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### Aim of the Project

To prepare and benchmark five surfaces for their resistance to protein adhesion: two tertiary amines, two amine *N*-oxides<sup>1</sup> and a triethyleneglycol benchmark. Quartz Crystal Microbalance (QCM) experimentation and water droplet contact angles are then used to characterise these thiol-gold surfaces for resistance to protein adhesion.

### The Need for Bio-inert Surfaces

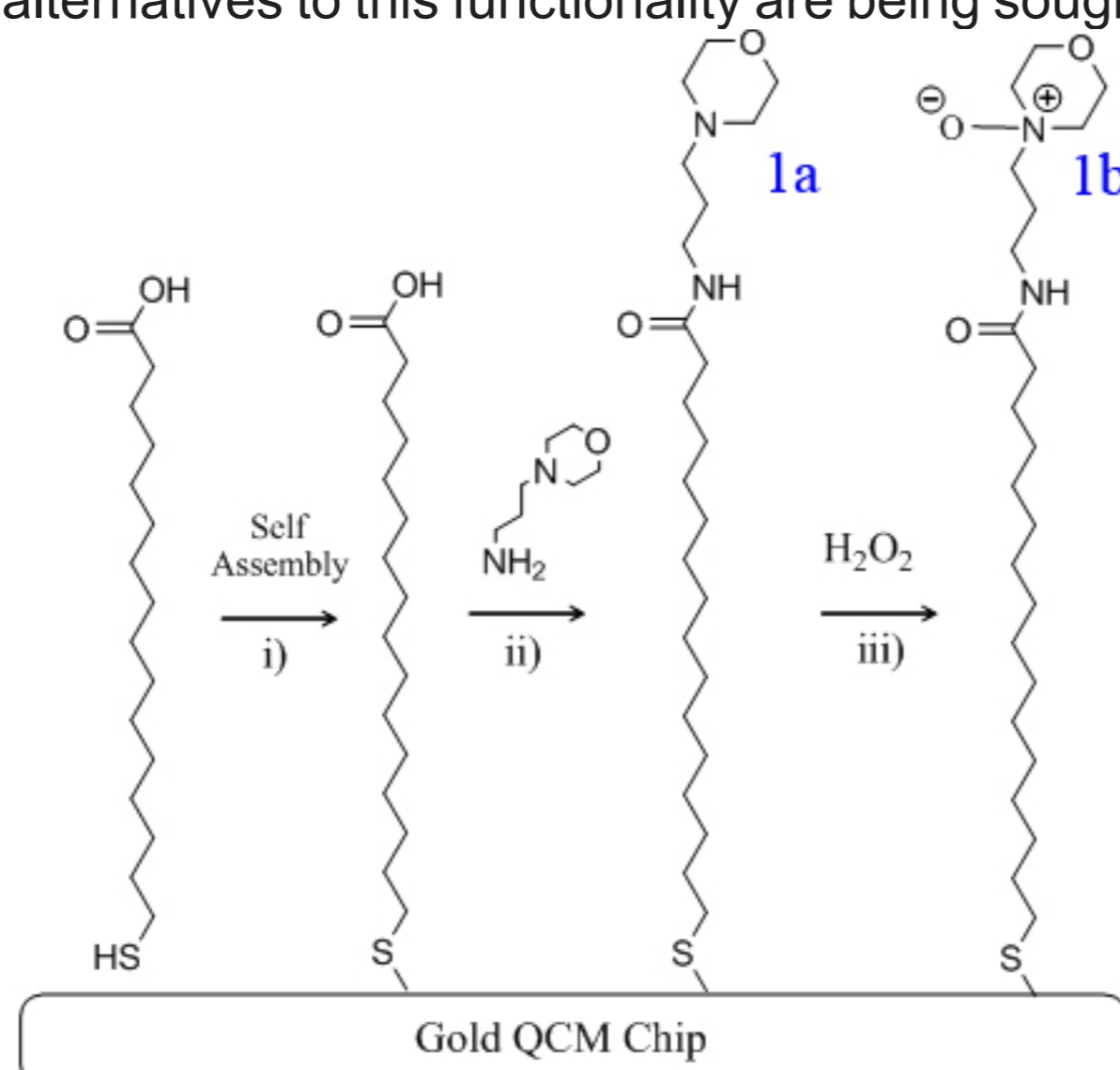
Protein absorption is the method by which cell adhesion occurs on an *in vivo* device, this process of repeated and undesirable cell adhesion is known as biofouling. The ability to prevent biofouling would be advantageous as the process can cause the activation of the host auto-immune response leading to inflammation.<sup>2</sup> This response has been seen on vascular implants, ultimately leading to the failure of the device. Ethyleneglycol bio-inert surfaces have applications in biosensors, contact lenses and drug delivery,<sup>3</sup> alternatives to this functionality are being sought by manufacturers.

### Surfaces Synthesised

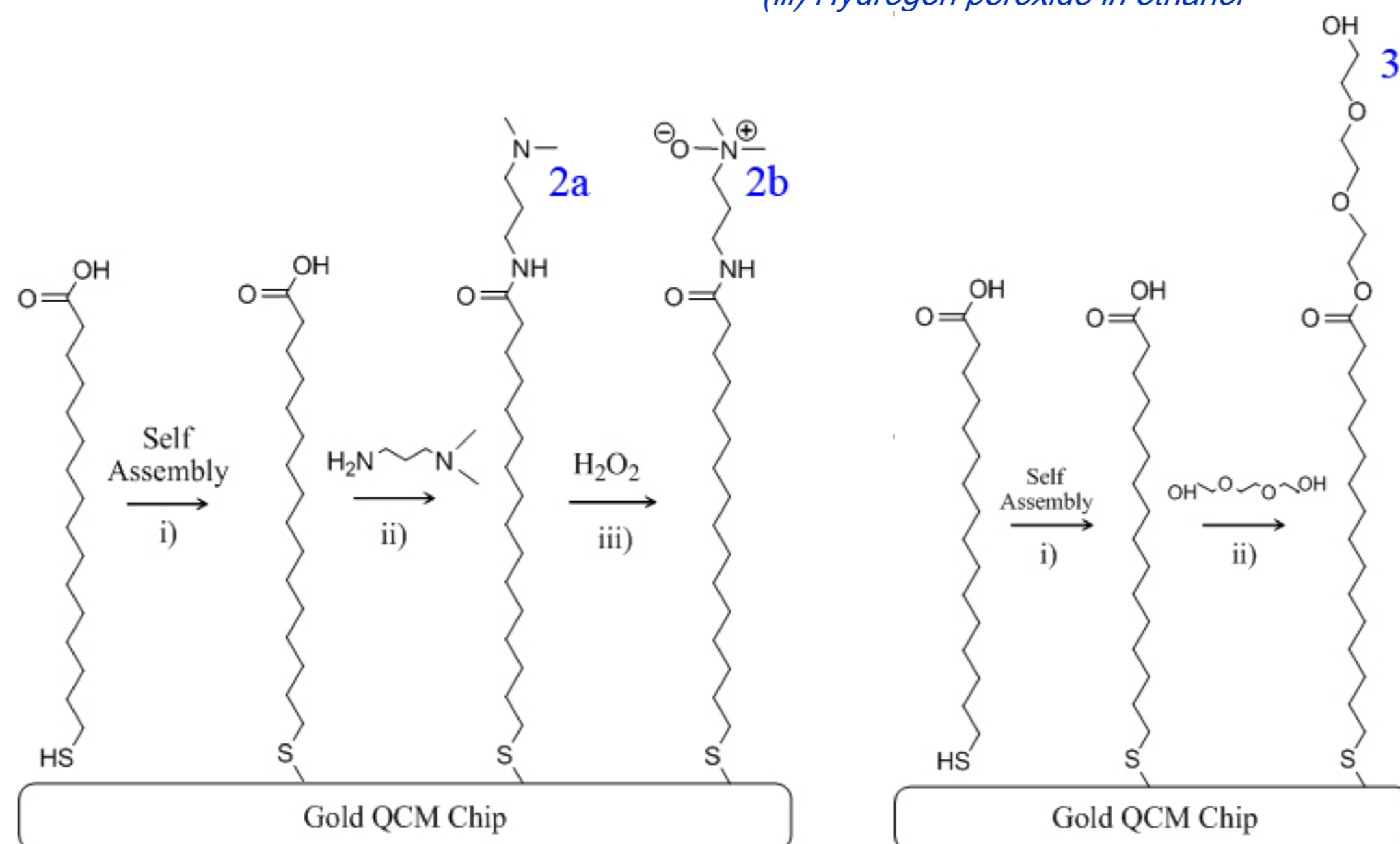
The surfaces in this work are supported on gold QCM chips.

The use of gold chips is desirable because it allows long chain thiol molecules to covalently bond to the chip by chemisorption, to produce a self assembled monolayer (SAM). The  $\omega$ -carboxylic acid is then available to couple to the amine or ethylene glycol reagent.

The tertiary amine is finally oxidised to the amine *N*-oxide by hydrogen peroxide.



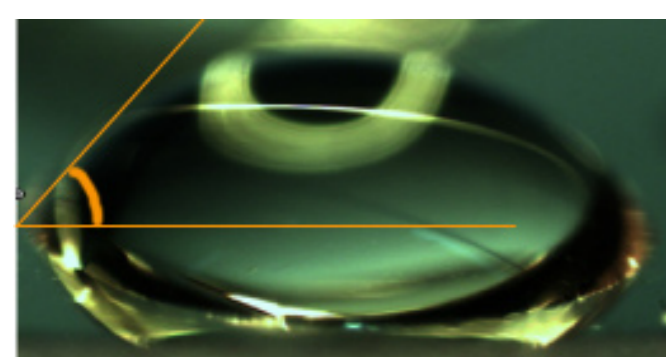
6-mercaptohexadecanoic (3-morpholin-4-yl-propyl) amide SAM **1a** and corresponding *N*-Oxide **1b**  
Reagents: (ii) Pybop in DMF, *N*-methylmorpholine.  
(iii) Hydrogen peroxide in ethanol



16-mercaptohexadecanoic (3-dimethylamino-1-propyl) amide SAM **2a** and corresponding *N*-Oxide **2b**  
Reagents: (ii) Pybop in DMF, *N*-methylmorpholine.  
(iii) Hydrogen peroxide in ethanol

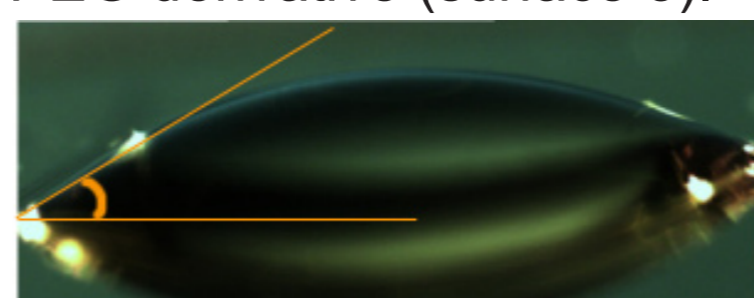
Triethyleneglycol surface **3**  
Reagents: (ii) EDC, DMAP in PBS

### Characterisation by Contact Angle



Contact angle for surface **1a**

The opposite is true for a hydrophilic surface; the water will be attracted and form a more disperse droplet with a lower contact angle. This is the case for the *N*-oxides.



Contact angle for surface **2b**

### Characterisation by Quartz Crystal Microbalance

The QCM equipment is used to investigate the extent of protein binding to the surfaces. This method allows the detection of tiny changes in mass. A quartz crystal is present at the centre of the gold chip, oscillating at its resonant frequency when a potential is applied. A decrease in resonance indicates an increase in mass, such as protein adsorption.

The protein adhesion was tested with fibrinogen and lysozyme on each surface.

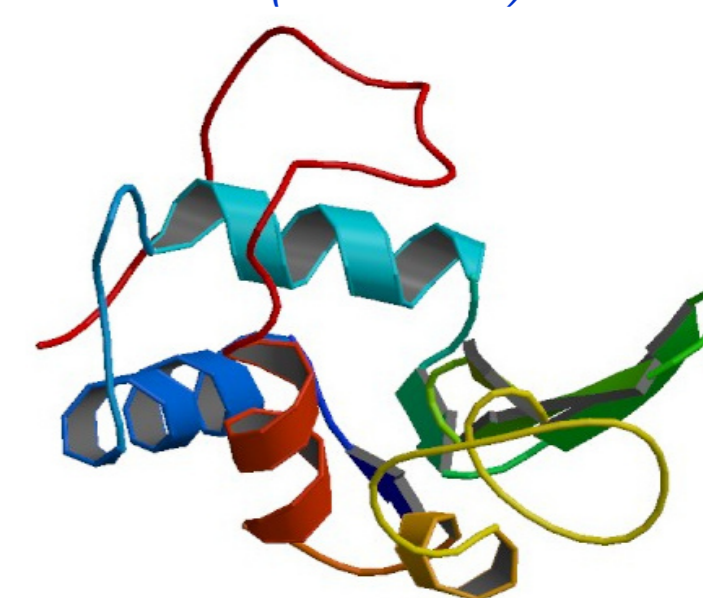
Fibrinogen is the inactive form of fibrin. It is a water soluble protein found in high concentrations in the blood and involved in blood clotting. There are abundant hydrophobic amino acid residues on the protein's surface so it adsorbs significantly onto many surfaces.

Lysozyme is a small enzyme that attacks the protective cell walls of bacteria causing them to burst. It is found in blood, mucus and tears. There are few hydrophobic amino acid residues on the surface so it adsorbs less than fibrinogen and is therefore much easier to remove from the surfaces.

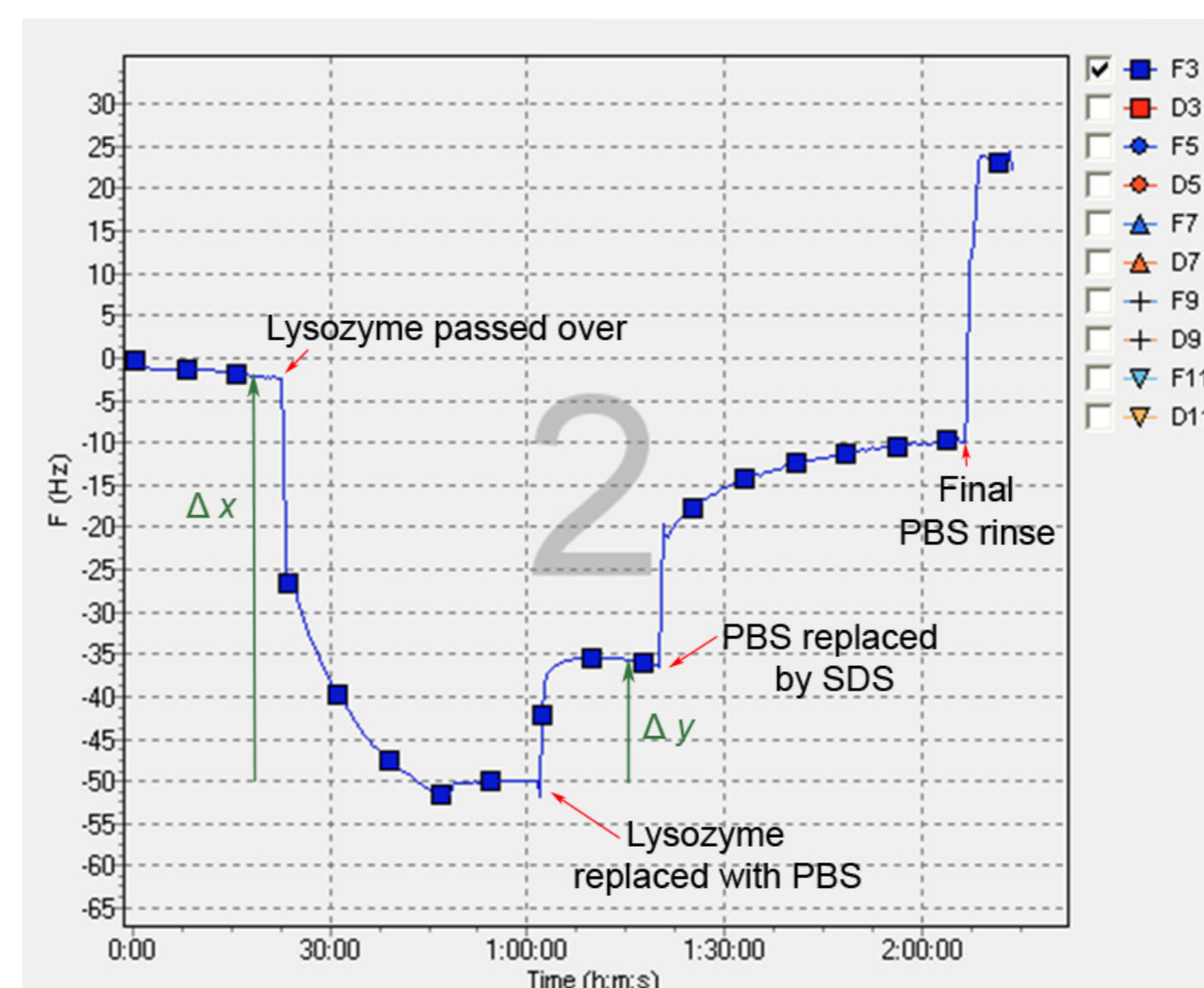
The protein resistance is quantified as the percentage removal efficiency by PBS. This is calculated as  $\Delta y / \Delta x$  multiplied by 100 to get a percentage. A smaller value indicates a less successful removal and thus a less bio-inert surface.



Fibrinogen crystal structure (PDB: 3E11)



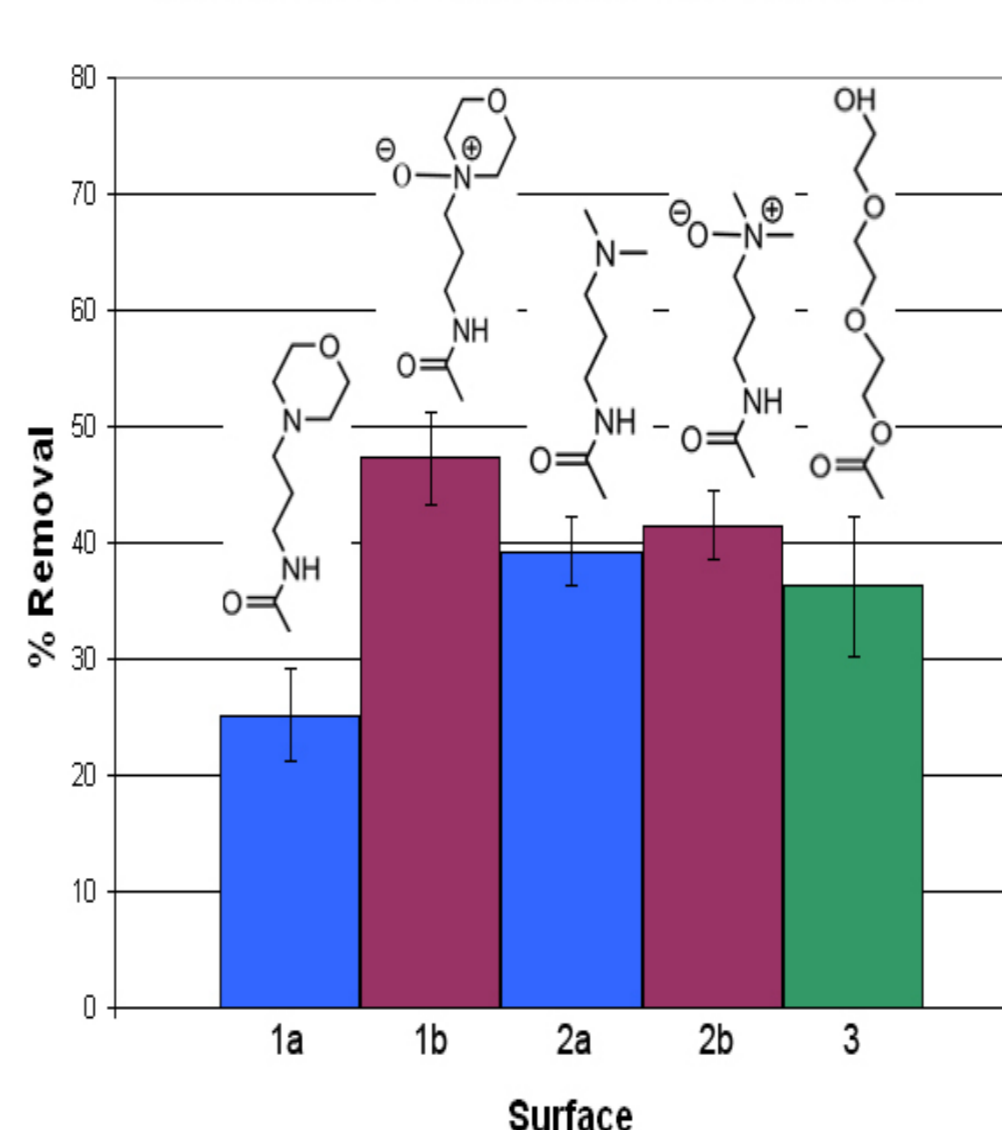
Lysozyme crystal structure (PDB: 6LYZ)



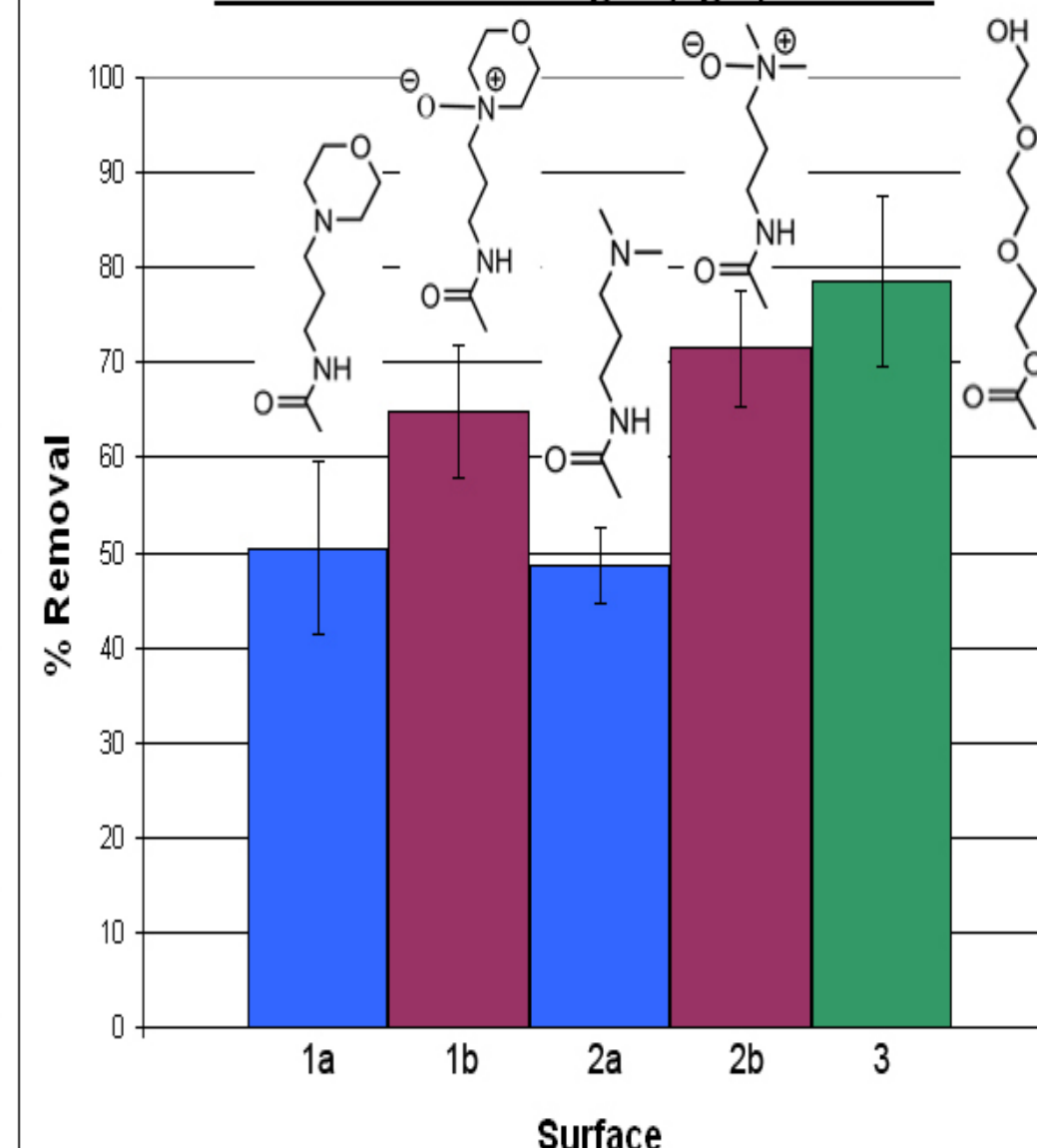
It appears that the fibrinogen was removed more easily than the lysozyme despite being more "sticky", however this may be due to a much less concentrated solution being used. This is because fibrinogen will not dissolve at 1mM however if lower concentration of lysozyme was used the protein adsorption would be limited by mass transport.<sup>4</sup>

Example run using lysozyme

% Removal of Lysozyme (14.6g/L) with PBS



% Removal of Fibrinogen (1g/L) with PBS



Average protein removal for each surface shown with standard errors.

### Conclusion

Ethylene glycol or PEG derivatives are often used to make surfaces bio-inert. The new amine oxides are found to be comparable in efficacy to this benchmark when challenged with either lysozyme or fibrinogen.

#### Acknowledgements

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#### References

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3 Heuberger R.; Sukhorukov G.; Voros J.; Textor M.; Mohwald H. *Adv. Funct. Mater.* 2005, 15, 357-366

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