An Establishment of peptide aggregation process

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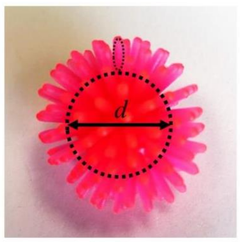
Background

Very little is known about the process of how proteins adhere to nanoscale solid surfaces. One thing that is known, though, is that amyloidogenic peptides effectively adsorb onto gold. Another thing that is known is that the amyloidogenic peptides (*e.g.*, amyloid beta: Aβ; beta 2 Microglubulin: β2M; and alpha-synuclein: α-syn) adsorb onto a gold surface through a sulfur atom of thiol (–SH) group. These amyloidogenic peptides conduct drastic protein structural change (protein folding) to form many units of toxic polymer that eventually combine to create a few micron sized fibers (*i.e.*, amyloid), causing neurodegenerative diseases. The adsorption of amyloidogenic peptides, amyloid beta 1-40 ($A\beta_{1\text{-}40}$), alpha-synuclein (α-syn), and beta 2 microglobulin (β2m), was attempted over the surface of nano-gold colloidal particles, ranging from d =10 nm and 100 nm in diameter (*d*). The spectroscopic inspection between pH 2 and pH 12 successfully extracted the critical pH point (pH_o) at which the color change of the amyloidogenic peptide coated nano-gold colloids occurred due to aggregation of the nano-gold colloids. The change in surface property caused by the degree of peptide coverage was hypothesized to reflect the ΔpH_o, which is the difference of pH_o between bare gold colloids and peptide coated gold colloids. The coverage ratio (Θ) for all amyloidogenic peptides over gold colloid of different sizes was extracted by assuming Θ = 0 at ΔpH_o = 0. Remarkably, Θ was found to have a nano-gold colloidal size dependence, however, this nano-size dependence was not simply correlated with *d*. The geometric analysis and simulation of reproducing Θ was conducted by assuming a prolate shape of all amyloidogenic peptides. The simulation concluded that a spiking-out orientation of a prolate was required in order to reproduce the extracted Θ.



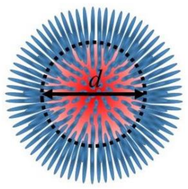


Figure 1. A picture and sketch demonstrating a peptide aligning over the surface of a gold colloidal particle with a diameter, *d*.

Since $A\beta_{1\text{-}40}$ coated gold colloid is dissolved in an aqueous solution, hydrophobic segments of $A\beta_{1\text{-}40}$ (sequences 23-40, C-terminal side) must be used for contacting the gold colloidal surface, causing hydrophilic segments of $A\beta_{1\text{-}40}$ (sequences 1-22, N-terminal side) to face outside, making it soluble in water. Among the hydrophobic sequences (23-40), only ^{28}Lysine (^{28}Lys, ^{28}K) can be positively charged at neutral conditions. Therefore, it is hypothesized that $–N^+–$ part of the ^{28}Lysine is responsible for contacting on the gold colloidal surface as shown Fig. 2

Method

The data from the spectral analysis of the gold and protein complexes confirmed by UV-Vis that the proteins were adhering to the surface of the gold particles. Peptide-colloid aggregation was confirmed by a spectrochemical shift from Blue to Red. The pH of this shift (ΔpH_0) denoted the exact change in conformation and was tested at various gold sizes (nm) and different pH conditions. The non-zero ΔpH_o values indicate that $A\beta_{1\text{-}40}$ is adsorbed on the gold surface and the surface charge potential was altered due to peptide coverage. $A\beta_{1\text{-}40}$ was then added to bare gold (20 nm) and a concentration dependence plot was constructed to find the rate of aggregation during the amyloidogenic process. The experimental procedure was repeated in of DMSO to investigate different conformations in aprotic solvents.

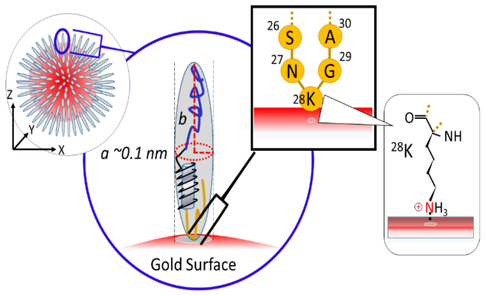


Figure 2: Schematic describing our hypothesis of a peptide orientation at the gold colloidal surface with spiking out orientation of a prolate. The inserts (upper left/ right) indicate a possible contacting sequence to the surface

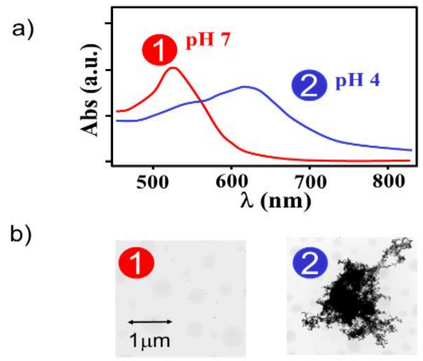


Figure 3: The absorption spectra at pH 7 or pH 10 (1) and that at pH 4 (2). **b)** TEM images correspond to spectrum (1) and (2).

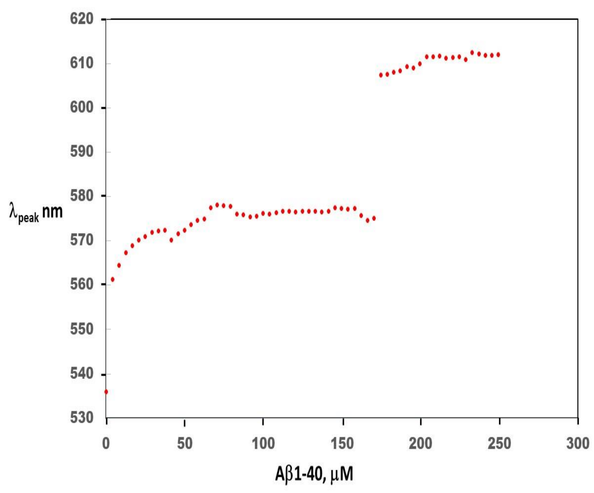


Figure 4: Concentration dependence of peptide coverage on 20 nm gold nanoparticles. Stepwise addition indicates a multiphase aggregation process.

Discussion

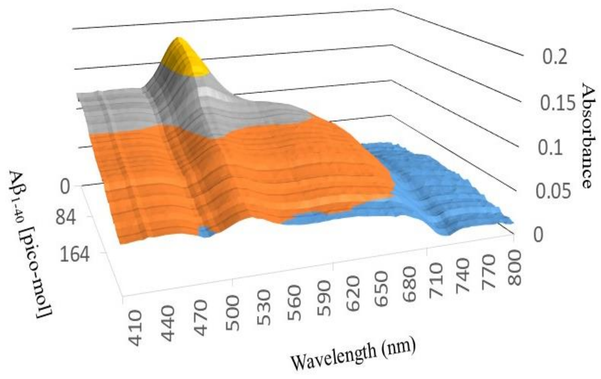


Figure 5: 3D UV-Vis spectrum of adsorption changes of $A\beta_{1\text{-}40}$ on 20 nm gold nanoparticles, indicating formation of amyloidogenic fibrils

UV-Vis spectrophotometry revealed conformation changes in response to different pH conditions along with physical aggregations confirmed by TEM (Figure 4). Analysis revealed a higher tendency of aggregations in lower pH and concentration dependence of added $A\beta_{1\text{-}40}$ on 20 nm Au showed initial curvature before adopting a sigmoidal curve of absorbance, indicating that peptide-colloid monomers aggerate in small clusters which then join into the amyloid plaque structure.

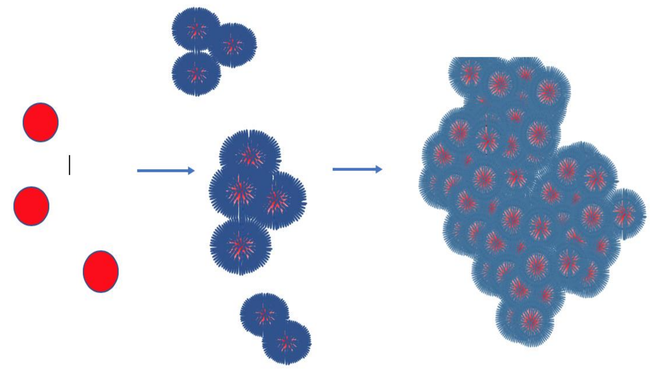


Figure 6: Model of stepwise amyloidogenic aggregation

Acknowledgments

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