

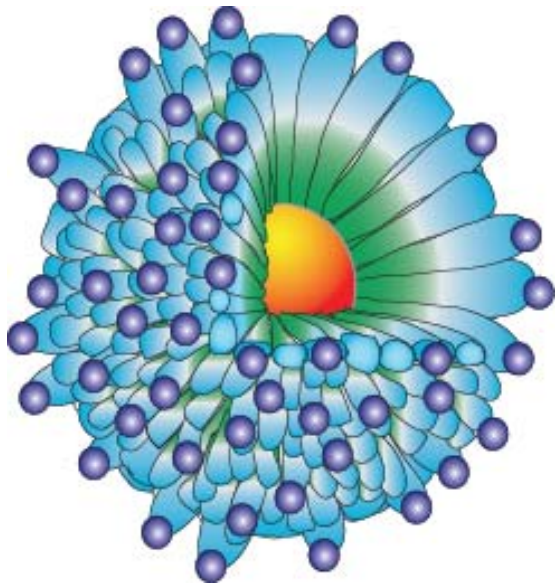
Mass Spectrometric Analyses of Functionalized Gold Nanoparticles and Applications

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Functionalized nanoparticles: applications in biology

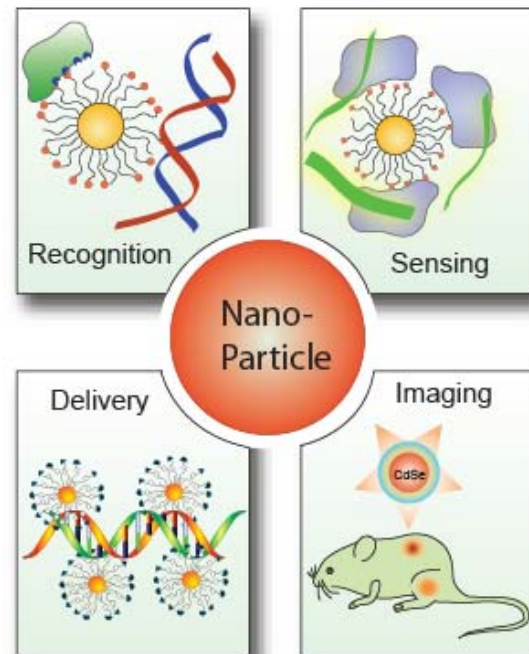


➤ Functionalized nanoparticles

- **Core materials:** Au, Pt, Fe₃O₄, CdSe, SiO₂...
- **Protection monolayer:** thiol, disulfide, carboxylate ...
- **Recognition groups:** interaction with biomolecules...

➤ Applications in biology

- Drug delivery and therapy
- *In vivo* and *in vitro* diagnostics
- Sensing and imaging



Functionalized gold nanoparticles (AuNPs)

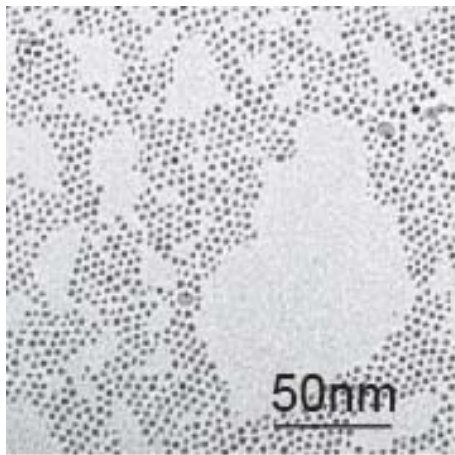
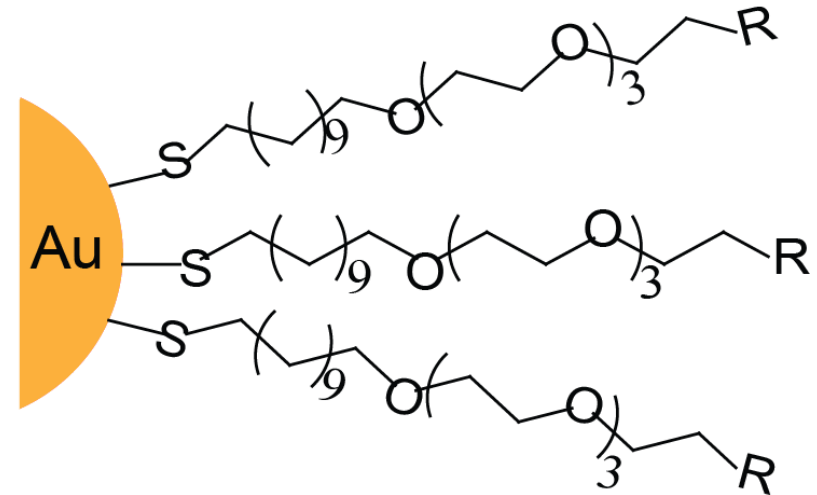
➤ Monolayer of AuNPs

- A hydrophobic part to stabilize core
- An OEG layer for biocompatibility
- End groups for **surface properties**

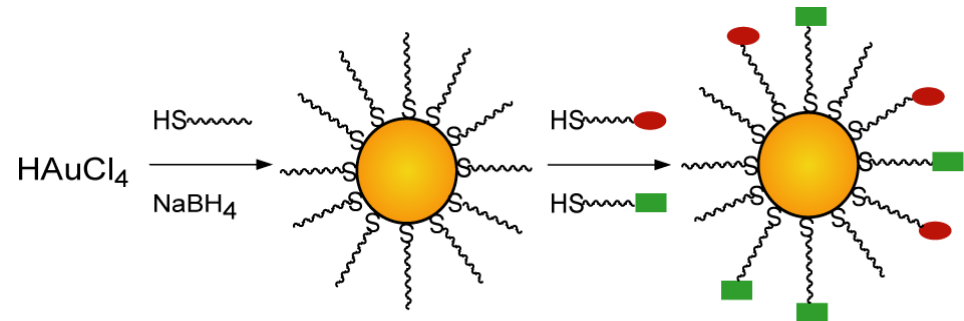
➤ Synthesize through ligand-exchange reaction

➤ Characterization methods:

- TEM, DLS, NMR, TGA ...



Size: 2.2 ± 0.3 nm



Brust, M. *et al. Chem. Commun.* **1994**, 801-802.

Murray, R. W. *et al. Langmuir* **1998**, 14, 17-30.

Mass spectrometry (MS)



M → **M^{z+}**

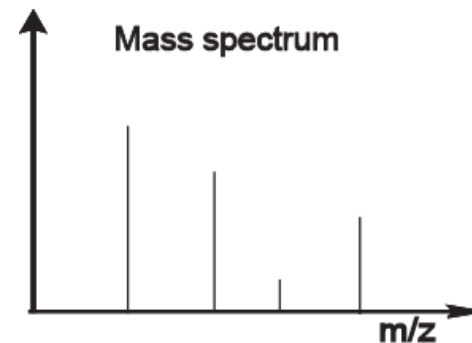
M^{•+}, M^{•-}, [M+H]⁺
[M-H]⁻ ...

MALDI, ESI,
FAB, EI, CI...

**Mass-to-charge ratio
(m/z)**

Magnetic sector
Time of flight
Ion trap ...

Ion abundance



- Mass spectrometry **ionize** chemical compounds to generate charged molecules or molecule fragments and measure their **mass-to-charge ratios (m/z)**.
- Sensitivity, specificity and universality
- Mass spectrometry has both **qualitative** and **quantitative** uses.

Mass spectrometric analyses of AuNPs



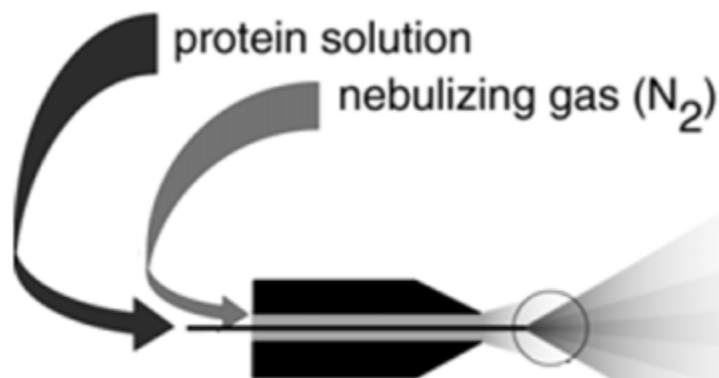
Electrospray
Ionization (ESI)

Matrix-assisted Laser
Desorption/Ionization
(MALDI)

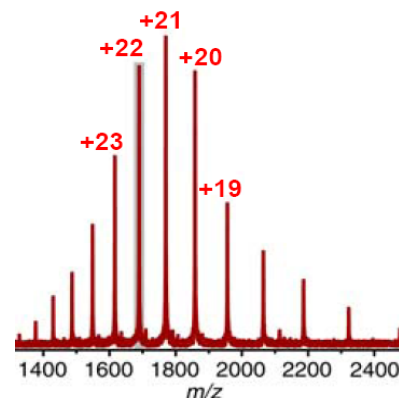
Laser Desorption/Ionization
(LDI)

- Three different ionization methods are used to ionize AuNPs
 - **ESI, MALDI, LDI**
- Generally, MS gives out:
 - **mass of AuNP**
 - **formula of AuNP**
 - **surface monolayers**

AuNP analyses with ESI-MS



ESI-MS spectrum of protein

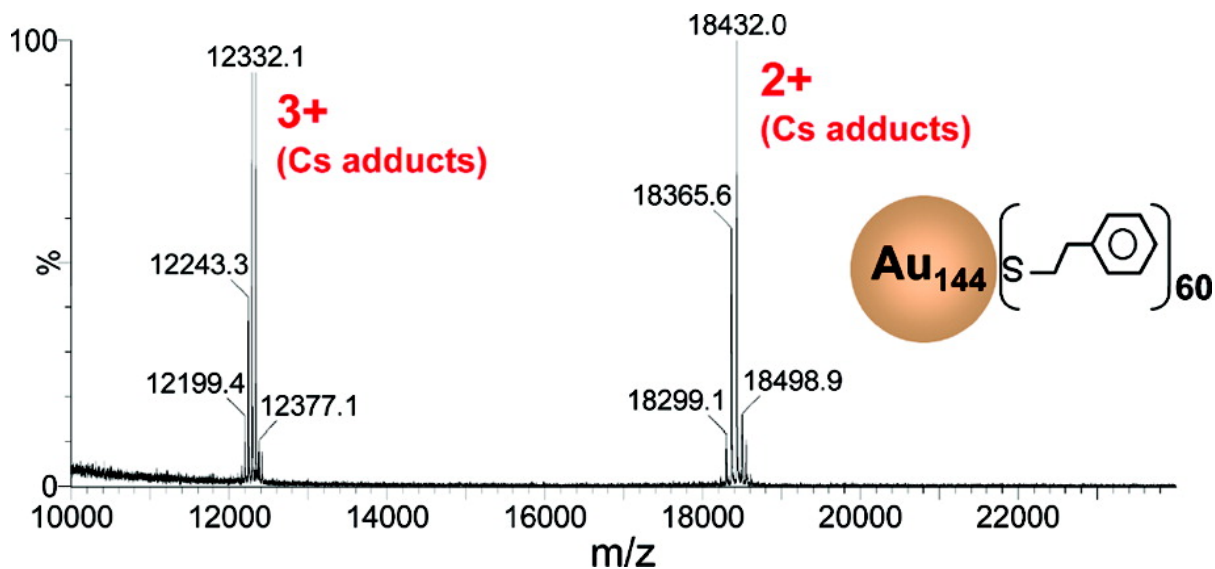


Au₁₄₀₋₁₄₆(SR)₅₀₋₆₀
(*JPCB* 1997, 101, 7885.)



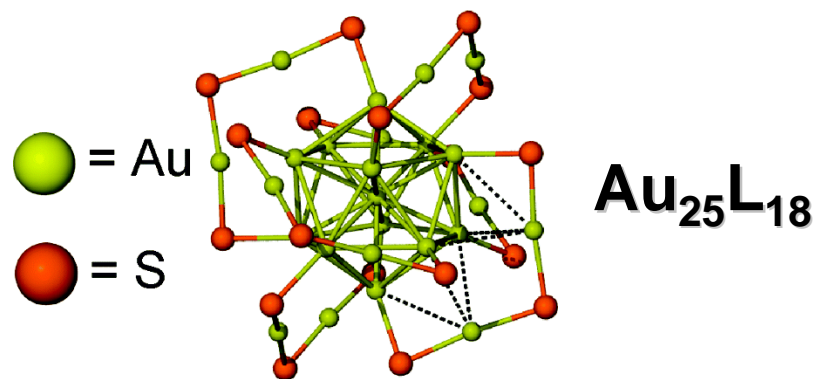
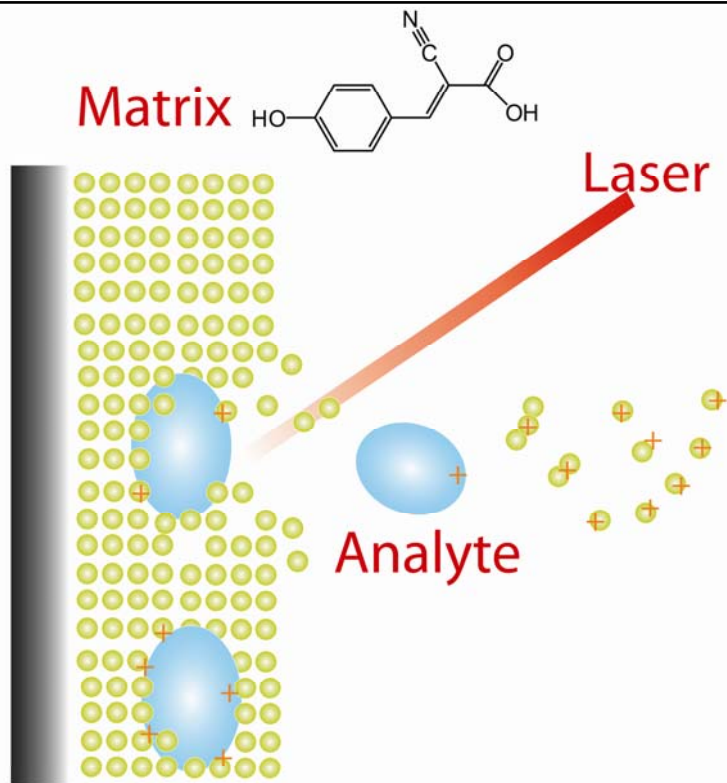
A 12-year
journey

Au₁₄₄(SR)₆₀
(*Nano Lett* 2009, ASAP)



➤ ESI-MS can determine AuNP formula with atomic precision

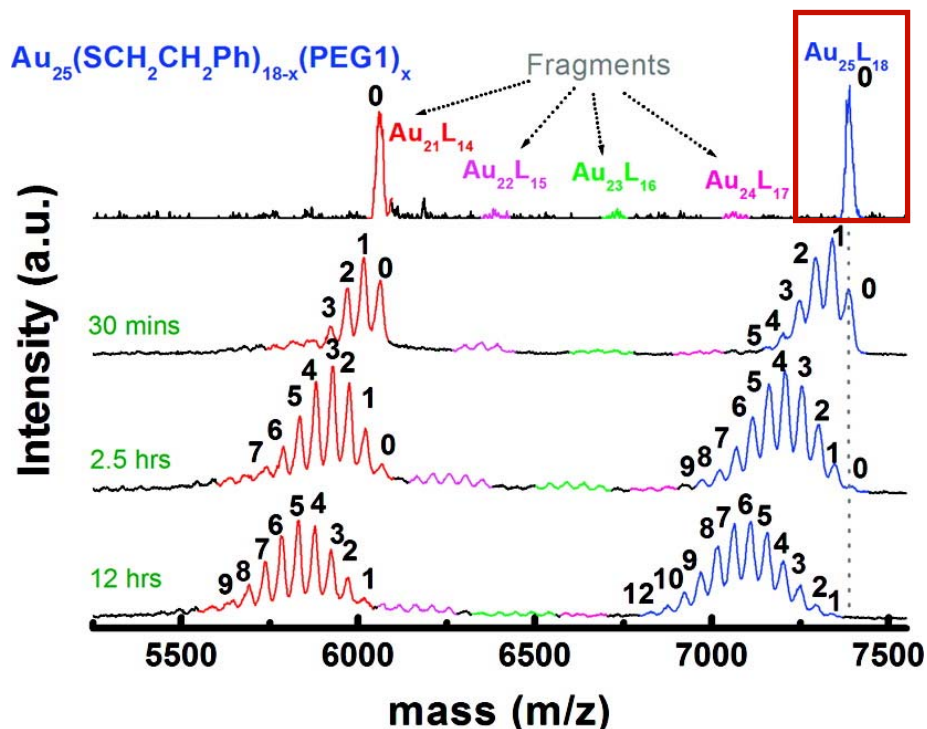
AuNP analyses with MALDI-MS



Murray, R. W. *et al.* *JACS*, **2008**, 130, 3754

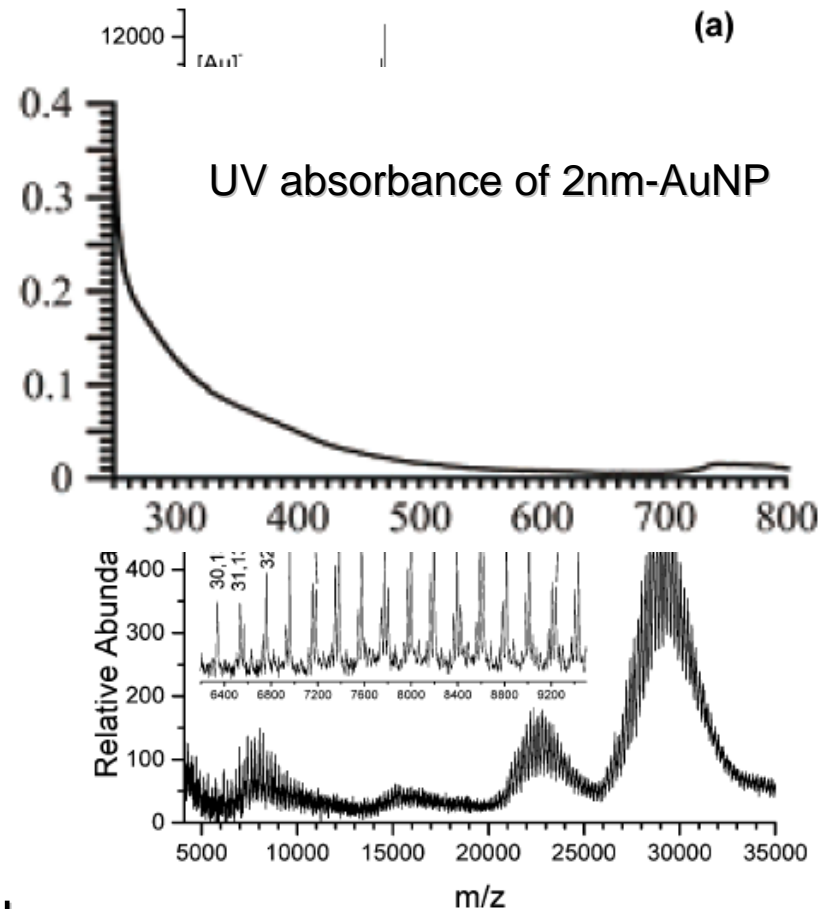
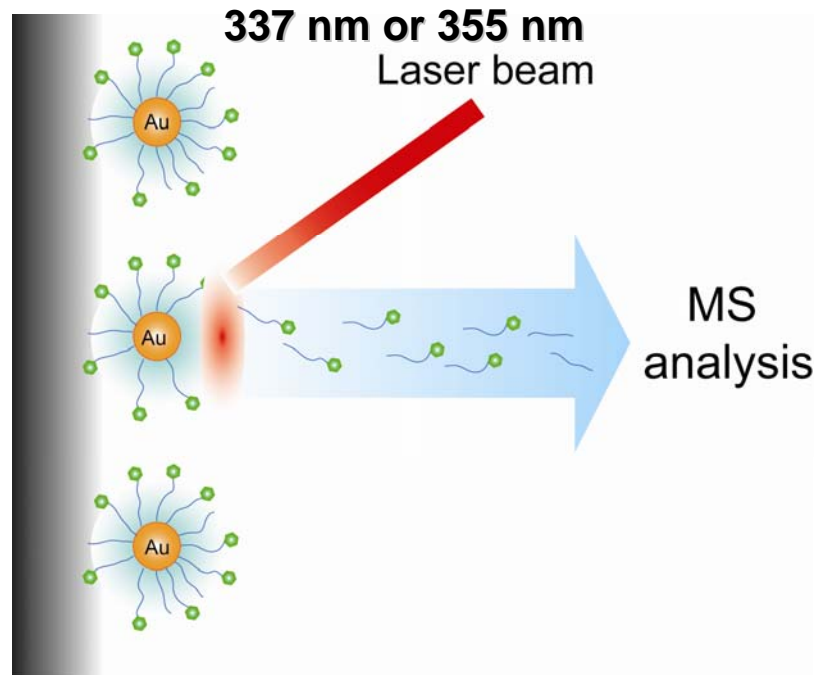
➤ MALDI-MS can detect AuNP and generate fragments

➤ Matrix interferes the detection of small molecules less than 500 Da



Murray, R. W. *et al.* *JACS*, **2008**, 130, 5940 7

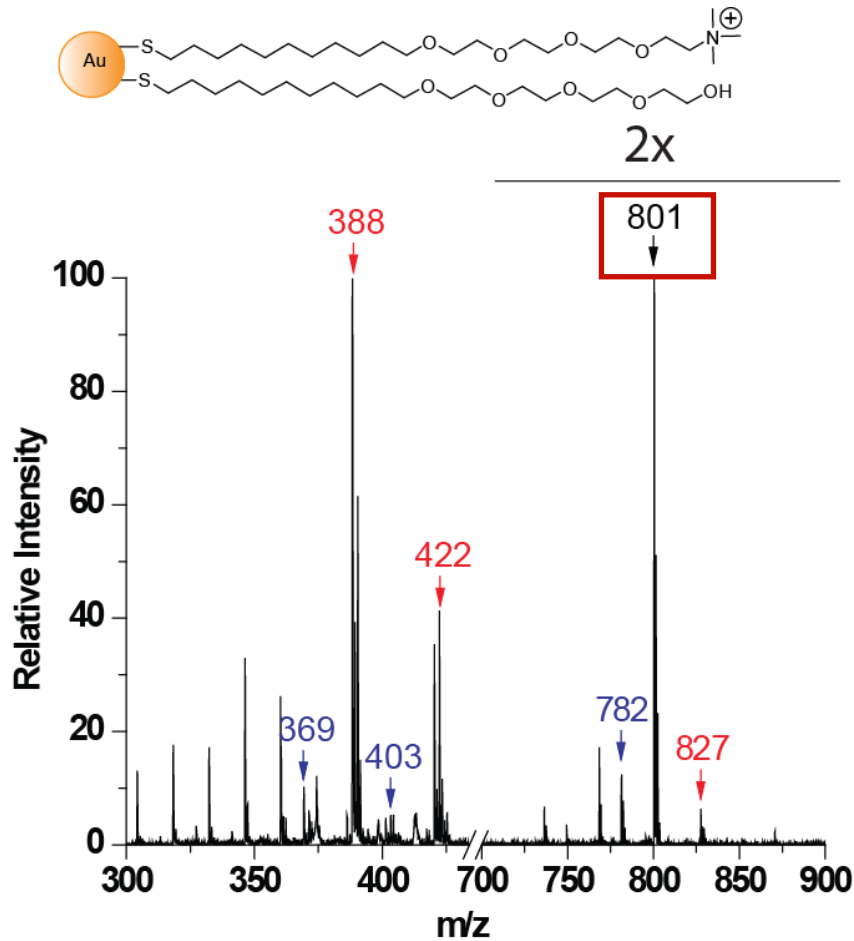
AuNP analyses with **LDI-MS (AuNP-assisted LDI-MS)**



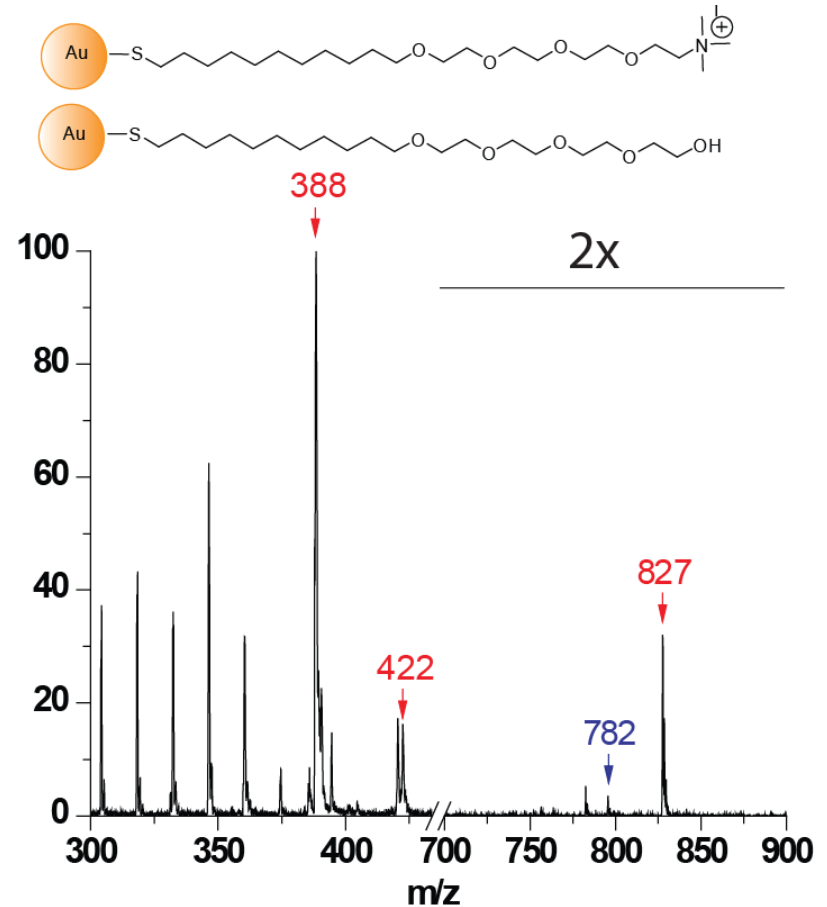
- **NO matrix**, so no matrix interferences
- **AuNPs work as matrix**: upon laser irradiation, AuNPs absorb laser energy and AuNPs can be desorbed and ionized or get fragmented
- LDI-MS gives out mass of AuNP (**low resolution**) and information of its fragments in **low mass region**

Schaaff, T.G. *Anal. Chem.* **2004**, 76, 6187

Identification of AuNPs with mixed monolayers



**Mixed monolayers
in one AuNP**

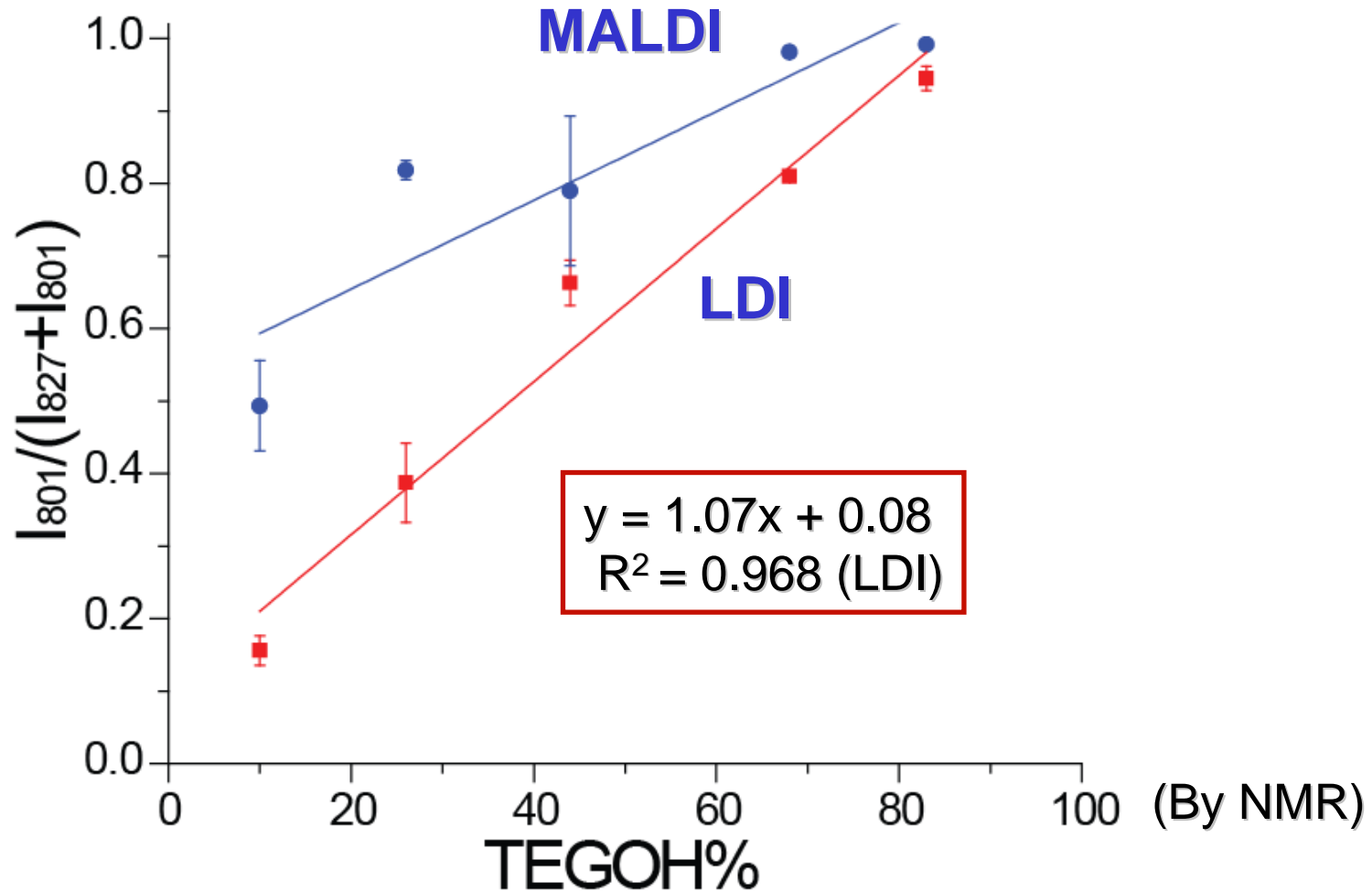


Mixed AuNPs

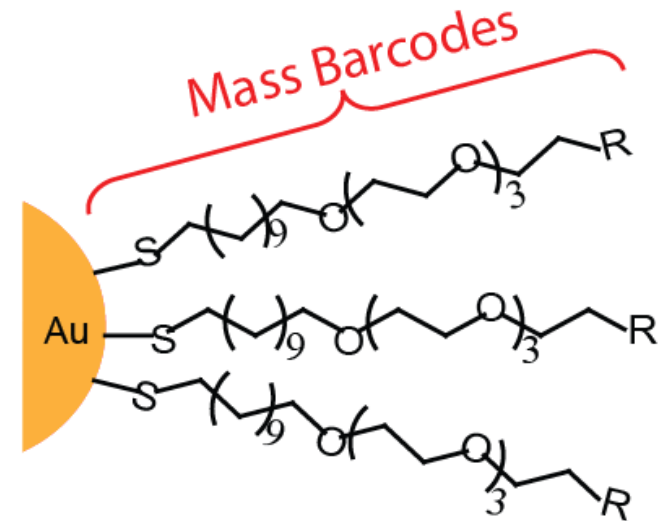
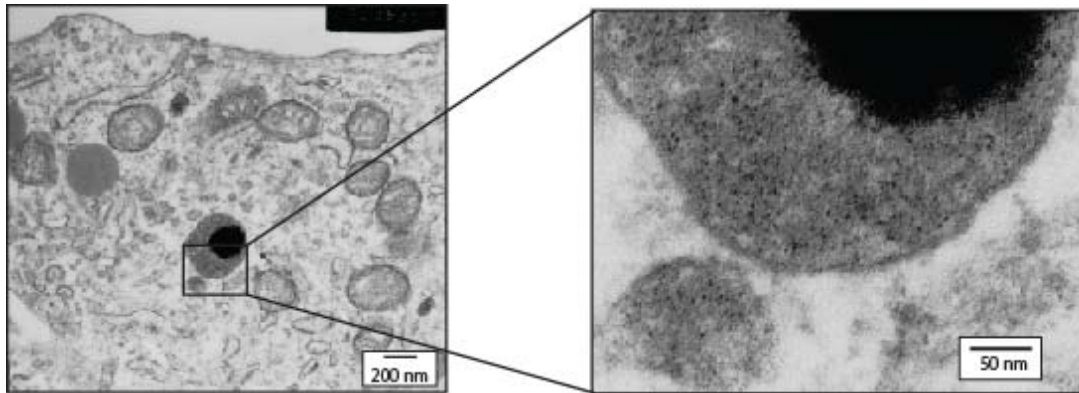
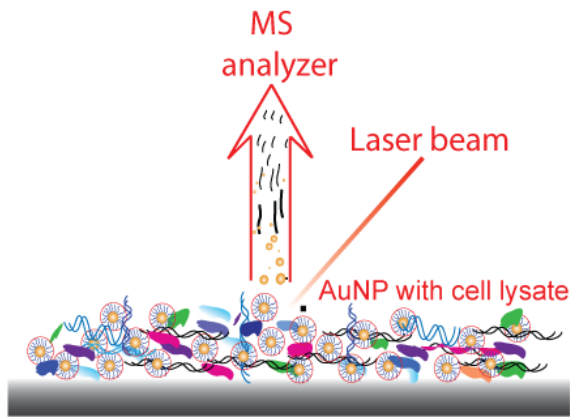
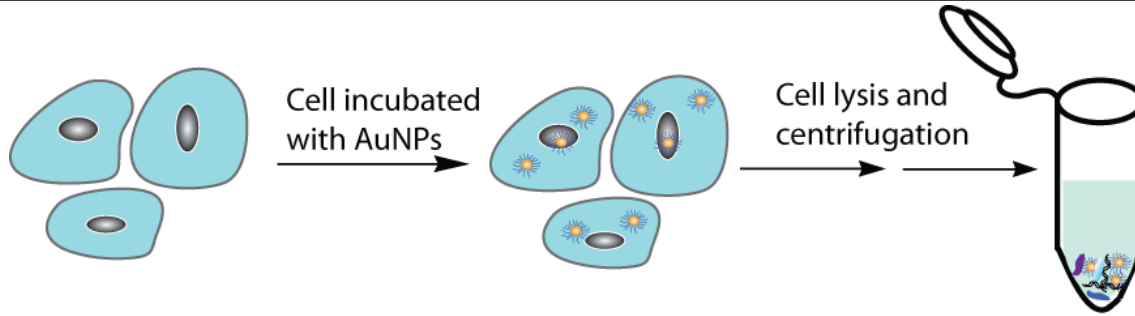
➤ Presence of **disulfide of two ligand molecules** help to identify AuNP with mixed monolayers

Quantification of AuNPs with mixed monolayers

Ratios of MS peaks

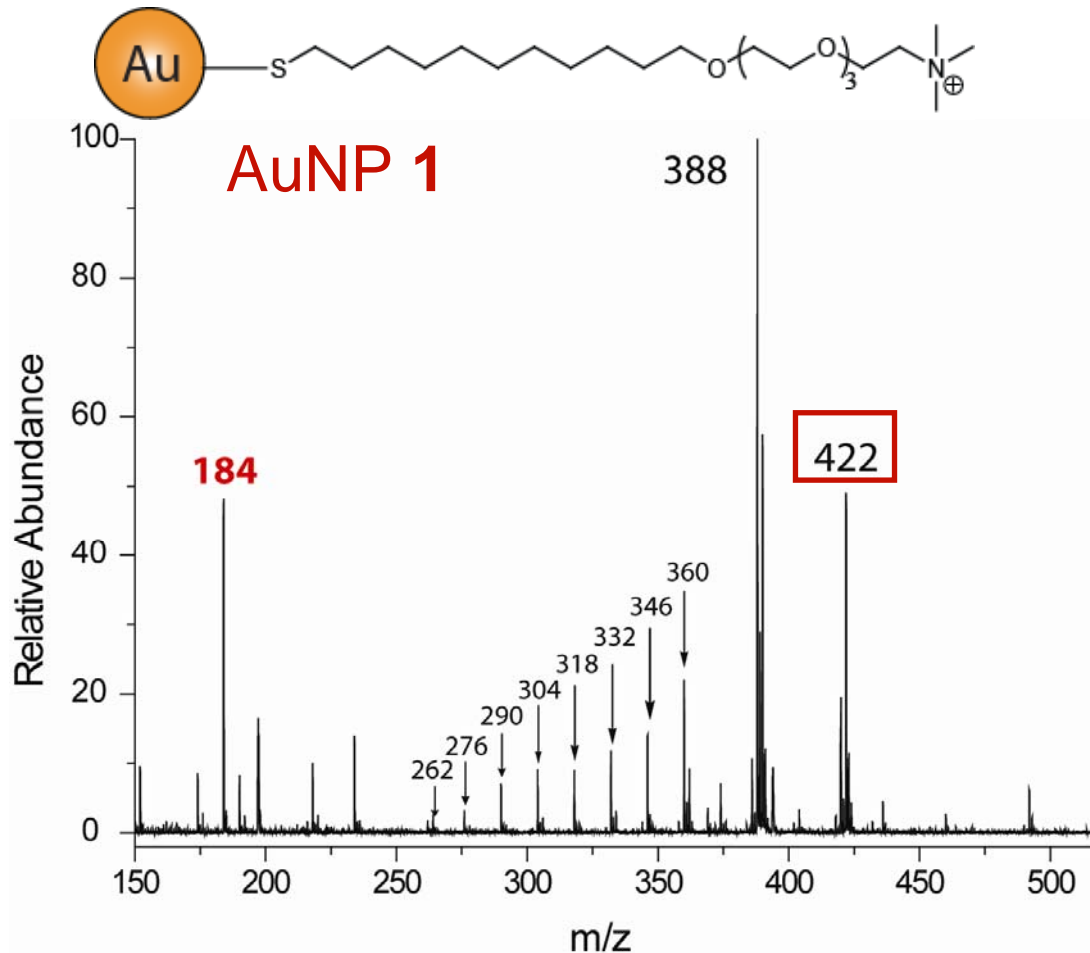


Multiplexed tracking of cellular uptake of AuNPs



- Mass Barcodes**
- AuNP 1: R= ---N^{\oplus} m/z 422
 - AuNP 2: R= ---N^{\oplus} -CH₃ m/z 436
 - AuNP 3: R= ---N^{\oplus} -CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂ m/z 492
 - AuNP 4: R= ---N^{\oplus} -CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂NH₂ m/z 465
 - AuNP 5: R= ---OH m/z 403

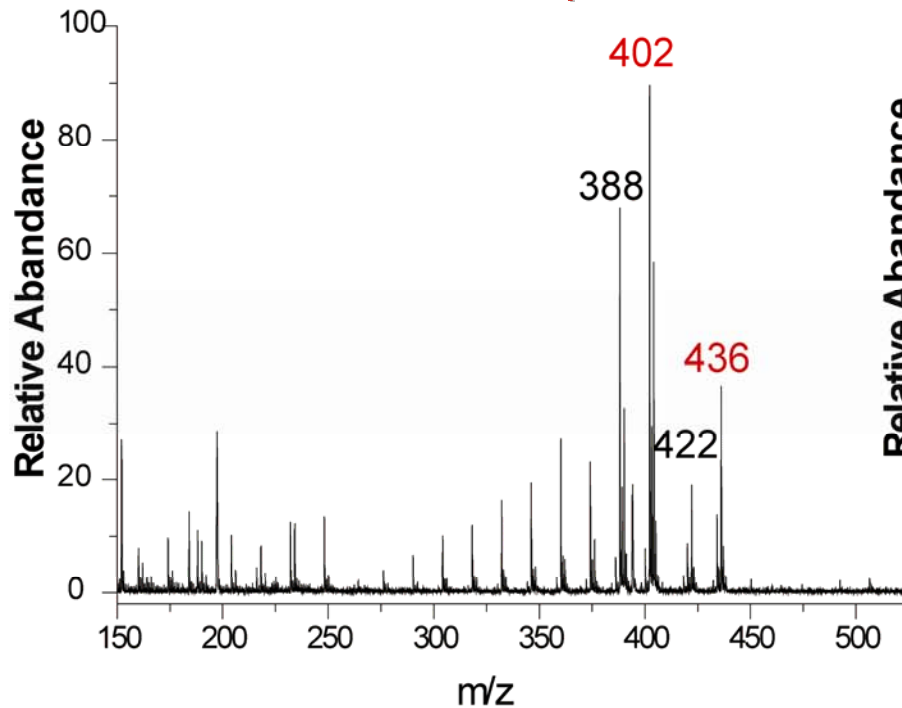
Detect cellular uptake of AuNPs by LDI-MS



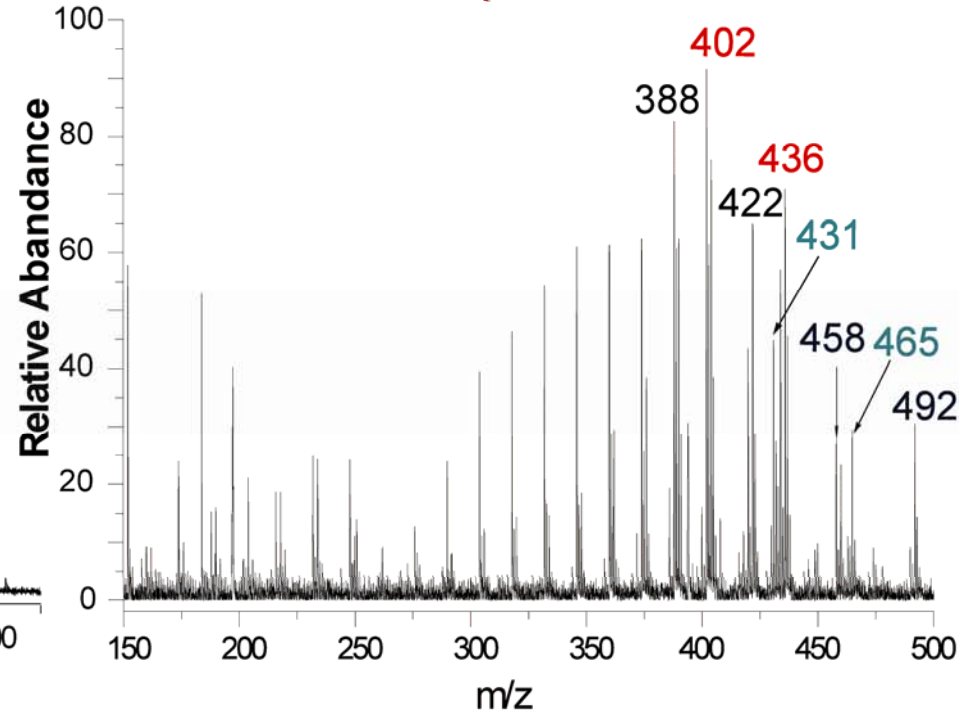
- LDI mass spectrum of COS-1 cell lysate after uptake of AuNP 1 (TTMA)
 - **m/z 422** corresponds to the molecular ion (M^+) of ligand
 - **m/z 184** is the head-group fragment of phosphatidylcholine (PC)
 - 30 pmol (50 nM) AuNP in cell culture could be detected with a S/N of 75

Multiplexed tracking of cellular uptake of AuNPs

AuNP 1 and 2 uptake

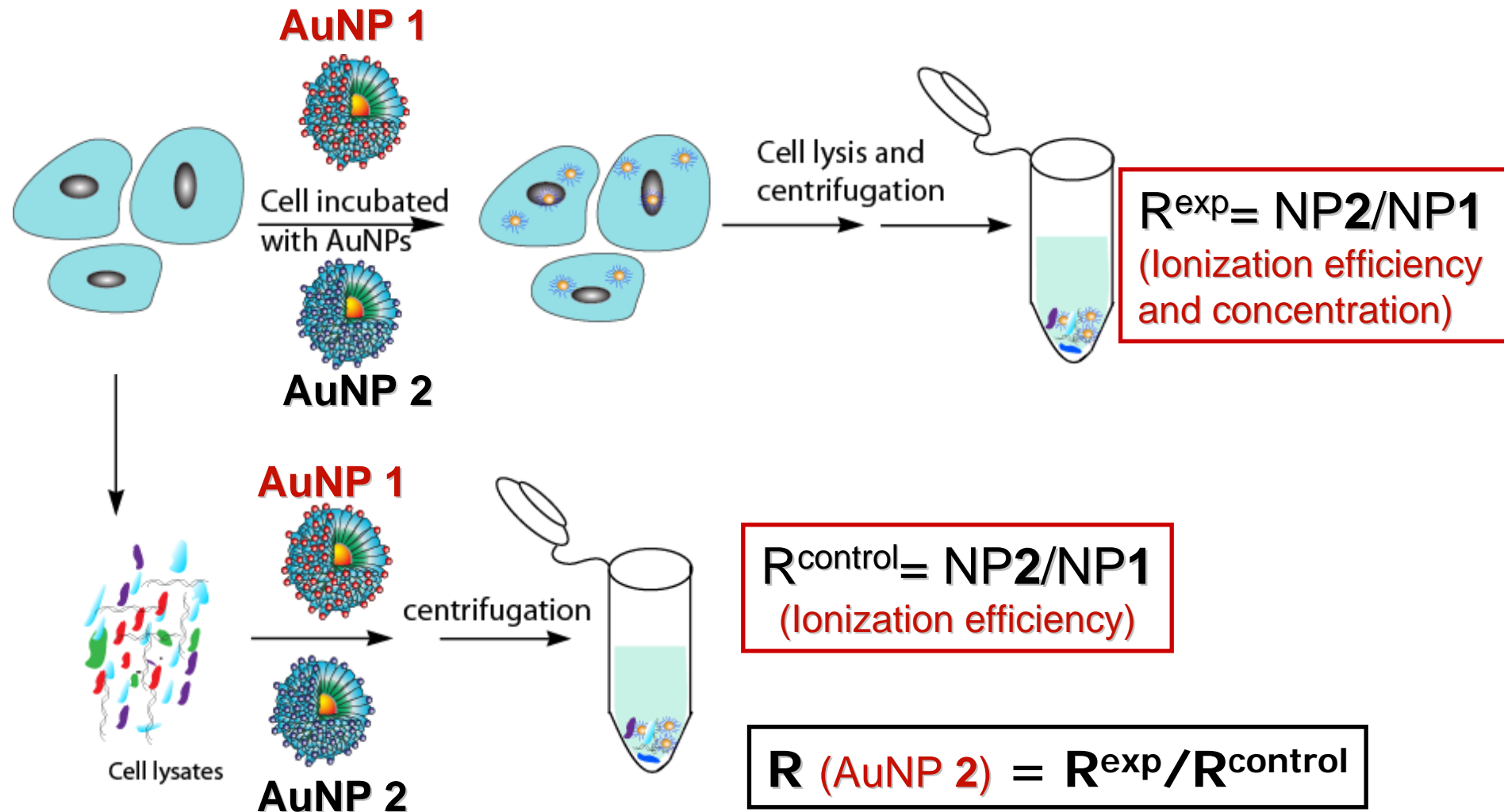


AuNP 1-4 uptake



- The different “mass barcodes” of each AuNP facilitate multiplexed tracking by LDI-MS
- Multiplexed tracking provides **side-by-side comparison of AuNP uptake**
- Two factors contribute to abundances of “mass barcode” ions:
ionization efficiency and AuNP concentration

Relative quantitation of AuNP cellular uptake



- Control: spike equal amounts of AuNPs in cell lysate, and then measure the ionization efficiency ratio

LDI-MS relative quantitation of AuNP (1-5) cellular uptake

	Mass barcodes (m/z)	Control Ratio [a]	Experimental Ratio [b]	Relative Ratio [c]
AuNP 2 / AuNP 1	436 / 422	1.41 ± 0.09	1.70 ± 0.10	1.20 ± 0.10
AuNP 3 / AuNP 1	492 / 422	0.72 ± 0.08	0.40 ± 0.10	0.50 ± 0.20
AuNP 4 / AuNP 1	465 / 422	0.19 ± 0.05	0.21 ± 0.03	1.10 ± 0.30
AuNP 5 / AuNP 1	369 / 388	0.26 ± 0.05	0.09 ± 0.02	0.34 ± 0.09


Ionization efficiency

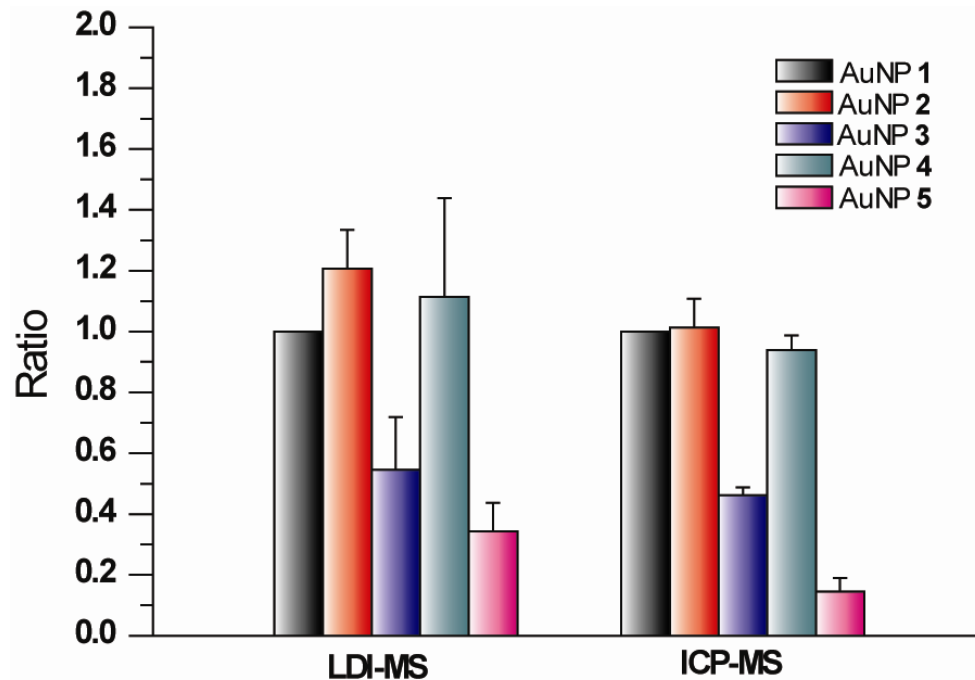
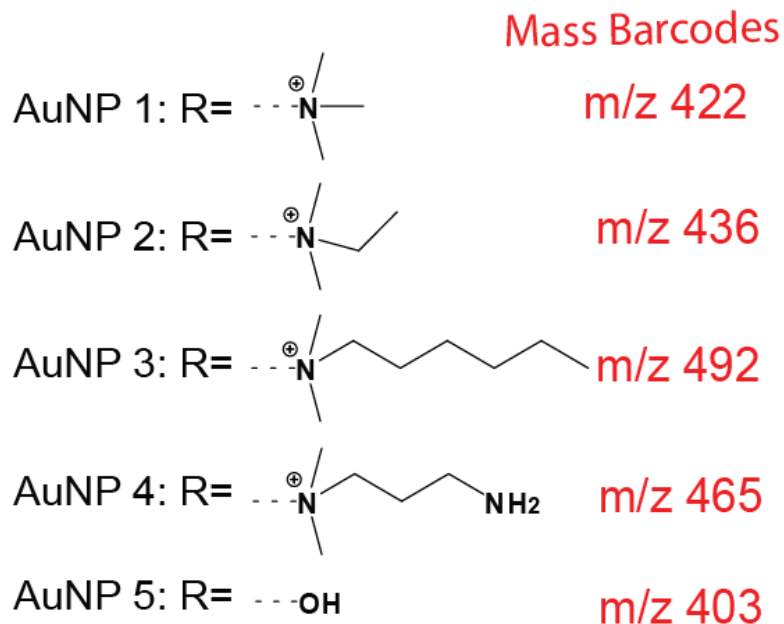

Concentration

[a] Equal mole amounts of each AuNP and AuNP 1 were mixed with lysed COS-1 cells and subjected to LDI-MS analysis.

[b] Equal mole amounts of each AuNP and AuNP 1 were cultured with living COS-1 cells.

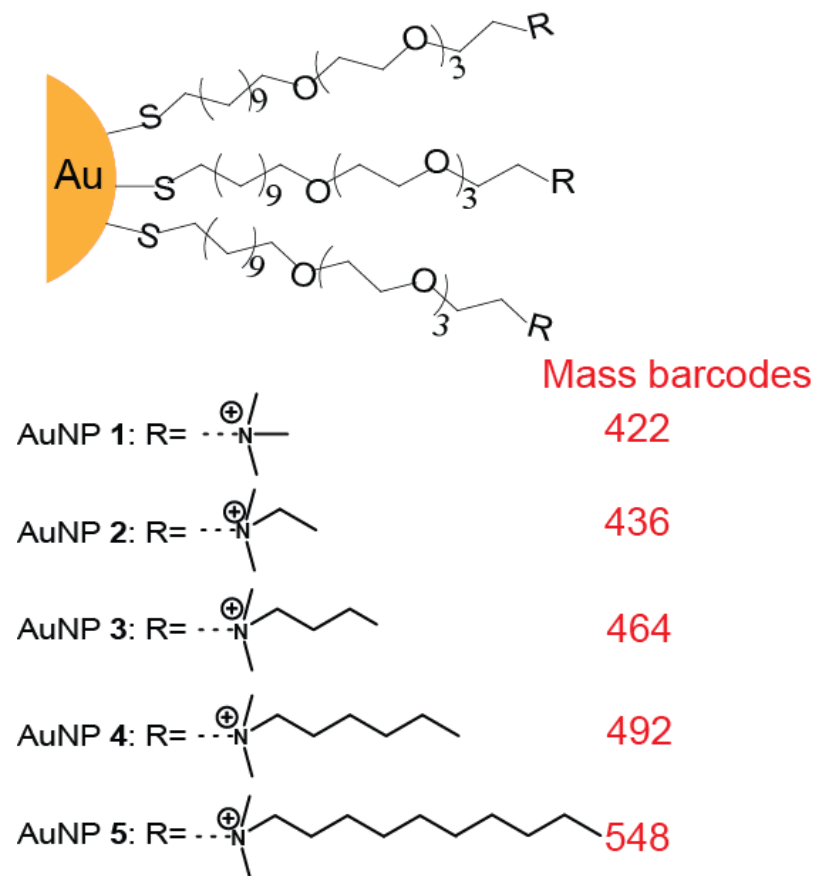
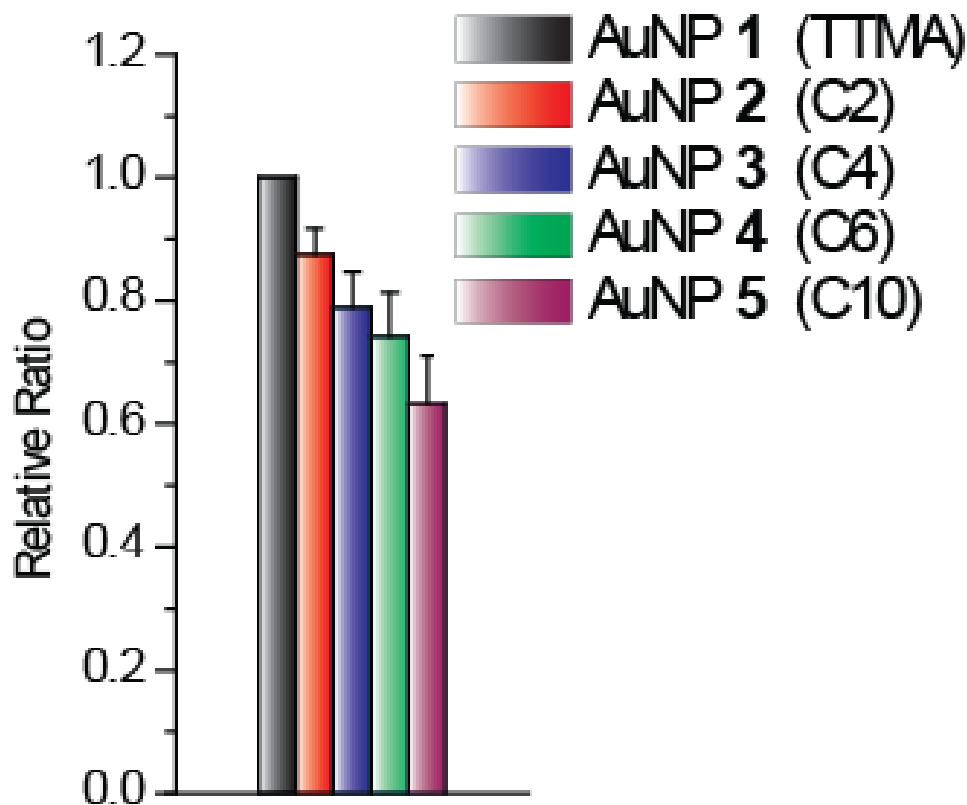
[c] Relative ratios are generated by comparing experimental ratios with control ratios.

LDI-MS relative quantitation of AuNPs validated by ICP-MS



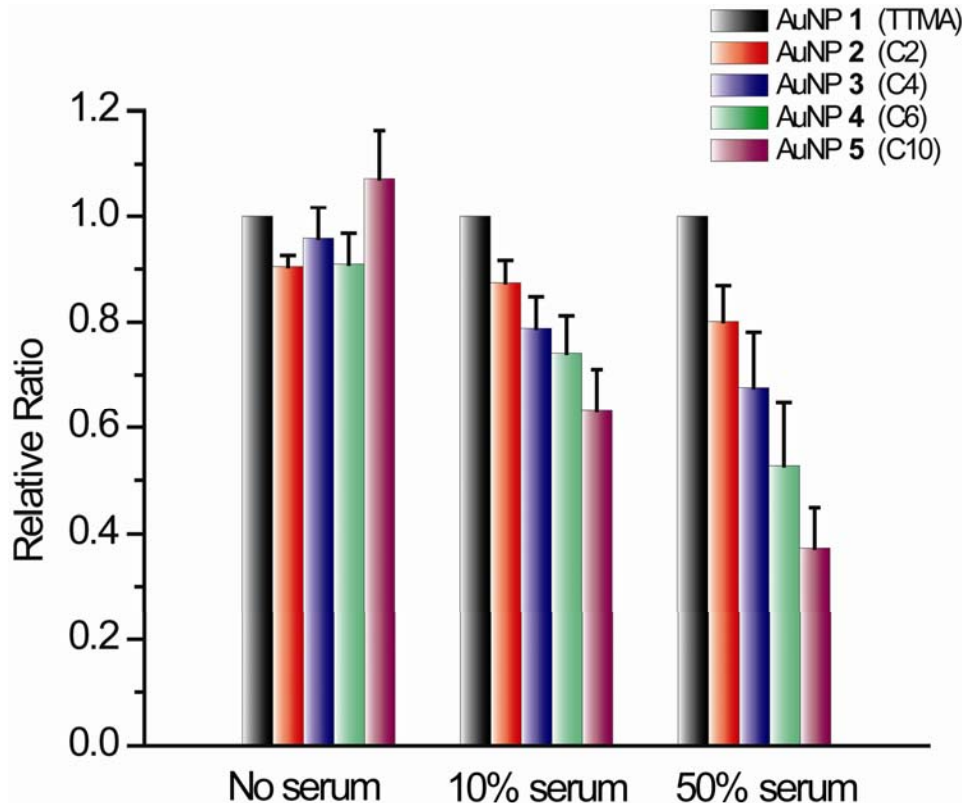
- The relative uptake amounts for each AuNP as determined by ICP-MS are very similar to the LDI-MS data
- Cellular uptake is *surface property dependent*:
 - Neutral AuNP 5 is taken up less than four cationic AuNPs (**Charge**)
 - AuNP 3 is taken up less than AuNP 1 and AuNP 2 (**Hydrophobicity**)

Surface property dependent cellular uptake of AuNPs



- **Hydrophobic AuNPs** tend to be taken up less by MCF-7 cells
- LDI-MS technique can help to design AuNP based drug carriers for **highly efficient cellular uptake**

Cellular uptake of AuNPs with different serum supplements

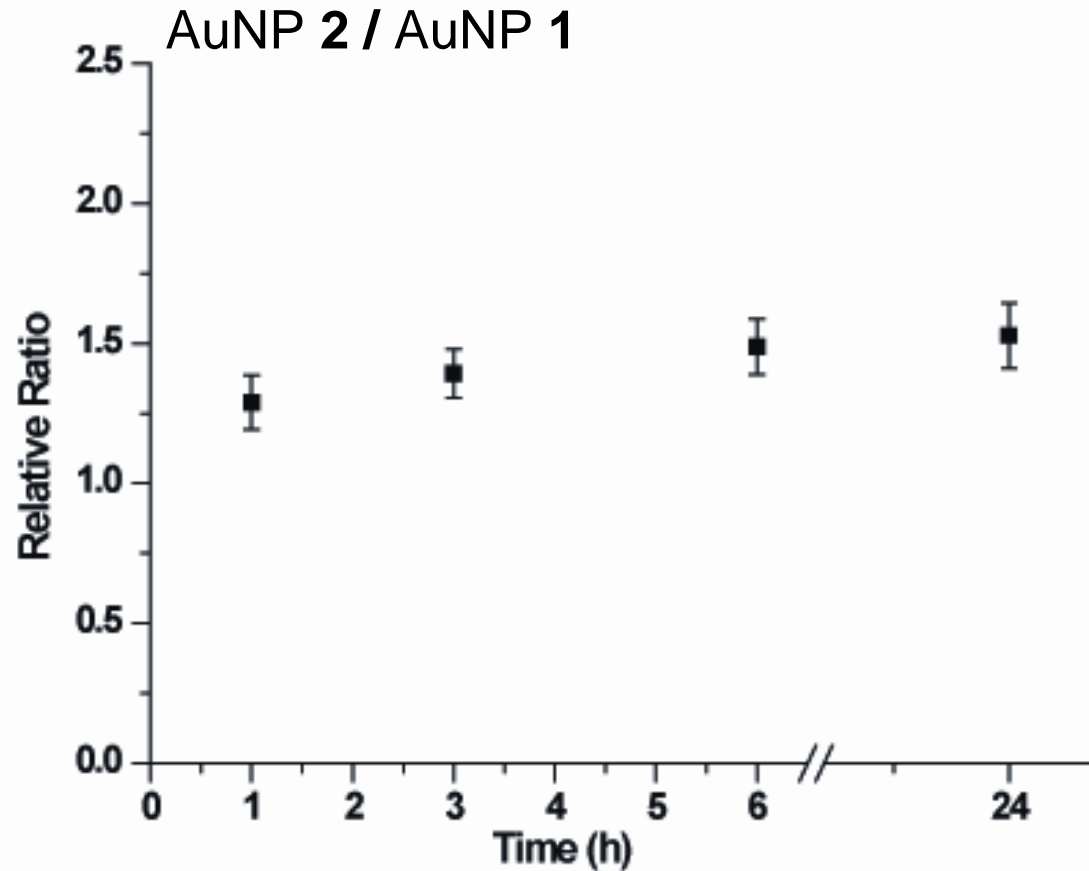


MCF-7 cell

(100 nM of each AuNP mixed with different percentages of serum and incubated with cells for 6 h)

- Cell culture media supplemented by **0%, 10%, 50%** fetal bovine serum (FBS)
- Serum proteins absorb on the surface of AuNPs, altering the surface properties of AuNPs
- The protein absorption depends on AuNP functionality
- With more serum present in cell culture media, **hydrophobic AuNPs** are taken up less into cells
- AuNP uptake into cells can be controlled

Stability of AuNPs in cellular environment



- Spike AuNP 1 and AuNP 2 into cell lysate, keep in 37°C
- These two AuNPs have the same stability in the cellular environment

The summary

- LDI-MS provide informative characterizations of AuNP monolayers
- “**Mass barcodes**” along with the LDI-MS technique can successfully track and quantify cellular uptake of AuNPs
- Cellular uptake of AuNPs depends on surface functionalities of AuNPs:
 - **Positively charged** AuNPs are better taken up than neutral AuNPs
 - **Very hydrophobic** AuNPs tend to be taken up less
 - **Serum in cell culture media** control cellular uptake of AuNPs

Thank you for your attentions !