorded during the embryonic phase (green plot), lag phase (red plot), elongation phase (blue plot) and saturation phase (black plot) occurring along the aggregation pathway of α-Syn, normalized to Phe band at 1003 cm$^{-1}$.

**Fig S4. Background subtraction analysis.** Background (BG) subtraction from raw α–Syn spectra obtained on the Au/mica substrate. (a) Full spectrum (averaged over the surface area of 15 by 15 µm, 225 spectra in total) and (b) close-up to the region of interest before and after polynomial subtraction. We used a third-order polynomial.
Fig. S5. AFM analysis of oligomeric and fibrillar aggregates of α-Syn when incubated with Cu\(^{2+}\) for period of 1-8 days. (a-h) AFM images recorded from day 2 till day 8 of α-Syn solution incubation with copper. (i) AFM height analysis of protofibrils detected on day 1 after incubating the α-Syn solution with copper. (a) Large-area AFM height image showing sparse distribution of short protofibrillar aggregates and spherical aggregates. (j) Sectional analysis of the protofibrillar aggregates and spherical aggregates (k) resolved in the AFM image shown in panel i. (l) Statistical analysis of protofibrillar height based on the AFM images recorded on day 1 of incubating α-Syn solution with copper. The protofibrillar aggregates were not very frequently detected on the surface of Au after day 1 of incubation of α-Syn solution with copper.
Fig. S6. AFM analysis of fibrillar aggregates of α-Syn when incubated with Cu²⁺ for period of 9-10 days.

S1. Benchmarking our findings against earlier physicochemical characterization of α-Syn. Although biochemical assays can quantify the amount of α-Syn in body fluids, more specific, diagnostic information on the polymorphic nature and diversity in aggregated assemblies can be obtained from microscopy and chemical spectroscopic techniques. The self-assembly of α-Syn follows a typical nucleation-elongation mechanism, whereby formation of a critical nucleus (smallest ordered oligomer capable of initiating elongation) is followed by an elongation phase (steady-state growth of oligomers until the population balance between monomers and aggregates reaches a thermodynamic equilibrium), although a secondary nucleation mechanism of α-Syn aggregation is also possible. Thus, diverse polymorphs with distinct topologies of α-Syn protofibrillar aggregates were recently identified in structural characterizations of both wild-type and familial mutant forms using cryo-electron microscopy (Cryo-EM) and solid-state nuclear magnetic resonance (ssNMR) with Angstrom level resolution under ultrahigh vacuum. A Greek-key topology of a single filament of α-Syn fibrils solved at 4.8 Å resolution by ssNMR (first reported by Tuttle et al.) spurred many follow-up investigations reporting “rod” and “twister” like topologies including a topology similar to the rod polymorph, and similar topologies have also been deduced independently by Guerrero-Ferreira et al. and Li et al. using high-resolution cryo-EM techniques. In the Greek key polymorph, the core of the α-Syn fibrils is formed by in-register cross-β sheets representing a Greek-key topology (the Greek-key like core spanning residues Thr44 to Lys96), stabilized by...
Glu46-Lys80 salt bridge, hydrophobic packing of Phe94 residue and a glutamine ladder (Gln79). By contrast, structural characterization of smaller, presumably more transient, α-Syn oligomers\textsuperscript{16} has been restricted to indirect studies of artificial covalently-linked designed oligomers\textsuperscript{17}. Also due to their inherent IDP nature, the structures of α-Syn monomers are resolved by solution NMR in their micelle-bound environment\textsuperscript{18,19}, or bound to membrane\textsuperscript{20,21} forming partially folded α-helical conformations, or captured by an engineered affinity antibody in their neurotoxic β-hairpin conformation\textsuperscript{22}.

Other techniques operating under standard laboratory conditions such as atomic force microscopy\textsuperscript{23-33} (AFM) and transmission electron microscopy\textsuperscript{34,35} (TEM) have also been demonstrated to resolve and quantify the size, shape and morphology of the transient nanostructures occurring along the natural and modulated aggregation pathway of α-Syn. Raman\textsuperscript{36-38} and infrared\textsuperscript{39} spectroscopy has revealed the spectrochemical signatures of α-Syn aggregates and distinguished oligomers from fibrillar assemblies based on analyses of amide I, II and III bands, to estimate the β-sheet content of the protein aggregates\textsuperscript{36,38}. These in vitro studies are generally conducted by drop-casting α-Syn from buffered aqueous solution onto either cleaved mica, Si, or metal-based TEM grids followed by gently rinsing the surface with clean water and then immediately proceeding with imaging after air-drying the adsorbed α-Syn protein aggregates. An alternative approach by A.Makki et al. involves creation of a thin water layer encompassing the α-Syn fibrils, obtained by leaving the samples to dry overnight in a humidity-controlled environment to preserve the aggregates in a partially hydrated state during AFM measurements\textsuperscript{40}. AFM-based structural analysis of fibrils of E46K mutant α-Syn was also conducted in a liquid environment and compared with height profiles of air-dried fibrils\textsuperscript{32}. To the best of our knowledge, only very few high-speed AFM based studies have been able to capture the conformational changes of soluble and insoluble α-Syn aggregates at the solid-liquid interface\textsuperscript{41,42} under conditions in which the protein aggregates remain covered in aqueous solution throughout the measurements. As α-Syn is an IDP in water, its conformation is known to be highly dependent on interfacial interactions\textsuperscript{33} and encompassing solution environment\textsuperscript{43} such as pH\textsuperscript{44}, salt concentration\textsuperscript{45}, and temperature\textsuperscript{43}. Furthermore, there is compelling evidence that using an AFM to measure air-dried amyloidogenic proteins can result in size variations (calculated from height profiles) as a result of shrinking effects due to sample dehydration\textsuperscript{46-50}.

Hence it merits consideration to re-examine α-syn aggregation pathway at the liquid-solid interface with similar spatial resolution as previous AFM reports\textsuperscript{23,25,26,28,32,40} at multiple
time intervals to track changes in α-Syn aggregate size and shape when incubated with metal ions in a hydrated state. Besides the encompassing chemical and physical environment, the underlying surface on which the proteins are imaged plays an important role in accurately deciphering the α-Syn size, shape, and morphological profile, which is why we chose to image on ultraflat gold, an electrochemically inert electrode.

In the present study, we employ ultra-flat gold with a surface roughness of ~0.2 nm as the platform to characterize the adsorbed α-Syn proteins in its free and when incubated with divalent copper ions (Cu$^{2+}$) from monomers to mature fibrils in both hydrated and air-dried state. Ultraflat gold is electrochemically inert, not a highly charged surface like mica, is less expensive than epitaxial graphene, and has fewer batch-to-batch variations in surface quality due to its smooth micron-sized terraces. To aid the room-temperature based experiments in mapping the aggregation pathway of α-Syn, we employed molecular dynamics (MD) simulations to clarify the aggregation promoting and inhibiting conformational shifts and stability of specific α-Syn monomers$^{51-55}$ and small aggregates$^{56-58}$ in solution at the gold–water interface. The α-Syn protein comprises three major domains (see Fig. S7a): a lipid-binding amphipathic N-terminus$^{21}$ (NT; residues Met1–Lys60) having imperfect repeat region, a central hydrophobic non-amyloid β component region (NAC, residues Glu61–Val95)$^{59}$ known to be critical for α-Syn aggregation$^{60}$, and a conserved acidic C-terminus (CT; residues Lys96–Ala140), which may be protective against α-Syn aggregation$^{51,52,57,61}$. A recent study using deletion and site-directed mutagenesis assays identified the role of different α-Syn regions in initial nucleation and subsequent elongation of the fibrillar fold, showing that NAC is the most important region for promoting fibril elongation$^{62}$. α-Syn trimers are one of the toxic species in the aggregation pathway$^{17,63}$, and may also constitute the minimum critical nucleus required for fibrillar elongation$^{64}$, which could further undergo self-assembly to form hexamers. Here, we identify the structural, morphological, dimensional and shape triggers of α-Syn elongation from the preformed fibrillar Greek-key fold$^{14}$ models of monomer and oligomers constituting dimers, trimers and hexamers on the chemically inert Au(111) sheet using atomistic MD simulations in their free and Cu$^{2+}$-coordinated states at μM and sub-μM Cu$^{2+}$ concentrations and predict the thermodynamic stabilities of different assemblies (see details under Methods). The Greek-key topology has been employed in MD studies to decipher the morphological switching of α-Syn protofibrils$^{65,66}$ due to familial mutations$^{67,68}$. In one very recent example, MD simulations of the α-Syn$_{44-96}$ core of protofibrillar oligomers
predicted the trimer as the critical nucleus for fibril formation, while tetramers were the most stable nucleus in the Greek-key morphology. In terms of putative roles for environmental biological ions in directing self-assembly, copper(II) binding to α-Syn monomers has been shown to accelerate the aggregation at physiologically relevant conditions and contribute to α-Syn oligomer toxicity. A number of Cu²⁺:monomer binding sites have been proposed, including: (1) The NT residues Met1, Asp2 and Met5/water, (2) Met1, Asp2 and His50, and (3) the acidic CT residues Asp119, Asp21, Asn122, and Glu123. The first coordination site towards the end of NT (with the fourth coordination site being Met5 or a water molecule) is a high-affinity Cu²⁺ binding site, while the other two are low-affinity sites, although full Cu²⁺ coordination to the CT was shown to promote self-assembly of α-Syn. However, the accessibility of His50 binding site in α-Syn to Cu²⁺ have been shown to be pH-dependent, given the near-7 pKa of the His imidazole sidechain. By contrast, the binding sites of Cu²⁺ to α-Syn fibrillar morphologies are not well characterised, although fibrillar structural polymorphism may be promoted due to Cu²⁺-mediated α-Syn aggregation. A recent study employing extensive experimental techniques, density functional theory (DFT) calculations and MD simulations demonstrated that Cu²⁺ is only able to bind His50 in the monomeric form of α-Syn, but not in the fibrillar form and instead the Cu²⁺ ions jumps between residues within the NT and the CT. This study investigated octameric constructs of α-Syn in two polymorphic fibrillar forms other than the popular Greek-key model, and thus to the best of our knowledge the Cu²⁺-induced morphological features in the Greek-key topology have not been deciphered to date.

S2. Supporting details on molecular dynamics simulations and analyses

S2.1 Preparation of the models. The initial structures of monomer, dimer, trimer and hexamer for molecular dynamics (MD) simulation were taken from the recently solved Greek-key structure of a decamer fragment of α-Syn fibril structure (ssNMR model, PDB code 2N0A). We modelled the monomer, dimer, trimer, and hexamer in the protofibrillar fold. A 15 nm x 15 nm Au(111) two-layer slab with thickness of 0.3 (see Fig. S7b) was used for the experimental atomically flat and defect free Au(111) surface. The infinite Au(111) surface was obtained by applying periodic boundary conditions, and the Au atoms were given net zero charges and held fixed in their crystallographic positions throughout the simulations. The initial peptide structures were oriented with the fibril axis parallel to the Au(111) surface, with part
of the Non-Amyloid-β Component59 (NAC; residues 61–95) exposed to the gold surface to create the initial interface between the monomer/oligomers and gold (see Fig. S7c-f, S7h-n). This orientation provided maximum surface exposure of α-Syn on gold. The structures were placed at a starting distance of at least 6 Å above the gold sheet.

The starting models for Cu2+-coordinated α-Syn protobrillar/profibrillar monomers, dimers, trimers and hexamers in the same Greek-key fold were constructed based on the high-affinity78,84 [2N2O] Cu2+ binding site of α-Syn monomer model with the initial Cu–coordinating residues interaction distances previously computed from density functional theory (DFT)83. In this model, Cu2+ is coordinated to amino (NH2) terminal group of Met1, deprotonated amide group of Asp2, carboxylic oxygen of Asp2 and the oxygen from a water molecule85 (see Fig. S7g). We note that another model used in the study83 computed by DFT has a low-affinity [3N1O] coordination site where the Cu2+ binds to Met1, Asp2, and His50. The authors found that Cu2+ did not bind His50 residue of α-Syn mature fibrils in octameric constructs. Following this observation and previous studies showing uncertainty in the binding affinity of Cu2+ to His5078,86, we did not model the lower affinity, minor [3N1O] coordination site.

S2.2 Molecular simulation details. The protein and the gold were represented by the CHARMM 36m87 force field, and solvated with CHARMM-modified TIP3P87 explicit water. The partial charges of Cu2+-coordinated monomeric and oligomeric assemblies (proteins) were derived using the Restrained Electrostatic Potential (RESP)88 scheme based on quantum mechanical calculations with Gaussian89, followed by charge fitting with Antechamber90. MD simulations were carried out using the Gromacs 2018.491 package with a time step of 2 fs using the Leap frog integrator. Bond lengths to hydrogen were constrained using the LINCS92 (protein) and the SETTLE93 (water) algorithms. Background ions were added to neutralize full-protein formal charges. Long-range electrostatics were treated by the Particle mesh Ewald94 (PME) method. Protein (and Cu2+ for copper-coordinated monomer and oligomers) and non-protein molecules (gold, water, and ions) were coupled separately to an external heat bath (298 K) with the coupling time constant of 1 ps using the velocity rescaling95 method. All systems were minimized for 100 ps, and equilibrated for 1 ns in constant volume NVT ensemble followed by another 1 ns of NPT equilibration with the reference pressure at 1 bar and a time constant of 4 ps using the Parrinello-Rahman96 barostat. The production runs were carried out for 200 ns in constant pressure NPT ensemble. Structures were saved every 20 ps. The
Supporting Information

simulated systems were α-Syn Monomer, Dimer, Trimer, Hexamer, Cu²⁺:Monomer (1:1), Cu²⁺:Dimer (1:2), Cu²⁺:Dimer (1:1), Cu²⁺:Trimer (1:2), Cu²⁺:Trimer (1:1), Cu²⁺:Hexamer (1:2), and Cu²⁺:Hexamer (1:1) (the ratios within parentheses correspond to Cu²⁺:Monomer ratios in the oligomer).

S2.3 MD data analyses. All analyses (main text Figs. 1g, h, Fig. 6 and Figs. S7 – S14 below) of root mean square deviation (RMSD), radius of gyration (R_g), interaction energies, hydrogen bonds and salt bridge analyses were performed using Gromacs tools. The protein shape profile was computed in terms of their ‘asphericity’ (Δ) by using Tcl/Tk scripting integrated with Visual Molecular Dynamics (VMD).\textsuperscript{21}

Asphericity describes the deviation of the protein shape away from a sphere, such that Δ = 0 is a sphere and Δ = 1 is a rod. Δ is expressed by the three principal moments of radius of gyration R_g1, R_g2 and R_g3 such that:

\[
\Delta = \frac{(R_{g1} - R_{g2})^2 + (R_{g2} - R_{g3})^2 + (R_{g1} - R_{g3})^2}{2 \times (R_{g1} + R_{g2} + R_{g3})^2}
\] (S1)

The maximum height and mean assembly length profiles of the α-Syn monomer and oligomers with or without Cu²⁺ coordination were computed using Tcl/Tk scripting in VMD. The maximum heights were computed by measuring the distance between the highest atom on the Au(111) surface and the highest atom of monomer and oligomers perpendicular to the gold surface. Mean oligomeric length along the gold sheet was calculated by considering the length of their stretch in the structured (38–97) region – the distance from one end monomer to the other end monomer of residues Leu38, Lys60, Thr81 and Lys97 (see Fig. S8l) were computed, and the average of these distances were considered as the mean length of oligomers.

The conformational energy was calculated by the Generalized Born using Molecular Volume (GBMV) implicit solvent model implemented in the CHARMM\textsuperscript{97} (v40b2) program. The single point energy of each conformation was calculated after a 200-cycle minimization using the GBMV II algorithm.\textsuperscript{98-100} The total conformational energy calculations included bonded energy, van der Waals (vdW) energy, electrostatic energy, and solvation energy. The dielectric constant of water was set to 80. The hydrophobic solvent accessible surface area (SASA) term factor was fixed to 0.00592 kcal.mol⁻¹ Å².

S2.4 Supplementary Analyses. Here we provide more details of the analyses of MD trajectories of α-Syn monomers and oligomers in their copper-free and copper-coordinated (Cu:monomer ratios of 1:2 and 1:1) states. The structural deviation through root mean square
deviation (RMSD) plots (Figs. S9a, b) of the backbone atoms show that all structures stabilize during the first 150 ns and so we obtain physically meaningful statistics by sampling equilibrated structures during the final 50 ns of dynamics. The monomer from an initial protofibrillar conformation immediately collapses on top of the gold sheet within the first 20 ns as evident from their maximum height profile (Fig. S9g) as a function of time. After this initial collapse, the NT and CT interact with Au(111) (see Fig. S11a for normalized per monomer α-Syn–gold interactions) remaining mostly in an extended coil conformation as seen from the steep rise in the radius of gyration, \( R_g \) after the first 20 ns (Fig. S9c). The maximum height value of α-Syn monomer obtained from MD simulation of ~3.6 nm is comparable to the height profile of monomers obtained from the AFM measurements (~3 nm). The NAC forms a stable β-hairpin conformation between intra-monomer domains most amenable to hydrogen bonds due to the initial Greek-key core (residues 44-96) morphology, and thus NAC remains loosely attached to the gold surface (Fig. S11a). To assess the shape parameters, we calculated the ‘asphericity’ (\( \Delta \); the tendency to become non-spherical particles, see details in Materials and Methods) property, where \( \Delta = 0 \) is a sphere and \( \Delta = 1 \) is a rod (see details in section S2.3 MD data analyses). All α-Syn oligomeric assemblies (including the Cu\(^{2+}\)–coordinated states) behave like spherical proteins with \( \Delta \) values close to 0 (Figs. S9e, f), while monomer tends to become slightly rod-like after 20 ns, with \( \Delta \) values above 0.1 usually considered aspherical. In presence of Cu\(^{2+}\), α-Syn monomer (Cu:Monomer (1:1)) undergoes similar collapse (Fig. S9h) as without Cu\(^{2+}\), but samples more compact conformations (Fig. S9d) owing mainly to significant Cu-mediated NT–CT (Fig. S10b) and direct CT–Cu\(^{2+}\) interactions (Fig. S12a) and less NT–gold and CT–gold interactions (Fig. S11b) than the monomer in absence of Cu\(^{2+}\).

α-Syn dimer also collapses on Au(111) with the NAC and CT of both monomer units lying flat, but to a lesser extent than monomer (Fig. S9g), and samples more compact conformations than monomer (Fig. S9c). There is evidence of CT anchoring first on gold (see CT–Au(111) interactions in Fig. S11c), an effect observed with the other oligomeric assemblies too, where the interaction between CT and gold is prominent and improves over time for trimer and hexamer (normalized energies in Figs. S11 f, i). Therefore, the CT in dimer is not available for interacting with NT (as was the case also for monomer) (Fig. S10c) but undergoes NAC–CT interactions. This CT anchoring provides support for the oligomers to create a stable orientation with fibril axis parallel to the gold sheet, suitable for elongation of the oligomeric nucleus along Au(111). The α-Syn dimers collapse less when coordinated to copper(II) at both Cu\(^{2+}\) concentrations (Fig. S9h) as also observed with other Cu–assembly
constructs (Fig. 6b). Moreover, at the higher Cu²⁺ concentration, the dimer samples smaller heights than at lower Cu²⁺ concentration. The computed structures show that the Cu²⁺:Dimer (1:1) complex creates a large-area interface with Au(111) (Fig. S11e), which also produces more compact conformations than the Cu²⁺:Dimer (1:2) complex (Fig. S9d).

α-Syn trimers follow a similar pattern as other oligomers, sampling conformations with larger height profiles (Fig. S9h) in presence of Cu²⁺ than in their absence (Fig. S9g) with Cu²⁺:Trimer (1:1) sampling lower heights than Cu²⁺:Trimer (1:2) owing mainly to a more favorable CT–Au(111) interactions at the higher Cu concentration (Fig. S11h). However, the R₇ values predict a more compact conformation of trimer at lower Cu²⁺ concentration than at higher Cu²⁺ concentration (Fig. S9d), the opposite of what was calculated for the dimers and hexamers. This is because of the trimer NT–CT interactions at 1:2 (Fig. S10g), which are sparse for the 1:1 model (Fig. S10h). Comparatively, the difference in maximum height is less significant for Cu²⁺:Trimer complexes (~8.6 nm for 1:2 vs. ~8 nm for 1:1). This is because the weaker NT–CT interaction at higher Cu²⁺ concentration (Fig. S10h) than at lower Cu²⁺ concentration (Fig. S10g) is compensated by NAC–Cu²⁺ interaction at high Cu²⁺ concentration (Fig. S12e) which is absent at low Cu²⁺ concentration (Fig. S12d).

The largest oligomer considered in our study, the α-Syn hexamers follow a similar trend to that of the dimers in terms of their dimensions (Figs. S9c, d) and maximum height on gold (Figs. S9g, h) profiles in the absence of Cu²⁺ and in Cu–coordinated hexamers. Hexamer does not become compact throughout the 200 ns dynamics (Figs. S9c, d) because the unfolded NT remains extended with more transient interactions with CT and NAC (Figs. S10j, k) than was the case for the trimer. We note from the distribution of heights that Cu²⁺:Hexamer (1:1) samples a broad range of heights (7.5–13 nm) with no prominent peak (Fig. 6b). It is observed that at higher Cu²⁺ concentration, the hexamer has the tendency to topple over on gold surface and re-orient itself from the starting orientation where the fibril axis is parallel to Au(111) (Fig. S7n) into an orientation having the fibril axis perpendicular to gold (Fig. S8k). This could be attributed to cross–interactions between NT and Cu²⁺ of two different monomers in hexamer (Fig. S12g; values are normalized per monomer) coupled with better CT–Cu²⁺ interactions than in the Cu²⁺:Hexamer (1:2) complex. The maximum height densities show peaks at 3.6 nm for Monomer, 3.4 nm for Cu²⁺:Monomer (1:1), 3.9 nm for Dimer, 6.4 nm for Cu²⁺:Dimer (1:2), 4.7 nm for Cu²⁺:Dimer (1:1), 7.3 nm for Trimer, 8.6 nm for Cu²⁺:Trimer (1:2), 8 nm for Cu²⁺:Trimer (1:1), 9.8 nm for Hexamer, 10.1 nm for Cu²⁺:Hexamer (1:2), and 8.8 nm for Cu²⁺:Hexamer (1:1). While the mean lengths of monomer, trimer and hexamer do not deviate
Supporting Information

Throughout the 200 ns dynamics (Fig. S9i), the dimer lateral length increases over time indicating their tendency to become disordered and slightly dissociate. Thus, the mean length of fibrils resolved on day 4 (D4) in Fig. 1f correspond to assemblies that are much larger (100-mer) than the size of hexamer. The mean assembly lengths of Cu–oligomer constructs show lateral compaction over time except the trimers which tend to slightly extend across the fibril core (Fig. S9j).

For α-Syn dimer, the initial protofibrillar core architecture is lost with the decay of β-sheet structure (see Fig. S13b), suggesting dimers may not be amenable to elongation by the addition of monomers. The Greek-key core structural integrity is retained for both trimer and hexamer, with a slight rotation of trimer around the fibril axis. On the other hand, the cross-β sheet morphology is more retained for assembly complexes with Cu²⁺ with slightly larger fraction of residues in β-sheet conformation for dimers (Fig. S13c), but not so much for trimers and hexamers. In fact, the Cu²⁺:Dimer (1:2) complex samples β-sheet content at par with the Cu²⁺:trimer complexes. The β-sheet is lost more for Cu²⁺:Trimer (1:1) than for Cu²⁺:Trimer (1:2) indicating the trimer may be more protofibrillar at lower Cu²⁺ concentration. However, the Cu²⁺:trimer complexes have overall more β-sheet content than Cu²⁺:hexamer complexes (Fig. S13c).

Based on our computed MD trajectories, we predicted several properties to estimate the thermodynamic stabilities of the oligomeric constructs and monomer in the protofibrillar fold. The first, and the most important is the stability of salt bridge between residues E46 and K80 (see Fig. S13a) that contributes most towards retaining the Greek-key morphology of the core. The E46 and K80 separation in the monomer increases over time, while the dimer fluctuates without sampling stable short E46-K80 distances. However, for both trimer and hexamer, the E46-K80 salt-bridge is extremely stable showing distances ~0.4 nm throughout the 200 ns dynamics. Trimers may thus be the smallest oligomer stable in the Greek-key fold as also estimated from the timeline showing the higher percentage of β-sheet secondary structure of trimer than hexamer (Fig. S13b). The assembly constructs with coordinated Cu²⁺ predicts E46–K80 separation distances which are unstable for Cu²⁺:Monomer (1:1) and Cu²⁺:Dimer (1:1), but more stable (with transient fluctuations) for Cu²⁺:Dimer (1:2) (Fig. S13a). For all Cu–trimer and Cu–hexamer complexes, the salt bridge is extremely stable throughout the 200 ns dynamics.

To complement the computed stabilities of the E46-K80 salt-bridge, we estimated the conformational energies of the n-mers by using the GBMV-II⁹⁸-¹⁰⁰ (Generalized Born using
Supporting Information

Molecular Volume) algorithm implemented in CHARMM97 (see Methods and section S2.3 MD data analyses for details). The timelines of normalized (per monomer) conformational energies (Fig. S13d) show monomer and dimer energies almost at par with each other and the hexamer energy being more favorable than trimer, as expected. We also computed the mean conformational energies (Fig. 6e) for the last 50 ns of simulation and normalized per monomer for direct comparison. The data reveals a prominent energy difference between dimer and trimer, which is not evident between monomer and dimer, and trimer and hexamer, highlighting the predominance of protofibrillar morphology in the trimer. With Cu²⁺, the Cu²⁺:Monomer (1:1), Cu²⁺:Dimer (1:2) and Cu²⁺:Dimer (1:1) thermodynamic stability becomes unfavorable after ~100 ns dynamics (Fig. S13e). Thus, despite the α-Syn dimer retaining significant percent of residues with β-sheets (Fig. S13c) at low concentration of Cu²⁺ (Cu²⁺:dimer (1:2)), its conformational energy (Fig. S13e) becomes unfavorable, coupled with a relatively unstable salt bridge (Fig. S13a). The conformational energies of Cu–trimer and Cu–hexamer complexes improve over time with the hexamer being more favorable than the trimer. However, Cu²⁺:Trimer (1:2) complex is more stable than Cu²⁺:Trimer (1:1), while for all other oligomeric constructs, the conformational energy improves with higher concentration of Cu²⁺ (Fig. 6e). We thus identify the Cu²⁺-coordinated trimer to form two distinct but interconvertible protofibrillar polymorphs, the assemblies of which are guided by the concentration of Cu²⁺.

An estimation of intra-monomer (normalized per monomer) and inter-monomer (for dimer, trimer, and hexamer) hydrogen bond (H-bond) numbers (Figs. S14a-j) reveals that hexamer has the strongest intra-peptide H-bonding followed by Cu–hexamer complexes (in a Cu²⁺ concentration dependent manner), with the H-bonds strengthening over time for all oligomers (Fig. S14a). The trimer and Cu–trimer complexes follow suit with the Cu²⁺:Dimer (1:2) complex sampling most number of H-bonds amongst dimers followed by monomers. For trimer (Fig. S14e) and hexamer (Fig. S14h), the number of all inter-monomer H-bonds are greater than in the dimer (Fig. S14b), confirming the stability of these larger oligomeric constructs. Similarly, the Cu–dimer complexes (Fig. S14c, d) sample more monomer-monomer H-bonds than dimer alone, but the Cu²⁺:Dimer (1:2) H-bonds (Fig. S14e) improve significantly over time. Cu–trimer inter-monomer H-bonds (Fig. S14f, g) are slightly less in number than trimer alone. Cu²⁺:Hexamer (1:1) shows larger differences between the inter-monomer H-bonds of edge dimers (M1–M2 and M5–M6) (Fig. S14j) than Cu²⁺:Hexamer (1:2) (Fig. S14i) revealing the propensity of hexamer to form twisted protofibrils at higher Cu²⁺ concentration.
Fig. S7. (a) Cartoon representation of α-Syn monomer with the Greek-key core solved by ssNMR in protofibrillar morphology (PDB code 2NA0\textsuperscript{14}). The disordered N-terminus and C-terminus in this model are coloured in red and green, respectively, and the Non amyloid-β component (NAC) is coloured in grey. (b) Atomic model of Au(111) slab used in this study from the side (15 nm X 15 nm) and top (0.3 nm thickness) views. (c – f) Starting models of monomer, dimer, trimer and hexamer in protofibrillar folds at Au(111)-water interface. The NAC region is shaded in grey. (g) The 2N2O Cu\textsuperscript{2+} coordination sphere of α-Syn monomer showing coordination to amine nitrogens of Met1 and Asp2, carboxylic acid oxygen of Asp2 and oxygen of a water molecule with their corresponding distances from Cu\textsuperscript{2+}. The model was constructed using UCSF Chimera\textsuperscript{101} by constraining the distances and angles obtained from ref.\textsuperscript{83} (h – n) Starting models of Cu\textsuperscript{2+}:monomer complexes and complexes of Cu\textsuperscript{2+} with dimer, trimer and hexamer at different Cu\textsuperscript{2+} concentrations (Cu\textsuperscript{2+}:oligomer ratios) on Au(111)-water interface. The NAC region is shaded in grey. The Cu\textsuperscript{2+} ions are shown as brown spheres coordinated to the N-terminal residues.
Fig. S8. (a–d) Representative models of monomer, dimer, trimer and hexamer on Au(111)–water interface without Cu$^{2+}$ and in complexes (e – k) with Cu$^{2+}$ at different Cu$^{2+}$:monomer ratios (within parentheses) obtained during 200 ns dynamics. The NAC region is shaded in grey. The Cu$^{2+}$ ions are shown as brown spheres coordinated to the N-terminal residues. (l) Example structure of a hexamer at the gold–water interface showing the residues considered in the stretch of the structured region to calculate mean monomeric and oligomeric lengths.
Fig. S9. Timelines of root mean square deviation (RMSD) of structural backbone of (a) monomer, dimer, trimer and hexamer, and (b) the assembly constructs in complex with Cu$^{2+}$ at different Cu$^{2+}$:monomer ratios. Timelines of radius of gyration ($R_g$) of (c) monomer and different oligomeric constructs, and (d) their complexes with Cu$^{2+}$ at different Cu$^{2+}$ concentrations. Timelines of asphericity ($\Delta$) of (e) monomer, dimer, trimer and hexamer, and (f) their monomeric and oligomeric complexes with Cu$^{2+}$. Timelines of maximum height of (g) monomers and oligomers, and (h) their complexes with Cu$^{2+}$ on the Au(111) surface. Timelines of maximum length of (i) monomers and oligomers, and (j) their complexes with Cu$^{2+}$ on the Au(111) surface.
Fig. S10. Intra-domain interaction energies ($\Delta E$; Coulomb electrostatics + van der Waals energy) of $\alpha$-Syn (a – k) monomer, dimer, trimer and hexamer and their respective complexes with Cu$^{2+}$ at 1:2 and 1:1 Cu$^{2+}$:monomer ratios. All oligomer energies are normalised per monomer.
Fig. S11. Interaction energies (ΔE; Coulomb electrostatics + van der Waals energy) of domains of α-Syn (a – k) monomer, dimer, trimer and hexamer and their respective complexes with Cu²⁺ at 1:2 and 1:1 Cu²⁺:monomer ratios. All oligomer energies are normalised per monomer.
Fig. S12. Interaction energies (ΔE; Coulomb electrostatics + van der Waals energy) of Cu$^{2+}$ with domains of α-Syn (a – g) monomer, dimer, trimer and hexamer in complexes with Cu$^{2+}$ at different Cu$^{2+}$:monomer ratios. All oligomer energies are normalised per monomer.
Fig. S13. (a) α-Syn hexamer in Greek-key core morphology showing the positions of E46 (red) and K80 (blue) that forms a salt bridge to stabilise the core structure and their corresponding timelines of E46–K80 minimum distances of monomer, dimer, trimer and hexamer and their complexes with Cu$^{2+}$ to assess the stability of the salt bridge in these constructs. (b) Timelines of percentage of residues sampling β-sheet secondary structure in monomer and oligomers and their complexes with Cu$^{2+}$. The values are normalised per monomer (c) Timelines of conformational energies of monomer and oligomers (normalised per monomer) and their complexes with Cu$^{2+}$ estimated from the GBMV-II$^{98-100}$ method (see Materials and Methods).
Fig. S14. (a) Comparison of intra-monomer (normalised per monomer for oligomers) hydrogen bonds number for monomer, dimer, trimer and hexamer and their Cu$^{2+}$ complexes as a function of time. (b–j) inter-monomer hydrogen bonds number as a function of time for dimer, trimer and hexamer and their complexes with Cu$^{2+}$. 
Supplementary References

1. Cremades, N., Chen, S. W. & Dobson, C. M. in International Review of Cell and Molecular Biology Vol. 329 (ed Massimo Sandal) 79-143 (Academic Press, 2017).

2. Stefani, M. & Dobson, C. M. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. J Mol Med (Berl) 81, 678-699, doi:10.1007/s00109-003-0464-5 (2003).

3. Buell, A. K. et al. Solution conditions determine the relative importance of nucleation and growth processes in alpha-synuclein aggregation. Proc Natl Acad Sci U S A 111, 7671-7676, doi:10.1073/pnas.1315346111 (2014).

4. Wood, S. J. et al. α-Synuclein Fibrillogenesis Is Nucleation-dependent: IMPLICATIONS FOR THE PATHOGENESIS OF PARKINSON’S DISEASE. Journal of Biological Chemistry 274, 19509-19512 (1999).

5. Ferrone, F. Analysis of protein aggregation kinetics. Methods Enzymol 309, 256-274 (1999).

6. Peduzzo, A., Linse, S. & Buell, A. K. The Properties of alpha-Synuclein Secondary Nuclei Are Dominated by the Solution Conditions Rather than the Seed Fibril Strain. ACS Chem Neurosci 11, 909-918, doi:10.1021/acschemneuro.9b00594 (2020).

7. Guerrero-Ferreira, R. et al. Cryo-EM structure of alpha-synuclein fibrils. Elife 7, doi:10.7554/eLife.36402 (2018).

8. Boyer, D. R. et al. Structures of fibrils formed by alpha-synuclein hereditary disease mutant H50Q reveal new polymorphs. Nat Struct Mol Biol 26, 1044-1052, doi:10.1038/s41594-019-0322-y (2019).

9. Sun, Y. et al. Cryo-EM structure of full-length alpha-synuclein amyloid fibril with Parkinson’s disease familial A53T mutation. Cell Res 30, 360-362, doi:10.1038/s41422-020-0299-4 (2020).

10. Li, Y. et al. Amyloid fibril structure of alpha-synuclein determined by cryo-electron microscopy. Cell Res 28, 897-903, doi:10.1038/s41422-018-0075-x (2018).

11. Li, B. et al. Cryo-EM of full-length alpha-synuclein reveals fibril polymorphs with a common structural kernel. Nat Commun 9, 3609, doi:10.1038/s41467-018-05971-2 (2018).

12. Guerrero-Ferreira, R. et al. Two new polymorphic structures of human full-length alpha-synuclein fibrils solved by cryo-electron microscopy. Elife 8, doi:10.7554/eLife.48907 (2019).

13. Schweighauser, M. et al. Structures of alpha-synuclein filaments from multiple system atrophy. Nature 585, 464-469, doi:10.1038/s41586-020-2317-6 (2020).

14. Tuttle, M. D. et al. Solid-state NMR structure of a pathogenic fibril of full-length human alpha-synuclein. Nat Struct Mol Biol 23, 409-415, doi:10.1038/nsmb.3194 (2016).

15. Li, B. et al. Cryo-EM of full-length α-synuclein reveals fibril polymorphs with a common structural kernel. Nature Communications 9, 3609, doi:10.1038/s41467-018-05971-2 (2018).

16. Bhattacharya, S., Xu, L. & Thompson, D. Revisiting the earliest signatures of amyloidogenesis: Roadmaps emerging from computational modeling and experiment. Wiley Interdisciplinary Reviews: Computational Molecular Science 8, e1359, doi:10.1002/wcms.1359 (2018).
17 Salveson, P. J., Spencer, R. K. & Nowick, J. S. X-ray Crystallographic Structure of Oligomers Formed by a Toxic beta-Hairpin Derived from alpha-Synuclein: Trimers and Higher-Order Oligomers. *J Am Chem Soc* **138**, 4458-4467, doi:10.1021/jacs.5b13261 (2016).

18 Ulmer, T. S., Bax, A., Cole, N. B. & Nussbaum, R. L. Structure and dynamics of micelle-bound human alpha-synuclein. *J Biol Chem* **280**, 9595-9603, doi:10.1074/jbc.M411805200 (2005).

19 Rao, J. N., Jao, C. C., Hegde, B. G., Langen, R. & Ulmer, T. S. A combinatorial NMR and EPR approach for evaluating the structural ensemble of partially folded proteins. *J Am Chem Soc* **132**, 8657-8668, doi:10.1021/ja100646t (2010).

20 Jao, C. C., Hegde, B. G., Chen, J., Haworth, I. S. & Langen, R. Structure of membrane-bound alpha-synuclein from site-directed spin labeling and computational refinement. *Proc Natl Acad Sci U S A* **105**, 19666-19671, doi:10.1073/pnas.0807826105 (2008).

21 Bartels, T. *et al.* The N-terminus of the intrinsically disordered protein alpha-synuclein triggers membrane binding and helix folding. *Biophys J* **99**, 2116-2124, doi:10.1016/j.bpj.2010.06.035 (2010).

22 Mirecka, E. A. *et al.* Sequestration of a beta-hairpin for control of alpha-synuclein aggregation. *Angew Chem Int Ed Engl* **53**, 4227-4230, doi:10.1002/anie.201309001 (2014).

23 Shin, E. J. & Park, J. W. Nanoaggregates Derived from Amyloid-beta and Alpha-synuclein Characterized by Sequential Quadruple Force Mapping. *Nano Letters* **21**, 3789-3797, doi:10.1021/acs.nanolett.1c00058 (2021).

24 Sweers, K. K. M., van der Werf, K. O., Bennink, M. L. & Subramaniam, V. Atomic Force Microscopy under Controlled Conditions Reveals Structure of C-Terminal Region of alpha-Synuclein in Amyloid Fibrils. *ACS Nano* **6**, 5952-5960, doi:10.1021/nn300863n (2012).

25 Sidhu, A., Segers-Nolten, I., Raussens, V., Claessens, M. M. A. E. & Subramaniam, V. Distinct Mechanisms Determine alpha-Synuclein Fibril Morphology during Growth and Maturation. *ACS Chemical Neuroscience* **8**, 538-547, doi:10.1021/acschemneuro.6b00287 (2017).

26 Fink, A. L. The aggregation and fibrillation of alpha-synuclein. *Acc Chem Res* **39**, 628-634, doi:10.1021/ar050073t (2006).

27 Ruggeri, F. S. *et al.* The Influence of Pathogenic Mutations in alpha-Synuclein on Biophysical and Structural Characteristics of Amyloid Fibrils. *ACS Nano* **14**, 5213-5222, doi:10.1021/acsnano.9b09676 (2020).

28 Ruggeri, F. S. *et al.* Identification and nanomechanical characterization of the fundamental single-strand protofilaments of amyloid alpha-synuclein fibrils. *Proceedings of the National Academy of Sciences* **115**, 7230-7235, doi:10.1073/pnas.1721220115 (2018).

29 Kumar, S. *et al.* Role of Sporadic Parkinson Disease Associated Mutations A18T and A29S in Enhanced alpha-Synuclein Fibrillation and Cytotoxicity. *ACS Chemical Neuroscience* **9**, 230-240, doi:10.1021/acschemneuro.6b00430 (2018).

30 Sharma, K. *et al.* Effect of Disease-Associated P123H and V70M Mutations on beta-Synuclein Fibrillation. *ACS Chemical Neuroscience* **11**, 2836-2848, doi:10.1021/acschemneuro.0c00405 (2020).
31 Ghosh, S., Mahapatra, A. & Chattopadhyay, K. Modulation of α-Synuclein Aggregation by Cytochrome c Binding and Hetero-dityrosine Adduct Formation. ACS Chemical Neuroscience 10, 1300-1310, doi:10.1021/acschemneuro.8b00393 (2019).
32 Sweers, K. K. M., Segers-Nolten, I. M. J., Bennink, M. L. & Subramaniam, V. Structural model for α-synuclein fibrils derived from high resolution imaging and nanomechanical studies using atomic force microscopy. Soft Matter 8, 7215-7222, doi:10.1039/C2SM25426C (2012).
33 Campioni, S. et al. The Presence of an Air–Water Interface Affects Formation and Elongation of α-Synuclein Fibrils. Journal of the American Chemical Society 136, 2866-2875, doi:10.1021/ja412105t (2014).
34 de Oliveira, G. A. P. & Silva, J. L. Alpha-synuclein stepwise aggregation reveals features of an early onset mutation in Parkinson’s disease. Communications Biology 2, 374, doi:10.1038/s42003-019-0598-9 (2019).
35 Han, J. Y., Choi, T. S. & Kim, H. I. Molecular Role of Ca2+ and Hard Divalent Metal Cations on Accelerated Fibrillation and Interfibrillar Aggregation of α-Synuclein. Scientific Reports 8, 1895, doi:10.1038/s41598-018-20320-5 (2018).
36 Apetri, M. M., Maiti, N. C., Zagorski, M. G., Carey, P. R. & Anderson, V. E. Secondary structure of alpha-synuclein oligomers: characterization by raman and atomic force microscopy. J Mol Biol 355, 63-71, doi:10.1016/j.jmb.2005.10.071 (2006).
37 Flynn, J. D., McGlinchey, R. P., Walker, R. L., 3rd & Lee, J. C. Structural features of α-synuclein amyloid fibrils revealed by Raman spectroscopy. The Journal of biological chemistry 293, 767-776, doi:10.1074/jbc.M117.812388 (2018).
38 Maiti, N. C., Apetri, M. M., Zagorski, M. G., Carey, P. R. & Anderson, V. E. Raman Spectroscopic Characterization of Secondary Structure in Natively Unfolded Proteins: α-Synuclein. Journal of the American Chemical Society 126, 2399-2408, doi:10.1021/ja0256176 (2004).
39 Zhou, L. & Kurouski, D. Structural Characterization of Individual α-Synuclein Oligomers Formed at Different Stages of Protein Aggregation by Atomic Force Microscopy-Infrared Spectroscopy. Analytical Chemistry 92, 6806-6810, doi:10.1021/acs.analchem.0c00593 (2020).
40 Makky, A., Bousset, L., Polesel-Maris, J. & Melki, R. Nanomechanical properties of distinct fibrillar polymorphs of the protein α-synuclein. Scientific Reports 6, 37970, doi:10.1038/srep37970 (2016).
41 Watanabe-Nakayama, T. et al. Self- and Cross-Seeding on α-Synuclein Fibril Growth Kinetics and Structure Observed by High-Speed Atomic Force Microscopy. ACS Nano 14, 9979-9989, doi:10.1021/acsnano.0c03074 (2020).
42 Zhang, Y. et al. High-speed atomic force microscopy reveals structural dynamics of α-synuclein monomers and dimers. J Chem Phys 148, 123322, doi:10.1063/1.5008874 (2018).
43 Ahmad, B., Chen, Y. & Lapidus, L. J. Aggregation of α-synuclein is kinetically controlled by intramolecular diffusion. Proceedings of the National Academy of Sciences 109, 2336-2341, doi:10.1073/pnas.1109526109 (2012).
44 Pálmadóttir, T., Malmendal, A., Leiding, T., Lund, M. & Linse, S. Charge Regulation during Amyloid Formation of α-Synuclein. Journal of the American Chemical Society 143, 7777-7791, doi:10.1021/jacs.1c01925 (2021).
Supporting Information

45 Ramis, R., Ortega-Castro, J., Vilanova, B., Adrover, M. & Frau, J. Unraveling the NaCl Concentration Effect on the First Stages of α-Synuclein Aggregation. Biomacromolecules 21, 5200-5212, doi:10.1021/acs.biomac.0c01292 (2020).
46 Relini, A. et al. Detection of Populations of Amyloid-Like Protofibrils with Different Physical Properties. Biophys J 98, 1277-1284, doi:https://doi.org/10.1016/j.bpj.2009.11.052 (2010).
47 Nirmalraj, P. N. et al. Complete aggregation pathway of amyloid β (1-40) and (1-42) resolved on an atomically clean interface. Science Advances 6, eaaz6014, doi:10.1126/sciadv.aaz6014 (2020).
48 Chiang, Y.-L. et al. Atomic Force Microscopy Characterization of Protein Fibrils Formed by the Amyloidogenic Region of the Bacterial Protein MinE on Mica and a Supported Lipid Bilayer. PLOS ONE 10, e0142506, doi:10.1371/journal.pone.0142506 (2015).
49 Mastrangelo, I. A. et al. High-resolution atomic force microscopy of soluble Abeta42 oligomers. J Mol Biol 358, 106-119, doi:10.1016/j.jmb.2006.01.042 (2006).
50 Kowalewski, T. & Holtzman, D. M. In situ atomic force microscopy study of Alzheimer’s β-amyloid peptide on different substrates: New insights into mechanism of β-sheet formation. Proceedings of the National Academy of Sciences 96, 3688-3693, doi:10.1073/pnas.96.7.3688 (1999).
51 Bhattacharya, S., Xu, L. & Thompson, D. Molecular Simulations Reveal Terminal Group Mediated Stabilization of Helical Conformers in Both Amyloid-β42 and α-Synuclein. ACS Chemical Neuroscience 10, 2830-2842, doi:10.1021/acscchemneuro.9b00053 (2019).
52 Bhattacharya, S., Xu, L. & Thompson, D. Long-range Regulation of Partially Folded Amyloidogenic Peptides. Sci Rep 10, 7597, doi:10.1038/s41598-020-64303-x (2020).
53 Dedmon, M. M., Lindorff-Larsen, K., Christodoulou, J., Vendruscolo, M. & Dobson, C. M. Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations. J Am Chem Soc 127, 476-477, doi:10.1021/ja044834j (2005).
54 Balupuri, A., Choi, K. E. & Kang, N. S. Computational insights into the role of alpha-strand/sheet in aggregation of alpha-synuclein. Sci Rep 9, 59, doi:10.1038/s41598-018-37276-1 (2019).
55 Yu, H., Han, W., Ma, W. & Schulten, K. Transient beta-hairpin formation in alpha-synuclein monomer revealed by coarse-grained molecular dynamics simulation. J Chem Phys 143, 243142, doi:10.1063/1.4936910 (2015).
56 Gurry, T. et al. The dynamic structure of alpha-synuclein multimers. J Am Chem Soc 135, 3865-3872, doi:10.1021/ja310518p (2013).
57 Xu, L., Bhattacharya, S. & Thompson, D. Re-designing the alpha-synuclein tetramer. Chem Commun (Camb) 54, 8080-8083, doi:10.1039/c8cc04054k (2018).
58 Xu, L., Bhattacharya, S. & Thompson, D. On the ubiquity of helical alpha-synuclein tetramers. Phys Chem Chem Phys 21, 12036-12043, doi:10.1039/c9cp02464f (2019).
59 Ueda, K. et al. Molecular cloning of CDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci U S A 90, 11282-11286, doi:10.1073/pnas.90.23.11282 (1993).
60 Periquet, M., Fulga, T., Myllykangas, L., Schlossmacher, M. G. & Feany, M. B. Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. J Neurosci 27, 3338-3346, doi:10.1523/JNEUROSCI.0285-07.2007 (2007).
61 Levitan, K. et al. Conserved C-terminal charge exerts a profound influence on the aggregation rate of alpha-synuclein. *J Mol Biol* **411**, 329-333, doi:10.1016/j.jmb.2011.05.046 (2011).

62 Gallardo, J., Escalona-Noguero, C. & Sot, B. Role of alpha-Synuclein Regions in Nucleation and Elongation of Amyloid Fiber Assembly. *ACS Chem Neurosci* **11**, 872-879, doi:10.1021/acschemneuro.9b00527 (2020).

63 Winner, B. et al. In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci U S A* **108**, 4194-4199, doi:10.1073/pnas.1100976108 (2011).

64 Zou, Y. et al. Critical nucleus of Greek-key-like core of alpha-synuclein protofibril and its disruption by dopamine and norepinephrine. *Phys Chem Chem Phys* **22**, 203-211, doi:10.1039/c9cp04610k (2019).

65 Yao, Y., Tang, Y. & Wei, G. Epigallocatechin Gallate Destabilizes alpha-Synuclein Fibril by Disrupting the E46-K80 Salt-Bridge and Inter-protofibril Interface. *ACS Chem Neurosci* **11**, 4351-4361, doi:10.1021/acschemneuro.0c00598 (2020).

66 Romo, T. D., Lewis, A. K., Braun, A. R., Grossfield, A. & Sachs, J. N. Minimal Nucleation State of alpha-Synuclein Is Stabilized by Dynamic Threonine-Water Networks. *ACS Chem Neurosci* **8**, 1859-1864, doi:10.1021/acschemneuro.7b00171 (2017).

67 Siegel, D. et al. Discovery and Synthesis of a Phosphoramidate Prodrug of a Pyrrolo[2,1-f][triazin-4-amino] Adenine C-Nucleoside (GS-5734) for the Treatment of Ebola and Emerging Viruses. *J Med Chem* **60**, 1648-1661, doi:10.1021/acs.jmedchem.6b01594 (2017).

68 Xu, L., Bhattacharya, S. & Thompson, D. The fold preference and thermodynamic stability of alpha-synuclein fibrils is encoded in the non-amyloid-beta component region. *Phys Chem Chem Phys* **20**, 4502-4512, doi:10.1039/c7cp08321a (2018).

69 Rasia, R. M. et al. Structural characterization of copper(II) binding to α-synuclein: Insights into the bioinorganic chemistry of Parkinson’s disease. *Proc Natl Acad Sci U S A* **102**, 4294-4299, doi:10.1073/pnas.0407881102 (2005).

70 Choi, T. S. et al. Supramolecular Modulation of Structural Polymorphism in Pathogenic alpha-Synuclein Fibrils Using Copper(II) Coordination. *Angew Chem Int Ed Engl* **57**, 3099-3103, doi:10.1002/anie.201712286 (2018).

71 Binolfi, A. et al. Interaction of alpha-synuclein with divalent metal ions reveals key differences: a link between structure, binding specificity and fibrillation enhancement. *J Am Chem Soc* **128**, 9893-9901, doi:10.1021/ja0618649 (2006).

72 Wright, J. A., Wang, X. & Brown, D. R. Unique copper-induced oligomers mediate alpha-synuclein toxicity. *FASEB J* **23**, 2384-2393, doi:10.1096/fj.09-130039 (2009).

73 Wang, X., Moualla, D., Wright, J. A. & Brown, D. R. Copper binding regulates intracellular alpha-synuclein localisation, aggregation and toxicity. *J Neurochem* **113**, 704-714, doi:10.1111/j.1471-4159.2010.06638.x (2010).

74 Valensin, D., Dell'Acqua, S., Kozlowski, H. & Casella, L. Coordination and redox properties of copper interaction with alpha-synuclein. *J Inorg Biochem* **163**, 292-300, doi:10.1016/j.jinorgbio.2016.04.012 (2016).

75 Wineman-Fisher, V., Bloch, D. N. & Miller, Y. Challenges in studying the structures of metal-amyloid oligomers related to type 2 diabetes, Parkinson’s disease, and Alzheimer’s disease. *Coordination Chemistry Reviews* **327**, 20-26 (2016).

76 Miller, Y., Ma, B. & Nussinov, R. Metal binding sites in amyloid oligomers: Complexes and mechanisms. *Coordination Chemistry Reviews* **256**, 2245-2252 (2012).
Supporting Information

77 Bortolus, M. et al. Structural characterization of a high affinity mononuclear site in the copper(II)-alpha-synuclein complex. *J Am Chem Soc* **132**, 18057-18066, doi:10.1021/ja103338n (2010).

78 Lee, J. C., Gray, H. B. & Winkler, J. R. Copper(II) binding to alpha-synuclein, the Parkinson’s protein. *J Am Chem Soc* **130**, 6898-6899, doi:10.1021/ja711415b (2008).

79 Abeyawardhane, D. L., Heitger, D. R., Fernandez, R. D., Forney, A. K. & Lucas, H. R. C-Terminal Cu(II) Coordination to alpha-Synuclein Enhances Aggregation. *ACS Chem Neurosci* **10**, 1402-1410, doi:10.1021/acschemneuro.8b00448 (2019).

80 De Ricco, R. et al. Remote His50 Acts as a Coordination Switch in the High-Affinity N-Terminal Centered Copper(II) Site of alpha-Synuclein. *Inorg Chem* **54**, 4744-4751, doi:10.1021/acs.inorgchem.5b00120 (2015).

81 Tian, Y. et al. Copper(2+) Binding to alpha-Synuclein. Histidine50 Can Form a Ternary Complex with Cu(2+) at the N-Terminus but Not a Macrochelate. *Inorg Chem* **58**, 15580-15589, doi:10.1021/acs.inorgchem.9b02644 (2019).

82 Lucas, H. R., Debeer, S., Hong, M. S. & Lee, J. C. Evidence for copper-dioxygen reactivity during alpha-synuclein fibril formation. *J Am Chem Soc* **132**, 6636-6637, doi:10.1021/ja101756m (2010).

83 Bloch, D. N. et al. Fibrils of alpha-Synuclein Abolish the Affinity of Cu(2+)-Binding Site to His50 and Induce Hopping of Cu(2+) Ions in the Termini. *Inorg Chem* **58**, 10920-10927, doi:10.1021/acs.inorgchem.9b01337 (2019).

84 Dudzik, C. G., Walter, E. D. & Millhauser, G. L. Coordination features and affinity of the Cu(2)+ site in the alpha-synuclein protein of Parkinson's disease. *Biochemistry* **50**, 1771-1777, doi:10.1021/bi101912q (2011).

85 Binolfi, A. et al. Bioinorganic chemistry of Parkinson's disease: structural determinants for the copper-mediated amyloid formation of alpha-synuclein. *Inorg Chem* **49**, 10668-10679, doi:10.1021/ic1016752 (2010).

86 Jackson, M. S. & Lee, J. C. Identification of the minimal copper(II)-binding alpha-synuclein sequence. *Inorg Chem* **48**, 9303-9307, doi:10.1021/ic901157w (2009).

87 Huang, J. et al. CHARMM36m: an improved force field for folded and intrinsically disordered proteins. *Nat Methods* **14**, 71-73, doi:10.1038/nmeth.4067 (2017).

88 Bayly, C. I., Cieplak, P., Cornell, W. & Kollman, P. A. A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model. *The Journal of Physical Chemistry* **97**, 10269-10280 (1993).

89 Gaussian 16 Rev. C.01 (Wallingford, CT, 2016).

90 Wang, J., Wang, W., Kollman, P. A. & Case, D. A. Automatic atom type and bond type perception in molecular mechanical calculations. *J Mol Graph Model* **25**, 247-260, doi:10.1016/j.jmgm.2005.12.005 (2006).

91 Van Der Spoel, D. et al. GROMACS: fast, flexible, and free. *J Comput Chem* **26**, 1701-1718, doi:10.1002/jcc.20291 (2005).

92 Hess, B., Bekker, H., Berendsen, H. J. & Fraaije, J. G. LINCS: a linear constraint solver for molecular simulations. *Journal of computational chemistry* **18**, 1463-1472 (1997).

93 Miyamoto, S. & Kollman, P. A. Settle: An analytical version of the SHAKE and RATTLE algorithm for rigid water models. *Journal of computational chemistry* **13**, 952-962 (1992).

94 Darden, T., York, D. & Pedersen, L. Particle mesh Ewald: An N· log (N) method for Ewald sums in large systems. *The Journal of chemical physics* **98**, 10089-10092 (1993).
Supporting Information

95 Bussi, G., Donadio, D. & Parrinello, M. Canonical sampling through velocity rescaling. *J Chem Phys* **126**, 014101, doi:10.1063/1.2408420 (2007).

96 Parrinello, M. & Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *Journal of Applied physics* **52**, 7182-7190 (1981).

97 Brooks, B. R. *et al.* CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *Journal of computational chemistry* **4**, 187-217 (1983).

98 Lee, M. S., Feig, M., Salsbury, F. R., Jr. & Brooks, C. L., 3rd. New analytic approximation to the standard molecular volume definition and its application to generalized Born calculations. *J Comput Chem* **24**, 1348-1356, doi:10.1002/jcc.10272 (2003).

99 Lee, M. S., Salsbury Jr, F. R. & Brooks III, C. L. Novel generalized Born methods. *The Journal of chemical physics* **116**, 10606-10614 (2002).

100 Feig, M. *et al.* Performance comparison of generalized born and Poisson methods in the calculation of electrostatic solvation energies for protein structures. *J Comput Chem* **25**, 265-284, doi:10.1002/jcc.10378 (2004).

101 Pettersen, E. F. *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-1612, doi:10.1002/jcc.20084 (2004).