Stabilization of α-Chymotrypsin at Air-Water Interface through Surface Binding to Gold Nanoparticle Scaffolds

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Supporting Information

Experimental Section

General. α-Chymotrypsin or ChT (type II from bovine pancreas) and *N*-benzoyl-tyrosine *p*-nitroanilide (BTNA) were purchased from Sigma and used as received. The synthesis of AuTCOOH was performed following a previous published procedure. The resulting nanoparticles were fully functionalized as determined by NMR. All experiments used sodium phosphate buffer (pH 7.4) and were conducted at 30 ˚C.

Circular Dichroism (CD). Experiments were performed with quartz crystal cuvettes with a 1-mm path length, using a Jasco 720 spectropolarimeter. Both samples of chymotrypsin (3.2 μM) and chymotrypsin with AuTCOOH (0.8 μM), were incubated for 30 minutes in a 8 ml glass vial, 1.5 cm diameter, 5.3 cm in length, (dimensions and volume will have an effect upon stirring and the vortex formed in the vial). Samples were subjected to vigorous stirring using a Fischer Scientific Isotemp Stir Plate, model 109U0920. Samples were stirred at a calibrated 1200 rpm, using a Fisherbrand stir bar, (1/2 x 1/8 inches). At predetermined time point, aliquots were transferred to a quartz crystal cuvette with minimal volume loss upon each measurement. Scans were recorded from 190 to 250 nm at a rate of 20 nm/min, sample interval of 0.2 nm, and an 8 second response.

To calculate the rate of unfolding at 232 nm, the fraction of ChT folded ($\mathbf{f_f}$) versus time stirred was measured. First, ellipticity (θ_{232nm}/mdeg) was converted to the fraction unfolded according to equation 1. We assumed at time point zero (no stirring), ChT was 100% folded ($\boldsymbol{\theta_n}$), while after 90-minute stirring, ChT was 0% folded, ($\boldsymbol{\theta_u}$). **L** is the pathlength, **C** is the protein concentration, and **n** is the total number of amino acids. The fraction that is folded over time stirred was calculated by assuming a unimolecular decay rate (**eq 3**).

$$f_u = \frac{\theta - \theta_n}{\theta_u - \theta_n} \qquad \text{where } \theta = [\theta] \times 10 \times L \times C \times n \qquad \text{(eq. 1)}$$

$$f_u = 1 - f_f \qquad \text{(eq. 2)}$$

F_t = fraction folded (f_f) at a given time stirred

$$F_t = e^{-kt_{(stirred)}} \qquad \text{(eq. 3)}$$

Activity Assay. Enzymatic activity assays were performed using a microplate reader (EL808IU, Bio-Tek Instruments, Winooski, VT). Stock solutions were made for ChT and BTNA, at 10 μM and 1.25 mM respectively**.** Solutions of ChT and AuTCOOH were preincubated for 30 minutes, using a constant final ChT concentration of 0.8 μM. The AuTCOOH concentration was varied at different ratios of ChT to AuTCOOH. The samples were stirred, as previously mentioned, in order to obtain air bubbles in solution. At predetermined time point, aliquots were withdrawn from the vial for activity assays. The enzymatic hydrolysis reaction was initiated by adding a substrate stock solution (16 μl) in EtOH to a preincubated and stirred ChT-AuTCOOH solution (184 μl) to reach a final substrate concentration of [BTNA]=100 μM. The hydrolysis of the substrate was measured at 405 nm. The assays were performed in triplicates and the standard deviation was usually less than 10%. The results were normalized to the solution at time zero and a decay curve was extrapolated against time stirring.

Electrophoresis. Agarose gels were prepared using 5 mM sodium phosphate buffer at a final agarose concentration of 1%. By placing a comb in the center of the gel, appropriately sized wells (40 μl) were formed. A ChT stock solution of 300 μM was used to prepare 30 μl of samples at the appropriate ChT to AuTCOOH ratios. After about 30-minute incubation, 3 μl of 80% glycerol was added to each well in order to ensure proper loading. A constant voltage (100 V) was applied for 30 minutes to achieve significant separation. Gels were stained (0.5% Coomassie blue, 40% methanol, 10% acetic acid aqueous solution) for 1 hour, followed by extensive destaining (40% methanol, 10% acetic acid aqueous solution) until protein bands were visible. Scans of the gels were performed prior to and immediately after staining to visualize AuTCOOH and ChT bands.

Surface Tension Analysis. Experiments were performed using a Fisher AutoTensiomat. Preparing a 1 ml stock solution of ChT (10 μM), AuTCOOH (10 μM), and a mixture of ChT and AuTCOOH (final concentration for both ChT and AuTCOOH was 10 μM), samples were placed into the subsequent teflon well for measurement. Samples were measured using the Du Nuoy Ring Method, (ring model no. 1495, circumference 6.025, R/r 53.9728386), at 0.05 in/min, 23.8 ˚C, and allowed a 5 minute incubation time before measurement. At least five trials were taken for each sample and the average was recorded. The data was then transformed to absolute surface tension, S, by using the following correction factor:

$$F = 0.7250 + \sqrt{\frac{(0.01452\ P)}{(C^2)} + 0.04534 - \frac{1.679\ r}{R}} \qquad \text{(eq. 4)}$$

Where **P** is the observed relative surface tension, **C** is the circumference of the ring, and **r/R** is the inverse of the **R/r** ratio.

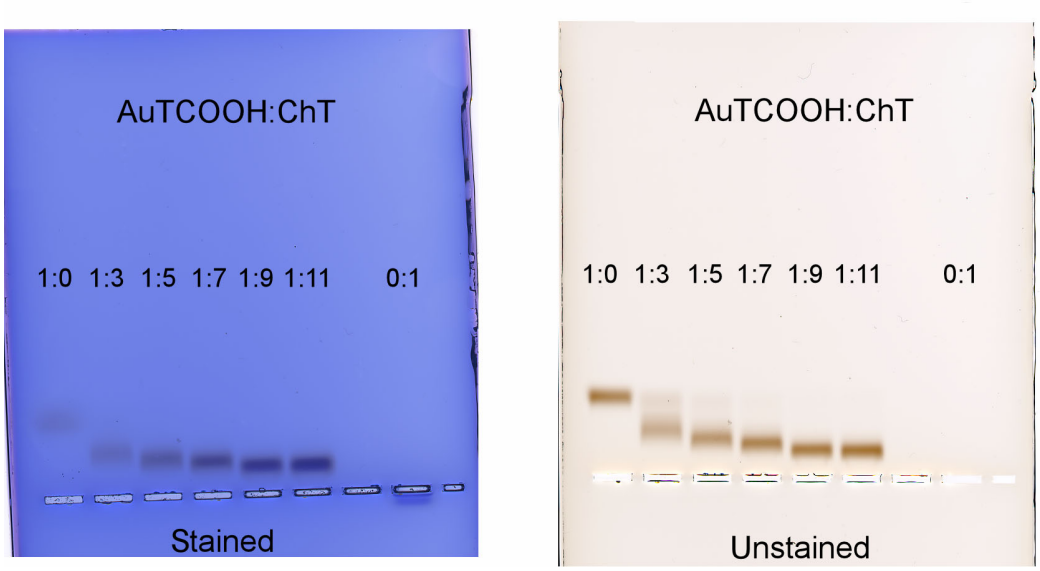

Figure S1. Gel electrophoresis for determining the binding ratio between ChT and AuTCOOH. Gel is stained for protein bands, (on left), and is unstained for AuTCOOH, (on right). Binding ratio appears to be 1:11, one AuTCOOH for every 11 ChT.

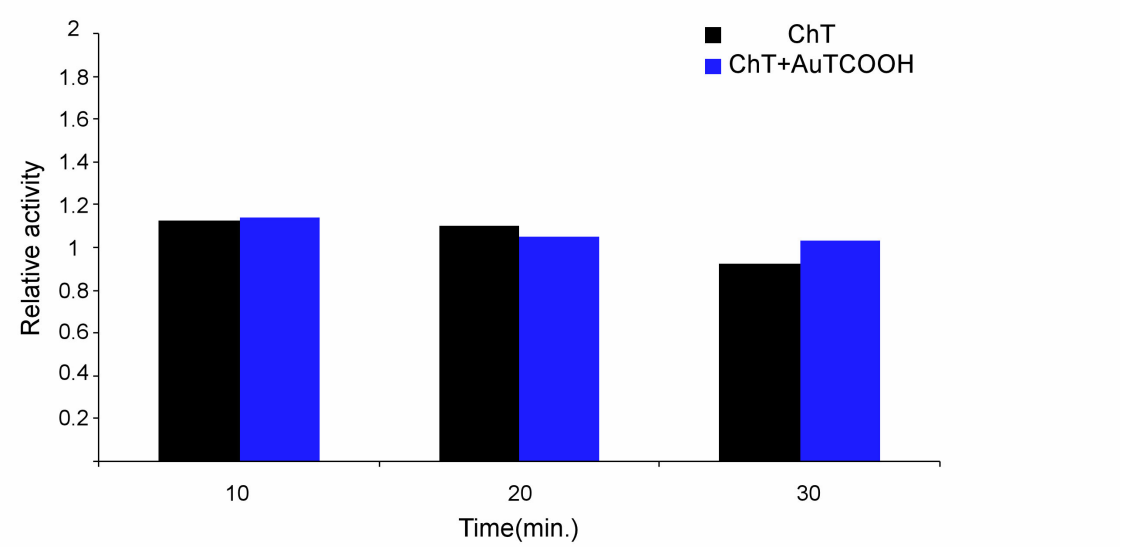

Figure S2. Comparison of relative ChT activity, (with and without addition of AuTCOOH), after being stirred for 30 minutes under degassed conditions, (Thermovac2 from Microcal, 25 ˚C, 28 Hg). ChT activity was measured via the formation of *p*-Nitroaniline, as previously mentioned. Under degassed conditions, mechanical stirring appears to have a negligible effect upon the activity of ChT.

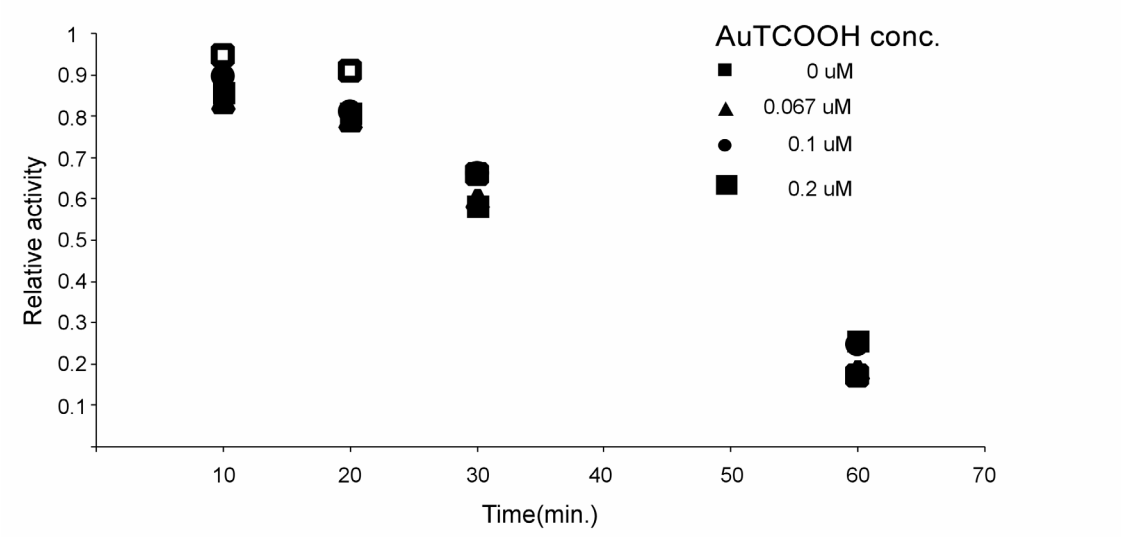

Figure S3 Comparison of relative ChT activity at different AuTCOOH ratios, upon the addition of 200 mM NaCl. The samples were stirred for over 60 minutes, according to the above procedure. The NaCl appears to disrupt the electrostatic binding between ChT and AuTCOOH, which results in loss of ChT stabilization via the AuTCOOH.

RATIO (AUTCOOH TO CHT)	DECAY RATE (K) S^{-1}	HALF-LIFE (MIN)
0 to 1	0.0049	2.4
1 to 11	0.0020	5.8
1 to 4	0.0016	7.2
1 to 1	0.0014	8.3

Table S1 Extrapolated ChT decay rates and half-lifes via curve fitting the exponential ChT decay from activity assays. Decay rates were measured at different ratios of AuTCOOH, relative to ChT. The R^2 values were always greater than 0.9.

	$NaPO_4$ buffer	ChT	AuTCOOH	ChT+AuTCOOH
SURFACE TENSION (dynes/cm)	69.4 ± 1.0	65.7 ± 0.7	56.4 ± 1.3	58.8 ± 1.3

Table S2 Absolute surface tension of ChT, AuTCOOH, and mixture of ChT + AuTCOOH solutions. Surface tension decreases for AuTCOOH which indicates a higher affinity for the air-water interface than the corresponding ChT.