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Internalization of phospholipid coated gold nanoparticles

Supplemental Information:

Characterization of gold nanoparticles (Wang and Petersen, Can J. Chem 93, 265-271, 2014) [1]:
Wang and Petersen reported the thorough characterization of these phospholipid coated gold nanoparticles and found the following:

1) Comparison of measured and calculated diffraction patterns showed that the phospholipid coated gold nanoparticles had a face-centred cubic lattice structure (Au (a-b=c=4.0789, Sys. Cubic, Fm-3m)).
2) High resolution transmission electron microscopy showed gold structures with sharp edges consistent with cubo-octahedral particles that would arise from growth along the 100 surface. They look either cubic or hexagonal, depending on the orientation at which they are observed.
3) That the synthetic procedure could control the particle size averages as determined by electron microscopy and dynamic light scattering. Examples of 30 nm and 42 nm average sizes were shown. They also showed larger cubic structures of 120-250 nm.
4) Three pieces of evidence supported that there is a coating of a phospholipid membrane on these particles
    a. High resolution transmission electron microscopy revealed a 4 nm thick low contrast material on the exterior of the high contrast gold particle (consistent with a 4 nm thick phospholipid bilayer).
    b. The zeta-potential was -65mV, which is more negatively charged than the citrate coated gold nanoparticles with about -40mV. This is consistent with a coating of negatively charged phospholipids (here phosphatidyl glycerol (PG)). This larger negative zeta-potential also contributes to the stability of these particles in solution since they are less prone to agglomeration.
    c. The plasmon resonance for these gold nanoparticles of both 30 nm and 42 nm particles are red-shifted by about 5 nm relative to the resonance measured by Link and El-Sayed [2] for citric acid coated nanoparticles, which according to theories by Liz-Marzan et al. [3] and calculations by Van Dijk [4] is consistent with a 4 nm coating of a material with a refractive index of 1.46 (which compares with the refractive index of phospholipids of 1.456).
5) They further found that the fluorescence of the NBD-labeled phospholipid was quenched by about 80% when the gold-nanoparticles are formed (found by exposing the particles to a detergent that removes the lipid layer). This quenching arose mostly from a quenching by the gold with some contribution from self-quenching in the lipid bilayer.
6) Finally, they determined that while the lipids could exchange from one gold nanoparticle to another, they were not removed from the gold nanoparticles when taken up by cells. This was established by mixing two types of gold nanoparticles labeled with different fluorescent lipids and observing energy transfer in solution and colocalization in cells.

In the present work, the synthetic processes were developed to prepare 20 nm gold nanoparticles with a lipid coating and their sizes were determined after each synthesis by dynamic light scattering as shown in Figure 1 in the main manuscript.

Uptake of gold nanoparticles previously studied by Wang and Petersen (BBA 1831, 20189-1097 (2013)) [5].

In their previous work, Wang and Petersen showed that the same gold nanoparticles could be taken up by A549 Cells and that they stayed with the gold nanoparticles and did not disperse into other membrane components in the cells. They also showed that the gold nanoparticle fluorescence was in acidic compartments, including the lysosomes, and that they were not found in mitochondria or peroxisomes. In that work, the acidic compartments were identified generically using Lyso Tracker Red and lysosome-RFP. They also demonstrated, using electron microscopy, that the gold nanoparticles were found in intracellular compartments, some of which were identified as lamellar bodies, structures that are unique to the A549 cells. Finally, they showed that the gold nanoparticles did not inhibit cell growth.

In contrast to and as an extension of that work, **the present work was aimed at determining specifically how the gold nanoparticles are taken up, how they distribute and what may happen to them with time, and to expand this analysis to another cell type that does not produce lamellar bodies.**

Calculation of correlation functions and determination of correlation function amplitudes:

Auto- and Cross-Correlation Function calculations of images:

The auto-correlation function can be calculated directly as the sum of the products of all the pair-wise intensities $i(x, y)$ and $i(x + \xi, y + \eta)$ as indicated by the following equation:

$$g(\xi, \eta) = \frac{\langle (i(x,y) - \langle i(x,y) \rangle)(i(x+\xi, y+\eta) - \langle i(x,y) \rangle \rangle}{\langle i(x,y) \rangle^2}$$

where the angular brackets indicate averaging over all spatial coordinates, x and y.

For images of any size, this becomes computationally prohibitive, so the alternative is to recognize that the auto-correlation function is the reverse Fourier Transform (FFT^{-1}] of the power spectrum of the intensities in the image and that the power spectrum can be calculated as the Fourier Transform [FFT] of the image multiplied by its complex conjugate [6]. Thus, the following applies:

$$\tilde{\imath}(\xi', \eta') = FFT[i(x, y)]$$

and

$$g(\xi,\eta) = \frac{FFT^{-1}[\tilde{\imath}(\xi',\eta')\tilde{\imath}^*(\xi',\eta')]}{\langle i(x,y)\rangle^2}$$

where the asterisk denotes the complex conjugate of the Fourier Transform.

Correspondingly, the cross-correlation function between two images, say red, r, and green, g, is calculated as the reverse Fourier Transform of the joint power spectrum [6], that is:

$$\tilde{\imath}_r(\xi',\eta') = FFT[i_r(x,y)]$$

and

$$g_{r,g}(\xi,\eta) = \frac{FFT^{-1}[\tilde{\imath}_r(\xi',\eta')\tilde{\imath}_g^*(\xi',\eta')]}{\langle i_r(x,y)\rangle\langle i_g(x,y)\rangle}$$

where the asterisk denotes the complex conjugate of the Fourier Transform. Importantly, it does not matter which image is used for the complex conjugate.

Once the auto- or cross-correlation functions have been calculated, they are fit to a two-dimensional Gaussian function to extract the amplitude of the correlation function, g(0,0). Thus,

$$g(\xi,\eta) = g(0,0)\, exp\left(-2(\xi^2+\eta^2)/_{w^2}\right) + g_0$$

where g_0 is a factor that allows for incomplete decay of the correlation function and w is the width of the Gaussian function. Note that the use of a Gaussian function is determined by the use of a laser beam whose cross-sectional profile is Gaussian, thus w is the width of the laser beam for the auto-correlation function and the geometric mean of the two laser beams for the cross-correlation function.

Triple cross-correlation function calculation and fitting to extract the amplitude.

As with the cross-correlation function of two images, it is possible to calculate a triple cross-correlation function of three images. In this case, the triple cross-correlation function is the reverse Fourier Transform of a bispectrum, which in turn is the product of the Fourier Transforms of two of the images multiplied with the complex conjugate of the Fourier Transform of the third image [7]. Once again, it does not matter which image is used as the complex conjugate. Thus, we can calculate the bispectrum as

$$\tilde{I}(\xi',\eta',\upsilon',\theta') = \tilde{\imath}_r(\xi',\upsilon')\,\tilde{\imath}_g(\eta',\theta')\,\tilde{\imath}_b^*(-\xi',-\upsilon',-\eta',-\theta')$$

where the subscripts refer to the red, green, and blue image as an example.

The triple cross-correlation function in now calculated as

$$g(\xi,\eta,\upsilon,\theta) = \frac{FFT^{-1}[\tilde{I}(\xi',\eta',\upsilon',\theta')]}{\langle i_r(x,y)\rangle\langle i_g(x,y)\rangle\langle i_b(x,y)\rangle}$$

Note that there are four lag parameters in this function since there are two between the first and the second image, and two between the second and the third image.

The amplitude of interest is found when all lag parameters approach zero, so the fitting function is now:

$$g(\xi,\eta,\upsilon,\theta) = g(0,0,0,0)\, exp\left(\frac{-(\xi^2 + \eta^2 + \upsilon^2 + \theta^2)}{w^2}\right) + g_0$$

where w is given by the geometric mean of the width of the three laser beams used, i.e.

$$w^2 = \sqrt[3]{w_r^2 w_g^2 w_b^2}$$

Using these equations, it is possible to develop the appropriate software to perform the calculations needed and extract the amplitudes used in the present work.

The detailed theory of the triple cross-correlation function calculations of images and their applications is planned to be published by Max Anikovski and Nils Petersen in the near future.

References

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