Supporting information for

Structural Confirmation and Quantification of Individual Ligands from the Surface of Multi-Functionalized Gold Nanoparticles

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Experimental Section

Chemicals were purchased from Acros Organics (Geel, Belgium) and were used without further purification.

Synthesis of N-(benzo[*d*][1,3]dioxol-5-ylmethyl)-5-(1,2-dithiolan-3-yl)pentanamide (1)

1-hydroxypyrrolidine-2, 5-dione (NHS, 172 mg, 1.50 mmol) was added to a solution of thioctic acid (206 mg, 1.00 mmol) and N, N'-methanediylidenedicyclohexanamine (DCC, 309 mg, 1.50 mmol) in THF (10 mL). The mixture was stirred at 25 °C for 2 hr. The precipitate was filtered and benzo[*d*][1,3]dioxol-5-ylmethanamine (227 mg, 1.50 mmol) was added to the filtrate. This mixture was stirred at room temperature overnight. After removal of the solvent under reduced pressure, the crude product was extracted between water and ethyl acetate. The organic layer was concentrated under reduced pressure and purified by chromatography to give N-(benzo[*d*][1,3]dioxol-5-ylmethyl)-5-(1,2-dithiolan-3-yl) pentanemide (**1**, SI-Scheme 1) ^{1}H NMR (400 MHz, DMSO) δ 8.23 (t, J = 5.8, 1H), 6.83 (d, J = 7.9, 1 H), 6.79 (d, J = 1.5, 1 H), 6.71 (dd, J = 1.5, 7.9, 1 H), 5.97 (s, 2 H), 4.15 (d, J = 5.9, 2 H), 3.66 – 3.54 (m, 1 H), 3.23 – 3.06 (m, 2 H), 2.40 (td, J = 6.4, 12.5, 1H), 2.11 (t, J = 7.3, 2H), 1.85 (dq, J = 6.8, 13.6, 1 H), 1.67 (m, 2 H), 1.54 (m, 2 H), 1.41 – 1.28 (m, 2 H).

Synthesis of N-(3,4-dimethoxyphenethyl)-5-(1,2-dithiolan-3-yl)pentanamide (2)

N-(3,4-dimethoxyphenethyl)-5-(1,2-dithiolan-3-yl) pentanamide (**2**) was prepared from thioctic acid and 2-(3,4-dimethoxyphenyl)- ethanamine according to the procedure described above.. ^{1}H NMR (400 MHz, DMSO) δ 7.81 (s, 1H), 6.85 (d, *J* = 8.2, 1H), 6.78 (s, 1H), 6.69 (d, *J* = 8.1, 1H), 3.74 (s, 3H), 3.71 (s, 3H), 3.64 – 3.52 (m, 1H), 3.28 – 3.05 (m, 4H), 2.62 (t, *J* = 7.2, 2H), 2.45 – 2.33 (m, 1H), 2.04 (t, *J* = 7.2, 2H), 1.93 – 1.79 (m, 1H), 1.63 (dd, *J* = 13.6, 7.4, 1H), 1.57 – 1.40 (m, 3H), 1.31 (dd, *J* = 15.4, 7.6, 2H). ^{13}C NMR (101 MHz, DMSO) δ 171.69, 148.48, 147.09, 131.89, 120.33, 112.42, 111.76, 56.04, 55.42, 55.29, 31.60, 34.05, 33.26, 28.11, 24.99.

Synthesis of Methyl 2-(5-(1,2-dithiolan-3-yl)pentanamido)-2-(4-hydroxy-

phenyl)acetate (3)

Methyl 2-(5-(1,2-dithiolan-3-yl)pentanamido)-2- (4-hydroxy-phenyl)acetate (**3**) was prepared from thioctic acid and methyl 2-amino-2-(4-hydroxyphenyl)acetate according to the procedure described above. ^{1}H NMR (400 MHz, DMSO) δ 9.53 (s, 1H), 8.50 (d, *J* = 6.8, 1H), 7.11 (d, *J* = 8.5, 2H), 6.74 (d, *J* = 8.5, 2H), 5.24 (d, *J* = 8.0, 1H), 3.67 – 3.53 (m, 4H), 3.25 – 3.05 (m, 2H), 2.40 (td, *J* = 12.4, 6.2, 1H), 2.17 (t, *J* = 7.3, 2H), 1.86 (td, *J* = 12.6, 6.3, 1H), 1.77 – 1.57 (m, 1H), 1.57 – 1.42 (m, 3H), 1.33 (m, 2H).

Synthesis of five double-functionalized GNPs: GNP-3 to GNP-7

The double functionalized GNPs (**GNP-3** to **GNP-7**) were synthesized using different ratio of ligand **1** and ligand **2**. The amount of each reagent used for experiment was listed in table 1. In a typical experiment, 1.5 mL of water containing chloroauric acid (25.0 mg, 0.063 mmol) was added to a solution of ligand **1** (10.7 mg, 0.032 mmol) and **2** (11.8 mg, 0.032 mmol) in MeOH (12.0 mL). After stirring for 30 min at room temperature, $NaBH_4$ (9.5 mg, 0.252 mmol) in 12 mL water was added to the mixture drop wise. The solution turned red immediately and the solution was stirred for 4 hrs at room temperature. 1M HCl was added to the reaction mixture drop wise to neutralize the excess $NaBH_4$. To remove the free ligands and solvent from the GNPs, the reaction mixture was centrifuged at 13000 rpm for 60 min. The colorless supernatant was decanted and the solid was dissolved in 20 mL methanol or deionized water alternatively followed by sonication and centrifugation. This wash-centrifugation cycle was repeated five times. After the final washing step, the double-functionalized GNPs were dried in vacuum at 50 ˚C for 12 hrs.

Synthesis of double functional GNPs 9-13

The double functional **GNPs 9-13** were synthesized using different amount of liand **1** and **3** (Scheme S1). The amount of each reagent used for experiment was listed in SI-Table 2. In a typical experiment, 1.5 mL of water containing chloroauric acid (25.0 mg, 0.063 mmol) was added to a solution of ligand **1** (10.7 mg, 0.032 mmol) and **3** (11.8 mg, 0.032 mmol) in MeOH (12.0 mL). After stirring for 30 min at room temperature, $NaBH_4$ (10 mg, 0.252 mmol) in 12.0 mL water was added to the mixture dropwise. The solution turned red immediately and was stirred for 4 hrs at room

temperature. 1M HCl was added to the reaction mixture dropwise to neutralize the excess sodium tetrahydroborate. To remove the free ligand and solvents from the GNPs, the reaction mixture was centrifuged at 13000 rpm for 30 min. The colorless supernatant was decanted and the solid was dissolved in 20 mL methanol or deionized water alternatively by sonication and centrifugation. This wash-centrifugation cycle was repeated 5 times. After the final washing step, the GNPs were dried in vacuum at 50 ˚C for 12 hrs.

Synthesis of triple-functionalized GNP-8

Ligand **1** (10.7 mg, 0.032 mmol), **2** (11.8 mg, 0.032 mmol), **3** (11.8 mg, 0.032 mmol) were dissolved in MeOH (12 ml). To this solution was added 1.5 mL of water containing chloroauric acid (25.0 mg, 0.063 mmol). After stirring at room temperature for 30 min, 12 mL water containing $NaBH_4$ (9.5 mg, 0.252 mmol) was added to the mixture drop wise. The solution turned red immediately and the solution was stirred for further 4 h at room temperature. 1M HCl was added to the reaction mixture drop wise to neutralize the excess sodium tetrahydroborate. To remove the free ligands from the GNPs, the reaction mixture was centrifuged at 13000 rpm for 30 min and washed as described above. After the final washing step, the triple-functionalized GNPs were dried in vacuum at 50 ˚C for 12 hrs.

Cleavage of ligands from GNPs with I_2

GNPs (2.0 mg) were dispersed in 100 μL MeOH and sonicated to make a uniform suspension. A solution of I_2 (100 μL, 13 mg/mL) was added and the mixture was shaken for 30 min at room temperature. The naked GNPs were removed by centrifugation at 13000 rpm for 30 min. A 20 μL of the supernatant was injected into HPLC/MS/UV/CLND system for analysis.

FTIR Spectroscopy

Approximately 1 mg of GNPs and 200 mg KBr were ground in a agate mortar with a pestle. A KBr pellet was pressed by a Carver 4350 Standard Press at 4 tons of pressure. IR spectra were acquired at 25^{o}C on a Nicolet 700 FTIR spectrometer (Thermo-Fisher Scientific, INC., Waltham, MA) with 32 scans and a resolution of 4 cm^{-1}. The background was a plain KBr pellet made from pure KBr powder.

HPLC/MS/UV/CLND

HPLC/MS/UV/CLND was performed on a Waters system equipped with a Waters 2795 separation module, a Waters 2996 PDA detector, a Micromass ZQ detector and a Antek 8060-C nitrogen detector. A C_{18} column (2.0 μM, 2.0 × 150 mm) was used for the separation. The eluent was a mixture of methanol/tetrahydrofuran and water containing 0.05% trifluoroacetic acid with a linear gradient from 45% to 55% (methanol-tetrahydrofuran)/H_2O (v/v) over 15 min at a flow rate of 1.0 mL/min. UV detection was at 214 nm. Mass spectra were recorded in positive ion mode using electrospray ionization. CLND was calibrated with 2,6-dichloropurine in the concentration range from 0.5 to 18 mM (Figure S3). The injection volume was 20 μL for both standard and samples.

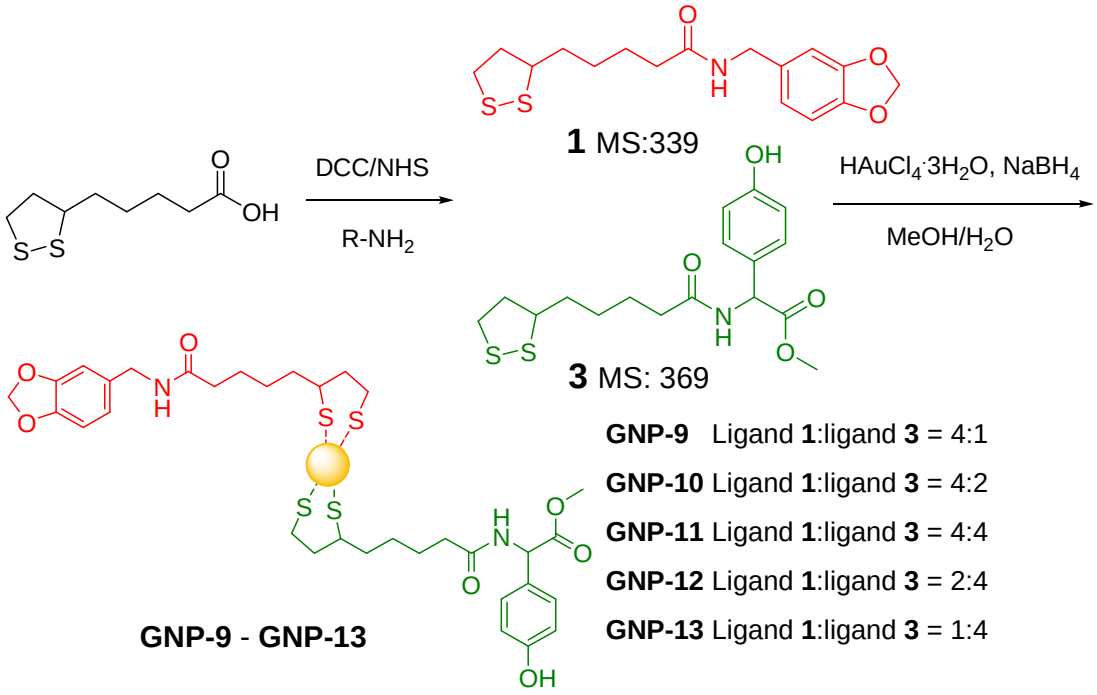
FTIR and HPLC/MS/UV/CLND data of GNPs 9-13

IR spectra were recorded for **GNP-1** and **GNP-Ligand 3** respectively and used as control for the exploration of the double functional GNPs. Both of the ligands had strong C=O stretching vibration at 1627 cm^{-1} to 1637 cm^{-1}. Specifically, **GNP-1** had signals for C-O stretching vibrations at 1042 cm^{-1} and 924 cm^{-1} while **GNP-Ligand 3** had signals for C=O stretching vibration at 1733 cm^{-1} (Figure S1a).

The ratios for ligand **1** and **3** in reactions **9-13** were 4:1, 4:2, 4:4, 2:4, and 1:4. FTIR spectra were recorded for double-functionalized GNPs using KBr method. Double-functionalized GNPs exhibited their characteristic peaks for ligand **1** at 1042 cm^{-1} and 924 cm^{-1} and ligand **3** at 1733 cm^{-1}. When the IR spectra of double functionalized GNPs were normalized by amide C=O bond at 1637 cm^{-1}, peak intensities correlated with their relative amount of each compound. The peak intensities of ether C-O bond at 1042 cm^{-1} and 924 cm^{-1} decreased from **GNP-9** to **GNP-13** as the percentage of ligand **1** on GNP surface decreased. On the contrary, the intensity of ester C=O bond at 1733 cm^{-1} increased by this order because the percentage of ligand **3** increased (Figure S1b).

When the ligands were cleaved from GNPs **9-13** by I_2 and subsequently checked by HPLC/MS/UV/CLND, the identity of each ligand was confirmed by the mass spectra (Figure S2, a and b). Evidence shown clearly the amount of ligand was

changed with the usage of the reactants (Figure S2, c and d). The result of CLND analysis was listed in SI-Table 2. The loading of ligand **1** on GNPs was from 0.05 mmol/g to 0.08 mmol/g from **GNP-3** to **GNP-7**, while the loading of ligand **3** was from 0.01 mmol/g to 0.14 mmol/g. It was noticeable that the highest loading of ligand **1** did not exist when highest amount of ligand **1** was used for synthesis, properly due to the minor difference for reaction conditions, such as the reaction temperature and the adding rate of $NaBH_4$. However, we still could conclude that competed with ligand **3**, ligand **1** had higher affinity with Au in all cases (for example, when the ratio of ligand **1** and **3** was 4:1 for synthesis, the loading of ligand **1** and **3** on GNP surface was 6.0:1.0 instead).



Scheme S1. Synthesis route for the double functionalized GNPs **9-13**

Table S1. Elemental analysis of naked **GNP-3** and **GNP-5**

Naked GNPs	C	H	N
Naked **GNP-3**	<0.5%	<0.5%	<0.5%
Naked **GNP-5**	<0.5%	<0.5%	<0.5%

Table S2. Amount of ligand **1** and **3** on GNPs determined by CLND and FTIR

GNPs	Amount of **1** for reactions (mmol)	Amount of **3** for reactions (mmol)	Ratio of **1**and **3** for reactions	CLND data			Ratio of **1** and **3** on GNPs by FTIR
				Amount of **1** on GNPs (mmol/g)	Amount of **3** on GNPs (mmol/g)	Ratio of **1** and **3** on GNPs	
GNP-9	0.032	0.008	4:1	0.06±0.01[a]	0.01±0.01	6.0:1.0	6.0:1.0[b,c]
GNP-10	0.032	0.016	4:2	0.07±0.01	0.04±0.01	3.5:2.0	6.4:2.0
GNP-11	0.032	0.032	4:4	0.05±0.01	0.03±0.01	6.8:4.0	6.7:4.0
GNP-12	0.016	0.032	2:4	0.08±0.02	0.12±0.02	2.0:3.0	2.0:2.3
GNP-13	0.008	0.032	1:4	0.05±0.01	0.12±0.02	1.0:2.4	1.0:2.5

[a]The standard deviations were based on three parallel experiments. [b]The ratio was based on peak at 924 cm^{-1} from ligand **1** and at 1733 cm^{-1} from ligand **3**. [c]The ratio was calculated by the area of characteristic peaks, and GNP-3 was defined the same ratio as CLND and used as control.

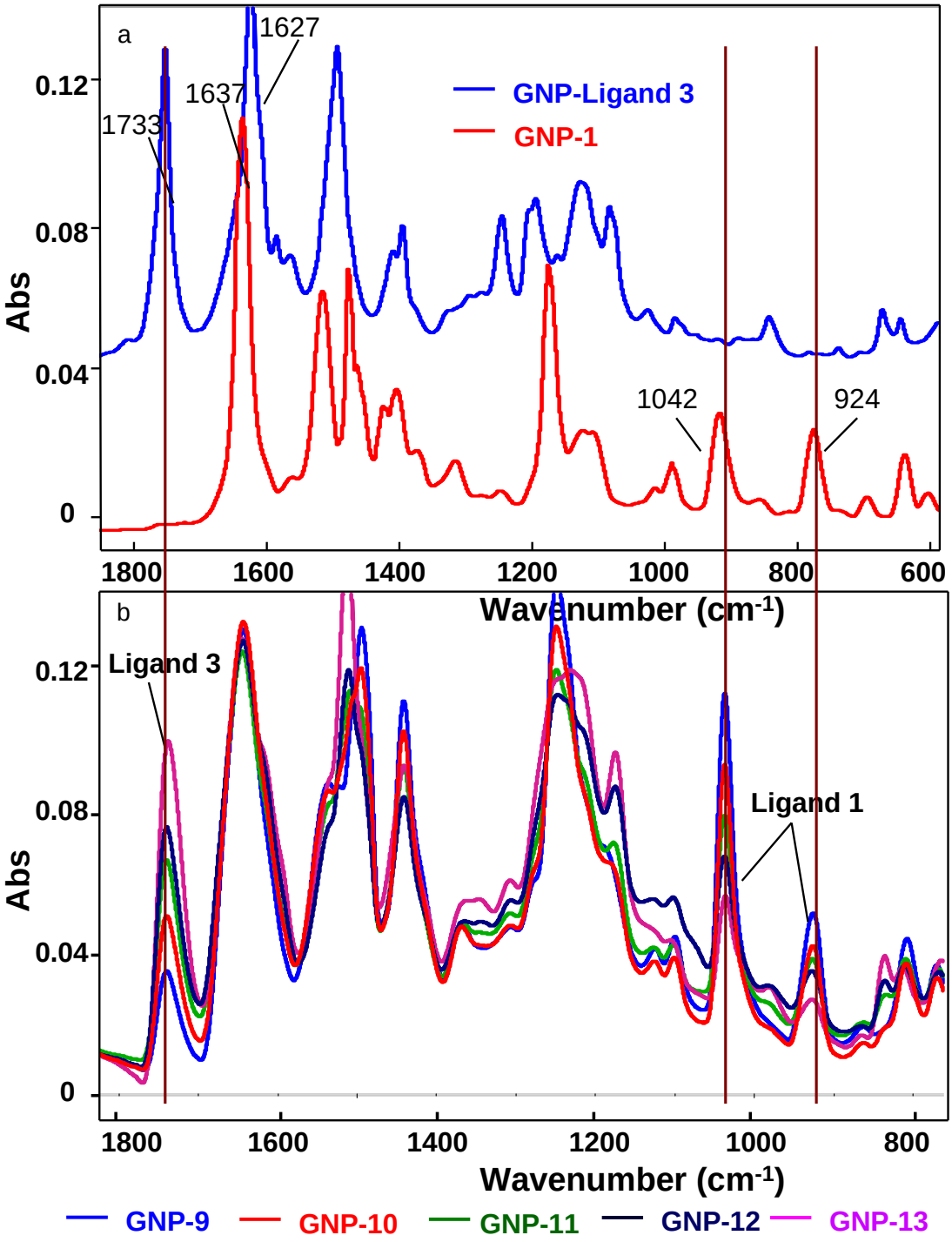


Figure S1. FTIR spectra of **GNP-1**, **GNP-Ligand 3** and double-functionalized GNPs **9-13**

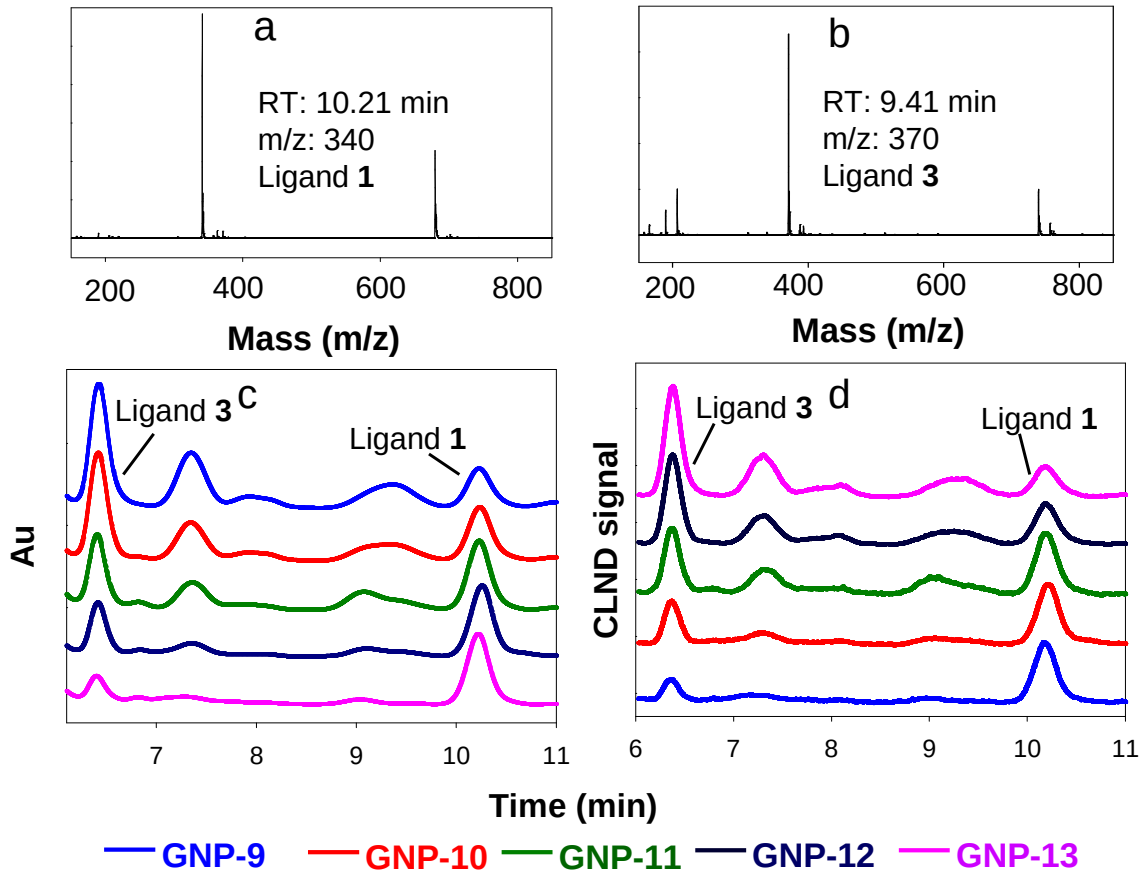


Figure S2. Structure identification and quantification of ligands cleaved from double-functionalized GNPs. Mass spectra and UV and CLND traces of ligands cleaved from **GNPs 9-13**.

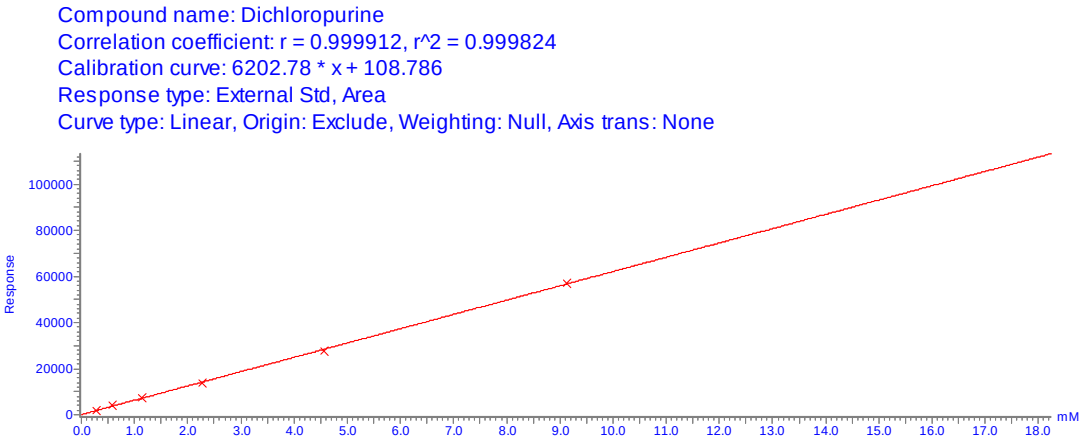


Figure S3. The CLND calibration curve. The standard curve was made using 2,6-dichloro-purine with concentration range from 0.5 to 18 mM nitrogen concentration and the curve had a correlation coefficient of 0.999912.