Supplementary Materials: Molecular Specific and Sensitive Detection of Pyrazinamide and Its Metabolite Pyrazinoic Acid by Means of Surface Enhanced Raman Spectroscopy Employing In Situ Prepared Colloids

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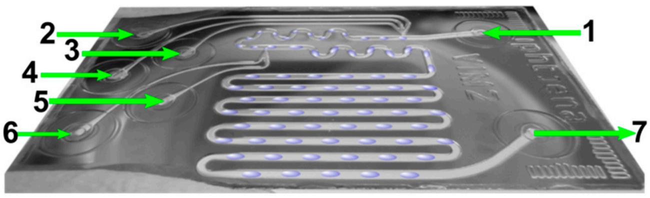

Figure S1. Droplet based microfluidic chip used for in situ nanoparticle synthesis and automated and reproducible SERS measurements. Port 1: Mineral oil, port 2: $AgNO_3/NH_3$, port 3: Sodium citrate, port 4: N_2H_4, port 5: Sample containing the target molecule, port 6: Aggregation agent and port 7: Waste.

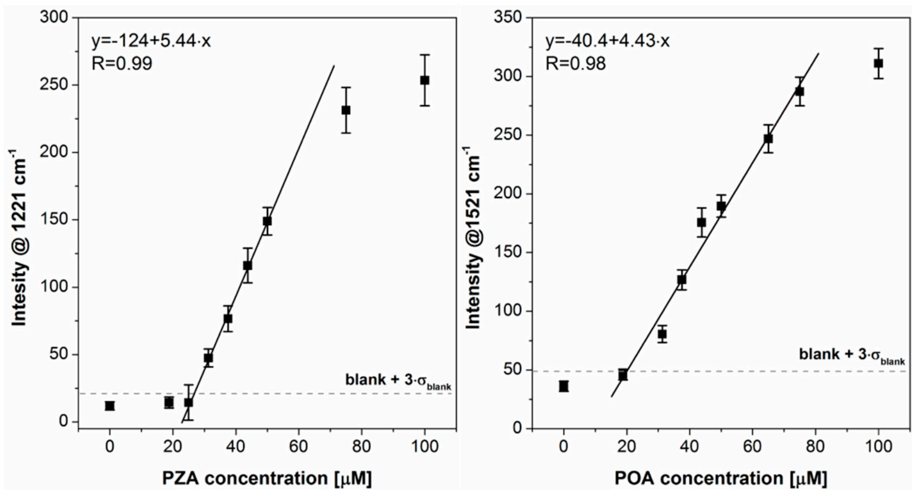

Figure S2. Determination for the limit of detection for PZA and POA solved in high purity water measured in LoC-SERS device applying in situ produced AgNPs (y-AgNPs).

Table S1. Band assignment for PZA and POA according to the cited literature.

PZA Wavenumber Experimental	Wavenumber Lit. B3LYP/6-31G(d) [1]	Assignment
471	497	NH_2 rocking
718	739	The OCN bending, out of plane
1021	1000	In-plane bending vibration of the pyrazine ring
1055	1037	
1218	1206	Symmetric stretching of the amide group
1270	1274	In-plane bending of the C-H groups
1366	1397	Ring vibration +NH_2 in-plane bending
1440	1457	NH_2 bending
1528	1556	CN stretching vibrations of the pyrazine ring
1580	1569	Symmetric ring stretching
3066	3075	CH stretching

POA Wavenumber Experimental	Wavenumber Lit. B3LYP/6-31G(d) [2]	Assignment
448	451	OCO rocking
734	739	The OCO bending, out of plane
852	852	Ring breathing
1021	1000	In-plane bending vibration of the pyrazine ring
1055	1037	
1186	1189	CH In plane bending
1380	1376	Sym. OCO vibration
1410	1411	Ring vibration
1528	1556	CN stretching vibrations of the pyrazine ring
1565	1565	Asym. OCO vibration
3066	3075	CH stretching

Measurement Overview for All Spectra Depicted in Figures 1–5 of the Main Manuscript:

Figure 1. Reference Raman and SERS spectra of PZA and POA.

For the Raman measurements the analyte in a powder form was placed on a microscope glass slide, the laser light was focused on the sample surface via a 20×/0.4 N.A. objective. The power incident on the PZA powder was 20 mW, whereas for POA 5 mW was used. Each spectrum is the result of 10 accumulations of 1 s each. For recording the SERS spectra the same objective, 20 mW and 1 s with 10 accumulations was employed.

Figure 2: Varying flowrates for mineral oil, $AgNO_3$ with NH_3 and N_2H_4 in the microfluidic chip device. The flowrates of PZA (10^{-4} M) and $NaNO_3$ were kept constant at 10 nl/s. With addition of citrate (10^{-5} M) depicted in black, without citrate plotted in gray. In light gray the standard deviation values are indicated.

Flow rates:

Port 1	Port 2	Port 3	Port 4	Port 5	Port 6
Mineral oil	$AgNO_3/NH_3$	Citrate 10^{-5} M	N_2H_4	Analyte	Na_2NO_3
5–30 nl/s	5–30 nl/s	5 nl/s	5–30 nl/s	10 nl/s	10 nl/s

Figures 3 and 4: Average LoC-SERS spectra of different PZA concentrations (Figure 3) and different POA concentrations (Figure 4) in water.

Flow rates:

Port 1	Port 2	Port 3	Port 4	Port 5	Port 6
Mineral oil	$AgNO_3/NH_3$	Citrate 10^{-5} M	N_2H_4	Analyte	Blocked
20 nl/s	20 nl/s	5 nl/s	20 nl/s	40 nl/s	

Figure 5: Average LoC-SERS spectra of mixtures of PZA and POA in water. Mixing ratios of analyte stock solutions (10^{-4} M) are indicated in the graph. Mixing was done before injection into the chip.

Flow rates:

Figure 5a

Port 1	Port 2	Port 3	Port 4	Port 5	Port 6
Mineral oil	$AgNO_3/NH_3$	Citrate 10^{-5} M	N_2H_4	Analyte PZA/POA	$NaNO_3/HNO_3$
20 nl/s	20 nl/s	5 nl/s	20 nl/s	30 nl/s	10 nl/s

References

1. Chiş, V., Pirnau, A., Jurca, T., Vasilescu, M., Simon, S., Cozar, O., David, L. Experimental and DFT study of pyrazinamide. *Chem. Phys.* **2005**, *316*, 153–163, doi: 10.1016/j.chemphys.2005.05.004
2. Castro, J.L., Montanez, M.A., Otero, J.C., Marcos, J.I. Enhanced Raman Scattering from Pyrazinoic Acid on Silver and Gold Sols. *Spectrosc. Lett.* **1993**, *26*, 237–244, doi: 10.1080/00387019308011528