# Comment on "Protein Corona Fingerprinting Predicts the Cellular Interaction of Gold and Silver Nanoparticles"

■ Numerous studies were dedicated to the protein/nanoparticle (NP) interaction.[1,2] Walkey *et al.*[3,4] studied the factors influencing the formation of the protein corona following interaction of nanoparticles and human fluids. They developed a useful paradigm predicting association of NP to cells by using not only the nanoparticle size, aggregation state, and surface charge but also the protein distribution in the nanoparticle corona. Nanoparticle/serum protein interaction is a critical parameter for cell uptake, giving some evidence for the so-called concept of "corona fingerprinting" of nanoparticles. The authors used 10 distinct surface-modified gold nanoparticles, with sizes varying from 15 to 90 nm. Recently, we obtained such a protein fingerprint with Eudragit nanoparticles, a copolymer of ethyl acrylate and methyl methacrylate, displaying a diameter of 65 nm and a $\zeta$-potential of $+51$ mV.[5,6] The corona signature of three other types of nanoparticles (silica and positively and negatively charged polystyrene nanoparticles) was in the meantime published.[2] However, corona proteins were eluted using different elution methods: neutral and anionic detergent (LDS) *versus* guanidine thiocyanate, a chaotropic agent allowing optimized elution.

Interestingly, in the three mentioned studies, a similar number of proteins was retrieved from coronæ of polymer, gold, silica, or positively and negatively charged polystyrene, namely, 178,[5] 144,[4] and 165.[2] Twenty one proteins (6%) were common in five coronæ.

As the nanoparticles have different physicochemical properties, it seems interesting to demonstrate, if any, some relationships between proteins to explain their presence in corona of different nanoparticles. Some of the proteins we analyzed (41/178) act through complex interplay and were linked at the action level as recognized in the String database.[5] Those proteins attract others not randomly but by affinity interaction as previously noted, for example, by ligand/receptor or protease/antiprotease complexes.[6] Moreover, the protein corona may induce NP aggregation and cell adhesion onto aggregated NP as was demonstrated previously.[3,5]

We propose to know more deeply the complex network of proteins adsorbed onto nanoparticles and forming coronæ. Therefore, we retrieved their InterPro (IP) domains and evaluated their relative abundance. A protein can possess 1−13 different domains and different proteins, as demonstrated by physiochemical and biological properties, and can share similar IP domains that are responsible for a similar function. By re-analyzing data (Table 1) from three studies,[2,4,5] we observed that within the 511 IP retrieved from proteins adsorbed at the coronæ of the five different NPs 389 (76%) are unique, 55 (11%) are common to two coronæ, and 15 (3%) and 14 (2.7%) are present in three and four different coronæ, respectively. The most striking information is that 38 (7.5%) IP are shared by all five coronæ (Table 2). As IP domains reflect the amino acid sequence of proteins, determining the relative abundance of the InterPro domain of corona proteins indicates a biological signature, namely, the previously mentioned "corona fingerprinting", describing better the complex interplay of nanoparticles with biological fluids. It would be interesting to use the paradigm described by Walkey *et al.*[4] to cluster the IP domains of all corona proteins. By the way, those coronae may drive the fate of the cells with which they are in contact. Using similar polymeric nanoparticles with three different cell lines, we evidenced three different cell fates: autophagy with NR8383 monocytes[7] cell proliferation with human monocytes,[8] and cell differentiation with HMEC cells.[6] Moreover, the knowledge of IP domains gives some information on protein functions and localization,

**TABLE 1. Corona Distribution, Number, and Percentage of InterPro Domains**

| number of coronae[a] | number of InterPro | percentage (%) |
|---|---|---|
| 1/5 | 389 | 76.1 |
| 2/5 | 55 | 10.9 |
| 3/5 | 15 | 2.9 |
| 4/5 | 14 | 2.7 |
| 5/5 | 38 | 7.4 |
| total | 511 | 100 |

[a] Coronomes of silica, polymer, gold, or positive and negative polystyrene NP.

**TABLE 2. Example of 10 on 38 IP Domains (IP Number and IP Name) with Their Function As Accepted and Annotated by Gene Ontology[a]**

| IP number | IP name | protein function (gene ontology) |
|---|---|---|
| IPR000010 | proteinase inhibitor I25 | cystatin |
| IPR000020 | anaphylatoxin/fibulin | anaphylatoxin/fibulin |
| IPR000074 | ApoA1_A4_E | apolipoprotein A/E |
| IPR000152 | EGF-type_Asp/Asn_hydroxyl_site | EGF-type aspartate/asparagine hydroxylation site |
| IPR000436 | sushi_SCR_CCP | sushi/SCR/CCP domain |
| IPR002035 | VWF_A | von Willebrand factor type A |
| IPR002395 | kininogen | HMW kininogen |
| IPR009048 | A-macroglobulin_rcpt-bd | Alpha-macroglobulin receptor-binding |
| IPR014760 | serum_albumin_N | serum albumin N-terminal |
| IPR018486 | hemopexin/matrixin_C | hemopexin conserved site |

[a] Coronomes shared by all NP: silica, polymer, gold, or positive and negative polystyrene.

as they are involved in extracellular, membrane, and cellular compartments (Table 2). The knowledge of such functions may guide researchers in explaining more accurately NP/cell interactions and may be useful in predictive and mechanistic studies achieved on NP.

Hence we propose the word "coronome", a contraction of "corona proteome", a biological endpoint that should be determined for each nanoparticle that is designed for human applications. Studying coronomes consists not only of identifying and quantifying proteins but also of determining the complex interplay of protein networks as given by the String database and analysis of relative abundance of IP domains in the corona. This endpoint seems as important to check as the more classical physicochemical parameters like chemical composition, ζ-potential, or diameter. It allows researchers to better understand corona protein interplay and functions as well as gives some clues on biological fate and action of nanoparticles *in vivo*.

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