

Spectroscopic kinetic determination of hard protein corona composition

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The protein content and composition of the NP protein corona (NP-PC) will determine the interaction of the NP-PC complex with other biological entities in-vivo and in-vitro[1-2]. Current methods for the composition determination, like spectroscopies or Mass Spectrometry[3], rely still on good technical skills and careful manipulation of the materials, and this is the main reason behind the controversies about the final PC composition. Here, we analyse the different profiles of NP-PC corona formation using the two model proteins (albumin and immunoglobulin) to develop a robust and reliable method, mainly based on NP colloidal stability study when the stabilizing protein excess is removed (**Figure 1**), for analysis of the PC formation, evolution and composition, and further compare them to serum. To do that, we employed time dependent UV-Visible absorption spectroscopy, Dynamic Light Scattering, and Z-Potential measurements, providing a simple and reliable approach for determining both density and composition of the resulting hard-PC. We observed different temporal behaviour for the nanoparticle-protein association depending on the affinity of each tested protein for the NP surfaces. Surprisingly, the expected evolution from a non-stable –soft- to stable –hard- protein corona was confirmed for serum and albumin, while immunoglobulin G inevitably and slowly led to nanoparticle aggregations, indicating lower affinity for the NP surface under the tested experimental conditions. Finally, different kinetic profiles allows for composition determination and the study of the kinetic evolution of the protein corona indicates the dominance of albumin. Mass spectrometry analysis of the digested hard-PC confirmed this observation.

References

- [1] Casals, E. & Puentes, V. F. Inorganic nanoparticle biomolecular corona: formation, evolution and biological impact. *Nanomedicine (Lond)*. 7, 1917–30 (2012).
- [2] Monopoli, M. P., Aberg, C., Salvati, A. & Dawson, K. A. Biomolecular coronas provide the biological identity of nanosized materials. *Nat. Nanotechnol.* 7, 779–86 (2012).
- [3] Tenzer, S. et al. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat. Nanotechnol.* 8, 772–781 (2013)

Figures

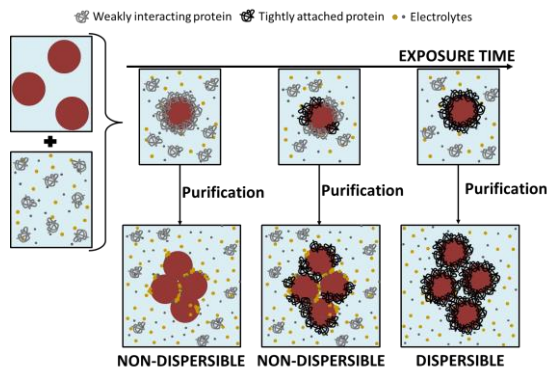


Figure 1: Analysis of the formation and evolution of the NP-PC at different exposure time. The colloidal stability of the NP is determined by UV-Vis spectroscopy and DLS.