Investigation of Ultra Trace Detection in Functionalised- Surface Enhanced Raman Spectroscopy (SERS)

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We have developed an explanation for ultra trace detection found when using Au/Ag SERS nanoparticles linked to biochemical affinity tags, e.g. antibodies. The nanoparticle structure is not as usually assumed and the aggregated nanoparticles constitute hot spots that are indispensable for these very low levels of analyte detection, even more so when using a direct detection method.

Indirect SERS detection using a sensitive Raman reporter incorporated into functionalised nanoparticles commonly provides very low levels of analyte detection. [1, 2] Figure 1 is typical of the illustrations of nanoparticles functionalised by glutaraldehyde linkage to a chemical/ biochemical affinity tag (e.g. antibody) given in articles.[2, 3] If the TEM characterisation of the nanoparticles is included only the bare nanoparticles are usually shown,[2] or only mentioned.[1] In an article where the finished nanoparticle TEM was shown,[4] the evident aggregation of nanoparticles is not mentioned, nor is an explanation or implications of this given. We propose an explanation and some implications here.

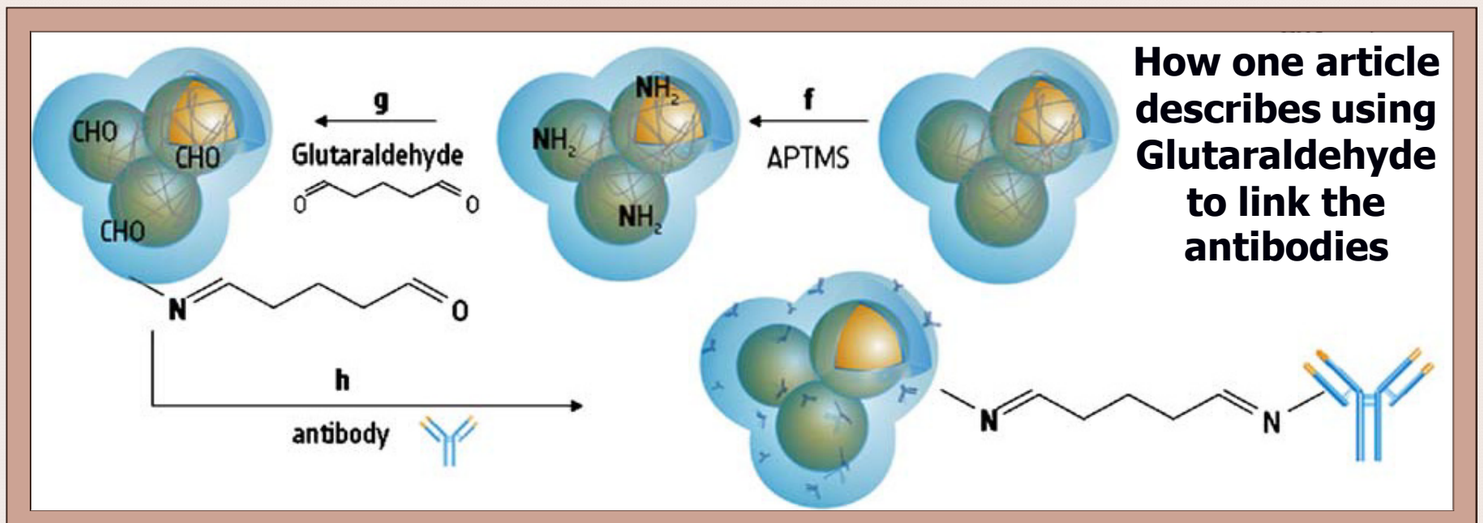

Fig. 1. Exerted from literature.[3] Structure after reaction g is equivalent to figure 2. final structure is typical of the structures show as functionalised nanoparticles

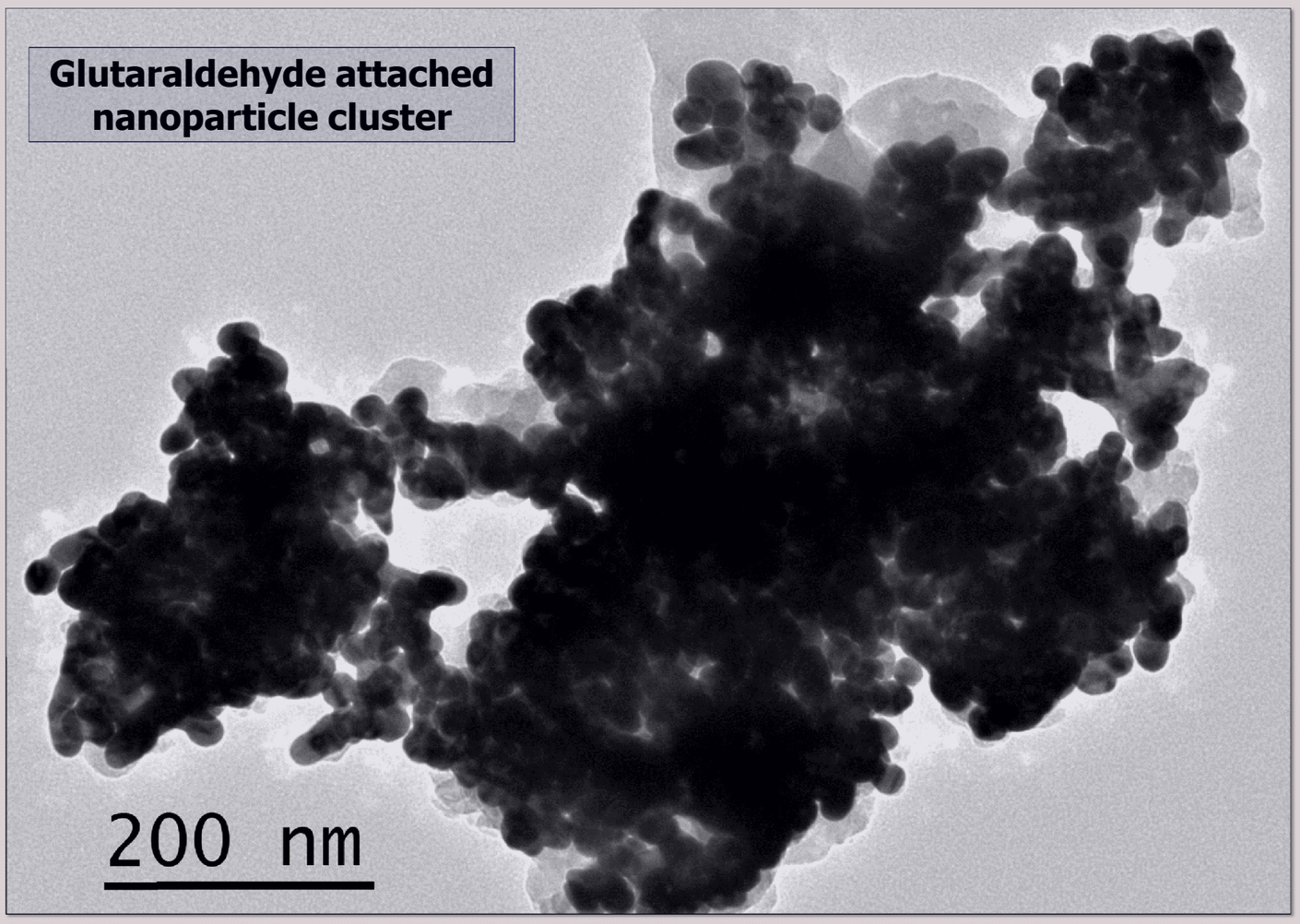

Fig. 2. TEM of Nanoparticles with glutaraldehyde linker attached prior to attachment of antibody.

We have found quantification limits down to 3.5×10^{-13}M, and ready detection of the analyte at 1×10^{-15} M, without the usual use of Raman reporters, i.e. direct SERS detection. On investigation of the TEM of Au nanoparticles after addition of the glutaraldehyde linker, we have found large aggregations of nanoparticles, see figure 2 and compare it with structure after reaction g in figure 1. Glutaraldehyde is a dialdehyde that readily links to primary amine groups on the amino silane modified nanoparticle coating and on antibodies; hence glutaraldehyde's common use as affinity tag linker. Other than for steric hindrance, there is no reason why the nanoparticles will not also link each other and consequently aggregate. Our TEM experiments are consistent with this concept, as no non-aggregated nanoparticles were found.

To further confirm this we used acid digestion on the glutaraldehyde attached nanoparticles . Washed and dried nanoparticles (3 aliquots) were resuspended in Millipore water, 1M HCL and concentrated (32%) HCl respectively. These suspensions were boiled for 1 hour, topping up with Millipore water as needed. Figure 3 is the area normalised UV-Vis spectrograph of these 3 suspensions and the insert photograph shows the suspensions after the boiling and cooling to room temperature. The blue-black to brown colour change (visible after 5 minutes for the concentrated acid) shows that the nanoparticle cluster changes with the digestion with more red coloured (smaller cluster) nanoparticles in the suspension. The UV-Vis shows that smaller nanoparticles are formed as the peaks at lower wavelengths (525 – 650 nm) grow while the higher wavelength (650 – 800 nm) peaks shrink.

SERS measurements are greatly enhanced when nanoparticles have sufficient proximity, i.e. aggregation, to form extra sensitive enhancement regions known as 'hotspots'; enhancing the Raman signal intensity in SERS by orders of magnitude from 10^4 for an individual nanoparticle to as high as 10^{12} for nanoparticle clusters. Glutaraldehyde holds the nanoparticles close enough (1nm) for this extra enhancement, which would inevitably form these hotspots. When an affinity tag is added to these large nanoparticle aggregations, the attached analytes would be within sufficient proximity to the hotspot to allow this extra enhancement to their Raman spectra (5nm).[5] Thus, the aggregated nanoparticles are fundamental to these very low levels of analyte detection.

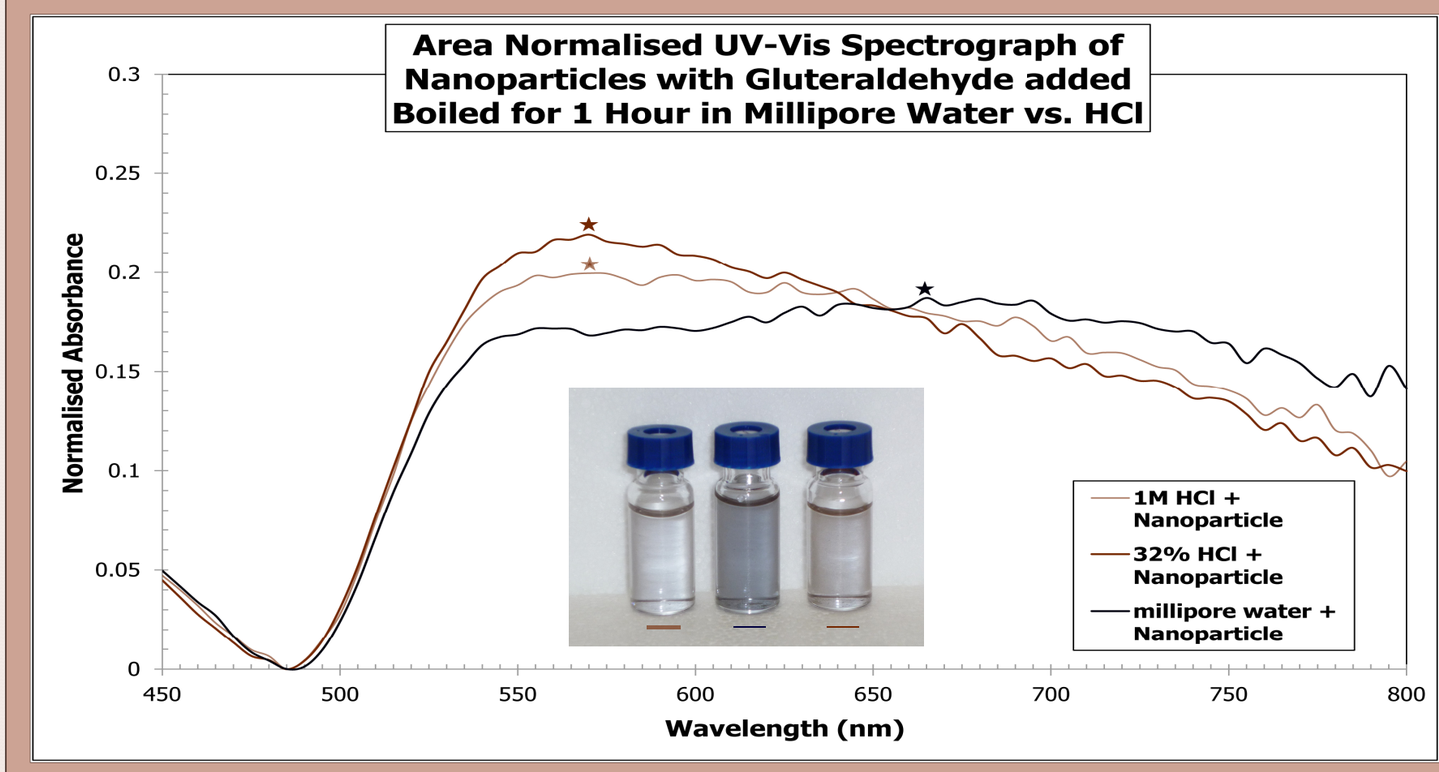

Fig. 3. UV-Vis normalised spectrograph of acid digested glutaraldehyde attached nanoparticles. Insert is a photograph of the nanoparticle suspensions after acid digestion. The bar colours under the vials refer to the key.

1. G. Shen and Y. Zhang *Anal Chim Acta* **674** 27 (2010).
2. Z. Chang, *et al. Electrochim Acta* **52** 575 (2006).
3. I. Freitag, *et al. Vib Spectrosc* **60** 79 (2012).
4. S. Kanimozhi and K. Perinbam *Mater Res Bull* **48** 1830 (2013).
5. K. Kneipp, H. Kneipp and J. Kneipp *Acc Chem Res* **39** (2006).