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Volumetric Interpretation of Protein Adsorption: Capacity Scaling with Adsorbate Molecular Weight and Adsorbent Surface Energy

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Abstract

Silanized-glass-particle adsorbent capacities are extracted from adsorption isotherms of human serum albumin (HSA, 66 kDa), immunoglobulin G (IgG, 160 kDa), fibrinogen (Fib, 341 kDa), and immunoglobulin M (IgM, 1000 kDa) for adsorbent surface energies sampling the observable range of water wettability. Adsorbent capacity expressed as either mass-or-moles per-unit-adsorbent-area increases with protein molecular weight (MW) in a manner that is quantitatively inconsistent with the idea that proteins adsorb as a monolayer at the solution-material interface in any physically-realizable configuration or state of denaturation. Capacity decreases monotonically with increasing adsorbent hydrophilicity to the limit-of-detection (LOD) near $\tau^o = 30$ dyne/cm ($\theta \sim 65^\circ$) for all protein/surface combinations studied (where $\tau^o \equiv \gamma_{lv}^o \cos \theta$ is the water adhesion tension, γ_{lv}^o is the interfacial tension of pure-buffer solution, and θ is the buffer advancing contact angle). Experimental evidence thus shows that adsorbent capacity depends on both adsorbent surface energy and adsorbate size. Comparison of theory to experiment implies that proteins do not adsorb onto a two-dimensional (2D) interfacial plane as frequently depicted in the literature but rather partition from solution into a three-dimensional (3D) interphase region that separates the physical surface from bulk solution. This interphase has a finite volume related to the dimensions of hydrated protein in the adsorbed state (defining “layer” thickness). The interphase can be comprised of a number of adsorbed-protein layers depending on the solution concentration in which adsorbent is immersed, molecular volume of the adsorbing protein (proportional to MW), and adsorbent hydrophilicity. Multilayer adsorption accounts for adsorbent capacity over-and-above monolayer and is inconsistent with the idea that protein adsorbs to surfaces primarily through protein/surface interactions because proteins within second (or higher-order) layers are too distant from the adsorbent surface to be held surface bound by interaction forces in close proximity. Overall, results are consistent with the idea that protein adsorption is primarily controlled by water/surface interactions.

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Impact Statement: This work illuminates how protein size and adsorbent surface energy control the amount of protein that adsorbs to surfaces from purified, single-protein solutions.

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Keywords

Protein adsorption; kinetics; adsorption competition

1.0 Introduction

Biophysical mechanism(s) of protein adsorption are of fundamental importance in biomaterials surface science because adsorbed proteins apparently catalyze, mediate, or moderate the biological response to artificial materials [1–6]. Protein adsorption is a complex phenomena for a variety of technical reasons, not the least of which is that proteins are relatively large polyelectrolytes with adsorption properties that might depend on interrelated factors such as protein type/size (pI, molecular weight, structure/conformation, *etc.*), solution concentration, adsorbent surface chemistry, and protein/surface interaction time. Thus, protein adsorption is a multifaceted problem in surface physical chemistry varying over broad time-and-length scales. Perhaps as a consequence of these factors, exacerbated by the wide variety of analytical methods employed to measure protein adsorption [7–9] that defy meaningful comparison [8], it has proven difficult to discern generalities underlying protein adsorption.

There are two related ways adsorption can be theoretically interpreted. The first and most popular paradigm arises from the earliest considerations of diffusion and mass transport, extended and applied by luminaries such as Milner and Langmuir (see ref. [10] and citations therein for a brief historical perspective). The basic idea is that adsorbate binds to a surface through strong adsorbent/adsorbate interactions leading to a pseudo-2D adsorbed layer such that, in the words of Irving Langmuir, “...the adsorbed film should not exceed one molecule in thickness...” [11]. Hence, adsorption comes to be measured on a per-unit-area basis, effectively ignoring the interfacial volume occupied by adsorbate and abandoning per-unit-volume concentration scaling. This core concept has been applied to the specific case of protein adsorption by elaboration of the Langmuir isotherm into the Random Sequential Adsorption (RSA) model [12,13]. Over time, RSA itself has been embellished to account for various factors such as reversible adsorption, lateral diffusion of adsorbed molecules, protein denaturation, and so on (see, for examples, refs. [13–16] and citations therein).

The above “2D paradigm” is related to an alternative “3D paradigm” that is premised on the idea that adsorbate collects in a near-surface (vicinal) region sometimes referred to as an interphase. The interphase is a discrete volume that separates the bulk-solution phase from the physical adsorbent surface phase (see refs. [10,17–20] and citations therein). Here, the thickness dimension is explicitly considered and adsorption is (or can be) measured in concentration units of per-unit-interphase-volume. In the limit of small-molecule adsorbates such as gas molecules or simple surfactants, 2D and 3D models are substantially equivalent. But as adsorbate dimensions increase, the distinction between the models becomes more important because the interfacial energy required to create a thick interphase becomes an increasingly significant component of the overall free energy of adsorption. The 3D interphase paradigm is hardly new to surface science (Guggenheim surface construction [21,22]), but has not been widely applied in the study of protein adsorption, or to the general problem of adsorption for that matter (see, for example, ref. [21] for a lucid comparison of Gibbs and Guggenheim constructions). Significant advantages of the interphase paradigm are retention of the concept of chemical activities (concentrations) essential for a complete understanding of adsorption energetics and consistency with standard surface thermodynamics [10]. Importantly, the interphase model easily accommodates multilayer-protein adsorption that has been shown to occur by a number of investigators using a variety of experimental methods over the last twenty years or so [6,23–35]. A disadvantage of the 3D model is that it requires

a slightly different way of thinking about adsorption than usually applied and a commensurately different theoretical treatment.

This paper examines adsorption of selected purified-blood proteins spanning three decades in molecular weight (MW) to silanized-glass adsorbent particles with surface energy sampling the observable range of water wettability. Full adsorption isotherms are measured for each protein/adsorbent pair, revealing systematic size-and-surface-energy dependence that is interpreted both in terms of 2D and 3D models. Adsorbent capacity measurements are shown to be inconsistent with the 2D model, strongly suggesting that the 3D model is a more appropriate construct for interpretation of protein adsorption.

2.0 Methods and Materials

2.1 Protein Solutions

Table 1 lists relevant details for proteins used in this work as received from the vendor without further purification. SDS-PAGE of purified protein solutions yielded single bands. Selected proteins were dissolved in phosphate buffer solution (PBS; Sigma; 0.14 M NaCl, 3mM KCl prepared in 18 M Ω water; pH = 7.2) to the desired concentration. A protein concentrate was freshly prepared before each depletion experiment (Section 2.3 below) from which 80:20 dilutions were made.

2.2 Preparation of Particulate Adsorbents

Adsorbents used in this work were 106 μ m nominal diameter glass particles (Sigma Aldrich) in either cleaned or silanized form. Surface area measured by the Brunauer–Emmett–Teller (BET) method (Micromeritics ASAP 2000 using nitrogen as the probe gas) was 0.38 ± 0.09 m²/g. Octadecyltrichlorosilane (OTS), 3-aminopropyltriethoxysilane (APTES), n-propyltriethoxysilane (PTES), vinyltriethoxysilane (VTES) (used as received from Gelest) were used to silanize glass particles to produce adsorbents with different surface chemistry and energy sampling the observable water-wettability range (Table 2). Nyebar solution (0.2% solution of 1,1-pentadecafluorooctylmethacrylate in trichlorotrifluoroethane, Nye Lubricants, Fairhaven, MA) was optionally used to render OTS-treated particles slightly more hydrophobic than the OTS silane layer. A glass cover slip (Fisher 22 $\times$ 30 $\times$ 0.1mm) witness sample was carried through the surface-treatment protocol described below with particles, providing a substrate suitable for reading PBS contact angles.

Glass particles and cover slips were first treated with piranha solution (a hot mixture of 30% H₂O₂ and concentrated H₂SO₄) by immersion for 30 min followed by 3X sequential washes in each of 18 M Ω de-ionized water and 100% ethanol. Piranha-solution oxidized glass was air dried and subsequently oxidized by air-plasma treatment of a single layer of particles (or coverslip) held in a 15 mm Pyrex glass petri dish (10 min at 100 W plasma; Herrick, Whippany, NY) directly before use in silanization procedures or adsorption measurements. This process yielded fully water-wettable “clean” glass surfaces. Clean-glass particles and cover-slip witness samples were silanized by 1.5 hr reaction with 5% OTS dissolved in chloroform. OTS-silanized samples were 3X rinsed with chloroform before curing in a vacuum oven at 110 $^{\circ}$ C for 12 hr. Cured-OTS samples were optionally immersed in Nyebar solution for 10 min and air dried. Adsorbents more hydrophilic than OTS but more hydrophobic than clean glass were obtained by silanization with APTES, PTES, VTES or by oxidation of OTS adsorbents with chromerge (H₂SO₄/Cr₂O₃; VWR). Different wetting characteristics were obtained using chromerge oxidation by immersing OTS-treated particles in 25%, 50% and 75% aqueous chromerge for between 5 and 25 mins. Oxidized surfaces were thoroughly washed with water and ethanol before drying in a vacuum oven at 110 $^{\circ}$ C overnight. Silanization with APTES was carried out in 95:5 ethanol-water mixtures containing 5% APTES that was allowed to

hydrolyze overnight before use as a silanizing reagent. APTES silanization was accomplished by immersing clean glass in APTES reagent for 20 mins. followed by washing with ethanol and drying in the above-mentioned vacuum oven overnight. Silanization with PTES and VTES followed the APTES procedure except that 90:10 ethanol-water containing 0.5 % glacial acetic acid was used.

PBS contact angles on glass cover-slip witness samples were measured on an automated contact-angle goniometer (First Ten Angstroms Inc., Portsmouth, VA). Advancing contact angles were measured using the captive-drop technique (see refs. [36,37] for comparison of goniometric techniques and discussion of experimental errors). Contact angles could not be read directly on glass particles but optical microscopy of the shape of the liquid meniscus of particles partly immersed in water on a microscope slide qualitatively confirmed that the treated particles were not different from the witness samples. Water wettability of the n^{th} surface type was expressed as (advancing) water adhesion tension τ_n^o ; where $\tau_n^o \equiv \gamma_{lv}^o \cos\theta$ is the product of pure-buffer interfacial tension $\gamma_{lv}^o = 71.97$ dyne/cm at 20 °C and cosine of the observed advancing contact angle θ , and n identifies the substrate type listed in Table 2.

2.3 Measurement of Adsorption Isotherms by the Solution-Depletion Method

Experimental details of using the solution-depletion method for measuring adsorption isotherms of both purified proteins and mixtures have been disclosed elsewhere [10,17–20]. Briefly, protein solutions (30 μ L) in PBS at various concentrations were mixed with a mass (surface area, Table 1) of adsorbent particles by gentle pipette aspiration. Solution and particles were allowed to stand undisturbed in 0.5 mL conical microtubes for 1 hr. before analysis. Cited prior work comparing adsorption from continuously-mixed and unmixed solutions revealed that mixing had no effect on the amount of protein adsorbed from purified-protein solution (see especially ref. [10]).

Two methods of quantifying protein-solution concentration were used in this work, electrophoresis and nanodrop spectroscopy. Electrophoresis methods have been detailed in above-cited work. Spectroscopic measurement of protein concentrations was performed using a Nanodrop 1000 spectrometer (Thermo Scientific). Following the basic protocol outlined by the vendor, 2 μ L protein solutions were pipetted onto the pedestal and a sample column (meniscus) was formed by closing the instrument sample arm. Adsorption was measured at 280 nm directly after closing the sample arm. Solution concentration of unknown samples were deduced from a previously-constructed linear calibration curve for each protein-surface combination. Calibration curves were fit to an equation of the form $y = mx + b$ by linear-least-squares regression yielding a statistically null intercept b , slope m with less than 3% standard-error-of-the-fit, and $R^2 > 99\%$ in all cases. We found that $0.85 < m < 1.0$ for IgG and Fib but $2.7 < m < 2.8$ for IgM.

2.4 Computational Methods

Statistical methods of analyzing depletion isotherms have been disclosed elsewhere [10,17–20]. Briefly reiterating essential details for the purposes of this paper, amount of the i^{th} protein adsorbed to the n^{th} adsorbent was calculated by difference $(D_i)_n$ in weight/volume (w/v, mg/mL) protein-solution concentrations before $W_{B_i}^o$ and after W_{B_i} adsorption to particulates (*i.e.* the solution depletion $(D_i)_n \equiv (W_{B_i}^o - W_{B_i})_n$, see further Section 4.1). In the absence of particulate adsorbent, $D_i < 0.1$ mg/mL for all proteins at surface-saturating bulk-solution concentrations $(W_{B_i}^o)^{\max}$ and decreased in proportion to decreasing $W_{B_i}^o$. This background adsorption, due to all sources of protein loss to tubes and pipette tips in handling procedures, represented less than 1–2% of experimental $(D_i)_n$ measured in the presence of particulate

adsorbent. Thus, it was concluded that background correction of depletion measurements was unnecessary within the $W_{B_i}^o$ range explored in this work [17]. The parameters $(D_i)_n^{\max}$ and $(W_{B_i}^o)_n^{\max}$ were extracted from least-squares statistical fitting of isotherm data to the so-called Chapman equation. Error estimates listed in columns 4 and 5 of Table 3 were determined from standard-error-of-the-fit as described in ref. [17]. Adsorbent capacities reported herein are normalized to 50 mg adsorbent corresponding to 190 cm² adsorbent surface area for direct comparison to HSA (see Table 1).

3.0 Results

The intent of this work was to compare experimental and theoretical adsorbent capacities for selected proteins spanning 3 decades of molecular weight (MW) for adsorbent surface energies sampling the observable range of water wettability. Table 1 compiles information on proteins used in this study (columns 1–4), including human serum albumin (HSA) for which adsorption measurements were taken from ref. [18]. Column 5 of Table 1 lists the mass (surface area) of adsorbent used in the measurement of adsorption isotherms by the solution-depletion method (see Section 2.3). We found it necessary to customize adsorbent mass (surface area) used in solution-depletion assays to accommodate adsorption characteristics of each protein. In particular, greater mass of larger proteins adsorbed from solutions require use of lower adsorbent masses (surface area) for the mg/mL solution-concentration range explored in this work. Table 2 summarizes basic data on glass-particle adsorbents used in this work prepared as described in Section 2.2.

3.1 Adsorption Isotherms by Solution Depletion

The standard depletion method described in Section 2.3 implemented with either electrophoresis or nanodrop spectroscopy was used to measure adsorption isotherms. Solution depletion met the need for unambiguous interpretation in a manner that was largely free of experimental artifacts; such as solute labeling, rinsing/drying, or complicated instrumentation. This method was sensitive to about 0.1–0.2 mg/mL (estimated to be equivalent to 0.3 mg/m² [18]). Previous work “certified” method and interpretive theory applied to steady-state adsorption (adsorption time $t \rightarrow 1$ hr.) by first studying adsorption of a broad range of single proteins to hydrophobic [17] surfaces (octyl sepharose and silanized glass) from aqueous-buffer solution, showing that results comported with thermochemically-measured free energies of adsorption and interfacial energetics measured by tensiometry (contact angle and wettability methods). Subsequently, HSA adsorption to silanized-glass adsorbent particles with incrementally-increasing hydrophilicity was measured [18], showing here that mass and energy balances for HSA adsorption were in full agreement. Consistent mass-and-energy balance obtained using very different analytical methods engendered confidence that the depletion method provided internally-consistent and accurate results. We have also demonstrated utility of these methods in studying protein-adsorption competition to the same adsorbent surface immersed in multi-component solutions [19] and protein-adsorption kinetics [10]. Depletion methods have been implemented using electrophoresis [10,17–20], radiometry [38], and spectroscopy (this work) yielding statistically identical results.

Fig. 1 compiles three IgM adsorption isotherms for Nyebar ($n = 1$), OTS ($n = 2$), and PTES ($n = 4$) adsorbents listed in Table 2. Horizontal line annotations indicate maximum adsorbent capacities $(D_{IgM})_n^{\max}$ corresponding to these n adsorbents and vertical lines estimate the solution concentrations $(W_{B_{IgM}}^o)_n^{\max}$ required to achieve $(D_{IgM})_n^{\max}$. Parameters estimated from isotherms for all protein and surface combinations studied in this work are collected in Table 3, where column 2 lists adsorbents in a “Type.Trial” format where the first digit refers to adsorbent type

keyed to Table 2 and Trial refers to the experimental trial with a particular adsorbent/protein pair. All adsorbent capacities reported in this work were normalized to account for the different adsorbent masses used in depletion measurements for different proteins listed in Tables 1 and 2.

3.2 Adsorbent Capacities

Fig. 2 plots maximum adsorbent capacities $(D_i)_{n=2}^{\max}$ of the hydrophobic OTS surface $n = 2$ for the various proteins studied in this work (designated by the i subscript, see Table 1). Normalized adsorbent capacities $(D_i)_n^{\max}$ converted to maximal adsorbed mass $(m_i)_n^{\max} = [V_B(D_i)_n^{\max}]$ are listed in column 5 of Table 3, where $V_B = 30 \mu\text{L}$ was the total solution volume used in the depletion assays. Column 7 converts $(m_i)_n^{\max}$ into $\Gamma^{\max}$ (mg/cm^2) using an adsorbent surface area $0.38 \pm 0.09 \text{ m}^2/\text{g}$ determined by BET. Fig. 3B plots experimental $\Gamma^{\max}$ for i proteins adsorbing to hydrophobic Nyebar-treated particles $n = 1$ for comparison to theoretical adsorbent capacities shown in Fig. 3A (see Section 4 for discussion).

Fig. 4 collects normalized adsorbent capacities for all proteins and adsorbents reported in Table 3. Adsorbent capacity decreased monotonically with increasing adsorbent hydrophilicity in the range $-40 < \tau^o < 30$ (where $\tau^o \equiv \gamma_{lv}^o \cos\theta$ is the water adhesion tension, γ_{lv}^o is the interfacial tension of pure-buffer solution, and θ is the buffer contact angle observed on glass-slide witness samples carried through silanization protocols with adsorbent particles). Adsorption fell dramatically to limit-of-detections (LOD) near $\tau^o = 30 \text{ dyne}/\text{cm}$ ($\theta \sim 65^\circ$) for all proteins studied, consistent with independent measurements of interfacial energetics. Decrease in adsorbent capacity for IgM was especially pronounced, making measurement of IgM adsorption isotherms for surfaces with wettability lying between $0 < \tau^o < 30$ experimentally problematic. Electrophoretic and spectroscopic depletion measurements for Fib are compared in Fig. 4 (open and closed triangles, respectively) and were found to be statistically identical. Inset of Fig. 4 expands adsorbent-capacity measurements in the region of fully-water-wettable clean-glass adsorbents, showing that adsorption was not detectable within the zone annotated “LOD”.

4.0 Discussion

4.1 Measuring Protein Adsorption

It is worthwhile to briefly define some nomenclature and discuss analytical methods of measuring protein adsorption before going engaging in the details of subsequent sections. We categorize all physicochemical events leading to an excess accumulation of a solute (*e.g.* a protein) at a surface immersed in a solution of that solute as adsorption [9,21]. The surface region is that portion of the system that separates the bulk-solution phase from the physical-adsorbent phase (a.k.a. interphase, see Section 1). Adsorbed excess may formally be positive or negative compared to bulk solution concentration [39], although protein adsorption is typically construed to be a positive excess in biomaterials literature. Interphase concentrations are equal to bulk-solution concentrations if no adsorption occurs, which is sometimes referred to as protein repellency even though this term is suggestive of negative excess. True protein repellency (expulsion from the interphase) requires a negative excess (interphase concentration less than bulk concentration), which must be small for dilute solutions [39] and is therefore presumably difficult to detect and differentiate from the no- adsorption case. Mechanistic descriptors such as binding, charge interactions, directed assembly, ion-exchange, and the like are nothing more than specific ways (mechanisms) surface-active solutes can adsorb to a surface [40] and are not different processes than adsorption [8].

Adsorption has been measured by a diverse array of surface-sensitive techniques (see refs. [7–9] for examples). This richness in analytical information is actually a burden in the

interpretation of protein adsorption because of the ensuing difficulty in comparing results on a consistent basis [8]. In particular, those analytical methods involving the removal of an adsorbent from adsorbate (protein) solution and/or rinsing of the surface to remove bulk solution (*e.g.* dip-rinse-measure protocols sometimes used in ellipsometry and radiometry) can introduce artifacts that might significantly compromise results (see, for example, refs. [38, 41] and citations therein). Protein bound to a surface after destruction of the interphase, as by rinsing or drying for example, falls outside of our definition of adsorption because removal of the adsorbent from solution can perturb the adsorption dynamic or steady-state. So-deposited protein may indeed be bound to an adsorbent surface, but comes to that state through processing over-and-above adsorption from solution. For example, we have found that radiometric dip-rinse-measure methods significantly underestimates the amount of protein adsorbed to hydrophobic surfaces because weakly (reversibly) adsorbed protein is removed by rinsing steps [38].

In effort to avoid experimental artifacts possibly involved in protein labeling or surface-rinsing steps, our work has emphasized use of either tensiometric (interfacial energetics) [6,23–25, 39,42–48] or solution-depletion (mass balance) methods [10,17–20] that do not perturb the interphase. The venerable solution-depletion method is among the most unambiguous measures of protein adsorption and is (or can be) essentially free from experimental artifacts or cumbersome interpretive theory. The basic idea behind the depletion method is to measure the concentration of protein in solution before- and-after contact with adsorbent particles (mass balance) while adsorbent is in contact with adsorbate solution. Concentration may be expressed in any convenient unit. For chemically-defined solutions of purified proteins, concentration in mass (or moles) per volume (*e.g.* mg/mL) allows the amount of protein adsorbed to be directly calculated from measured solution depletion $D \equiv (W_B^o - W_B)$; where W_B^o and W_B are concentrations before and after contact with adsorbent, respectively, and D is expressed as per-unit-volume of bulk solution. The total adsorbed mass or moles is the product DV_B , where V_B is the solution volume. Any number of methods can be used to quantify W_B^o and W_B , such as electrophoresis [10,17–20], radiometry [38], spectroscopy (this work for example), or non-specific dye uptake of proteins (such as the Bradford assay) [49]. Commercial availability of micro- or nano-drop spectroscopic instruments has greatly reduced solution volumes required in analysis, greatly extending use of spectroscopic methods for study of adsorption from single-protein solutions. Separation and quantification by electrophoresis permits multiple proteins to be used in the depletion assay [19,20].

However protein adsorption is measured, complete interpretation in terms of adsorption mechanisms requires construction of adsorption isotherms. A few arbitrarily-chosen solution concentrations is usually an insufficient basis on which to draw general conclusions. And, when comparing adsorption of any two-or-more proteins with different molecular weights to a particular adsorbent, it is not at all obvious whether that comparison should be made on mass or molar basis, as will be discussed further in this section. Finally, the choice between per-unit-surface-area or per-unit-interphase-volume as intensive scaling parameters is not obvious for reasons outlined in Section 1. In fact, the following section confirms that per-unit-surface-area scaling of protein adsorption does not account for measured adsorbent capacities, suggesting that scaling by interphase concentration is a more appropriate measure of adsorption for large adsorbate molecules such as proteins.

4.2 Adsorption Isotherms by Solution Depletion

IgM adsorption isotherms of Fig. 1 supplement isotherms for lysozyme (15 kDa), α -amylase (51 kDa), HSA (66 kDa), prothrombin (FII, 72 kDa), IgG (160 kDa), Fib (341 kDa) adsorbing to various surfaces including octyl sepharose particles (a surface-modified hydrogel), silanized-glass particles (a surface-modified impermeable ceramic), and ion-exchange resins

(functionalized sepharose-based hydrogels) we have published previously [10,17–20]. IgM isotherms are similar to others in that solution depletion increases in direct proportion to solution concentration $W_{B_{IgM}}^o$, up to a maximum value $(D_{IgM})_n^{\max}$ occurring at $W_{B_{IgM}}^o = (W_{B_{IgM}}^o)_n^{\max}$, after which further increase in solution concentration does not lead to continued adsorption from solution (subscript n keys to adsorbent type listed in Table 2). Thus, adsorption isotherms obtained using the depletion method for a wide variety of proteins spanning three decades in MW adsorbing from mg/mL solutions to different kinds of adsorbent surfaces sampling the observable range of water wettability are shown to approximate simple Henry-type isotherms (adsorbed mass in proportion to solution concentration). Adherence to a Henry-type isotherm within the mg/mL solution-concentration range suggests that proteins adsorb as noninteracting particles up to saturating-surface concentrations (*i.e.* no cooperativity among identical proteins). Furthermore, we have correlated mass balance obtained from depletion isotherms with energy balance obtained using tensiometry [18]. Overall, we find no evidence for chemical specificity in protein adsorption or dependence on adsorbent surface type, over-and-above the direct relationship with adsorbent surface energy to be discussed in Section 4.5. A notable exception to this generality are surfaces bearing strong Lewis acid/base functional (charged) groups that adsorb protein by an ion-exchange mechanism [20].

We have further found from depletion isotherms that adsorbent capacity $(D_i)_n^{\max}$ varies systematically with protein size as measured by MW [17]. This work corroborates and extends cited observations to the 1000 kDa protein IgM by showing that surface saturation increases more than 6-fold over HSA at constant surface area of hydrophobic OTS adsorbent $n = 2$ of Table 2, as summarized in Fig. 2. The guideline connecting data points of Fig. 2 speculates that adsorbed proteins occupy a succession of adsorbed layers inferred from interfacial energetics of adsorption [25] (see further Section 4.4). However interpreted, it is clear from the experimental data of Fig. 2 that adsorbent capacity depends not only on adsorbent surface area but also adsorbate size. The relationship between adsorbent capacity and adsorbate size is diagnostic of how adsorbed proteins arrange within the surface region, as discussed in the following sections.

4.3 Surface Area as an Intensive Scaling Parameter

Contemporary theories or models of protein adsorption premised on the basic idea that protein collects in a pseudo-two-dimensional (2D) layer at the solution-material interface typically compute the amount adsorbed in units of mass-or-moles-per-unit-area of adsorbent surface (represented by the symbol Γ herein). Variants of the RSA model propose that protein adsorbs to a surface in a single-layer (monolayer) arrangement due to strong protein/surface interactions up to a ‘jamming limit’ of about 55% coverage [13,50,51]. Adsorption from solution ceases when available adsorbent surface area is filled to jammed capacity. As a consequence, adsorption is capped at a certain number of molecules-per-unit-area governed by protein dimensions/configurations. This scaling is considered in the following sub-section 4.3.1, compared to experimental capacity measurements in sub-section 4.3.2, and contrasted to the per-unit-interphase-volume scaling in Section 4.4.

4.3.1 Theoretical 2D Surface Capacity—As size of adsorbed proteins increase, the occupied surface area also increases [52], causing a jammed monolayer of large proteins to contain fewer molecules than a jammed monolayer of smaller proteins. Globular blood proteins are oblate spheroids in solution with core-protein radius following $r_v = 6.72 \times 10^{-8} MW^{1/3}$ to a very good approximation (packing-volume radius in cm for molecular weight MW expressed in kDa; see refs. [53–59] for basic information regarding spherical dimensions of proteins). As a direct consequence of spheroidal shape, the partial-specific volume v^o of globular proteins falls within a conserved range of $0.70 \leq v^o \leq 0.75 \text{ cm}^3/\text{g}$ protein [60] ($v^o = 0.77 \text{ cm}^3/\text{g}$ for a

perfectly-spherical protein; see Appendix A). Assuming momentarily that proteins retain spheroidal shape in a hypothetical jammed monolayer, the excluded projected area A_p will be that of a circle with radius r_v or $A_p = \pi r_v^2 = 1.42 \times 10^{-14} MW^{2/3} (\text{cm}^2)$. Hence, a unit area will fill with $(0.55/A_p)$ proteins at the maximum jammed-sphere-limit. It follows that maximum jammed-surface capacity $\Gamma^{\max}$ for spheroids will scale as $MW^{-2/3}$ (moles/cm²) or $MW^{1/3}$ (g/cm²).

Comparison of any two spherical proteins i and j with $MW_i \neq MW_j$ adsorbed to identical adsorbents at the jammed limit reveals that the adsorbed (mole) ratio scales with $(MW_j/MW_i)^{2/3}$. For example, if protein i = HSA and protein j = IgM at 66 and 1000 kDa, respectively, then a jammed layer of HSA contains a factor of $(1000/66)^{2/3} = 6.1$ more HSA molecules than IgM molecules. The jamming-limit adsorbed mass ratio $(m_j/m_i) = (MW_i/MW_j)^{2/3} \bullet (MW_j/MW_i) = (MW_j/MW_i)^{1/3} = 2.3$, meaning that a jammed monolayer of IgM contains 2.3X more mass than a jammed monolayer of HSA molecules, even though the jammed HSA monolayer contains more molecules. This occurs because mass increases linearly with MW whereas size-excluded area increases only as $MW^{2/3}$.

Of course many other protein-sphere arrangements on a 2D plane may be contemplated, such as hexagonal close-packed (HCP) monolayers [25,52], but all size-limited-packing arguments lead to qualitatively-similar conclusions. Focusing on jammed spheres for illustration, Fig. 3A plots the $MW^{-2/3}$ (moles/cm², left-hand ordinate) and $MW^{1/3}$ (g/cm², right-hand ordinate) scaling relationship, illustrating the sharp decrease in molar $\Gamma^{\max}$, especially at the lower-end of the MW scale, compared to a relatively shallow increase in mass $\Gamma^{\max}$. Effectively then, protein adsorption falls into two broad categories: low-MW proteins (*e.g.* MW < 200 kDa such as albumin, lysozyme, casein, *etc.*) for which relatively high-molar capacity changes rapidly with molecular size (shaded bar of Fig. 3A) and high-MW proteins (*e.g.* MW > 200 kDa such as IgG, fibrinogen, IgM, *etc.*) for which relatively low-molar capacity changes slowly with molecular size. Much of the research on protein adsorption has focused on the low-MW category of proteins for which sharp changes in adsorbent capacity might amplify minor differences in proteins and adsorbents used by various investigators, possibly accounting for some of the inconsistency within the protein-adsorption literature.

4.3.2 Experimental Surface Capacity—Fig. 3B plots experimentally-measured adsorbent capacities (molar and mass scaling on left and right ordinates, respectively) for proteins of Table 1 adsorbing to the hydrophobic Nyebar-treated surface $n = 1$ and Tables 2–3. It is apparent that the theoretical scaling anticipated from Fig. 3A roughly describes trends in data (smooth curves drawn through the data will be discussed subsequently), especially for the low-MW protein HSA. But experimental adsorbent capacities are much higher than theory for high-MW proteins. In fact, detailed inspection of the corresponding data of Table 3 reveals that the theoretical jammed limit ratio $(m_{\text{IgM}}/m_{\text{HSA}}) = 2.3$ is 4–5 fold smaller than the experimentally-measured adsorbent capacity (see also Fig. 2).

Ostuni *et al.* [52] observed similar disparity between sphere-packing arguments and experiment for α -galactosidase (a 540 kDa tetramer), carbonic anhydrase (30 kDa), lysozyme (14 kDa) and RNase A (14 kDa). Ostuni considered both jammed or HCP monolayers. Table 3 column 9 expands our jammed-limit analysis discussed above to the HCP-packing case considered by Ostuni. All taken together, Table 3 corroborates Ostuni's conclusion that the surface area occupied by protein adsorbed from high-concentration (surface saturating) solutions significantly exceeds that anticipated by simple monolayer adsorption of spheres in a jammed or HCP arrangement.

Ostuni *et al.* proposed that the discrepancy between theory and experiment was due to protein denaturation in the adsorbed state by which change in adsorbed-protein conformation permits greater packing into an adsorbed monolayer than might otherwise be expected based on packing hard spheres with rigid excluded volumes. It is of interest to explore this denaturation idea with blood proteins studied in this work, especially for a protein as large as IgM falling well outside the low-MW category that receives little attention in protein-adsorption studies [23]. A very unrealistic upper bound on protein denaturation would be complete loss of globular-protein structure whereupon the entire available adsorbent area is covered with a contiguous layer of “melted protein” with density equal to that of close-packed amino acids comprising the primary structure [20]. Accepting this purely-hypothetical upper-bound for the sake of continued discussion, a fully-denatured layer would have a density of 1.30 g/cm^3 ($1/\nu^o$ at $\nu^o = 0.77 \text{ cm}^3/\text{g}$; see further Appendix A; Ostuni used 1.34 g/cm^3 ; see Table 1 of ref. [52]). According to the computational method outlined in Appendix B, such a fully-denatured layer of IgM adsorbed to hydrophobic surfaces studied herein would be 8.5 nm thick – more than 60% of the diameter of an IgM molecule (adsorbent 1.6, column 10 of Table 3). Similarly, large proteins such as Fib and IgG would require a denatured layer more than half of a molecular diameter (experiments 1.4 and 1.5, column 10 of Table 3). The improbability of a fully-denatured protein layer of this kind requiring seemingly-impossible adsorbed thickness, coupled with the failure of simple sphere-packing models to predict quantitative experimental trends in adsorbent capacity, forces the conclusion that proteins do not adsorb to surfaces in the manner contemplated by the 2D planar model in any physically-realizable configuration.

4.4 Interphase Concentration as an Intensive Scaling Parameter

As discussed in Section 1, the alternative to the standard 2D model of adsorption premises that protein collects in a three-dimensional 3D interphase separating bulk-solution from the physical adsorbent surface (see refs. [10,17–20] and citations therein). According to this perspective, adsorbed proteins arrange within an interphase volume $V_I = A\Omega_I$, where A the adsorbent is surface area and Ω_I is the thickness dimension of a layer defined by protein dimensions in the adsorbed state. Adsorption into an interphase region can be further interpreted as a partitioning process [9,21] that distributes protein molecules between the bulk-solution phase and an interphase. This distribution is measured by a partition coefficient P equal to the ratio of interphase and bulk-solution concentrations W_I and W_B , respectively, such that $P \equiv (W_I/W_B)$. We find that P varies systematically with protein MW and ranges from about 500 for low-MW proteins such as lysozyme to 100 for high-MW proteins such as Fib [17] for adsorption to a hydrophobic surface. In other words, the interphase is 100–500X more concentrated than bulk solution at steady state. In consideration of such high interphase concentrations, it becomes apparent that interphase water is less concentrated than in bulk solution. Of course, this occurs because, when a hydrated protein enters the interphase, it must displace an equivalent volume of interphase water (referred to as interphase dehydration). We and others [61] find that displacement of interphase water by adsorbing peptides or proteins is a key energy step in the adsorption process.

We further contend that energetics of interphase dehydration establishes a maximal w/v (not molar) adsorbed-protein concentration $W_I^{\max}$ (mg/mL, maximum adsorbent capacity). $W_I^{\max}$ is proposed to be characteristic of adsorbent water-wetting properties and not identity of protein or proteins adsorbed within the interphase [17,18,25]. Dependence on adsorbent surface energy occurs because displacement of interphase water by adsorbing protein requires increasing energy with increasing adsorbent hydrophilicity. As a consequence, hydrophobic adsorbents exhibit higher capacity than hydrophilic adsorbents (exceptions include adsorbents with strong Lewis acid/base functionalities with authentic ion-exchange properties [20] and possibly hydrogels that can ABsorb proteins). Complete justification of this so-called ‘volumetric interpretation’ of protein adsorption is beyond the intended scope of this paper, so let it suffice

only to say that this theory seems to adequately explain both energy-and-mass balance of adsorption of a wide variety of blood proteins adsorbing to surfaces spanning the full range of observable surface energy [6,10,17–20,23–25,39,42–48].

4.4.1 Theoretical 3D Surface Capacity—Combining the interphase model with the idea that different proteins adsorb from surface-saturating solution concentrations to the same maximal interphase concentration $W_l^{\max}$ leads to a simple relationship among adsorbent surface area A , maximum interphase thickness $\Omega_l^{\max}$, and adsorbent capacity for the n^{th} surface $\Gamma_n^{\max} \equiv (n_l^{\max}/A)$ (moles/cm²) or $\Gamma_g^{\max} \equiv (m_l^{\max}/A)$ (grams/cm²) [25]:

$$W_l^{\max} = \left(\frac{n_l^{\max} MW}{A \Omega_l^{\max}} \right) = \Gamma_g^{\max} / \Omega_l^{\max} \quad (\text{grams/cm}^2) \quad (1)$$

where the ‘max’ superscript emphasizes the adsorbent-saturated condition and n_l is the number of moles adsorbed. Comparison of any two proteins i and j with $MW_i \neq MW_j$ adsorbed to identical adsorbents a surface saturation reveals that both interphase thickness ratio and adsorbed mass ratio varies directly with molecular-weight ratio:

$$\left(\Omega_{l_j}^{\max} / \Omega_{l_i}^{\max} \right) = \left(\Gamma_{n_j}^{\max} / \Gamma_{n_i}^{\max} \right) (MW_j / MW_i) = \left(\Gamma_{g_j}^{\max} / \Gamma_{g_i}^{\max} \right) = \left(\frac{(D_j)_n^{\max}}{(D_i)_n^{\max}} \right) \quad (2)$$

Thus, fixed $W_l^{\max}$ for a particular adsorbent implies that the interphase expands or contracts to accommodate mass (volume) of adsorbing protein. As a consequence, much more protein can adsorb to a given surface than anticipated based on the 2D monolayer paradigm discussed in Section 4.3 by occupying as many layers as necessary to achieve the $W_l^{\max}$ characteristic of the adsorbent.

4.4.2 Experimental Surface Capacity—Fig. 2 plots adsorbent capacity for OTS surface $n = 2$ for which HSA capacity is conveniently close to 1 mg/mL and capacity for all other proteins can be approximately read as a ratio to HSA directly from the graph. It is evident that capacity for Fib is 3.5X HSA, IgG 4X HSA, and IgM 6.5X HSA. Furthermore, according to eq. (2), capacity ratio is identical to the layer-thickness ratio $(\Omega_{l_j}^{\max} / \Omega_{l_i}^{\max})$. Hence, if HSA occupies a single adsorbed layer as is inferred from various studies (see refs. [17,25] and citations therein), then Fig. 2 suggests that surface saturation of a hydrophobic surface by IgG requires more than two complete layers, in agreement with quartz-crystal microbalance measurements [34]. Expectations that Fib and IgM require more than two layers corroborates our previous work using depletion [17,20], tensiometry [25], and interfacial rheology [23].

If interphase thickness is interpreted to be strictly proportional to spherical blood-protein dimensions, then $\Omega_l = 2\chi r_v$; where χ is an unknown proportionality constant related to protein packing density within the interphase [25]. It follows then that

$$(\Omega_{l_j}^{\max} / \Omega_{l_i}^{\max}) = (\chi_j / \chi_i) (MW_j / MW_i)^{1/3}. \quad \text{Thus, both } (\Omega_{l_j}^{\max} / \Omega_{l_i}^{\max}) \text{ and } (\Gamma_j^{\max} / \Gamma_i^{\max}) \text{ follow}$$

$(MW_j / MW_i)^{1/3}$ scaling if (χ_j / χ_i) is a constant across the molecular weight range. Thus it is apparent that, both 2D and 3D interpretations of protein adsorption predict that maximum adsorbent capacity $\Gamma^{\max}$ will scale as $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²). Smooth curves drawn through the data of Fig. 3B correspond to non-linear statistical fit of the data to power laws of the form $\Gamma^{\max} = a + b \cdot MW^{-2/3}$ or $\Gamma^{\max} = a + b \cdot MW^{1/3}$ for molar (moles/cm²) and mass (g/cm²) capacities, respectively; where a and b are arbitrary adjustable parameters. Data at

hand is insufficient to derive a quantitative relationship between $\Gamma^{\max}$ and MW and the physical meaning of adjustable parameters is unclear at this writing, but the fact that fitted curves reproduce general trends in the limited data at hand suggests (but does not prove) that adsorption capacity indeed follows $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²) scaling. Hence, we conclude that measured adsorbent capacities for a particular hydrophobic surface are generally consistent an adsorption model based on the packing of hydrated spheroids into an interphase volume at fixed $W_l^{\max}$. The following section argues that fixed $W_l^{\max}$ is the only feasible way that $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²) scaling can occur in a 3D model of adsorption.

4.3.3 Alternatives to a Characteristic Fixed Surface Capacity—Setting aside our surface-energy rationalization of a fixed $W_l^{\max}$ that is characteristic of adsorbent but not adsorbate for the moment, it is worthwhile to consider alternative options that might lead to $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²) scaling for $\Gamma^{\max}$ that is anticipated by both 2D and 3D paradigms. It is evident from Eq. (1) that MW scaling depends on the molecular-weight dependence of $\Omega_l^{\max}$. For example, $\Gamma^{\max}$ (g/cm²) will also scale as $MW^{1/3}$ if it is supposed that $\Omega_l^{\max}$ is a constant (rather than $W_l^{\max}$) and that $W_l^{\max}$ follows $MW^{1/3}$ (rather than $\Omega_l^{\max}$). This idea is impractical, however, because it demands formation of the thickest conceivable interphase regardless of the size of adsorbing protein -- $\Omega_l^{\max}$ cannot be smaller than the largest protein. Furthermore, the free energy of adsorption ($\Delta G_{ads}^o = -RT \ln P$) for large proteins would be necessarily larger than that of small proteins, which is opposite to experimental evidence [17]. Other options involve the proposition that $\Omega_l^{\max}$ scales with MW through a functional relationship of the form $\Im(MW)^x$; where $x > 0$ (constant $W_l^{\max}$ corresponds to $x = 1/3$). However, any $x > 1/3$ forces $W_l^{\max}$ into an inverse power law following $\Im(MW)^{1/3-x}$, implying sharply higher $\Gamma^{\max}$ for low-MW proteins and sharply lower $\Gamma^{\max}$ for high-MW proteins. This latter proposition can be rejected out-of-hand because it is clearly not supported by the data (Table 3 or Fig. 2) and is inconsistent with measured ΔG_{ads}^o [17]. We conclude, therefore, that $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²) scaling for $\Gamma^{\max}$ requires a maximal w/v adsorbent capacity $W_l^{\max}$ that is characteristic of adsorbent surface energy and independent of the chemical identity of the protein adsorbate. That is to say, the interphase model with imposed $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²) scaling requires a constant characteristic $W_l^{\max}$. Experimental verification that $\Gamma^{\max}$ indeed follows $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²) scaling for globular proteins will require adsorbent capacity measurements for a much broader range of proteins than investigated in this work, incrementally sampling proteins falling within the range $1000 > MW > 30$ adsorbing to different types of surfaces.

4.5 Adsorbent Capacity Scaling with Surface Energy

Fig. 4 shows that adsorbent capacity decreases with increasing adsorbent hydrophilicity to within detection limits (see LOD annotation) near $\tau_n^o = 30$ dyne/cm for all proteins studied. This observation is completely consistent with adsorption energetics measured using tensiometry [39,46–48] and trends in protein-surface adhesion determined by AFM [62,63]. Interestingly, the rate-of-change of adsorption capacity with adsorbent wettability was sharper for larger proteins than smaller proteins. In particular, IgM adsorbent capacity falls so rapidly with adsorbent hydrophilicity that obtaining isotherms at different adsorbent surface energies is experimentally problematic (see Fig. 1). Our interpretation is that the multilayer structure required to support $W_l^{\max}$ for large proteins at hydrophobic surfaces (estimated to be 300 mg/mL [17]) decays sharply with adsorbent hydrophilicity [17].

5.0 Conclusions

Experimentally-measured silanized-glass-particle adsorbent capacities for human serum albumin, IgG, fibrinogen, and IgM cannot be reconciled with a theory premised on the idea that proteins adsorb in a pseudo-2D monolayer on a planar surface for any physically-realizable protein configuration or state of denaturation. An alternative interpretation accounts for measured excess capacity over monolayer coverage by asserting that protein partitions from solution into a 3D interfacial volume (a.k.a. interphase) in one-or-more layers depending on protein size. Both 2D (monolayer) and 3D (volumetric) theories predict adsorption capacity will follow $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²), but only if 3D theory additionally assumes that adsorbent capacity is a constant for a particular adsorbent surface energy and is independent of adsorbing-protein MW, as we have proposed previously [17–20,25]. Experimental adsorbent-capacity measurements are consistent with, but not quantitatively diagnostic of, $MW^{1/3}$ (g/cm²) or $MW^{-2/3}$ (moles/cm²) scaling. Adsorbent capacity decreases monotonically with adsorbent hydrophilicity, falling precipitously for large proteins such as Fib and IgM. Adsorbent capacity decreases to limits of detection near a water contact angle of 65°, corroborating independent measurement of interfacial energetics by time-and-concentration-dependent tensiometry [39,46–48]. Displacement of interphase water by adsorbing protein is an energy-limiting step that controls adsorbent capacity.

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Appendices

Appendix A: Arithmetic of the Spheres

Molecular volume of a hypothetical protein sphere $V_p = 4/3\pi r_v^3$ where r_v is molecular radius in cm/molecule. Consequently, molar volume $\bar{V}_p = 4/3\pi r_v^3 N_A$ in $\text{cm}^3/\text{molecule}$ if N_A is the Avogadro number. The specific volume $v^o = [\bar{V}_p / (MW \cdot 10^3)]$ in cm^3/g , where MW is molecular weight in kDa. Using the relationship $r_v = 6.72 \times 10^{-8} MW^{1/3}$ (see Section 4.3.1), it is apparent

$$\text{that } v^o = 4/3\pi N_A \left[\frac{((6.72 \times 10^{-8} MW^{1/3})^3)}{10^3 MW} \right] = \left(\frac{4\pi(6.72 \times 10^{-8})^3}{3 \times 10^3} \right) (6.02 \times 10^{23}) = 0.77 \text{ cm}^3/\text{g}.$$

Appendix B: Thickness of A Hypothetical, Fully Denatured Protein Layer

A contiguous slab of protein adsorbed to a surface with area A at a density of $1/v^o \text{ g/cm}^3$ would require a thickness $t = (mv^o/A)$, where m is the total mass of adsorbed protein. The total adsorbed mass measured by the depletion D (mg/mL bulk solution) is given by $m = DV_B$, leading to the conclusion that $t = (DV_B v^o/A)$. Using $v^o = 0.77 \text{ cm}^3/\text{g}$ (Appendix A) and $D \sim 7 \text{ mg/mL}$ for IgM adsorbing to a hydrophobic surface (Fig. 1 and Table 3) with $A = 190 \text{ cm}^2$ implies that:

$$t = \left[\frac{(7 \times 10^{-3} \text{ g})(30 \times 10^{-3} \text{ mL})(0.77 \text{ g} \cdot \text{cm}^{-3})}{190 \text{ cm}^2} \right] = 8.51 \times 10^{-7} \text{ cm} = 8.51 \text{ nm} \quad (3)$$

This value is to be compared to the diameter of an IgM molecule of $2r_v = 2 (6.72 \times 10^{-8} MW^{1/3} = 2 (6.72 \times 10^{-8})(1000)^{1/3} = 13.5 \text{ nm}$.

Figure 1.

Three adsorption isotherms for IgM obtained by the depletion method implemented with anodrop spectroscopy plotting solution depletion $(D_{IgM})_n$ against solution concentration $(W_{B_{IgM}}^o)$. Surfaces with increasing hydrophilicity are identified by a numeric code n keyed to Table 3 and Table 2. Maximum adsorbent capacities $(D_{IgM})_n^{\max}$ (dotted horizontal-line annotations) occurring at $W_{B_{IgM}}^o = (W_{B_{IgM}}^o)_n^{\max}$ (dashed vertical-line annotations) are estimated from the plateau of the adsorption isotherm. Estimated limit of detection is indicated by a double-headed arrow labeled LOD at the lower-right hand corner. Notice that the amount of IgM adsorbed from solution decreases with increasing adsorbent hydrophilicity (curved-arrow annotation).

Figure 2.

Maximum adsorbent capacity $(D_i)_{n=2}^{max}$ for surface $n = 2$ of Table 2 corresponding to the i proteins listed in Table 1 obtained by the depletion method implemented with electrophoresis (for HSA only) or nanodrop spectroscopy, plotting solution depletion $(D_i)_{n=2}^{max}$ against protein molecular weight MW. Adsorbent capacity increases with increasing MW. The guideline connecting data points of Fig. 2 speculates that adsorbed proteins occupy a succession of adsorbed layers inferred from interfacial energetics of adsorption, giving rise to a stair-case-like rise in adsorbent capacity (see further Section 4.2).

Figure 3.

Theoretical (Panel A) and experimental (Panel B) scaling of adsorbent capacity expressed as $\Gamma^{\max}$ in either moles/cm² (left-hand ordinate) or grams/cm² (right-hand ordinate) as indicated by arrow annotations. Theory corresponds to expectations based on a jammed monolayer of proteins. Shaded box corresponds to relatively low-molecular-weight proteins for which molar capacity decreases sharply with molecular weight. Experimental adsorbent capacities for the *i* proteins listed in Table 1 (open circles = moles/cm², closed circles = grams/cm²) adsorbing to Nyebare-treated particles (*n* = 1, Tables 2 and 3). Panel A and B axes scaling are identical. Note that experiment and theory are in close agreement for low-molecular-weight proteins but deviation increases with increasing molecular weight.

Figure 4.

Maximum adsorbent capacities $(D_i)_n^{\max}$ for n surfaces listed in Tables 2 and 3 sampling the observable range of surface energy. Adsorbent surface energy is measured as water adhesion tension $\tau^o \equiv \gamma_{lv}^o \cos \theta$, where γ_{lv}^o is the interfacial tension of pure-buffer solution, and θ is the buffer advancing contact angle. Adsorption capacities were estimated from adsorption isotherms for each protein surface combination (closed circles = HSA, closed squares = IgG, closed and open triangles = Fib, stars = IgM, see Table 1). Electrophoretic and spectroscopic depletion measurements for Fib are compared in Fig. 4 (open and closed triangles, respectively) and were found to be statistically identical. Lines through the data are guides to the eye indicating decreasing adsorbent capacity with increasing adsorbent hydrophilicity. Note that adsorbent capacity decreases precipitously near $\tau^o = 30$ dyne/cm (dashed vertical line) to limits of detection indicated by a double-headed arrow labeled LOD at the lower-right hand corner. Inset expands adsorption measurements corresponding to fully-water-wettable surfaces near $\tau^o = 72$ dyne/cm.

Table 1**Proteins and Adsorbent Mass Used in Measurement of Adsorption Isotherms by Solution Depletion**

Protein (identifying number)	Molecular Weight (kDa)	Vendor	Form (purity)	Adsorbent mass, mg (surface area, cm²)
Human Serum Albumin (HSA) (<i>i</i> = 1)	66.3	Sigma Aldrich	Powder (96–99%)	50 (190 cm ²)
Fibrinogen (Fib) (<i>i</i> = 2)	341	Sigma Aldrich	Powder (80% clottable)	30 (114 cm ²)
Immunoglobulin G (IgG) (<i>i</i> = 3)	160	Sigma Aldrich	Powder >95%	30 (114 cm ²)
Immunoglobulin M (IgM) (<i>i</i> = 4)	970–1000	Scipac (UK)	Solution 2.2 mg/mL >96%	4 (15.2 cm ²)

Notes: Adsorbent surface area based on $0.38 \pm 0.09 \text{ m}^2/\text{g}$ by BET. Total adsorbent mass used was optimized for each protein and listed value for a particular protein was used for each of adsorbent listed in Table 2.

Table 2

Glass Particle Adsorbents

Adsorbent Treatment	Number Designation (n)	Advancing Contact Angle Range (degrees)
Nyebar	1	110 – 115
OTS	2	100–110
APTES	3	50–67
PTES	4	70–80
Chromerge	5	40–102
Clean Glass	6	0

Notes: Nyebar = 1,1- pentadecafluorooctylmethacrylate deposited on OTS-treated particles. OTS = octadecyltrichlorosilane. PTES = n-propyltriethoxysilane. Chromerge = timed oxidation of OTS-treated particles with H₂SO₄/Cr₂O₃ solutions. Clean glass = as received glass particles oxidized in piranha solution (30% H₂O₂ in concentrated H₂SO₄). See Section 2 for details. Contact angle range refers to maximum and minimum advancing contact angle range observed on a glass cover slip carried through surface-treatment procedures with glass particles. See also Table 3 for adsorbent-specific wettabilities.

Table 3

Experimental and Theoretical Adsorbent Capacities

Protein	Adsorbent (Type,trial)	τ_n^o (dyne/cm)	$(D_i)_{\text{max}}$ (mg/mL) (N)	$\left(\frac{W_B^o}{M_H}\right)_{\text{max}}$ (mg/mL)	$(m_i)_{\text{max}} \times 10^{-3}$ (mg)	$\Gamma_{\text{max}} \times 10^{-3}$ (mg/cm ²)	Jammed Capacity proteins/cm ² (moles/cm ²)	HCP Capacity proteins/cm ² (moles/cm ²)	Denatured Layer Thickness Nm (# protein diameters)
HSA	1.1	-30.4	1.61 ± 0.33 (12)	7.16 ± 1.51	48.3 ± 9.9	0.25 ± 0.05	23.6 × 10 ¹¹ (2.6 × 10 ⁻¹⁰)	39.1 × 10 ¹¹ (4.3 × 10 ⁻¹⁰)	1.96 (0.4)
	1.2	-30.4	1.32 ± 0.10 (8)	4.50 ± 0.42	39.6 ± 3.0	0.21 ± 0.02			1.60 (0.3)
	1.3	-28.3	1.33 ± 0.06 (19)	3.88 ± 0.29	39.9 ± 1.8	0.21 ± 0.01			1.61 (0.3)
	2.1	-20.3	1.11 ± 0.02 (8)	4.60 ± 0.26	33.3 ± 0.6	0.18 ± 0.003			1.35 (0.25)
	2.2	-19.2	0.91 ± 0.12 (8)	4.65 ± 0.64	27.3 ± 3.6	0.14 ± 0.02			1.11 (0.2)
	3.1	29.0	0.56 ± 0.11 (8)	5.31 ± 1.19	16.8 ± 3.3	0.10 ± 0.02			0.68 (0.12)
	3.2	31.0	0.2 (8)		6.0	0.03			-
	5.1	-15.2	0.98 ± 0.02 (8)	4.87 ± 1.37	29.4 ± 0.6	0.15 ± 0.003			1.19 (0.22)
	5.2	42.5	0.3 (8)		9.0	0.05			-
	5.3	54.5	0.2 (17)		6.0	0.03			-
IgG	5.4	72.0	0.2 (7)		6.0	0.03	13.1 × 10 ¹¹ (3.5 × 10 ⁻¹⁰)	21.7 × 10 ¹¹ (5.8 × 10 ⁻¹⁰)	-
	1.4	-28.4	3.66 ± 0.05 (14)	12.22 ± 0.78	109.8 ± 1.5	0.58 ± 0.01			4.45 (0.6)
	2.3	-20.3	3.40 ± 0.06 (14)	10.85 ± 0.67	102.0 ± 1.8	0.54 ± 0.01			4.13 (0.56)
	4.1	-0.2	2.73 ± 0.07 (13)	10.71 ± 0.11	82.9 ± 2.1	0.44 ± 0.01			3.32 (0.45)
	3.3	44.5	0.3 (13)		9.0	0.05			-
	6.1	72.0	0.3 (9)		7.8	0.04			-
	1.5	-34.9	5.51 ± 0.20 (12)	7.55 ± 0.58	165.3 ± 6.0	0.87 ± 0.03			6.70 (0.7)
	2.4	-24.6	4.03 ± 0.22 (10)	6.32 ± 0.61	120.9 ± 6.6	0.64 ± 0.04			4.90 (0.52)
	3.4	23.4	3.03 ± 0.18 (8)	6.06 ± 0.59	90.9 ± 5.4	0.48 ± 0.03			3.68 (0.4)
	3.5	44.1	0.6 (12)		18.9	0.10			-
Fib	5.6	7.5	3.40 ± 0.14 (12)		102.0 ± 4.2	0.54 ± 0.02	7.9 × 10 ¹¹ (4.5 × 10 ⁻¹⁰)	13.1 × 10 ¹¹ (7.4 × 10 ⁻¹⁰)	4.13 (0.44)
	5.7	52.0	0.6 (9)		17.4	0.10			-
	4.2	-2.9	2.90 ± 0.05 (10)	3.4 ± 0.13	87.0 ± 1.5	0.46 ± 0.01			3.52 (0.37)
	6.2	72.0	0.3 (8)		9.0	0.05			-
	1.6	-22.5	7.00 ± 0.04 (10)	1.95 ± 0.13	210.0 ± 1.2	1.11 ± 0.01			8.51 (0.63)
	2.5	-18.1	6.38 ± 0.03 (10)	1.7 ± 0.08	191.4 ± 0.9	1.01 ± 0.003			7.75 (0.57)
IgM	4.3	-5.3	3.00 ± 0.01 (10)	1.48 ± 0.02	90.0 ± 0.3	0.47 ± 0.002	3.8 × 10 ¹¹ (6.4 × 10 ⁻¹⁰)	6.4 × 10 ¹¹ (1.1 × 10 ⁻¹¹)	3.65 (0.27)
	6.3	72.0	1.6 (8)		48.0	0.252632			1.94 (0.14)

Notes: Type:trial designation refers to adsorbent type keyed in Table 2 and experimental trial with the particular adsorbent/protein pair. Water adhesion tension $\tau_n^o = \gamma_v^o \cos \theta$ is calculated from measured advancing contact angles θ of buffer on glass-coverage witness samples. Estimated in θ is $\pm 1^\circ$. $(D_i)_{\text{max}}$ and error estimated from fitting described in Section 2. Estimated adsorbed quantities below LOD are listed without error. Adsorbent surface area = $0.38 \pm 0.09 \text{ m}^2/\text{g}$ by BET.