# Surface Functionalization of Nanoparticles and Protein Adsorption Studied Using Fluorescence Correlation Spectroscopy

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## Background

The use of nanoparticles in biomedicine is a rapidly evolving area of study with tremendous potential. A large barrier to the effectiveness of nanoparticles in drug delivery is the formation of the protein corona, which forms when proteins adsorb to the nanoparticle once it enters the physiological environment. The protein corona alters the functions and interactions of the nanoparticles because it leads to adverse reactions with the immune system. For nanomedicine to progress, it is essential to study the formation of the protein corona in order to limit and prevent unwanted proteins from interacting with the nanoparticles. Fluorescence correlation spectroscopy (FCS) offers a unique and effective method to study nanoparticles and protein coronas. FCS is a very sensitive spectroscopic technique which can measure fluorescence intensity fluctuations at the molecular level. FCS is particularly useful for the study of protein coronas because it can be used to study them in situ and with minimal invasiveness. The diffusion times gathered from FCS also allow for the determination of spatial and temporal analysis of nanoparticles and protein coronas. In FCS, fluorescently labeled species diffuse in and out of the confocal volume, and the diffusion times are recorded. From the diffusion times, the size of diffusing species can be determined and interactions of nanoparticles and protein coronas can be analyzed. When the protein corona is formed around the nanoparticle, the diffusion times are longer because larger molecules take longer to diffuse. The diffusion coefficient can be analyzed in FCS studies which allows for the calculation of the hydrodynamic radius, and the size of the protein coronas can be determined. The calculation of the hydrodynamic radius is useful for protein corona studies that involve nanoparticle coatings. Nanoparticles need to be provided with a stealth coating in order to limit formation of the protein corona. The determination of hydrodynamic radii through FCS can be utilized to determine the effectiveness of the stealth molecules used to coat nanoparticles and the extent to which they are able to limit the formation of protein coronas in situ.

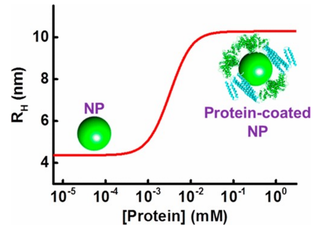

Figure 1. Hydrodynamic Radius of Nanoparticles

## Zwitterionic polymer ligands: an ideal surface coating to totally suppress protein-nanoparticle corona formation?

Debayle et al. used fluorescence correlation spectroscopy (FCS) as one of the methods to study the formation of the protein corona on CdSe/CdS/ZnS quantum dots when zwitterionic polymers were used as the stealth coating. The polybetaines studied were phosphorylcholine (PC, anionic phosphate group), carboxybetaine (CB, anionic carboxyl group), and sulfobetaine (SB, anionic sulfonate group)

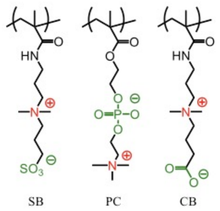

Figure 2. Sulfobetaine (SB), phosphorylcholine (PC) and carboxybetaine (CB)

## Results

The study found that SB-coated nanoparticles had no significant increase in hydrodynamic radius (Rh) when incubated with increasing concentrations of bovine serum albumin (BSA) or with human serum (HS). This means there was essentially no protein corona formation or protein aggregation on these nanoparticles. The CB-coated nanoparticles had a slightly significant increase in Rh at a BSA concentration of 500 µm, but there was no significant increase in Rh at any other BSA concentrations. The main difference between CB-coated nanoparticles and SB-coated nanoparticles was that there was a very significant increase in Rh when CB-coated nanoparticles were incubated with HS. Next, the PC-coated nanoparticles were shown to have a significant increase in Rh when incubated with increasing concentrations of BSA and a very significant increase when incubated with HS.

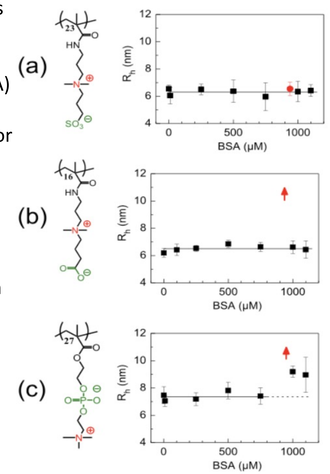

Figure 3. Hydrodynamic radius (Rh) measurements of Sulfobetaine (a), carboxybetaine (b) and phosphorylcholine (c) in BSA (black) or HS (red)

## Zwitterion and Oligo(ethylene glycol) Synergy Minimizes Nonspecific Binding of Compact Quantum Dots

Although zwitterions are able to form strong hydration shells, they are also potentially able to attract counterions which can lead to nonspecific binding due to electrostatic interactions and this can form a protein corona. Han et al. utilized a nanoparticle coating technique which included the use of both oligo-ethylene glycol (OEG) and zwitterionic amino acids. The 5 nanoparticle coatings studied were polymers functionalized with cationic groups, anionic groups, zwitterionic amino acid groups, OEG groups, or a combination of zwitterionic amino acid and OEG groups.

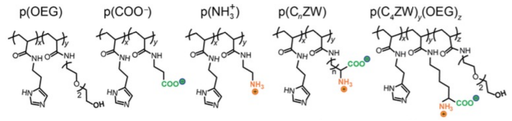

Figure 4. Polymers functionalized with cationic groups p($NH_3$+), anionic groups p(COO-), zwitterionic groups p($C_4$ZW), oligo-ethylene glycol p(OEG), or a combination of zwitterionic and OEG groups p($C_4ZW)_xOEG)_z$

## Results

The study found that quantum dots treated with a dual zwitterionic amino acid and OEG coating were the most resistant to protein corona formation and aggregation compared to the other coatings studied. In these quantum dots, a protein corona did not form until around 0.4% BSA and this was the smallest protein corona formed compared to the other coatings. Also, at around 1% BSA, only about 23% of the mixture consisted of aggregated quantum dots. Both of these findings proved that zwitterionic amino acids and OEG had a synergistic effect in reducing the BSA adsorption and aggregation

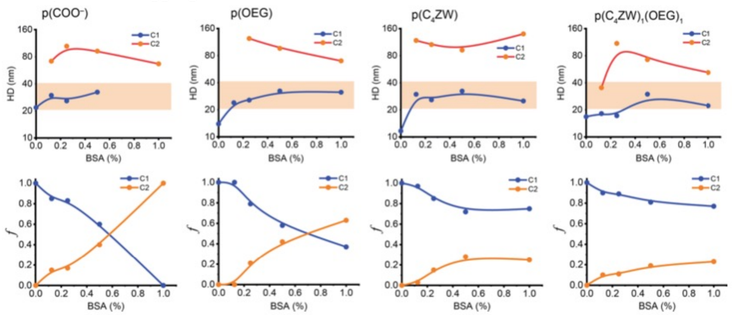

Figure 5. Hydrodynamic radius measurements for polymers functionalized with anionic groups p(COO-), zwitterionic groups p($C_4$ZW), oligo-ethylene glycol p(OEG), or a combination of zwitterionic and OEG groups p($C_4ZW)_xOEG)_z$. C1 (blue line) represents nanoparticles that had a protein corona or were nonaggregated and C2 (orange line) regpresents nanoparticles that were aggregated.

## Potential Future Applications

Zwitterions form a strong hydration shell and attract counterions, which are very effective at limiting hydrophobic interactions. Poly-ethylene glycol (PEG) is effective at limiting electrostatic interactions because these polymers interact with water through hydrogen bonding and this makes binding by hydrophilic macromolecules unfavorable because ordering of the polymer reduces entropy. A new nanoparticle coating which could be tested would be sulfobetaine with PEG. When zwitterionic amino acids are used in conjunction with oligo ethylene glycol they become even more effective at repelling protein corona formation and aggregation. Sulfobetaine has proven to be one of the most effective zwitterionic coatings and PEG is more effective than OEG, so the synergistic effect of sulfobetaine and PEG could potentially lead to an even greater prevention of protein corona formation and aggregation than zwitterionic amino acids with OEG. FCS would be utilized to study the effectiveness of this nanoparticle coating by using a fluorescently labeled nanoparticle or a fluorescent quantum dot in order to determine the hydrodynamic radii at different concentrations of bovine serum albumin.

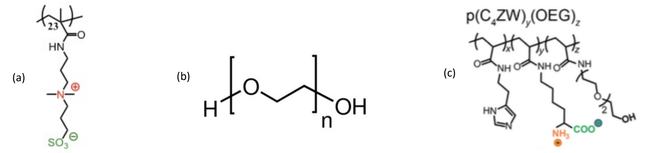

Figure 6. Sulfobetaine (a), Poly-ethylene glycol (PEG) (b), and nanoparticle coating with combination of zwitterionic and OEG groups p($C_4ZW)_xOEG)_z$ (c)

## References

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