Electronic Supplementary Information for

Alignment of Au Nanorods Along de novo Designed Protein Nanofibers Studied with Automated Image Analysis

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Materials and Methods

Reagents

$HAuCl_4.3H_2O$, ≥99.9% trace metals basis, and L-Ascorbic acid, BioXtra, ≥99.0% cyristalline, were purchased from Sigma Aldrich. Hexadecyltrimethylammonium bromide (CTAB) , >98.0%, and 5-Bromosalicylic acid(5-BromoSA), >98.0%, were purchased from TCl America. $AgNO_3$, 99.9%-Ag, and $NaBH_4$, 98%, were purchased from Stream Chemicals.

Synthesis

Synthesis of Au Nanorods

The synthesis of Au nanorods were followed by using literature procedure[1]. The seed solution for Au nanorods was prepared as reported previously. A 5 mL amount of 0.5 mM $HAuCl_4$ was mixed with 5 mL of 0.2 M CTAB solution. A 0.6 mL portion of fresh 0.01 M $NaBH_4$ was diluted to 1 mL with water and was then injected into the Au(III)-CTAB solution under vigorous stirring (1200 rpm). The color of the solution was changed from yellow to brownish-yellow, and the stirring was stopped after 2 min. The seed solution was aged at room temperature for 30 min before use. To prepare the 25-ml of growth solution, 0.9 g of CTAB together with 5-

bromosalicylic acid, 0.11 g were dissolved in 25 mL of warm water (50-70 °C) in a 125 mL Erlenmeyer flask. The solution was allowed to cool to 30 °C, when a 4 mM $AgNO_3$ solution as detailed in Table S1, was added. The mixture was kept undisturbed at 30 °C for 15 min, after which 25 mL of 1 mM HAuCl4 solution and, if necessary, a small amount of HCl (37 wt % in water, 12.1 M) was added. After 15 min of slow stirring (400 rpm),0.2 ml 0.064 M ascorbic acid (Table S1) was added, and the solution was vigorously stirred for 30 s until it became colorless. The growth solution had a CTAB concentration of about 0.05 M and was used right after preparation. Finally, 0.8 mL of seed solution was injected into the growth solution. The resultant mixture was stirred for 30 s and left undisturbed at 30 °C for 12 h for Au nanorod growth. The reaction products were isolated by centrifugation at 8500 rpm for 25 min followed by removal of the supernatant. The precipitates were re-dispersed in 10 mL of water.

Sample name	Seed solution (ml)	4 mM of Ag solution (ml)	12.1 M of Conc. HCl (μl)	Au Seed solution (ml)	CTAB (g)	5-BromoSA (g)	0.064 M of L-Ascorbic acid (ml)
AR=2	0.06	1.2	0	25	0.9	0.11	0.2
AR=2.5	0.04	1.2	0	25	0.9	0.11	0.2
AR=4	0.04	1.2	420	25	0.9	0.11	0.2

Table S1. Initial amounts of each precursor for the synthesis of Au nanorods.

Synthesis of De novo designed protein fibers

The *de novo* designed protein fibers were synthesized were followed by using literature procedure[2].

Assembly of Au nanorods and protein fibers

Silane coated ITO was washed with ethanol then dry with N_2 blower. Protein Fibers (PF) (18 μM) were diluted 5 times with Tris buffer solution (25 mM Tris and 75 mM NaCl, pH 8). 20 mL, 4 μM of Protein Fiber (PF) was drop casted on silane coated ITO and washed with 500 ml of same solution for dilution and then with 500 ml of water gently and dried by hand shaking. A glass side with chamber was filled with Au NRs (check Table S1). 70 mL of Au NRs solution were diluted with 1000 ml of water and waited 30 min. To remove excess CTAB, centrifugated 5 min at 14000 rpm, removed supernatant part and resuspended until 70 mL of total volume.
Silane ITO with PF were put on the top of the chamber (upside-down, see Figure 1) and waited 5 min and then gently washed with water and dried with N_2 blower for further characterization.

Structural and Optical Characterization

The SEM images were obtained using TFS Apreo-S with Lovac Scanning Electron Microscope operating at 2kV and 13 pA. The UV-Vis data were obtained using an Agilent 8453 UV−vis spectroscopic system. The zeta potential measurement was performed using a Malvern Zetasizer with a 633 nm laser.

Method

Data analysis

All images were analyzed using the automated image analysis tool. This Jupyter notebook is publicly available at https://github.com/yamanmy/Automated_image_tool_for_Au_PF_image.

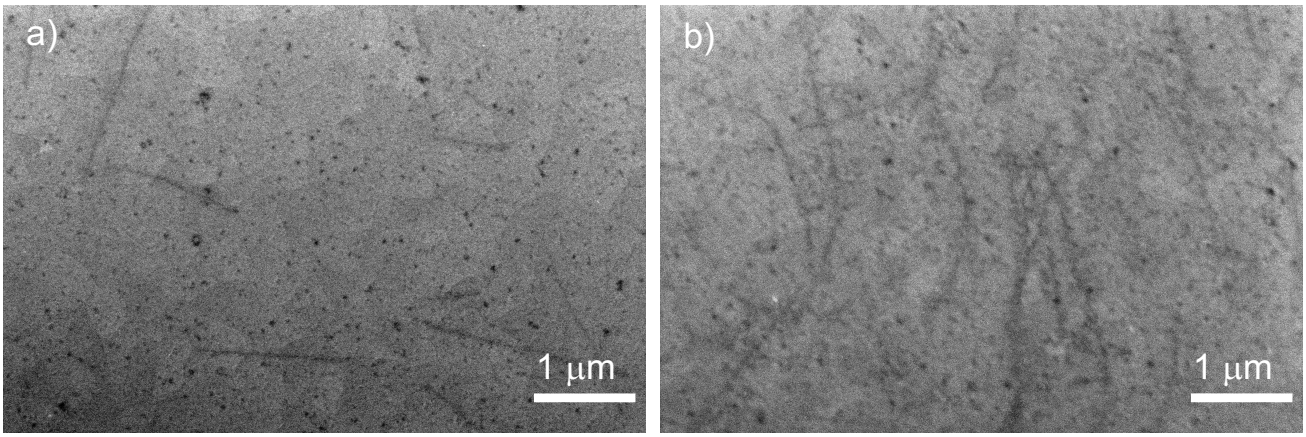

Fig. S1 A SEM image of the protein nanofibers attach to substrates (a) without silane treatment, (b) with silane treatment.

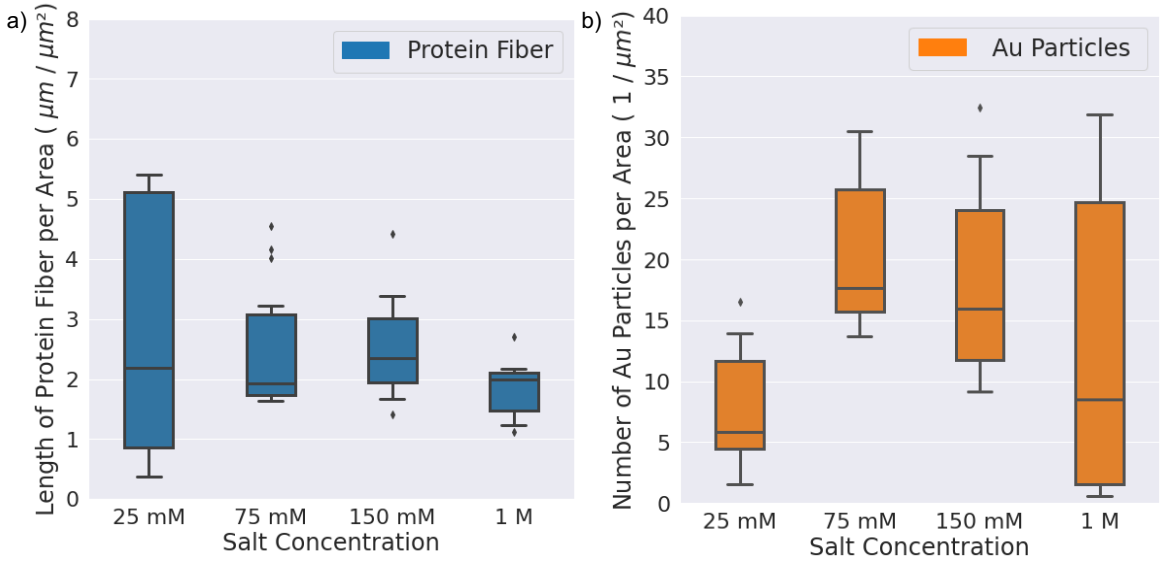

Fig. S2 a) Boxplot of quantity results for the density of protein fibers on substrate at different salt concentration. b) Boxplot of quantity results for the density of Au particles on substrate at different salt concentration.

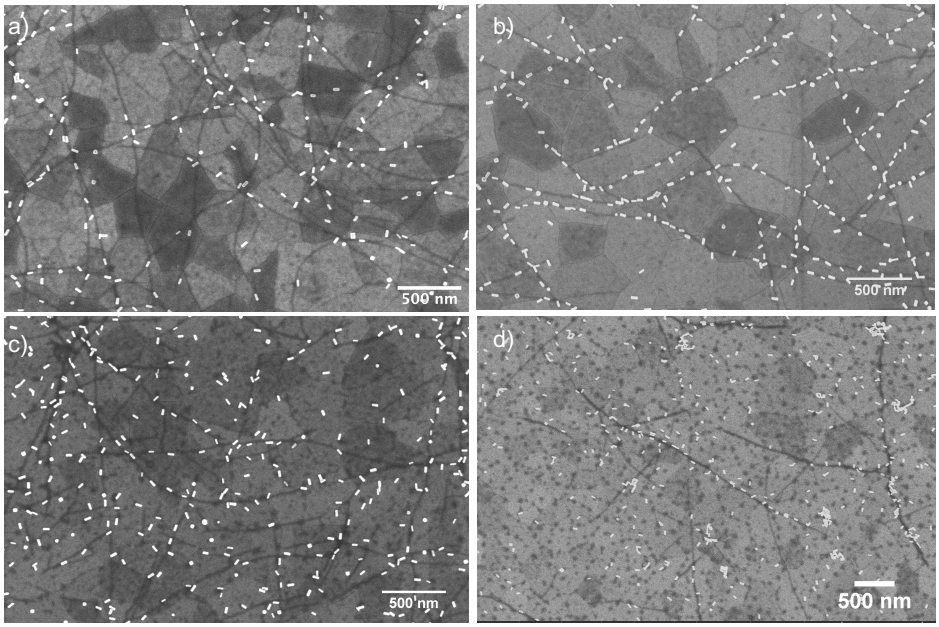

Fig. S3. Raw SEM images at different salt concentration from 25 mM (a), 75mM (b), 150mM (c), to 1M (d).

Sample name	Zeta Potential (mV)	Width of Au Nanorods (nm)	Height of Au Nanorods (nm)	Aspect Ratio of Au Nanorods
AR=2	45 ± 1	18 ± 5	37 ± 8	2.1 ± 0.4
AR=2.5	43 ± 1	19 ± 5	45 ± 9	2.4 ± 0.3
AR=4	43 ± 1	17 ± 7	56 ± 13	3.8 ± 1.2

Table S2. Quantity analysis of different Au nanorod solution.

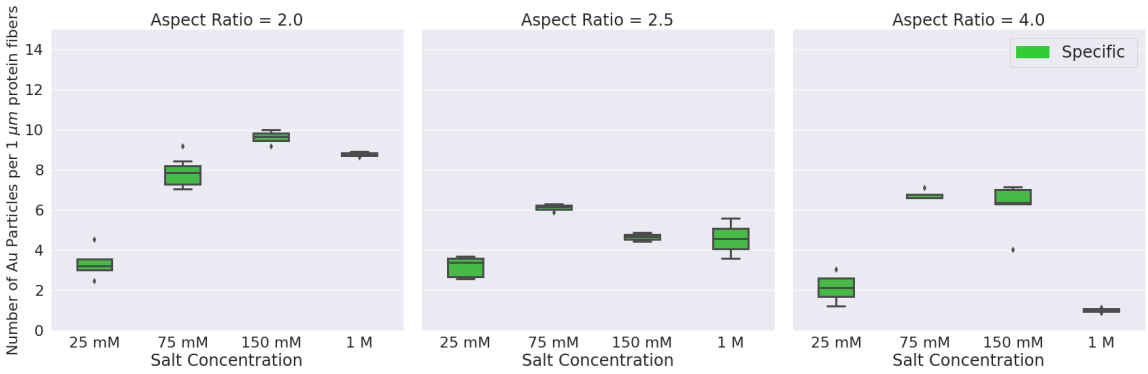

Fig. S4. a) Boxplot of quantity results for Au attachment on protein fibers at different aspect ratio of Au solution. The y-axis shows the number of attached Au particles per 1 μm protein fiber. The green bins show specific Au particle attachment to the fibers.

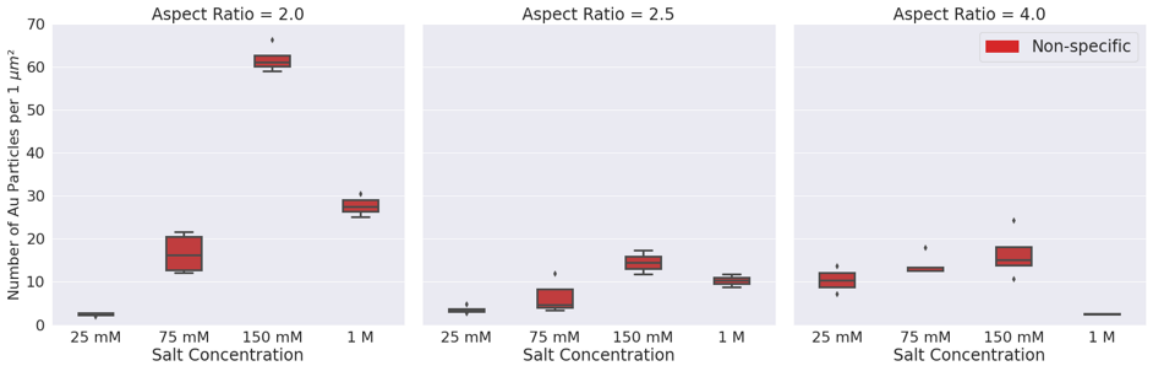

Fig. S5. a) Boxplot of quantity results for above images with automated image tool. The y-axis shows the number of attached Au particles to the substrate. The red bins show non-specific Au particle attachment to the substrate.

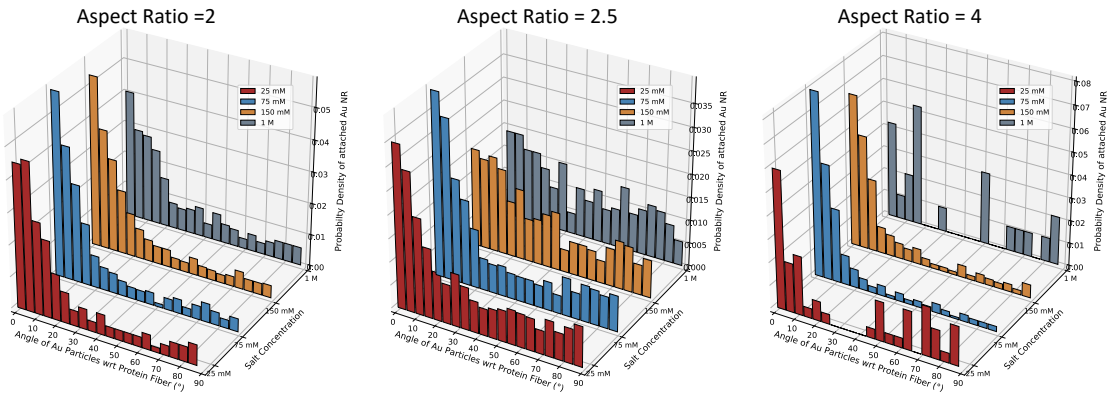

Fig. S6. 3D plot of angle distribution of attached Au nanoparticles on the protein fiber at different Au aspect ratios (increase from 2 to 4). The x-axis shows the angle of Au nanorods with respect to the protein fiber, the y-axis shows the different salt concentration and the z-axis shows the probability density of attached Au NRs.

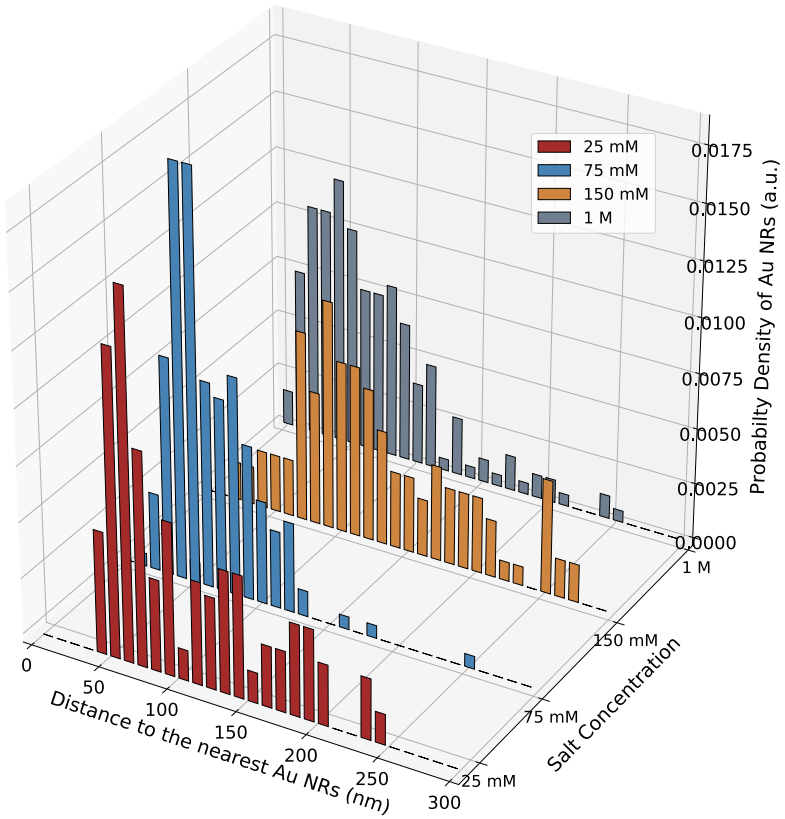

Fig. S7. 3D plot of pair distribution of Au nanorods. The x-axis shows the distance of Au nanorods to the nearest Au NRs, the y-axis shows the different salt concentration and the z-axis shows the probability density of the distance of Au nanorods.

Notes and References

1 X. Ye, L. Jin, H. Caglayan, J. Chen, G. Xing, C. Zheng, V. Doan-Nguyen, Y. Kang, N. Engheta, C. R. Kagan and C. B. Murray, Improved Size-Tunable Synthesis of Monodisperse Gold Nanorods through the Use of Aromatic Additives, *ACS Nano*, 2012, **6**, 2804–2817.

2 H. Shen, J. A. Fallas, E. Lynch, W. Sheffler, B. Parry, N. Jannetty, J. Decarreau, M. Wagenbach, J. J. Vicente, J. Chen, L. Wang, Q. Dowling, G. Oberdorfer, L. Stewart, L. Wordeman, J. de Yoreo, C. Jacobs-Wagner, J. Kollman and D. Baker, De novo design of self-assembling helical protein filaments, *Science*, 2018, **362**, 705.