Electronic Supplementary Material (ESI) for Analyst

Analysis of Intracellular Enzyme Activity by Surface Enhanced Raman Scattering

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Supplementary Figures and Text

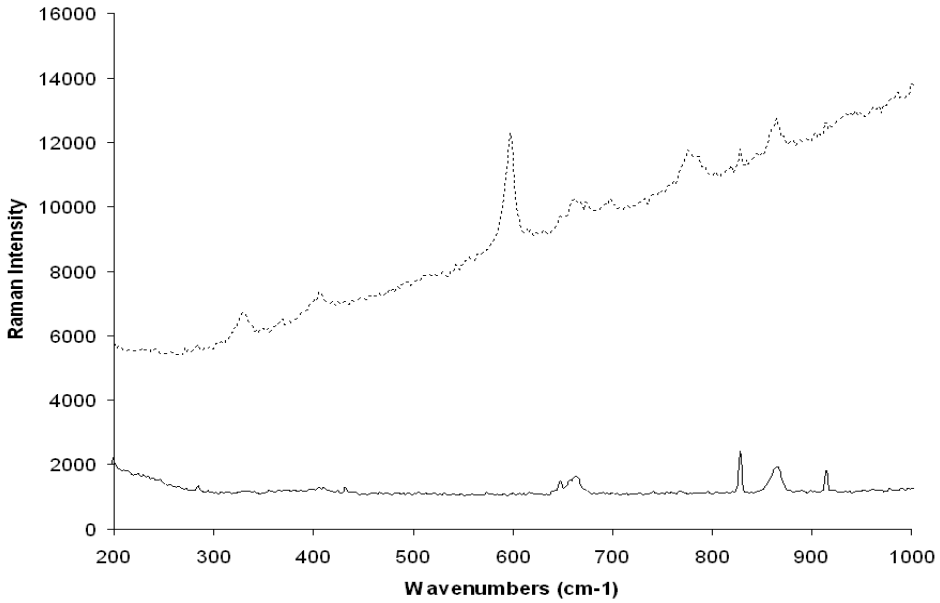

Figure S1. SERS analysis of X-Gal hydrolysis. The sprectra observed from Au nanoparticles with X-Gal is shown (bottom, solid line) compared to the same mixture after a 30 min incubation with galactosidase (top, dashed line). Results recorded on a Renishaw InVia 632.8 nm, using a 10s acqusition with peak centred at 600 cm^{-1}.

Figure S2

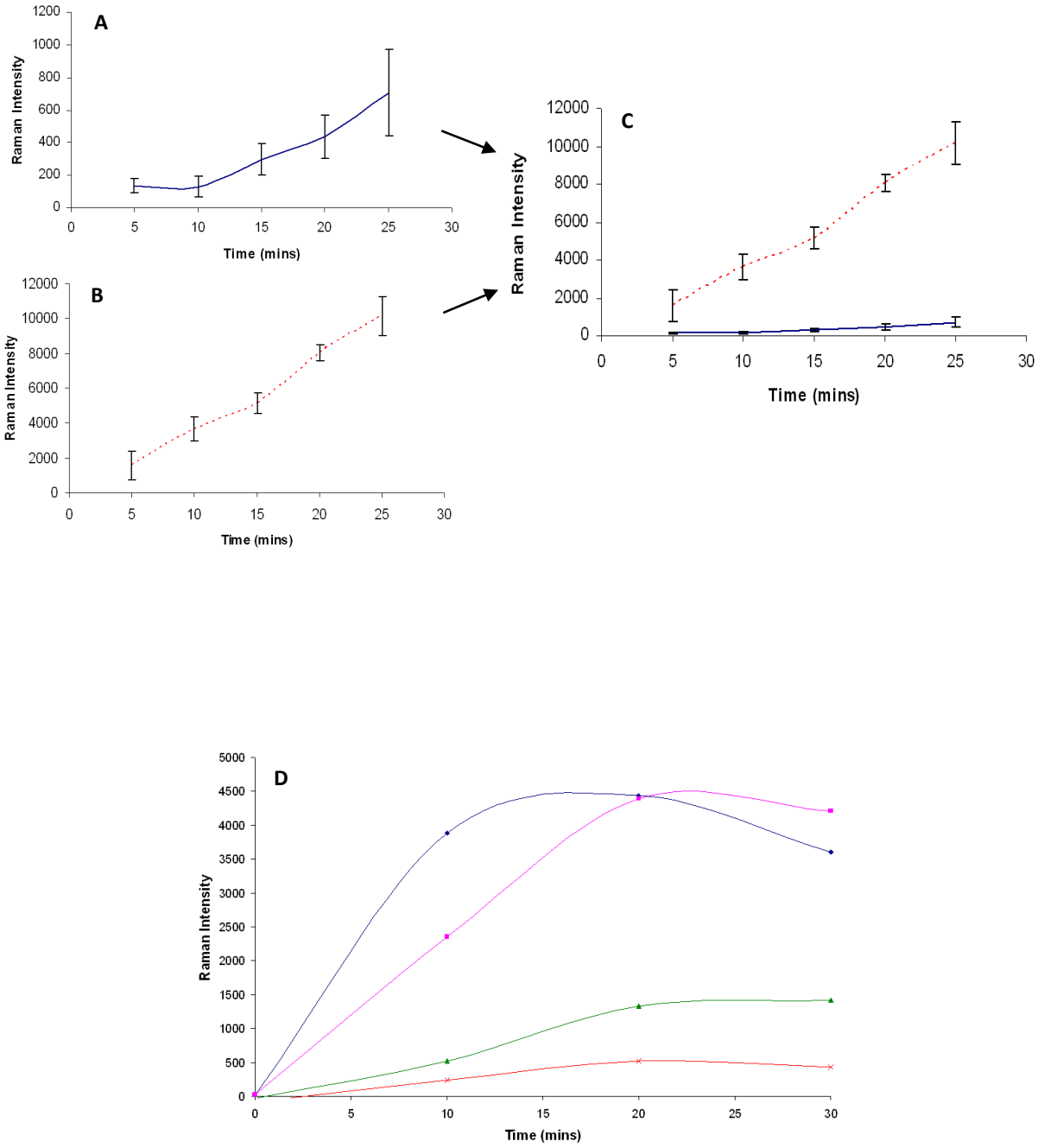

Figure S2. Temporal analysis of product formation. By monitoring the change in peak at 598 cm^{-1}, it is possible to observe the emergence of a Raman (A) and surface enhanced Raman (B) signal. A comparison of the two techniques is shown (C). (D) shows the comparisons of different sized gold (20 nm blue ◆, 40 nm pink ■) and silver colloids (EDTA 42 nm green ▲and citrate 50 nm red **X**).

Figure S3

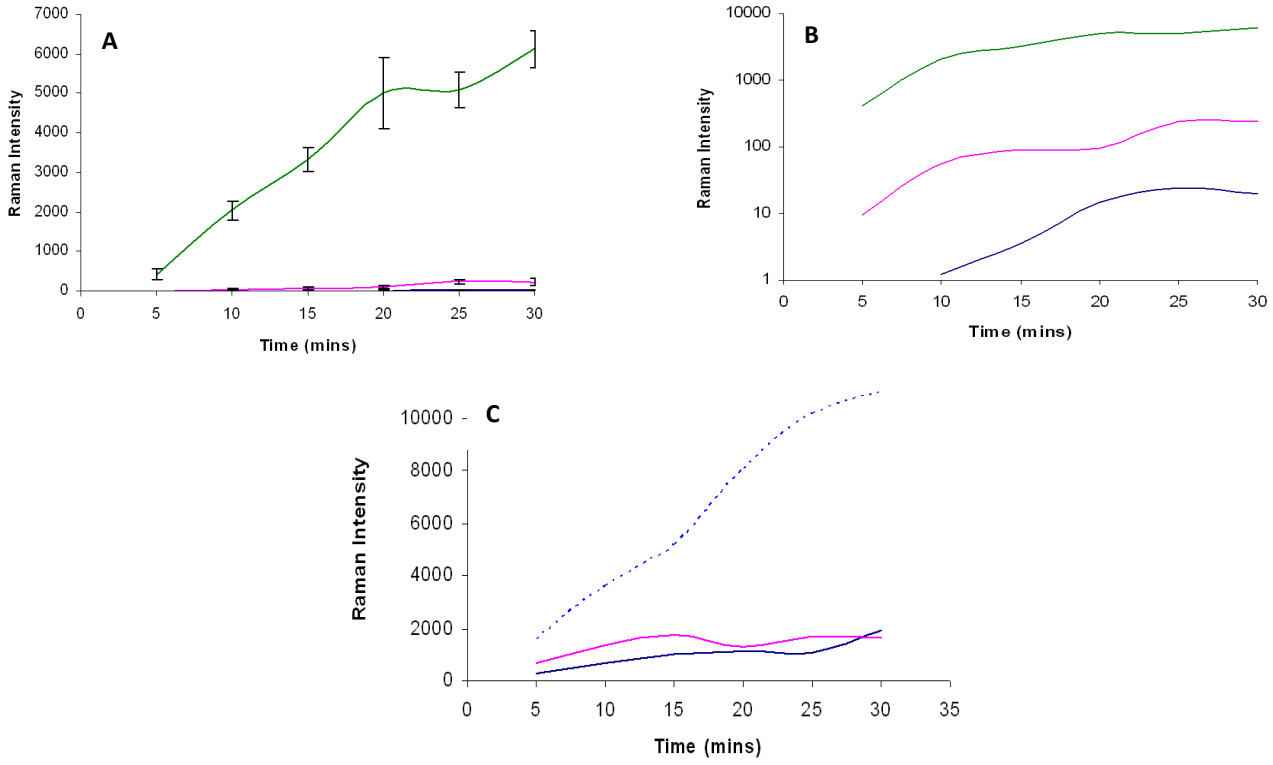

Figure S3. Effect of inhibitor concentration on the Raman peak at 598 cm^{-1}. The effect of phenylethyl-β-*D*-galactopyranoside (PEG) inhibitor concentration of the SERS response over a defined time course is illustrated in (A) and (B). Phenylethyl-β-D-thiolgalactopyranoside (PETG) gave similar data. Concentrations of PEG 10 mM (blue), 1 mM (pink) and 0.1 mM (green) were analysed in triplicate, with (B) showing the same response but in a logarithmic scale. (C) shows the SERS response using an uninhibited sample over time (dashed) compared to 10 mM of either PEG (blue) of PETG (pink).

Figure S4

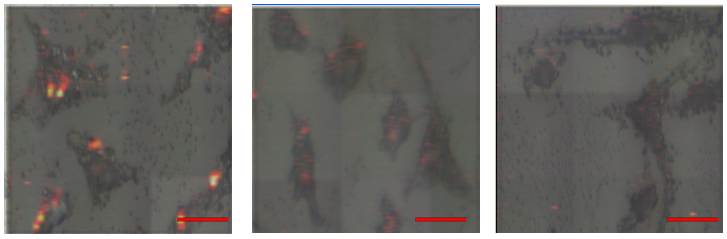

Figure S4. Concentration dependant cellular SERS. False colour images overlaid on bright field maps can be used to show the variance in SERS from cells when changing the concentration of X-Gal. NP concentration was maintained at 0.1 nM and X-Gal concentration diluted logarithmically from 10 mM to 0.1 mM. A 20 µm scale bar has been superimposed on each figure.

Figure S5

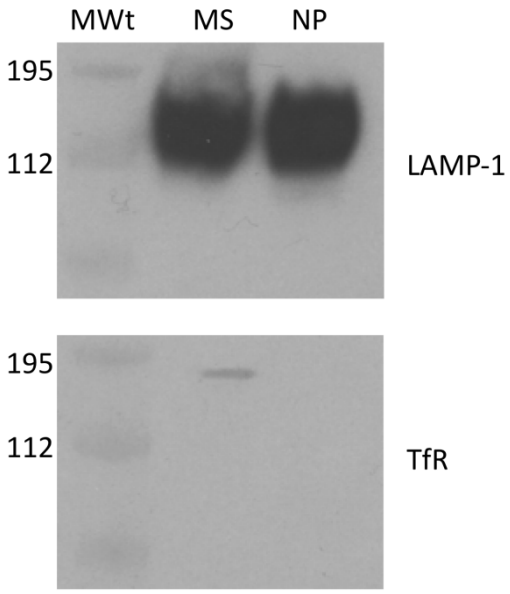

Figure S5. Western Blot analysis of intracellular compartments containing nanoparticles. Lane 1 (MWT) shows the molecular weight ladder with representative values shown on the left hand side (kDa). Microsome extract (MS) and nanoparticle extracts (NP) were blotted against LAMP-1 and TfR (transferrin) antibodies.

Isolation and Analysis of Nanoparticle containing microsomes

Macrophages (2.5 x10^{7}) were pulsed for 4 h with nanoparticles (5 x 10^{-11} M) and chased overnight. Microsomes were isolated following the protocol described previously (Tjelle, T. E., Saigal, B., Froystad, M. & Berg, T. Degradation of phagosomal components in late endocytic organelles. *J Cell Sci* **111**, 141-148 (1998)). Briefly, cells were harvested in homogenization buffer (0.5 mM EDTA, 0.5 mM EGTA, 20 mM HEPES, 0.05% gelatin and 250 mM sucrose), and passed repeatedly through a 25 G syringe. Whole microsome extract (MS) was separated from nuclei by centrifuging at 100 × *g*. The microsome containing supernatant was ultracentrifuged at 100 000 × g for 45 min at 4˚C. The pellet was resuspended in 17% Percoll (Amersham Biosciences, Buckinghamshire, UK) loaded onto a 64% sucrose cushion.The suspension was centrifuged at 27,000 × g for 30 min and the nanoparticle containing microsomes (NP) were isolated at the bottom of the sucrose cushion. Nanoparticle (NP) and whole microsome (MS) extracts were adjusted for protein concentration and underwent non-reducing electrophoresis using a NuPage pre-cast gel (Invitrogen, UK) followed by transfer to a nitrocellulose membrane. Blots were probed with primary antibodies, anti-TfR/FITC and anti-LAMP-1/FITC (both Invitrogen, UK) overnight at 4 ˚C. Blots were washed for 5 min with TBS-Tween (x 6) and then probed with primary antibodies using anti-FITC horseradish peroxidase (HRP; Molecular Probes) and visualised using enhanced chemiluminenscence (ECL) (Amersham Biosciences, UK).

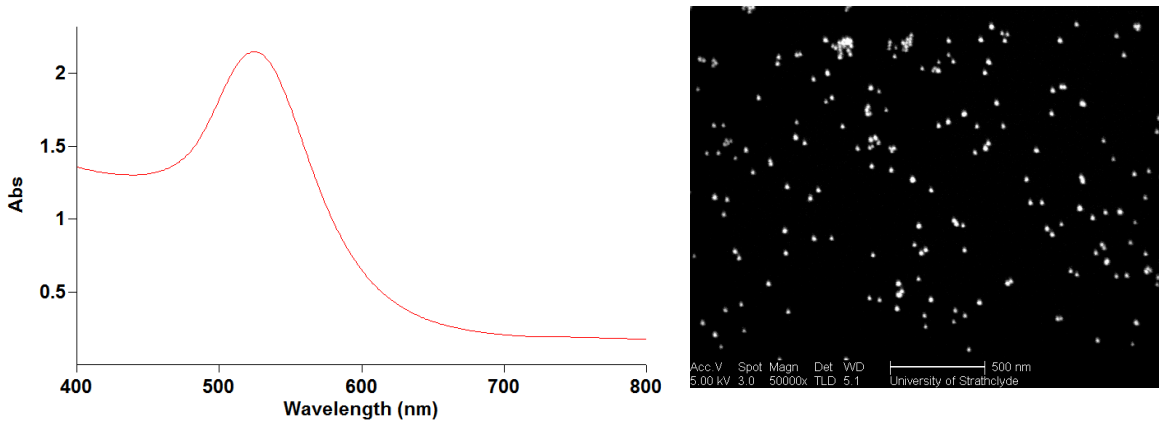

Figure S6. Extinction and SEM analysis of 20 nm gold nanoparticles