Electronic Supplementary Material (ESI) for Chemical Communications

Acoustically Controlled Enhancement of Molecular Sensing to Assess Oxidative Stress in Cells

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Supplementary Material

A- Materials and Methods:

1. Interdigitated Transducer

The IDT were microfabricated on piezoelectric 128° Y-cut X-propagating 4-inch double-sided polished (transparent) $LiNbO_3$ wafers. The devices consisted of 20 pairs of electrodes to form an inter-digitated transducer (IDT) with a pitch of 200 μm, 100 μm width, and a 5 mm aperture, yielding a frequency of ~10 MHz for the propagating SAW. The IDT was connected to an Agilent Technologies MXG Analog Signal Generator N5181A in conjunction with a Mini Circuits ZHL-5W-1, 5-500MHz amplifier and a 3A, ±24V DC power supply. The device was used at the third harmonic frequency (~30 MHz).

A hydrophilic spot of 3 mm in diameter was patterned using standard lithography. The rest of the surface was made hydrophobic by a silane (FOTS, Sigma), obtained by immersing the photoresist-patterned (AZ4562) wafer in a 1.6 mM silane solution in heptane (Sigma, H9629) for 10 min and dissolving the resist in acetone.

2. Nanoparticle Preparation and Functionalisation

Colloidal silver nanoparticles (mean diameter 63 nm) were prepared according to the literature.[1] To functionalise nanoparticles with 4-mercaptobenzoic acid (4-MBA), 50 μl of a 300 μM 4-MBA ethanol solution was added to a 1 ml solution of colloid. Before use, the mixture was left to stand at room temperature for 2 h, allowing thiol chemisorption onto the metal surface. Dilutions were carried out in DI water.

3. Raman Microscopy

All Raman spectra were recorded using a Horiba Jobin Yvon LabRam inverted microscope spectrometer. SAW devices and samples were mounted atop a precision motorised X-Y-Z sample stage, onto which laser light (533 nm, 70 mW) was focused using a 20 × Nikon UPlanFL objective (NA =0.5). Spectra were recorded between 1000-2000 cm^{-1} with an acquisition time of 15 s. All spectra were processed using LabSpec 5 software.

4. Cell culture.

Jurkat cells (ATCC - CRL-2063) were cultured in RPMI 1640 with 10% heat-inactivated foetal calf serum and 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cultures were incubated at 37°C in humidified air with 5% carbon dioxide. Cells were transferred into a 5 ml culture flask at a concentration of 1 million cells/ml, before stimulation with the appropriate concentration of H_2O_2 (Sigma, 216763) (from a stock solution at 10 mM in water). After an overnight incubation, the cells were washed once in PBS and resuspended in a low conductivity solution (8.5 % w/v sucrose, 0.3 % w/v glucose).[2] The pH was adjusted to 7 with NaOH before introducing the cells.

The cells were lysed acoustically using SAW (9.4 MHz, 1 W) in 10 μl drops. 2.5 μl of the lysed sample was mixed with 2.5 μl of colloid solution on the SAW-aggregation platform and the SERS spectra obtained.

B- Supplementary Figures

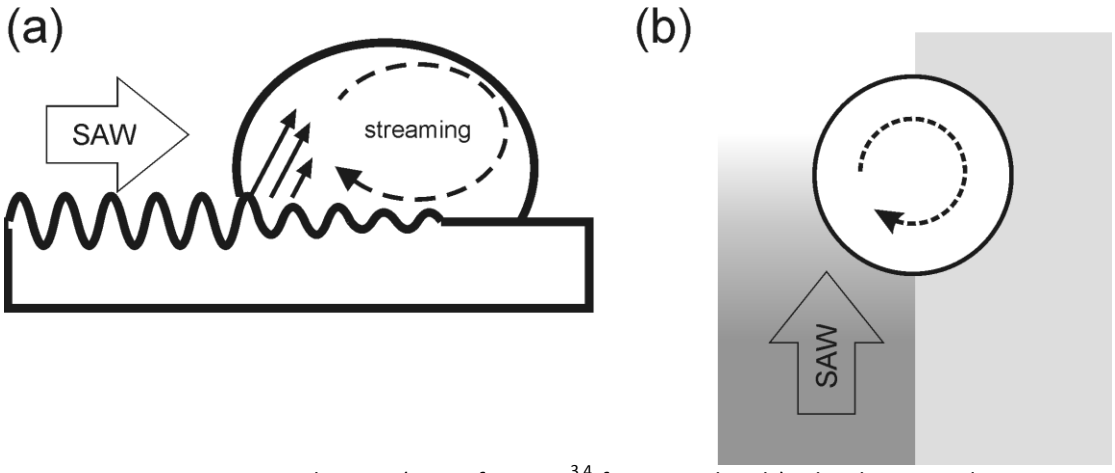

Figure. S1. Aggregation mechanism (see references[3,4] for more details). The drop sample containing the colloids is positioned half way over the propagating path of the SAW, so that only one side is interfaced with the acoustic energy. The mechanical vibrations of the waves refract into the liquid as longitudinal waves, leading to recirculation (a) and angular momentum (b). The flow patterns created share characteristics with the 'tea leaf effect', as described by Batchelor,[5] and lead to the concentration of particles in the centre of the drop. This effect is dependant on the size[6] and the mechanical properties of particles[7].

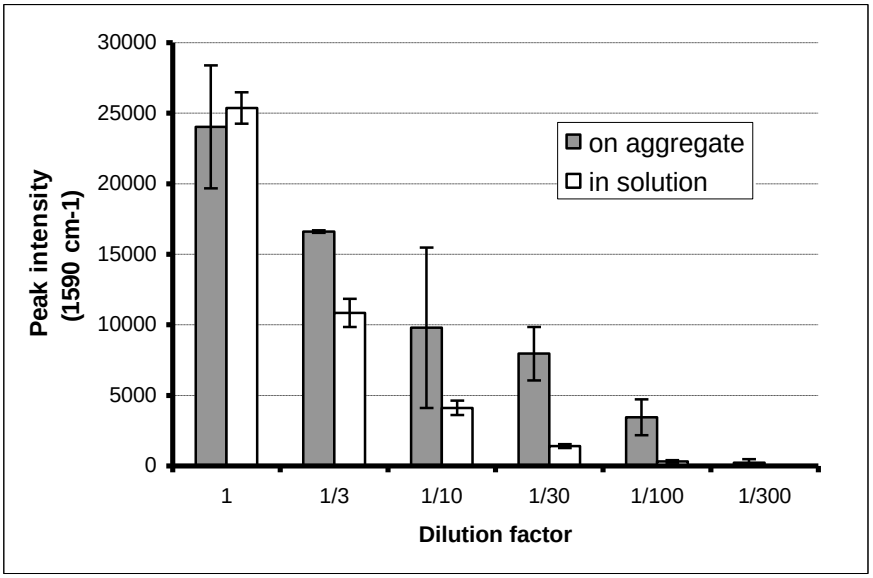

Figure S2. Average intensity of the peak at 1590 cm^{-1} for different dilutions of the silver colloid solution after SAW aggregation and in the bulk solution. This data was used to calculate the ratios presented in Fig 2a within the main text. Error bars are standard deviation over a minimum of 3 experiments.

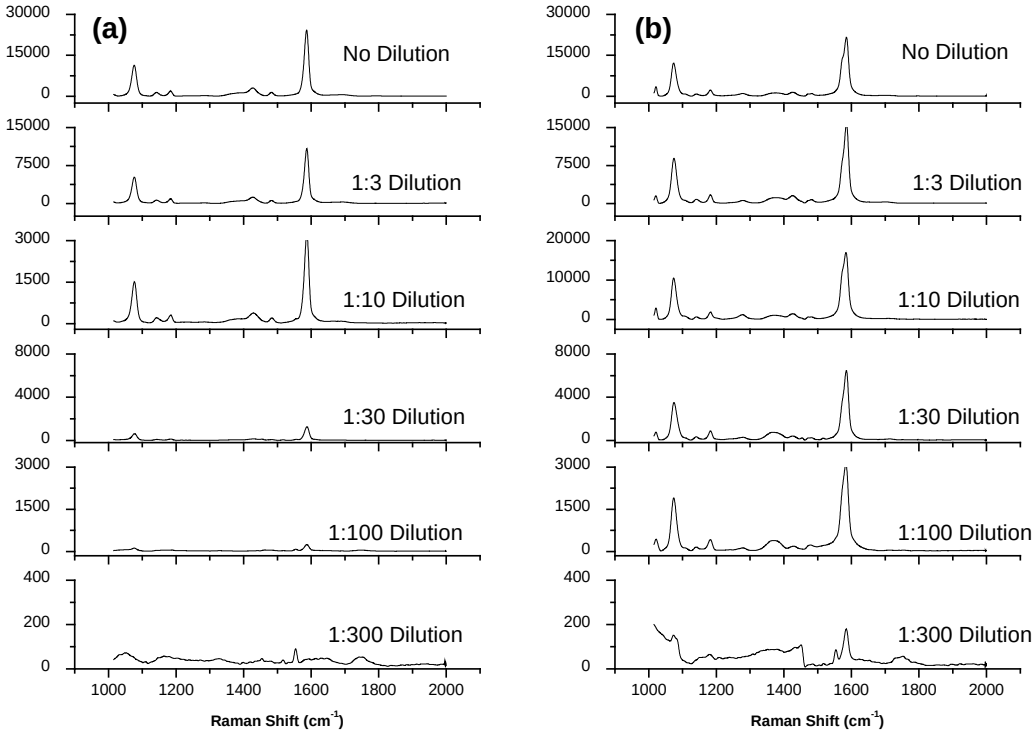

Figure S3. A representative spectrum for each dilution of the colloid solution in the bulk of the solution (a) and after SAW aggregation (b). The y-axis of spectra in (b) have been scaled to show the maximum of data, while spectra in (a) use the same y-axis as the corresponding spectra in (b), to demonstrate the enhancement due to SAW aggregation.

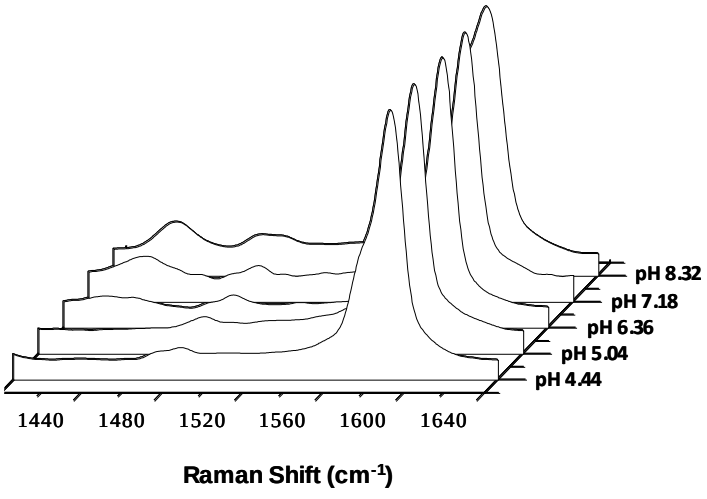

Figure S4. Representative spectra for the pH solutions used for calibration, obtained after SAW-aggregation. The spectra have been normalised using the peak at 1590 cm^{-1} and clearly show the increased intensity of the peak at 1430 cm^{-1} as the pH increases.

Supplementary Movies – Legends

Movie M1. Shows the aggregation of Ag colloids (at a dilution of 1/10), as a top view of a 5 μl drop on the $LiNbO_3$ substrate. SAW were propagated at 30.6 MHz (0.12 W). At the beginning of the movie the voltage is turned off. The bright rings on the drop are reflections of the illumination. Upon supplying the voltage, the SAW actuate the drop, leading to capillary waves on the surface and a periodic deformation of the spherical cap structure. As the acoustic energy is refracted within the droplet, rotational momentum is imparted to the fluid, leading to concentration flows that aggregate the colloid within the drop (the location is shown in Fig. 1 in the main text). This concentration effect creates an aggregate within the first few seconds (<3 s), and it can be seen more clearly when the actuation is turned off after ca. 5s at the end of the movie.

Movie M2. Shows the same process as Movie M1, with a colloid solution that has not been functionalised with 4-MBA, confirming that the aggregation process is not due to the nature of the colloids surface.

C- Additional References

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2. P. R. C. Gascoyne, X.-B. Wang, Y. Huang, and F. F. Becker, *IEEE Transactions on Industry Applications*, 1997, **33**, 670 –678.
3. L. Y. Yeo and J. R. Friend, *Biomicrofluidics*, 2009, **3**, 012002.
4. W. Achim, *Superlattices and Microstructures*, 2003, **33**, 389–396.
5. G. K. Batchelor, *Q J Mechanics Appl Math*, 1951, **4**, 29–41.
6. P. R. Rogers, J. R. Friend, and L. Y. Yeo, *Lab Chip*, 2010, **10**, 2979–2985.
7. Y. Bourquin, A. Syed, Lisa C., Ranford-Cartwright, Michael P., Barrett, and Jonathan M., Cooper, *submitted*, 2013.