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Charge and Hydrophobicity of Amyloidogenic Protein/Peptide Templates Regulate the Growth and Morphology of Gold Nanoparticles

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Experimental methods

All the reagents and chemicals were purchased from commercial suppliers. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$) (Catalog no. 27988-77-8) was purchased from Loba-Chemie (India). 4-Dimethyl amino pyridine (DMAP) (Catalog no. 8.51055), Wang resin (100–200 mesh) (Catalog no. 8.55002) were purchased from Nova-biochem (Germany). Proteinase K (Catalog no. 49936) was purchased from SRL chemicals, India. The α-synuclein LB 509 antibody (Catalog no. AB27766) was purchased from Abcam, USA. *N, N*'-Diisopropylcarbodiimide (DIC) (Catalog no. D4781), Triisopropylsilane (TIPS) (Catalog no. 233781), 1-Hydroxybenzotriazole hydrate (HOBt) (Catalog no. 157260), Trifluoroacetic acid (TFA) (Catalog no. T6508) and Poly-L-Lysine (PLL) (M_r 70,000-150,000 Da) (Catalog no. 5988-63) were purchased from Sigma Aldrich (USA). *N, N*-Dimethylformamide (DMF) (Catalog no. 8.22275.2521), Diethyl ether (Catalog no. 1.07026.0521), Dichloromethane (DCM) (Catalog no. 1.94508.2521), Phenol (Ph) (Catalog no. 33517), OC antibody (Catalog no. AB2286), the anti-mouse horseradish peroxidase-conjugated secondary antibody (Catalog no. 40125) and anti-rabbit horseradish peroxidase-conjugated secondary antibody (Catalog no. 401353) were purchased from Merck Millipore.

α-Syn/ α-Syn-core expression and purification. The expression of wild-type (WT) full length α-Syn and the α-Syn-core (30-110) were performed using *E.coli*-BL21 (DE3) strain and purification was performed according to the previously reported protocols with little modifications.[1,2] Briefly, transformed *E.coli*-BL21 (DE3) cells with pRK 172 containing α-Syn and α-Syn-core constructs, were grown in Luria Broth solution under shaking at 200-230 rpm at 37 ºC temperature till the optical density (OD_{600nm}) reaches to 0.6-0.8. The cells were then induced using 1 mM isopropyl β-D-1 thiogalactopyranoside (IPTG) for 4 h at 37 ºC at 200 rpm followed by centrifugation at 4000*g* for 30 minutes. The pellet was resuspended in the lysis buffer (50 mM Tris buffer, pH 8, 10 mM EDTA and 150 mM NaCl) with a protease inhibitor cocktail (Roche scientific) to avoid any proteolytic cleavage. The resuspended cell pellet was further sonicated using a probe sonicator at 40% amplitude with 3 sec on and 1 sec off pulse for 10 minutes followed by heating in boiling water at 95 ºC for 20 minutes. The solution was further centrifuged at 14000*g* for 30 minutes and the supernatant was collected. The 10 % streptomycin sulfate (136 µL/mL of supernatant) and glacial acetic acid (228 µL/mL of supernatant) were added to the supernatant and

incubated for 30 minutes at 4 ºC followed by centrifugation at 14000*g* for 30 minutes. Further, an equal volume of saturated ammonium sulfate was added to the supernatant to allow protein precipitation. The precipitated protein was washed with ammonium sulfate (1:1 v/v saturated ammonium sulfate and water). The washed pellet was resuspended in 100 mM ammonium acetate and reprecipitated using an equal volume of absolute ethanol. This step was repeated twice. Finally, the pelleted protein was redissolved in a minimal volume of 100 mM ammonium acetate and lyophilized. The lyophilized protein powder was stored at -20˚C for further use.

Preparation of low molecular weight (LMW) of α-Syn/ α-Syn-core. The lyophilized WT α-Syn/α-Syn-core protein powder was resuspended in 20 mM Glycine-NaOH buffer, pH 7.4. To dissolve the protein, a few drops of 2 N NaOH were added to obtain a clear solution and the pH was readjusted to 7.4 using 2 N HCl. The protein solution was then dialyzed overnight using 12.4 kDa molecular weight cut-off (MWCO) for WT α-Syn and 3.5 kDa MWCO for α-Syn-core. Both proteins were dialyzed against the same buffer at 4°C. LMW protein was prepared using molecular weight cut-off, 100 kDa MWCO for WT α-Syn and 50 kDa MWCO for α-Syn-core, as per previously established protocol.[2] For α-Syn-core, the protein solution was further ultracentrifuged at 41900*g* to remove undesired protein aggregates before nanoparticle synthesis. The resulting LMW solution in the supernatant was used for further studies.

Peptide synthesis. All the small peptides were synthesized using the solid-phase peptide synthesis (SPPS) method based on the Fmoc strategy. We activated the carboxyl groups by HOBt using DIC as a coupling agent. In a typical synthesis method, wang resin (100 mg) was allowed to swell for 3 h using 1 mL of DMF: DCM (1:9) mixture. The swelled resin was then transferred to the solid-phase vessel (10 mL) and a mixture of the amino acid (0.5 mM) and HOBt (0.5 mM) dissolved in1 mL of DMF was transferred to the resin vessel. The reaction mixture was vortexed for 15 minutes and then 0.5 mM of DIC was added to the reaction vessel and it was incubated for 2-3 h for coupling of amino acid to resin. After incubation, the reaction was further washed several times with DMF/DCM. Subsequently, N-terminal Fmoc protection was removed using 30% piperidine in 1 mL DMF. Using a similar coupling method, peptide synthesis was done and the coupling steps were performed till the desired sequence of peptides were obtained. The peptides were then

cleaved from the resin using a cleavage reagent (1 mL of TFA:Ph:H_2O:TIS, 88:5:5:2). The cleaved peptides were precipitated using ice-cold ether and ether was evaporated. The peptides were then dissolved in 10 mL of 50 mM NH_4HCO_3 solution and lyophilized. The lyophilized peptide powder was used for further studies.

Formation of amyloid fibrils from α-Syn/ α-Syn-core/peptides. For fibrillization of α-Syn and α-Syn-core protein, 300 μM LMW protein was used. The concentration of LMW protein solution was measured by UV-vis spectroscopy. The concentration has been determined by taking absorption at 280 nm, using molar absorptivity (ε) coefficient 5960 and 1490 M^{-1} cm^{-1} for α-Syn and α-Syn-core, respectively. 300 μM protein in 20 mM Glycine-NaOH buffer, pH 7.4 were incubated at 37°C with a slight rotation of ~50 rpm for 5 days. Finally, the fibrillization was monitored using circular dichroism (CD) and morphological characterization was done using TEM.

To achieve the fibrillization of peptides, the lyophilized peptides of Fmoc/F-(KLMEI/KLLDI) were dissolved in 20 mM sodium phosphate buffer (PB), pH 7.4 at a concentration of 1 mg/ 200 μL of PB. The peptides were solubilized by the addition of a few microliters of 2 N NaOH. This step was repeated so that the peptides were dissolved completely in the PB and a clear solution is obtained. After peptides were fully soluble, the pH of the solution was readjusted to pH 7.4 by the addition of 2N HCl. Finally, 10 μL of 5 M NaCl was added to the 200 μL peptide solution in PB. The reaction was incubated for 15 minutes for fibril formation. TEM was performed to confirm the fibril formation. For fibrillization of Poly-L-Lysine (PLL) polypeptide, 1 mg of PLL (M_r 70,000-150,000 Da) was dissolved in 200 μL of 20 mM PB and the pH was adjusted to 11.5. The solution was then heated in the water bath at 85 ºC for 6-8 h to facilitate fibril formation. Further, the solution was cooled down and stored at room temperature. The fibril formation was confirmed using CD spectroscopy and TEM analysis.

Circular Dichroism analysis. For the analysis of secondary structural features of protein templates (α-Syn and α-Syn-core proteins), circular dichroism of protein and peptides was performed. For circular dichroism measurement, 150 μL of 10 μM protein in 20 mM Glycine-NaOH buffer was used. The spectra scanning was done from wavelength 198 nm to 260 nm. For

PLL polypeptide, 10 μL of PLL (5 mg/mL) was added to 140 μL 20 mM PB and the CD spectrum was recorded. Each spectrum was recorded and scanned three times and an average was plotted. The raw data was processed according to the manufacturer's instructions. The CD experiments were performed using the JASCO-1500 instrument with the path length set to 0.1 cm quartz cell at room temperature.

Synthesis of gold nanoparticles (without protein templates). The AuNPs were synthesized according to the previously established green synthesis method using lemongrass (LG) extract as a reducing agent.[3] In the typical synthesis, LG extract was prepared using 5 gm of thoroughly washed LG leaves, which were finely cut and autoclaved with 25 mL of sterile water at 120 ºC for 20 minutes. The extract was filtered using Whatman filter paper 42 and used for further steps.
To synthesize AuNPs, 940 μL of 1 mM $HAuCl_4$ (Chloroauric acid) solution in Milli-Q water was added to the 1.5 mL eppendorf tube and 60 μL lemongrass extract in water was added to the reaction. The volume was adjusted to 1.2 mL with further addition of Milli-Q water and incubated for 48 h to form AuNPs. The color of the reaction changed from yellow to ruby red as the reaction progressed. The formation of NPs was confirmed using UV-vis spectroscopic analysis and the morphological differences were analyzed using TEM. The time dependent NPs growth was analyzed by taking small aliquots from the reaction and analyzed with UV-vis spectroscopy and TEM.

Synthesis of the gold nanoparticles using amyloid fibrils. The 50 μL aliquots of various fibrils prepared in respective buffers were taken in a 1.5 mL of eppendorf tube. The various amyloid fibrils used were, α-Syn/α-Syn-core fibrils, prepared in 300 μM in 20 mM Gly-NaOH buffer, pH 7.4; Fmoc/F-(KLMEI/KLLDI) peptide fibrils (5 mg/mL) prepared in PB, pH 7.4 and PLL (5 mg/mL) fibrils prepared in PB, pH 11.5. Before the addition of chloroauric acid to the template solutions, the pH of the fibril solutions was adjusted to pH 2.5 or pH 7.5 for α-Syn, pH 6 or pH 10 for α-Syn-core, pH 2.5 or pH 7.5 for Fmoc/F-(KLMEI/KLLDI) peptides and pH 6.5 or pH 11.5 for PLL polypeptide using 2 N HCl or 2 N NaOH. Further, 940 μL of 1 mM chloroauric acid solution prepared by dissolving $HAuCl_4$ in Milli-Q water was added to the eppendorf tubes containing individual protein/peptide fibril solution in the respective buffer. The amyloid-

chloroauric acid solutions were mixed for 30 sec to achieve homogeneous dispersion of the chloroauric acid solution. The reaction was further incubated at room temperature for 24 h. The solution was then centrifuged at 6500*g* for 5 minutes to isolate the fibrils-bound chloroauric acid as pellet fractions. The unbound chloroauric acid in the supernatant was removed. The extent of gold bound to fibrils and remaining in solution was analyzed using the ICP-AES method (see below). Further, 60 μL LG extract was added to the eppendorf tube containing fibril-bound gold pellets and the volume was adjusted to 1.2 mL with Milli-Q water. This mixture was kept for another 24 h at room temperature for nanoparticle synthesis. The color of the reaction changed from yellow to ruby red as the reaction progressed. The formation of NPs was confirmed using UV-vis spectroscopic analysis and the morphological differences were analyzed using TEM.

Inductive coupled plasma atomic emission spectroscopic (ICP-AES) analysis. For this analysis, the α-Syn fibrils were incubated with chloroauric acid at pH 2.5 and pH 7.5. After 24 h, the unbound chloroauric acid was removed from the fibril templates using centrifugation at 6500*g*. The pelleted α-Syn fibril and supernatant solutions were then transferred into separate glass vials and subsequently, 1 mL aqua regia (HCl: HNO_3 3:1) was added to both the vials. Further, the volume of both reactions was adjusted to 5 mL using Milli-Q water. After 48 h of reaction, the concentration of atomic gold was measured using ARCOS simultaneous ICP spectrometer, Germany. Three independent measurements were taken for each sample and the observed concentrations (in ppm) of bound and unbound gold to the α-Syn fibrils were plotted using Prism 9 software.

Gold nanoparticles synthesis using monomeric protein/peptides. The 50 μL aliquots of various proteins in the monomeric form prepared in respective buffers and pHs were taken in a 1.5 mL of eppendorf tube. Various proteins taken were α-Syn/α-Syn-core prepared in 300 μM in 20 mM Gly-NaOH buffer, pH 7.4 and PLL (5 mg/mL) prepared in PB, pH 11.5. Before the addition of chloroauric acid to the protein solutions, the pH of the protein solutions was adjusted to pH 2.5 or pH 7.5 for α-Syn, pH 6 or pH 10 for α-Syn-core and pH 6.5 or pH 11.5 for PLL polypeptide using 2 M HCl or 2 NaOH. Further, 940 μL of 1 mM chloroauric acid solution, prepared by dissolving $HAuCl_4$ in Milli-Q water was added to the eppendorf tubes containing individual protein/peptide

solutions in respective buffers. The resulting solutions were mixed for 30 sec to achieve homogeneous dispersion of proteins and chloroauric acid solution. Finally, 60 µL LG extract prepared in Milli-Q water was added to the eppendorf tube and the final volume was adjusted to 1.2 mL with Milli-Q water. The reaction was kept for 24 h and the color change from yellow to ruby red was observed as the reduction reaction progressed. The formation of NPs was confirmed using UV-vis spectroscopy analysis and the morphological differences were observed using TEM.

Synthesis of gold seed nanoparticles (AuSNPs) for Surface Plasmon Resonance (SPR) study. To synthesize AuSNPs, 940 µL of 1 mM chloroauric acid solution prepared by dissolving $HAuCl_4$ solid in Milli-Q water was taken in a 1.5 mL eppendorf tube. Subsequently, 80 µL LG extract was added to the reaction solution. The volume was adjusted to 1.2 mL with Milli-Q water and incubated for 5 h to form AuSNPs. The formation of NPs was confirmed using UV-vis spectroscopic analysis and TEM imaging.

TEM analysis. The samples were prepared by drop-casting 20 µL of 30 µM α-Syn and α-Syn-core (fibrils and monomers) and amyloid-gold nanocomposites (α-Syn-Au, α-Syn-core-Au, Fmoc/F-Au (KLMEI/KLLDI) and PLL-Au) on carbon-coated copper grids. The samples were then incubated for 10 minutes and unbound fractions were washed with autoclaved Milli-Q water. The samples were stained with uranyl formate 10 µL (1 mg/mL) and incubated for another 5 minutes. Finally, the excess uranyl formate was removed and the sample was dried for 30 minutes. Before the TEM analysis, the samples were dried under an IR lamp for 5 minutes. The samples were subjected to TEM for imaging and the imaging was done using (Phillips TEM-CM- 200 kV), FEG-TEM (FEI Tecnai) at 200 kV and FEG-TEM (Philips, 300 kV) with 6000X to 27000X magnifications.

X-ray diffraction analysis of nanoparticles. The 200 µL aliquots of the solution containing AuNPs supported with α-Syn-fibrils at pH 2.5 and 7.5 were drop-casted on a round coverslip of 10 mm thickness. The sample was dried at 37 °C overnight and was finally exposed to IR light for 5 mins before analysis. The X-ray diffraction has been performed with Cu-K-α as

an X-ray source with 1548Å λ using Smartlab, Rigaku diffractometer (USA) instrument. The diffraction was analyzed using Adxv software and the data was plotted using Prism 9 software.

Protein X-ray diffraction study of fibrils. For this study, the protein fibrils and fibrils-gold composites were loaded into a clean capillary (~0.7 mm) and dried for 24 h under vacuum. The capillary with the dried sample was mounted in the path of the X-ray beam at 1.2 kW for 300 sec. The micrographs were captured using the Rigaku R Axis IV^{++} detector (Rigaku, Japan). Finally, the diffraction data was analyzed using Adxv software.

Nile-red (NR) binding assay. The NR stock solution (1 mM) was prepared in dimethyl sulfoxide (DMSO). The binding assay was performed by mixing 0.2 μL of 1 mM NR solution with 10 μL each of 300 μM α-Syn and α-Syn-core fibrils or monomers (both at pH 2.5 and pH 7.5), in 190 μL of 20 mM Glycine-NaOH buffer. Similarly, for NR binding of peptides, 10 μL aliquots of Fmoc/F-KLMEI, Fmoc/F-KLLDI (5 mg/mL) fibrils at pH 2.5 and pH 7.5 and PLL fibrils or monomers (5 mg/mL) at pH 6.5 or 11.5 prepared in 20 mM PB were mixed with 0.2 μL of 1 mM NR solution in 190 μL of Glycine-NaOH buffer. The reaction was incubated for 5 minutes at room temperature and then fluorescence measurement was performed. The excitation of NR was kept at 550 nm and emission spectra were recorded from 565 nm to 700 nm. Spectra were recorded using a spectrofluorometer (JASCO FP 8500, USA). The NR fluorescence value at 630 nm was plotted.

Surface plasmon resonance (SPR) analysis. The SPR was performed using SPR Bia-CORE T200 (GE Health Care, USA) instrument. Both monomeric and fibrillar forms of α-Syn (pH 2.5 and 7.5) and α-Syn-core protein (pH 6 and 10) were dissolved in 20 mM Glycine NaOH buffer and used for this study. To perform the SPR study, both fibrillar and monomeric proteins were immobilized on the CM5 Bia-Core sensor chip, where an immobilization level in the range of ~1400-1500 was achieved. All the AuSNPs solutions in the concentration range of 10−400 μM were injected into the microfluidic channel at a flow rate of 30 μL/min in 10 mM PB (pH 7.4) for the binding. The contact time and dissociation time were as follows: for α-Syn-core, 90 sec and 600 sec respectively; for α-Syn, 90 sec and 300 sec, respectively. All the kinetic binding experiments were performed at 25 °C. The surface was regenerated by injecting 100 mM NaCl

over 60 sec at a flow rate of 20 µL/min. The response unit of the blank run was considered as a baseline and was subtracted from the response unit of the sample. The resultant response unit after blank correction was fitted using a steady-state affinity model and the dissociation constant (K_D) was calculated. The response unit curve obtained for each sample was plotted with respect to time.

Fourier transform infrared spectroscopy (FTIR). The secondary structural conformation of the α-Syn-core monomer before and after NPs formation was determined using FTIR. 10 µL of 30 µM α-Syn-core monomer and 10 µL of α-Syn-core monomer NPs as prepared were spotted on a compressed KBr pellet. The background spectrum was corrected using 10 µL of 20 mM Glycine-NaOH buffer, pH 7.4. The samples were dried using an IR lamp and the spectra were recorded in the range of 1500-1800 cm^{-1} using Vertex 80 FTIR system equipped with a DTGS detector (Bruker, Ettlingen, Germany). The raw spectra were obtained by averaging 32 scans and deconvoluting the amide-I region (1600-1700 cm^{-1}) by the Fourier self-deconvolution method. The deconvoluted spectra were fitted by the Lorentzian curve-fitting procedure using Opus-65 software.

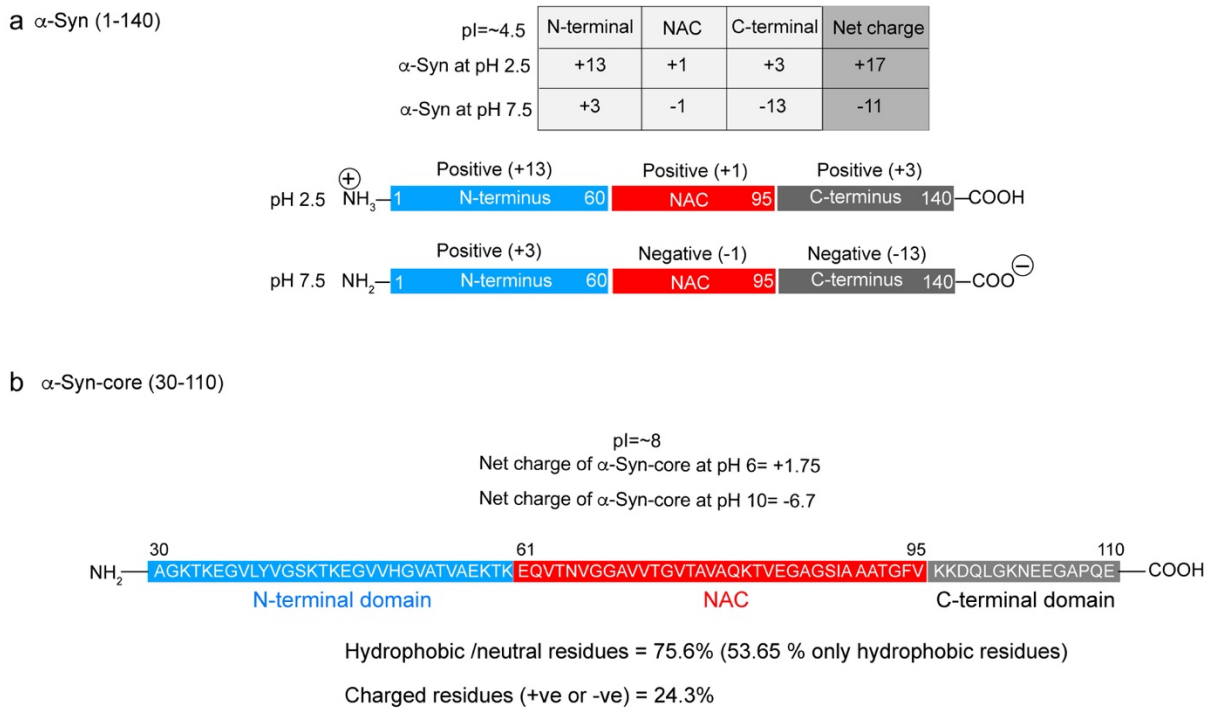
Zeta potential measurement. The analysis was done at 25 °C with 60 s equilibration time using Malvern's Zeta-seizer Ultra instrument. The cuvette was cleaned with Milli-Q and methanol followed by nitrogen gas purging. The 500 µL aqueous solution of AuSNPs and AuTNPs was then added to the cleaned cuvette and the zeta-potential was measured. Three independent measurements were recorded per analysis with three acquisitions.

Proteinase K digestion (PK). For PK digestion, the 500 µL aliquots of prepared amyloid-gold nanocomposites were taken into 2 mL eppendorf tubes. Subsequently, 100 µL of 20 mg/mL PK solution in milli-Q water was added to each reaction tube and the volume was adjusted to 1 mL using autoclaved milli-Q water to make the final PK concentration 2 mg/mL. For control, 500 µL of 10 µM of α-Syn fibrils were aliquoted to a 2 mL eppendorf tube, and subsequently, 100 µL of 20 mg/mL PK solution in milli-Q was added to each reaction tube. The final volume was adjusted to 1 mL using autoclaved milli-Q water. The reaction was kept at 37 °C for 4 h to digest the fibrils. The solution was then heated in a dry bath for 10 minutes at 100 °C to halt the reaction. The digested solutions were centrifuged at 6500*g* to isolate the NPs from the digested solution. The

extent of digestion of fibrils was further confirmed by TEM analysis and dot-blot assay (see below).

Dot blot assay: After PK digestion as discussed above**,** dot blot assay was performed to detect the presence of α-Syn protein using LB 509 antibody and amyloid fibrils using OC antibody.[4] Each sample (from PK digestions, 2 μL) was spotted on a nitrocellulose membrane (NC-Cytiva Amersham™ Protran™), and was left to air dry. The membrane was then washed with Tris-base saline buffer with 0.01% Tween (TBST) for 5 min followed by blocking with 5% non-fat milk (Himedia, Mumbai, India) in TBST for 1.5 h at room temperature. The blots were incubated with the α-Syn-specific antibody LB509 (1:1500 dilutions in TBST) and fibril-specific OC antibody (1:1000 dilutions in TBST) overnight at 4 °C with gentle rocking, followed by three TBST washes. The anti-mouse horseradish peroxidase-conjugated secondary antibody against LB509 and anti-rabbit horseradish peroxidase-conjugated secondary antibody against OC was then treated with the membrane (1:5000 dilutions in TBST). The blots were incubated with respective secondary antibodies at room temperature with mild shaking on a rocking platform for 2h. Blots were developed by subjecting them to a chemiluminescent substrate after three TBST washes.

Supporting Figures:



Schematic S1. Charge distributions at different domains of α-Syn protein at different pH conditions and structural composition of α-Syn-core protein. (a) The table and schematic representation showing charge distribution across the three subdomains of α-Syn protein at pH 2.5 and pH 7.5. The isoelectric point (pI) of α-Syn protein is ~4.5, thus at pH 2.5, the N-terminal domain is highly positively charged (+13); while the C-terminal domain is less positively charged (+3). Due to the combination of NAC domains charge (+1), the resulting net charge of the protein is (+17) at pH 2.5. In contrast, at pH 7.5, the C-terminal is highly negative (-13) while N-terminal is less positively charged (+3). In combination with the NAC domain's charge (-1), the net charge of the protein is (-11). The NAC domain comprises mostly hydrophobic amino acid residues; thus, it shows less response toward variable pH-dependent charge contribution. (b) Sequence of α-Syn-core protein and its structural composition in combination with hydrophobic/neutral and charged amino acids. The pI of α-Syn-core protein is ~8, thus at pH 6, the net charge of the protein is less positively charged (+1.75) while, at pH 10, the protein shows net negative charge (-6.7). The total count of charged residues in α-

Syn-core is 24.3% while the remaining amino acid residues are hydrophobic and neutral. The protein charges at given pHs were calculated by "Prot-pi" protein tool.

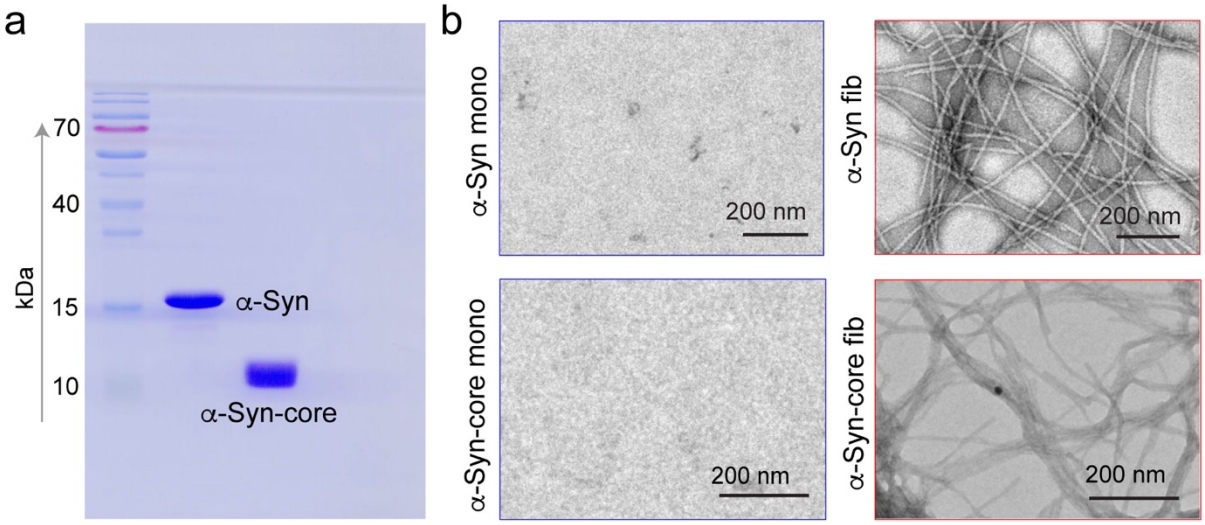


Figure S1. Biophysical characterization of α-Syn and α-Syn-core proteins. (a) SDS-PAGE image showing protein bands of α-Syn and α-Syn-core corresponding to their molecular weights ~14.4 and ~8 kDa, respectively. (b) TEM images of α-Syn and α-Syn-core monomers (*left*) and their respective fibrillar form (*right*). The fibrillization was achieved by rotating α-Syn/ α-Syn-core monomer (300 μM) in 20 mM Glycine-NaOH buffer, pH 7.4 at 37 ºC for 120 h. The "Fib" and "Mono" represents fibrils and monomeric form, respectively.

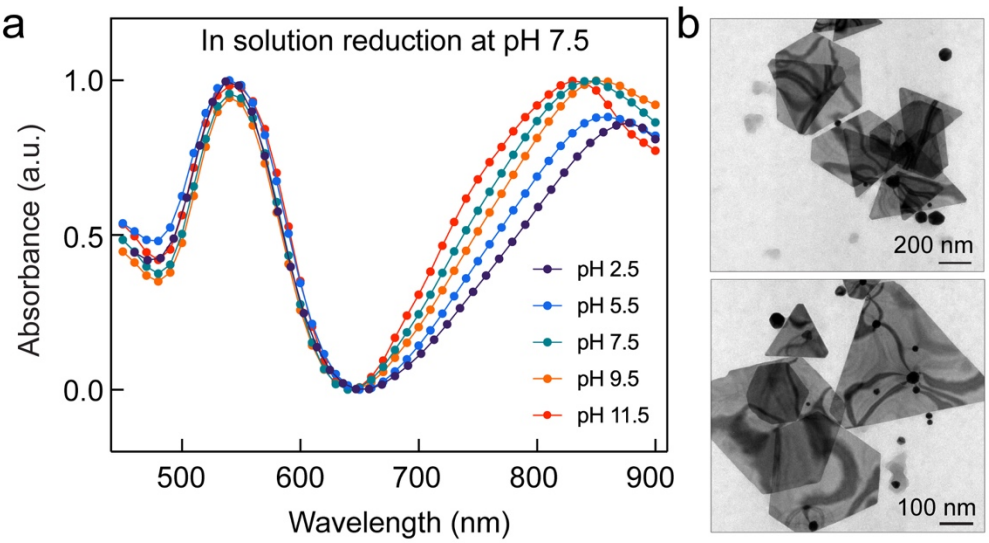


Figure S2. In-solution (without protein) synthesis of gold nanoparticles with variable pH conditions. (a) UV-vis spectra of gold nanoparticles (AuNPs) synthesized at different pH conditions (2.5, 5.5, 7.5, 9.5 and 11.5) showing two distinct plasmonic bands i.e. one at ~535 nm and another at ~850 nm, representing the transverse and longitudinal plasmonic modes of nanoparticles, respectively. These two plasmonic bands illustrate the anisotropic nature of synthesized nanoparticles. (b) TEM micrographs of nanoparticles showing majorly triangular-shaped morphology (AuTNPs), synthesized at pH 7.5 in the aqueous medium.

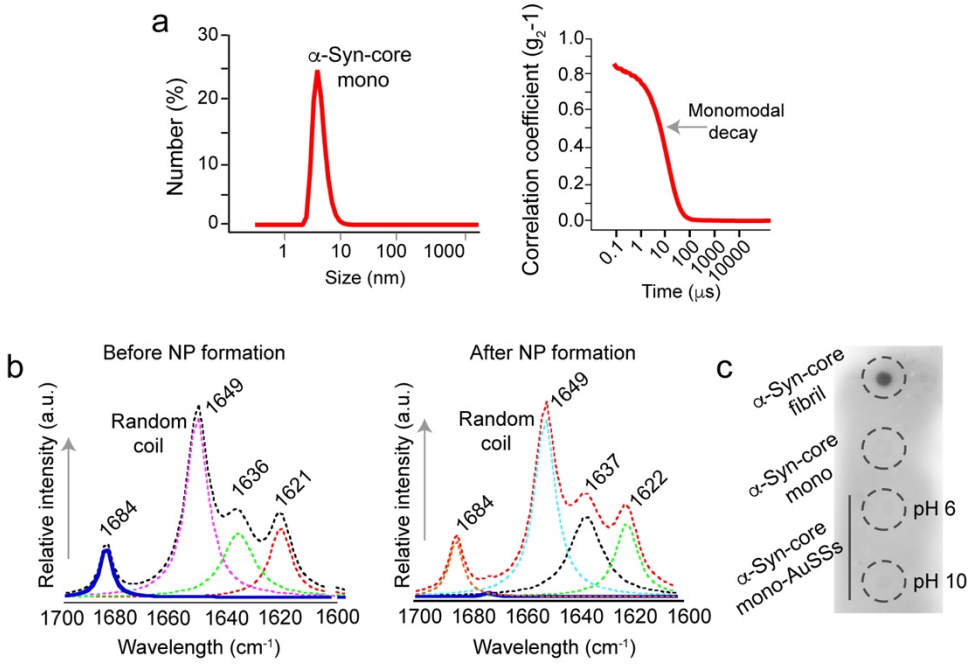


Figure S3. Biophysical characterization of α-Syn-core monomer. (a) Dynamic light scattering (DLS) analysis was performed before NPs synthesis. The DLS profile showing single population distribution of α-Syn-core monomer and the corresponding DLS autocorrelation curve showing a single-step decay profile of α-Syn-core monomer indicating a dominant monomeric population. (b) Fourier transform infrared (FTIR) spectroscopic analysis of α-Syn-core monomer before NPs formation (*left*)and after NPs formation (*right*) showing the major abundance of random coil structure (~1648 cm^{-1}) at pH 6. (c) Dot-bot assay performed with amyloid specific OC antibody showing high intense signal for the positive control α-Syn-core fibrils, while no signal was detected for monomeric α-Syn-core before and after AuSSs formation at pH 6 and pH 10. This suggests the persistent amyloid free state of α-Syn-core monomers before and after the NPs formation.

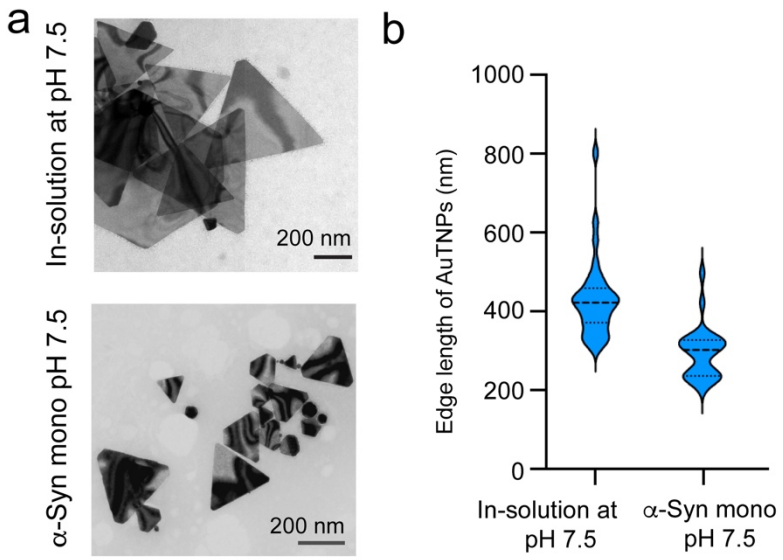


Figure S4. **The size distributions of triangular-shaped gold nanoparticles**. (a) TEM images showing AuTNPs synthesized in aqueous solution (without any template, at pH 7.5) and with α-Syn monomer at pH 7.5. (b) The violin plot showing the calculated edge lengths of AuTNPs formed in solution (without protein) and with α-Syn monomer at pH 7.5.

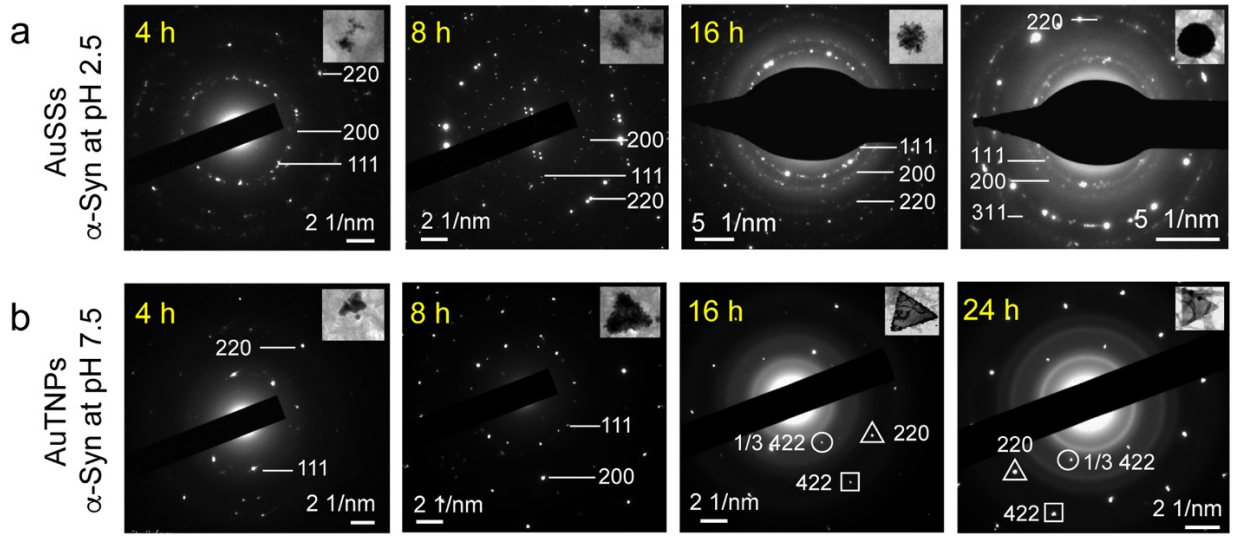


Figure S5. Structural evolution of gold nanostructures formed with amyloid fibril bound chloroauric acid at different pH conditions. (a) The selected area electron diffraction (SAED) pattern of gold NPs supported by α-Syn fibrils at pH 2.5, showing the disordered polycrystalline nature of AuNPs formed at the early stage of reaction (mainly spherical NPs as shown in TEM inset, 4 h). As the reaction progressed the SAED pattern of self-assembled gold supra-spheres (AuSSs) (8 h to 24 h) showed the ordered face-centered cubic (fcc) crystal planes in {111}, {200}, {220} direction. This suggests the polycrystalline nature of AuSSs oriented ring confinement of fcc crystal lattice. (b) The SAED pattern of AuTNPs supported by α-Syn fibrils at pH 7.5, indicating the weak polycrystalline nature of AuNPs at the early stages of the reaction (4 h to 8 h). Further, the crystal growth progressed in an anisotropic direction (as shown in the TEM inset, 16 h to 24 h). The corresponding SAED pattern exhibiting fcc crystal planes in 1/3{422}, {220} and {422} Bragg facets of the fcc crystal lattice represented by circled, triangular, and cubic spots, respectively. This suggests the single-crystalline nature of AuTNPs (The TEM inset micrographs represent the corresponding SAED patterns).

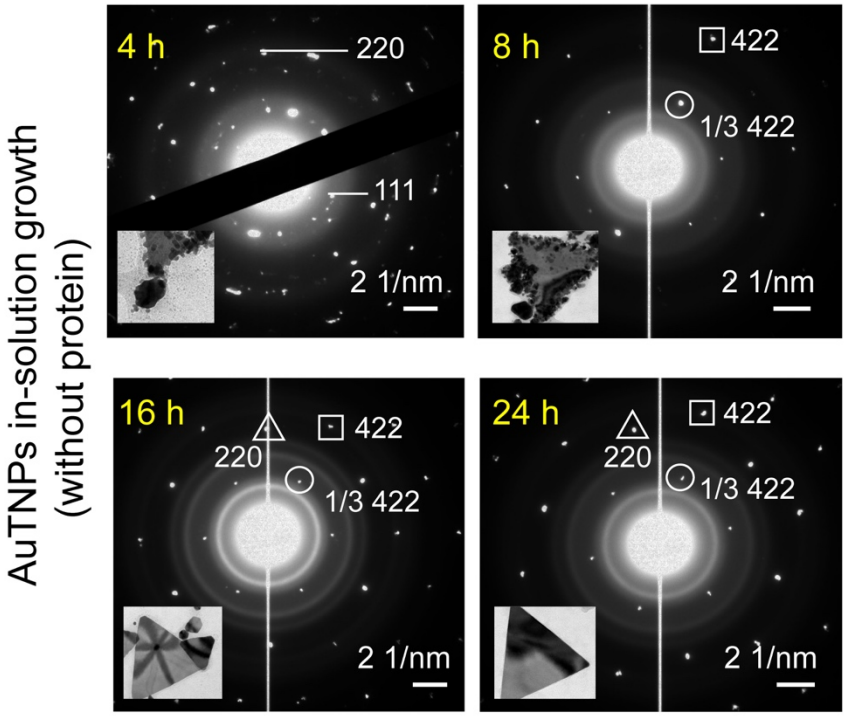


Figure S6. Structural evolution of gold nanostructures synthesized in an aqueous medium (without protein) at pH 7.5. The crystal growth examined with the SAED pattern showing weak polycrystalline nature of initially formed AuNPs (4 h). The further crystal growth progressed in anisotropic direction (8 h to 24 h) and the fcc crystal planes emerged in 1/3 {422} and {422} direction. At 16-24 h, the crystal growth showed the formation of AuTNPs and the corresponding SAED pattern exhibited bright spots of 1/3{422}, {220} and {422} Bragg facets corresponding to the fcc crystal lattice represented by a circle, triangular, and cubic spots, respectively. The data suggest the single-crystalline nature of AuTNPs. (The inset TEM micrographs represent the corresponding SAED patterns).

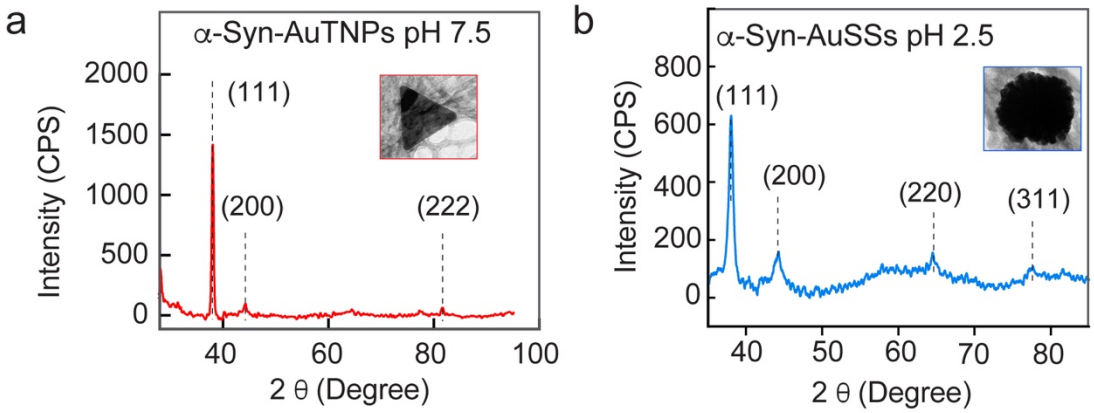


Figure S7. XRD analysis of different gold nanoparticles supported by α-Syn amyloid fibrils. The XRD patterns of α-Syn-AuNPs synthesized under different amyloid templating conditions illustrate the spacing and crystal packing of the nanoparticles at pH 7.5 and pH 2.5. Inset showing the corresponding TEM images of respective diffraction patterns. (a) XRD pattern of AuTNPs showing preferred (111) plane with low intense fcc planes (200) and (220), illustrating the high anisotropic nature of the nanostructures. (b) XRD pattern of AuSSs nanoparticles showing (111), (200), (220) and (311) planes corresponding to fcc crystal lattice, suggesting the polycrystalline nature of nanostructures.

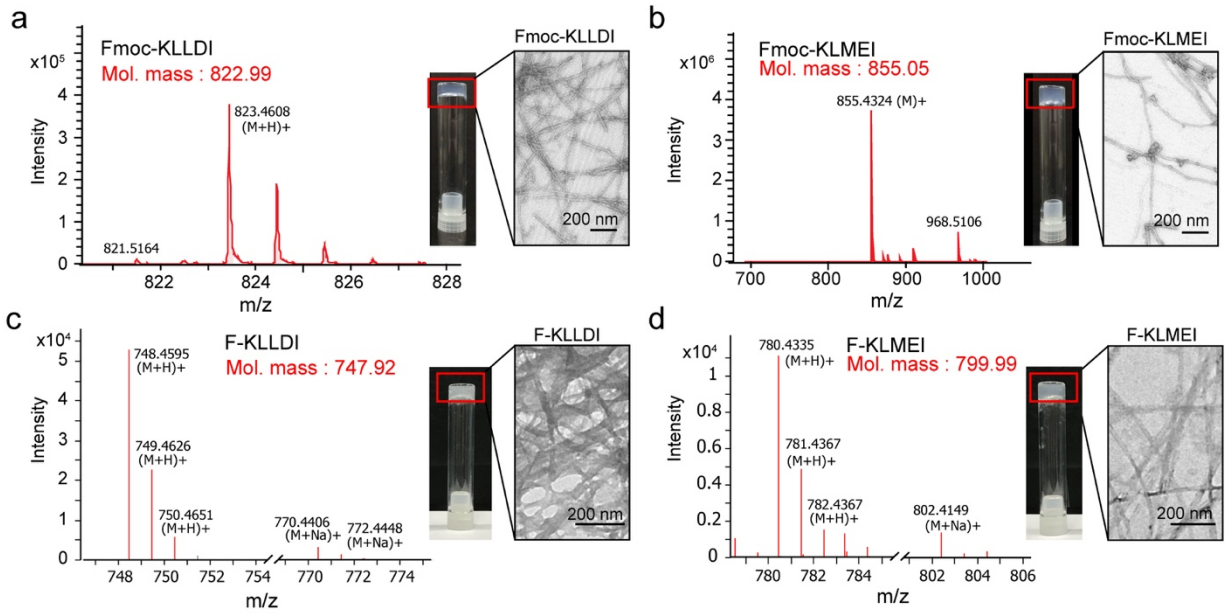


Figure S8: Peptide amyloids synthesis and characterization. Electrospray ionization mass spectrometry (ESI-MS) profile of (a) Fmoc-KLLDI, (b) Fmoc-KLMEI, (c) F-KLLDI and (d) F-KLMEI peptides. The high-resolution mass (HR-MS) profiles showing the theoretical and corresponding observed mass of synthesized peptides. The inverted vessels showing the amyloid hydrogel formation and their corresponding TEM micrographs illustrating the fibrillar morphology of synthesized peptides.

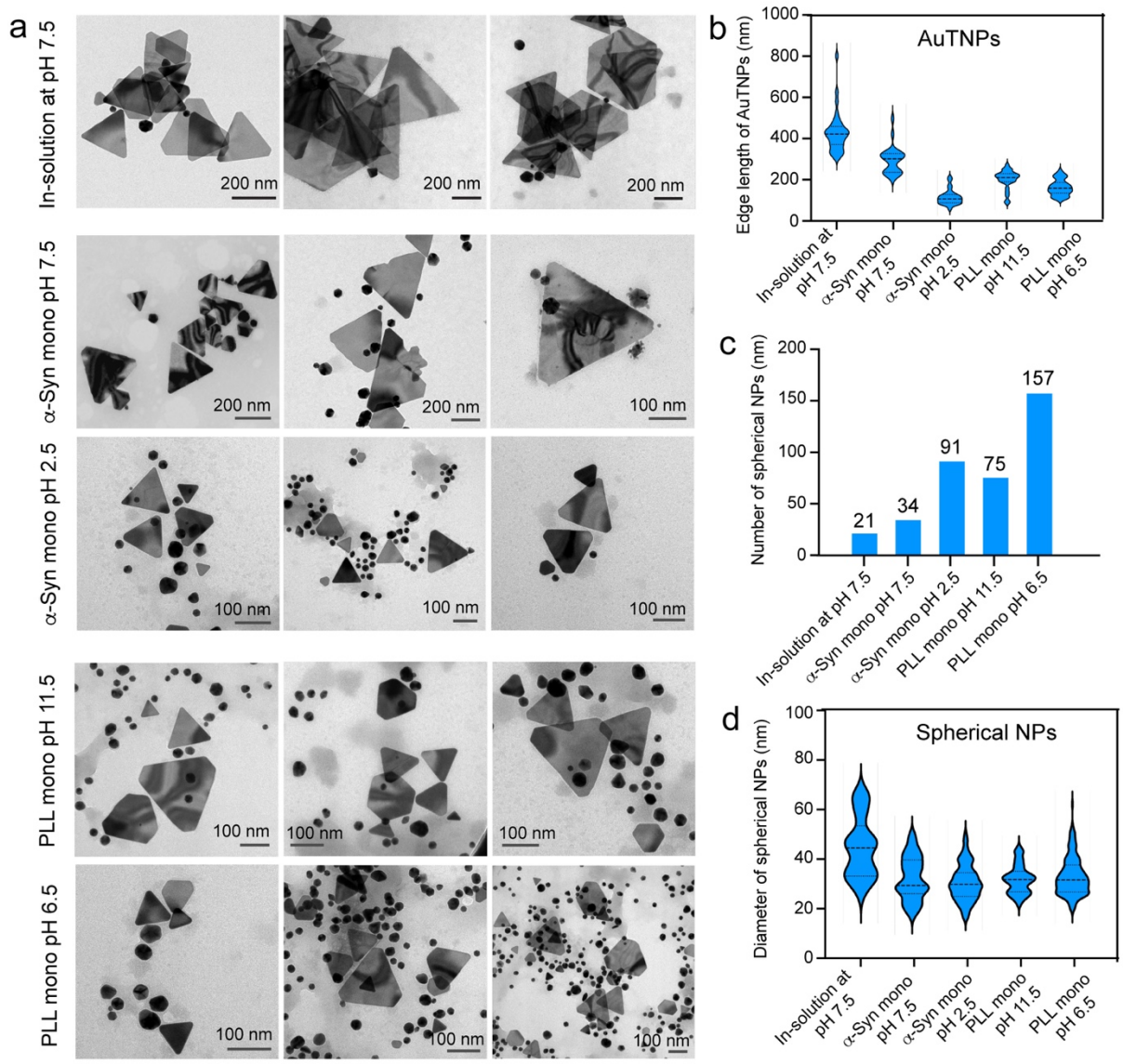
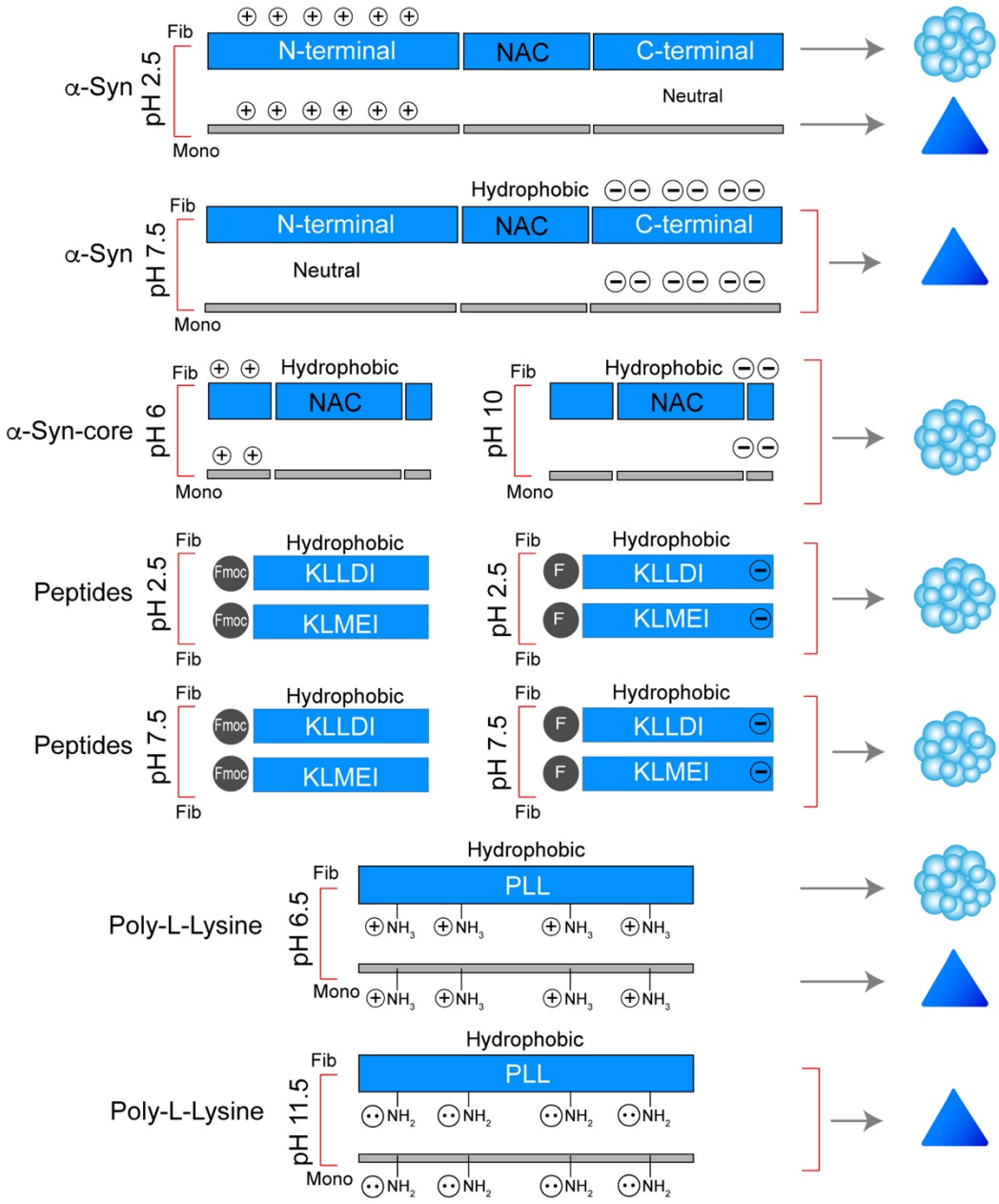


Figure S9. Templating effect of monomeric α-Syn and PLL for AuTNPs growth. (a) TEM micrographs of AuTNPs synthesized in solution at pH 7.5 (without protein), with α-Syn at pH 2.5 or 7.5 and PLL monomeric polypeptide at pH 6.5 or 11.5 conditions. (b) The violin plot showing the edge length of AuTNPs formed in solution at pH 7.5 (without protein), with α-Syn and PLL monomeric polypeptide at respective pHs. (c) The bar plot showing the number distribution of spherical NPs observed along with AuTNPs in different regions of TEM images and the corresponding size distribution profile of spherical AuNPs profile (d). The distribution analysis was done using ImageJ 1.53k software.



Schematic S2: Graphical illustration of the shape-controlled synthesis of gold nanoparticles with different templating conditions. All proteins, polypeptides and their corresponding fibrils at different pHs exhibit different surface charge distributions, which produce different morphologies of gold crystals under reduced conditions. The blue color represents hydrophobic amyloid form (Fibrils) while the grey color represents the corresponding monomeric form of proteins.

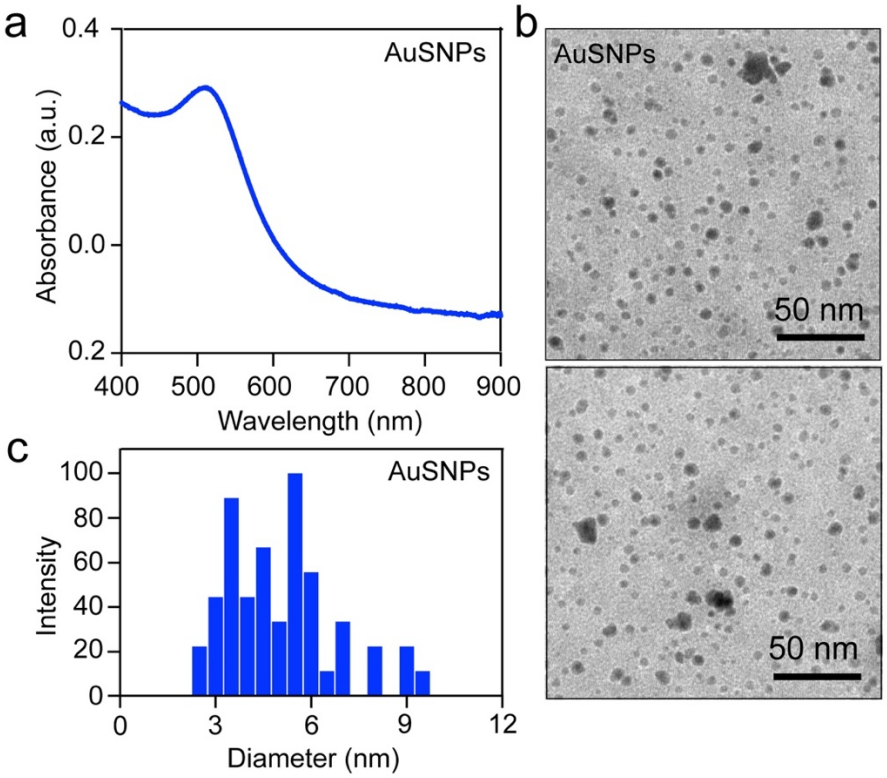


Figure S10: Synthesis of gold seed nanoparticles (AuSNPs). (a). UV-vis spectrum of AuSNPs synthesized by reducing 1 mM of aqueous chloroauric acid ($HAuCl_4$) solution with LG extract for 5 h. The spectrum showing transverse (out-of-plane dipole) corresponding to the small spherical AuSNPs. (b) TEM images and (c) size distribution profile of AuSNPs, showing the average diameter of AuSNPs ~5.07 (±1.7) nm.

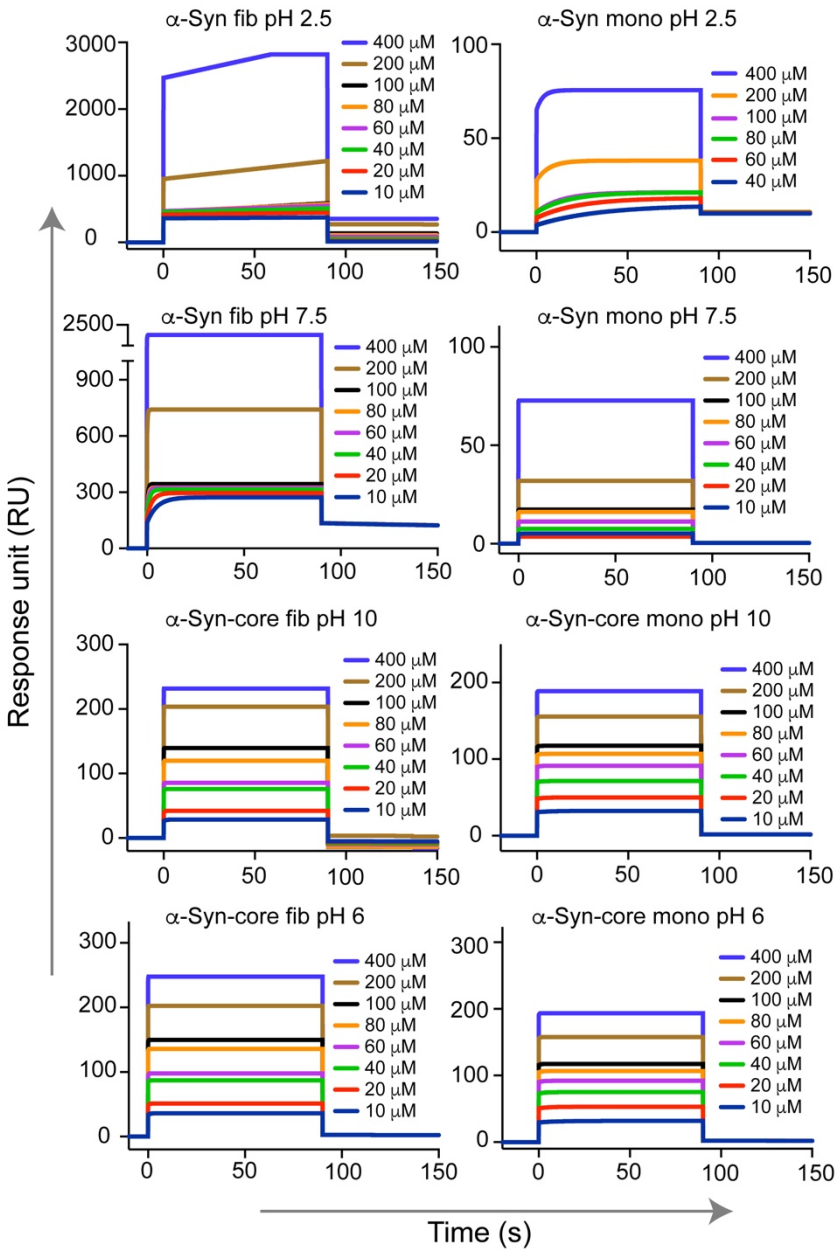


Figure S11. Interaction of AuSNPs and different protein templates using surface plasmon resonance spectroscopy (SPR). Protein samples (both fibrillar and monomeric) were immobilized on SPR chip (CM5) and the concentration gradient of AuSNPs solutions (10−400 μM) were allowed to flow on the immobilized chip through the microfluidic channel. The resultant response unit after blank correction was fitted using a steady-state affinity model and the dissociation constant (K_D) was calculated. The response unit curve obtained for each sample was plotted with respect to time.

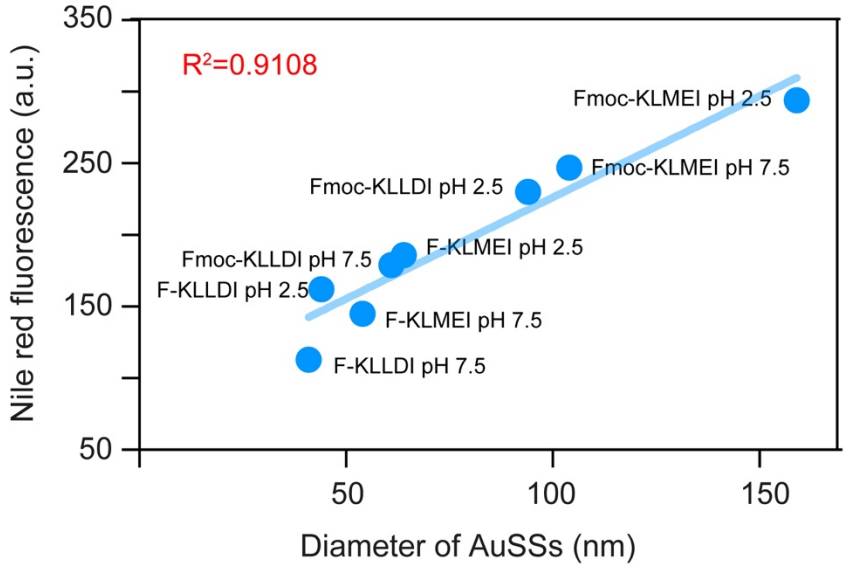


Figure S12. Correlation plot of Nile red (NR) fluorescence and diameter of AuSSs. The correlation plot showing a linear relationship (R^2=*0.9108*) between the NR fluorescence (extent of the exposed hydrophobic surface of the Fmoc/F-peptide templates) and the resulting AuSSs size (diameter).

Table S1. Zeta potential measurement of gold nanoparticles showing the negative charge of synthesized nanostructures.

Sample	Zeta potential (mV)	Standard deviation
Gold seed (AuSNPs)	-10	(± 2.0)

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