Supporting Information to

Carboxylate-Imidazole Cooperativity in Dipeptide-functionalized Gold Nanoparticles with Esterase-like Activity

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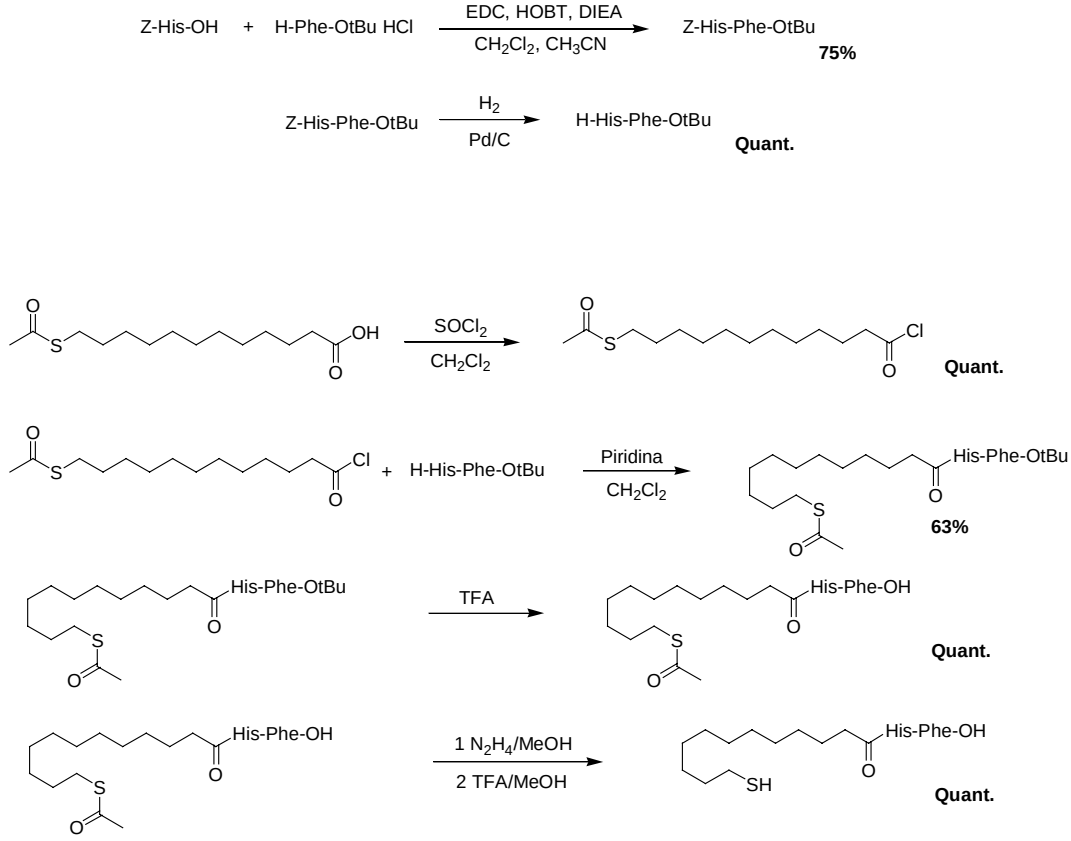
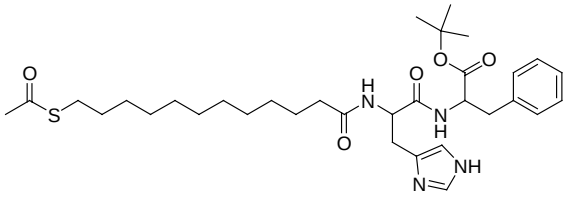
Synthesis of the dipeptide-functionalized thiol 2.

Synthetic Scheme.



Synthetic procedure.



To a round bottom 100 ml flask have been introduced 580 mg of H-His-Phe-OtBU (1.62 mmol), dissolved in 10 ml of a CH_3CN/CH_2Cl_2 1/1 v/v mixture (anhydrous); to this solution has been added DIEA (300 µl, 1.72 mmol). Subsequently *S*-acetil-12-mercaptododecanoyl chloride (570 mg, 1.94 mmol) in dichloromethane (10 ml) has been added. The pH of the reaction mixture was kept above 10 by occasional addition of DIEA. After 3 days the solvent has been evaporated and the residue taken up with methanol (10 ml). The solution was rotaryevaporated again, the residue taken up with AcOEt (50 ml) and washed with $KHSO_4$ 10% (2 x 10 ml); $NaHCO_3$ 5% (2 x 10 ml) and water (2 x

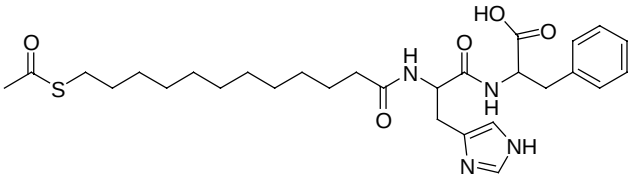
10 ml). The organic layer gave an oily material that was eluted down a column (SiO_2 eluent EP/AcOEt 10/1 v/v). The product (625 mg) was obtained in 63 % yield.

^{1}H-NMR (250 MHz, $CDCl_3$) δ: 7.65 (d, 2H, J = 7.3, NH); 7.44 (ba, 1H); 7.26-7.02 (m, 6H, C_6H_5 and NH); 6.72 (ba, 1H); 4.70-4.50 (m, 2H, $2C^{\alpha}H$); 3.2-2.9 (m, 6H, $3CH_2$); 2.82 (t, J = 7.3, 2H); 2.32 (s, 3H, CH_3); 2.15 (t, J = 7.9, 2H); 1.8-1.2 (m, 27H, CH_2 and tBu).

^{13}C-NMR (62.90 MHz, $CDCl_3$) δ: 196.15, 173.62, 171.13, 170.24, 136.0, 135.0, 129.4, 128.32, 126.9, 82.0, 54.0, 52.5, 37.7, 36.5, 30.6, 29.43, 29.37, 29.27, 29.18, 29.10, 29.15, 28.75, 27.85, 25.5.

IR (KBr) ν: 3276, 2914, 2844, 1734, 1687, 1646, 1535, 1447, 1366, 1249, 1226, 1150, 952, 841.

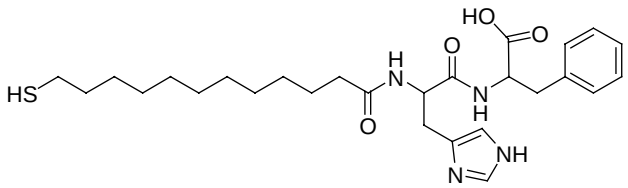
ESI-MS (1mg/ml, MeOH) m/z: 615.2 (100, $M+H^+$).



To a round bottom 50 ml flask have been introduced 100 mg (0.162 mmol) of the above material dissolved in 3 ml of dichlorometaeo; to this solution 1.5 ml of TFA have been added. The reaction mixture was kept under stirring at r.t. for 3 hours, the solvent was then evaporated under reduced pressare to give 110 mg of product as a yellowish oil in quantitative yield.

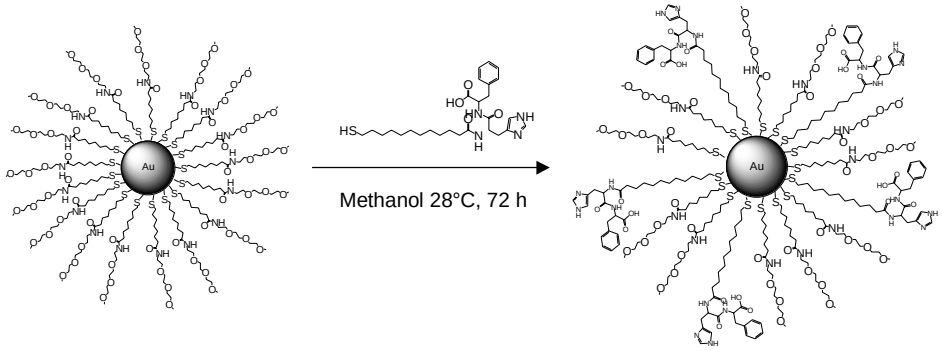
^{1}H-NMR (250 MHz, $CDCl_3$) δ: 7.65 (d, 2H, J = 7.3, NH); 7.44 (ba, 1H); 7.26-7.02 (m, 6H, C_6H_5 and NH); 6.72 (ba, 1H); 4.70-4.50 (m, 2H, $2C^{\alpha}H$); 3.2-2.9 (m, 6H, $3CH_2$); 2.82 (t, J = 7.3, 2H); 2.32 (s, 3H, CH_3); 2.15 (t, J = 7.9, 2H); 1.8-1.2 (m, 18H, CH_2).

IR (KBr) ν: 3295, 2919, 2847, 1744, 1692, 1655, 1641, 1621, 1541, 1260, 1211, 1185, 1080, 823, 698, 624.



Thiol **2** was prepared immediately before the exchange reaction with the nanoparticles. In a polyethylene vial have been introduced 10 mg of the protected thiol dissolved in 0.5 ml of deoxygenated methanol. To this solution have been added 25 μl of a 2M hydrazine solution in oxygen free methanol. The mixture was kept for 2 hr with occasional stirring and the pH was then adjusted to 4-5 by adding a few drops of a 2M TFA solution in deoxygenated methanol. The reaction is quantitative. The resulting solution has been used directing in the site exchange reaction. ESI-MS (1 mg/ml, MeOH) m/z: 517.4 (100, $M+H^+$).

Site exchange protocol.



In a jacketed reactor kept at 28 °C containing 15 mg of Au-MPC of thiol **1** in 15 ml of oxygen free methanol have been introduced 4 mg of freshly deprotected (see above) thiol **2**. The reaction mixture was kept under argon for 72 hr under stirring. After this time the solvent was partly evaporated (1/5 of the original volume) and the solution passed down a Sephadex LH-60 column eluting with methanol. 17 mg of nanoparticles **3** have been obtained. They are highly soluble in methanol even after prolonged storage at the solid state.

^{1}H-NMR (250 MHz, CD_3OD) δ: 8.53 (ba); 8.05 (ba); 7.28 (ba); 4.75 (ba); 4.58 (ba); 3.66-3.10 (ba); 2.26 (ba); 2.03-1.20 (ba).

IR (KBr) ν: 3448, 3287, 2920, 2844, 1732, 1638, 1555, 1462, 1410, 1241, 1109, 956, 724.

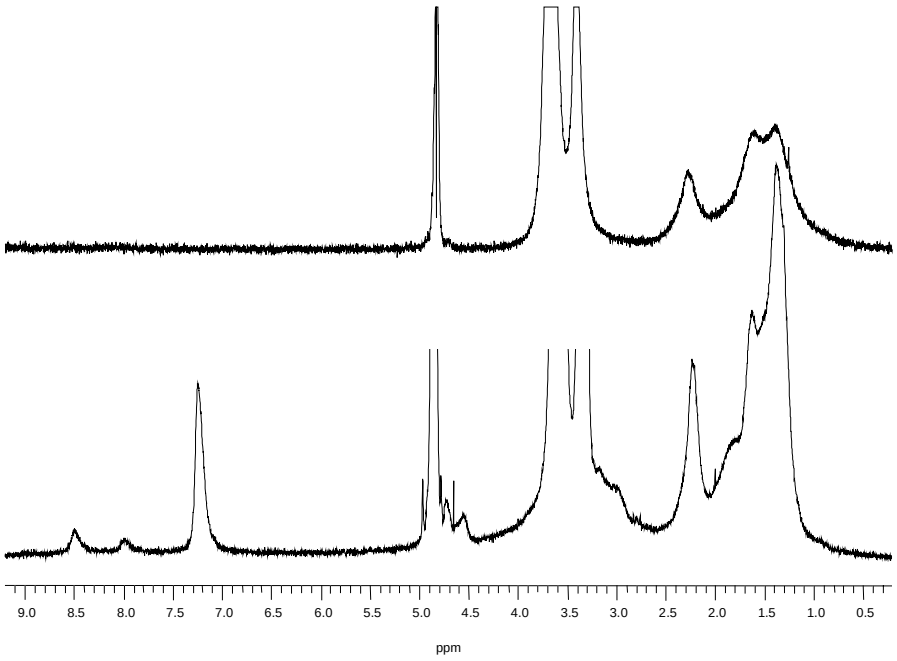


Figure S1. ^{1}H-NMR (250 MHz) of Au-MPC functionalized with thiol **1** (top) and of **3** (bottom) at 25°C in methanol d_4.

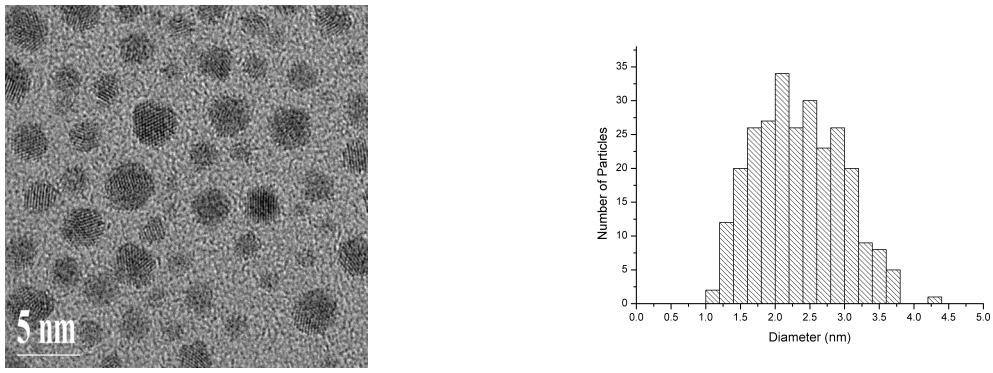


Figure S2. TEM image of gold nanoparticles **3** and histogram of size distribution; average diameter 2.22 nm, sigma = 0.6 nm and number of measured nanoparticles N = 233.

Kinetic experiments.

Kinetic of hydrolysis have been followed at 400 nm and 25 ± 0.5 °C by adding the appropriate amount of substrate in acetonitrile to the buffer solution containing the catalysts. Buffer used (10 mM) are: CAPS (pH = 10.00); CHES (pH = 9.00); EPPS (pH = 8.00); HEPES (pH = 7.00 and 6.50); MES (pH = 6.00 and 5.5); acetic acid/sodium acetate (pH = 4.00 and 4.8); citric acid/sodium citrate (pH = 3.00).

Inhibition experiments.

Inhibition experiments in the presence of iodoacetamide

Typical procedure: Nanoparticles **3** were incubated with iodoacetamide at pH 7, 8 and 9 with a 200 fold molar excess of iodoacetamide for 3 to 12 hours, final concentration of His-Phe units: 9.25×10^{-4} M, final concentration of iodoacetamide 0.18 M, buffers 20-30 mM. Under these conditions double alkylation of histidine cannot be ruled out. Afterwards aliquots of these solutions were buffered at pH 4 and 5.5 and used to run kinetics.

Table S1. Kinetic data for the hydrolysis of DNPB after reaction with iodoacetamide.

pH	Incubation pH	Incubation time	Inhibited reactivity[a)]	Not inhibited reactivity[b)]	% of retained reactivity[c)]
4.0	7.0	12 hours	1.46 M^{-1} s^{-1}	1.61 M^{-1} s^{-1}	90%
4.0	8.0	3 hours	1.0 M^{-1} s^{-1}	1.61 M^{-1} s^{-1}	62%
4.0	9.0	3 hours	0.98 M^{-1} s^{-1}	1.61 M^{-1} s^{-1}	61%
5.5	7.0	12 hours	2.96 M^{-1} s^{-1}	3.09 M^{-1} s^{-1}	96%
5.5	8.0	3 hours	2.5 M^{-1} s^{-1}	3.09 M^{-1} s^{-1}	81%
5.5	9.0	3 hours	1.85 M^{-1} s^{-1}	3.09 M^{-1} s^{-1}	60%

a) Inhibited activity is given as the apparent second order rate constant measured after reaction with iodoacetamide.
b) Second order rate constant for the hydrolysis of DNPB in not inhibited conditions.
c) Ratio between the rate constant measured after inhibition and that measured in not inhibited experiments.

Table S2. Kinetic data for the hydrolysis of DNPB at pH 4.0 and 5.5 after reaction with iodoacetamide at pH 9 for 12 hours.

pH	Incubation pH	Incubation time	Inhibited reactivity[a)]	Not inhibited reactivity[b)]	% of retained reactivity[c)]
4.0	9.0	12 hours	Precipitation[§]	1.61 M^{-1} s^{-1}	-----
5.5	9.0	12 hours	1.57 M^{-1} s^{-1}	3.09 M^{-1} s^{-1}	51 %

a) Inhibited activity is given as the apparent second order rate constant measured after reaction with iodoacetamide.
b) Second order rate constant for the hydrolysis of DNPB in not inhibited conditions.
c) Ratio between the rate constant measured after inhibition and that measured in not inhibited experiments
[§] The functionalised nanoparticles become sparingly soluble at pH 4 after treatment with iodoacetamide

Inhibition experiments in presence of diethylpyrocarbonate

Nanoparticles **3** were incubated with diethylpyrocarbonate at pH 8 with a 17-fold molar excess of diethylpyrocarbonate for 30 minutes. Afterwards, a stoichiometric amount of imidazole was added to quench the excess of C. Final concentration of His-Phe units: 7.58×10^{-4} M, diethylpyrocarbonate 0.013 M, Imidazole, 0.013 M. Afterwards, aliquots of this solution were buffered at pH 4 and 5.5 and used to run kinetics.

Table S3. Kinetic data for the hydrolysis of DNPB at pH 4.0 and 5.5 after reaction with diethylpyrocarbonate.

pH	Incubation pH	Inhibited Reactivity[a)]	Not inhibited reactivity[b)]	% of retained reactivity[c)]
4.0	8.0	1.1 M^{-1} s^{-1}	1.61 M^{-1} s^{-1}	68%
5.5	8.0	2.2 M^{-1} s^{-1}	3.09 M^{-1} s^{-1}	71%

a) Inhibited activity is given as the apparent second order rate constant measured after reaction with diethylpyrocarbonate.
b) Second order rate constant for the hydrolysis of DNPB in not inhibited conditions.
c) Ratio between the rate constant measured after inhibition and that measured in not inhibited experiments