Chapter 3 – Characterization of 5 kDa PEG-SH

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April 18, 2012

In the spring of 2011, several experiments were performed to characterize the 5 kDa PEG-SH (PS), which is used to provide protection to the Au nanospheres. The PS was characterized in three ways: a titration, a time study, and a protection study. These demonstrated that a number concentration of 50, 000 PS molecules per Au nanosphere, after 24 hours of incubation, should provide complete long-term protection to the Au nanospheres in a salt-rich environment.

0.1 Titration Study

A titration curve was made by adding 5 kDa PS to 90 nm gold nanospheres (R=53 nm) in varying concentrations, from 1000 PS per nanosphere to 10^7 PS per nanosphere. The hydrodynamic radii of these PEGylated nanospheres were then measured using the Dynamic Light Scattering (DLS) instrument, taking 15 30-second acquisitions of each solution and discarding the first three to account for temperature acclimation. This procedure was performed three separate times; a plot of the results is shown in Figure 1.

This plot shows the behavior of a rapid rise followed by a plateau that is expected of a species forming a monolayer on a surface. The plateau begins at approximately 50, 000 PS molecules per nanosphere with R=60–61 nm, and has an elbow at around 10, 000 PS per nanosphere.

A single 5 kDa PEG-SH molecule, with a density of $1.11\,\frac{\mathrm{g}}{\mathrm{cm}^3}$, has a volume of

$$\mathrm{V}_{5\,\mathrm{kDa\ PEG-SH}} = \frac{5\mathrm{kDa}}{1.11\frac{\mathrm{g}}{cm^3}} = 7.5\,\mathrm{nm}^3$$

This means that a 5 kDa PEG-SH monolayer has, based on the change in hydrodynamic radius,

$$\#_{5\,\mathrm{kDa\ PEG-SH}} = \frac{\frac{4}{3}\pi((60.5\,\mathrm{nm})^3 - (51.5\,\mathrm{nm})^3)}{V_{5\,\mathrm{kDa\ PEG-SH}}} = 47,500\ 5\,\mathrm{kDa\ PEG-SH}$$

This indicates that the measurement of the plateau as beginning at 50,000 PEG-SH per nanosphere is correct. This also allows us to get a sense of the effective `width`(Prof. Haskell: word choice?) of a PEG-SH molecule as it sits on the gold. At monolayer concentration, the area on the surface of the gold taken up by each PEG-SH molecules is

$$A_{\mathrm{PS}} = \frac{4\pi(51.5\,\mathrm{nm})^2/\mathrm{Au}}{47,500\,\frac{\mathrm{PS}}{\mathrm{Au}}} = .70\mathrm{nm}^2 = 70^2$$

This size is determined partially by the atomic size of the sulfur atom (= 2), but mostly by the extent to which the PEG chain is bunched; clearly, most of the effective width comes from the bunching.

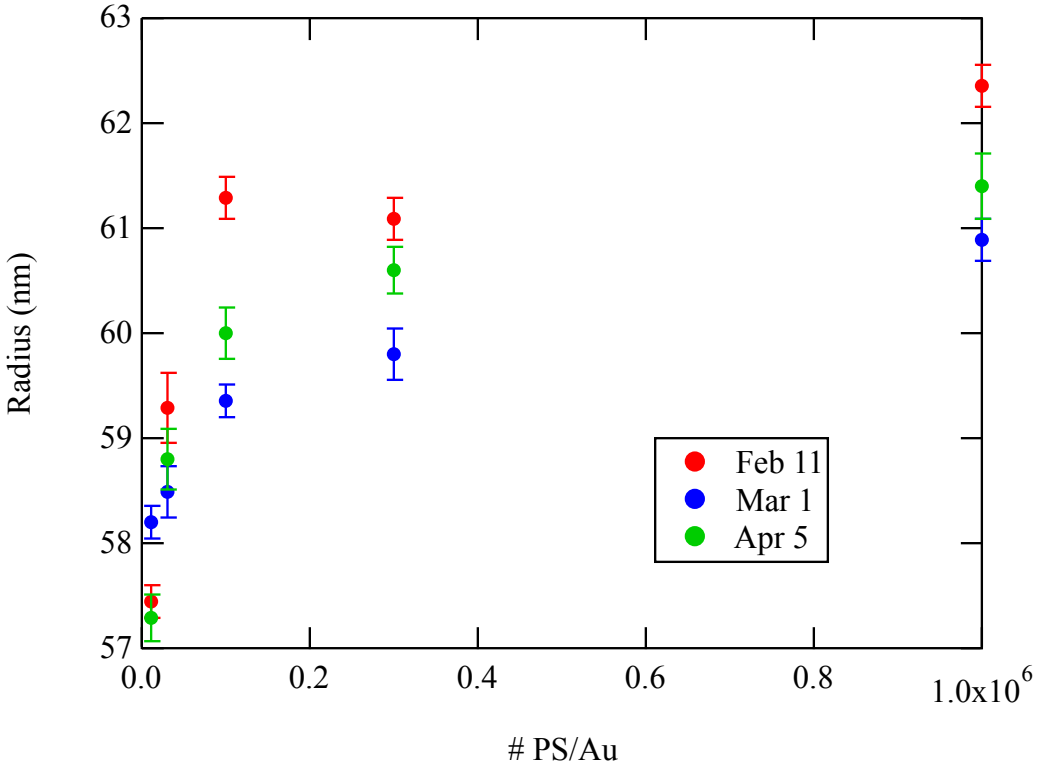

Figure 1: Plot of hydrodynamic radius of Au nanospheres with at various concentrations less than 30 minutes after addition of PS.

0.2 Time Study

The same samples used in the titration study were measured again after 48–72 hours of incubation, producing the radii shown in Figure 2.

The plateau in this graph is much sharper than in Figure 1, indicating that van der Waals forces and thiol bonding processes are in competition, with van der Waals binding dominating immediately after addition, but the lower-energy thiol bonds dominating after incubation time. This leads to the increased radius of the lower-radius samples and the decreased radius of the higher-radius samples. However, the plateau region still has R=60–61 nm, as a second indication of a monolayer. Clearly, it is essential that PEG-SH be allowed to incubate with spheres to allow for the PEG-SH binding to reach equilibrium.

0.3 Protection Study

As mentioned above, the main reason for using 5 kDa PEG-SH is to prevent the Au nanospheres from conglomeration. The Au nanosphere solution includes a negatively charged capping agent that makes the spheres repel each other; when the solution is buffered at pH $\sim$7.5 to prevent antibodies from denaturing during the full immunogold procedure, positive ions are introduced into the solution that neutralize the capping agents, causing the gold

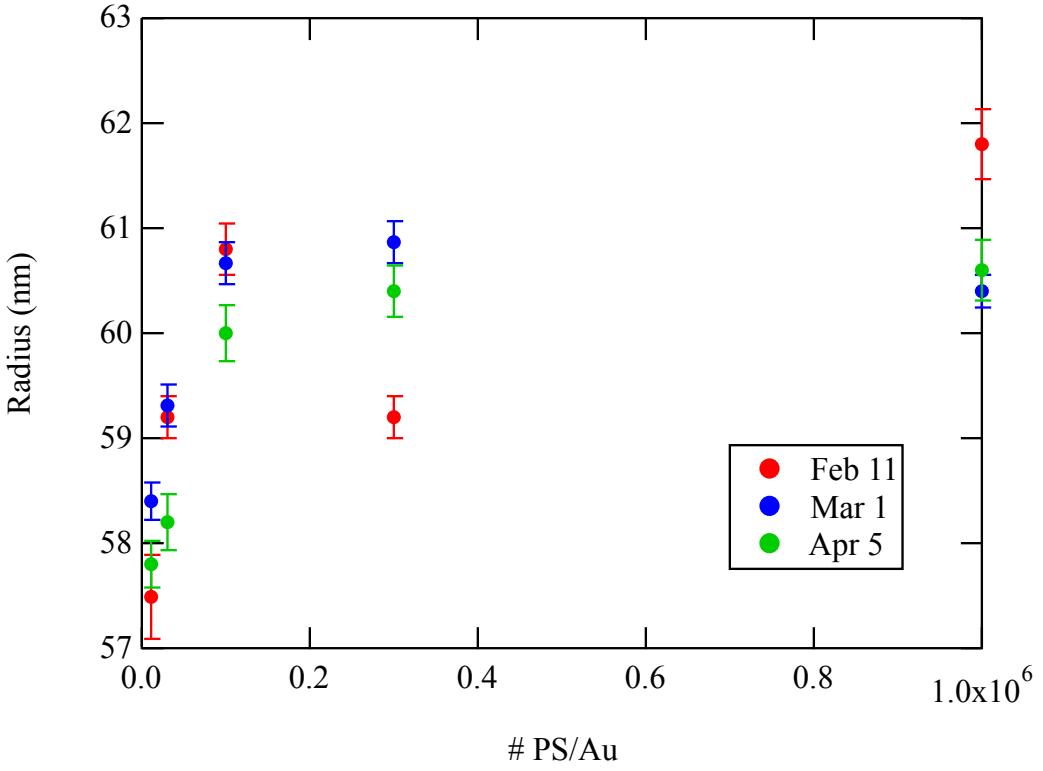

Figure 2: Plot of hydrodynamic radius of Au nanospheres with at various concentrations 48–72 hours after addition of PS.

nanospheres to agglomerate. Theoretically, PEG-SH would prevent this from happening, but 1 kDa PEG-SH does not, as shown in Figure 3.

Therefore, the protection capabilities of 5 kDa PS were tested by adding PEG-SH to the Au nanospheres in various concentrations, then mixing those solutions in equal volumes with commercial phosphate buffered saline. The spheres were allowed to incubate for at least 30 minutes with the PBS, then measured in the DLS. Selected results from those measurements are shown in Figure 4.

For all but the naked gold, almost all acquisitions are within 3 nm of the mean; this is also true of the 300k PS/Au without PBS from Summer 2010. However, with just 10k 5 kDa PS/Au, the width of the distribution barely widens when PBS is added–a stark contrast to the addition of PBS to 300k 1 kDa PS/Au. Furthermore, there was a $\sim$15 nm increase in average radius between the 1 kDa PS spheres with and without PBS. In the case of the 10k 5 kDa PS/Au, the difference in average radius was negligible: 0.09 nm.

From observing the lack of change in both average radius and the change in radial distribution when using the 5 kDa PEG-SH, it is clear that the 5 kDa PEG-SH fully protects the Au nanospheres against capping agent neutralization. There is, however, a noticeable difference between the 10k and 300k PS/Au samples; the 300k is slightly narrower, indicating that it offers slightly more protection, consistent with the 300k PS/Au solution being on the plateau while the 10k PS/Au is still on the rising part of the titration curve

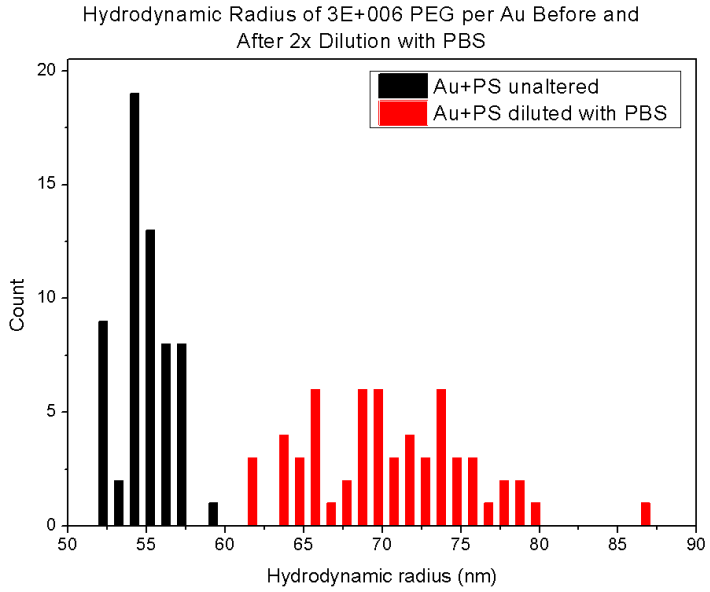

Figure 3: Histogram of radii of acquisitions of 300k 1 kDa PS/Au with and without PBS from Summer 2010. The distribution is noticeably shifted to the right and flattened after the addition of PBS. Data taken by Ellis and Hoidn [1].

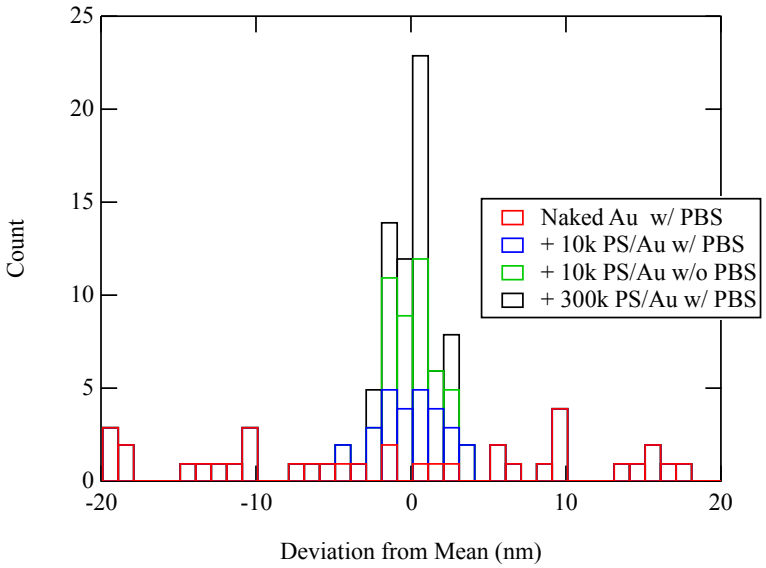

Figure 4: Stacked histograms of differing concentrations of Au-PS with and without PBS. The naked gold is significantly broader than any of the other distributions.

Bibliography

[1] Perry Ellis and Oliver Hoidn. Pegylation of antibodies to gold nanospheres. August 2010.