Supporting Information for

SERS spectrum in the regime of one and a few molecules of acid 4- mercaptobenzoic in AgNP enabled by hot spots generated by hydrogen bonding.

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S1. The atomic coordinates for the optimized structure of model complex [AgNP-(4-MBA)$_2$-AgNP].

	X	Y	Z
C_1	5.6051420	-0.6721180	1.6644390
C_2	5.2594700	0.6844570	1.7312970
C_3	3.9435920	1.0896960	1.5570090
C_4	2.9415970	0.1458610	1.3151630
C_5	3.2792040	-1.2094590	1.2508750
C_6	4.5934610	-1.6133410	1.4240910
H_7	6.0287920	1.4251840	1.9209390
H_8	3.6906580	2.1423660	1.6086060
H_9	2.5015360	-1.9415170	1.0634820
H_{10}	4.8461350	-2.6668600	1.3702460
C_{11}	1.5330990	0.5479770	1.1284220
O_{12}	0.6315170	-0.2767130	0.9344550
O_{13}	1.3055660	1.8427080	1.1833020
H_{14}	0.3221960	2.0418210	1.0493180
S_{15}	7.2796390	-1.2040710	1.9519720
C_{16}	-6.2120540	2.8404700	0.2736050
C_{17}	-5.8623450	1.4769190	0.2442800
C_{18}	-4.5459880	1.0685270	0.3758850
C_{19}	-3.5218300	2.0080780	0.5467110
C_{20}	-3.8544360	3.3683400	0.5805690
C_{21}	-5.1702590	3.7750980	0.4468080
H_{22}	-6.6363050	0.7259560	0.1180160

H_{23}	-4.3099520	0.0100740	0.3483180
H_{24}	-3.0671410	4.1027170	0.7131970
H_{25}	-5.4095720	4.8332610	0.4743030
C_{26}	-2.1192530	1.6000160	0.6927400
O_{27}	-1.1980860	2.4152850	0.8515630
O_{28}	-1.8996720	0.2997450	0.6448360
H_{29}	-0.9196720	0.0991390	0.7549820
S_{30}	-7.8692280	3.4123500	0.1111320
Ag_{31}	8.7674300	0.3728400	0.5779310
Ag_{32}	10.1219240	-0.4854190	-1.6638400
Ag_{33}	7.9345630	-2.3552700	-0.2112610
Ag_{34}	10.4301170	2.0480250	-0.7830180
Ag_{35}	-8.8474140	-1.3775380	-0.2426790
Ag_{36}	-9.3349430	1.4156160	-0.3334180
Ag_{37}	-11.2991500	-0.3115960	-0.9098000
Ag_{38}	-0.9098000	-2.9452950	0.3589430

S2. UV-Vis spectra of AgNP and AgNP at pH= 4.

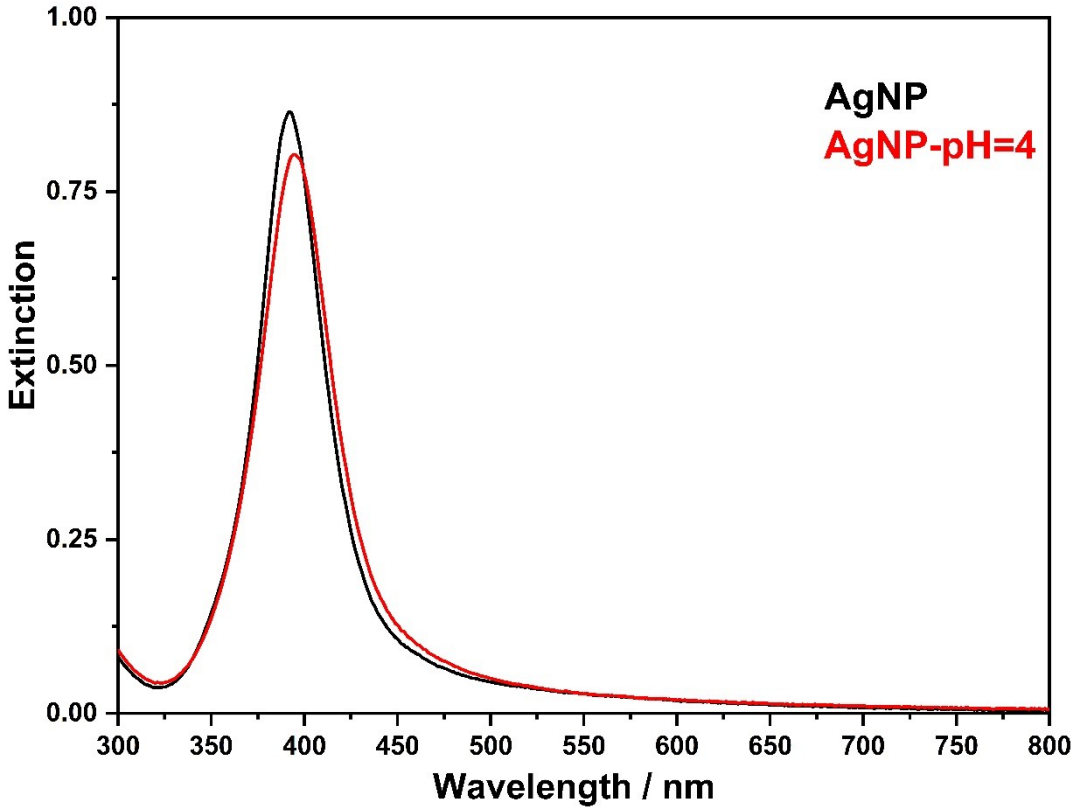

Figure S2: Extinction spectrum of Ag colloid compared to Ag colloid after the addition of 5 µL of HCl 0.1 mol L^{-1} (pH=4).

S3. Evolution of UV-Vis spectra of 4-MBA (5.0×10^{-7} mol L^{-1}) adsorbed on AgNP after the addition of 50.0 µL of HCl 0.1 mol L^{-1} as a function of time.

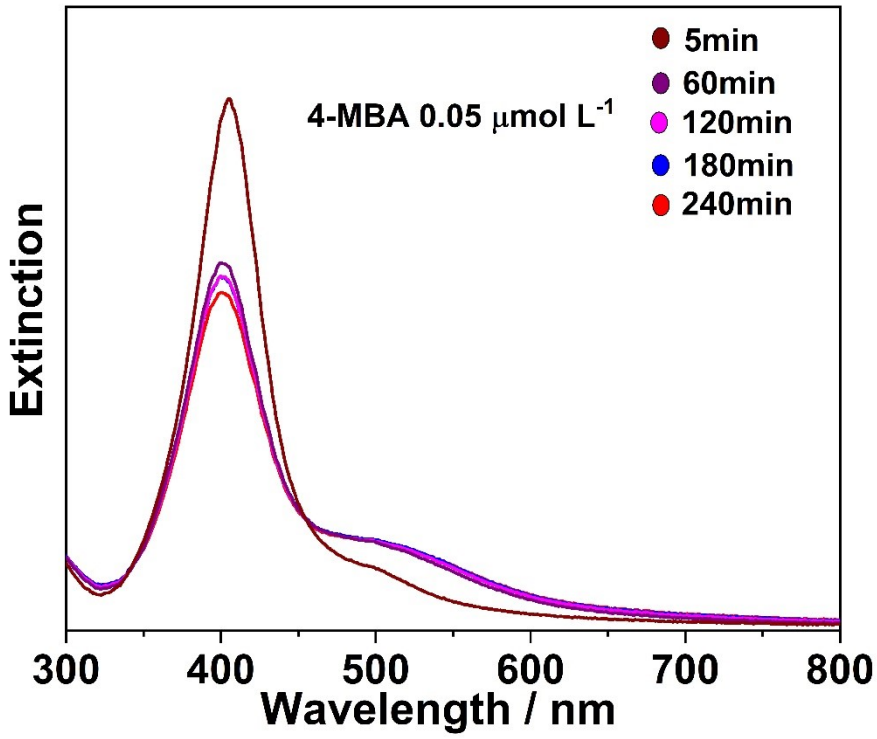

Figure S3: UV-Vis spectra of 0.05 µmol L^{-1} 4-MBA with added HCl to pH=4 as a function of time after adding the acid.

S4. DLS results of the size distribution for the AgNP suspensions with 4-MBA (5.0×10^{-7} mol L^{-1}) in pH=4, 6, and 9.

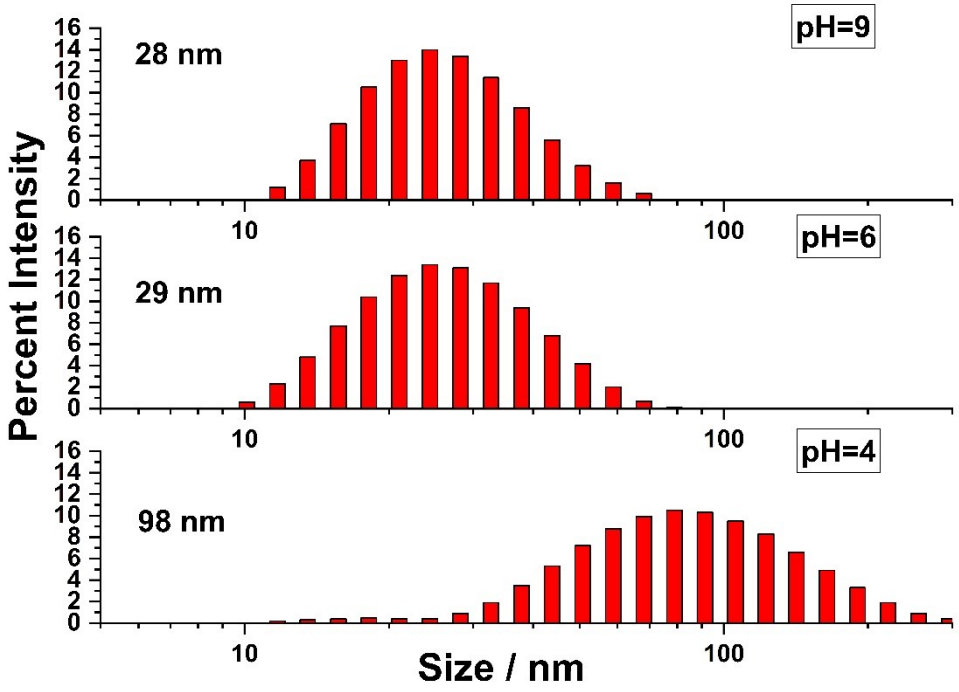

Figure S4. AgNP size distribution was measured by DLS in pH=9, 6, and 4, respectively.

S5. DLS analysis of the size distribution for the AgNP suspensions with 4-MBA in different concentrations.

Confidence intervals (CI) were estimated for the size distributions of AgNP as a function of the concentration of 4-MBA obtained from the DLS analysis compared to AgNP without the addition of 4-MBA. An asymmetrical sample distribution can be seen in the histograms, *i.e.*, a Log-Normal distribution. For a 95% confidence level, the confidence interval a Log-Normal sample distribution is given by the lower and upper limits described by Equation S1.[1]

$$e^{(\ln(\bar{d}) - (1.96 * \sigma))} \leq CI \leq e^{(\ln(\bar{d}) + (1.96 * \sigma))}$$

Equation S1

Where $\bar{d}$ is an average diameter, σ the standard deviation and the 95% confidence level is 1.96. The confidence intervals are shown in Figure S5.

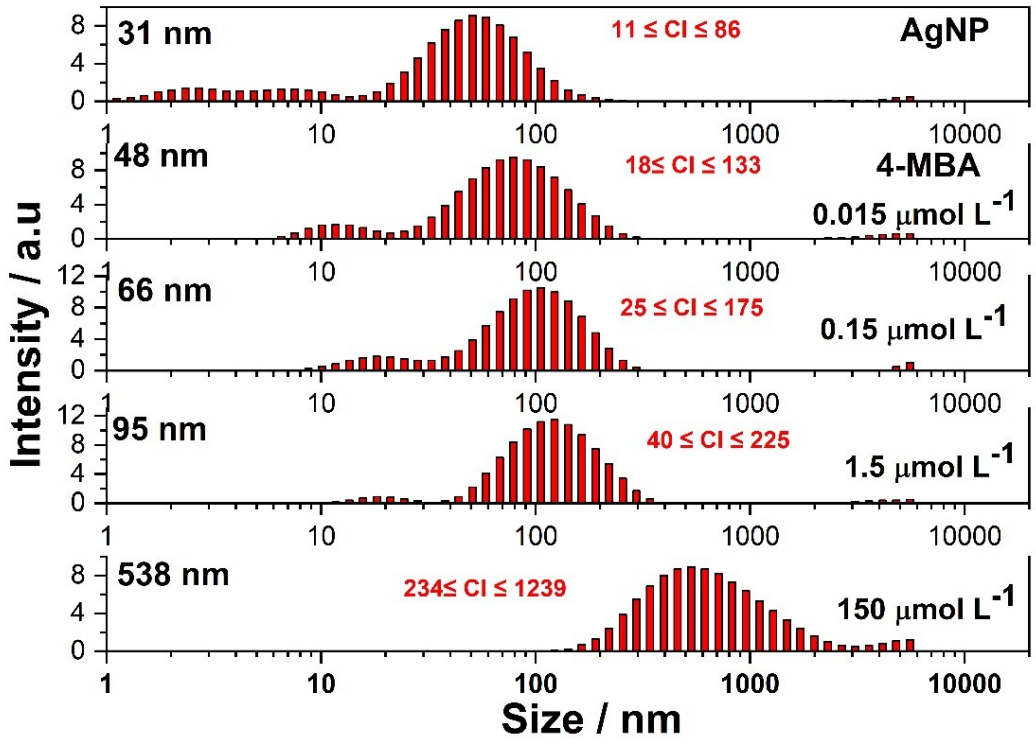

Figure S5: Size distribution of AgNP nanostructures as a function of 4-MBA concentration obtained from DLS analysis. The first result was obtained for AgNP without any 4-MBA. Each result presents the average size measured in the analysis.

S6. The Raman spectrum of the SERS substrate in a 1:1 ratio (AgNP: water) at pH=4.

To overcome the difficulties of obtaining the SERS spectrum of 4-MBA in a single-molecule regime, a SERS substrate that does not present a signal in the same region as the adsorbate was used. Figure S6 shows the spectrum of the SERS substrate obtained in a 1:1 ratio (AgNP: water), with pH adjustment to 4 with HCl 0.1 mol L^{-1}. Note a low-intensity band at 1638 cm^{-1} referring to the water in the solution, attributed to the angular deformation of the water. Other Raman bands are not observed in the obtained spectrum. Therefore, AgNP was used to obtain the SM-SERS spectrum.

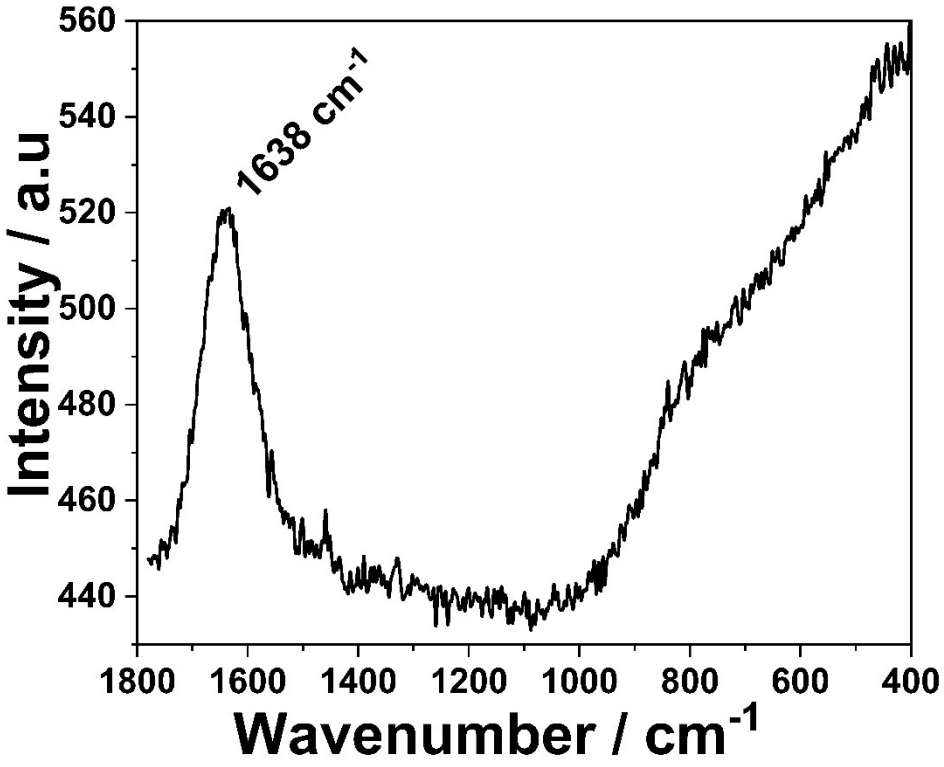

Figure S6: Spectrum of the SERS substrate in a 1:1 ratio (AgNP: water) at pH=4.

S7. Classical electrodynamics simulations.

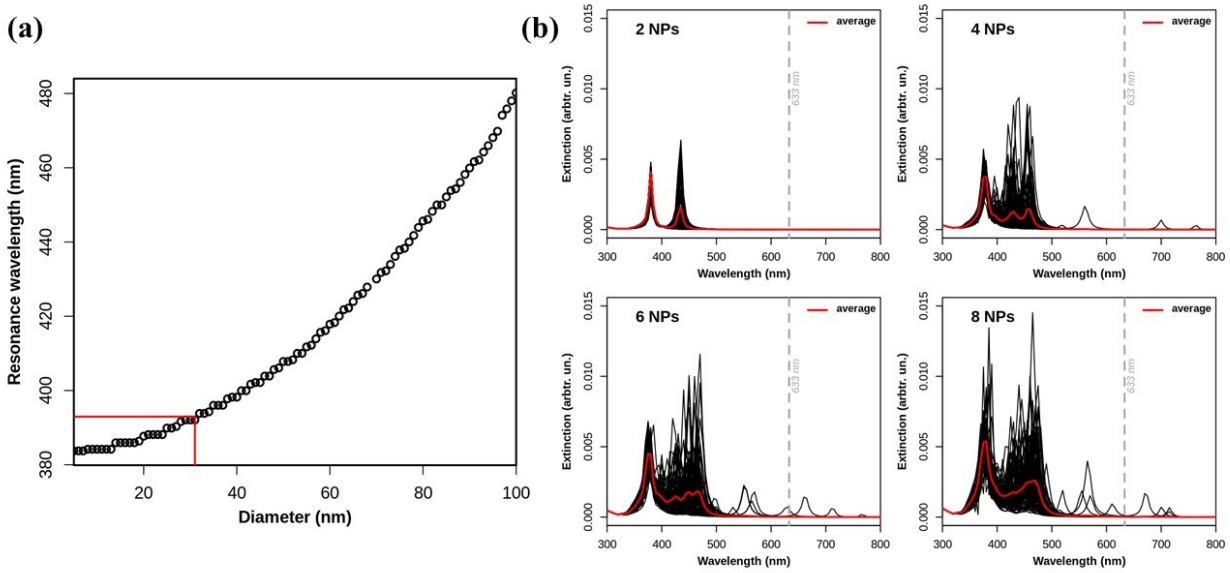

Figure S7: (a) The diameter nanoparticles of the 30 nm was obtained from fitting of ressonance wavelength versus diameter by simulated via classical electrodynamics using the generalized mie theory (GMT) and (b) Simulated extinction spectrum for different aggregates randomly formed by 30 nm nanoparticles.

References

1. E. Taylor and T. J. Webster, Reducing infections through nanotechnology and nanoparticles., *Int. J. Nanomedicine*, 2011, **6**, 1463–1473.