Fluorescence Imaging of Single Gold Nanoclusters

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Abstract
We have fabricated red-emitting single gold clusters of around 25 atoms in bovine serum albumin (BSA) and studied their unusual fluorescence at the single cluster level for use in biological imaging. In contrast to larger gold nanoparticles, small metal nanoclusters of sizes comparable to the Fermi wavelength of electrons (ca. 0.7 nm), do not possess plasmon resonance, but have molecule-like properties including size-dependent emission and discrete electronic states. Gold nanoparticles of size 2-3 nm are required to be specially shaped e.g. nanorods, in order to fluoresce, and then two-photon excitation is required. Unlike nanorods, 25 atom gold clusters can be excited by one-photon excitation and have advantages in imaging over conventional organic dyes. Gold nanoparticles are more photostable, are more sensitive as FRET acceptors because they have a larger critical transfer distance, and less cytotoxic. The BSA-Au nanoclusters are produced by reduction of HAuCl₄ solution.²

Synthesis and Spectroscopy of Gold Nanoclusters
Gold Nanoclusters were synthesised by the addition of gold (III) chloride to bovine serum albumin at 37°C for 2 minutes and shaken firmly using a magnetic stirrer or Eppendorf Comfort mixer. Sodium hydroxide was then added as a catalyst. Solutions were made to have a final composition of 23.81 g 10⁻³ bovine serum albumin and 4.76 mM of gold (III) chloride. The standard sample had a final composition of 47.62 mM of sodium hydroxide. The sample was stirred for 8 hours at 37°C and then left to incubate.

Figure 1: Schematic of the formation of BSA-Au Nanoclusters.¹ Emission spectra during mixing and incubation period of the standard sample was measured in a HORIBA Scientific Fluorolog 3. Visual changes in the sample are observed. Initially the sample takes on the pale yellow colour of the gold (III) chloride solution. After the sodium hydroxide is added it changes to an orange colour. The mixture is stirred for a period of 8 hours at 37°C and then left to incubate.

Single Molecule Blinking Characteristics
One main challenge in light microscopy is the ability to distinguish features that are close together; the best optical resolution is ~250 nm due to the diffraction limit. This is too large to observe the interplay of molecules and structures within cells.

Figure 4: The image of a single molecule (A) can be analysed and fitted with a 2D gaussian function (B) to determine the actual localisation with nanometer precision (C). This is the principle behind the localisation-based super-resolution approaches, such as dSTORM (direct stochastic optical reconstruction microscopy) and PALM (photodetected localisation microscopy).

One of the main limitations holding back localisation microscopy techniques is the types of dyes available; ideally a dye should possess a low duty ratio and not photobleach. Preliminary work at NPL shows that Au-BSA nanoclusters may possess desirable traits for use as probes.

Figure 5: Preliminary dSTORM of 90.91 mM Au-BSA (A-D). Multiple images are recorded ~10,000. In each image a random subset of gold nanoclusters are excited. Localisation of each blink (red circles) is carried out and processed to create a (E) a final super-resolution image. Scale bars are 3 µm.

A localisation precision of ~70 nm was obtained in these experiments however further optimisation of the system optics would decrease the width of the point spread functions of the particles and hence further increase the resolution obtained. In addition since precision is intimately related to the number of photons collected, excitation with a 488 nm laser opposed to the a 561 nm laser which was used should give higher precision and therefore a higher resolution.

Preliminary work also seems to suggest the duty (on/off) ratio my be related to the pH/stage the nanoclusters were formed at. A low duty ratio is desired for dSTORM.

Figure 6: Time reslife of an Au-BSA prepared to a final concentration of 90.91 mM NaOH sample in comparison to that prepared to 35.05 mM. The y scale bar is 3 µm and the x scale bar is 1000 ms and 10000 ms respectively. Visually it can be seen that the 90.91 mM sample has a much lower duty ratio. This is more desirable as the majority of the blink lifetimes are of the order of the cycle time 10-20 ms which is what is used for currently employed dyes such as Alexa 647.

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References