Supplementary Information

Integration of protein tethering in a rapid and label-free SERS screening platform for drugs of abuse

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Material and Methods

Chemicals:

$AgNO_3$ (99.5%), sodium citrate (99%) and human serum albumin were purchased from Sigma Aldrich, USA and was used without further modification. The drugs, namely butalbital, alpha-hydroxyalprazolam, pseudoephedrine and benzoylecgonine, were obtained from Cerilliant, USA.

Preparation of plasmon-tuned nanoparticles:

The silver (Ag) colloids were synthesized by the Lee-Meisel method.[1] The core-shell nanoparticles were synthesized by diluting 12.5 ml Ag colloid solution with 10 ml Millipore water and adding 6.25 x 10^{-3} M NH_2OH: HCl (Sigma Aldrich) and 4.65 x 10^{-4} M $HAuCl_4$ (Sigma Aldrich) dropwise with constant stirring till the absorption maximum reaches 830 nm. The Au^{3+} ions were reduced by hydroxylamine hydrochloride using Ag nanoparticles as seeds.[2] All the reagents were used as received from the vendor and glassware was cleaned thoroughly with Millipore water and air-dried before use in experiments.

The nanoparticles were characterized using UV-Vis spectroscopy (StellarNet Inc. FL, USA) to investigate the surface plasmon resonance. Transmission electron microscope (TEM) imaging was used for structural analysis of the synthesized nanoparticles by a Philips EM300 at an accelerating voltage of 300 kV on a Lacey Carbon Films on 300 Mesh Copper Grids (Ted Pella, Inc.). 2D finite difference time domain (FDTD) simulations (Lumerical Solutions Inc., Vancouver, Canada) were employed for calculating the near field intensity around the

nanoparticles. A plane wave polarized light of wavelength 830 nm, corresponding to the laser wavelength in our experiments, was used as the source with incidence along y-axis while perfectly matched layers were used along the x-axis.

Molecular docking:

For the docking studies crystal structure of human serum albumin were obtained from Protein Data Bank with codes 1AO6 and 1LZ1, respectively. Structures of the drug molecules were obtained from Drugbank. Docking analysis was performed with Autodock 4.213[3] using Lamarkian genetic algorithm (GA) and a grid spacing of 0.375 Å. Docking was performed with flexible ligand and 100 GA runs, a population size of 300, 25 x 10^5 evaluations and a maximum of 27000 generations. The figures were generated using the PyMOL molecular graphics system (Schrödinger, LLC).

Data processing:

The spectral data was processed using built-in principal component analysis (PCA) module and in-house partial least squares (PLS) code developed in the MATLAB R2014a environment (MathWorks Inc., Natick, MA). The spectral range chosen for analysis was 680-1870 cm^{-1}. The statistical data (relative error of prediction REP, relative standard deviation RSD, limit of detection LOD, Correlation), were calculated from the PLS prediction results. The radial visualization plots were prepared using Orange ver 2.7 data mining software.

Table S1: Tentative band assignment of SERS spectra of human serum albumin based on previous literature reports. [4,5]

Human Serum Albumin (cm^{-1})	Band assignment
733	C-S stretch
865	Tryptophan
973	$\rho(CH_3)$, $\delta(CCH)$
1015	Phenylalanine/Tyrosine
1077	$\nu(CC)$ skeletal
1156	C-C, C-N stretching
1228	Amide III
1252	Amide III
1290	C-H bending, CH_2 deformation
1373	Tryptophan
1480	Amide II/C=N stretching
1649	Amide I

Table S2: Vibrational modes of butalbital,[6] alpha-hydroxyalprazolam,[7,8] pseudoephedrine[9,10] and benzoylecgonine.[11,12]

Butalbital (cm^{-1})	Alpha-hydroxy Alprazolam (cm^{-1})	Pseudoephedrine (cm^{-1})	Benzoylecgonine (cm^{-1})
500		618	
617	686	753	650
645		834	
841		910	
925		1002	
1040	1000	1025	1000
1140	1158	1173	1020
1300	1304	1208	
1410		1334	
	1595	1390	1595
1642		1462	1650
1726		1603	1715
1746			1730

Table S3: Residue names and numbers of human serum albumin forming hydrogen bonds with the drug molecules obtained from docking simulations.

	Alpha-hydroxyalprazolam	Butalbital	Benzylecgonine	Pseudoephedrine
Human serum albumin	Ala-176, Asp-183	Glu-167, Asp-173, Thr-515, Arg- 521	Lys-190, Lys 432	Val-120, Asp-121

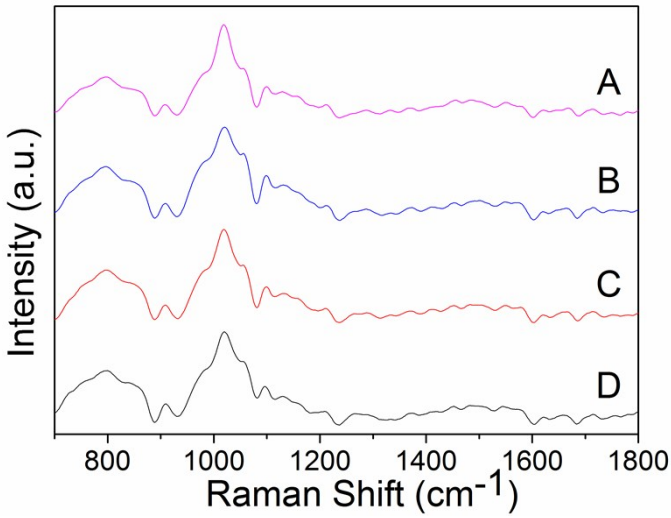

Figure S1: SERS of (A) alpha-hydroxyalprazolam, (B) butalbital, (C) benzoylecgonine and (D) pseudoephedrine (at the highest measured concentration of 1mg/ml) in the absence of HSA.

References:

1. P. C. Lee and D. Meisel, *The Journal of Physical Chemistry*, 1982, **86**, 3391-3395.
2. G.V.P. Kumar, S. Shruthi, B. Vibha, B. A. A. Reddy, T. K. Kundu and C. Narayana, *Journal of Physical Chemistry C*, 2007, **111**, 4388-4392.
3. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, *Journal of computational chemistry*, 2009, **30**, 2785-2791.
4. Z. Movasaghi, S. Rehman and I. U. Rehman, *Applied Spectroscopy Reviews*, 2007, **42**, 493-541.
5. G. Fabriciova, S. Sanchez-Cortes, J. V. Garcia-Ramos and P. Miskovsky, *Biopolymers*, 2004, **74**, 125-130.
6. L. Lin, J. Lv, Y. Ji, J. Feng, Y. Liu, Z. Wang and W. Zhang, *Analytical Letters*, 2013, **46**, 2890-2898.
7. E. L. Doctor and B. McCord, *Analyst*, 2013, **138**, 5926-5932.

8. G. Montalvo, L. López-Melero, F. Ortega-Ojeda, M. Á. Peña and C. García-Ruiz, *Analytical Methods*, 2014, **6**, 9536-9546.
9. A. E. Guimarães, M. T. T. Pacheco, L. Silveira Jr, D. Barsottini, J. Duarte, A. B. Villaverde and R. A. Zângaro, *Journal of Spectroscopy*, 2006, **20**, 185-194.
10. M.-L. O'Connell, A. G. Ryder, M. N. Leger and T. Howley, *Applied spectroscopy*, 2010, **64**, 1109-1121.
11. B. Sägmüller, B. Schwarze, G. Brehm, G. Trachta and S. Schneider, *Journal of molecular structure*, 2003, **661**, 279-290.
12. C. Shende, F. Inscore, P. Maksymiuk and S. Farquharson, Smart Medical and Biomedical Sensor Technology III, 2005.