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Review article

Dissociation coefficients of protein adsorption to nanoparticles as quantitative metrics for description of the protein corona: A comparison of experimental techniques and methodological relevance

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ABSTRACT

Protein adsorption to nanoparticles is described as a chemical reaction in which proteins attach to binding sites on the nanoparticle surface. This process is defined by a dissociation coefficient, which tells how many proteins are adsorbed per nanoparticle in dependence of the protein concentration. Different techniques to experimentally determine dissociation coefficients of protein adsorption to nanoparticles are reviewed. Results of more than 130 experiments in which dissociation coefficients have been determined are compared. Data show that different methods, nanoparticle systems, and proteins can lead to significantly different dissociation coefficients. However, we observed a clear tendency of smaller dissociation coefficients upon less negative towards more positive zeta potentials of the nanoparticles. The zeta potential thus is a key parameter influencing protein adsorption to the surface of nanoparticles. Our analysis highlights the importance of the characterization of the parameters governing protein–nanoparticle interaction for quantitative evaluation and objective literature comparison.

Contents

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1. Introduction

Proteins in solution may adsorb to surfaces. This phenomenon has been investigated with a long history for extended (and in general planar) surfaces. One example in this direction are intended protein coatings of cell culture substrates to improve cell growth such as fibronectin, laminin, polyornithine (Hindie et al., 2011; Keselowsky et al., 2003; Jung et al., 2009; Min et al., 2013). Even when cell culture substrates are not coated intentionally, proteins from serum-containing media adsorb to the surface and thus provide coating. Formation of such protein films can be for example measured with ellipsometry (Vroman and Lukosevicius, 1964). With nanotechnological tools such coatings can nowadays even be directly visualized, for example by scratching the surface with the tip of an atomic force microscope (AFM) (Domke et al., 2000). Protein coatings can drastically change the physicochemical properties of surfaces (Vroman, 1962). Hereby already early work has demonstrated that protein coatings are not a static entity, but may undergo continuous adsorption and desorption, in which originally adsorbed proteins desorb and other adsorb. This is nowadays described in the context of the Vroman effect (Jung et al., 2003; Noh and Vogler, 2007). The order in which proteins may adsorb to a surface has been quantified for many examples. For example human fibrinogen (FIB) binds stronger than human serum albumin (Vroman and Adams, 1969).

From the physicochemical point of view adsorption and desorption can be interpreted as a chemical reaction

$$n \cdot P + S \leftrightarrow P_n S \tag{1}$$

in which the educts are proteins P in solution and the free surface S, and the product is a protein-surface complex P_nS in the form of a protein-layer bound to the surface. Such chemical reaction can be quantified in terms of a dissociation equilibrium coefficient or apparent dissociation coefficient

$$K_D = \frac{c^n(P) \cdot c(S)}{c(P_n S)} \tag{2}$$

which describes in dependence of the protein concentration $c(P)$ *etc.* whether in equilibrium the reaction will be shifted towards free or adsorbed proteins (del Pino et al., 2014). According to the Hill model (Hill et al., 1910) in equilibrium N of N_{max} binding sites of the surface for proteins will be occupied.

$$\frac{N}{N_{max}} = \frac{1}{1 + (K'_D/c(P))^n} \tag{3}$$

Hereby

$$K'_D = (K_D)^{1/n} \tag{4}$$

is the protein concentration at which half of the surface is saturated with proteins. In this way also K'_D can be used in as similar way as K_D as quantifier for protein adsorption to surfaces. In the context of Eq. (3), n is the Hill coefficient. Dissociation coefficients are thus a convenient way to quantify protein adsorption of different surfaces.

While being textbook knowledge for planar surfaces, these concepts have gained new interest in the context of colloidal nanoparticles (NPs). In case NPs are dispersed in protein containing media, also for this geometry protein adsorption may occur, which is termed the formation of a protein corona (Cedervall et al., 2007; Docter et al., 2015a). In the last decade, it has been demonstrated experimentally that the protein corona plays a crucial role in the interaction of the NPs with cells. As outermost part of the NP the protein corona largely determines the "biological identity" of a NP (Fadeel et al., 2013; Docter et al., 2015b). Adsorption of proteins (and likely other macromolecules) from the host changes the actual physico-chemical nature of the NPs, which can change general features of the nanosurface "seen" by soluble and cellular biological actors, like surface charge or hydrophobicity. Dependence of the corona formation on several NP parameters such as size (Goy-Lopez et al., 2012; Lundqvist et al., 2008; Deng et al., 2011; Huang et al., 2013), charge (Hühn et al., 2013; Feliu et al., 2012), shape (Albanese et al., 2012), surface chemistry (Pelaz et al., 2015), *etc.* and on external parameters such as pH (Moerz et al., 2015), temperature (Mahmoudi et al., 2013; Lesniak et al., 2010) *etc.* has been investigated. It is also known that the protein corona is no static entity, but undergoes dynamic changes during the lifetime of a NP from the first contact with extracellular medium until having reached the final location inside cells (Casals et al., 2010; Tenzer et al., 2013; Chanana et al., 2013).

However, besides changing the physico-chemical properties of NPs the protein corona may also directly interfere with biological signal cascades. Indeed, virtually any biological macromolecule is "full" with information, encoded by its structural determinants, substantially dictating its ability to bind to other molecules, free in solution or expressed on cells. In this respect, it is to be remembered that often protein-mediated binding to other molecules leads to the activation of normally inactive self-amplifying cascades of biological and pathological paramount relevance, like, for example, the coagulation (Tavano et al., 2010) and the complement cascades (Banda et al., 2014). Another related aspect to be closely monitored when considering new nanoparticle properties induced by the protein corona, is the interaction with phagocytic cells (Segat et al., 2011; Fedeli et al., 2013, 2015). Indeed, there may be induction of an improved or a reduced ability to evade, or obstacle, the clearance of the formed nanoentity by macrophages placed in organs like the liver and the spleen. This is of utmost importance, since the intensity of these phenomena influences the blood circulation half-life and hence the efficacy of a nanotheranostic formulation.

Despite joint efforts by many research groups to investigate the protein corona there are still some important questions. This involves for example the geometry of the protein corona. While in some studies formation of monolayers are claimed, others report thick coronas and multiple shell formation (Maiolo et al., 2015). One general problem for unravelling the remaining secrets is that though numerous studies exist, many of them are hard to compare, as there is a lack of quantitative parameters which could be used as metrics for direct comparison.

In Fig. 1, transmission electron microscopy (TEM) data are presented, in which the protein corona adsorbed to NPs is visualized with negative staining. These data correspond nicely with the monolayer hypothesis, as shown in Table 1.

Similar to planar surfaces, also in case of NPs the strength with which proteins bind to the surface of NPs can be quantified by determining the protein concentration $K'_D = (K_D)^{1/n}$ at which half of the NP surface is saturated with proteins (del Pino et al., 2014). K'_D values thus could be used as quantifier to compare protein corona formation for different NPs and different proteins under equilibrium conditions. K'_D values regarding the protein corona have been determined by several groups. The aim of this review article is to describe the different methods with which these values have been experimentally obtained and to compare the results obtained for different NPs and proteins.

2. Description of experimental techniques

2.1. Fluorescence correlation spectroscopy (FCS) and dynamic light scattering (DLS)

Protein adsorption to NPs can be directly observed by measuring changes in hydrodynamic diameter d_h. The more proteins adsorb on the surface of one NP, the bigger the NP-protein complex and thus the hydrodynamic diameter of the NP becomes. Hydrodynamic