Antibody Adsorption and Solution Structural Changes –

Recent advances and future development

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BBSRC – STFC Facility Access Funding for Bioscience Partnerships: Briefing Event, 5 Dec 2022

BBSRC remits - **our industrial collaborations** - STFC neutron reflection and scattering

- Our industrial collaboration area:
  - **Antibody adsorption and solution stability (AstraZeneca)**

- BBSRC strategic priorities
  - Biomolecular science underpins the development of new tools and approaches, including effective and efficacious vaccines and diagnostics

- Biointerfaces and membranes – neutron reflection and scattering play a crucial role

Industrial challenge: mAb instability during storage

1. MAbs and other protein drugs may deform or even unfold.

2. Sequence modifications are common in bioengineered protein therapeutics – altering their stability – functions?

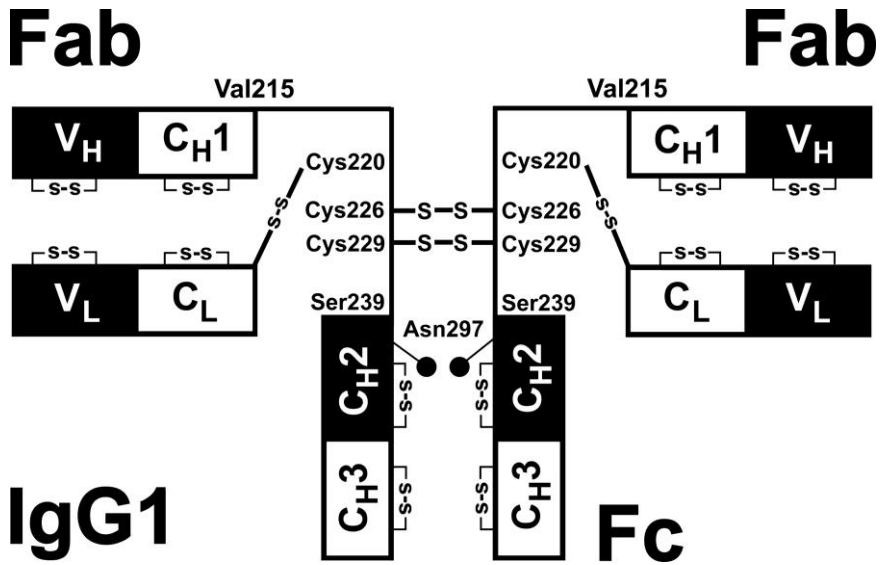
1. Surface adsorption – desorption – deformation/unfolding – seeding for aggregation?

2. Relevant to situations in separation, purification and storage

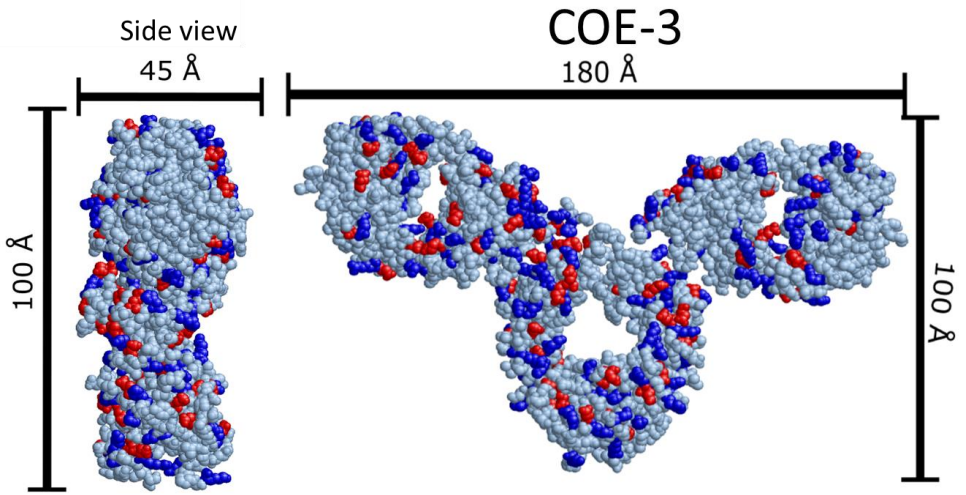
3. How to mitigate protein adsorption and desorption and devise predictable models?

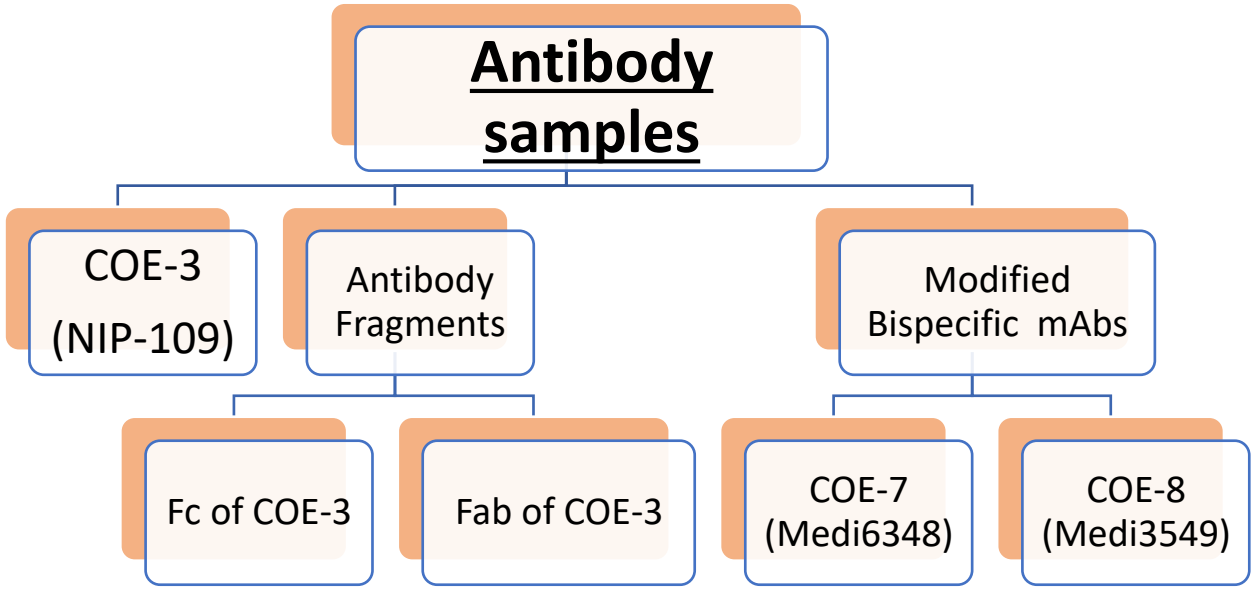
4. Effects of excipients (pH, ions, additives, surfactants) and interfaces

The human IgG1 domain structure – structural instability



Lucy E. Rayner et al. J. Biol. Chem. 2015;290:8420-8438





Sources of instability:

- Sequence modifications
- Interactions with interfaces
- Ions, surfactants

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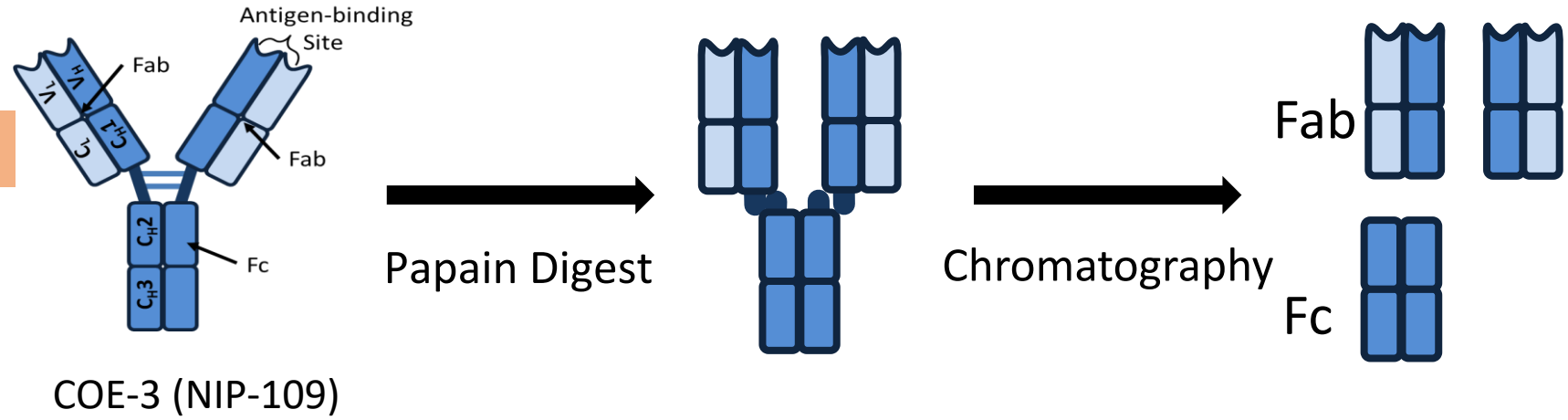
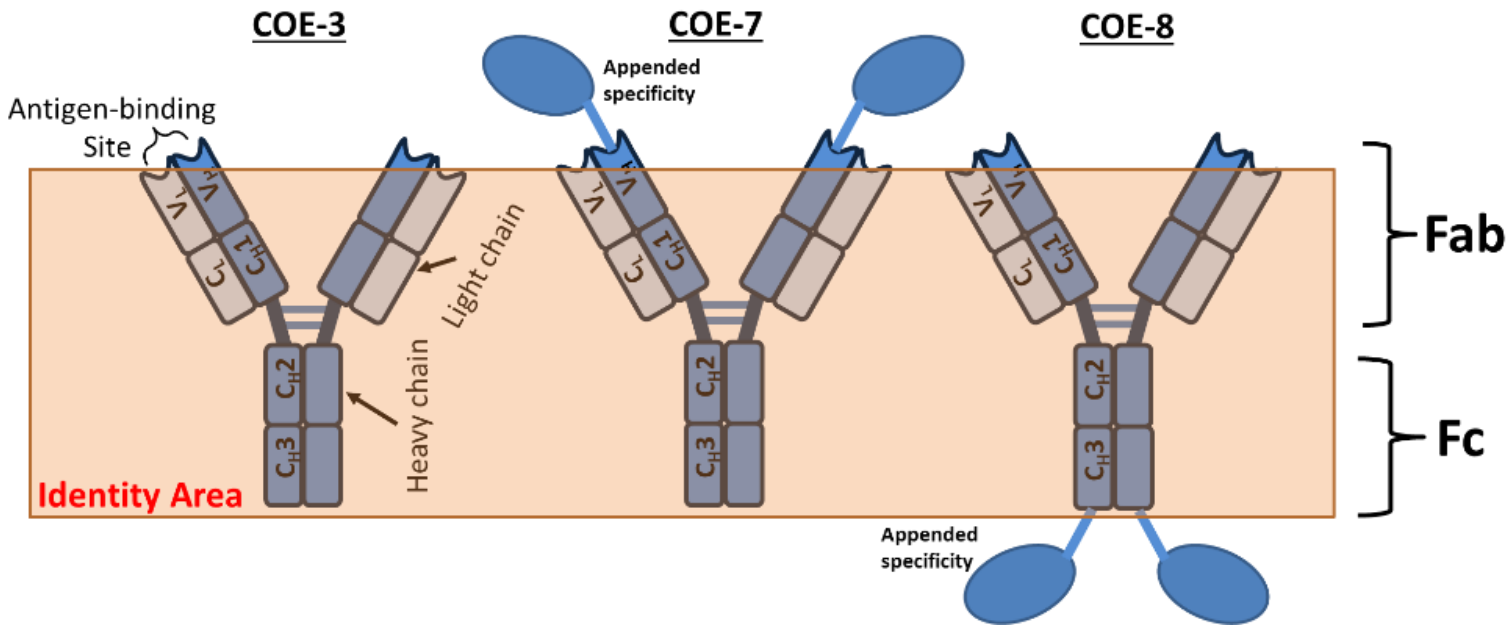
Antibody Fragments



COE-3 (NIP-109)

Bispecific mAbs



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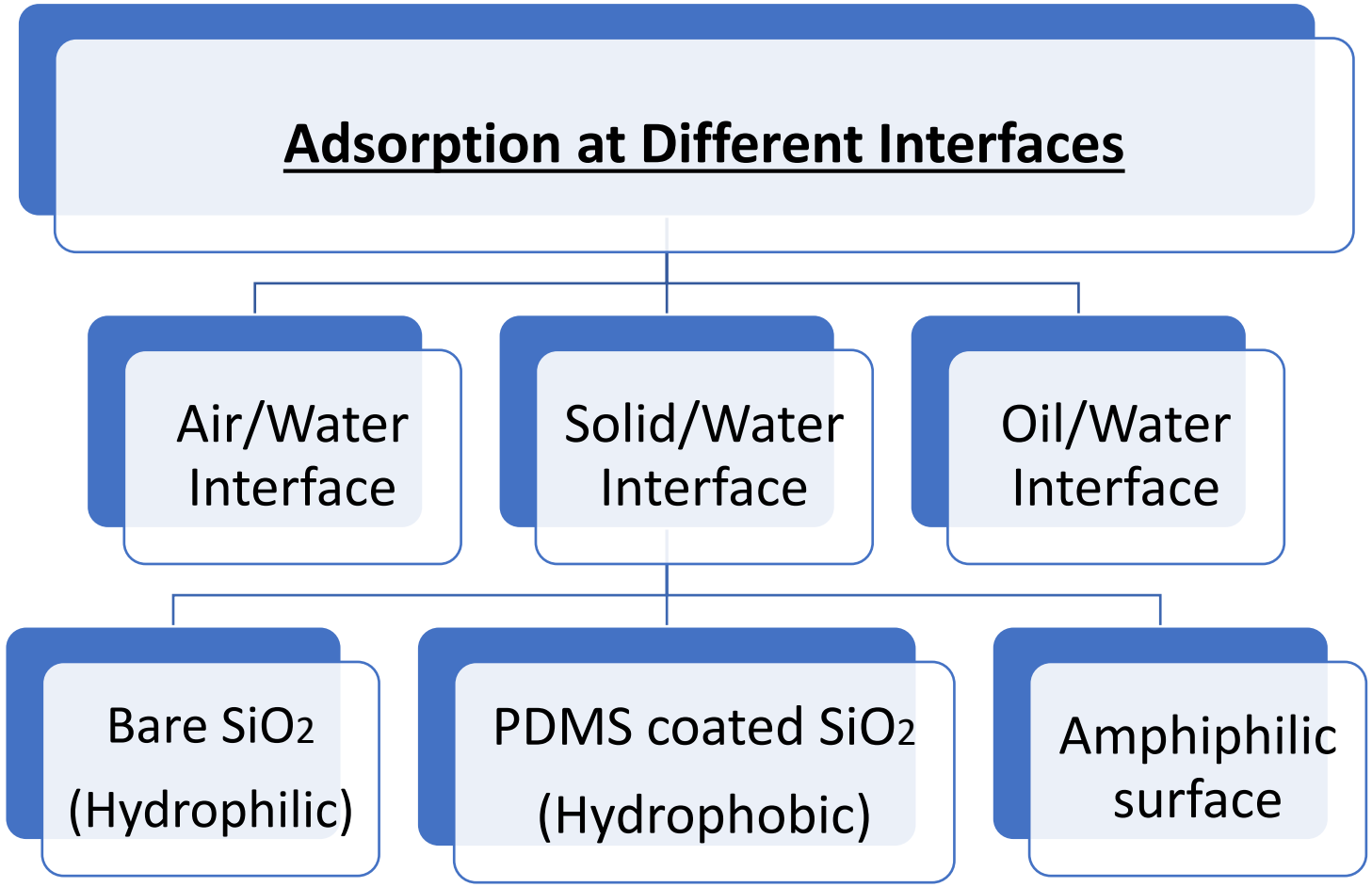
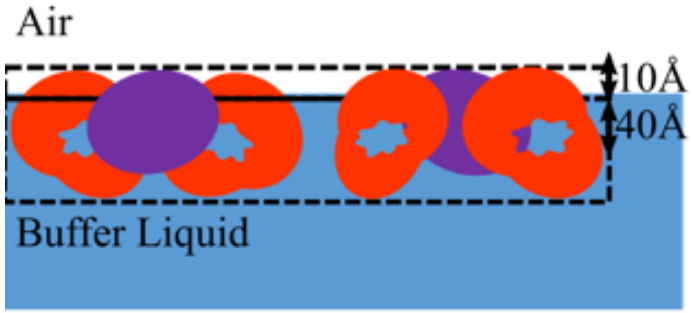
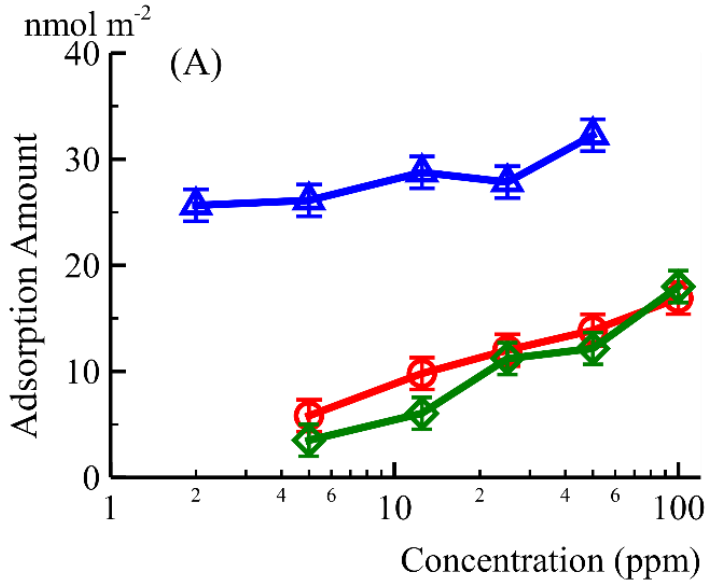
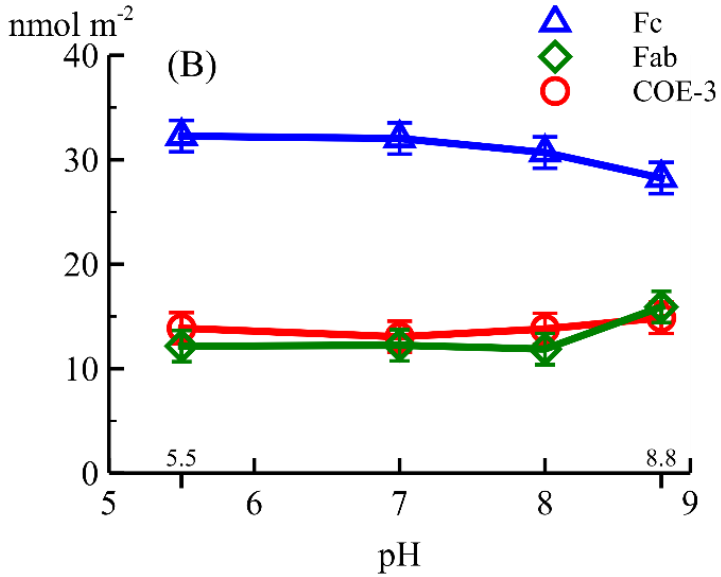

Adsorption at Different Interfaces
Air/Water Interface
Solid/Water Interface
Oil/Water Interface
Bare SiO_2 (Hydrophilic)
PDMS coated SiO_2 (Hydrophobic)
Amphiphilic surface

Air/Water Interface



Neutron reflection reveals COE-3 molecules adopted a ‘flat-on’ conformation with their short axes projected perpendicular to the interface.



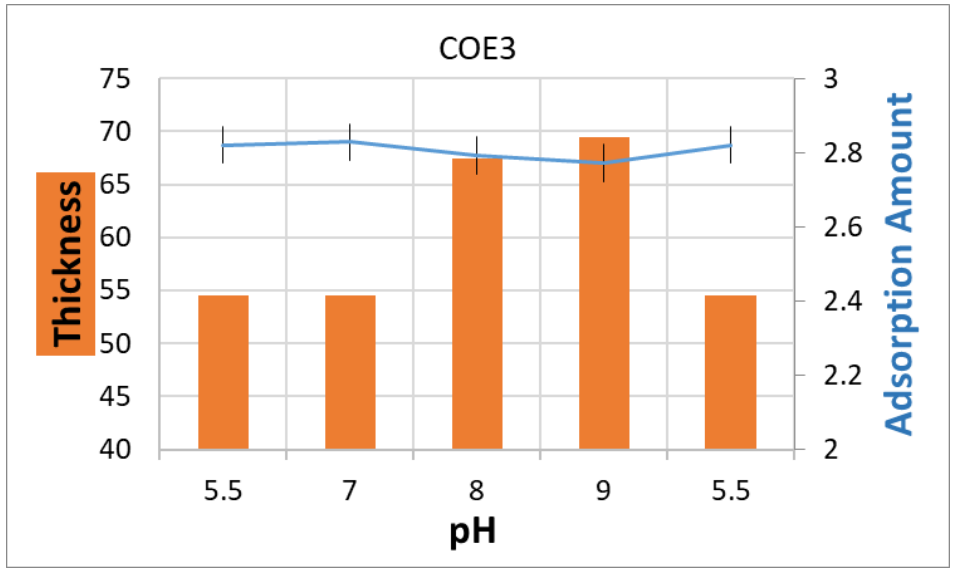


- Fc is more surface active than Fab, cause Fc carries fewer charges.
- The adsorption of the whole mAb was dominated by its Fab fragments.
- pH is not a key factor for air/liquid interface adsorption.

Z. Li, R. Li, C. Smith, F. Pan, M. Campana, J.R.P. Webster, C.F. van der Walle, S. Uddin, S.M. Bishop, J. Warwicker, J.R.Lu, R.Narwal, Neutron reflection study of surface adsorption of Fc, Fab and the whole mAb, ACS Appl Mater Inter, 2017, 9(27), pp 23202-23211

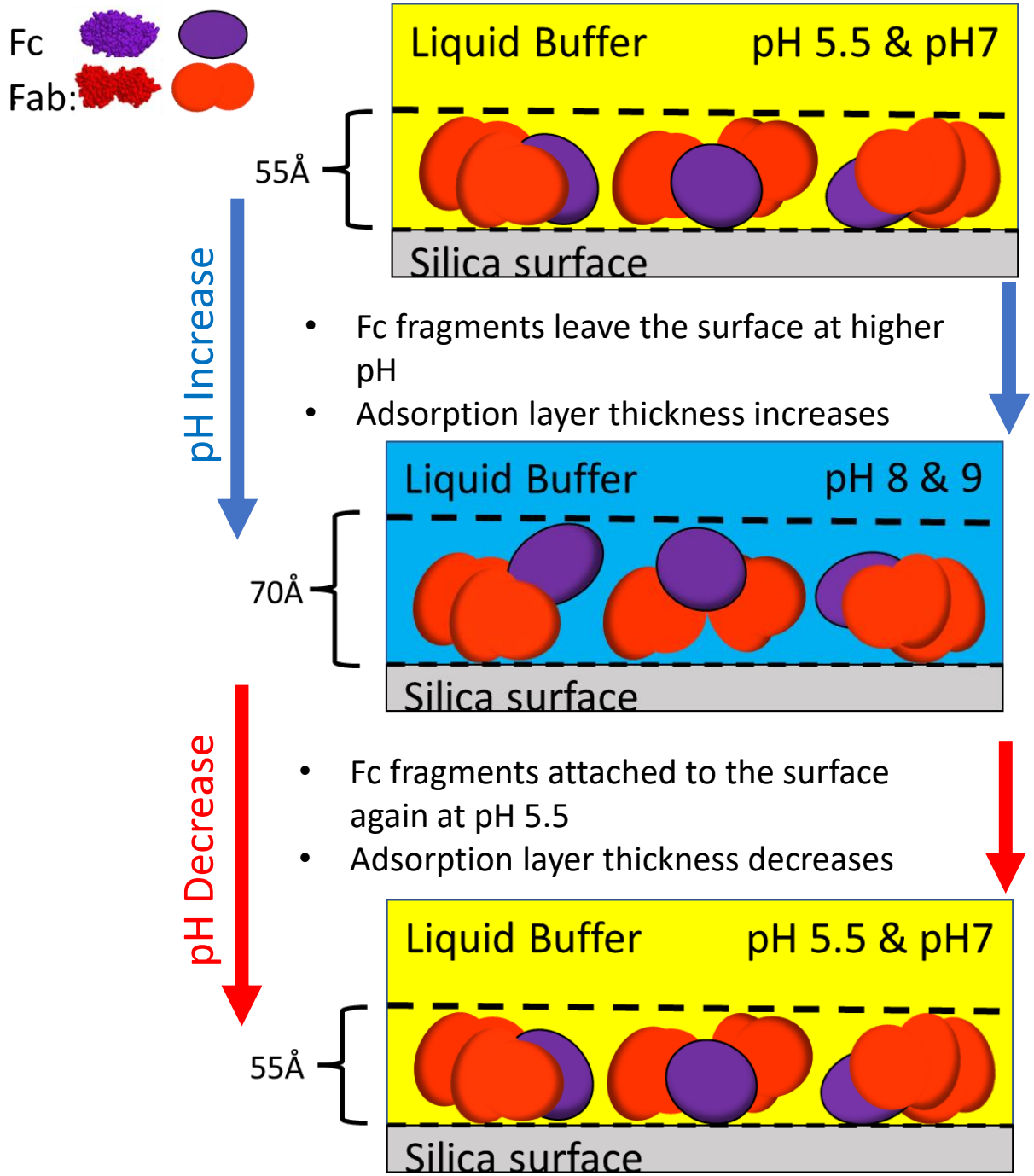
SiO_2/Water Interface

pH Effect

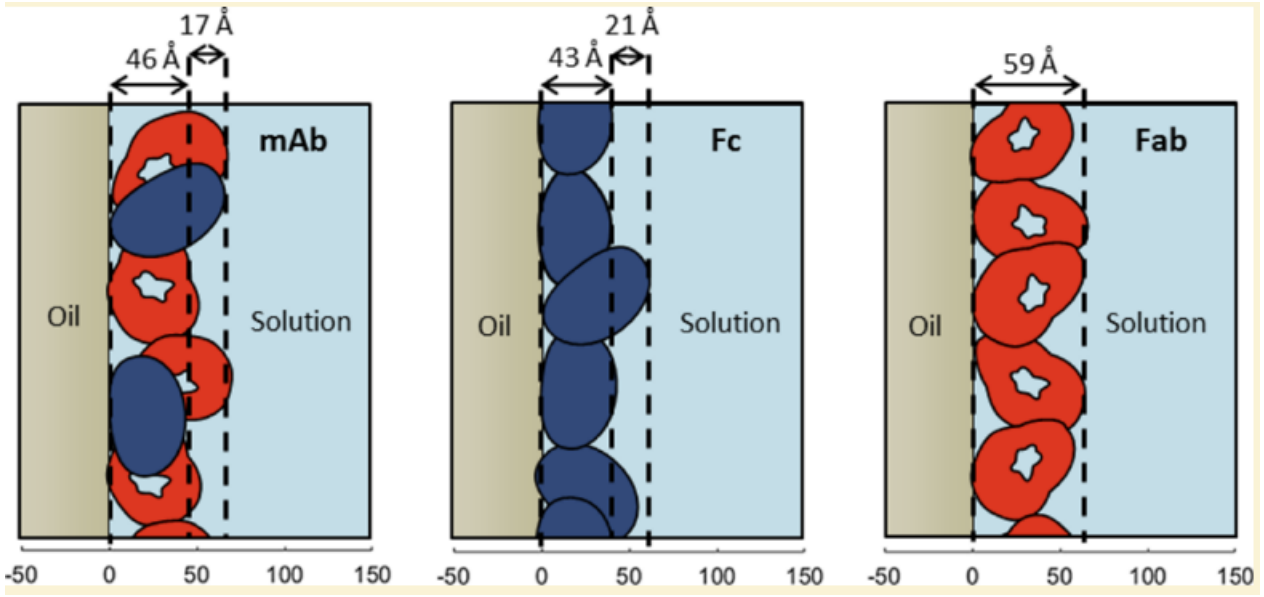


Buffer pH: 5.5 -> 7 -> 8- > 9 -> 5.5

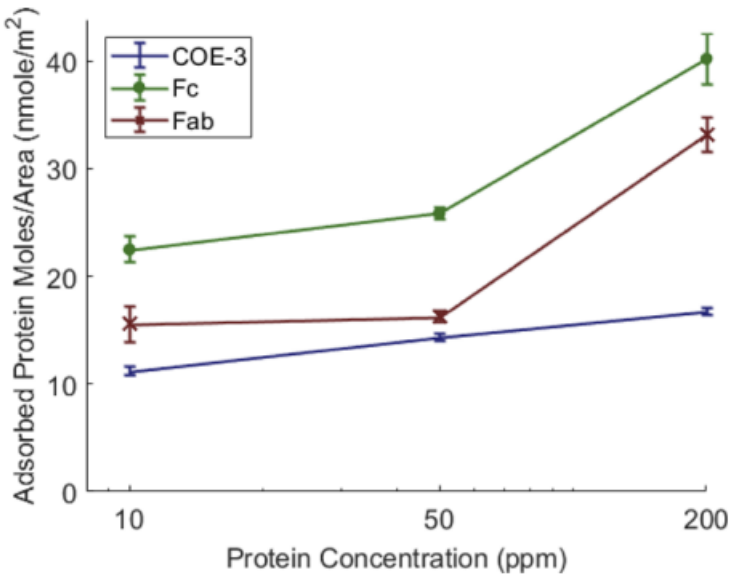
- Adsorption amount did not change with pH
- Thickness increases from 55 Å at lower pH to 70 Å at higher pH
- Thickness change due to Fc desorbed at higher pH condition
- COE-3 orientation is affected by pH change.



Oil/Water Interface



- COE-3 and Fc were found to adsorb flat-on to the interface, with denser 45 and 42 Å inner layers, respectively, in contact with the oil and a more diffuse 17−21 Å outer layer caused by fragments adsorbing in a tilted manner.
- Fab fragments formed a uniform 60 Å monolayer



- COE-3 had a higher affinity to the interface than either of its constituent fragments, while Fab had a lower interfacial affinity consistent with its higher net surface charge.

Ruane, S.; Li, Z.; Campana, M.; Hu, X.; Gong, H.; Webster, J.R.P.; Uddin, F.; Kalonia, C.; Bishop, S.M.; Van Der Walle, C.F.; et al. Interfacial Adsorption of a Monoclonal Antibody and Its Fab and Fc Fragments at the Oil/Water Interface. Langmuir 2019.

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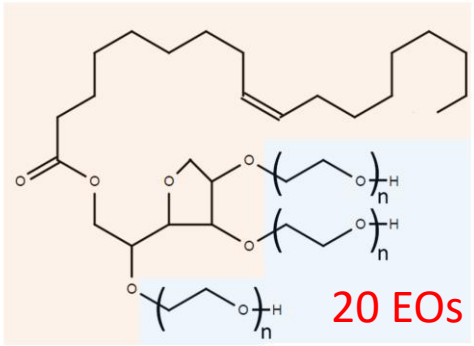
Surfactant-Antibody Mixture

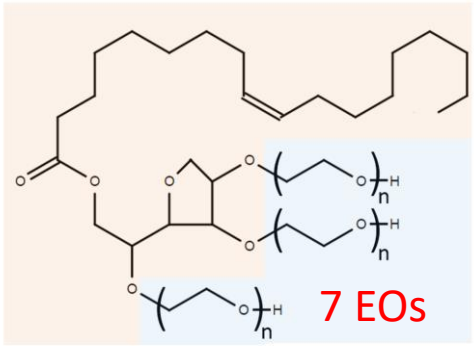
- Polysorbate-80 (Tween80)
- Effects in Solution
- Effects on interface Adsorption

20EO



HLB=14.3

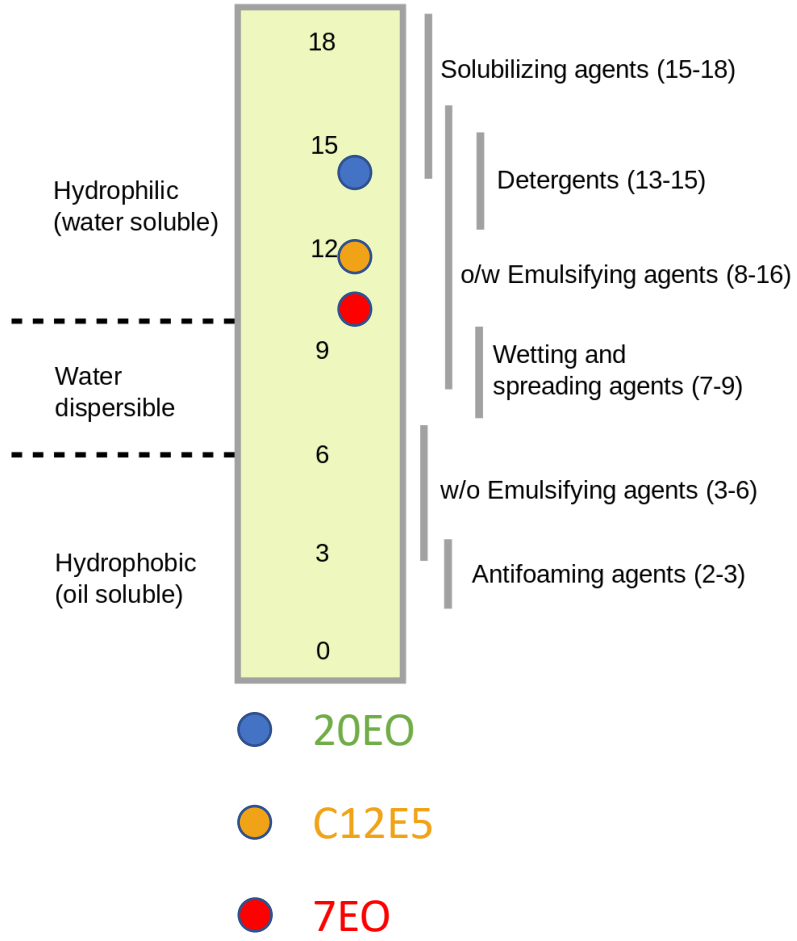
7EO



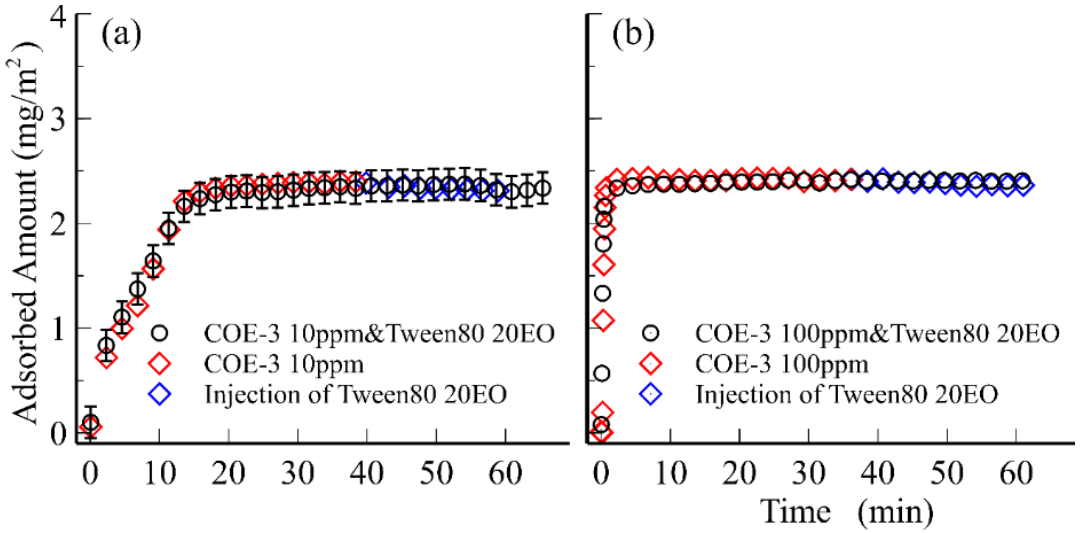
HLB=9.9

Hydrophilic-lipophilic balance (HLB)

$$\mathrm{HLB} = 20\frac{M_h}{M_w}$$

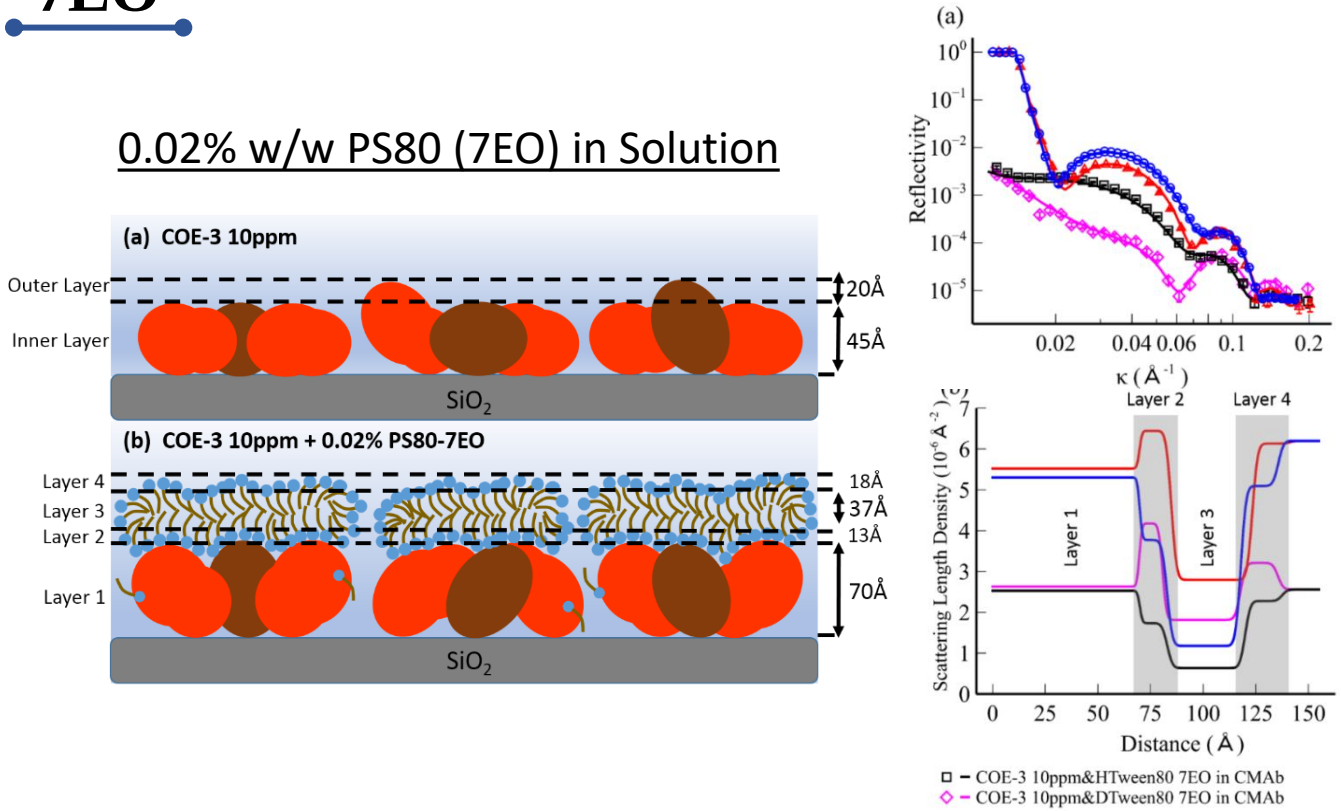


20EO



- PS80 (20EO) has no influence on the adsorption behavior of COE-3 at the SiO_2/water interface.

7EO

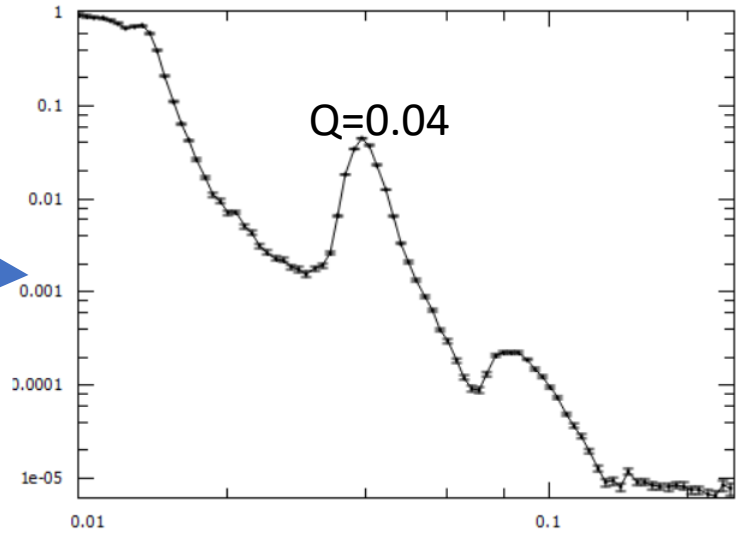


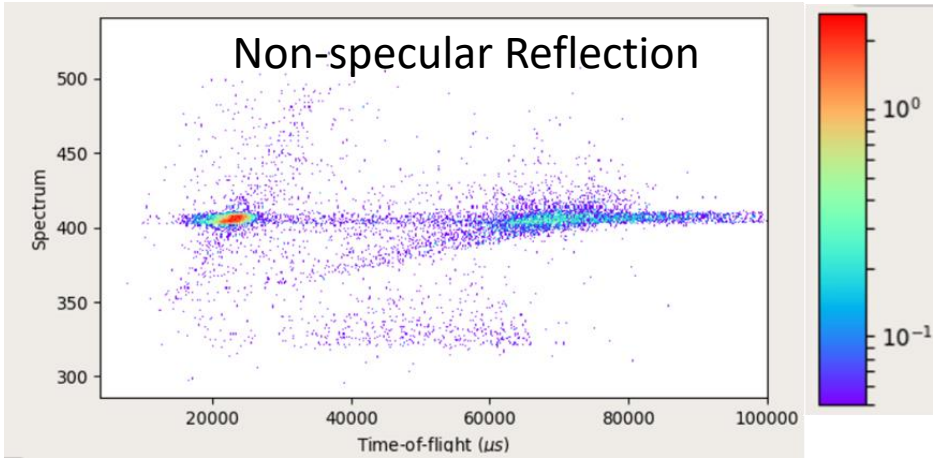
- PS 80 (7EO) cannot prevent antibody adsorption, but it could self-assemble onto the adsorbed antibody layer and form a surfactant bilayer.
- The surfactant bilayer can prevent further mAb adsorption.

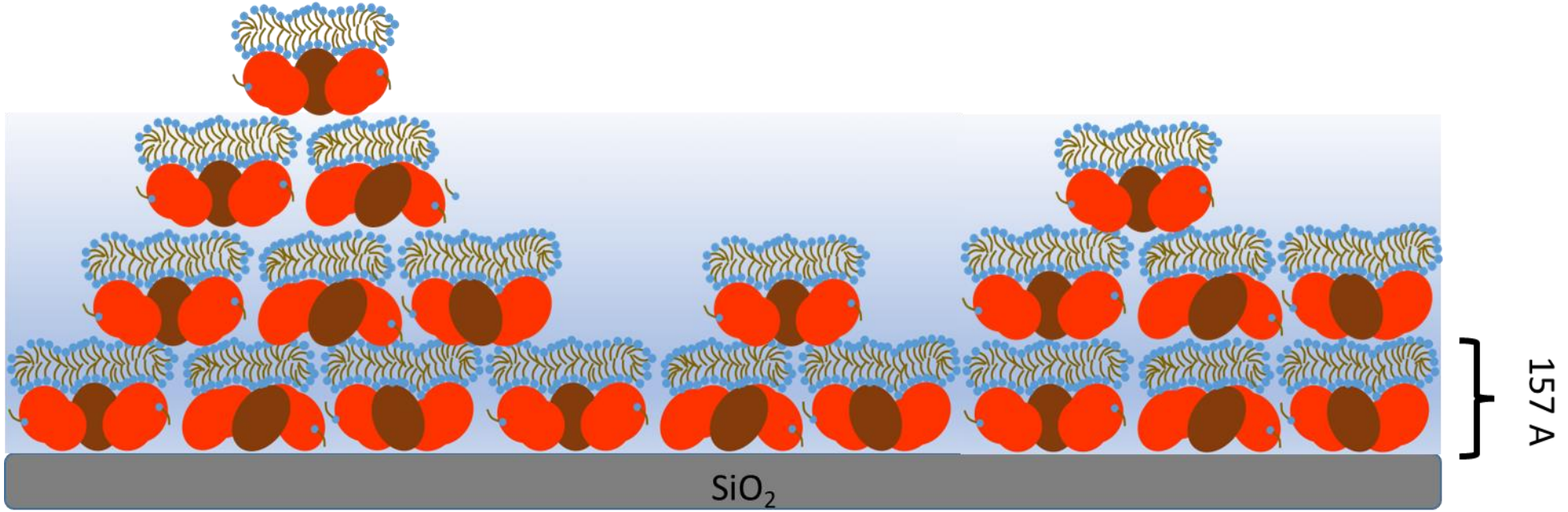
Zongyi Li, Fang Pan, Ruiheng Li, Elias Pambou, Xuzhi Hu, Sean Ruane, Daniela Ciumac, Peixun Li, Rebecca J.L. Welbourn, John R.P. Webster, Steven M. Bishop, Rojaramani Narwal, Christopher F. van der Walle, and Jian Ren Lu, ACS Applied Materials & Interfaces, 2018 10 (51), 44257-44266

In-solution structure related to structure of adsorption layer at SiO_2/Water Interface

PS80-7EO at 100CMC
COE-3 at 2500ppm



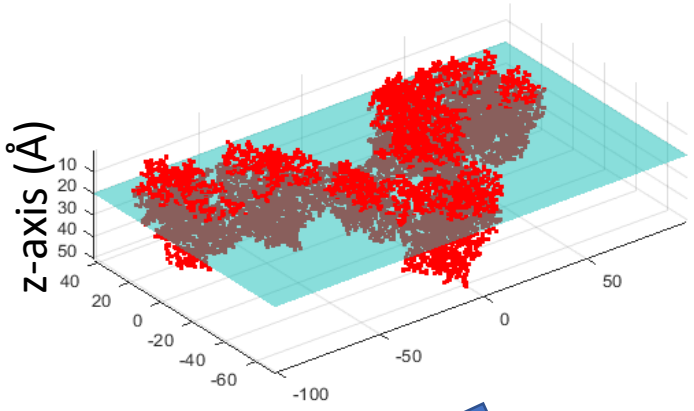


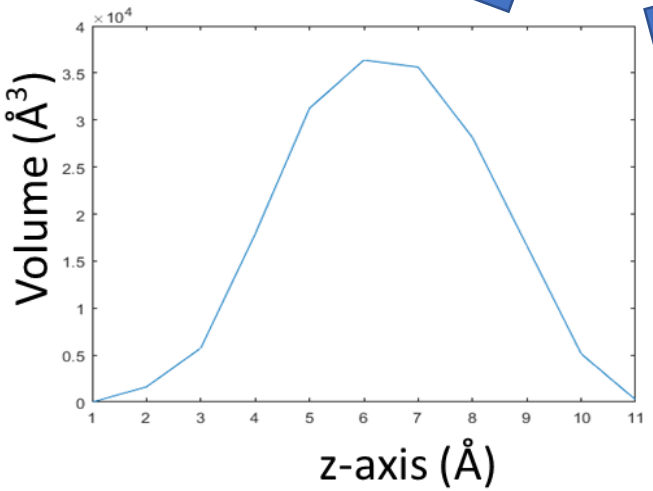


4. *New Method of Analysis for Neutron Reflectivity Data*

Simulated NR profiles can be calculated based on the PDB files and MD simulation results

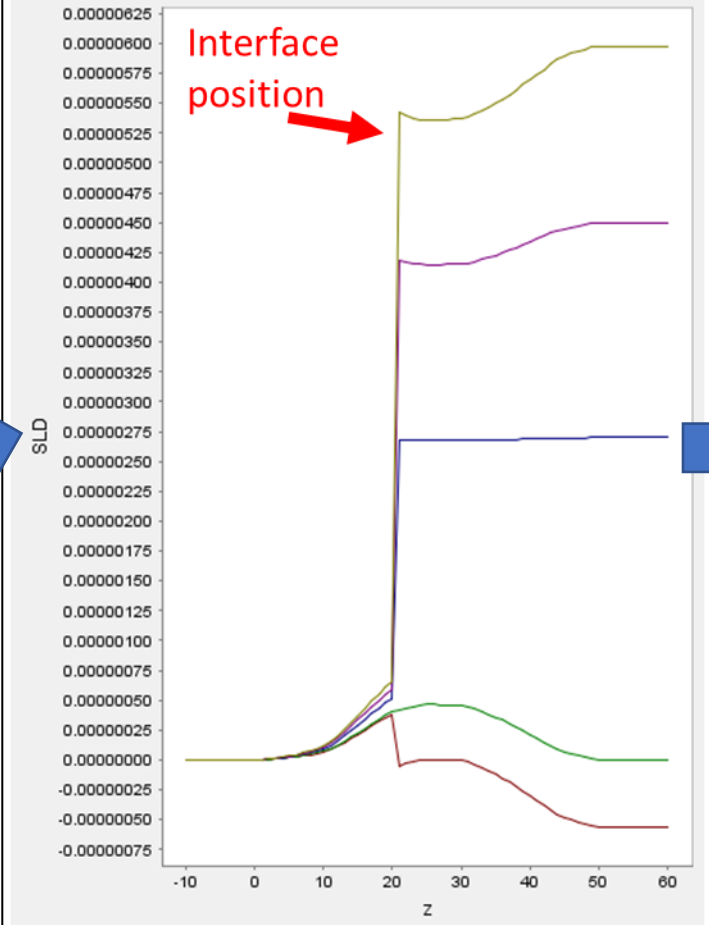
PDB file obtained from MD simulation



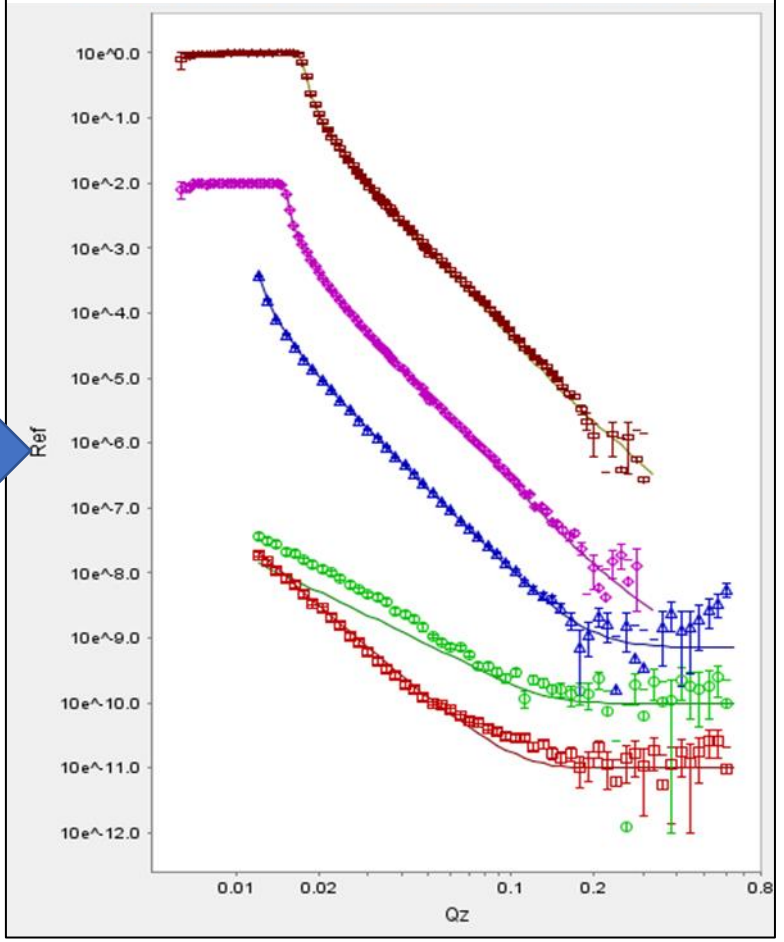


Volume Density on z-direction

SLD profiles in different contrasts



NR profiles in different contrasts



Future challenges

- How do surface charged and hydrophobic amino acids affect protein folding, interfacial adsorption and solution stability?
- How do certain short amino acid sequences affect adsorption and solution stability?
- How to develop models to predict adsorption and solution instability?

https://iop.eventsair.com/aass22