Supplementary information

Synthetic "Chaperones": Nanoparticle-Mediated Refolding of Thermally Denatured Proteins

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Materials:

All the reagents, α-Chymotrypsin (Type II from bovine pancreas, ChT), Lysozyme (from chicken egg white), Papain (from *Carica papaya*), *N*-succinyl-L-phenylalanine *p*-nitroanilide (SPNA) and Nα-benzoyl-L-arginine *p*-nitroanilide (BAPNA) was purchased from Sigma and used as received.

Synthesis of ligand and nanoparticle:

Synthesis of 2-(10-mercaptodecyl)-malonic acid (**1**) ligand and fabrication of 2 nm gold particle was done according to the reported procedure from our group.[1] In brief, 1,10-dibromodecane was added to the solution of malonic diethyl ester and sodium ethoxide solution in ethanol. After the completion of the reaction, ethanol was evaporated and residues are redissolved in water-dichloromethane mixture. After the separation the crude product was purified using silica gel column chromatography to obtain 2-(10-bromodecyl)-malonic acid diethyl ester (**2**). The compound **2** was refluxed with sodium thioacetate in ethanol for overnight for the thioacetate substitution followed by aqueous NaOH hydrolysis to produce the 2-(10-mercaptodecyl)- malonic acid (**1**) which was purified using silica gel column chromatography.

Pentanethiol coated gold nanoparticles ($d = 2$ nm) were prepared according to the previously reported protocol.[2] Place-exchange reactions[3] were carried out to replace the 1-pentanethiol ligand on the nanoparticle surface with malonic acid functionalized ligand **1** to obtain the **AuDA** nanoparticle.

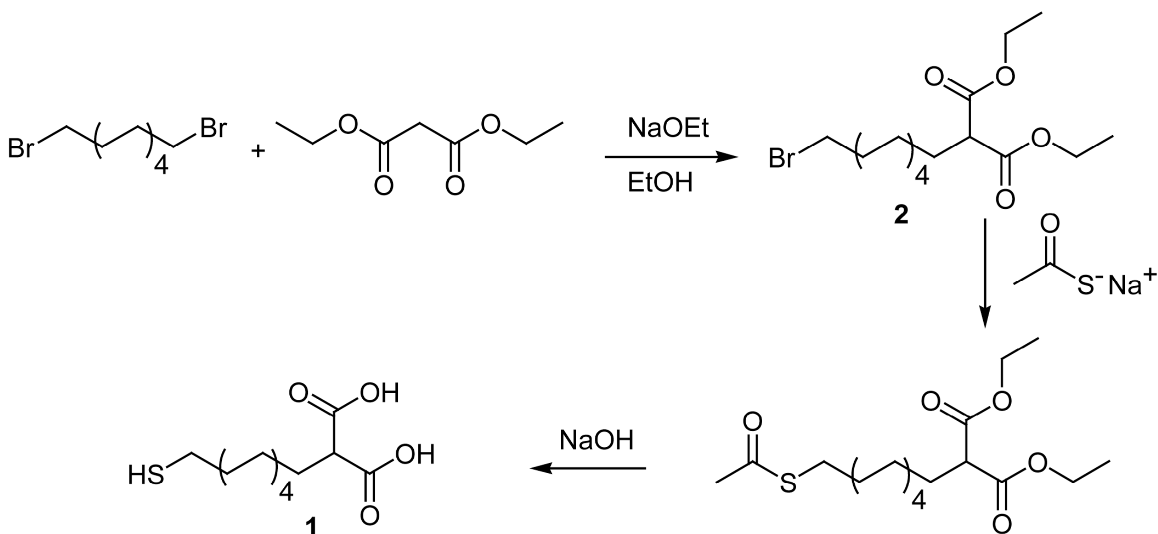

Fig. 1S. Synthesis of malonic acid derivative for AuDA nanoparticle.

Thermal denaturation of proteins:

Proteins were dissolved in aqueous 5 mM sodium phosphate buffer solution (Na_2HPO_4 / NaH_2PO_4), pH 7.4 to make a concentration of 10 μM. The protein solutions were thermally denatured by incubating at 60°C (for lysozyme) to 80°C (for α-chymotrypsin and papain) for 30 min in airtight micro-centrifuge tubes. Following incubation, the proteins were transferred to an icewater bath to quench the denaturation process and the protein solution was used for activity assay and Circular dichroism study.

Activity assay:

Activity assay of α-Chymotrypsin. Thermally denatured α-Chymotrypsin (3.48 μM) was incubated with AuDA (0.87 μM) in 5 mM sodium phosphate buffer (pH 7.4)

for 4h, then at 100 mM NaCl concentrations for 1h. Thereafter, 184 μL of the each solution were taken in a 96-well flat bottom microplate along with native and denatured α-Chymotrypsin followed by the addition of 16 μL of 25.9 mM SPNA dissolved in ethanol, resulting in final concentrations of 3.2 μM for ChT, 0.8 μM for NPs and 2.0 mM for SPNA. Activity was followed by monitoring the absorption changes at 405 nm with an Ultra Microplate Reader (EL808 Bio-Tek Instruments, Inc.). The obtained activity was normalized to that of ChT without NPs. Each sample was measured in triplicate and the average was reported.

Activity assay of Papain. Papain enzyme activity was also assayed with the chromogenic reagent BAPNA. The enzyme was activated by incubating in 2-mercaptoethanol at concentration of 142 mM. In this experiment thermally denatured papain was incubated with AuDA for 4h and then 1h with 100 mM NaCl at same concentration of ChT activity assay. Similarly 182 μL of each solution with 16 μL BAPNA and 2 μL mercaptoethanol was taken in a 96-well flat bottom microplate. The activity of papain on BAPNA was followed spectrophotometrically at 405 nm using same microplate reader. The obtained activity was normalized to that of native papain. Each sample was measured in triplicate and the average was reported.

Circular dichroism:

Far-UV circular dichroism (CD) spectra of ChT (3.2 μM) were measured on a JASCO J-720 spectropolarimeter with quartz cuvettes of 1 mm path length at 25 °C. The spectra were recorded from 190 to 250 nm as an average of 3 scans at a rate of 20 nm/min. The concentration of proteins is fixed at 5 μM and 0.8 μM for AuDA. The final

spectra were obtained by subtracting the blank to eliminate background effects. The resultant CD spectra was fitted into secondary structure algorithm CDSSTR (protein ref. set 7 comprising of 49 proteins) using DICHROWEB to determine the change in secondary structures (Table S1).

Table S1. The percentage of secondary structure elements of native proteins and thermally denatured proteins in the absence and presence of gold nanoparticles as estimated from far-UV CD spectra using CDSSTR method.[4]

Sample	α−Helix	β−Sheets	β−Turn	Random coil
ChT	14.1	23.1	14.6	48.1
DChT	6.5	15.2	14.7	63.7
DChT-AuDA	8.3	26.4	17.6	47.7
Lys	37.0	16.2	19.1	27.3
DLys	6.1	19.2	14.2	60.5
DLys-AuDA	28.0	25.3	17.5	28.2
Pap	12.6	27.6	17.5	42.3
DPap	4.1	18.8	18.6	58.5
DPap-AuDA	9.1	30.1	18.3	42.4

Zeta Potential:

AuDA gold nanoparticles were dissolved in 5 mM sodium phosphate buffer, pH 7.4 to make a 1 μM solution and their zeta potentials were measured on a MALVERN Zetasizer Nano ZS instrument. The similar experiment was repeated with three denatured proteins at 4 μM concentration. Three rounds of assays of each solution have been performed and the average values were reported.

Table S2. Zeta-potentials for AuDA nanoparticle and in presence of three denatured proteins in 5 mM sodium phosphate buffer, pH 7.4 at 25.0 °C

Sample	**zeta-potential / mV**
AuDA	−44.7±1.5
AuDA + α-Chymotrypsin	−42.8±1.4
AuDA + Papain	−30.6±2.5
AuDA + Lysozyme	−22.1±1.5

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