Supplementary Information for

Investigating the applicability of HEPES-AuNPs for SERS measurements on individual functional red blood cells in saline

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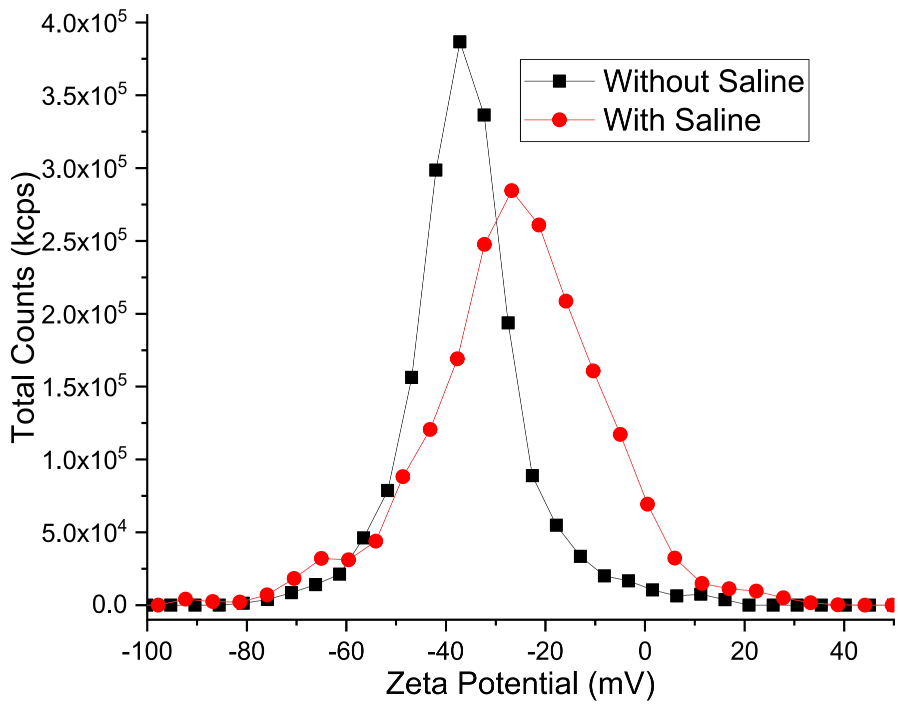

Figure S1: Zeta potential distribution of AuNPs with and without saline treatment.

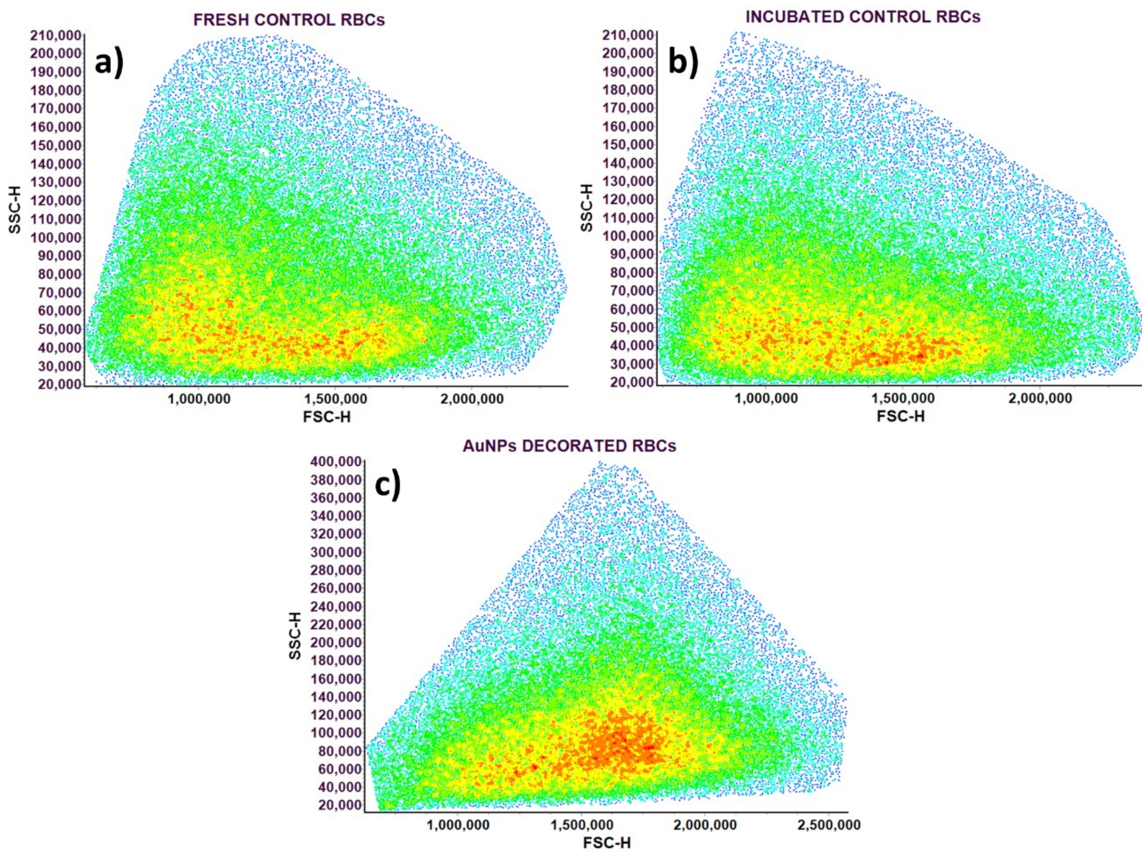

Figure S2: Flow cytometry results showing FSC-H v/s SSC-H population plot for a) fresh control RBCs, b) incubated control RBCs, and c) AuNPs decorated RBCs.

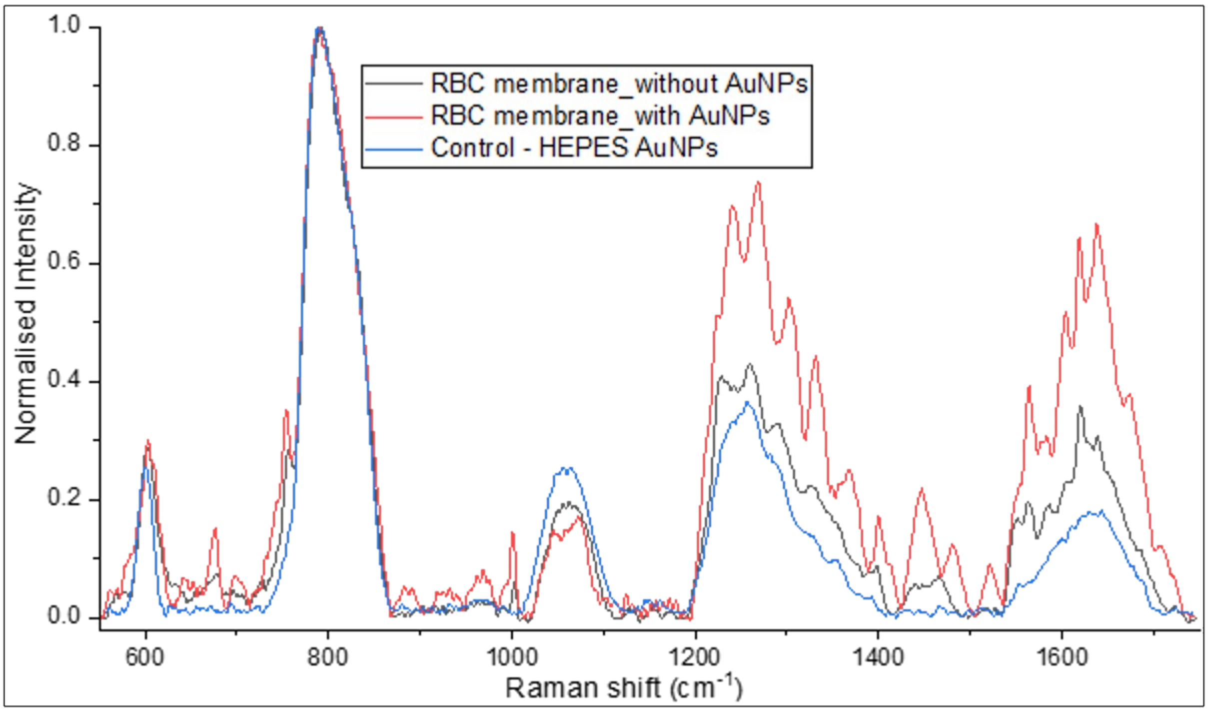

Figure S3: Comparison of the Raman spectrum of the RBC membrane with and without HEPES-AuNPs of optically trapped single live RBC membrane and control – HEPES AuNPs recorded using a vortex beam.

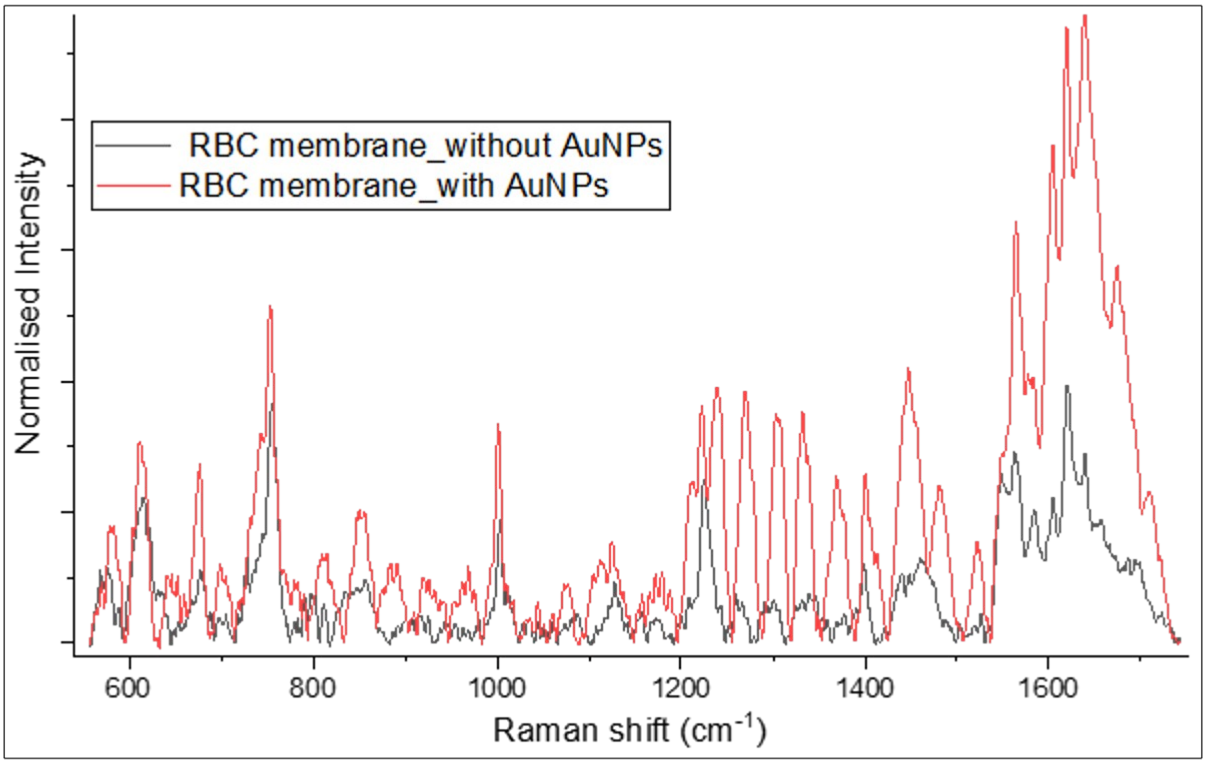

Figure S4: Comparison of the background-subtracted Raman and SERS spectra of RBCs used for calculating enhancement factors for various Raman frequencies.

Table S1: SERS frequencies and corresponding band assignments for RBC trapped using vortex beam at power density (~ 9.8 ×10^4 W/cm^2)

Sl. No.	Raman Frequency	Assignments				
		Heme [1]	**Protein**	**Lipids [2, 3]**	**Carbohydrates [4-7]**	**Coordinates**
1	574	$\nu(Fe\text{-}O_2)$ [8]				
2	584	ν_{48}				δ(pyr deform)$_{sym}$
3	591					
4	647				Sia	δ(ring) + $\rho(CH_2)$
5	652		Cys [8], Met [9]			ν(C-S)
6	659		p:C-S str [10]		Gal	β(CCC), β(CCO), β(OCO)
7	670		p:C-S str [10]		Fuc	
8	674	ν_7	p:C-S str [10]			ν(pyr def)$_{sym}$
9	680		Cys			C_β-S_γ [9]
10	698		Met			ν(C-S) [9]
11	731		Amide V [11]	PS		Serine residue
12	744		Phe			$\omega(C_\zeta$-H) [12]
13	754	ν_{15}, γ_1	Trp			ν (pyr breathing), Pyr foldasym $C_{\varepsilon 2}$-C_2 [12]

14	761					
15	847		Tyr, Lys		Sia	$\omega(C_{\varepsilon2}$-H) [12], C_γ-twist [13], $\delta(CH, CH_2)$
16	876			PC, SM	Fuc	
17	884		Trp		Man, Sia	$C_{\varepsilon3}$-$C_{\zeta3}$-C_η [12], ν(C–O–C)
18	891			PC, PI, Palmitic acid	Gal	ν(CC)
19	896		p: C-C skeletal [14]		Sia	$\delta(C–H) + \tau(C–H_2)$
20	918		Glut, Ire, Thr, Lys			ν(CC)
21	921					
22	925		p: N–C_α–C [10]			
23	931		p: N–C_α–C [10], Glut		Man, Ace-Glu	γ(OH) [15]
24	940	ν_{32}				$\delta(\text{pyr deform})_{asym}$
25	944				Sia	ν(C–O–C) + ν(C–O)
26	954	γ(OH)				???
27	962		Glut			ν(CC)
28	968					

29	975	$\nu(C_c\text{-}C_d)$				$\nu(C_c\text{-}C_d)$
30	987		Pro			Ring str [16]
31	992					
32	1000	C_β-vinyl str	Phe		Gal, Sia	$C_\gamma\text{-}C_{\delta1}$ [12] β(CCH, CCO),
33	1209	$\nu_{5+}\ \nu_{18}$				$\delta(C_mH)$
34	1224	ν_{13}				$\delta(C_mH)$
35	1232	ν_{42}				$\delta(C_mH)$
36	1240		Amide III (β- sheet) [10, 17]			40% ν(C–N)+30% δ(N–H)
37	1244		Amide III (β- sheet) [10] Irregular/turns [17]		Gal	40% ν(C–N)+30% δ(N–H) τ(CH2)
38	1251		Tyr			C_ζ-O-H [12]
39	1259		Trp		Fuc, Ace-Glu, Sia	$C_{\varepsilon2}\text{-}N_{\varepsilon1}$ [12]
40	1268		Amide III (α-helix) [10], Tyr, Hist	PC, PS, PI	Man, Gal-Amine	C_ζ-O [12], $N_\delta\text{-}C_\varepsilon$-H, Im. δ(C–H) [18]
41	1279					
42	1291		Lys, Arg			C_β-twist, C_γ-rock [13]
43	1296			PS, PE, SM	Glu	

44	1301	$\delta(C_aH=)_2$				
45	1309		Glut, p: C_α-H def [10]		Gal	$\omega(CH_2)$
46	1328		Val			$\omega(CH_2)$
47	1332		Trp		Gal, Fuc, Glu	C_β-rock [12], $\omega(CH_2)$
48	1337		Phe, Tyr			C_γ-$C_{\delta1}$-H, C_γ-$C_{\delta2}$ [12]
49	1348					
50	1358		Ala, Hist, Lys, Arg			N_δ-C_ε [18], C_δ-twist [13], $\delta(CH)$, CH_3 symmetric
51	1368		???			
52	1377				Gal, Sia	$\omega(CH_2)$
53	1391		???			
54	1400	ν_{40}				ν(pyr quarter-ring)
55	1405	ν_{29}				ν(pyr quarter-ring)
56	1413		Phe, Glut			C_β-C_α-H [12]
57	1443		Arg	PC, PE, PI		$N_{\eta1}$-C_ζ-$N_{\eta2}$ Asym str [13]
58	1447		p: CH_2 and CH_3 asym. def.	PC, PS, PE, PI: CH def	Fuc	

59	1455		Glut	CH_2 and CH_3 asym. def.	Man, Glu, Sia	$\delta(CH_2)$, $\delta(CH_2)$
60	1480		Hist			Im. ring str. + im. δ(C–H) [19]
61	1499		Hist			C_ε- N_ε [18]
62	1521	ν_{38}				$\nu(C_\beta C_\beta)$
63	1548	ν_{11}				$(C_\beta C_\beta)$ [1]
64	1553		Trp, Amide II,		Ace-Glu	C_γ-$C_{\delta1}$ [12]
65	1564	ν_2				$\nu(C_\beta C_\beta)$
66	1569		???			
67	1577		Trp			$C_{\delta2}$ -$C_{\varepsilon3}$ [12]
68	1583	ν_{37}	---	---		$\nu(C_\alpha C_m)_{asym}$
69	1595					
70	1604	ν_{19}	Phe	---		$\nu(C_\alpha C_m)_{asym}$, $C_{\delta2}$ -$C_{\varepsilon2}$ [12]
71	1619	$\nu(C_a{=}C_b)_{vinyl}$	Tyr			$C_a{=}C_b$, $C_{\delta1}$-$C_{\varepsilon1}$ [12]
72	1632	$\nu(C_a{=}C_b)_{vinyl}$				
73	1637		Amide I (β-sheet) [20]			

74	1652		Amide I (α-helix) [20]			80% ν(C=O)
75	1663		Amide I (α-helix) [20]	PC, PS, PI	Sia	80% ν(C=O), ν(C=C), ν(C=O)
76	1674		Amide I (turn) [20]			80% ν(C=O)
77	1707		Amide I			
78	1721		Amide I			

*__Amino Acids__: Trp-Tryptophan, Phe-Phenylalanine, Tyr-Tyrosine, , Hist-Histidine, Arg-Arginine, Lys-Lysine, Cys- Cysteine, Pro-Proline, Met- Methionine, Glut-Glutamic Acid, and Thr-Threonine, Val- Valine, Ala- Alanine, Ire- Isoleucine ; #**Lipids**: PC-phosphatidylcholine, PE-phosphatidylethanolamine, PS-phosphatidylserine, PI-phosphatidylinositol, and SM-Sphingomyelin; $**Carbohydrates**: Gal-galactose, Man-mannose, Glu-glucose, Fuc-fucose, and Sia-sialic, (Ace-Glu)-(acid N-acetyl-D-glucosamine) and, (Ace-Gal)-(N-acetyl-D-galactosamine).

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