The protein corona of nanoparticles: structure responsiveness upon corona formation .

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ABSTRACT

AuNPs have been extensively explored for biomedical applications. In particular, fluorinated NPs are proposed as excellent candidates in the field of ^{19}F MRI based diagnosis [1]. In addition, the incorporation of fluorine atoms into the design of new biomedical products has been investigated as a strategy to enhance the therapeutic performance of drug candidates. Fluorine atoms impacts the therapeutics performance of drug candidates, enhancing the pharmacokinetic properties, metabolic stability and cellular uptake. Moreover, fluorine does not naturally occur in the human body which makes it an excellent candidate to develop contrast agents for MRI. Today it is well established that when NPs get in contact with the bloodstream, proteins adhere to their surface, forming the so-called protein corona [2]. This interaction can alter the physicochemical properties of the NPs and affect their internalization, circulation time,final destination and toxicity [3]. The changes in the original physicochemical properties of NPs after protein corona formation are often addressed *in vitro* using optical methods, however they often present several limitations [2]. As an alternative, we study the interaction of our fluorinated gold NPs (Au NPs) with corona-forming proteins (FBS; Fetal Bovine Serum and HSA; human serum albumin) by small angle X-ray scattering (SAXS) and Microfluidic diffusional sizing (MDS) [2]. Depending on the fluorinated ligands used in the NP (**Figure1**), different structural effects in the PEGylated shell were observed due to protein corona formation. Previous ^{19}F diffusion NMR studies were carried out to study the formation of protein corona on AuNPs, showing no change or reduction on AuNPs after being exposed to relevant media [3]. However, this technique only provides information on size variations based on the fluorine signal. To complement ^{19}F diffusion NMR studies, we perform MDS (microfluidic diffusion studies) to quantify potential changes on protein diffusion. While ^{19}F NMR allows us to observe diffusion changes on fluorinated AuNPs, MDS adds a new insight on diffusion changes on proteins induced by the protein adsorption into AuNPs. Diffusion is used as an indicator of protein binding, such that protein-nanoparticle binding induces changes in the protein diffusion profile and consequently in the protein hydrodynamic radius. Shows protein hydrodynamic radius obtained by MDS. No significant protein size increase was observed even for the highest protein:NP ratio, indicating a lack of adsorption of proteins into the AuNPs surface.

With ^{19}F diffusion NMR and MDS, we are only getting information about diffusion changes in AuNPs and proteins, but not molecular or structural information. To obtain this information, we perform SAXS to observe and quantify potential changes induced by relevant protein (HSA and FBS) adsorption into AuNPs PEG shell. SAXS pattern for the most hydrophobic AuNPs, NP-PhF , showed diffraction peaks indicating the formation of a 3D ordering of self-assembled particles. Analysis of peak position suggests the arrangement of NPs into a FCC lattice. When NP-PhF are incubated with increasing concentrations of HSA, SAXS curves maintained the strong peaks corresponding to FCC lattice. Moreover, the size of the smallest repeating unit, the unit cell, remained constant, meaning protein molecules do not penetrate inside the ordered assembly of

NPs. In contrast, the appearance of this type of arrangement disappears when high concentrations of proteins are added (10% FBS). For NP-F, NP-F/NH$_2$, NP-F/COOH and NP-/MeO, diffraction peaks are no longer observed, suggesting increase colloidal stability. Afterwards, the addition of protein on NP-F induces the appearance of diffraction peaks, however these peaks are not very pronounced, suggesting a coexistence of disordered NPs suspension and ordered NPs domains. Besides, the addition of proteins induces the presence of a slight upturn at low q range in the SAXS pattern, suggesting the formation of aggregates due to the attractive interactions between NP-F and proteins .In contrast, for NP-COOH, NP-NH$_2$and NP-MeO, where the hydrophobicity is reduced, after NPs incubation with increasing concentrations of proteins diffraction peaks shown in NP-F are no longer observed. This corroborate previous studies which shown that the introduction of a higher number of hydrophobic groups to the PEG end-groups promotes attractive intermolecular interactions between the protein and the hydrophobically terminated polymer that do not occur when the polymer is hydroxyl-terminated [4]. In addition, quantitative information was extracted after fitting SAXS curves with models representing a core-shell structure. From this fitting the mean radius of gold core of AuNPs were obtained and aligns well with Au core radius obtained from TEM. For all NPs, the thickness corresponding to the PEG shell does not show significant changes upon incubation with excess HSA or FBS, this corroborates PEG capacity to prevent corona formation even after the introduction of hydrophobic atoms as fluorine.

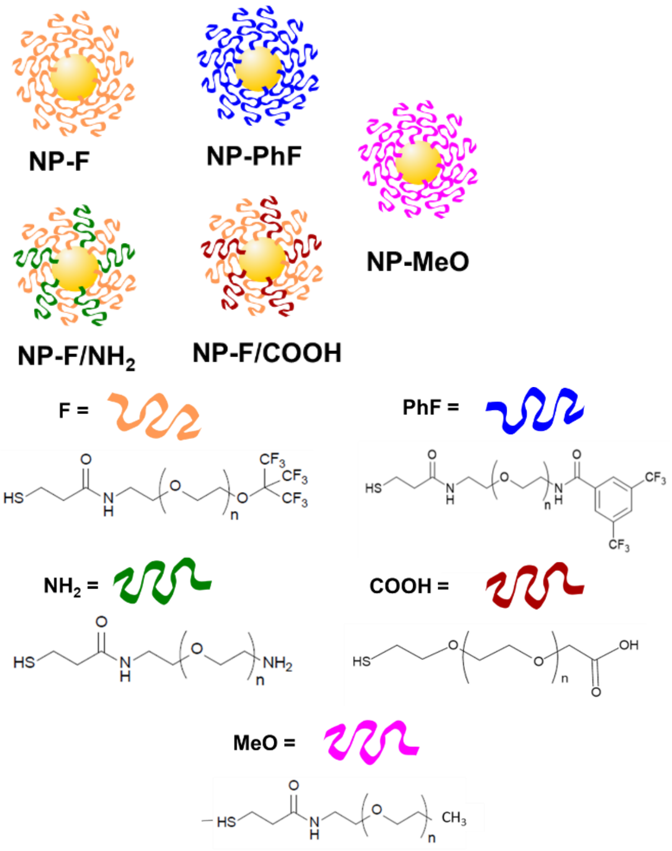

Figure 1:schematic PEG AuNPs.

References

[1] Arango JM, Padro D, Blanco J, et al. *ACS Appl. Mater. Interfaces* **13**, 12941‑1294 (2021).
[2] Carrillo-Carrion, C., Carril, M. & Parak, W. J. Curr. Opin. Biotechnol. 46, 106‑113 (2017).
[3] Carril, M., Padro, D., del Pino, P. *et al.*, Nat Commun 8,1542 (2017).
[4] Bekale, L and Agudelo, D and Tajmir-Riahi, HA, Colloids and Surfaces B: Biointerfaces 130, 141—148 (2015).