Electronic Supplementary Information

Light-induced inhibition of chymotrypsin using photocleavable monolayers on gold nanoparticles

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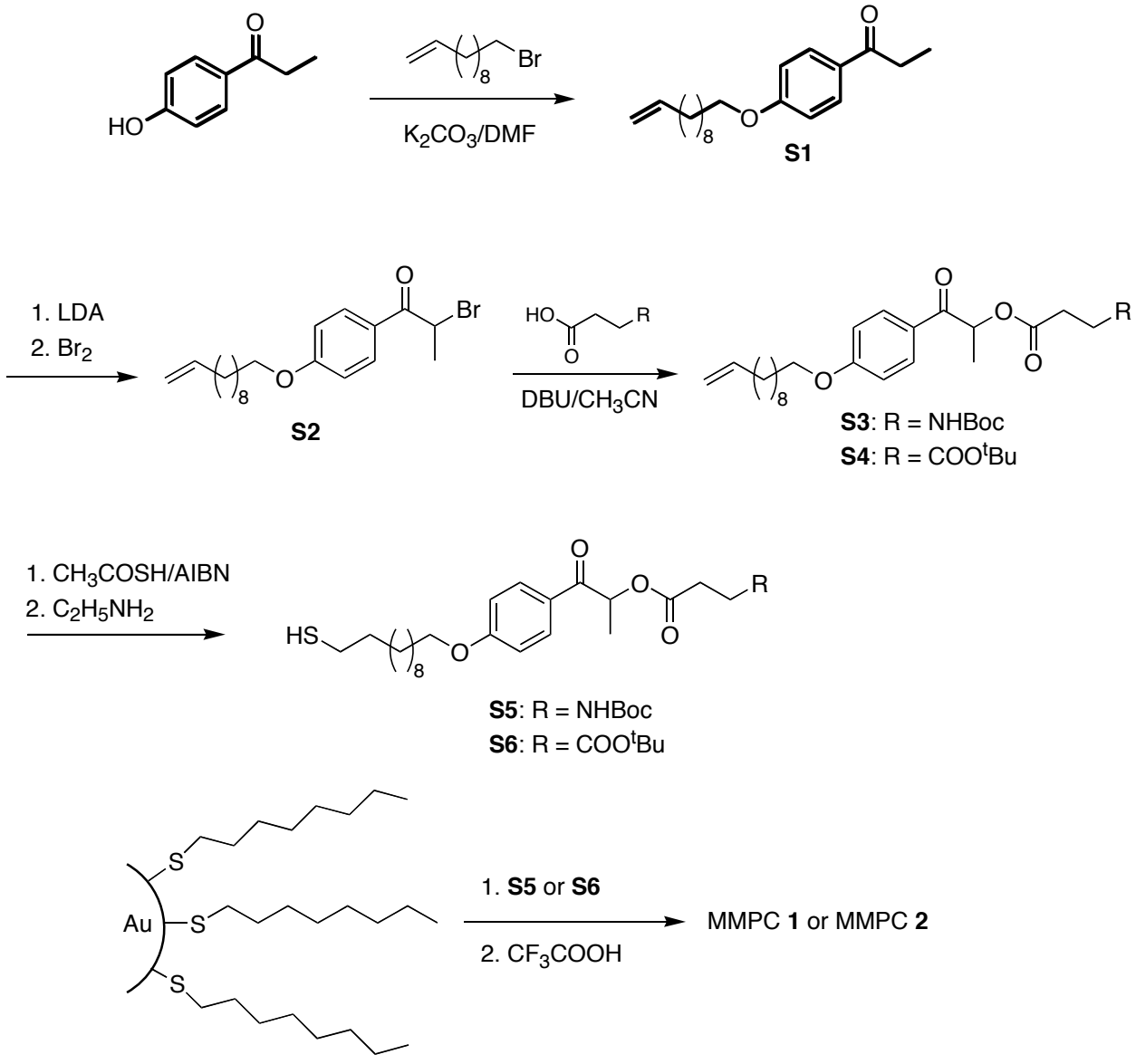
General. α-Chymotrypsin from bovine pancreas (EC 3.4.21.1) and benzoyl tyrosine *p*-nitroanilide (BTNA), were purchased from Sigma. All the other chemicals were obtained from Aldrich. All the solvents were purchased from VWR and were used as received unless specified otherwise.

Synthesis.



Synthesis of MMPCs (LDA: lithium diisopropylamide; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; AIBN: 2,2'-azobisisobutyronitrile).

1-(4-Undec-10-enyloxy-phenyl)-propan-1-one (S1). In a 250 mL round-bottom flask, 4-hydroxy-propiophenone (1 equiv) and K_2CO_3 (3 equiv) were dissolved in DMF, and 11-bromo-1-undecene (1.2 equiv) was then added to the solution. The reaction was stirred overnight under argon at r.t. and then added to a separatory funnel containing Et_2O and H_2O. The aqueous phase was extracted with Et_2O and the combined organic fractions were washed twice with H_2O, once with a saturated aqueous $NaHCO_3$ solution and twice with brine before being dried over $MgSO_4$. Column chromatography on silica gel afforded the product as a colorless liquid (96% yield). ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.94 (d, 2H, J = 9 Hz), 6.91 (d, 2H, J = 9 Hz), 5.82 (m, 1H), 4.96 (m, 2H), 4.01 (t, 2H, J = 6 Hz), 2.95 (q, 2H, J = 7 Hz), 2.04 (q, 2H, J = 7 Hz), 1.80 (qu, 2H, J = 7 Hz), 1.31 (m, 12H), 1.21 (t, 3H, J = 7 Hz). IR (neat): ν 3075, 2926, 2854, 1660, 1601, 1509, 1460, 1419 cm^{-1}.

2-Bromo-1-(4-undec-10-enyloxy-phenyl)-propan-1-one (S2). In a 250 mL round-bottom flask, LDA (1.5 M solution in cyclohexanes, 1.5 equiv) was added dropwise to a stirred solution of aryl-alkyl ether (**S1**) (1 equiv) in dry THF cooled to –78°C under argon. After 30 min of stirring at low temperature, bromine (1 equiv) was added and the reaction was allowed to warm to r.t. and stirred for an additional 2h. The reaction mixture was then slowly poured into aqueous $Na_2S_2O_4$ solution, extracted with EtOAc and washed once with a saturated $NaHCO_3$ solution and twice with brine before being dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography on silica gel yielding (**S2**) as a yellow liquid in 59% yield. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.00 (d, 2H, J = 9 Hz), 6.94 (d, 2H, J = 9 Hz), 5.82 (m, 1H), 5.27 (q, 1H, J = 7 Hz), 4.96 (m, 2H), 4.03 (t, 2H, J = 6 Hz), 2.04 (q, 2H, J = 7 Hz), 1.89 (d, 3H, J = 7 Hz), 1.81 (q, 2H, J = 7 Hz), 1.31 (m, 12H). IR (neat): ν 3075, 2926, 2855, 1679, 1600, 1511, 1467, 1422 cm^{-1}. EI-HRMS (m/z) calcd for $C_{20}H_{29}BrO_2$ (M^+) 380.1351, found 380.1308.

3-*tert*-Butoxycarbonylamino-propionic acid 1-methyl-2-oxo-2-(4-undec-10-enyloxy-phenyl)-ethyl ester (S3). To a solution of (**S2**) (1 equiv) in CH_3CN under argon, 3-tert-butoxycarbonylamino-propionic acid (1.1 equiv) and then DBU (1.1 equiv) were added and the reaction stirred under argon for 3h. The mixture was then added to a separatory funnel containing EtOAc and a saturated aqueous $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc, and the combined organic fractions were washed with saturated aqueous $NaHCO_3$ solution and twice with brine before being dried over $MgSO_4$. Concentration *in vacuo* afforded pure product as a yellow oil in 99% yield. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.92 (d, 2H, J = 9 Hz), 6.94 (d, 2H, J = 9 Hz), 5.97 (q, 1H, J = 7 Hz), 5.82 (m, 1H), 5.29 (bs, 1H), 4.96 (m, 2H), 4.02 (t, 2H, J = 6 Hz), 3.44 (q, 2H, J = 6 Hz), 2.61 (t, 2H, J = 6 Hz), 2.04 (q, 2H, J = 7 Hz), 1.81 (q, 2H, J = 7 Hz), 1.53 (d, 3H, J = 7 Hz), 1.44 (s, 9H), 1.31 (m, 12H). IR (neat): ν 3382, 3075, 2929, 2855, 1714, 1693, 1601, 1511 cm^{-1}.

Succinic acid *tert*-butyl ester 1-methyl-2-oxo-2-(4-undec-10enyloxy-phenyl)-ethyl ester (S4). To a solution of (**S2**) (1 equiv) in CH_3CN under argon succinic acid mono-*tert*-butyl ester (1.1 equiv) and then DBU (1.1 equiv) were added and the reaction stirred under argon for 3h. The mixture was then added to a separatory funnel containing EtOAc and a saturated aqueous $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc, and the combined organic fractions were washed with saturated aqueous $NaHCO_3$ solution and twice with brine before being dried over $MgSO_4$. Concentration *in vacuo* afforded pure product as a yellow oil in 99% yield. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.91 (d, 2H, J = 9 Hz), 6.93 (d, 2H, J = 9 Hz), 5.97 (q, 1H, J = 7 Hz), 5.80 (m, 1H), 4.96 (m, 2H), 4.02 (t, 2H, J = 6 Hz), 2.73 (m, 2H), 2.55 (m, 2H), 2.04 (q, 2H, J = 7 Hz), 1.80 (qu, 2H, J = 7 Hz), 1.52 (d, 3H, J = 7 Hz), 1.44 (s, 9H), 1.31 (m, 12 H). IR (neat): ν 3075, 2927, 2854, 1735, 1686, 1601, 1511 cm^{-1}. EI-HRMS (m/z) calcd for $C_{28}H_{42}O_6$ (M^+) 474.2981, found 474.2914.

3-tert-Butoxycarbonylamino-propionic acid 2-[4-(11-mercapto-undecyloxy)-phenyl]-1-methyl-2-oxo-ethyl ester (S5). In a 100 mL round-bottom flask, (**S3**) (1 equiv) was dissolved in toluene, and AIBN (0.25 equiv) and thiolacetic acid (3 equiv) were added. The orange solution was then refluxed for 3h, and after cooling added to a separatory funnel containing EtOAc and a saturated aqueous $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc, and the combined organic fractions were washed with saturated aqueous $NaHCO_3$ solution and twice with brine before being dried over $MgSO_4$. The crude product was purified by chromatography on silica gel providing thioacetate intermediate as a yellow, viscous oil in 72% yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.92 (d, 2H, J = 9 Hz), 4.94 (d, 2H, J = 9 Hz), 5.97 (q, 1H, J = 7 Hz), 5.29 (bs, 1H), 4.02 (t, 2H, J = 6 Hz), 3.44 (q, 2H, J = 6 Hz), 2.86 (t, 2H, J = 7 Hz), 2.61 (t, 2H, J = 6 Hz), 2.32 (s, 3H), 1.81 (qu, 2H, J = 7 Hz), 1.53 (d, 3H, J = 7 Hz), 1.44 (s, 9H), 1.29 (bm, 16 H). IR (neat): ν 3374, 2928, 2854, 1690, 1600, 1511 cm^{-1}. EI-HRMS (m/z) calcd for $C_{30}H_{47}NO_7S$ (M^+) 565.3073, found 565.3090.In a 25 mL round-bottom flask, thioacetate intermediate (150 mg, 0.27 mmol) was dissolved in a mixture of 3 mL THF and 1.5 mL MeOH, and argon was bubbled through the solution for 60 min. To this was added 5 mL of a 2M solution of $EtNH_2$ in THF which had been purged with argon for 60 min. The reaction was stirred overnight under argon and quenched by addition of excess saturated aqueous NH_4Cl solution. The resulting slurry was extracted with EtOAc, and the organic layer was washed twice with brine and dried over $MgSO_4$. Column chromatography on silica gel afforded the product as a colorless liquid (68 % yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.92 (d, 2H, J = 9 Hz), 6.94 (d, 2H, J = 9 Hz), 5.97 (q, 1H, J = 7 Hz), 5.30 (bs, 1H), 4.02 (t, 2H, J = 6 Hz), 3.44 (q, 2H, J = 6 Hz), 2.61 (t, 2H, J = 7 Hz), 2.53 (q, 2H, J = 7 Hz), 1.81 (qu, 2H, J = 7 Hz), 1.53 (d, 3H, J = 7 Hz), 1.44 (s, 9H), 1.30 (bm, 17 H). IR (neat): ν 3382, 2926, 2853, 2572, 1711, 1686, 1600, 1510 cm^{-1}. EI-HRMS (m/z) calcd for $C_{28}H_{45}NO_6S$ (M^+) 523.2968, found 523.2944.

Succinic acid 2-[4-(11-mercapto-undecyloxy)-phenyl]-1-methyl-2-oxo-ethyl ester *tert*-butyl ester (S6). The same procedure as for the preparation of **S5** was used. Starting from **S4**, title compound was obtained as a colorless liquid (55 % yield over 2 steps). ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.91 (d, 2H, J = 9 Hz), 6.93 (d, 2H, J = 9 Hz), 5.97 (q, 1H, J = 7 Hz), 4.02 (t, 2H, J = 7 Hz), 2.69 (m, 2H), 2.58 (m, 2H), 2.53 (q, 2H, J = 7 Hz), 1.81 (qu, 2H, J = 7 Hz), 1.52 (d, 3H, J = 7 Hz), 1.44 (s, 9H), 1.30 (bm, 17 H). IR (neat): ν 2928, 2855, 2575, 1731, 1693, 1601, 1512 cm^{-1}. EI-HRMS (m/z) calcd for $C_{28}H_{44}O_6S$ (M^+) 508.2859, found 508.3004.

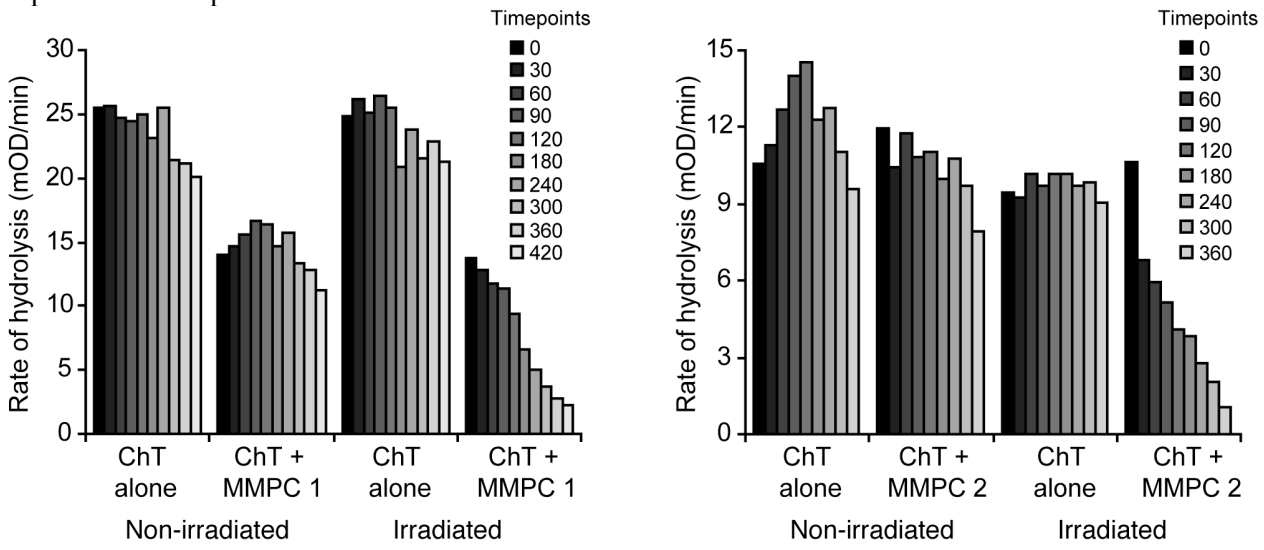
MMPC 1. To a solution of 20 mg of octanethiol-functionalized Au nanoparticles (2 nm) in 5 mL CH_2Cl_2, 55 mg (0.105 mmol) of thiol ligand (**S5**) were added. The reaction mixture was stirred 48h under argon, then 3 mL TFA was added causing the complete precipitation of the colloids within 10 min. The reaction was stirred an additional 90 min, then all volatile compounds were removed *in vacuo* and the colloid was resuspended in CH_2Cl_2 and stirred for another 30 min. All reactions were carried out at room temperature. The nanoparticles were then filtered using a colloid filter and washed extensively with CH_2Cl_2 before being dried. The resultant colloids were sparingly soluble in D_2O and could be readily dissolved in alcohols and DMSO. Concentrated EtOH stocks were stable for weeks at 4°C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ (ppm) 7.87 (bs), 6.90 (bs), 5.95 (bs), 3.89 (bs), 2.97 (bs), 2.74 (bs), 1.22 (bm), 0.78 (bs).

MMPC 2. To a solution of 20 mg of octanethiol-functionalized Au nanoparticles in 5 mL CH_2Cl_2, 60 mg (0.118 mmol) of thiol ligand (**S6**) were added. The reaction mixture was stirred 48h under argon, then 3 mL TFA was added causing slight precipitation of the colloids. The reaction was stirred an additional 90 min, then all volatile compounds were removed *in vacuo* and the colloid, now insoluble in CH_2Cl_2, was suspended in CH_2Cl_2 and stirred for another 30 min. All reactions were carried out at room temperature. The nanoparticles were then filtered using a colloid filter and washed extensively with CH_2Cl_2 before being dried. Colloids were only soluble in alcohols and DMSO, but concentrated EtOH solutions could be readily diluted with H_2O (final EtOH concentration: 2%) without causing aggregation of the nanoparticles. Concentrated EtOH

stocks were stable for weeks at 4°C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ (ppm) 12.21 (bs), 7.87 (bm), 7.58 (bm), 6.88 (bs), 5.90 (bs), 3.88 (bm), 1.28 (bm).

UV Irradiation. Samples were irradiated in quartz cuvettes using a Rayonet photochemical reactor (Southern N.E. Ultraviolet Co., Middletown, CT). A glass cooling mantle connected to a cooling bath was used to maintain sample temperature at 23°C and filter all wavelengths below 300 nm. Irradiation spectra of ChT-MMPC samples were recorded on a UV-spectrophotometer (HP 8452A). Identical spectra were observed with MMPCs alone. Irradiation had no effect on ChT spectra.

Activity Assay. ChT (1.6 μM) was incubated with MMPCs (0.4 μM) in either MilliQ H_2O (MMPC **1**) or 5 mM sodium phosphate buffer (pH 7.4. MMPC **2**) at 23°C. At indicated time points, 10 μL of 2 mM benzoyl tyrosine *p*-nitroanilide (BTNA) stock was added to 140 μL of ChT-MMPC sample, resulting in a final BTNA concentration of 133 μM. Activity was followed by monitoring *p*-nitroaniline formation every 20 sec for 5 min at 405 nm with a microplate reader (EL808IU, Bio-Tek Instruments, Winooski, VT). Samples of ChT alone (v_o, +/- irradiation) were used to normalize ChT-MMPC data (v_i). Reported results are averages of three separate experiments in duplicate.



Raw activity data of ChT upon irradiation in the presence or absence of MMPCs.