In-situ derivatization of Au nanoclusters via aurophilic interaction of triphenylphosphine gold (I) salt with neurotransmitters and its rapid MALDI-TOF-MS detection in mice brain tissue extracts

1. Optimization of [Ph₃PAu]₃O⁺BF₄⁻ matrix

1.1. Effect of [Ph₃PAu]₃O⁺BF₄ concentration

To optimize the concentration of $[Ph_3PAu]_3O^+BF_4^-$, 5 to 100 µM concentrations of $[Ph_3PAu]_3O^+BF_4^-$ solutions have been prepared in acetonitrile solvent, and added into 10 µM of DA solution for MALDI-TOF-MS measurements. According to the MALDI-TOF-MS results in S1a, the abundance of $[Ph_3PAu]^+$ adduct have been gradually increased until the concentration of $[Ph_3PAu]_3O^+BF_4^-$ reaches 50 µM. But after 50 µM, the abundance of $[Ph_3PAu]^+$ became constant at 1.0×10^5 %. In S1b&c, the abundance of $[(Ph_3PAu)_2NR]^+$ dimer and $[(Ph_3PAu)_3NR]^+$ trimer have been gradually increased until the concentration of $[Ph_3PAu]_3O^+BF_4^-$ reaches 100 µM. After 100 µM, the abundance of dimer and trimer have started to decrease, it might be due to the less availability of nitrogen center than $[Ph_3PAu]^+$ adduct for auration at extremely higher concentrations of $[Ph_3PAu]_3O^+BF_4^-$. Therefore, 100 µM of $[Ph_3PAu]_3O^+BF_4^-$ have been considered as an optimum concentration for MALDI-TOF-MS detection of neurotransmitters.





S1: Calibration plot drawn between (a) concentration of $[Ph_3PAu]_3O^+BF_4$ and $[Ph_3PAu]^+$, (b) concentration of $[Ph_3PAu]_3O^+BF_4$ and $[Ph_3PAu]_2N^+R$ dimer and (c) concentration of $[Ph_3PAu]_3O^+BF_4$ and $[Ph_3PAu]_3N^+R$ trimer in the presence of 10 μ M DA.

1.2. Effect of solvent

To investigate the effect of various solvents, we have used acetonitrile (ACN), acetone (ACE), isopropyl alcohol (IPA) and methanol (MA) to dissolve $[Ph_3PAu]_3O^+BF_4^-$, and compared the MALDI-TOF-MS results. The concentration of $[Ph_3PAu]_3O^+BF_4^-$ and neurotransmitter DA was kept as constant at 100µM, respectively. According to the obtained results in S2, it is confirmed that the abundance of $[Ph_3PAu]_2N^+R$ dimer is higher than that of $[Ph_3PAu]_+^+$ adduct while using ACN as solvent. The overall abundance for $[Ph_3PAu]_2N^+R$ is also higher with ACN as compared with other solvents. Therefore, we have considered ACN as suitable solvent for further experiments to dissolve $[Ph_3PAu]_3O^+BF_4^-$ salt.



S2: Calibration plot drawn between various solvents and the relative abundance of $[Ph_3PAu]^+$, $[Ph_3PAu]_2N^+R$ and $[Ph_3PAu]_3N^+R$ products in the presence of $100\mu M DA$ into $100\mu M [Ph_3PAu]_3O^+BF_4^-$

1.3. Effect of common matrix

To investigate the effect of various common matrices, we have used CHCA, DHB and SA as an additional matrix along with $[Ph_3PAu]_3O^+BF_4^-$. The concentration of each matrix was used at $10\mu g/\mu L$. The concentration of $[Ph_3PAu]_3O^+BF_4^-$ and neurotransmitter DA was kept as constant at $100\mu M$, respectively. In addition, the already optimized ACN was used as solvent. According to the obtained results in S3, it is confirmed that CHCA is well suited for the detection of neurotransmitters. Hence, we have used CHCA as additional matrix for further experiments.



S3: Calibration plot drawn between various matrices and the relative abundance of $[Ph_3PAu]^+$, $[Ph_3PAu]_2N^+R$ and $[Ph_3PAu]_3N^+R$ products in the presence of $100\mu M DA$ into $100\mu M [Ph_3PAu]_3O^+BF_4^-$



2. MALDI-TOF-MS detection of various neurotransmitters using [Ph₃PAu]₃O⁺BF₄⁻ matrix



$[Ph_3PAu]_3O^+BF_4^- + GABA$





3. SEM and EDS analysis





Element	Weight%	Atomic%
СК	49.94	94.24
Au M	50.06	5.76
Totals	100.00	

S5: SEM and EDS analysis of as formed Au NPs in the presence of $100 \mu M DA$

4. [Ph₃PAu]⁺Cl⁻ and [CH₃]₃O⁺BF₄⁻ as matrix for MALDI-TOF-MS detection of neurotransmitters





S6: MALDI-TOF-MS spectra of (a) only $[Ph_3PAu]^+Cl^-$, (b) $100\mu M [Ph_3PAu]^+Cl^-$ in the presence of $100\mu M DA$ and (c) $[CH_3]_3O^+BF_4^-$ in the presence of $100\mu M DA$

5. Simultaneous MALDI-TOF-MS detection of various neurotransmitters



S7: MALDI-TOF-MS spectra of $100 \mu M [Ph_3PAu]_3O^+BF_4$ in the presence

50µM various neurotransmitters







S8: MALDI-TOF-MS spectra of 100mM $[Ph_3PAu]_3O^+BF_4^-$ in the presence of various region

of mice brain extracts