Protein at liquid solid interfaces: Toward a new paradigm to change the approach to design hybrid protein/solid-state materials

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Protein at liquid solid interfaces : toward a new paradigm to change the approach to design hybrid protein/solid-state materials

Diego Coglitore[1], Jean-Marc Janot[2], Sebastien Balme[2]*

[1]*CNR NANOTEC - Institute of Nanotechnology c/o Campus Ecotekne, Lecce, via Monteroni, 73100*
[2]*Institut Européen des Membranes, ENSCM, CNRS, Université de Montpellier,* Place E. Bataillon 34095 Montpellier France

*Corresponding Author E-mail: sebastien.balme@umontpellier.fr

Abstract:

This review gives an overview of protein adsorption at solid/liquid interface. Compared to the other ones, we have focus on three main questions with the point of view of the protein. The first question is related to the kinetic and especially the using of Langmuir model to describe the protein adsorption. The second question is about the concept of hard and soft protein. In this part, we report the protein structural modification induced by adsorption regarding their intrinsic structure. This allows formulating of a new concept to classify the protein to predict their behavior at solid/liquid interface. The last question is related to the protein corona. We give an overview about the soft/hard corona and attempt to make correlation with the concept of hard/soft protein

Keywords: protein adsorption; soft protein; hard protein; nanoparticle corona; CATH

1. Introduction

The protein interactions with solid interfaces have a long history, from the origin of life to the new high performance materials for industry. That is the raison why the topic has been of interest for numerous research teams for a long time. From a material point of view, the protein adsorption depends on its structure and its surface properties. Whatever the material, numerous applications have been targeted such as biosensors, implants, environment for pollutant degradation, biofuel production, energy, food[1]. To illustrate the importance of such hybrid protein/materials, we can mention enzymes that catalyst the reaction involved in biological regulation and metabolism. These proteins ensure that kinetics and thermodynamics of chemical reactions are compatible with life condition[2]. Numerous nanostructured materials such as nanoparticles, nanofibers and mesoporous materials were extensively studied to load enzymes for applications in energy, health, sensor, storage, separation, catalysis etc[3–5]. Materials doped with enzymes have a real interest to the market for their reusability[6]. Whatever the applications and the materials, the goal is to improve the stability and/or the enzyme activity. To do so, different strategies of immobilization as well as *in-situ* entrapment, are reported[7]. However, despite extensive studies and the improved understanding of protein structure, the approaches to synthesize such hybrid materials are empirical as perfectly describe by Hudson et al.[8,9], or Bolivar et al.[10]. The methodology described by Secundo takes into account the protein conformation but the approach is still random[11]. Adsorption of proteins on solid surfaces has been studied for several decades and the general concepts are nowadays well established. To improve our knowledge in the active field of enzyme immobilization a clear view about how the interface modulates the conformation and activity of the enzymes is crucial. Beside the conformation, the orientation that influences the accessibility of active site and the ability of internal motion are also important factor in the enzyme activity. Although no general rules can be formulated, one challenge would be to be able to predict the protein behavior on liquid/solid interface regarding their folding energy and the physical-chemistry properties of material. Conversely the fundamental understanding of the protein adsorption processes is not only essential to design and optimize these materials, but also to evaluate the impact of the material on health such as the use of nanoparticles[12], dialysis membrane, scaffold etc…

The protein adsorption is also the first step in the response to artificial material. Despite the importance of this process in regenerative medicine and the effort provided by numerous groups around the world, many question are still open, such as the quantitative description of structure/properties relationship, the protein selectivity and the role of absorbed proteins on the response to artificial materials[13]. As exemple, the fibrinogen is a key factor in the thrombogenicity of numerous biomaterials. The prediction of its behavior on material is essential because its adsorption limits the platelet adhesion[14]. The conformation of the material layer on a solid surface can also play a role. As an example, a high density of polyethylene glycol (PEG) that adopts a brush conformation is more efficient to prevent the protein adsorption than a mushroom conformation. Similar trends was also reported for stimuli responsive polymer such as poly(N-isopropylacrylamide) (*PMIPAM*) [15–17]. The wettability and surface charge are also involved on the protein process[18]. The salt concentration is also important for the protein adsorption[19]. As exemple the albumin allow to prevent the nanoparticle aggregation on salted medium[20–23]. The protein detection using single solid-state nanopore is one topic where the understanding of protein adsorption is crucial. In this field, several papers mention that the protein translocation are slower and the number of event lowers that the expected one[24,25]. To explain this, Balme and co-workers have demonstrated a

correlation between the number of event and the affinity between the protein and the nanopore surface[26].

In most of the cases, the protein adsorption is principally evaluated from the point of view of the material properties rather than the protein one. Indeed we can find reviews specifically focused on bioceramics for the application such as implants[27], metal organic framework for enzyme immobilisation[4][28][29], nanoparticles[1,30–34], mesoporous materials for energy and environmental applications[35] as well as polymeric surfaces[36] . Others are focused on specific types of protein such as enzymes for catalysis applications or on the impact of confinement on enzymatic reaction[5,37–40], as well as their stabilization[7,41] or fibrinogen adsorption on biomaterials[42]. All these reviews agree with the fact that protein adsorption is highly complicate and more investigations are required to go toward predictive models.

As we mentioned, the approach to immobilize proteins (especially enzymes) on a material is almost completely empirical. It consists in the optimization of the material taking into account external factors[6]. However the protein-material interactions were extensively investigated in numerous type of surfaces, aiming to understand the effect of the immobilization conditions on the protein conformation, stability, activity and effectiveness[11]. However, despite these efforts, basic questions about the structural details of the absorbed protein are still difficult to answer[11,43]. Thus, no predictive models emerged to forecast if an adsorbed protein has chance to maintain its biological activity. This is re-enforced by the fact that the time scale to optimize the protein conformation on the surface is not compatible with molecular dynamic simulations.

In the literature, we can find a first attempt to categorize the protein behavior on the surface. The concept of “hard” and “soft” protein based on empirical observations is useful but not based on clear intrinsic protein properties. In other word, it is not possible to a priori classify a protein in one of the two classes. With the recent advances in the understanding of the protein structure, their folding and the development of bio-computing, it could be possible to propose a way to predict the protein behavior on materials.

In this review, we will report the protein adsorption from the protein point of view, with a particular attention to the structural changes. We connect the new advances on protein structure with the literature on the protein adsorption on a large number of materials. We aim to open a new way to go for a predictive model to evaluate the protein behavior on materials. This review is divided in three main sections. The first one deals with the kinetic point of view. We will discuss about several discrepancies often found in literature such as the use of the Langmuir isotherm. The second section is focus on the protein structure point of view where a discussed the relation with the protein conformation change with the CATH classification. The last section deals with the protein corona on nanoparticle and attempts to make some correlation with the concept of hard and soft protein.

2. Kinetics point of view

2.1. Forces involved in the protein adsorption processes

The protein adsorption on a solid-state surface was wisely qualified as a ”common but very complicated phenomenon” by Nakanishi and co-authors[44]. The complexity of this process is inherent to the protein structure. The adsorption is the result of various interactions between the

surface, the protein but also the solvent and other small molecules and ions present in solution[45]. From the protein point of view, the interactions which drive the adsorption process are the same than those who maintain its tridimensional structure. They cover a large scale of energy from covalent bonds (i.e. disulfide bridge 320 kJ mol^{-1}), electrostatic interactions (35 to 90 kJ mol^{-1}), hydrogen bonds (8 to 40 kJ mol^{-1}), van der Wall interactions and hydrophobic interactions (4 to 12 kJ mol^{-1}). Regardless the mechanism, the spontaneous protein adsorption (at constant pressure and temperature) on material can occur only if the Gibbs energy $\Delta_{ads}G$ of the system decreases.

$$\Delta_{ads}G = \Delta_{ads}H - T\Delta_{ads}S < 0 \quad (1)$$

where $\Delta_{ads}H$ and $\Delta_{ads}S$ are the variation of enthalpy and entropy during the adsorption process. At the native state in solution, the conformational entropy of protein is low. The passage from solvated to the adsorbed state induces a loss of configurationally entropy[46]. In the equation 1, several phenomena are involved which can be decomposed as subprocess: (i) change in the state of hydration both for the solvent and the protein surface, (ii) redistribution of charged groups and (iii) rearrangement in the protein structure. All these subprocesses contribute to $\Delta_{ads}G$[45]. The complexity of the protein behavior makes difficult to predict its adsorption process on a solid state surface.

The approaches to explain the protein adsorption on hydrophilic and charged materials are more focused on electrostatic and hydrophilic considerations. It is well-known that the electrostatic interaction has a role on the protein adsorption[47]. However, as demonstrated by simulations, the electrostatics cannot fully explain the protein adsorption process[48,49]. Usually, any explanation of the protein adsorption underestimates the importance of hydrophobic interactions. A perfect illustration was the adsorption enhancement of methyled Bovin Serum Albumin (BSA) on montmorillonite reported by Stauton and Quiquampoix. The author have highlighted a real contradiction between the hydrophilic character of material (a clay mineral), on which the adsorption of hydophobic protein has promoted.[50]. The pH plays a role in the protein structure and its global charge. Its effect depends on the kind of interaction between the protein and the material. If the electrostatic interactions dominate the adsorption, the dependence on the pH will be high[51]. Even if we cannot enounce a general law, there is a general tendency for all proteins to be pH dependent in terms of adsorption. For pH<isoelectric point (IEP), the adsorption increases with pH until the IEP. For pH>IEP, it decreases with the pH. It was reported that the maximum of interfacial concentration is reached at pH close to IEP, for instance, for DNA polymerase[52], Monomeric Bt CryA1[53], and Laccase[54] This behavior is assigned to the protein neutral net charge which minimize the electrostatic repulsion effect[53]. As previously mentioned, the electrostatic attraction is easily counterbalanced by the optimization of the number of weak interactions relative to the protein unfolding. This general description does not claim to predict the behavior of all proteins. In the case of Bt CryA1, it was reported that the optimal pH for the adsorption is close to the isoelectric point for Mt and kalonite[53]. However opposite results were reported by Zhou et *al.* where the optimal pH is 9 for clay mineral[55].

2.2 Kinetics of adsorption

The kinetics of the protein (P) adsorption on a surface (C) can be represented as follow:

$$P + C \underset{k_{des}}{\overset{k_{ads}}{\rightleftharpoons}} P_C$$

where k_{ads} and k_{des} are, respectively, the kinetic constants of adsorption and desorption. The general kinetic equation implies that the adsorption process is reversible.

$$\frac{d\Gamma}{dt} = k_{ads} C_i \phi - k_{des} \Gamma \qquad (2)$$

where C_i is the protein concentration in solution close to the solid interface, Γ is the interfacial concentration of protein, the parameter ϕ is relative to the surface coverage. In the case of irreversible adsorption, a frequent case talking of clay mineral, we have $k_{des} = 0$, which limits the kinetics to the first term of equation 1.

The protein adsorption on solid state surface is composed of three steps. The first one is the protein transport from the bulk to the interface. This step involves the diffusion and other motion forces (convection, stirring)[56]. The second one is the adsorption and the last one is the future of the protein. The first step usually controls the kinetic of adsorption when the protein transport to the interface is slower than the interfacial reaction. If the protein transport is only driven by the diffusion, the kinetics follows the Smoluchowski model.

$$\Gamma(t) = 2C_b\sqrt{\frac{Dt}{\pi}} \qquad (3)$$

where is $\Gamma(t)$ the interfacial concentration, D the diffusion coefficient of the protein and C_b the protein concentration in the bulk. The Leveque model is often more suitable to follow the protein adsorption kinetics since it takes into account the convection. When the protein adsorption is limited by the diffusion and/or convection, the protein concentration close to the interface tends to zero. This depletion is usually theoretically determined by calculation of the Levêque distance[57,58]. The distance of depletion is a key point in the membrane science or in the coating process by proteins since it will influence the contact time required for an optimal protein covering. Despite the interest to characterize the depletion zone, the latter was never experimentally observed, even if confocal setups should be suitable to solve this issue[59]. In certain cases, the adsorption process is limited by the interfacial reaction. Here the protein concentration close to the interface is quasi-uniform and no depletion occurs. In order to describe the passage from the transport controlled by the diffusion to the one controlled by the interfacial reaction, one approximation is based on the addition of the characteristic time of both processes. However an exact solution was proposed in the case of slit geometry[58].

Several models were used to follow the protein adsorption taking into account the three steps. The most commonly used is the Langmuir model. The latter assumes that surface has a definite number of adsorption sites which are energetically equivalent and the adsorption process is reversible. In addition, at the equilibrium, desorption and adsorption occur and surface sites are occupied by a homogeneous monolayer. The Langmuir isotherm is the most used to describe the protein adsorption when equilibrium state is reached.[60,61] However, from a fundamental point of view, this model is not acceptable for several reasons (i) the protein adsorption is often irreversible, (ii) the surface of the material is rarely homogeneous and (iii) after adsorption protein unfolding and/or reorientation can occur.[62] For the heterogeneous materials (i.e. clay mineral), the empirical Freundlich equation is commonly reported to describe the protein adsorption. Compared to the

Langmuir one, it does not assume that the surface is homogenous which is more realistic for material where the protein can adsorb on different non-equivalent sites. However, it assumes that the saturation state is never reached that is not realistic. It is also interesting to notice that for a similar material the protein absorption was reported as Langmuir or Freundlich by different authors, for instance, for the BSA on natural Kaolinite [63–65], the lipase on Bentonite Na^+ or exchanged with surfactant[60,61]. Both Langmuir and Freudlich models have strong weakness in describing the protein adsorption and thus several models were proposed aiming a description that could take in account both the heterogeneity of the surface and the maximum of surface coverage. Sposito and coworker proposed an alternative to Freundlich equation which includes the maximum of surface coverage[66]. Another model called "Freundlich-Langmuir" derivated from Langmuir model adds an empirical factor of heterogeneity. The hydrid approach "Freundlich-Langmuir" seems particularly efficient to describe the gelatin adsorption on calcium and sodium bentonite[67]. It is less employed than Langmuir and Freundlich model despite their theoretical approximations. However, none of these models take into account the fundamental problem of the reversibility of the protein adsorption. Thus, we can ponder about the relevance to use these models because they are based on erroneous the assumption. The fact is that there are not simple model to provide a real description of the complexity of protein adsorption on clay mineral. Isotherms can be used to provide a rough "quantification" of a pseudo-equilibrium constant or a maximum of the interfacial concentration, which allow the comparison of the influence of different experimental conditions, intercalated molecule, material, etc. However, they should not be employed to explain the adsorption mechanism. In other we can reword the Latour's conclusion[62], the protein adsorption look like a Langmuir (or Freundlich) adsorption process and the data are nicely fitted by these models but it is not a simple Langmuir (or Freundlich) adsorption process excepted may be in extremely rare cases.

In the case of homogenous surfaces, the random sequential adsorption (RSA) was developed to overcome the weakness of Langmuir model. In this model the protein adsorption is still considered irreversible but it does not assume an organized and optimal surface coverage[68]. The model is useful if the protein keeps its structure and orientation on the surface. Indeed it takes into account the protein unfolding, surface rearrangement and desorption. In this case, the multiple-stage model are more suitable to describe a reversible adsorption process that is often observed experimentally[69]. It also allows to consider the modification of the protein structure. The rollover model is allowed to describe the reorientation (typically end-on/side-on) of a protein after adsorption on surface. It was reported for fibrinogen at high concentration where the second stage consists in the rearrangement of the protein to minimize its energy[70]. Other more complex kinetics models such as surface cluster or tracking model were also reported[69].

3. The protein structure point of view

3.1 Energy landscape of protein

Proteins are complex macromolecules composed by a linear chain of amino acids. Their conformations result from several level of structuration. The first one corresponds to the linear chain of amino acids. The secondary structure is the local folding in regular structure such as α-helix or β-sheet. The tertiary structure is the global folding of the protein. Finally the quaternary structure is the aggregation of monomer units of a same polypeptide chain. The 3D structure of monomeric proteins is driven by the secondary and tertiary structure. It results from a sum of intra-chain

interactions which are necessary to minimize the energy of the protein chain. The native structure is the operating form which usually corresponds to a low energetic state. However, the structure of the protein cannot be reduced to a native and an unfolded state[71]. They can adopt numerous transition states of folding corresponding to low energy states [72–74]. These states of misfolding protein are also involved in the aggregation processes [75]. The passage between these different conformations requires additional energy (temperature or irradiation) or a modification of the energy landscape by chemical agent (pH, molecule, ionic strength, solvent). The protein design is based on the success of the structural prediction[76,77]. The protein denaturation corresponds to a modification of the secondary and tertiary structure. In the case of enzymes, it is often followed by a loss of activity. Even if the energy landscape model seems to have a general consensus, the protein folding is still an open scientific question[78]. Indeed, the folding can be driven by a wide range of interactions that can be competitive, such as short range hydrophilic, hydrophobic and long range electrostatic[79]. Additionally, the polypeptide chain offers thousand degrees of freedom related to their bond angles and to the motion of the solvent[80]. In order to describe the different states of the protein folding, the protein adsorption can also be represented by an energy landscape where the different conformations have different energetic levels. In 2012, Pan and his co-workers have opened the way to this concept. They described the protein GB1 adsorption on a latex particle (80 nm diameter) following its unfolding[81]. This concept can be extended to the protein adsorption (Figure 1). Indeed, we can imagine that a protein can be adsorption on surface with numerous conformations corresponding to several minimum of energy. The lowest energetic state would correspond to protein conformation where the weak interactions with the surface are optimized, typically, an unfolded state. The other states have higher energy. Similarly to the protein energy landscape in solution, the passage to a stable conformation requires to overcome an energy barrier that mainly depends on the intrinsic protein energy, or in other word its unfolding energy. As in solution, these barriers can be modulated with the environmental condition, such as the solvent condition, the presence of surfactant and the material surface and size properties.

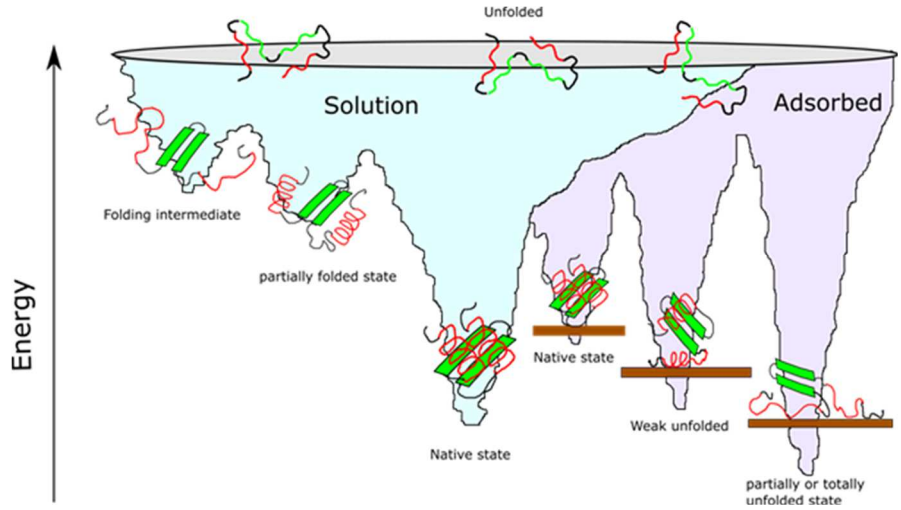

Figure 1 : sketch of protein energy landscape of adsorption

3.2 Hard/soft proteins: how improve the classification

The concept of “hard” and “soft” protein was proposed by Takaaki and Norde[82]. This classification assumes that the conformational rigidity, or “hardness”, of a protein drives the adsorption phenomena. More than 25 years after, it is used with the aim to provide a roughly prediction of the adsorption process and the conformational modification of proteins.

The “hard” proteins have high structural stability (Figure 2). Their adsorptions are mainly driven by the electrostatic interactions. They are sensitive to both the sorbent and the protein hydration. The hard protein adsorption occurs on hydrophilic surfaces only when electrostatic interactions are favorable. In this case, the process is driven by the electrostatic attraction $\Delta H < 0$. The ΔS due to the charge redistribution induces by the adsorption can play an additional role. On the contrary under electrostatic repulsion, the $T\Delta S$ contribution has to overcome the $\Delta H > 0$. This explains that hard protein adsorption on a surface with similar charge is usually not favorable. “hard” proteins absorb on hydrophobic surfaces when ΔH can be positive or negative but anyway small, and thus the driving force will be the enthalpy $T\Delta S > 0$.

On the contrary than hard proteins, “soft” proteins have low structural stability (Figure 2). Their adsorption process involve additional driving forces, related to their structural modification.[83] Compared to the “hard” protein, the thermodynamic prediction of the main process driving the adsorption is not easy because both ΔH and $T\Delta S$ components have to be added [84]. The protein unfolding increases entropy ($T\Delta S > 0$) and allows the protein internal regions to form additional contacts with the material sites optimizing the interaction with the surface. This explains that soft proteins can be adsorbed on both hydrophobic and hydrophilic surfaces, whatever the electrostatic interaction (attraction or repulsion) in contrast with the hard protein behavior. For a clay mineral, the location for the adsorption is also affected by the protein hardness. The ‘soft’ proteins occupy the interlayer space and external surface area, while for the hard one the adsorption occurs primarily on the external surface area[85].

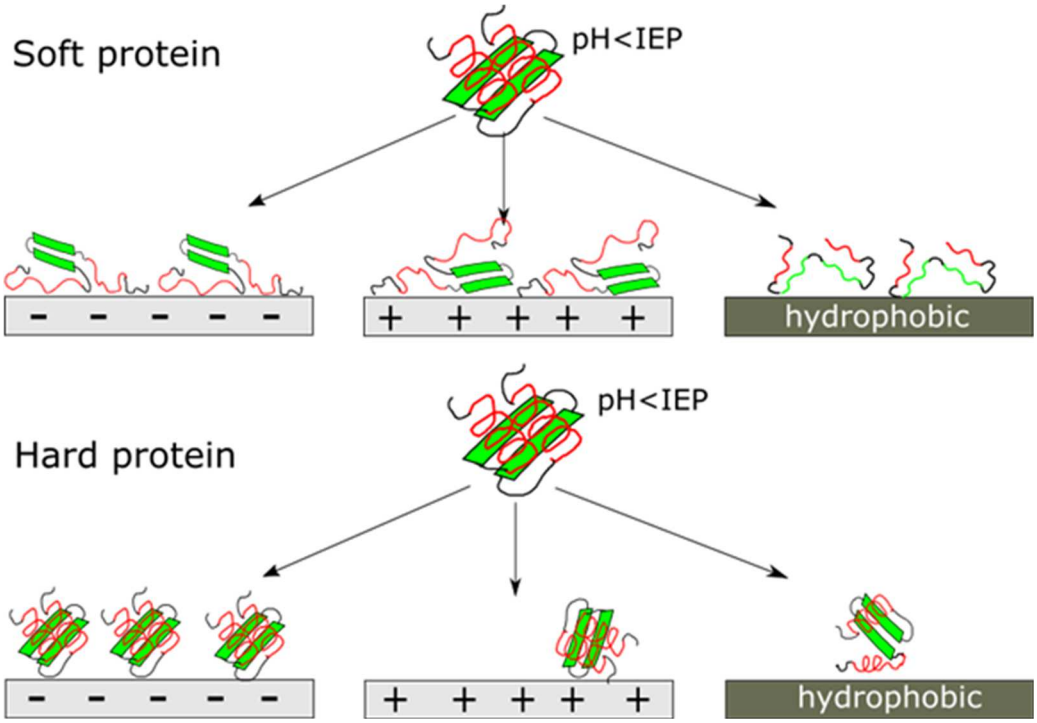

Figure 2 : illustration of hard and soft protein behavior on different kind of surface

Whatever the protein, it adopts a preferential conformation which aims to minimize its energy with the solid interface. The “hard” protein keeps roughly its structure. Thus, it makes a sense to speak about orientation “side-on” or “end-on”. The preferential orientation is driven by the charge distribution and the dipole moment on the protein surface in the case of electrostatic interaction[86]. As exemple the lysozyme can adopt different orientation on anionic clay and cationic layered double hydroxide [87]. After adsorption the protein can move on the solid surface. For hard protein, it is possible a reorientation end-on to side-on, characterized by overshooting adsorption kinetics[88,89]. Another difference is that at pH higher than IEP, the decrease in the adsorption is sharper for hard proteins than for soft proteins, because they are less prone to the unfolding. Thus the compensation of the electrostatic repulsive effect is not counterbalanced by the optimization of hydrophobic interaction with the material as occurs for the soft protein[90].

The assignment in the categories “hard” and “soft” was empirically stated several years ago from the ability to a protein be adsorbed on hydrophobic surface. Presently, there is no method to predict the behavior of the protein adsorption. Actually, less than 25 proteins were clearly assigned to one category. The majority of proteins are excluded from this classification due to their complexity, such as the fibrinogen[91], or never been clearly assigned to a category, such as glucose oxidase or lipase which are extensively used with numerous material for supported catalyst. The lack of predictive model implies that the immobilization of a protein to generate an hybrid material is only empirically considered as a succession of experimental go/no-go [8,9]. Thus, a method to wisely choose a protein according to the expected material properties would be useful.

The concept of “hard” and “soft” protein is related to their ability to modified their structure on a surface, and thus from a thermodynamic point of view, to the Gibbs energy of the protein ($\Delta G°_{unf}$) unfolding, dependent on its intra-chain interactions. From those, the protein adopts a secondary structure where the folding energy will be mainly subject to the α-helix and β-sheet content. Note that, $\Delta G°_{unf}$ also involves many external parameters such as pH, ionic straight, or the kosmotropic or chaotropic character of ions. Typically, the $\Delta G°_{unf}$ is larger for hard proteins (60 kJ mol^{-1} for lysozyme) than for soft proteins (21 kJ mol^{-1} for α-lactalbumin)[92].

From a structural point of view, α-helix are more compressive than β-sheet and thus their unfolding requires less energy explaining why for soft proteins the ratio α-helix/β-sheet is higher than for “hard” proteins. Based on that, one question is: how to assign *a priori* one protein to the soft or the hard category? A partial response can be found in the protein structure classification CATH. Proposed in 1997 by Oregon et al.[93], it is based on a structural hierarchy where the two first levels (C: Class and A: Architecture) are related to the secondary structure and especially the α-helix, the β-sheet and their tridimensional arrangements. On the table 1, we have reported many proteins commonly investigated in interaction with material. It is particularly interesting to note that all soft proteins belong to the mainly-α class. The hard proteins are shared between two class mainly-β and α/β-mix with a layer sandwich architecture.

Table 1. list of protein used in interaction with material

Class	Architecture	Protein	CATH (code)	category
Mainly β	β -barrel	Avidin	2.40.128.30	Hard

		streptavidin	2.40.128.30	Hard
		β-lactoglobulin	2.40.128.20	Unassigned
		α-chymotrypsin	2.40.10.10	Hard
		coagulation factor XII	2.40.10.10	Unassigned
	sandwich	Laccase	2.60.40.420	Unassigned
		α-amylase	2.60.40.1180	Unassigned
		concanavalin A	2.60.120.20	Unassigned
		Globulin		
		immunoglobulin G	2.60.40.10	Unassigned
	Roll	urease	2.30.40.10	Unassigned
		Ovalbumine	2.30.39.10	Unassigned
Mainly α-	Othrogonal bundle	BSA / HSA	1.10.246.10	Soft
		myglobin	1.10.490.10	Soft
		hemoglobin	1.10.490.10	Unassigned
		α-synuclein	1.10.287.70	Unassigned
		α-lactalbumin	1.10.530.10	Soft
	Up-down bundle	Fibrinogen	1.20.5.50	Unassigned
Mix α-β	Roll	protein G1	3.10.20.10	Unassigned
		Ribonuclease A	3.10.130.10	Unassigned
	α-β barrel	glucocerebrosidase	3.20.20.80	Unassigned
	layer sandwich	subtilisin Carlsberg	3.30.60.30	Unassigned
		DNA polymerase	3.30.70.370	Unassigned
		α-chemotrypsin	3.30.228.10	Hard
	Layer (aba) sandwich	Alkaline phosphatase	3.40.720.10	Unassigned
		Lipase	3.40.50.1820	Unassigned
		Cytochrome C	3.40.228.10	Hard
		Lysozyme	3.40.80.10	Hard
		pyruvate kinase	3.40.1380.20	Hard
		subtilisin	3.40.50.20	Hard
		transferrin	3.40.190.10	Unassigned
		RNase	3.40.50.1010	Hard
		glucose dehydrogenase	3.40.50.720	Unassigned
		β-D-glucosidase	3.20.20.80	Unassigned
		butyrylcholinesterase	3.40.50.1820	Unassigned

		formaldehyde deshydogenase	3.40.50.720	Unassigned
	layer(bba) sandwich	Glucose oxydase	3.50.50.60	Unassigned

3.2 Correlation between the protein classification and its structural modification at liquid-solid interface

Combining the CATH classification with the concept of “hard” and “soft” proteins seems convenient to predict potential structural modification after their adsorption (Figure 3). This is particularly true in the case of a biocatalyst where the integrity of protein structure has to be maintained. In this case, a hard protein will be more suitable because the adsorption induces less structural alterations than for the soft proteins. However, reality is more complicated and consequently also the external factors which influence the protein folding. The integrity of the protein structure is provided by a subtle entropy-enthalpy balance. There are proteins that have "hard" behavior under condition of adsorption and a "soft" one on other conditions. This is the case when the conditions favor the liberation of hydrophobic groups.

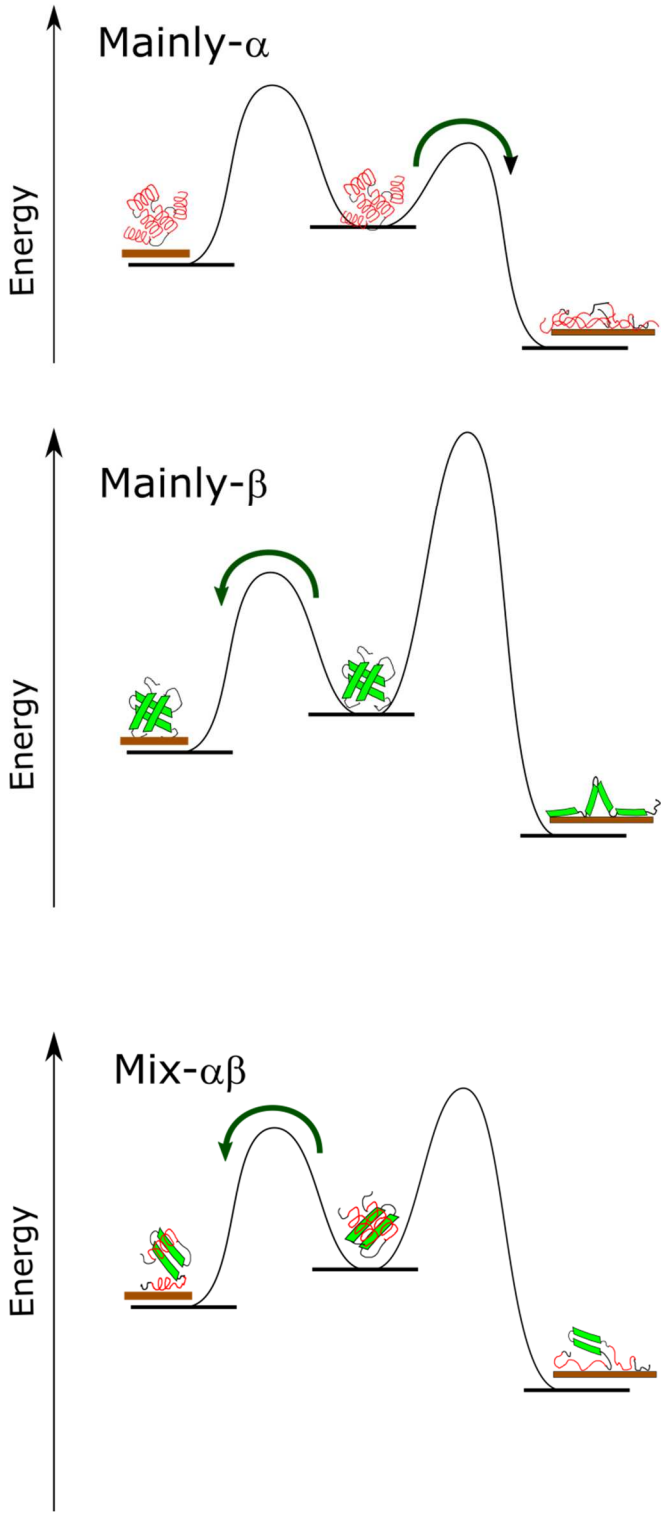

Figure 3 : Sketch about the protein energy barrier of adsorption.

Mainly-α proteins : The Albumin is known as soft protein. Its abundance in blood has made it probably the most studied protein. For the bovin serum albumin, it was reported that whatever the pH, the adsorption induces a decrease in the α-helix ratio. At pH close to IEP, external and internal α-helix domains are unfolded in order to provide a maximum of hydrophobic interaction with the material. The maximum of the BSA interfacial concentration is due to the cooperation of the unfolding and the reduction of surface coverage which occurs at pH above the IEP[50]. At pH lower

than IEP (2.9) the behavior of the absorbed BSA follows the same trend and the protein is mainly unfolded. At pH greater the IEP (6.7), the BSA negative charge leads to a less favorable adsorption. Despite this electrostatic repulsion, the adsorption occurs due the unfolding of the α-helix peripheral domains[94]. In this case the protein unfolding is only partial. There are examples where the use of the CATH classification can support the experimental results from isothermal titration calorimetry that provides information about the conformational change induced by adsorption process. The enthalpies of BSA and β-lactoglobulin (mainly-β) adsorption on Buthyl-sephadose 4FF column, respectively 215 kJ/mol and 77.9 kJ/mol, are directly connected to the large structural modification of the BSA due to its large α-helix structure[95]. On the hydrophobic surface the albumin loss α-helix while the percentage of β-sheet increases.[96] The Fibrinogen is another protein essentially composed by α-helix. With the Albumin and Immunoglobulin G, it is one of the most preponderant protein in blood and thus understand it behavior remain a huge medical interest[97]. As previously mentioned the fibrinogen is a key factor of thrombogenicity because, its adsorption limits the platelet adhesion. On the alkanethiol self-assembled monolayer (SAMS) functionalized with different moieties, the fibrinogen adsorption is enhanced on hydrophobic surface inducing large α-helix loss[98]. Fibrinogen and BSA has several common points. On mesoporous silica material, the secondary structure of human serum albumin and human fibrinogen are modified. The α-helix and β-sheet ratio decreases in benefit to the random one, conversely to the immunoglobulin G (mainly-β) whose keeps it secondary structure[99]. After adsorption on different surface, for the BSA and fibrinogen the ratio α-helix/β-sheet is dependent on the end-on, side-on orientation [100]. As soft proteins BSA and fibrinogen are more prone to adsorbed on hydrophobic because the adhesion force are significantly higher on poor wettable surface than highly wettable. This is due to their ability due unfolding to optimize their interaction with the surface. Conversely the coagulation factor XII (mainly-β) the structural difference is not significant[101]. Even if albumin and fibrinogen show very similar behavior, it exist some difference. On nanoparticle, the secondary structure of albumin becomes less organized (increase of random coil/loop ratio) with the increase of particle size conversely to the fibrinogen. However, for both proteins the trends are more pronounced in the case of hydrophobic material that hydrophilic one[70]. The albumin has much higher affinity with hydrophobic surface due to its low degree of ordered structure. Conversely, the fibrinogen adsorbs quickly on hydrophobic and hydrophilic surface as well[102]. The globin is a family of mainly-α protein, the myoglobin has a ratio α-helix/β-sheet of 86/1[103]. The Horse methemoglobin and MnmE unfold on hydrophobic surface of bioglass. The proteins expose their hydrophobic part inducing a decrease of α-helix to less order structures such as loops and random coils.[104] This was also reported for the myoglobin from equine hearts on different hydrophilic SBA-15.[105] On the same material functionalized with alkyl, the structural modification are more pronounce since the α-helix ratio decrease from 77% until 20% and the β-sheet increase from 5% to 27%. **Mainly-β protein :** Proteins mainly composed by β-sheet are supposed to have a high internal energy and thus a hard behavior. The immunoglobulin G loaded on the mesoporous silica does not show modification of the secondary structure[99]. However, another study on silica surface functionalized with different chemical function reports a systematic decrease of the β-sheet content[100]. The concanavalin A loses its ability of bind specifically oligosaccharide on a very hydrophobic surface (graphen), This was interpreted as due to the protein denaturation but without experimental support[106]. Conversely, the concanavalin A is often used on hydrophilic silica or Au surface to performed quartz crystal microbalance (QCM) experiment without activity loss. The β-lactoglobulin is usually under dimer form, that is very stable. The applied pressure modifies the conformation in a “swollen state” that

presents less repulsive moieties contributing to modify its adsorption properties [107]. With the combination of fluorescence spectroscopy and NMR, Assifaoui et al shown that β-lactogolubulin losses β-sheet when it is loaded on clay minerals[108]. The most surprising result is the evidence of protein structure dependence with the interfacial concentration.Many enzymes were successfully loaded on materials. Numerous of them have mainly-β. The α-chemotrypsin is known to be a hard protein with a ratio α-helix/β-sheet 12/34[109]. Thus no structural modification occurs after adsorption on Au-NP. At pH below or close to IEP, the adsorption of α-chemotryptin does not induce a strong modification of the secondary structure. At pH above IEP the electrostatic repulsion let think that the adsorption process is not possible. However, it occurs entailing a weak peripheral structural modification of the protein to optimize the interaction with the material. This partial unfolding combined with the internal hydration in the vicinity of the catalytic center are at the origin of the loss of enzymatic activity, reported at pH 10[110]. After adsorption on many kind of clay minerals, the optimal pH activity of enzymes such as catalase[111] or urease[112], significantly shifts of more than one unit. There are several interpretations to explain the optimal pH shift. One assumption is the value of interfacial pH due to presence of protons in the diffusive double layer which compensates the negative charge of the clay. The second assumption is an enzyme structural modification which is more relevant[113]. One interest of the enzyme adsorption on material is that its activity is often less sensitive than free in solution to the temperature as reported for acid phosphatase[114] or catalase[115]. The adsorption allows also the enzyme reuse as shown for the laccase[54]. This was explained by the optimization of the protein interactions with the material which stabilize its conformational structure, and consequently, its unfolding requires more energy.

Mix α-β protein : As shown previously, the majority of enzymes reported as active after adsorption on a material belong to the mainly-β, but also αβ mix. The most studied “hard” protein is the lysozyme that has a ratio 40/19[116]. As a very hard protein, it is expected that the adsorption process does not strongly reduces the α-helix content, that is usually reported when the interfacial concentration reaches the maximum coverage [117]. But at low surface coverage, unexpected structural modifications can occur[118]. Structural rearrangement at low ionic strength was reported and assigned to the reduction in the enthalpy transition[119]. The switching from hard to soft behavior was also demonstrated in the case of lysozyme under pressure[92]. Thus, the notion of “very hard protein” for lysozyme should be, in someway, modified[91]. As a hard protein, we could expect that lysozyme keeps its native state when the enzymatic activity is maintained. Actually, the reality is more complex since the protein structure cannot be sum-up to a native structure which is active and a denurated state which is not active. Some structural modification can occur without activity loss. A perfect illustration is the lysozyme that after fibrillation, as an amyloid, keeps partially its antibacterial activity. In most of the cases, the enzyme activity decreases after adsorption[52,114] that can be explain weak structural modifications leading to a partial loss of activity, as reported for the antibacterial activity of lysozyme[120]. Indeed, the adsorption does not induce a large structural modification[121]. Actually, the reality may probably be more complex and the question is still open to understand this partial loss of activity. Indeed two assumptions can be formulated. The first one consists in a homogenous weak structural modification of all the adsorbed protein, which induces a partial loss of activity. In the second one, considering two populations of protein, (i) one keeps its native structure and thus its activity and (ii) the other one is partially unfolded and loses totally its activity. By now, this question is not solved for any protein. Even for the lysozyme, several study report large structural modification after adsorption. As an example, the adsorption on SiO_2

nanoparticle causes a decrease of α-helicility leading to a partial loss of activity. However, this study opens new questions. For instance, it was reported that for NP with diameter of 100 nm, the structural loss is more important than for smaller NP (4 nm and 20 nm)[122]. On magnetic nanocluster, structural modification of lysozyme was also reported without any quantification.[123] As for the lysozyme, the subtilisin Carlsberg (ratio α-helix/β-sheet 30/17[124]) on silica nanoparticle has no significant structural modification.[125]

The protein included in the mix α-β class are often complex, making them more difficult to assign on the "hard" of "soft" behavior. The lipase has an enzyme active at the aqueous/organic interface. It has a complex structure allowing large structural modification. Its adsorption on hydrophobic octyl-agarose stabilizes the open structure[126]. On hydrophilic surface can result in a significant deformation of the protein structure[127]. As for many enzymes, the optimal pH activity of lipase is shifted[128]. In addition the enzymatic activity decreases for a pH higher and lower that the IEP. Usually this phenomena is reversible but not always as shown in the case of β-D-glucosidase on montmorillonite[129]. The adsorption of lipase clay mineral makes its activity less sensitive to the temperature[128]. The glucose dehydrogenase shows a partial unfolding of the side-chains interacting with hydrophobic surfaces[130], requiring a linker to overcome this problem[131,132]. The formaldehyde deshydrogenase loaded on mesoporous zirconia shows an increase of the β-sheet content, but the enzymatic activity is improved[133]. The adsorption of protein G1 on latex particle induces a decrease of α-helix content[81].

For the enzymes, many studies try to connect the modification of activity with the structural modification, but the location and orientation are also the key factors. The adsorption of alkaline phosphatase (IEP 5.7) at pH 7 on homoionic-exchanged saponite reveals a larger interfacial concentration interfacial concentration for Ca^{2+} than Na^{+}, while, the enzymatic activity is better for Na-Sap[134]. This behavior was interpreted by the structural effect of cation on clay structures. However, a similar experiment performed on horseradish peroxidase shows that the amount of adsorbed protein is 3 times higher on Na-Montmorillonite than on Ca-Montmorillonite due to a partial interaction interlayer space. This intercalation dramatically inhibits the enzymatic activity due to inaccessibility of the substrate[135].

To sum up, the literature of the mainly-α protein has clearly a soft protein behavior. The "hard" proteins are mainly-β and mix α-β. We can notice that structural modifications happen even for "hard" proteins, such as lysozyme, but also for more complex proteins, such as lipase, or other enzymes, without a total loss of the enzymatic activity. However, this interesting correlation between the protein structures that can easily found with the CATH database and their unfolding behavior at solid/liquid interface could improve the "hard/soft" classification, and thus could allow a *a-priori* choice of the suitable protein.

4. Protein adsorption on nanoparticle: point of view of corona formation

Due to the quick growth of nanoscience during the past decade, the interaction of proteins with nanoparticles (NPs) has attracted great attention due to the numerous potential applications. Indeed, nanoparticles and nanomaterials are widely used in consumer products, such as cosmetics, food, electronics, inks and paints. Their application is not only limited to these fields, as they are

increasingly considered for biomedical applications, such as imaging, drug delivery or diagnosis. The particularity of nanoparticle comes from their size that can be in the same order than the protein. Thus, the general concept based on the protein energy to predict their conformational changes has to be confronted with the literature on NP. Among the NP, the gold ones (Au-NP) are an interesting model because of the oscillation of their conduction electrons upon interaction with light (surface plasmon resonance), can be easily detected in the visible and near infrared spectral region[136,137]. Moreover, low level of toxicity were found for gold nanoparticles around 20 nm[138].

From a protein point of view, globular proteins are relatively stable colloids, because of the risks related to protein aggregation, even though they oscillate in a wide range of different conformations [139]. The interaction with the nanoparticle surface can induce a change in the protein conformation, with a consequent unfolding[140]. The unfolded protein exposes the hydrophobic moieties of the polypeptide chain to the nanoparticle surface[141]. Among the unwanted biological side effects[142], one of the most relevant is the loss of biological activity due to the conformational changes[122,143]. The unfolding of proteins upon contact with nanoparticles can stabilize the NPs, which easily aggregate in ionic media[144,145]. Nanoparticles are usually stabilized by electrostatic or steric repulsion, so that a repulsive barrier screen the particle preventing aggregation [146,147]. The proteins absorbed by the nanoparticle surface, forming the corona, can stabilize the suspension even in the presence of physiological electrolyte concentration[148]. This colloidal stability is often attributed to steric stabilization[140]. This was illustrated with Au-Np were the salt addition generate the aggregation for the one coated with the lysozyme conversely to the BSA[22]. This could a direct consequence of the optimization of surface coverage by the BSA due to its low internal energy. This is supported by a recent study where the author shown that the salt addition AuNP loaded with BSA bind with resveratrol induce their aggregation due to the desorption of protein[149]. Isothermal titration calorimetry[150,151], surface plasmon resonance [152], quartz crystal microbalance[153] and differential centrifugal sedimentation [154] are typical experimental techniques employed to highlight the affinities between proteins and nanoparticles. Fluorescence correlation spectroscopy (FCS) has been used to determine the binding constant of protein to NPs [155–158]. Langmuir absorption isotherms have been employed in many experimental works to measure the equilibrium binding constants of the adsorption process. However, the use of equilibrium concepts, such as adsorption isotherms, may not be suitable in the protein-nanoparticle interaction. The increasing evidence of the irreversibility of the protein adsorption on microscopic time-scales[159–161] seems to confirm the previous sentence.

4.1 Correlation between the protein classification and its structural modification at nanoparticle surface

Previously, we shown that the CATH classification allow to assign a “hard” of “soft” behavior to the proteins at liquid/solid interface. We discuss now if the same approach can be used in the case of the solid is a nanoparticle with a size comparable to the protein.

Mainly-α protein : Ideal candidates for the understanding of the protein-nanoparticle interaction is BSA even if the human serum albumin (HAS) is the most interesting in the point of view of medical application. BSA sharing almost its whole amino acid sequence with its human equivalent[162], has been widely employed for the studies on protein-nanoparticle interaction and often used for the stability of gold nanoparticles suspensions[163]. Gold nanoparticles are usually stabilized by citrate,

since they are typically synthetized through a citric acid reduction reaction[164]. The citrate layer gives a negative surface charge to the gold nanoparticles, which results in mutual repulsion of the colloids and a consequent electrostatic stabilization. But this stability fails when citrate-stabilized gold nanoparticles are dispersed in a saline solution, precipitating and aggregating at NaCl concentrations below representative values of blood plasma or other biofluids[165]. The mechanisms governing the interaction between bovine serum albumin and gold nanoparticle can involve two possibilities. The first is based on electrostatic interactions between the positive lysine groups of the protein and the citrate layer. The second one takes into account the bovine serum albumin that, unfolding, exposes its hydrophobic residues to the gold surface, letting them interacting with the nanoparticle and, consequently, causing the displacement of the citrate from the gold surface. The latter contribution provides colloidal stability much more than the electrostatic part [153]. On the (Au-NP) the albumin loss α-helix while the percentage of β-sheet increases [166]. This was also reported on silver nanoparticle (Ag-NP)[148]. Structural modification was also reported on Fe-nanoculster[123]. Nevertheless, opposite results were reported on Au-NP (diameter about 10 nm) with a different surface functionalization[167]. The interaction HSA/NP was also extensively studied. It was reported that the structural modification occurs after adsorption on AU-NP with size from 5 to 100 nm using circular dichroism[161] and fluorescence[168]. The decrease of ellipticity values was interpreted by a limited and localized conformational change. This was also reported for the fibrinogen and insulin. The hemoglobulin on hydroxylated multi-wall carbon nanotubes loss its α-helix content from 100% to 77%[169]. This conformational change was reported to be more pronounce with the increase of the gold-NP size in a range between 5 and 100 nm [170]. On ZnO nanoparticle the α-lactalbumin exhibit a change about 7 % of α-helix content[171]. However, there are few cases when the NPs–protein interaction did not necessarily induce the conformational change of protein, as for hemoglobin absorbed onto Fe_2O_3 nanoparticles, where the binding process was found to be an enthalpy-driven and exothermic process [172]. Conversely on Diamond nanoparticle a decrease of content of α-helix and an increase of β-sheet and random coil is observed. As for many other examples more the mass ratio protein/NP is low as the structure is strongly affected[173]. The α-synuclein is a small protein composed by one α-helix. Its adsorption on silica nanoparticle transforms the less ordered structure to β-sheet[174]. This structural modification mediates the formation of aggregation and thus could influence the amyloidogenesis.

Mainly-β protein. On au NP 7 nm, the chymotrypsin, a hard protein, lost its secondary structure while the cytochrome C maintains its native one making the chymotrypsin more sensitive to proteolyse[175]. On CuO nanoparticle with a diameter 5 nm, a decrease of α-helix of β-galactosidase was reported. However, the basic structure of the protein is kept intact after adsorption[176]. The α-chymotrypsin adsorbed on Au-NP smaller than 10 nm protected with mercaptoundecanoic acid loss its native structure since the ordered structure shift toward random coil content[177]. The ovalbumin has a modified structure when adsorbed on silver nanoparticle (AgNP) with a diameter 5-30 nm[178]. The β-lactoglobulin on silica nanoparticle (10 nm) reveals an unfolded conformation. The latter is more pronounced at low surface concentration of protein[179]. The urease adsorbed on AgNPs with diameter range 10-15 nm has significant structural changes at 1:1 (v/v) ratio becoming drastic for lower ratio protein/NP[180]. Typically, free in solution the urease is composed by 45 % of α-helix and 10 % of β-sheet. This content reaches 4 % of α-helix and 44 % of β-sheet for a ratio urease AgNP 1:4. This explains the loss of enzymatic activity.

Mix α-β protein. Lysozyme absorbed onto silica and gold nanoparticles, respectively, have shown a rapid change of the secondary and tertiary structures, with a loss of the α-helical content and a relevant increase of the β-sheets[181]. This was also reported in TiO_2 nanoparticle[169]. On negatively charged Silica nanoparticle at pH 7 a decrease of α-helical content was reported[182]. Conversely on ZnO nanoparticle 4-7 nm an increase of α-helical content bout 4% was reported[183]. The transferrin on hydroxylated multi-wall carbon nanotubes loss its α-helix content from about 50 %[169]. The lysozyme adsorbed on polystyrene nanoparticle was reported as strongly dependent on the surface charge. A modification of secondary structure was observed for polystyrene (PS) and PS-COOH while no structural change was reported on PS-NH_2. The authors have concluded that the opposite charge undergoes the structural change. However another parameter could be taken into account since the diameter of PS and PS-COOH are 23 and 24 nm respectively and PS-NH_2 57 nm[184]. The adsorption on silica nanoparticle (4-15 nm) does not lead to a significant reduction of the α-helicity of ribonuclease A, indicating that the protein secondary structure does not strongly change after the binding with the nanoparticle [185]. Such stability in the protein secondary structure can be ascribed to weak interactions such as van der Waals and hydrogen bond of NPs with proteins, which did not perturb the secondary structure of the protein.

The nanoparticle has different influence on the protein conformation compared to other material. Indeed, whatever the protein structural category, the protein is often prone to a change of its conformation in order to interact with NPs, leading to a decrease of the α-helix content and consequently an increase of β-sheet. This is mainly due to the curve radius that oblige to the protein to adopt another conformation. The enhancement of ordered structural element as β-sheet and thus fibrillation, generating amyloid-like fibril that are commonly associated with neuro-degenerative diseases[32]. Conversely, other report shows that NPs prevent the amyloid aggregation[186]. Actually this is still debated and as for all question relative to protein/material interaction, none general case can be done. However the investigation on the changes in the protein behavior due to the presence of the nanoparticle is important from a nanotoxicologic point of view [187]. The increase of β-sheet content is not a general case, in some case the adsorption can be caused by an increase of the α-helicity, as for β-lactoglobulin on Silica nanoparticle [179] or for lysozyme on ZnO nanoparticle [183], indicating that the binding of the protein with the particle can result in some hydrophobic regions becoming more compact [188]. The alteration in protein conformation by NPs depends anyway also on particle size, hydrophobicity, surface charge and interaction time.

4.2 Protein adsorption on NP under serum condition

As previously mentioned on interest of NP, especially the gold one are their using for theragnostic application. Thus, once released into the human body, their interactions with biological systems have to be deeply understood for safe and efficient applications. Generally speaking, proteins in a biological environment always cover the material upon contact[189]. The issues of protein/NP interaction are specific in biological or serum environment. Indeed, nanoparticles travelling in the blood plasma can encounter more than 3500 different proteins, with a wide range of different concentrations[190], leading to a competition where, initially, the most abounding proteins will be absorbed on the surface and subsequently replaced by proteins with higher affinity [191,192]. Compared to the classical report on the protein material interaction, here the problematic more difficult because the proteins are in crowded environment, they can enter in competition[100] and/or bind each-other and the high concentration make the multilayer formation possible.

Nanoparticles, dispersed in a biological fluid, are rapidly covered by biomolecules, such as proteins and lipids, forming a dynamic layer, the protein corona, that effectively screens the nanoparticle, changing its properties compared to the bare material[32], providing a new identity to the particle, that effectively interacts with the biological system[193]. The protein corona changes the properties of the nanomaterial in terms of size, shape, charge and surface and governs the physiological response in terms of transport, cellular uptake or toxicity[181]. It forms the biocorrona seen by the cell[154]. It also make the nanoparticle invisible to the immune system[170].For instance, silica NPs functionalized with transferrin lost their targeting capacities in biological fluids, probably because of the corona formation [194]. The characteristics of this biolayer have to be deeply understood for their implications in the physiological mechanisms, cell internalization and nanotoxicity [195] The direct exposure of humans to nanoparticles is still a concern. Indeed nanoparticles internalized could release toxic metals inside the cell, with a mechanism known as “Trojan horse” effect, for which the nanoparticle is wrongly recognized by the cell receptors; the formation of the protein corona could alter the cell signalling process, leading to cell inflammation [196].

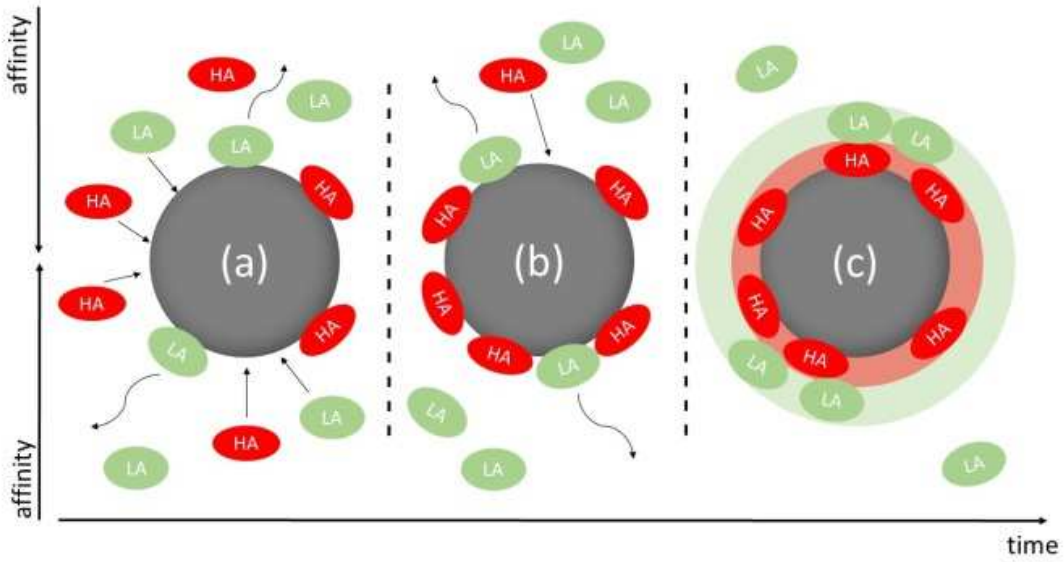

Figure 4 : "time evolution of the protein corona: (a) Proteins initially interact with the nanoparticle and are absorbed on its surface; (b) proteins with lower affinity (LA) can desorb from the nanoparticle surface, while proteins with higher affinity (HA) are generally tightly bound; (c) proteins with higher affinity are good candidate to form the hard corona, while protein with less affinity could form a second, more dynamic and transient, layer, the soft corona

In the native conformation of a protein, a basic role is played by the hydrophobic residues, that allow a close packaging of the cores[197], but their stability can be disrupted by the interactions with a surface[32]. Proteins binding with higher affinity, directly on the nanoparticle surface, are imputed for the formation of the “hard” corona, on the top of which there is a “soft” corona, consisting of less tightly bound proteins, interacting mostly by weak protein-protein interactions[198], and showing much higher exchange rates[155]. The corona is chemically composed of a mixture of distinct

proteins. Different aspects of the corona formation have been already clarified by experimental and theoretical studies but, due to the complexity of the process, its understanding is still incomplete. The formation of the protein layer is usually very fast, but the whole process may take hours or even days due to the slow reconfiguration and exchange of proteins[199]. In real applications, such as human blood, a large number of distinct proteins with different abundancies are present in the biological fluid but, due to the competition for the binding sites, the corona typically contains only some of them[200]. For instance in blood plasma only 20 proteins were found to form a layer around silica NPs [193]. Generally, proteins with higher affinity initially interact with NPs forming the hard corona (Figure 4). Other proteins may secondarily interact with NPs as a consequence of the presence of the hard corona proteins, rather than the core NP surface, forming a second and less tightly bound layer, the soft corona[195]. However, the mechanism of interaction is still under debate. Indeed other studies suggested that soft and hard corona proteins can interact with NPs based on different binding affinities. Cedervall et al. reported that the proteins forming the soft and hard layer bind to NPs, respectively, for only few minutes while in the second case for several hours, where proteins with the smallest molecular weight having the highest affinity [201]. Because of the longer interaction time with NPs for the hard corona proteins, Walkey et al. proposed that this first layer may be more important than the soft corona proteins in defining the biological identity and response of the nanomaterials [198]. Most of the literature focuses on the hard corona, because of the challenges in snapping and characterizing the more transient and dynamic soft corona. In this sense, iron oxide NPs were incubated in fetal bovine serum to study the soft corona, resulting as composed of mostly complement proteins, such as antithrombin and alpha-antiproteinase [202]. In another work, iron oxide NPs was incubated in human blood or lymph serum. Here, soft corona specific molecules identified were angiotensinogen, annexins, cathepsins and collagen-based. Interestingly, complement proteins were mainly found in the hard rather than in the soft corona [203] as in previous study on iron oxide NPs [202]. On the other hand components of the hard corona are more accurately identified and well established. Apolipoproteins, serum albumin, fibrinogen, and immunoglobulins are generally the most common, even looking to different nanoparticles, such as metalloid [204], liposome [205] and polymer [206] ones. The abundance of the fibrinogen and albumin (mainly-α) lead the first layer around the NP. Their ability to unfold due their low internal energy allows to optimize their interaction with NP making the corona “harder”. Typically the soft protein generates the base of “hard” corona. The hardness of corona has also an influence on the protein desorption[12]. The protein corona is not only protein dependent but also involve the nature of the material[190]. Another effect that has to be considered from a biological point of view is the so-called “Vroman effect” [207]. The Vroman effect, from a protein corona perspective, describes how the proteins, that initially associate with the nanoparticle-protein corona, can be substituted over the time by a new set of proteins with higher affinities [208] .This exchange of proteins may occur in a short time, seconds or minutes, for the soft corona or longer, even hour, for hard corona proteins [209].

Physical and chemical properties of the NPs, such as shape, size and charge, can influence the formation of the protein corona on NPs. The effect of nanoparticle shape, using gold nanorods and nanostars, on the protein corona was studied in vivo. In this work, were initially incubated in mouse blood, and consequently proteins specific to each nanoparticle were analyzed in order to characterize the composition of the protein corona [210]. β-globulin and plasminogen were found to be unique for the nanorods, while murinoglobulin-2, serine protease inhibitor A3N and

apolipoprotein A-I where found only on the nanostars. However, the majority of the corona constituents were shared by the nanorods and nanostars, but different abundances demonstrated that NP shape was a critical factor in the corona composition. The impact of nanoparticle size on the protein corona was investigated using iron oxide particles with different diameters, 30, 200, and 400 nm incubated in human plasma [211]. Only a quarter of corona proteins were shared among the three sizes, pointing out the importance of the NP size in forming the corona. Iron oxides with a diameter of 30 nm were the only one associated with cell cycle proteins, whereas the 200 nm particles were found to bind proteins with different functionality such as reproduction, localization, and homeostatic. The biggest ones, i.e. the 400 nm NPs, were found to be not related to any specific functionality. The three sizes of iron oxide NPs were also characterized by different protein abundance in the protein corona. To investigate nanoparticle charge, positive-(amine-conjugated) and negatively-charged (unmodified or carboxylated) polystyrene NPs incubated in human plasma were employed by Lundqvist et al. for an investigation on the effect of the NP charge on the corona formation[212]. Relevant differences were observed. Indeed corona proteins specific to positive NPs were apolipoprotein F, complement C1r, and mannose-binding protein, while the polycomb proteins specific to negative NPs were the majority of complement, Ig-γ and Ig-κ. Nonetheless, the corona confers to the proteins-NP complex a zeta potential in the range of −10 mV to −20 mV that seems independent of the physical and chemical characteristics of the bare NP. Coronal dynamics can also be reflective of coronal layers (a.k.a. thickness). The quick dynamic of the soft corona led to a new definition, the “protein cloud”[213], which pictures a weak and time-changing layer. The soft corona could consist even of protein aggregates, because generally plasma proteins generally have diameters of 3–15 nm, while coronas were found to be even over 30 nm thick. Moreover, the soft corona or protein cloud’s thickness can be much larger than the hard corona, since higher number of proteins at higher concentrations occupies the soft corona rather than the hard corona.

5. Summary and outlook

Protein at solid/liquid interface is really “common but very complicated phenomenon” even if numerous advances were done in the last three decades. However, the approach to functionalize materials with proteins is still empirical. The concept of “hard” and “soft” protein can help to reach a better understanding and anticipate the protein behavior after its loading on a material. The assignation of a protein of one or the other category can be based on the structural properties and thus the CATH database could be useful. From the protein category, several behaviors can be predicted. However, going through the literature, we can see that the issue is more complex than one can expect. Indeed, the hard protein can modify their conformation too. Thus, despite extensive investigations, numerous questions are still open. For the kinetics, a direct characterization of the depletion, occurring when the adsorption is limited by the diffusion, could allow the validation of the Leveque model. This could be possible with the high resolution of confocal microscopy. Such informations will allow to make huge advances to the conception of antifouling surface. Another effort have to be made to define a new isotherm model to replace the Langmuir and Freundlich, that has no physical meaning in the case of protein adsorption. Such model should include the heterogeneity of the material, the possible different protein orientation and conformation. To design hybrid protein/artificial material, we have to go further on the prediction of protein behavior at liquid/solid interface. A prediction of the systematic correlation between the protein conformational

change and the protein internal structure should be of an essential importance. From it, a database of protein at solid/liquid interface could be created to rationalize this knowledge and may be improve the prediction of adsorbed protein behavior. The following step will be to extend the prediction to other interfaces, such as liquid/air and aqueous/organic. In general point of view, the concept of hard and soft protein is useful but the proteins are described only at the equilibrium state. This does not involve degrees of freedom of protein that probably drive the real adsorption process. Indeed, many questions are still open about how a protein finds its way to optimize its conformation on material. This involves numerous intermediates: adsorption, structural modification, desorption, migration on surface and/or re-adsorption and so-on. Here, the Levinthal's paradox is also truth since to find a conformational stability the time scale is quite short because the equilibrium state is often reached after min/hours scale including the diffusion until surface. In solution, the protein folding can be solving by simulation due to the short time scale (µs). For the adsorption, this is not reasonable due to the time scale. Another question is it about the homogeneity of structural conformation of protein at liquid-solid interface and how are affected the free motion of the different protein chain. All these questions will improve our understanding and may be allow to reconcile the approach equilibrium and folding thermodynamics with the structural classifications for soft and hard proteins. The most important effort will be to predict the adsorption on the NP. Indeed, the exact composition and the role of the corona have to be elucidated to go further in nanomedicine and theragnostic. Among the open question, the exact role of albumin and the assembly with other serum protein (fibrinogen, IgG etc.) have to be solved. Because the adsorption of high order protein on NP seems to increase the β-sheet content, the involvement of NP on amyloid growth has to be more deeply investigated from a fundamental to a clinical point of view. Indeed, the increase of nanoplastic in the sea water let think to future sanitary problem. To conclude this review, we can say that the issue of protein at solid/liquid interface is far to be totally understood and remains a fascinating topic of research.

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REFERENCES

[1] Meryam Sardar RA. Enzyme Immobilization: An Overview on Nanoparticles as Immobilization Matrix. Biochem Anal Biochem 2015;04. doi:10.4172/2161-1009.1000178.

[2] Keller MA, Piedrafita G, Ralser M. ScienceDirect The widespread role of non-enzymatic reactions in cellular metabolism. Curr Opin Biotechnol 2015;34:153–61. doi:10.1016/j.copbio.2014.12.020.

[3] Lee ES, Kwon MJ, Lee H, Kim JJ. Stabilization of protein encapsulated in poly(lactide-co-glycolide) microspheres by novel viscous S/W/O/W method. Int J Pharm 2007;331:27–37. doi:10.1016/j.ijpharm.2006.09.008.

[4] Mehta J, Bhardwaj N, Bhardwaj SK, Kim KH, Deep A. Recent advances in enzyme immobilization techniques: Metal-organic frameworks as novel substrates. Coord Chem Rev 2016;322:30–40. doi:10.1016/j.ccr.2016.05.007.

[5] Cipolatti EP, Silva MJA, Klein M, Feddern V, Feltes MMC, Oliveira JV, et al. Current status and

trends in enzymatic nanoimmobilization. J Mol Catal B Enzym 2014;99:56–67. doi:10.1016/j.molcatb.2013.10.019.

[6] Zhang Y, Ge J, Liu Z. Enhanced Activity of Immobilized or Chemically Modified Enzymes. ACS Catal 2015;5:4503–13. doi:10.1021/acscatal.5b00996.

[7] Mateo C, Palomo JM, Fernandez-Lorente G, Guisan JM, Fernandez-Lafuente R. Improvement of enzyme activity, stability and selectivity via immobilization techniques. Enzyme Microb Technol 2007;40:1451–63. doi:10.1016/j.enzmictec.2007.01.018.

[8] Hudson SP, Cooney JC, Magner E. Proteins in mesoporous silicates. Angew Chemie Int Ed 2008;47:8582–94. doi:10.1021/bk-2008-0986.ch002.

[9] Hudson S, Magner E, Cooney J, Kieran B. Methodology for the immobilization of enzymes onto mesoporous materials. J Phys Chem B 2005;109:19496–506. doi:10.1021/jp052102n.

[10] Bolivar JM, Eisl I, Nidetzky B. Advanced characterization of immobilized enzymes as heterogeneous biocatalysts. Catal Today 2016;259:66–80. doi:10.1016/j.cattod.2015.05.004.

[11] Secundo F. Conformational changes of enzymes upon immobilisation. Chem Soc Rev 2013;42:6250–61. doi:10.1039/c3cs35495d.

[12] Walkey CD, Chan WCW. Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. Chem Soc Rev 2012;41:2780–99. doi:10.1039/c1cs15233e.

[13] Vogler EA. Protein adsorption in three dimensions. Biomaterials 2012;33:1201–37. doi:10.1016/j.biomaterials.2011.10.059.

[14] Zhang L, Casey B, Galanakis DK, Marmorat C, Skoog S, Vorvolakos K, et al. The influence of surface chemistry on adsorbed fibrinogen conformation, orientation, fiber formation and platelet adhesion. Acta Biomater 2017;54:164–74. doi:10.1016/j.actbio.2017.03.002.

[15] Xue C, Yonet-Tanyeri N, Brouette N, Sferrazza M, Braun P V., Leckband DE. Protein adsorption on poly(N -isopropylacrylamide) brushes: Dependence on grafting density and chain collapse. Langmuir 2011;27:8810–8. doi:10.1021/la2001909.

[16] Yu Q, Zhang Y, Chen H, Wu Z, Huang H, Cheng C. Protein adsorption on poly(N-isopropylacrylamide)-modified silicon surfaces: Effects of grafted layer thickness and protein size. Colloids Surfaces B Biointerfaces 2010;76:468–74. doi:10.1016/j.colsurfb.2009.12.006.

[17] Zhao T, Chen H, Zheng J, Yu Q, Wu Z, Yuan L. Inhibition of protein adsorption and cell adhesion on PNIPAAm-grafted polyurethane surface: Effect of graft molecular weight. Colloids Surfaces B Biointerfaces 2011;85:26–31. doi:10.1016/j.colsurfb.2010.10.047.

[18] Molino PJ, Higgins MJ, Innis PC, Kapsa RMI, Wallace GG. Fibronectin and bovine serum albumin adsorption and conformational dynamics on inherently conducting polymers: A QCM-D study. Langmuir 2012;28:8433–45. doi:10.1021/la300692y.

[19] Han L, Yan B, Zhang L, Wu M, Wang J, Huang J, et al. Tuning protein adsorption on charged polyelectrolyte brushes via salinity adjustment. Colloids Surfaces A Physicochem Eng Asp 2018;539:37–45. doi:10.1016/j.colsurfa.2017.12.004.

[20] Dominguez-Medina S, Blankenburg J, Olson J, Landes CF, Link S. Adsorption of a protein monolayer via hydrophobic interactions prevents nanoparticle aggregation under harsh

environmental conditions. ACS Sustain Chem Eng 2013;1:833–42. doi:10.1021/sc400042h.

[21] Sabela M, Balme S, Bechelany M, Janot J-M, Bisetty K. A Review of Gold and Silver Nanoparticle-Based Colorimetric Sensing Assays. Adv Eng Mater 2017. doi:10.1002/adem.201700270.

[22] Lepoitevin M, Lemouel M, Bechelany M, Janot J-M, Balme S. Gold nanoparticles for the bare-eye based and spectrophotometric detection of proteins, polynucleotides and DNA. Microchim Acta 2015;182. doi:10.1007/s00604-014-1408-1.

[23] Barbero F, Russo L, Vitali M, Piella J, Salvo I, Borrajo ML, et al. Formation of the Protein Corona: The Interface between Nanoparticles and the Immune System. Semin Immunol 2017;34:52–60. doi:10.1016/j.smim.2017.10.001.

[24] Plesa C, Kowalczyk SW, Zinsmeester R, Grosberg AY, Rabin Y, Dekker C. Fast translocation of proteins through solid state nanopores. Nano Lett 2013;13:658–63. doi:10.1021/nl3042678.

[25] Oukhaled A, Cressiot B, Bacri L, Pastoriza-Gallego M, Betton JM, Bourhis E, et al. Dynamics of completely unfolded and native proteins through solid-state nanopores as a function of electric driving force. ACS Nano 2011;5:3628–38. doi:10.1021/nn1034795.

[26] Balme S, Coulon PE, Lepoitevin M, Charlot B, Yandrapalli N, Favard C, et al. Influence of adsorption on proteins and amyloid detection by silicon nitride nanopore. Langmuir 2016;32. doi:10.1021/acs.langmuir.6b02048.

[27] Wang K, Zhou C, Hong Y, Zhang X. A review of protein adsorption on bioceramics. Interface Focus 2012;2:259–77. doi:10.1098/rsfs.2012.0012.

[28] Gkaniatsou E, Sicard C, Ricoux R, Mahy JP, Steunou N, Serre C. Metal-organic frameworks: A novel host platform for enzymatic catalysis and detection. Mater Horizons 2017;4:55–63. doi:10.1039/c6mh00312e.

[29] Lian X, Fang Y, Joseph E, Wang Q, Li J, Banerjee S, et al. Enzyme-MOF (metal-organic framework) composites. Chem Soc Rev 2017;46:3386–401. doi:10.1039/c7cs00058h.

[30] Assfalg M, Ragona L, Pagano K, D'Onofrio M, Zanzoni S, Tomaselli S, et al. The study of transient protein-nanoparticle interactions by solution NMR spectroscopy. Biochim Biophys Acta - Proteins Proteomics 2016;1864:102–14. doi:10.1016/j.bbapap.2015.04.024.

[31] Liu J, Peng Q. Protein-gold nanoparticle interactions and their possible impact on biomedical applications. Acta Biomater 2017;55:13–27. doi:10.1016/j.actbio.2017.03.055.

[32] Lynch I, Dawson K a. Protein-nanoparticle interactions. Nano Today 2008;3:40–7. doi:10.1016/S1748-0132(08)70014-8.

[33] Pino P Del, Pelaz B, Zhang Q, Maffre P, Nienhaus GU, Parak WJ. Protein corona formation around nanoparticles - From the past to the future. Mater Horizons 2014;1:301–13. doi:10.1039/c3mh00106g.

[34] Mahmoudi M, Lynch I, Ejtehadi MR, Monopoli MP, Bombelli FB, Laurent S. Protein À Nanoparticle Interactions : Opportunities and Challenges 2011:5610–37. doi:10.1021/cr100440g.

[35] Jiang J, Babarao R, Hu Z. Molecular simulations for energy, environmental and pharmaceutical applications of nanoporous materials: From zeolites, metal-organic frameworks to protein

crystals. Chem Soc Rev 2011;40:3599–612. doi:10.1039/c0cs00128g.

[36] Firkowska-Boden I, Zhang X, Jandt KD. Controlling Protein Adsorption through Nanostructured Polymeric Surfaces. Adv Healthc Mater 2018;7:1–19. doi:10.1002/adhm.201700995.

[37] Küchler A, Yoshimoto M, Luginbühl S, Mavelli F, Walde P. Enzymatic reactions in confined environments. Nat Nanotechnol 2016;11:409–20. doi:10.1038/nnano.2016.54.

[38] Ansari SA, Husain Q. Potential applications of enzymes immobilized on/in nano materials: A review. Biotechnol Adv 2012;30:512–23. doi:10.1016/j.biotechadv.2011.09.005.

[39] Talbert JN, Goddard JM. Enzymes on material surfaces. Colloids Surfaces B Biointerfaces 2012;93:8–19. doi:10.1016/j.colsurfb.2012.01.003.

[40] Rodrigues RC, Ortiz C, Berenguer-Murcia Á, Torres R, Fernández-Lafuente R. Modifying enzyme activity and selectivity by immobilization. Chem Soc Rev 2013;42:6290–307. doi:10.1039/c2cs35231a.

[41] Kim J, Grate JW, Wang P. Nanostructures for enzyme stabilization. Chem Eng Sci 2006;61:1017–26. doi:10.1016/j.ces.2005.05.067.

[42] Horbett TA. Fibrinogen adsorption to biomaterials. J Biomed Mater Res - Part A 2018;106:2777–88. doi:10.1002/jbm.a.36460.

[43] Gray JJ. The interaction of proteins with solid surfaces. Curr Opin Struct Biol 2004;14:110–5. doi:10.1016/j.sbi.2003.12.001.

[44] Nakanishi K, Sakiyama T, Imamura K. On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. J Biosci Bioeng 2001;91:233–44. doi:10.1016/S1389-1723(01)80127-4.

[45] Haynes CA, Norde W. Globular proteins at solid/liquid interfaces. Colloids Surfaces B Biointerfaces 1994;2:517–66. doi:10.1016/0927-7765(94)80066-9.

[46] Garland A, Shen L, Zhu X. Mobile precursor mediated protein adsorption on solid surfaces. Prog Surf Sci 2012;87:1–22. doi:10.1016/j.progsurf.2012.02.001.

[47] Hartvig RA, Van De Weert M, Østergaard J, Jorgensen L, Jensen H. Protein adsorption at charged surfaces: The role of electrostatic interactions and interfacial charge regulation. Langmuir 2011;27:2634–43. doi:10.1021/la104720n.

[48] Kubiak-Ossowska K, Mulheran PA. What governs protein adsorption and immobilization at a charged solid surface? Langmuir 2010;26:7690–4. doi:10.1021/la101276v.

[49] Kubiak-Ossowska K, Mulheran PA. Mechanism of hen egg white lysozyme adsorption on a charged solid surface. Langmuir 2010;26:15954–65. doi:10.1021/la102960m.

[50] Staunton S, Quiquampoix H. Adsorption and Conformation of Bovine Serum-Albumin On Montmorillonite - Modification of the Balance Between Hydrophobic and Electrostatic Interactions By Protein Methylation and Ph Variation. J Colloid Interface Sci 1994;166:89–94. doi:10.1006/jcis.1994.1274.

[51] Causserand C, Jover K, Aimar P, Meireles M. Modification of clay cake permeability by adsorption of protein. J Memb Sci 1997;137:31–44. doi:10.1016/S0376-7388(97)00181-6.

[52] Vettori C, Calamai L, Yoder M, Stotzky G, Gallori E. Adsorption and binding of AmpliTaq?? DNA

polymerase on the clay minerals, montmorillonite and kaolinite. Soil Biol Biochem 1999;31:587–93. doi:10.1016/S0038-0717(98)00162-X.

[53] Helassa N, Quiquampoix H, Noinville S, Szponarski W, Staunton S. Adsorption and desorption of monomeric Bt (Bacillus thuringiensis) Cry1Aa toxin on montmorillonite and kaolinite. Soil Biol Biochem 2009;41:498–504. doi:10.1016/j.soilbio.2008.12.008.

[54] Hu X, Zhao X, Hwang H min. Comparative study of immobilized Trametes versicolor laccase on nanoparticles and kaolinite. Chemosphere 2007;66:1618–26. doi:10.1016/j.chemosphere.2006.08.004.

[55] Zhou X, Huang Q, Chen S, Yu Z. Adsorption of the insecticidal protein of Bacillus thuringiensis on montmorillonite, kaolinite, silica, goethite and Red soil. Appl Clay Sci 2005;30:87–93. doi:10.1016/j.clay.2005.04.003.

[56] Vasina EN, Déjardin P. Adsorption of α-chymotrypsin onto mica in laminar flow conditions. Adsorption kinetic constant as a function of tris buffer concentration at pH 8.6. Langmuir 2004;20:8699–706. doi:10.1021/la0490588.

[57] Déjardin P, Vasina EN. Initial Adsorption Kinetics in a Rectangular Thin Channel, and Coverage-Dependent Structural Transition Observed by Streaming Potential. Proteins at Solid-Liquid Interfaces, 2006. doi:10.1007/3-540-32658-8_3.

[58] Déjardin P, Vasina EN. An accurate simplified data treatment for the initial adsorption kinetics in conditions of laminar convection in a slit: Application to protein adsorption. Colloids Surfaces B Biointerfaces 2004;33:121–7. doi:10.1016/j.colsurfb.2003.09.005.

[59] Balme S, Janot J-M, Déjardin P, Vasina EN, Seta P. Potentialities of confocal fluorescence for investigating protein adsorption on mica and in ultrafiltration membranes. J Memb Sci 2006;284. doi:10.1016/j.memsci.2006.07.034.

[60] Ghiaci M, Aghaei H, Soleimanian S, Sedaghat ME. Enzyme immobilization: Part 2. Immobilization of alkaline phosphatase on Na-bentonite and modified bentonite. Appl Clay Sci 2009;43:308–16. doi:10.1016/j.clay.2008.09.011.

[61] Dong H, Li Y, Li J, Sheng G, Chen H. Comparative study on lipases immobilized onto bentonite and modified bentonites and their catalytic properties. Ind Eng Chem Res 2013;52:9030–7. doi:10.1021/ie4001986.

[62] Latour RA. The Langmuir isotherm: A commonly applied but misleading approach for the analysis of protein adsorption behavior. J Biomed Mater Res - Part A 2015;103:949–58. doi:10.1002/jbm.a.35235.

[63] Duarte-Silva R, Villa-García MA, Rendueles M, Díaz M. Structural, textural and protein adsorption properties of kaolinite and surface modified kaolinite adsorbents. Appl Clay Sci 2014;90:73–80. doi:10.1016/j.clay.2013.12.027.

[64] Barral S, Villa-García MA, Rendueles M, Díaz M. Interactions between whey proteins and kaolinite surfaces. Acta Mater 2008;56:2784–90. doi:10.1016/j.actamat.2008.02.009.

[65] Alkan M, Demirbaş Ö, Doğan M, Arslan O. Surface properties of bovine serum albumin - adsorbed oxides: Adsorption, adsorption kinetics and electrokinetic properties. Microporous Mesoporous Mater 2006;96:331–40. doi:10.1016/j.micromeso.2006.07.007.

[66] Sposito G. Derivation of the Freundlich Equation for Ion Exchange Reactions in Soils. Soil Sci

Soc Am J 1980;44:652. doi:10.2136/sssaj1980.03615995004400030045x.

[67] Shamsuddin RM, Verbeek CJR, Lay MC. Producing protein intercalated bentonite - Equilibrium, kinetics and physical properties of gelatin-bentonite system. Appl Clay Sci 2014;87:52–60. doi:10.1016/j.clay.2013.11.023.

[68] Schaaf P, Voegel J-C, Senger B. From Random Sequential Adsorption to Ballistic Deposition: A General View of Irreversible Deposition Processes. J Phys Chem B 2002;104:2204–2214. doi:10.1021/jp9933065.

[69] Rabe M, Verdes D, Seeger S. Understanding protein adsorption phenomena at solid surfaces. Adv Colloid Interface Sci 2011;162:87–106. doi:10.1016/j.cis.2010.12.007.

[70] Roach P, Farrar D, Perry CC. Surface tailoring for controlled protein adsorption: Effect of topography at the nanometer scale and chemistry. J Am Chem Soc 2006;128:3939–45. doi:10.1021/ja056278e.

[71] Vendruscolo M, Dobson CM. Towards complete descriptions of the free-energy landscapes of proteins. Philos Trans A Math Phys Eng Sci 2005;363:433–52. doi:10.1098/rsta.2004.1501.

[72] Jahn TR, Radford SE. The Yin and Yang of protein folding. FEBS J 2005;272:5962–70. doi:10.1111/j.1742-4658.2005.05021.x.

[73] Malhotra P, Udgaonkar JB. Tuning Cooperativity on the Free Energy Landscape of Protein Folding. Biochemistry 2015;54:3431–41. doi:10.1021/acs.biochem.5b00247.

[74] Tzul FO, Vasilchuk D, Makhatadze GI. Evidence for the principle of minimal frustration in the evolution of protein folding landscapes. Proc Natl Acad Sci 2017;114:E1627–32. doi:10.1073/pnas.1613892114.

[75] Yu H, Dee DR, Liu X, Brigley AM, Sosova I, Woodside MT. Protein misfolding occurs by slow diffusion across multiple barriers in a rough energy landscape. Proc Natl Acad Sci 2015;112:8308–13. doi:10.1371/journal.pone.0000531.

[76] Pontiggia F, Pachov D V., Clarkson MW, Villali J, Hagan MF, Pande VS, et al. Free energy landscape of activation in a signalling protein at atomic resolution. Nat Commun 2015;6:1–14. doi:10.1038/ncomms8284.

[77] Min D, Jefferson RE, Bowie JU, Yoon TY. Mapping the energy landscape for second-stage folding of a single membrane protein. Nat Chem Biol 2015;11:981–7. doi:10.1038/nchembio.1939.

[78] Mallamace F, Corsaro C, Mallamace D, Vasi S, Vasi C, Baglioni P, et al. Energy landscape in protein folding and unfolding. Proc Natl Acad Sci 2016;113:3159–63. doi:10.1073/pnas.1524864113.

[79] Tsai MY, Zheng W, Balamurugan D, Schafer NP, Kim BL, Cheung MS, et al. Electrostatics, structure prediction, and the energy landscapes for protein folding and binding. Protein Sci 2016;25:255–69. doi:10.1002/pro.2751.

[80] Neupane K, Manuel AP, Woodside MT. Protein folding trajectories can be described quantitatively by one-dimensional diffusion over measured energy landscapes. Nat Phys 2016;12:700–3. doi:10.1038/nphys3677.

[81] Pan H, Qin M, Meng W, Cao Y, Wang W. How do proteins unfold upon adsorption on

nanoparticle surfaces? Langmuir 2012;28:12779–87. doi:10.1021/la302258k.

[82] Arai T, Norde W. The behavior of some model proteins at solid-liquid interfaces 1. Adsorption from single protein solutions. Colloids and Surfaces 1990;51:1–15. doi:10.1016/0166-6622(90)80127-P.

[83] Norde W. The behavior of proteins at interfaces, with special attention to the role of the structure stability of the protein molecule. Clin Mater 1992;11:85–91. doi:10.1016/0267-6605(92)90032-O.

[84] Lyklema J, Norde W. Interfacial behaviour of biomacromolecules. Prog. colloids Polym. Sci., 1996, p. 9–17.

[85] Rangel-Porras G, Rangel-Rivera P, Pfeiffer-Perea H, Gonzalez-Muñoz P. Changes in the characteristics of acid-treated clay after the inclusion of proteins. Surf Interface Anal 2015;47:135–41. doi:10.1002/sia.5685.

[86] Hristova SH, Zhivkov AM. Adsorption of cytochrome c on montmorillonite nanoplates: Protein concentration dependence. J Colloid Interface Sci 2015;446:252–62. doi:10.1016/j.jcis.2015.01.039.

[87] Balme S, Guégan R, Janot J-M, Jaber M, Lepoitevin M, Dejardin P, et al. Structure, orientation and stability of lysozyme confined in layered materials. Soft Matter 2013;9. doi:10.1039/c3sm27880h.

[88] Wertz CF, Santore MM. Adsorption and reorientation kinetics of lysozyme on hydrophobic surfaces. Langmuir 2002;18:1190–9. doi:10.1021/la0108813.

[89] Daly SM, Przybycien TM, Tilton RD. Coverage-dependent orientation of lysozyme adsorbed on silica. Langmuir 2003;19:3848–57. doi:10.1021/la026690x.

[90] Tran ATT, James BJ. A study the interaction forces between the bovine serum albumin protein and montmorillonite surface. Colloids Surfaces A Physicochem Eng Asp 2012;414:104–14. doi:10.1016/j.colsurfa.2012.08.066.

[91] Brandes N, Welzel PB, Werner C, Kroh LW. Adsorption-induced conformational changes of proteins onto ceramic particles: Differential scanning calorimetry and FTIR analysis. J Colloid Interface Sci 2006;299:56–69. doi:10.1016/j.jcis.2006.01.065.

[92] Koo J, Erlkamp M, Grobelny S, Steitz R, Czeslik C. Pressure-induced protein adsorption at aqueous-solid interfaces. Langmuir 2013;29:8025–30. doi:10.1021/la401296f.

[93] Orengo C, Michie A, Jones S, Jones D, Swindells M, Thornton J. CATH - a hierarchic classification of protein domain structures. Structure 1997;17:1093–109. doi:10.1016/S0969-2126(97)00260-8.

[94] Servagent-Noinville S, Revault M, Quiquampoix H, Baron M. Conformational Changes of Bovine Serum Albumin Induced by Adsorption on Different Clay Surfaces: FTIR Analysis. J Colloid Interface Sci 2000;221:273–83. doi:10.1006/jcis.1999.6576.

[95] Ueberbacher R, Rodler A, Hahn R, Jungbauer A. Hydrophobic interaction chromatography of proteins: Thermodynamic analysis of conformational changes. J Chromatogr A 2010;1217:184–90. doi:10.1016/j.chroma.2009.05.033.

[96] Sivaraman B, Latour RA. The Adherence of platelets to adsorbed albumin by receptor-

mediated recognition of binding sites exposed by adsorption-induced unfolding. Biomaterials 2010;31:1036–44. doi:10.1016/j.biomaterials.2009.10.017.

[97] Charbgoo F, Nejabat M, Abnous K, Soltani F, Taghdisi SM, Alibolandi M, et al. Gold nanoparticle should understand protein corona for being a clinical nanomaterial. J Control Release 2018;272:39–53. doi:10.1016/j.jconrel.2018.01.002.

[98] Sivaraman B, Latour RA. The relationship between platelet adhesion on surfaces and the structure versus the amount of adsorbed fibrinogen. Biomaterials 2010;31:832–9. doi:10.1016/j.biomaterials.2009.10.008.

[99] Ma Z, Bai J, Wang Y, Jiang X. Impact of shape and pore size of mesoporous silica nanoparticles on serum protein adsorption and RBCS hemolysis. ACS Appl Mater Interfaces 2014;6:2431–8. doi:10.1021/am404860q.

[100] Hasan A, Waibhaw G, Pandey LM. Conformational and Organizational Insights into Serum Proteins during Competitive Adsorption on Self-Assembled Monolayers. Langmuir 2018;34:8178–94. doi:10.1021/acs.langmuir.8b01110.

[101] Xu LC, Siedlecki CA. Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces. Biomaterials 2007;28:3273–83. doi:10.1016/j.biomaterials.2007.03.032.

[102] Roach P, Farrar D, Perry CC. Interpretation of protein adsorption: Surface-induced conformational changes. J Am Chem Soc 2005;127:8168–73. doi:10.1021/ja042898o.

[103] Unneberg P, Merelo JJ, Chacón P, Morán F. SOMCD: method for evaluating protein secondary structure from UV circular dichroism spectra. Proteins 2001;42:460–70. doi:10.1002/1097-0134(20010301)42:4%3C460::AID-PROT50%3E3.0.CO;2-U.

[104] Gruian C, Vanea E, Simon S, Simon V. FTIR and XPS studies of protein adsorption onto functionalized bioactive glass. Biochim Biophys Acta - Proteins Proteomics 2012;1824:873–81. doi:10.1016/j.bbapap.2012.04.008.

[105] Sang L-C, Coppens M-O. Effects of surface curvature and surface chemistry on the structure and activity of proteins adsorbed in nanopores. Phys Chem Chem Phys 2011;13:6689. doi:10.1039/c0cp02273j.

[106] Alava T, Mann JA, Théodore C, Benitez JJ, Dichtel WR, Parpia JM, et al. Control of the graphene-protein interface is required to preserve adsorbed protein function. Anal Chem 2013;85:2754–9. doi:10.1021/ac303268z.

[107] Kurpiewska K, Biela A, Loch JI, Świątek S, Jachimska B, Lewiński K. Investigation of high pressure effect on the structure and adsorption of β-lactoglobulin. Colloids Surfaces B Biointerfaces 2018;161:387–93. doi:10.1016/j.colsurfb.2017.10.069.

[108] Assifaoui A, Huault L, Maissiat C, Roullier-Gall C, Jeandet P, Hirschinger J, et al. Structural Studies of Adsorbed Protein (Betalactoglobulin) on Natural Clay (Montmorillonite). RSC Adv 2014;4:61096–103. doi:10.1039/C4RA11607K.

[109] Simon LM, Kotormán M, Garab G, Laczkó I. Structure and activity of alpha-chymotrypsin and trypsin in aqueous organic media. Biochem Biophys Res Commun 2001;280:1367–71. doi:10.1006/bbrc.2001.4282.

[110] Baron M, Revault M, Servagent-Noinville S, Abadie J, Quiquampoix H. Chymotrypsin Adsorption on Montmorillonite: Enzymatic Activity and Kinetic FTIR Structural Analysis. J

Colloid Interface Sci 1999;214:319–32. doi:10.1006/jcis.1999.6189.

[111] Calamai L, Lozzi I, Stotzky G, Fusi P, Ristori GG. Interaction of catalase with montmorillonite homoionic to cations with different hydrophobicity: Effect on enzymatic activity and microbial utilization. Soil Biol Biochem 2000;32:815–23. doi:10.1016/S0038-0717(99)00211-4.

[112] Garcia-segura JM, Cid C, De JM, Gavilanes G. Sepiol ite-su pported Urease 1987;19:517–22.

[113] Leprince F, Quiquampoix H. Extracellular enzyme activity in soil: effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus Hebeloma cylindrosporum. Eur J Soil Sci 1996;47:511–22. doi:10.1111/j.1365-2389.1996.tb01851.x.

[114] Rao MA, Violante A, Gianfreda L. Interaction of acid phosphatase with clays, organic molecules and organo-mineral complexes: Kinetics and stability. Soil Biol Biochem 2000;32:1007–14. doi:10.1016/S0038-0717(00)00010-9.

[115] Cengiz S, Çavaş L, Yurdakoç K. Bentonite and sepiolite as supporting media: Immobilization of catalase. Appl Clay Sci 2012;65–66:114–20. doi:10.1016/j.clay.2012.06.004.

[116] Gorga JC, Dong a, Manning MC, Woody RW, Caughey WS, Strominger JL. Comparison of the secondary structures of human class I and class II major histocompatibility complex antigens by Fourier transform infrared and circular dichroism spectroscopy. Proc Natl Acad Sci U S A 1989;86:2321–5.

[117] Kumar C V., Chaudhari A. Proteins immobilized at the galleries of layered α-zirconium phosphate: Structure and activity studies. J Am Chem Soc 2000;122:830–7. doi:10.1021/ja993310u.

[118] Norde W, Favier JP. Structure of adsorbed and desorbed proteins. Colloids and Surfaces 1992;64:87–93. doi:10.1016/0166-6622(92)80164-W.

[119] Larsericsdotter H, Oscarsson S, Buijs J. Thermodynamic Analysis of Proteins Adsorbed on Silica Particles: Electrostatic Effects. J Colloid Interface Sci 2001;237:98–103. doi:10.1006/jcis.2001.7485.

[120] Bouaziz Z, Soussan L, Janot J-M, Lepoitevin M, Bechelany M, Djebbi MA, et al. Structure and antibacterial activity relationships of native and amyloid fibril lysozyme loaded on layered double hydroxide. Colloids Surfaces B Biointerfaces 2017;157. doi:10.1016/j.colsurfb.2017.05.050.

[121] Gagner JE, Lopez MD, Dordick JS, Siegel RW. Effect of gold nanoparticle morphology on adsorbed protein structure and function. Biomaterials 2011;32:7241–52. doi:10.1016/j.biomaterials.2011.05.091.

[122] Vertegel AA, Siegel RW, Dordick JS. Silica nanoparticle size influences the structure and enzymatic activity of adsorbed lysozyme. Langmuir 2004;20:6800–7. doi:10.1021/la0497200.

[123] Yang Q, Luo B, Zhu Y, Lan F, Wu Y, Gu Z. Conformational changes of adsorbed and free proteins on magnetic nanoclusters. Colloids Surfaces B Biointerfaces 2018;170:664–72. doi:10.1016/j.colsurfb.2018.05.056.

[124] Janes RW. Bioinformatics analyses of circular dichroism protein reference databases. Bioinformatics 2005;21:4230–8. doi:10.1093/bioinformatics/bti690.

[125] Wang J, Jensen UB, Jensen G V., Shipovskov S, Balakrishnan VS, Otzen D, et al. Soft interactions at nanoparticles alter protein function and conformation in a size dependent manner. Nano Lett 2011;11:4985–91. doi:10.1021/nl202940k.

[126] Manoel EA, dos Santos JCS, Freire DMG, Rueda N, Fernandez-Lafuente R. Immobilization of lipases on hydrophobic supports involves the open form of the enzyme. Enzyme Microb Technol 2015;71:53–7. doi:10.1016/j.enzmictec.2015.02.001.

[127] Hoarau M, Badieyan S, Marsh ENG. Immobilized enzymes: Understanding enzyme-surface interactions at the molecular level. Org Biomol Chem 2017;15:9539–51. doi:10.1039/c7ob01880k.

[128] Yeşiloğlu Y. Utilization of bentonite as a support material for immobilization of Candida rugosa lipase. Process Biochem 2005;40:2155–9. doi:10.1016/j.procbio.2004.08.008.

[129] Quiquampoix H. A stepwise approach to the understanding of extracellular enzyme activity in soil. I. Effect of electrostatic interactions on the conformation of a beta-D-glucosidase adsorbed on different mineral surfaces. Biochimie 1987;69:753–63. doi:10.1016/0300-9084(87)90197-0.

[130] Thudi L, Jasti LS, Swarnalatha Y, Fadnavis NW, Mulani K, Deokar S, et al. Adsorption induced enzyme denaturation: The role of protein surface in adsorption induced protein denaturation on allyl glycidyl ether (AGE)-ethylene glycol dimethacrylate (EGDM) copolymers. Colloids Surfaces B Biointerfaces 2012;90:184–90. doi:10.1016/j.colsurfb.2011.10.022.

[131] During MJ. NIH Public Access 2012;14:324–38. doi:10.1016/j.cmet.2011.06.020.White.

[132] González-Fernández E, Staderini M, Avlonitis N, Murray AF, Mount AR, Bradley M. Effect of spacer length on the performance of peptide-based electrochemical biosensors for protease detection. Sensors Actuators, B Chem 2018;255:3040–6. doi:10.1016/j.snb.2017.09.128.

[133] Masuda Y, Kugimiya S ichi, Kato K. Improvement of thermal-stability of enzyme immobilized onto mesoporous zirconia. J Asian Ceram Soc 2014;2:11–9. doi:10.1016/j.jascer.2013.12.003.

[134] Carrasco M., Rad JC, Gonzalez-Carcedo S. Immobilization of Alkaline-Phosphatase By Sorption on Na-Sepiolite. Bioresour Technol 1995;51:175–81.

[135] Lozzi I, Calamai L, Fusi P, Bosetto M, Stotzky G. Interaction of horseradish peroxidase with montmorillonite homoionic to Na 1 and Ca 2 1 : effects on enzymatic activity and microbial degradation 2001;33:1021–8.

[136] Tcherniak A, Ha JW, Dominguez-Medina S, Slaughter LS, Link S. Probing a century old prediction one plasmonic particle at a time. Nano Lett 2010;10:1398–404. doi:10.1021/nl100199h.

[137] Chang WS, Willingham B, Slaughter LS, Dominguez-Medina S, Swanglap P, Link S. Radiative and nonradiative properties of single plasmonic nanoparticles and their assemblies. Acc Chem Res 2012;45:1936–45. doi:10.1021/ar200337u.

[138] Connor EE, Mwamuka, Judith, Anand Gole, Catherine J. Murphy MDW. Gold Nanoparticles Are Taken Up by Human Cells but Do Not Cause Acute Cytotoxicity. Small 2005;1:325–7.

[139] Nienhaus GU, Müller JD, McMahon BH, Frauenfelder H. Exploring the conformational energy landscape of proteins. Phys D 1997;107:297–311. doi:10.1016/S0167-2789(97)00097-3.

[140] Kittler S, Greulich C, Gebauer JS, Diendorf J, Treuel L, Ruiz L, et al. The influence of proteins on the dispersability and cell-biological activity of silver nanoparticles. J Mater Chem 2010;20:512–8. doi:10.1039/B914875B.

[141] Wang H, Lin Y, Nienhaus K, Nienhaus GU. The protein corona on nanoparticles as viewed from a nanoparticle-sizing perspective. Wiley Interdiscip Rev Nanomedicine Nanobiotechnology 2017:1–9. doi:10.1002/wnan.1500.

[142] Monopoli MP, Bombelli FB, Dawson KA. Nanobiotechnology: Nanoparticle coronas take shape. Nat Nanotechnol 2011;6:11–2. doi:10.1038/nnano.2011.267.

[143] Deng ZJ, Liang M, Monteiro M, Toth I, Minchin RF. Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nat Nanotechnol 2011;6:39–44. doi:10.1038/nnano.2010.250.

[144] Jiang J, Oberdörster G, Biswas P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. J Nanoparticle Res 2009;11:77–89. doi:10.1007/s11051-008-9446-4.

[145] Gebauer JS, Treuel L. Influence of individual ionic components on the agglomeration kinetics of silver nanoparticles. J Colloid Interface Sci 2011;354:546–54. doi:10.1016/j.jcis.2010.11.016.

[146] Segets D, Marczak R, Schäfer S, Paula C, Gnichwitz JF, Hirsch A, et al. Experimental and theoretical studies of the colloidal stability of nanoparticles?a general interpretation based on stability maps. ACS Nano 2011;5:4658–69. doi:10.1021/nn200465b.

[147] Kohut A, Voronov A, Peukert W. An effective way to stabilize colloidal particles dispersed in polar and nonpolar media. Langmuir 2007;23:504–8. doi:10.1021/la062465u.

[148] Gebauer JS, Malissek M, Simon S, Knauer SK, Maskos M, Stauber RH, et al. Impact of the nanoparticle-protein corona on colloidal stability and protein structure. Langmuir 2012;28:9673–9. doi:10.1021/la301104a.

[149] Coglitore D, Giamblanco N, Kizalaité A, Coulon PE, Charlot B, Janot JM, et al. Unexpected Hard Protein Behavior of BSA on Gold Nanoparticle Caused by Resveratrol. Langmuir 2018;34:8866–74. doi:10.1021/acs.langmuir.8b01365.

[150] Lindman S, Lynch I, Thulin E, Nilsson H, Dawson KA, Linse S. Systematic investigation of the thermodynamics of HSA adsorption to N-iso-propylacrylamide/N-tert-butylacrylamide copolymer nanoparticles. Effects of particle size and hydrophobicity. Nano Lett 2007;7:914–20. doi:10.1021/nl062743+.

[151] Chakraborty S, Joshi P, Shanker V, Ansari ZA, Singh SP, Chakrabarti P. Contrasting effect of gold nanoparticles and nanorods with different surface modifications on the structure and activity of bovine serum albumin. Langmuir 2011;27:7722–31. doi:10.1021/la200787t.

[152] Bousquet Y, Swart PJ, Schmitt-Colin N, Velge-Roussel F, Kuipers ME, Meijer DKF, et al. Molecular mechanisms of the adsorption of a model protein (human serum albumin) on poly(methylidene malonate 2.1.2) nanoparticles. Pharm Res 1999;16:141–7. doi:10.1023/A:1018843401077.

[153] Brewer SH, Glomm WR, Johnson MC, Knag MK, Franzen S. Probing BSA binding to citrate-coated gold nanoparticles and surfaces. Langmuir 2005;21:9303–7. doi:10.1021/la050588t.

[154] Walczyk D, Bombelli F. What the cell “sees” in bionanoscience Supporting Info. J … 2010;353:1–19.

[155] Röcker C, Pötzl M, Zhang F, Parak WJ, Nienhaus GU. A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles. Nat Nanotechnol 2009;4:577–80. doi:10.1038/nnano.2009.195.

[156] Maffre P, Nienhaus K, Amin F, Parak WJ, Nienhaus GU. Characterization of protein adsorption onto fept nanoparticles using dual-focus fluorescence correlation spectroscopy. Beilstein J Nanotechnol 2011;2:374–83. doi:10.3762/bjnano.2.43.

[157] Shao L, Dong C, Sang F, Qian H, Ren J. Studies on interaction of CdTe quantum dots with bovine serum albumin using fluorescence correlation spectroscopy. J Fluoresc 2009;19:151–7. doi:10.1007/s10895-008-0396-0.

[158] Jiang X, Weise S, Hafner M, Röcker C, Zhang F, Parak WJ, et al. Quantitative analysis of the protein corona on FePt nanoparticles formed by transferrin binding. J R Soc Interface 2010;7. doi:10.1016/j.jtcvs.2011.09.068.

[159] Deng ZJ, Mortimer G, Schiller T, Musumeci A, Martin D, Minchin RF. Differential plasma protein binding to metal oxide nanoparticles. Nanotechnology 2009;20. doi:10.1088/0957-4484/20/45/455101.

[160] Fertsch-Gapp S, Semmler-Behnke M, Wenk A, Kreyling WG. Binding of polystyrene and carbon black nanoparticles to blood serum proteins. Inhal Toxicol 2011;23:468–75. doi:10.3109/08958378.2011.583944.

[161] Lacerda SHDP, Park JJ, Meuse C, Pristinski D, Becker ML, Karim A, et al. Interaction of Gold Nanoparticles with Common Human Blood Proteins. ACS Nano 2010;4:365–79. doi:10.1021/nn9011187.

[162] He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature 1992;358:209–15. doi:10.1038/358209a0.

[163] Lundqvist M, Augustsson C, Lilja M, Lundkvist K, Dahlbäck B, Linse S, et al. The nanoparticle protein corona formed in human blood or human blood fractions. PLoS One 2017;12:1–15. doi:10.1371/journal.pone.0175871.

[164] Turkevich J, Stevenson PC, Hillier J. The formation of colloidal gold. J Phys Chem 1953;57:670–3. doi:10.1021/j150508a015.

[165] Dominguez-Medina S, Kisley L, Tauzin LJ, Hoggard A, Shuang B, D. S. Indrasekara AS, et al. Adsorption and Unfolding of a Single Protein Triggers Nanoparticle Aggregation. ACS Nano 2016;10:2103–12. doi:10.1021/acsnano.5b06439.

[166] Tsai DH, Delrio FW, Keene AM, Tyner KM, MacCuspie RI, Cho TJ, et al. Adsorption and conformation of serum albumin protein on gold nanoparticles investigated using dimensional measurements and in situ spectroscopic methods. Langmuir 2011;27:2464–77. doi:10.1021/la104124d.

[167] Huang R, Carney RP, Ikuma K, Stellacci F, Lau BLT. Effects of surface compositional and structural heterogeneity on nanoparticle-protein interactions: Different protein configurations. ACS Nano 2014;8:5402–12. doi:10.1021/nn501203k.

[168] Bhirde AA, Hassan SA, Harr E, Chen X. Role of albumin in the formation and stabilization of

nanoparticle aggregates in serum studied by continuous photon correlation spectroscopy and multiscale computer simulations. J Phys Chem C 2014;118:16199–208. doi:10.1021/jp5034068.

[169] Xu Z, Liu XW, Ma YS, Gao HW. Interaction of nano-TiO2 with lysozyme: Insights into the enzyme toxicity of nanosized particles. Environ Sci Pollut Res 2010;17:798 – 806. doi:10.1007/s11356-009-0153-1.

[170] Goy-López S, Juárez J, Alatorre-Meda M, Casals E, Puntes VF, Taboada P, et al. Physicochemical Characteristics of Protein–NP Bioconjugates: The Role of Particle Curvature and Solution Conditions on Human Serum Albumin Conformation and Fibrillogenesis Inhibition. Langmuir 2012;28:9113–9126. doi:10.1021/la300402w.

[171] Chakraborti S, Sarwar S, Chakrabarti P. The effect of the binding of zno nanoparticle on the structure and stability of α-lactalbumin: A comparative study. J Phys Chem B 2013;117:13397–408. doi:10.1021/jp404411b.

[172] Zolghadri S, Saboury AA, Amin E. Iranian Chemical Society A Spectroscopic Study on the Interaction between Ferric Oxide Nanoparticles and Human Hemoglobin 2010;7:145–53.

[173] Pishkar L, Taheri S, Makarem S, Alizadeh Zeinabad H, Rahimi A, Saboury AA, et al. Studies on the interaction between nanodiamond and human hemoglobin by surface tension measurement and spectroscopy methods. J Biomol Struct Dyn 2017;35:603–15. doi:10.1080/07391102.2016.1155172.

[174] Vitali M, Rigamonti V, Natalello A, Colzani B, Avvakumova S, Brocca S, et al. Conformational properties of intrinsically disordered proteins bound to the surface of silica nanoparticles. Biochim Biophys Acta - Gen Subj 2018;1862:1556–64. doi:10.1016/j.bbagen.2018.03.026.

[175] Worrall JWE, Verma A, Yan H, Rotello VM. “Cleaning” of nanoparticle inhibitors via proteolysis of adsorbed proteins. Chem Commun 2006;22:2338–40. doi:10.1039/b517421j.

[176] Rabbani G, Jahir M, Ahmad A, Yusof M, Hasan R. Colloids and Surfaces B : Biointerfaces Effect of copper oxide nanoparticles on the conformation and activity of ␢ -galactosidase. Colloids Surfaces B Biointerfaces 2014;123:96–105. doi:10.1016/j.colsurfb.2014.08.035.

[177] Fischer NO, McIntosh CM, Simard JM, Rotello VM. Inhibition of chymotrypsin through surface binding using nanoparticle-based receptors. Proc Natl Acad Sci 2002;99:5018–23. doi:10.1073/pnas.082644099.

[178] Joshi D, Soni RK. Laser-induced synthesis of silver nanoparticles and their conjugation with protein. Appl Phys A Mater Sci Process 2014;116:635–41. doi:10.1007/s00339-013-8210-5.

[179] Wu X, Narsimhan G. Characterization of secondary and tertiary conformational changes of β-lactoglobulin adsorbed on silica nanoparticle surfaces. Langmuir 2008;24:4989–98. doi:10.1021/la703349c.

[180] Ponnuvel S, Subramanian B, Ponnuraj K. Conformational Change Results in Loss of Enzymatic Activity of Jack Bean Urease on Its Interaction with Silver Nanoparticle. Protein J 2015;34:329–37. doi:10.1007/s10930-015-9627-9.

[181] Id CREF, Uln ML, Library O. Protein- Nanoparticle Interactions. 2013.

[182] Lee WK, McGuire J, Bothwell MK. Concentration effects on adsorption of bacteriophage T4 lysozyme stability variants to silica. J Colloid Interface Sci 2002;252:473–6.

doi:10.1006/jcis.2002.8473.

[183] Chakraborti S, Chatterjee T, Joshi P, Poddar A, Bhattacharyya B, Singh SP, et al. Structure and activity of lysozyme on binding to ZnO nanoparticles. Langmuir 2010;26:3506–13. doi:10.1021/la903118c.

[184] Cukalevski R, Lundqvist M, Oslakovic C, Dahlbäck B, Linse S, Cedervall T. Structural changes in apolipoproteins bound to nanoparticles. Langmuir 2011;27:14360–9. doi:10.1021/la203290a.

[185] Shang W, Nuffer JH, Dordick JS, Siegel RW. Unfolding of ribonuclease a on silica nanoparticle surfaces. Nano Lett 2007;7:1991–5. doi:10.1021/nl070777r.

[186] Debnath K, Pradhan N, Singh BK, Jana NR, Jana NR. Poly(trehalose) Nanoparticles Prevent Amyloid Aggregation and Suppress Polyglutamine Aggregation in a Huntington's Disease Model Mouse. ACS Appl Mater Interfaces 2017;9:24126–24139. doi:10.1021/acsami.7b06510.

[187] Elsaesser A, Howard CV. Toxicology of nanoparticles. Adv Drug Deliv Rev 2012;64:129–37. doi:10.1016/j.addr.2011.09.001.

[188] Xia YN, Sun YG. Shape-controlled synthesis of gold and silver nanoparticles. Science (80-) 2002;298:2176–9.

[189] Lynch I, Cedervall T, Lundqvist M, Cabaleiro-Lago C, Linse S, Dawson KA. The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. Adv Colloid Interface Sci 2007;134–135:167–74. doi:10.1016/j.cis.2007.04.021.

[190] Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Baldelli Bombelli F, et al. Physical-Chemical aspects of protein corona: Relevance to in vitro and in vivo biological impacts of nanoparticles. J Am Chem Soc 2011;133:2525–34. doi:10.1021/ja107583h.

[191] Vroman L, Adams AL, FIscher GC, Munow PC. Interaction of High Molecular Weight Kininogen, Factor XII, and Fibronogen in Plasma at Interfaces. Blood 1980;55:156–9.

[192] Cedervall T, Lynch I, Foy M, Berggård T, Donnelly SC, Cagney G, et al. Detailed identification of plasma proteins adsorbed on copolymer nanoparticles. Angew Chemie - Int Ed 2007;46:5754–6. doi:10.1002/anie.200700465.

[193] Monopoli MP, Aberg C, Salvati A, Dawson K a. Biomolecular coronas provide the biological identity of nanosized materials. Nat Nanotechnol 2012;7:779–86. doi:10.1038/nnano.2012.207.

[194] Salvati A, Pitek AS, Monopoli MP, Prapainop K, Bombelli FB, Hristov DR, et al. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. Nat Nanotechnol 2013;8:137–43. doi:10.1038/nnano.2012.237.

[195] Walczyk D, Baldelli Bombelli F, Monopoli MP, Lynch I, Dawson KA. What the cell [ldquo]sees[rdquo] in bionanoscience. J Am Chem Soc 2010;132:5761–8.

[196] Wang Z, Liu S, Ma J, Qu G, Wang X, Yu S, et al. Silver nanoparticles induced RNA polymerase-silver binding and RNA transcription inhibition in erythroid progenitor cells. ACS Nano 2013;7:4171–86. doi:10.1021/nn400594s.

[197] Word JM, Lovell SC, LaBean TH, Taylor HC, Zalis ME, Presley BK, et al. Visualization and quantification of molecular goodness-of-fit: small probe contact dots with explicit hydrogens. J Mol Biol 1999;285:1711–33.

[198] Walkey CD, Chan WCW. Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. Chem Soc Rev 2012;41:2780–99. doi:10.1039/C1CS15233E.

[199] Vilanova O, Mittag JJ, Kelly PM, Milani S, Dawson KA, Rädler JO, et al. Understanding the Kinetics of Protein-Nanoparticle Corona Formation. ACS Nano 2016;10:10842–50. doi:10.1021/acsnano.6b04858.

[200] Anderson NL, Anderson NG. The Human Plasma Proteome. Mol Cell Proteomics 2002;1:845–67. doi:10.1074/mcp.R200007-MCP200.

[201] Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, Nilsson H, et al. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc Natl Acad Sci 2007;104:2050–5. doi:10.1073/pnas.0608582104.

[202] Sakulkhu U, Mahmoudi M, Maurizi L, Salaklang J, Hofmann H. Protein corona composition of superparamagnetic iron oxide nanoparticles with various physico-Chemical properties and coatings. Sci Rep 2014;4:1–9. doi:10.1038/srep05020.

[203] Bonvin D, Chiappe D, Moniatte M, Hofmann H, Mionić Ebersold M. Methods of protein corona isolation for magnetic nanoparticles. Analyst 2017;142:3805–15. doi:10.1039/c7an00646b.

[204] Izak-Nau E, Voetz M, Eiden S, Duschl A, Puntes VF. Altered characteristics of silica nanoparticles in bovine and human serum: the importance of nanomaterial characterization prior to its toxicological evaluation. Part Fibre Toxicol 2013;10:56. doi:10.1186/1743-8977-10-56.

[205] Pozzi D, Colapicchioni V, Caracciolo G, Piovesana S, Capriotti AL, Palchetti S, et al. Effect of polyethyleneglycol (PEG) chain length on the bio-nano- interactions between PEGylated lipid nanoparticles and biological fluids: From nanostructure to uptake in cancer cells. Nanoscale 2014;6:2782–92. doi:10.1039/c3nr05559k.

[206] O'Connell DJ, Bombelli FB, Pitek AS, Monopoli MP, Cahill DJ, Dawson KA. Characterization of the bionano interface and mapping extrinsic interactions of the corona of nanomaterials. Nanoscale 2015;7:15268–76. doi:10.1039/c5nr01970b.

[207] Vroman L. Effect of Adsorbed Proteins on the Wettability of Hydrophilic and Hydrophobic Solids. Nature 1962;196:476–7.

[208] Zhdanov VP, Cho NJ. Kinetics of the formation of a protein corona around nanoparticles. Math Biosci 2016;282:82–90. doi:10.1016/j.mbs.2016.09.018.

[209] Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntes VF. Hardening of the nanoparticle-protein corona in metal (Au, Ag) and oxide (Fe 3O 4, CoO, and CeO 2) nanoparticles. Small 2011;7:3479–86. doi:10.1002/smll.201101511.

[210] García-Álvarez R, Hadjidemetriou M, Sánchez-Iglesias A, Liz-Marzán LM, Kostarelos K. In vivo formation of protein corona on gold nanoparticles. the effect of their size and shape. Nanoscale 2018;10:1256–64. doi:10.1039/c7nr08322j.

[211] Hu Z, Zhang H, Zhang Y, Wu R, Zou H. Nanoparticle size matters in the formation of plasma protein coronas on Fe3O4nanoparticles. Colloids Surfaces B Biointerfaces 2014;121:354–61. doi:10.1016/j.colsurfb.2014.06.016.

[212] Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci U S A 2008;105:14265–70. doi:10.1073/pnas.0805135105.

[213] Docter D, Westmeier D, Markiewicz M, Stolte S, Knauer SK, Stauber RH. The nanoparticle biomolecule corona: lessons learned - challenge accepted? Chem Soc Rev 2015;44:6094–121. doi:10.1039/c5cs00217f.