Thermodynamic Perspectives on the Design of Protein Adsorption Resistant Surfaces

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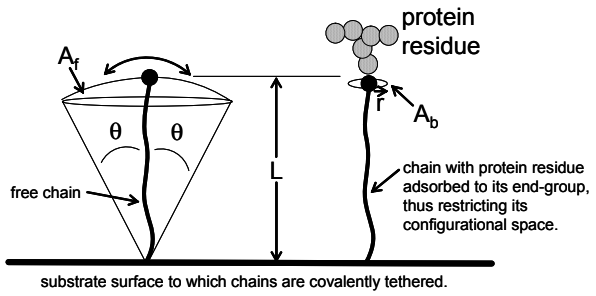
Introduction: The design of protein adsorption resistant surfaces is of great interest for blood-contacting biomaterials applications as a potential means of preventing platelet adhesion, activation, and subsequent thrombus formation. Numerous studies have been conducted to investigate how surface-tethered chains (STCs), such as oligo-ethylene glycol (OEG, $[-(CH_2)_2\text{-}O-]_n$), can be designed to provide protein adsorption resistance. These studies, however, have focused solely on the interaction of water with STCs while neglecting interactions with the protein. The objective of this study was therefore to use statistical mechanics and molecular modeling to provide a thermodynamic basis for protein adsorption-resistance that includes the protein. It is hypothesized that protein adsorption resistance can be provided by combining chain length with the presence of functional groups that are designed such to inhibit hydrogen bonding (H-bonding) with protein residues while being readily accessible for H-bonding with water.

Methods: In order for a surface to be highly resistant to protein adsorption, the change in system free energy for protein adsorption should be greater than zero (i.e., $\Delta G_{ads} = \Delta H_{ads} - T\Delta S_{ads} \geq 0$). The change in entropy (ΔS_{ads}) when a protein adsorbs to an STC was approximated using simple statistical mechanics relationships in the microcanonical ensemble by considering the change in the configurational space of the end-group of an STC when it is free (A_f) vs. when a protein adsorbs to it (A_b), as depicted in Fig. 1.



$$A_f = 2\pi L^2(1-\cos\theta),\quad A_b = \pi r^2;\quad \Delta S = R\ln\left(\frac{A_f}{A_b}\right) = h + 2R\ln L;$$

$$\text{with } h = R\{\ln[2(1-\cos\theta)] - 2\ln(r)\}\ ;\quad (\text{R} = \text{ideal gas constant})$$

Fig. 1 Calculation of ΔS_{ads} for protein adsorption to an STC

The change in enthalpy (ΔH_{ads}) when a protein adsorbs to an STC compared with water was investigated by semi-empirical quantum chemical calculations using MOPAC 2002 (v.2.5.0, BiomedCAChe software, Fujitsu, Inc., Beaverton, OR) with PM3 parameterization combined with COSMO to represent an aqueous solution environment. Molecular models (Fig. 2) were constructed to represent two types of STC structures known to exhibit high protein adsorption resistance[1]: A methoxy-capped OEG (MEG) and a dimethylamine-capped OEG (DMA). Each STC was represented as a set of 3 STCs in a close-packed array with the base CH_3 group of each chain locked in position while the remaining atoms were free to move. Two cases were then considered representing either water or a serine residue (methanol side-chain) hydrogen bonded to one of the STCs. After positioning the water or serine residue, the molecular geometry of each system was adjusted to find its minimum energy configuration using the eigenvalue-following method. The solute was then removed from the energy-minimized structure and a self-consistent field calculation was performed (i.e., energy of the system with all atoms fixed in position) to calculate the heat of formation (HoF) of the STCs themselves without the presence of the solute. Calculations were conducted for ten different H-bonded configureations of each solute and the mean and stnd. dev. for the HoF of the STCs were determined and compared by Student's t-test. This difference represents the increase in strain energy of the STCs (enthalpic effect) required for a protein to H-bond with the STC compared to water.

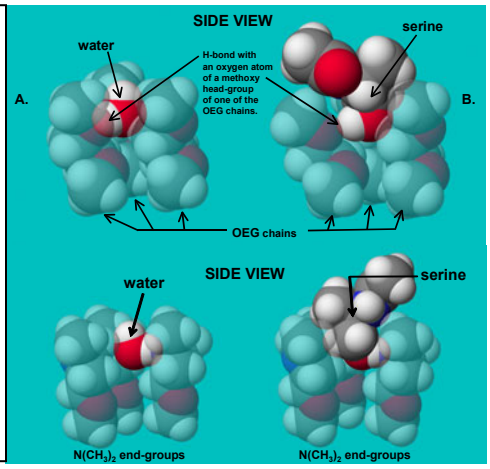


Fig. 2. Water (A) and serine (B) H-bonded to an ether O atom of an MEG chain(top) and an N atom of a DMA chain (bottom)

Results & Discussion: As shown in Fig. 1, the entropic penalty that results when a protein residue forms a stable bond with an STC logarithmically increases with chain length; this is in agreement with findings that OEG chains must have $n \geq 3$ to be resistant to protein adsorption.[1] The results from the calculations of the STC strain energy when H-bonded to water vs. a serine residue are shown in Table 1. As indicated, the H-bonding of serine results in a significant increase in strain energy in the STCs, which provides an entalpic penalty for protein adsorption.

Table 1	MEG (kcal/mol)		DMA (kcal/mol)	
(N=10)	Water	Serine	Water	Serine
Mean	3.87	4.52	0.28	1.27
Std.Dev.	0.27	0.27	0.08	0.37
t value	5.38 ($p < 0.01$)		8.27 ($p < 0.01$)	

Conclusions: Protein adsorption resistance is provided by an increase in STC length and functional-group design strategies that limit a protein's access to H-bond with the STC compared to water. These two effects combine to provide an overall increase in ΔG for protein adsorption.

Ref 1: Ostuni et al., Langmuir, 17: 5605-5620 (2001).