Electronic Supplementary Material (ESI) for Nanoscale.

New Insight on the Aptamer Conformation and Aptamer/protein Interaction by Surface Enhanced Raman Scattering and Multivariate Statistical Analysis

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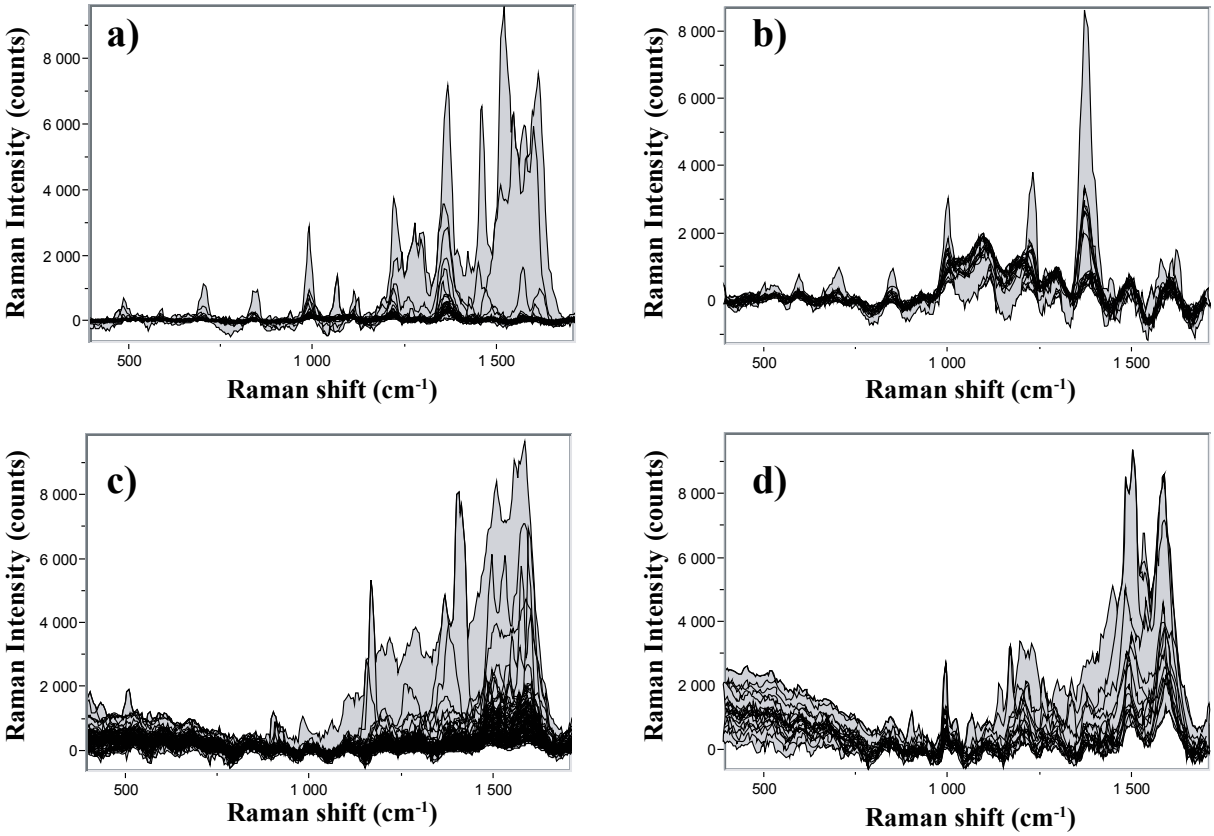

Figure S1: Whole sets of SERS spectra recorded for the aptamer alone (Apt-wT and Apt-w/oT) in the different experimental conditions (in air and in buffer). a) Apt-wT in air (accumulation time: 5s and laser power: 1.4mW, 60 spectra), b) Apt-wT in buffer (accumulation time: 45s and laser power: 1.4mW, 42 spectra), c) Apt-w/oT in air (accumulation time: 15s and laser power: 1.4mW, 150 spectra), d) Apt-w/oT n buffer (accumulation time: 30s and laser power: 1.4mW, 100 spectra). The SERS spectra were baseline corrected using the automatic function of the Labspec 6 software (Xplora Raman spectrophotometer, Horiba Scientific)

The oscillations that can be observed on the Raman spectra of the figures S1 and S2 can be assigned to the Raman signal from the raw substrate. They are observable on the corrected Raman spectra as the baseline subtraction are not perfect (see S6 to get the Raman spectrum recorded on the raw SERS substrate without molecules deposited on it and the baseline corrected spectrum). Indeed as the calculated baseline does not fit perfectly the spectrum one, the baseline of the new spectrum is not linear. This is especially observable when the SERS signal is low. Even if one can make a better baseline fit on one individual spectra, it is no more possible on a whole mapping as this process is done automatically for all Raman spectra of one mapping.

Such oscillations has also no influence on the assignment of the bands as the band intensities are much larger than the oscillation intensity, especially on the figure S1. For figure S2, the band intensity is lower than for figure S1 but as we only observe the intensity decrease with the MnSOD concentrations, the assignment is done unambiguously on the most intense spectra (S2a) and reproduce for the other spectra obtained with other MnSOD concentrations.

In any case, the most important thing is that such oscillations have no influence on the study or on the analysis of the data because they do not appear in the first components (no observation of these oscillations in the intensities of PC1 to 3, see S5). This means that their intensities remain constant over the whole mapping and over all the measured spectra (which can be seen on figures S1 and S2). They have no contribution to the spectral variations measured during the PCA analysis.

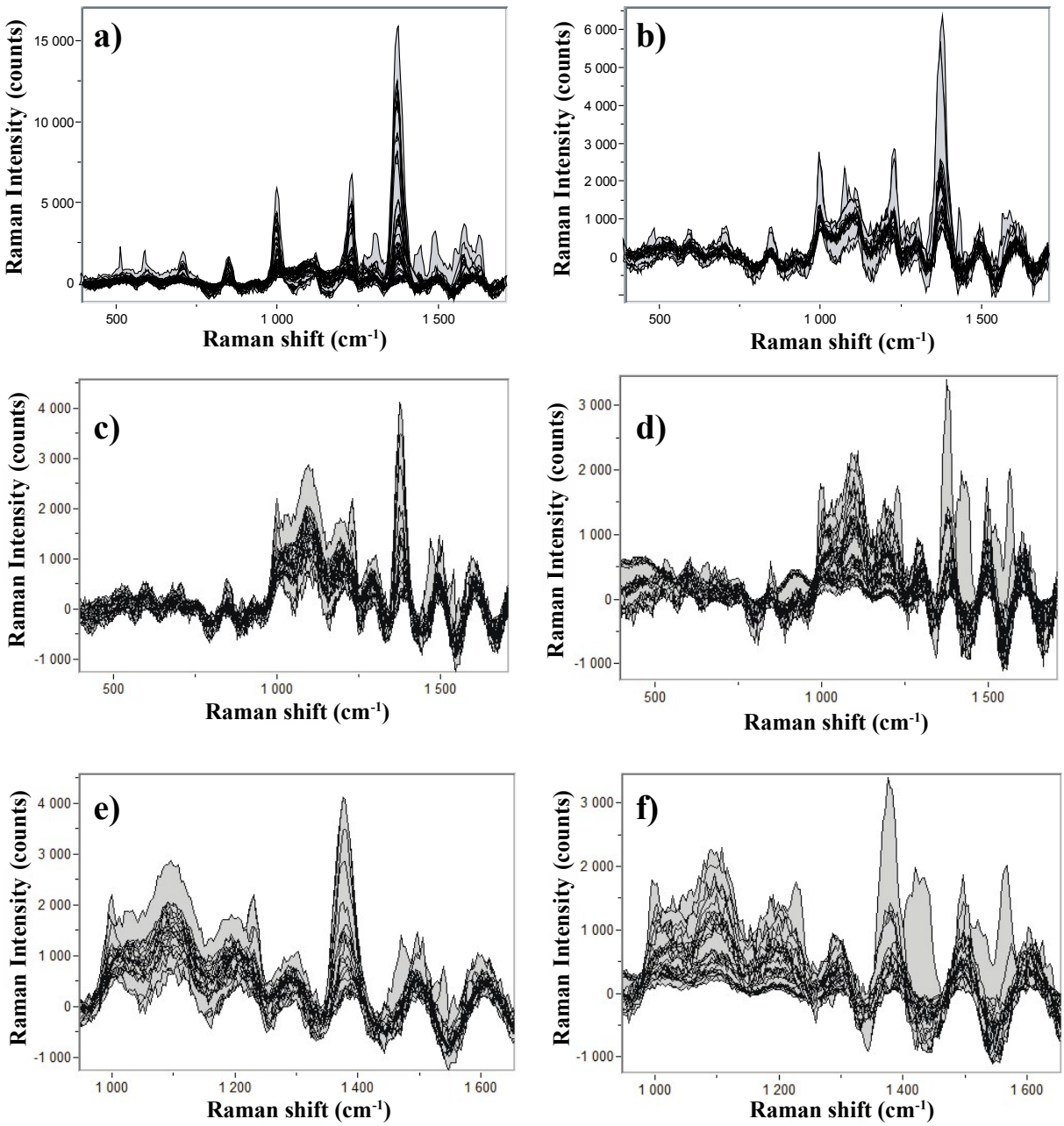

Figure S2: Whole sets of SERS spectra recorded for the Apt-wT in interaction with different concentrations of MnSOD from 0 up to 1000 nM in buffer. a) 1 nM (accumulation time: 40s and laser power: 1.4mW, 108 spectra), b) 10 nM (accumulation time: 40s and laser power: 1.4mW, 104 spectra), c) 100 nM (accumulation time: 40s and laser power: 1.4mW, 93 spectra), d) 1000 nM (accumulation time: 60s and laser power: 1.4mW, 72 spectra), e) and d) zoom of the figures c and d, respectively. The SERS spectra were baseline corrected using the automatic function of the Labspec 6 software (Xplora Raman spectrophotometer, Horiba Scientific)

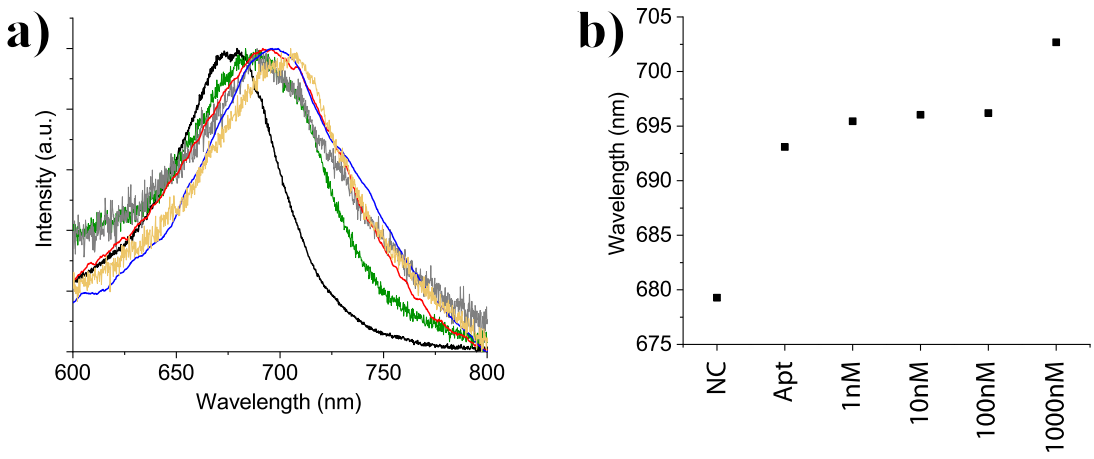

Figure S3: a) Extinction spectra of the SERS substrate for different concentrations of MnSOD (black spectrum: nanocylinders alone, green spectrum: nanocylinders with aptamers, grey spectrum: nanocylinders with aptamers and with 1nM of MnSOD, red spectrum: nanocylinders with aptamers and with 10nM of MnSOD, blue spectrum: nanocylinders with aptamers and with 100nM of MnSOD, brown spectrum: nanocylinders with aptamers and with 1000nM of MnSOD), b) Evolution of the plasmon resonance before (NC) and after the grafting of the aptamer (Apt) and after the deposition of the different concentrations of MnSOD (1nM, 10nM, 100nM and 1000nM).

The LSPR analysis were done in dry condition and one can notice successive redshifts of the LSPR with the grafting of the aptamers and the increase of the MnSOD concentrations. This is a clear indication of the increase of the number of molecules (aptamers or MnSOD) at the nanocylinder surface. Moreover, one can observe that from the aptamer alone to the largest concentration of MnSOD, the LSPR shift follows a profile similar to a langmuir isotherm with a redshift between the 100 nM and 1000 nM MnSOD concentrations much larger than between the other concentrations (1, 10 and 100 nM). This means that the coverage rate of the aptamer layer by the MnSOD at 1000 nM is largely higher than the one at 100 nM as we cross the affinity constant value (330 nM) between 100 nM and 1000 nM. With such behaviour, we assume that there is no removal of the aptamers from the nanocylinder surface during the whole experiments, especially in the buffer condition. Indeed, a removal of the aptamers would have decreased the number of molecules at the nanocylinder surface and thus would have induced a blueshift of the LSPR that is not observed. Consequently, there is no loss of aptamers between two experiments. As all the SERS measurement are done on the same substrate successively in dry and wet conditions, the decrease of the SERS intensity could not be due to the aptamer removal but to a modification of the orientation of the aptamers in interaction with the MnSOD.

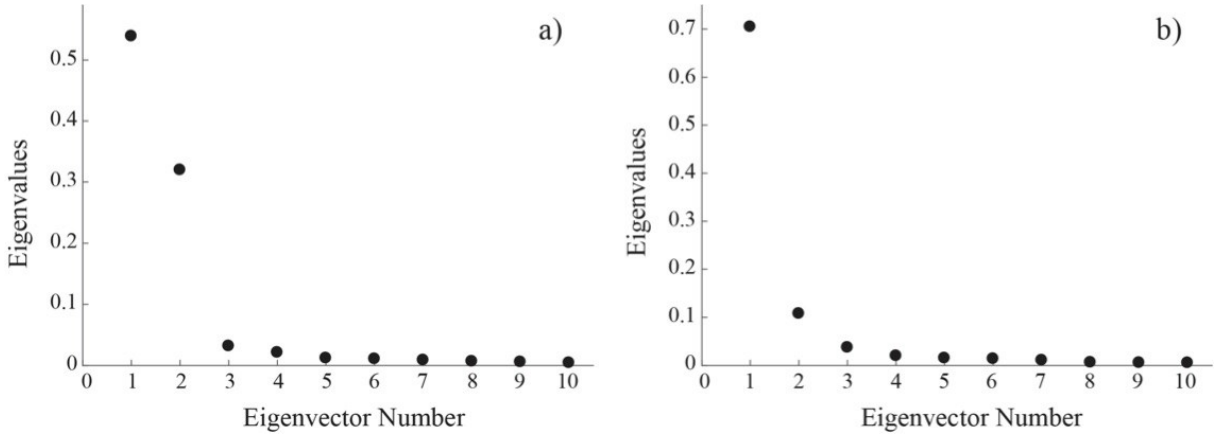

Figure S4: a) Eigenvalues of the different components determined by the PCA for the aptamer alone (Apt-wT and Apt-w/oT in air and in buffer), b) Eigenvalues of the different components determined by the PCA for the aptamer in interaction with different concentrations of MnSOD from 0 up to 1000 nM.

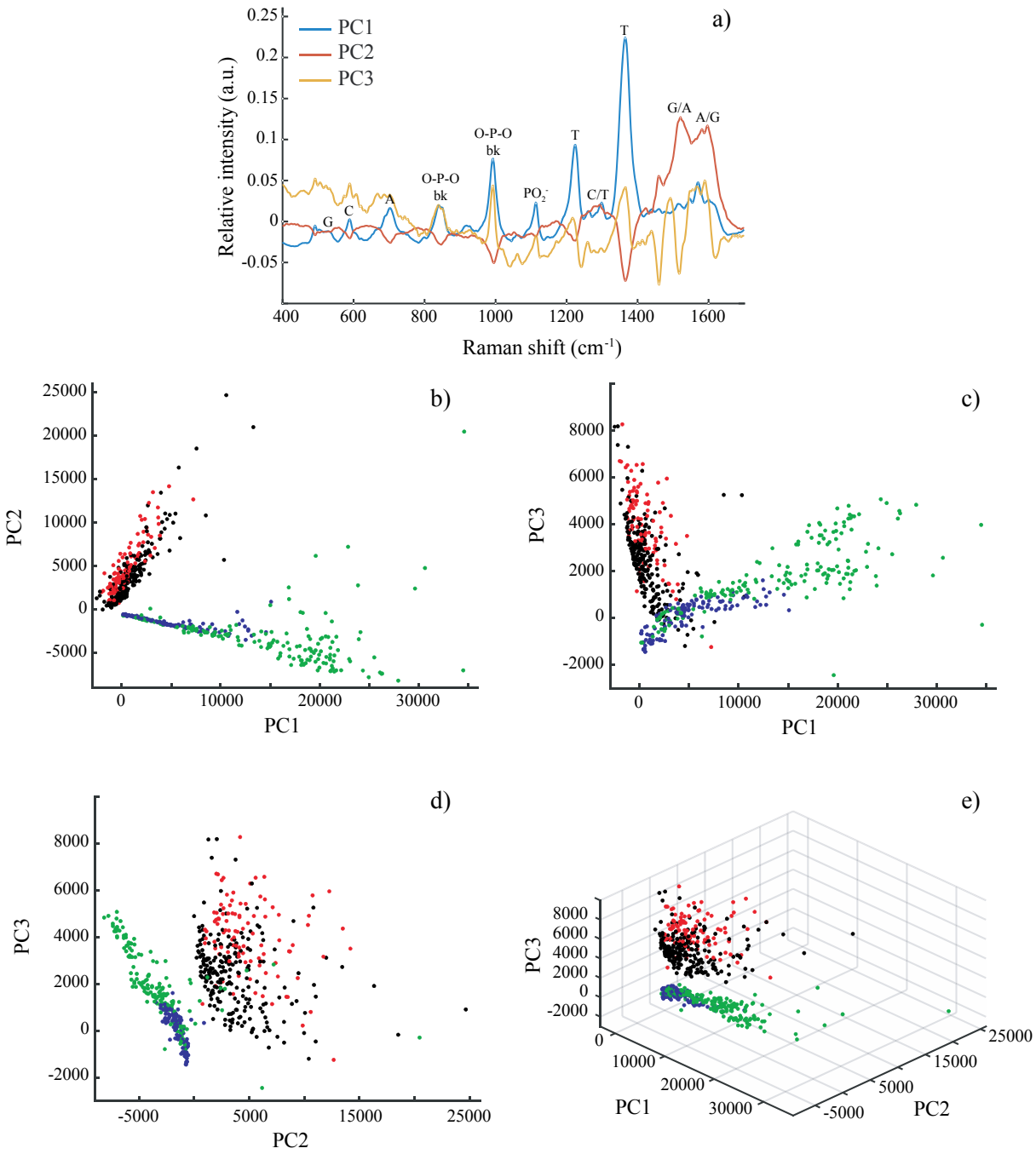

Figure S5: a) Three first principal components for the analysis of aptamer with and without T spacer in air and in buffer (blue curve: PC1, red curve: PC2, orange curve: PC3), b-e) PC scores for aptamer with and without T spacer in air and in buffer (black dots: aptamer without T spacer in air, red dots: aptamer without T spacer in buffer, green dots: aptamer with T spacer in air, blue dots: aptamer with T spacer in buffer) represented for different components (b - PC1 and PC2, c - PC1 and PC3, d - PC2 and PC3 and e - PC1, PC2 and PC3)

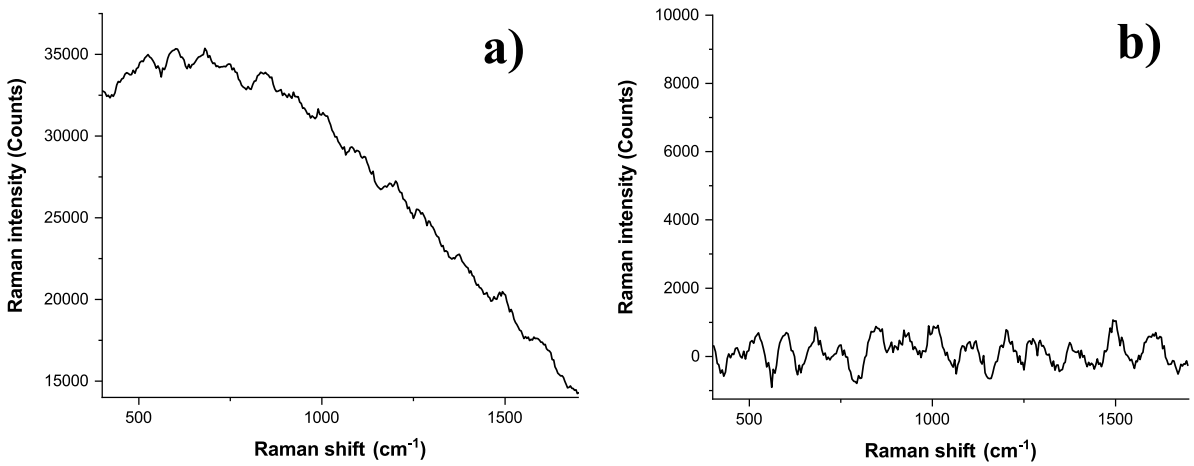

Figure S6: a) Raman spectrum of the raw SERS substrate without molecules deposited on it (excitation wavelength: 638 nm, accumulation time: 10s and laser power: 1.4mW), b) The SERS spectrum after baseline corrected using the automatic function of the Labspec 6 software (Xplora Raman spectrophotometer, Horiba Scientific)

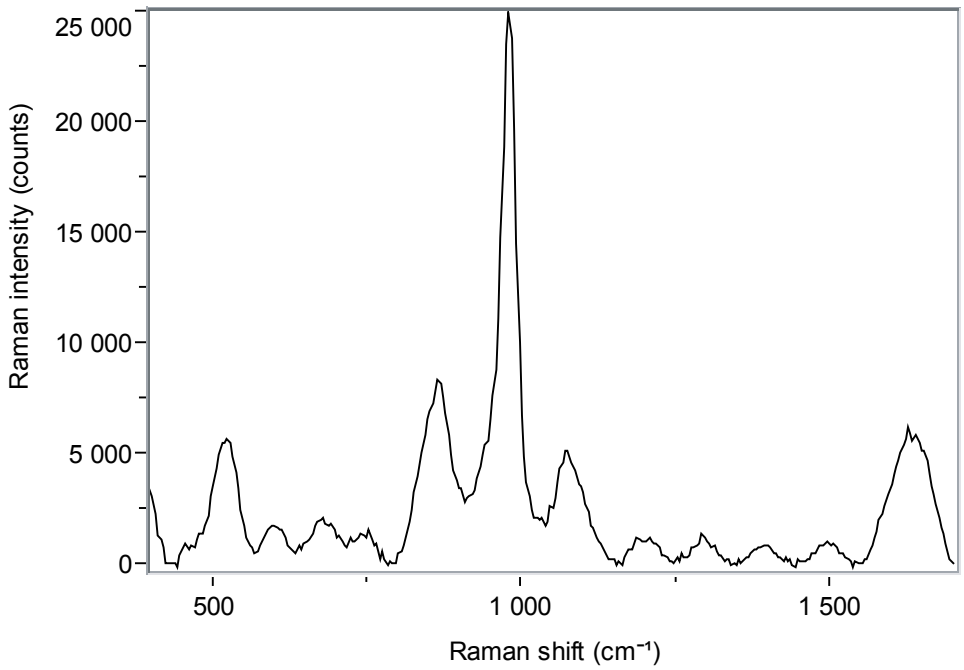

Figure S7: Reference Raman spectrum of the PBS buffer dried on a CaF_2 substrate. (excitation wavelength: 638 nm, accumulation time: 130s and laser power: 14mW) As no signal of the buffer can be recorded in solution, the buffer has been dried on the substrate and the signal was recorded on a crystal formed at the substrate surface.

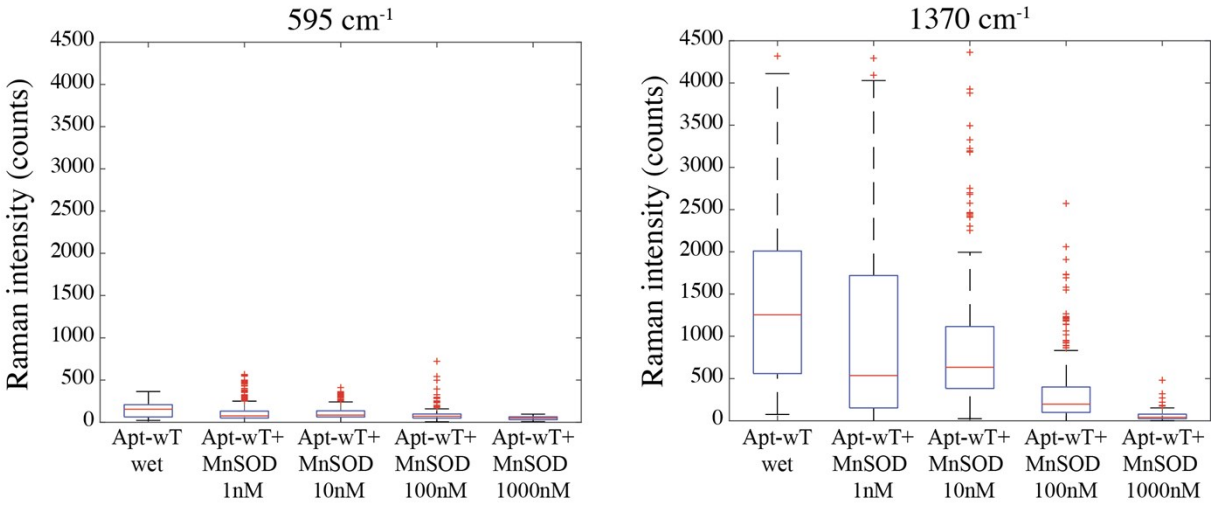

Figure S8: Evolution of the intensity of the bands at 595 cm^{-1} and 1370 cm^{-1} for the aptamer with T spacer in interaction with different concentrations of MnSOD (apt-wT wet: 0nM, apt-wT wet+MnSOD 1nM: 1nM, apt-wT wet+MnSOD 10nM: 10nM, apt-wT wet+MnSOD 100nM: 100nM, apt-wT wet+MnSOD 1000nM: 1000nM). For each figure, the red line corresponds to the median (50% of the population), the blue box to the quartile deviation (± 25% of the population around the median), the black lines to 1.5 time the quartile deviation and the red dots to the outliers.

Band position (cm^{-1})	Apt-wT	Apt-w/oT	References
500-536	Guanine (ring mode)		3,5
600-612	Cytosine (ring mode)		1,3
700-720	Adenine (ring mode)		3,4
854-856	Desoxyribose phosphate backbone (νO-P-O)		4,5
1006	Desoxyribose phosphate backbone		5
1113-1126	νPO_2^-		3–5
1220		Cytosine + Guanine	1,3
1237	Thymine (ν, ring mode))		1,4,5
1272-1310	Cytosine + Thymine (in-plane)		1,2,5
1378	Thymine (in-plane, ring mode)		1,2,4,5
1503		Guanine + Adenine (ring mode)	1,3-5
1527	Guanine (in-plane, νC=C)		1,2
1590-1600	Adenine + Guanine (ring mode)		3-5
1620	Thymine + Cytosine (νC=O)		1,3,6

Table S1: assignment of the bands observed on the Apt-wT and Apt-w/oT SERS spectra.

References:

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MnSOD concentration (nM)	Median angle (°)	Occupation rate
0	20°	0%
1	30°	14%
10	29°	13%
100	56°	51%
1000	90°	100%

Table S2: Median angle of the dot dispersion and occupation rates calculated for the different concentrations of MnSOD

The uptake rate was estimated using two simple assumptions: (i) this rate is proportional to the median angle calculated from the angular dot dispersion; (ii) the 0% rate corresponds to the median angle (20°) calculated with the dot dispersion obtained for the aptamer alone whereas the 100% rate corresponds to the median angle (90°) calculated with the dot dispersion obtained for the 1000 nM MnSOD solution assuming that the surface is saturated by the MnSOD at this concentration.