Mathematics in Medicine Study Group Problem:

Designer Materials to Control Competitive Protein Binding

Paul Roach, Institute for Science and Technology in Medicine, Keele University

Email: p.roach@keele.ac.uk; Tel +44(0)1782-555-226

Background

Broadly speaking *biological activity* at a material's interface is dictated by the ability of the surface to support protein adhesion. Specific motifs presented by various protein molecules are known to drive cell attachment and regulate function, such as the well studied arginine-glycine-aspartic acid (RGD) tri-peptide which interacts in a lock and key event with cell membrane receptors to allow cell attachment to surfaces.[1] Varying classes of activity are of key importance in a vast range of situations, from anti-fouling properties required in marine technology manufacture, solar cell technology and implantable or point-of-care biotechnology, to the other extreme where bio-fouling is required for cell attachment and tissue build-up – one definition of biocompatibility.

With this in mind there has been a huge amount of research on the design of surface modifications to provoke desired biological (cellular) responses, although much of this work has focussed either on single protein-surface interactions[2] or on cell response to surfaces conditioned using normal serum containing media which comprises hundreds of proteinatious species in competition to populate the surface.[3] One of the directions which we are taking is to examine the more complex biological environment, trying to unpick how cells might perceive and act to mediate their environment by secreting required conditioning proteins, and how a multi-protein system organises itself to form a mixed protein population at a surface. Further, we aim to clarify important aspects dictating the composition of this layer towards being able to design next generation materials that can control specific biological interactions.

The composition of a protein layer adsorbing from a mixed populations of proteins is dependent upon the concentration of each species and the affinity between each adsorbate and the surface.[4] Protein adsorption occurs through a dynamic equilibrium, with more abundant proteins initially populating the surface but being replaced by lesser abundant but higher affinity proteins.[5] This interaction can be altered by changing the characteristics presented at the surface, Figure 1.

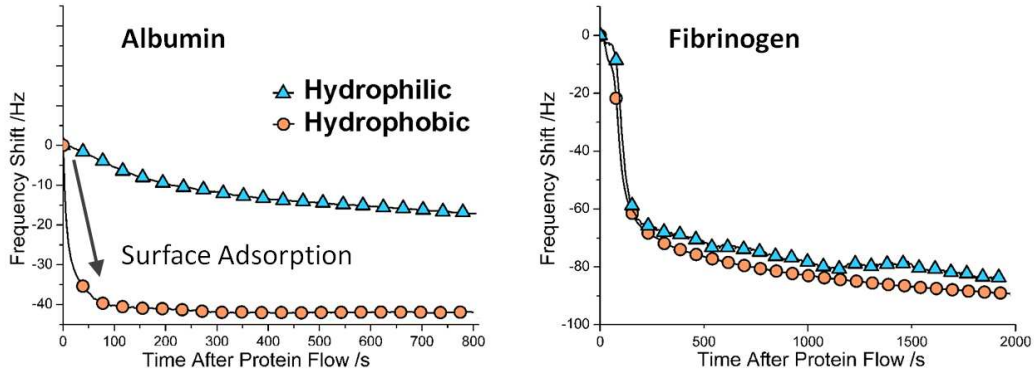


Figure 1: Adsorption of albumin and fibrinogen onto flat hydrophilic and hydrophobic surfaces, measured using frequency dependant sensors. Negative frequency indicates adsorption.

Surface chemistry is well known to sway protein adsorption characteristics dependant largely upon the hydrophobic and charge compensating interaction between an adsorbing molecule and the surface,[2] whilst surface curvature on the same length-scale as a protein molecule has been shown to alter the structure of the molecule upon adsorption,[6] Figure 2, and therefore alter the interaction affinity.

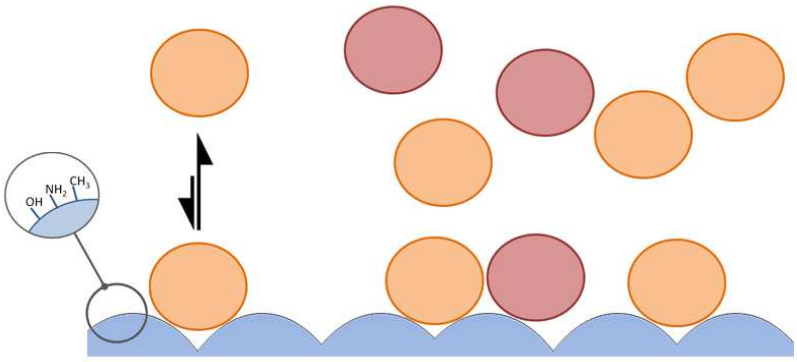
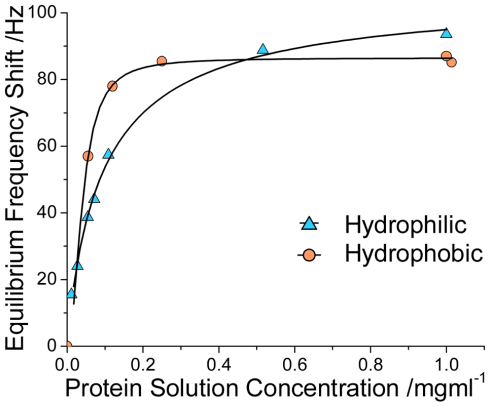


Figure 2: Model adsorbing protein layer with composition defined by molecular affinity, dependent upon presented surface chemistry and nano-scale features.

Mathematical Modelling

A basic molecular adsorption model, discounting molecular conformation and/or orientation (and changes upon adsorption), would be of adherent spheres interacting with a flat surface. Each sphere would be the model of a protein molecule having an affinity towards any given surface which can be derived experimentally, Figure 3.



	Affinity Constant	Saturation Values /Hz
Fg **OH**	10.9	102.0
Fg **CH_3**	36.0	92.6
BSA **OH**	5.3	47.2
BSA **CH_3**	5.4	40.9

Figure 3. Langmuir adsorption isotherm of fibrinogen alongside tabulated data.

Taking the problem at two levels, it would be interesting to model the adsorbing protein layer at the **macro-scale** and also the interaction of a single protein molecule at the **nano-scale**.

Macro-Scale

It is our hope that such a model can be used to highlight how surface and protein solution properties change the composition of a protein layer with respect to time, e.g. from our experimental data wherein the affinity constants describing specific protein-surface interaction (derived from Langmuir isotherms of equilibrium binding) may be used to build up a more complex model of multiple proteins competing for surface sites. A model of protein adsorption incorporating affinity constants, bulk protein concentrations and diffusivity constants will allow us to estimate the effects of these parameters on the compositional changes of an adsorbed protein layer with respect to time.

Nano-Scale

This is a more complex element to the problem, serving to give us more information regarding the probable interaction mechanisms occurring at the single molecule level. By modelling a protein molecule as a single deformable spheroid which deforms differently dependent upon its interaction strength with a surface (and/or the nano-scale curvature presented by the surface), an estimation of molecular deformity may be established. Models may also incorporate a level of protein surface characteristics such as charge and/or hydrophobic domain distribution such that orientational modelling of the adsorbing molecule may also be evaluated.

By using this model we may predict how designer surfaces presenting nano-scale features of varying chemical functionality can be used to steer protein layer composition such that they are cell non-/adherent. Such proof of principle data may be used in support of future grant applications which may involve more specific control over cellular mechanisms.

---

[1] Johansson, S., Svineng, G., Wennerberg, K., Armulik, A., Lohikangas, L., *Front. Biosci*. **1997**, 2, 126-146

[2] Roach, P., Farrar, D., Perry, C.C., *J. Am. Chem. Soc*., **2005**, 127, 8168-8173

[3] Wang, X., Gan, H., Zhang, M.X., Sun, T.L., *Langmuir*, **2012**, 28, 5, 2791-2798

[4] Vroman, L., Adams, A.L., *J. Biomed. Mater. Res.*, **1969**, 3, 43-67

[5] Kasemo, B. *Adv. Dental Res*., **1999**, 13, 1-20

[6] Roach, P., Farrar, D., Perry, C.C., *J. Am. Chem. Soc*., **2006**, 128, 3939-3945