Microbial Adhesion and Molecular Interactions

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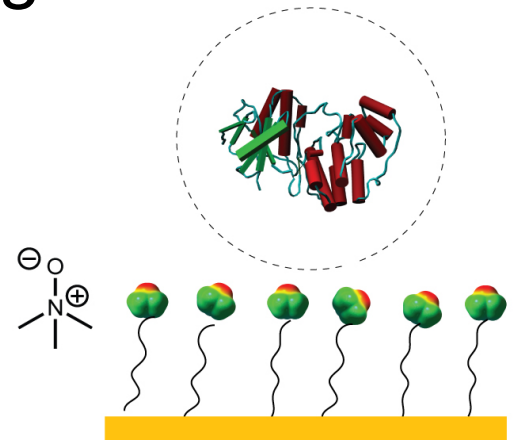
@marshgroup

Overview

- SiO_2, PS, PEGA beads ⋯ (phage-λ genomic display library)

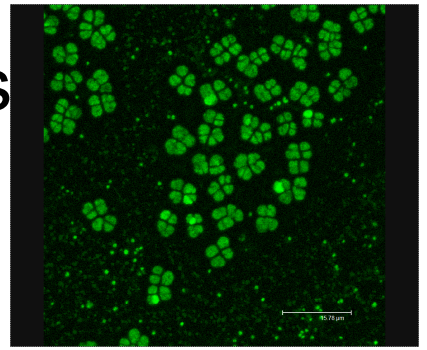
Langmuir, **2006**, *22*, 8144

Langmuir, **2013**, *29*, 2961



- Au, Si planar substrates with monolayers (lysozyme, fibrinogen)
- 1 μm microspheres, 100-200 μm beads (*Methanosarcina barkeri*)

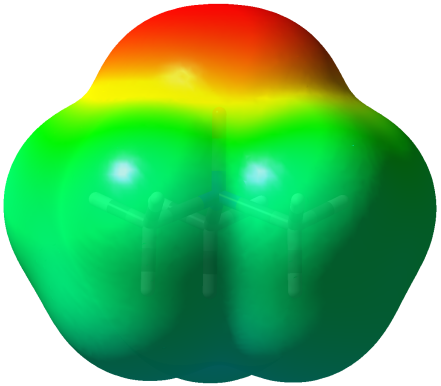
D Dobrzanska, *PhD Thesis,* University of Warwick **2014**

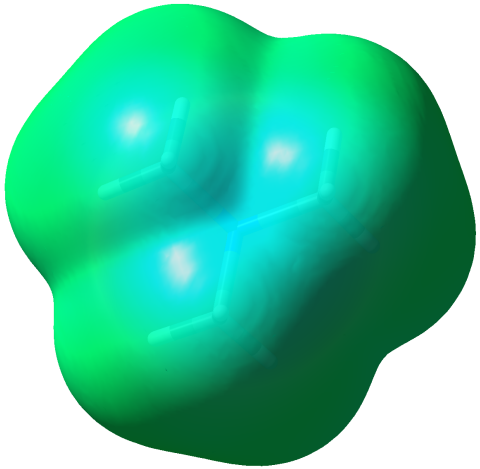


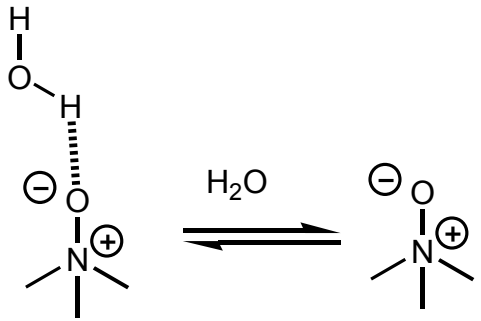
- polyurethane foam (cattle slurry, fruit and veg anaerobic digester sampling)

D Dobrzanska, *PhD Thesis,* **2014**

Trimethylamine *N*-oxide: a ‘kosmotrope’







μ = 5.07 D (calc.) 5.02 D (expt., benzene)
cf. 1.85 D for water, 0.61 D Me_3N

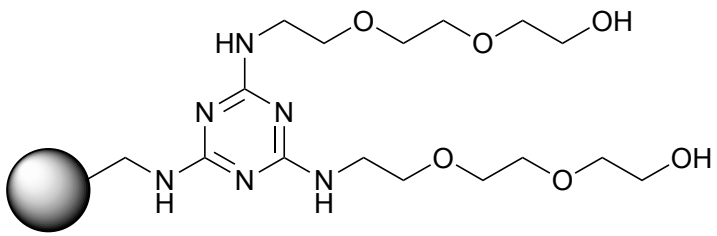
pK_a (Me_3N^+O-H) = 4.5 (R P Bell, W C E Higginson *Proc. Royal Soc.* **1949**, *197*, 141)

pKa (Me_3N^+-H) = 9.76 (H K Hall, Jr. *J. Am. Chem. Soc.* **1957**, *79*, 5441.

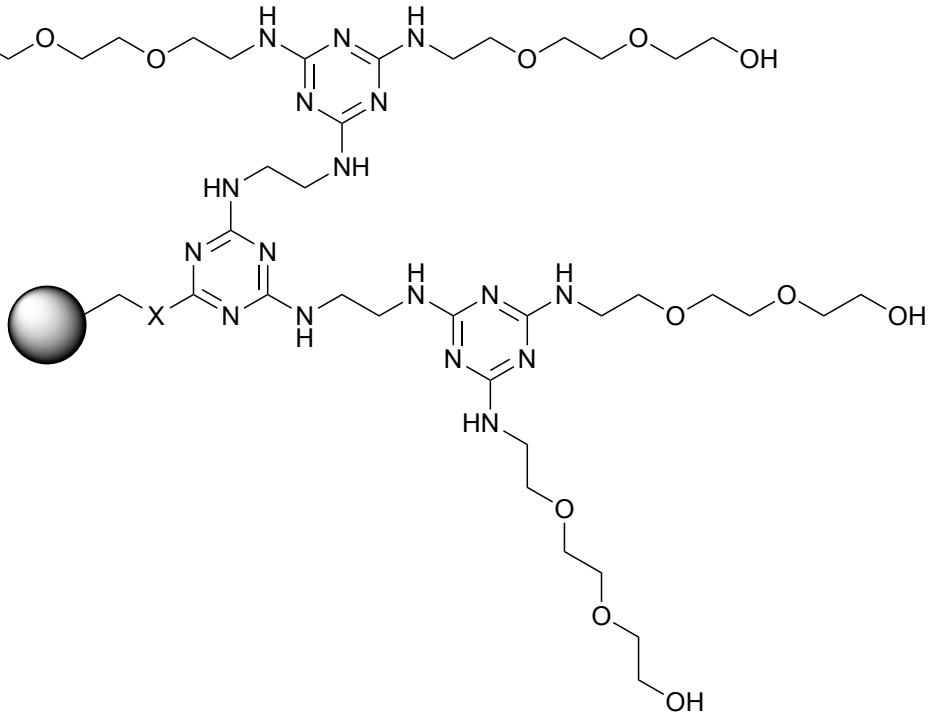
Calculation performed at MP2/aug-cc-pVDZ level using *Gaussian03* T R Walsh

Counteracts denaturing effect of urea on proteins, stabilises macromolecular structure
D W Bolen, I L Baskakov *J Mol Biol* **2001**, *310*, 955

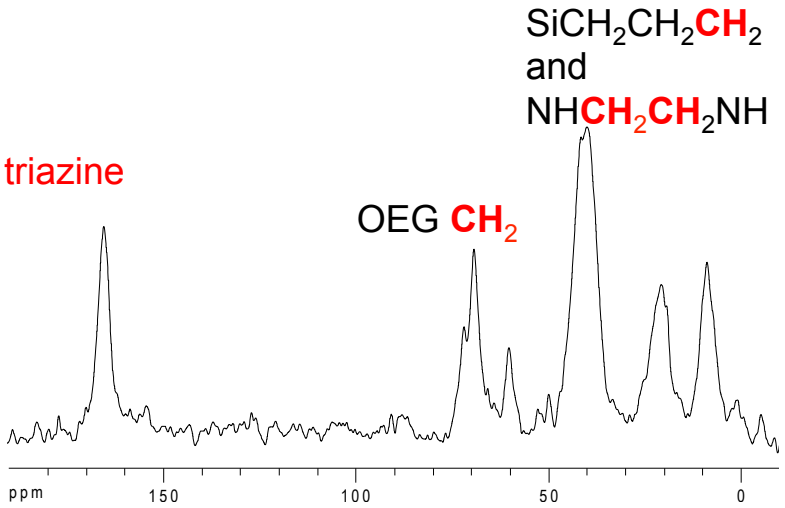
New substrates prepared: dendrimeric OEG



1a SynPhase™ Lanterns
1b Aminopropyl silica



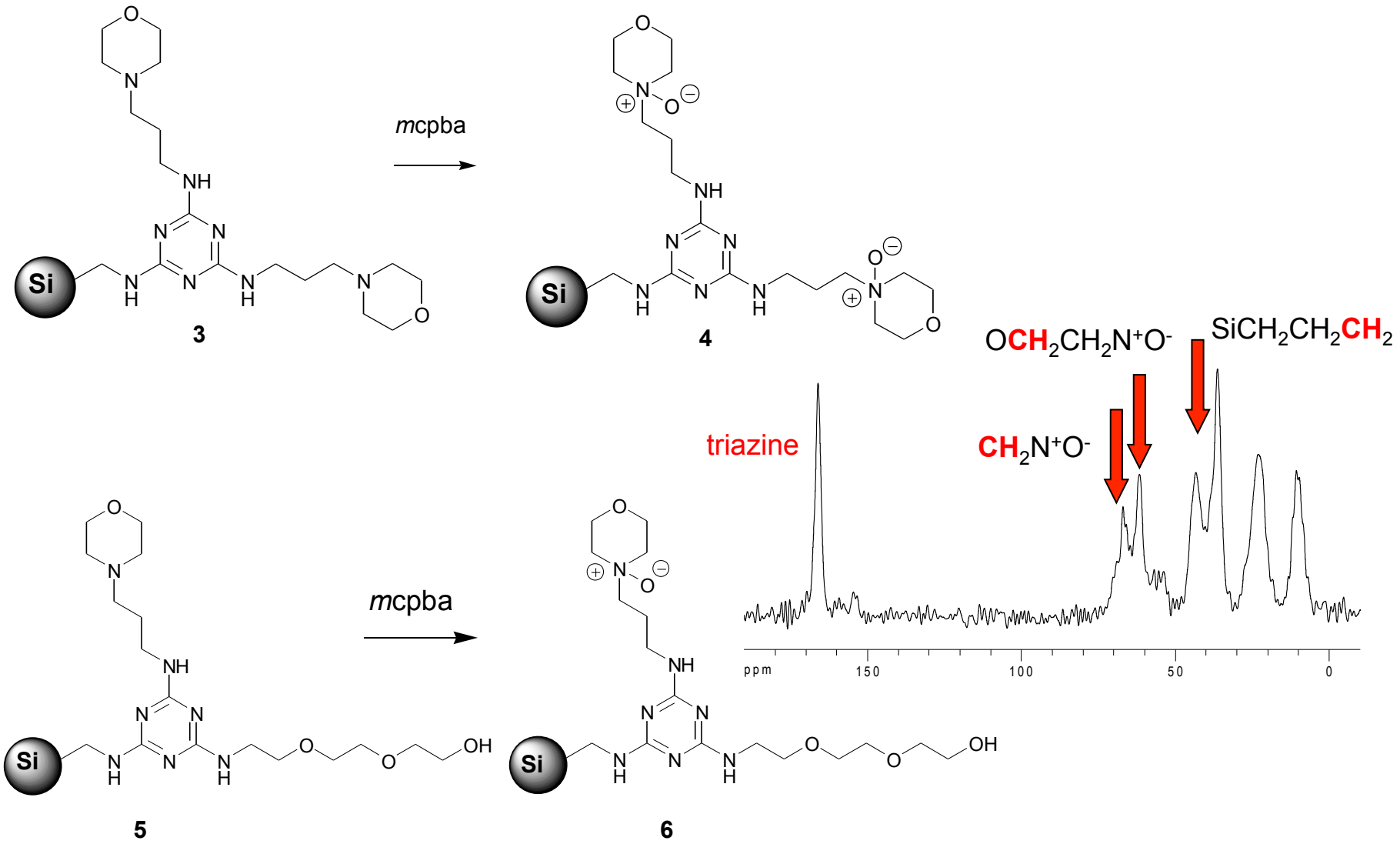
2a X = NH; SynPhase™ polyamide Lanterns
2b X = NH; Aminopropyl silica
2c X = O; Wang resin



^{13}C CP-MAS NMR

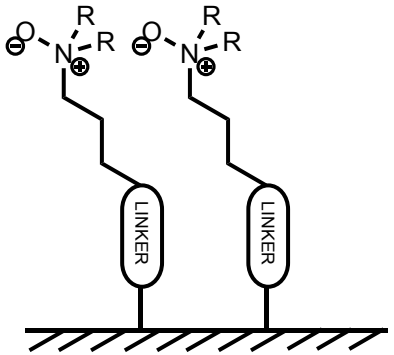
S J Dilly, S J Carlisle, A J. Clark, A R Shepherd, S C Smith, P C Taylor, A Marsh *J Pol Sci: Pol Chem (Part A)* **2006**, *44*, 2248
Dilly SJ, Beecham MP, Brown SP, Griffin JM, Clark AJ, Griffin CD, Marshall J, Napier RM, Taylor PC, Marsh A. *Langmuir* **2006***, 22,* 8144.

New substrates prepared: tertiary amine oxides



Dilly SJ, Beecham MP, Brown SP, Griffin JM, Clark AJ, Griffin CD, Marshall J, Napier RM, Taylor PC, Marsh A. *Langmuir* **2006**, *22,* 8144.

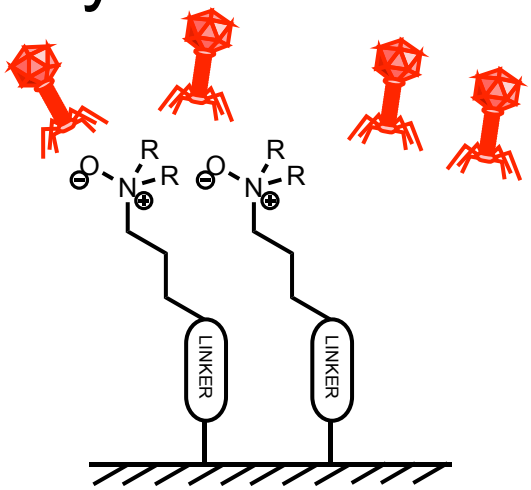
Testing non-specific adhesion using plant genome phage-λ display library



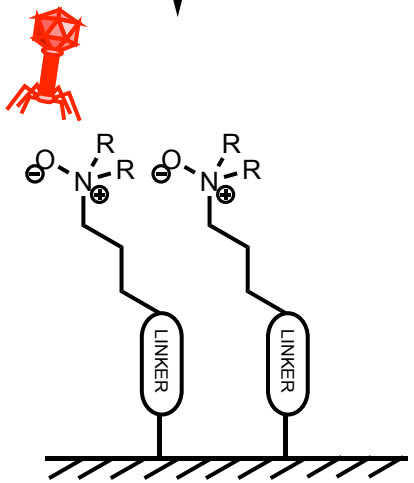
Support = Tentagel, silica, PEGA, PS

phage-λ genomic display library

diversity >10^6



wash



add

E Coli

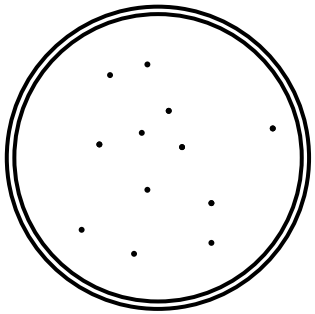
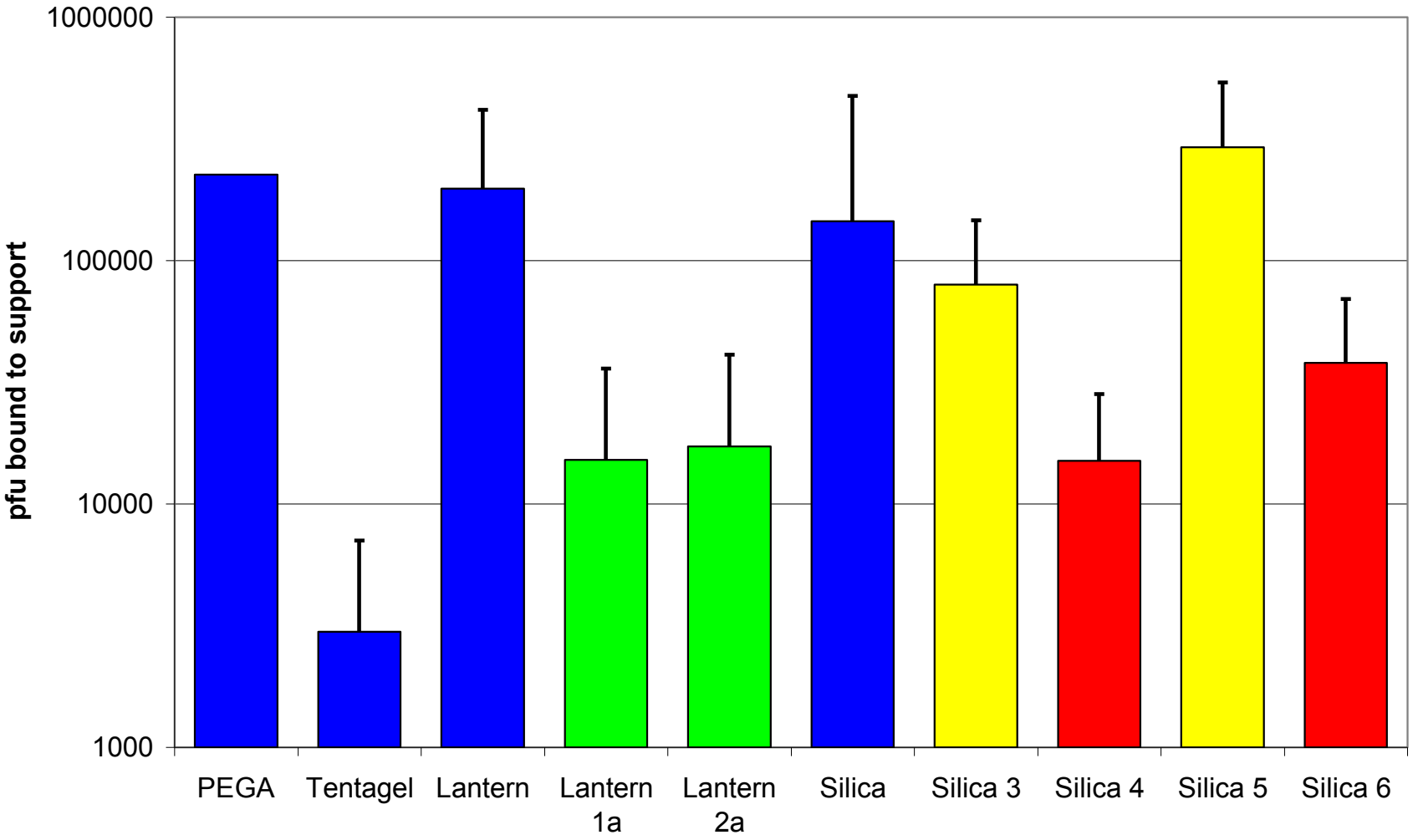


Plate out and count phage plaques

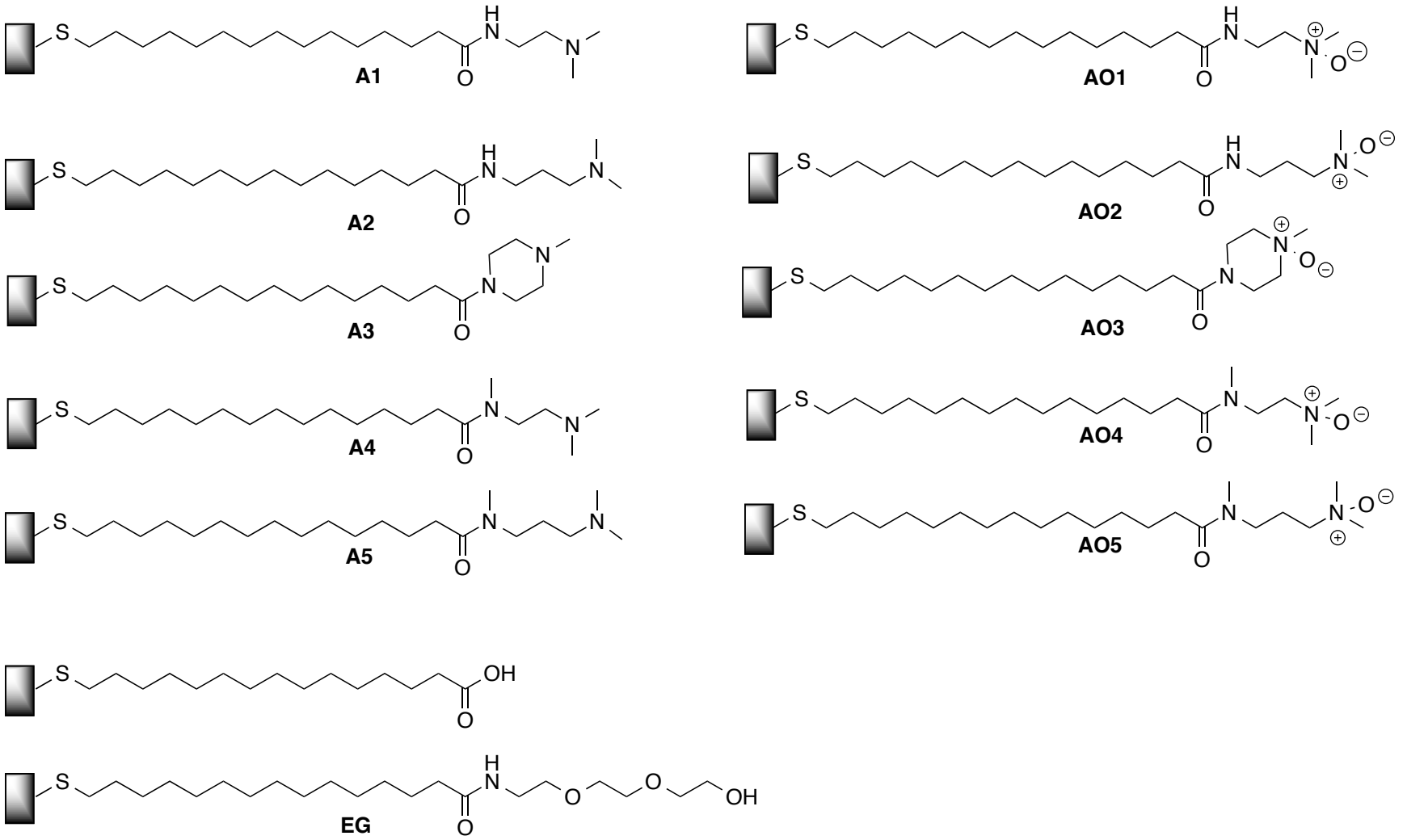
Langmuir **2006**, *22,* 8144.

Results of non-specific protein adhesion tests



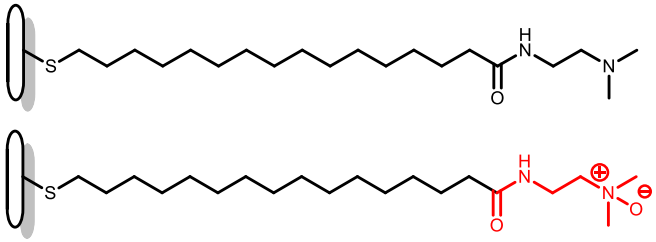
- Low binding levels desirable

Dilly SJ, Beecham MP, Brown SP, Griffin JM, Clark AJ, Griffin CD, Marshall J, Napier RM, Taylor PC, Marsh A. *Langmuir* **2006***, 22,* 8144.



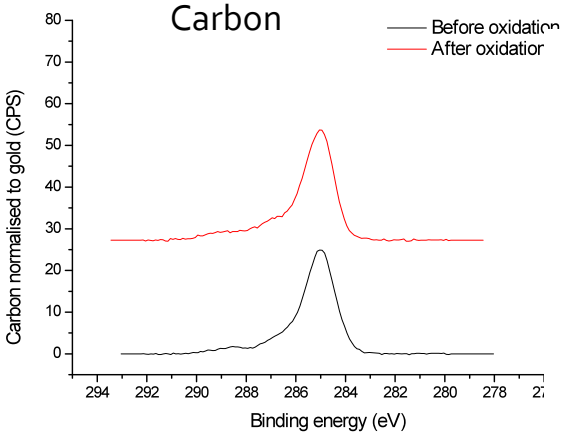
D. A. Dobrzanska … A. Marsh *Langmuir*, **2013**, *29*, 2961–2970

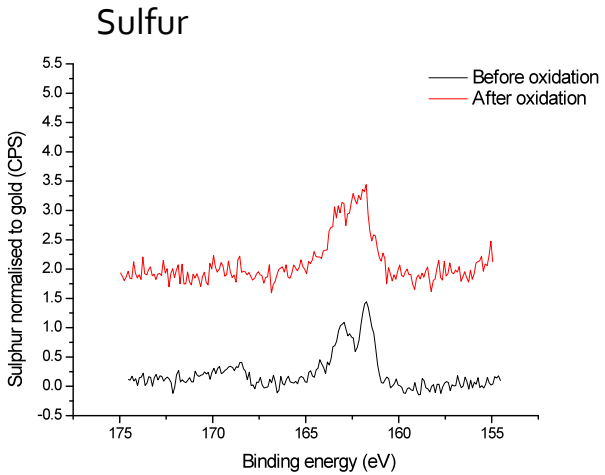
XPS and water contact angle

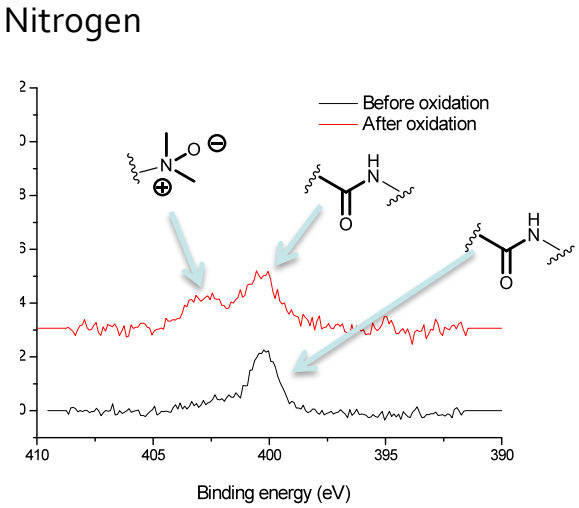


average contact angle/° 34±3

average contact angle/° 25±3







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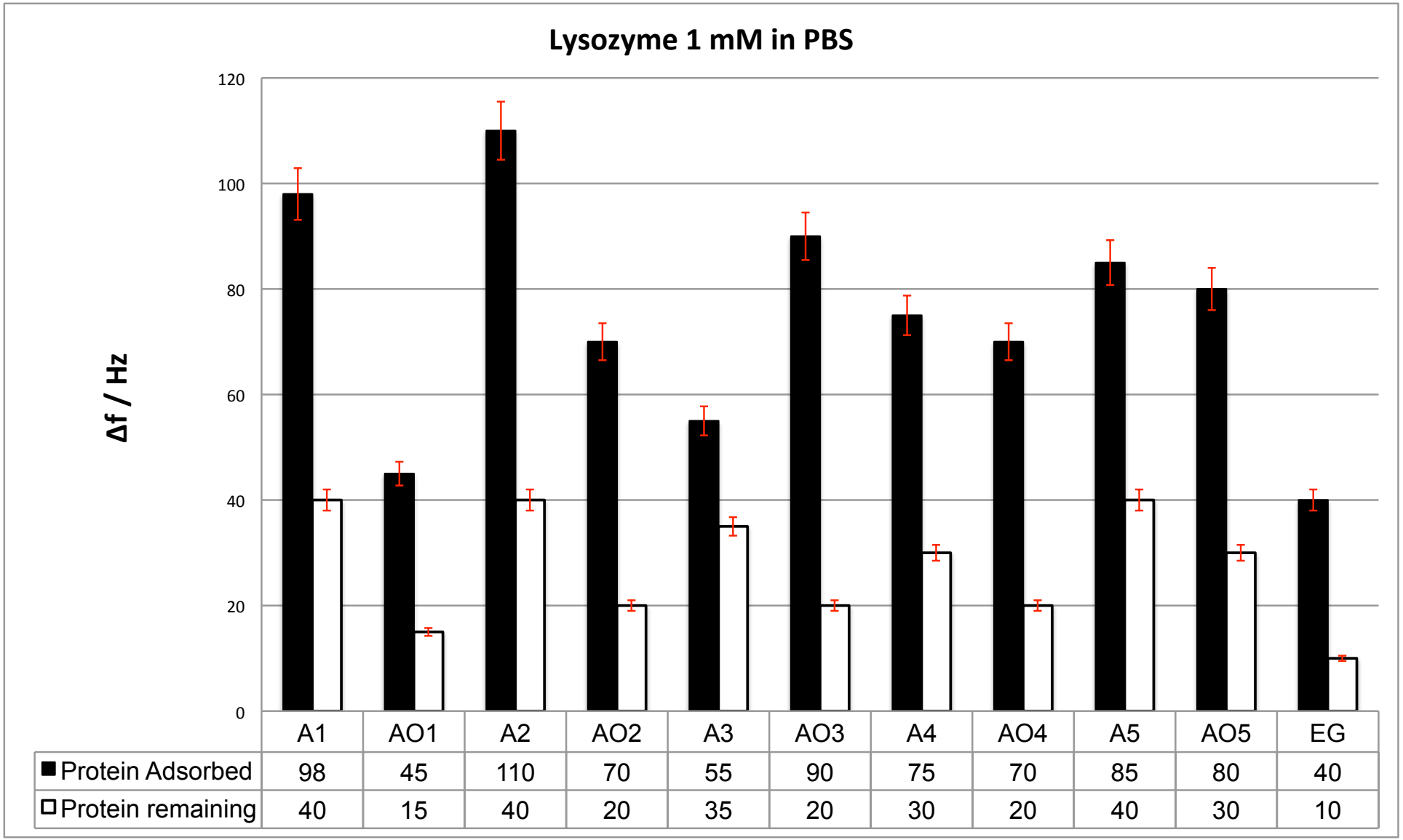
QCM study of lysozyme and fibrinogen adhesion to surfaces



	A1	AO1	A2	AO2	A3	AO3	A4	AO4	A5	AO5	EG
■ Protein Adsorbed	98	45	110	70	55	90	75	70	85	80	40
□ Protein remaining	40	15	40	20	35	20	30	20	40	30	10

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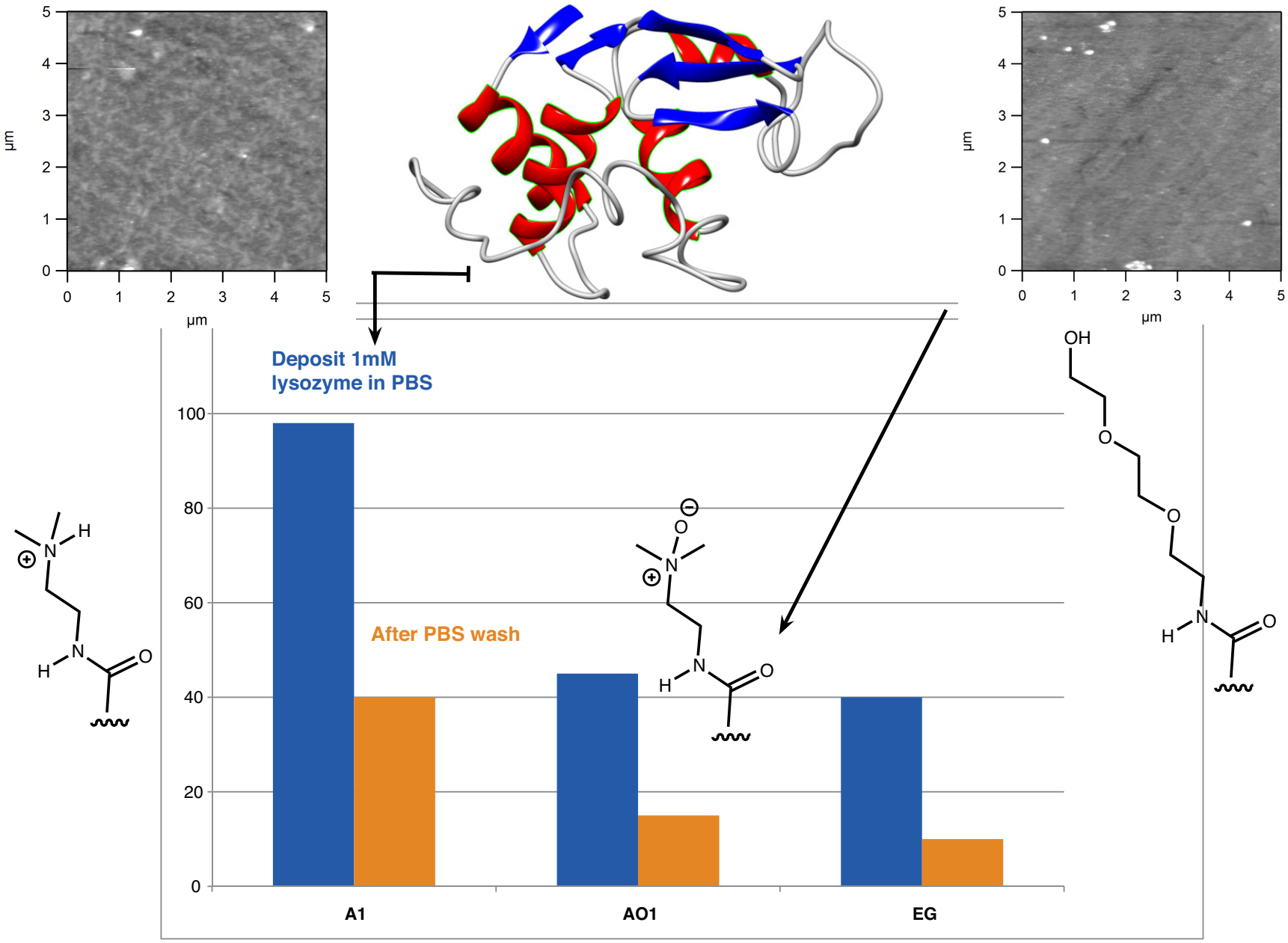
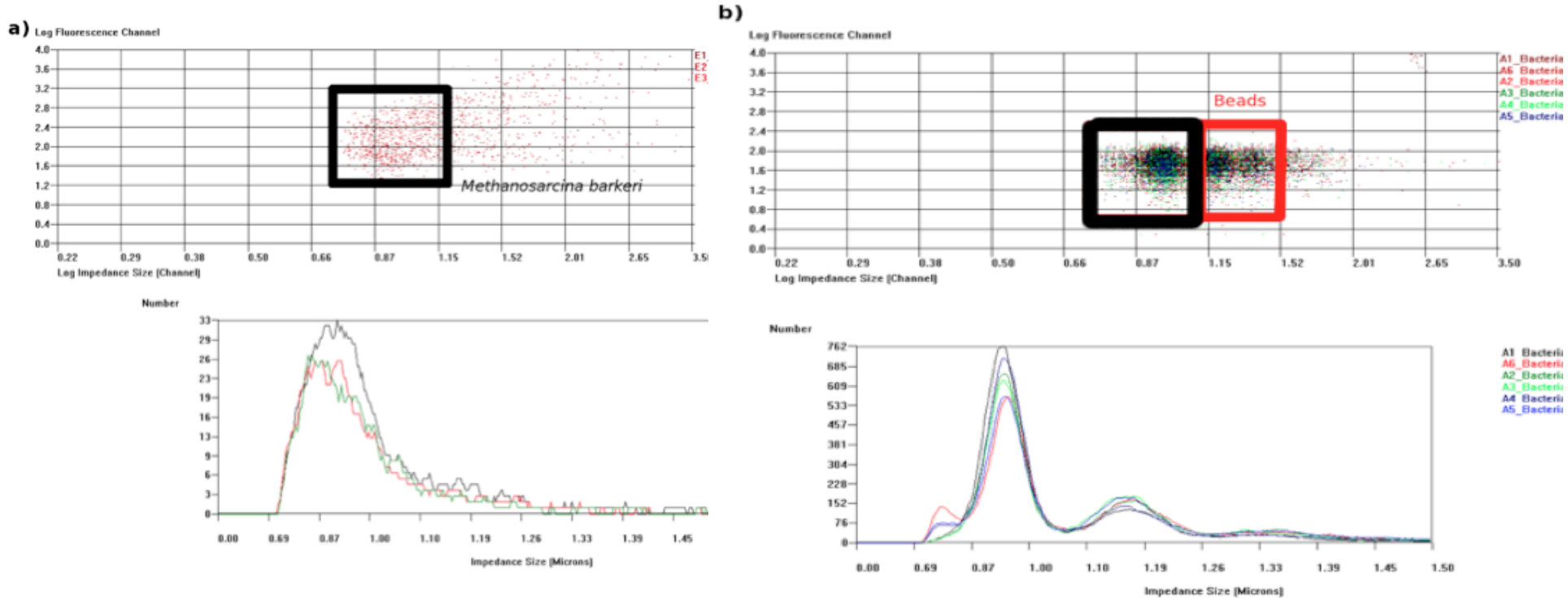
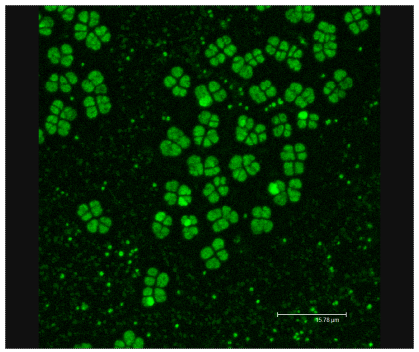
Tertiary amine *N*-oxide derivatised gold surfaces to control protein adhesion



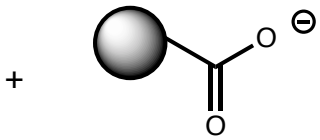
D A Dobrzanska … A Marsh *Langmuir*, **2013**, *29*, 2961–2970

Flow cytometry: *M. barkeri* and 1 µm PS beads





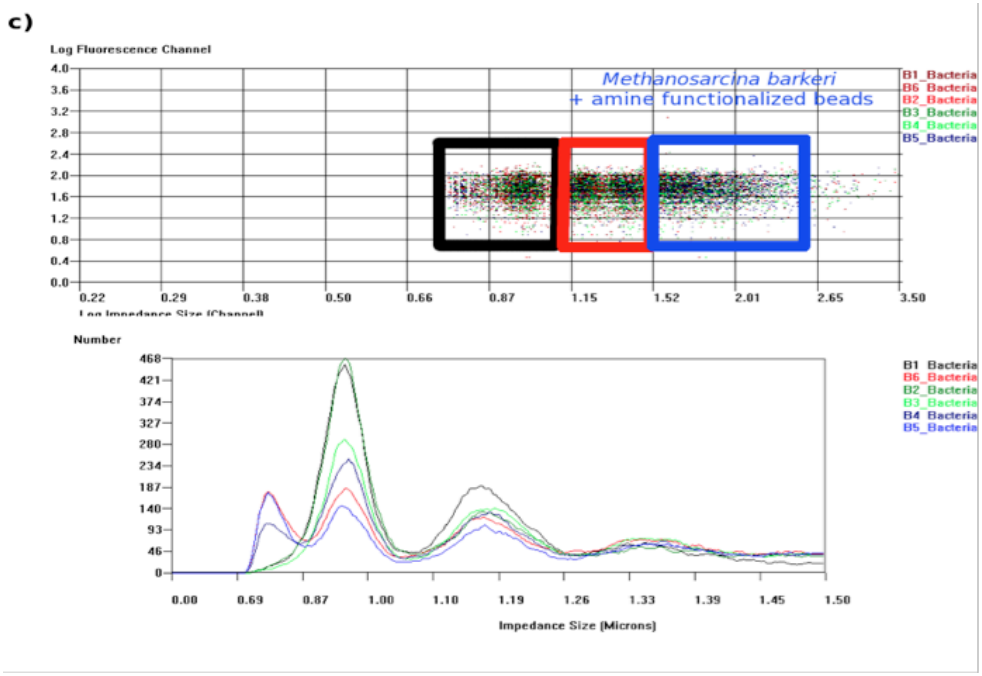
Methanosarcina barkeri

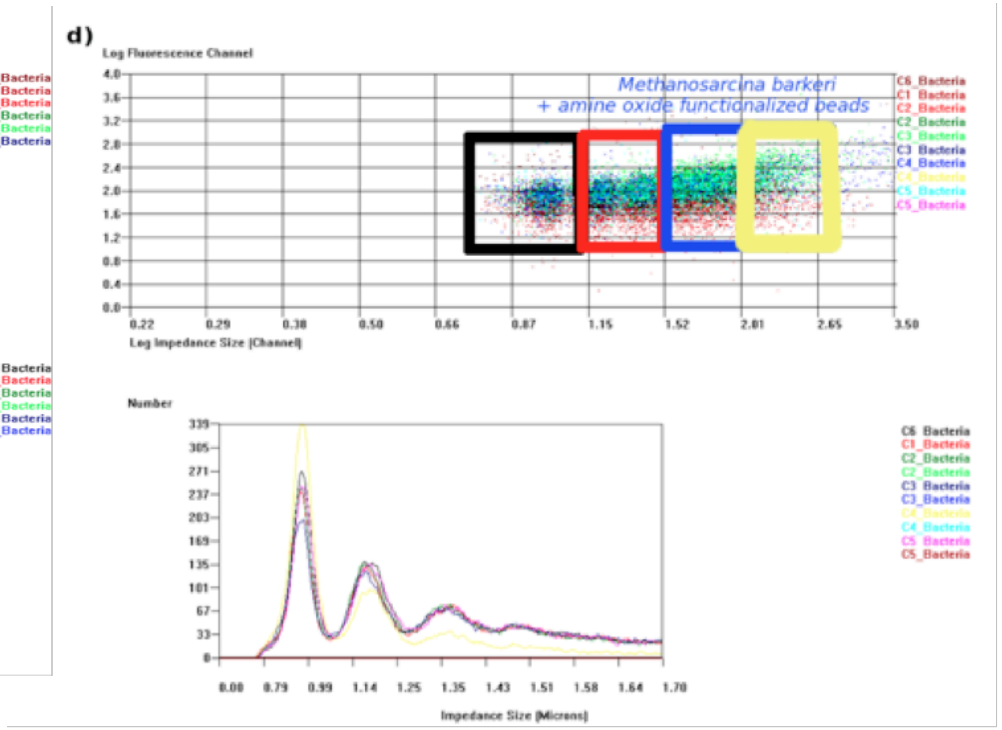


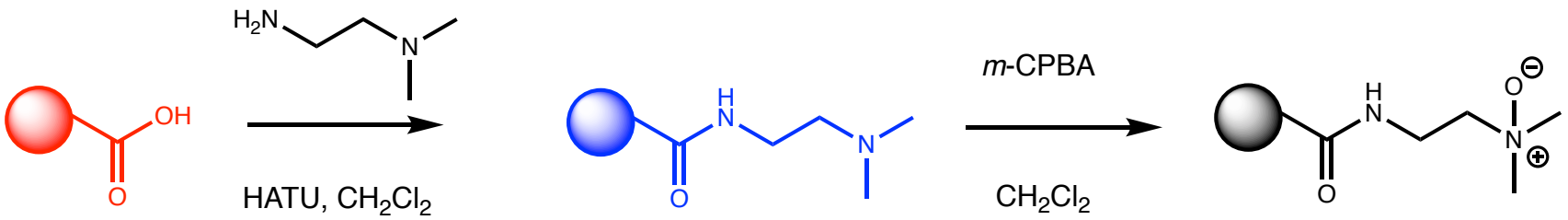
1µm polystyrene beads

D Dobrzanska, *PhD Thesis,* **2014**

Dimethylamino- and amine *N*-oxides

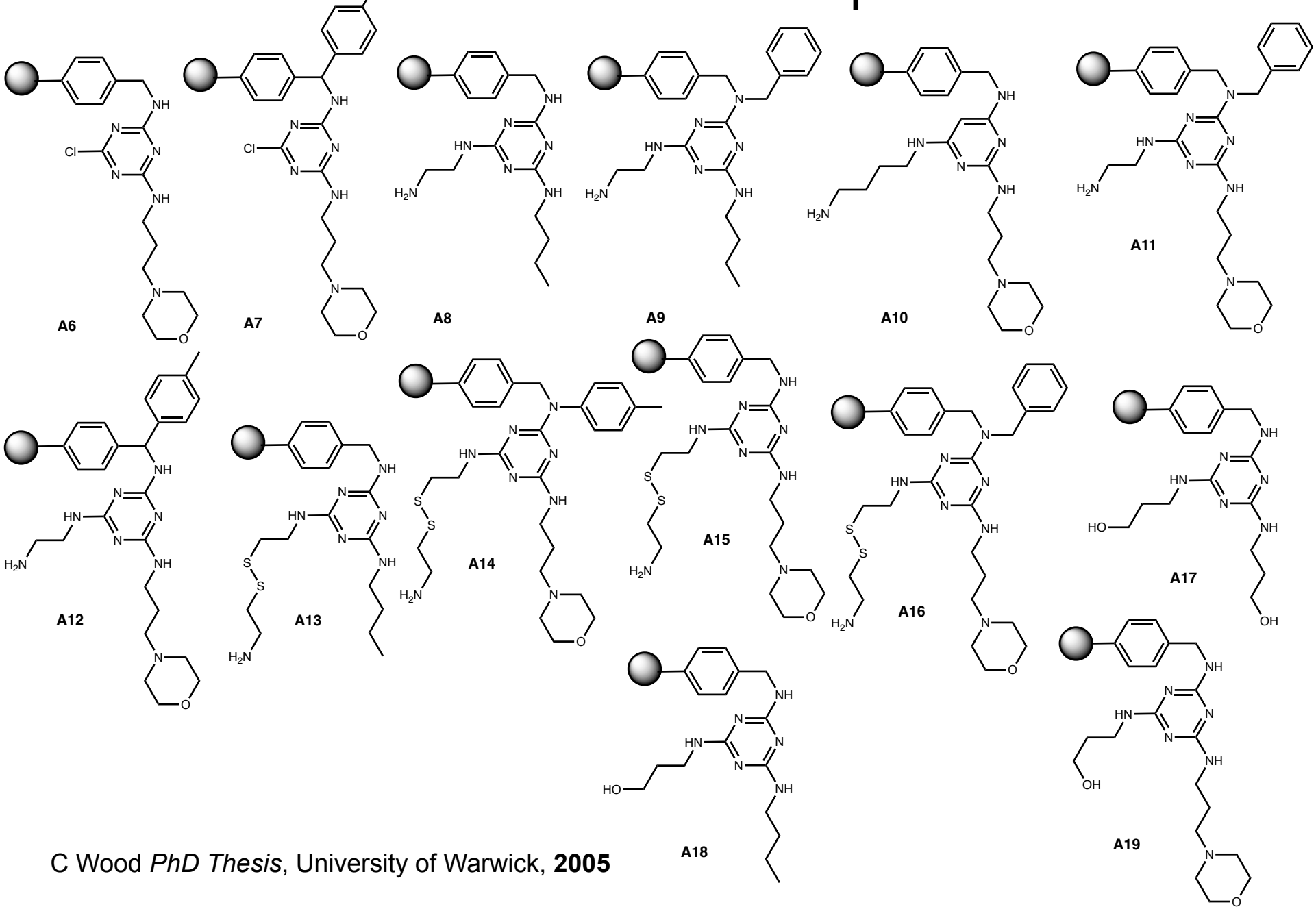






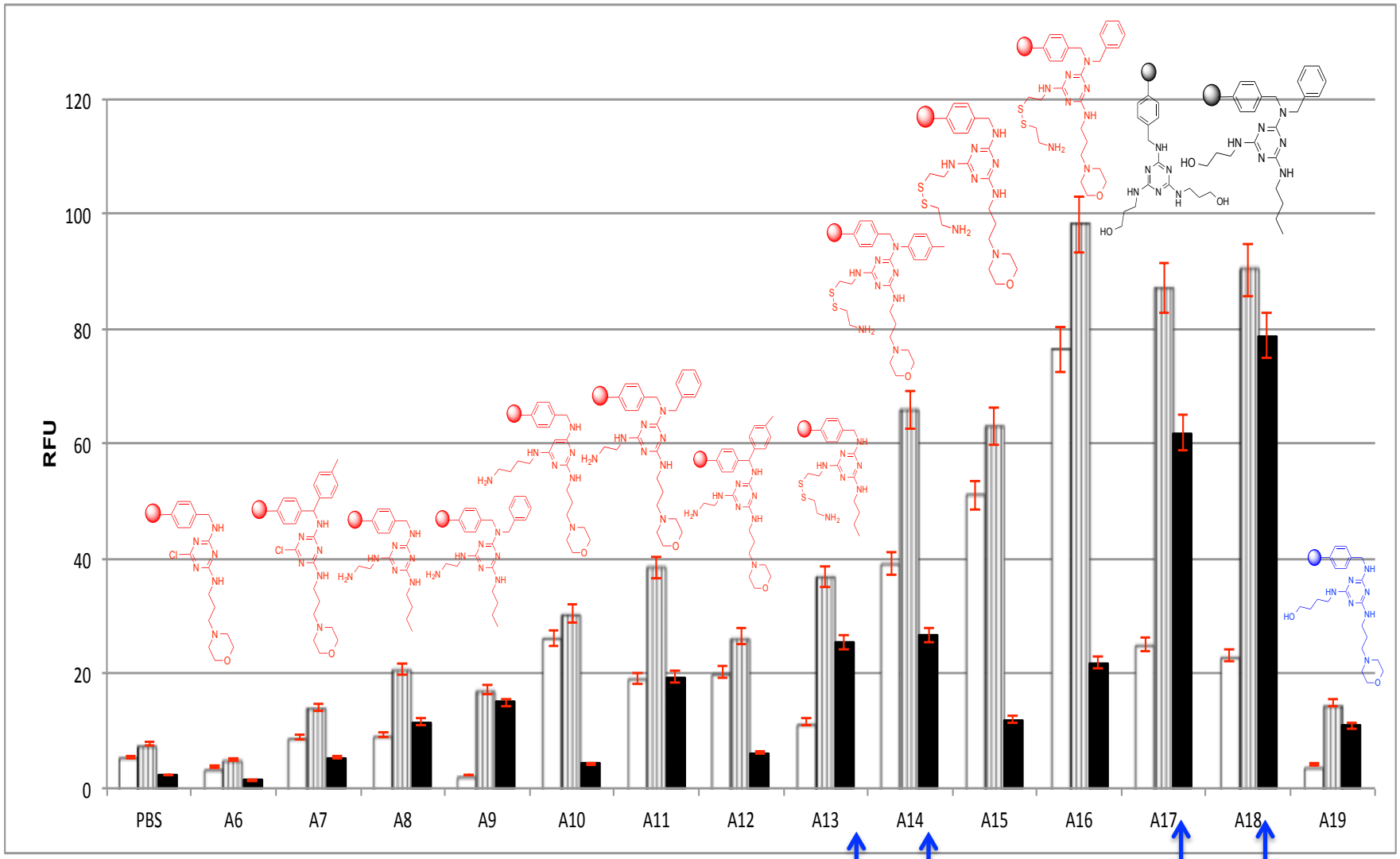
D Dobrzanska, *PhD Thesis,* **2014**

Triazine decorated 100-200 μm PS beads



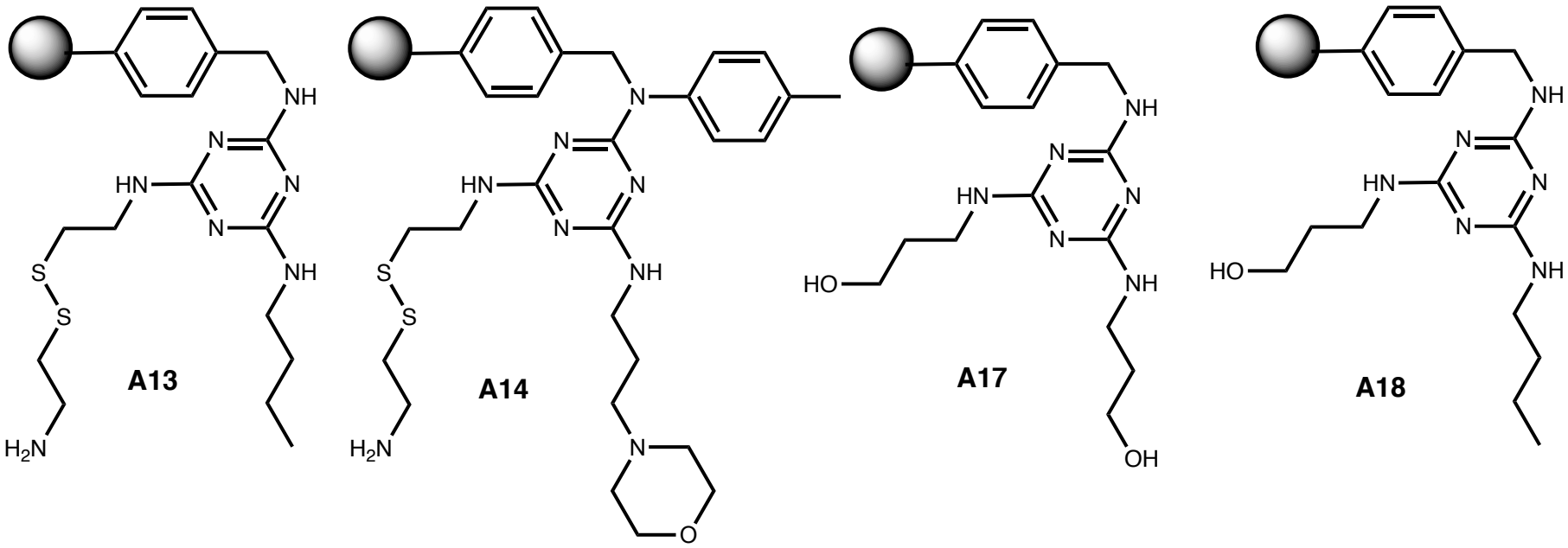
C Wood *PhD Thesis*, University of Warwick, **2005**

Fluorescence plate reader assay



Fluorescence intensity of:
(a) **White** PS beads in PBS buffer after 12h, anaerobic chamber.
(b) **Grey** *M. barkeri* with PS beads in PBS buffer
(c) **Black** *M. barkeri* intrinsic fluorescence by subtraction *i.e.* proportional to concentration

PS beads binding *M. barkeri*



Calc. pKa 9.6 (NH_3^+)	9.6 (NH_3^+)	8 (triazineNH^+)	6.2 (triazineNH^+)
at pH 7.4	7 (morpholineNH^+)		8 (triazineNH^+)

Best interactors neutral hydrophobic with −OH

D Dobrzanska, *PhD Thesis, University of Warwick* **2014**

Methanogen cell envelope structures

Plausible interaction with hydrophobic cell envelope. Possibility of more specific molecular interactions with glycoproteins.

For overview of archaea cell envelope structure see:

Putative cell envelope and adhesion proteins on the outer layer of *Methanobrevibacter ruminantium* Leahy, S C *et al.* PLOS ONE 2010 *5*: e8926

Methanosarcina acetivorans cell envelope M. A. Arbing *et al. Proc. Natl. Acad. Sci USA* **2012**, *109*, 11812-11817

Supports used for Anaerobic Digester biofilm stabilization

Fixed support	**Advantage**	**Disadvantage**
PVC sheet media	- easy install - low cost - no loss of material	- fouling if rag removal inadequate
Fabric web	- easy install - no loss of material	- prone to brandling worm blooms
Dispersed support		
Polypropylene cylinders	- excellent mixing - high surface area	- media loss - maintenance of aeration system
Polyurethane foam	- excellent mixing - high surface area	- media loss - maintenance of aeration system

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High-throughput 16S rRNA sequencing of surface bound microbes

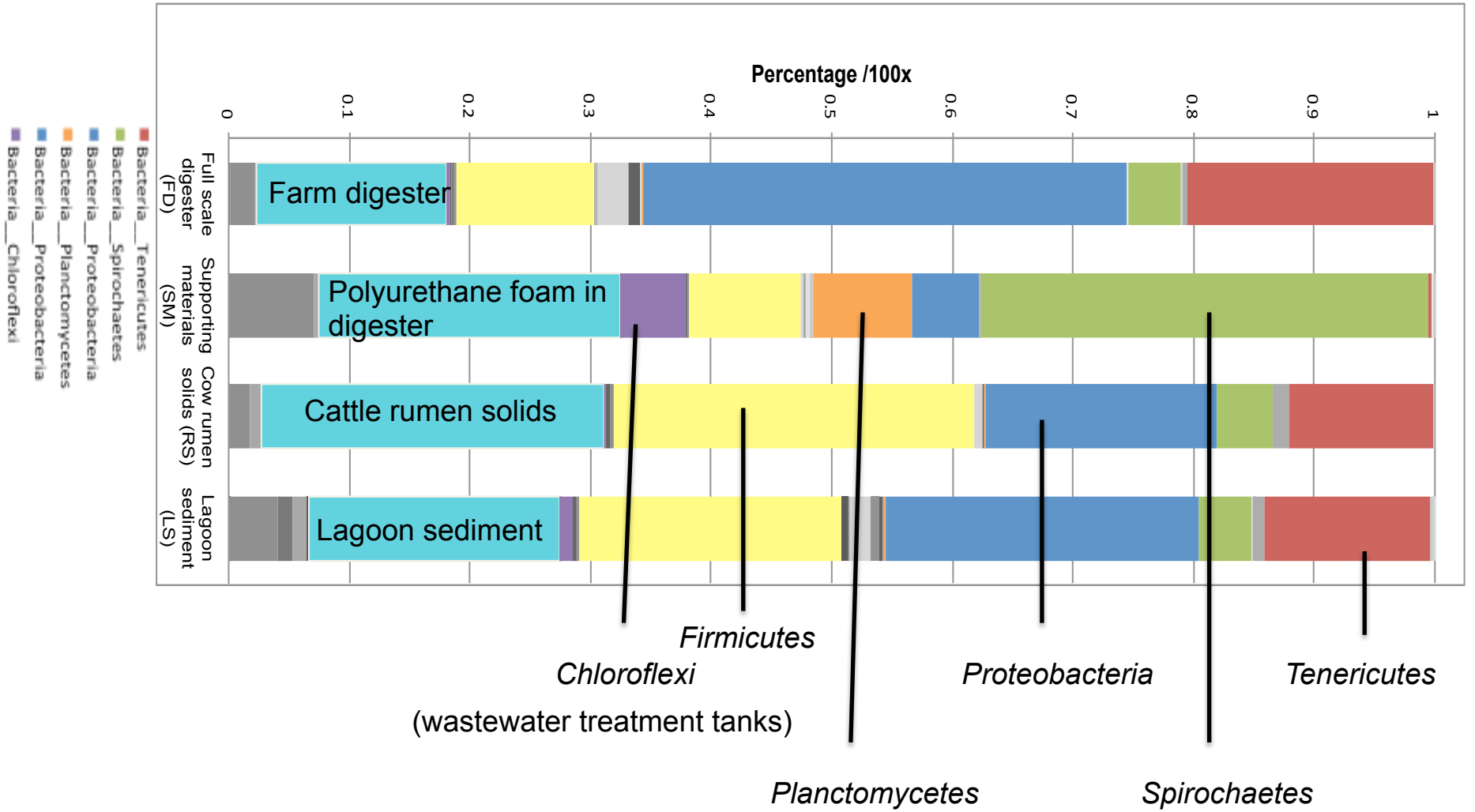
Extracted from microbes:

- In farm scale digester liquid
- On polyurethane foam surfaces suspended in farm scale digester
- Cattle rumen solids
- Cattle slurry lagoon

Sequences searched using QIME pipeline against `greengenes` 16S RNA database to identify *bacteria* and *archaea*

D A Dobrzanska, C G Dowson, P C Taylor, A Marsh, manuscript to be submitted.

Bacteria from cattle slurry at phylum level using16S rRNA sequencing



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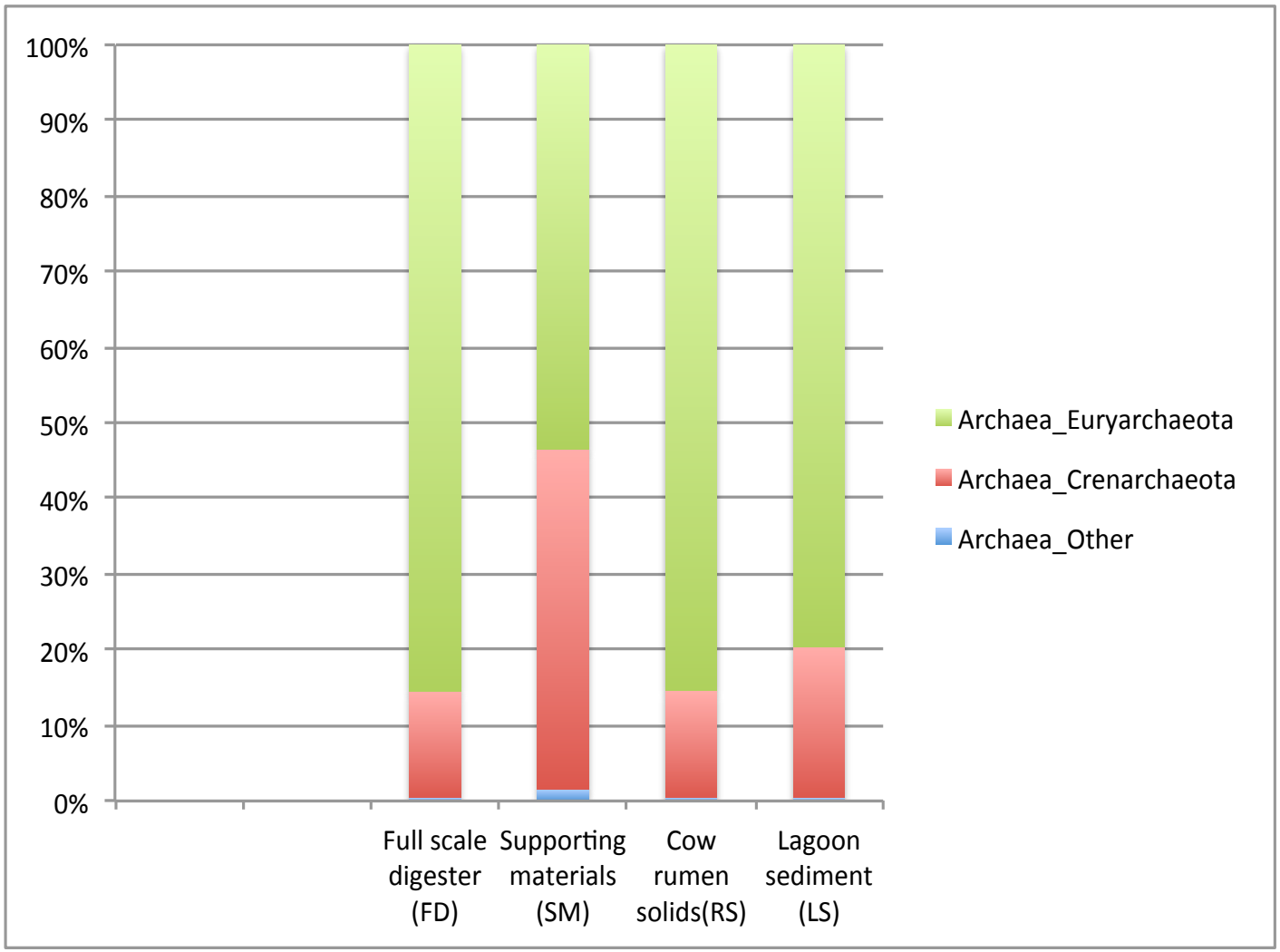
Archaea at phylum level from 16S rRNA sequencing



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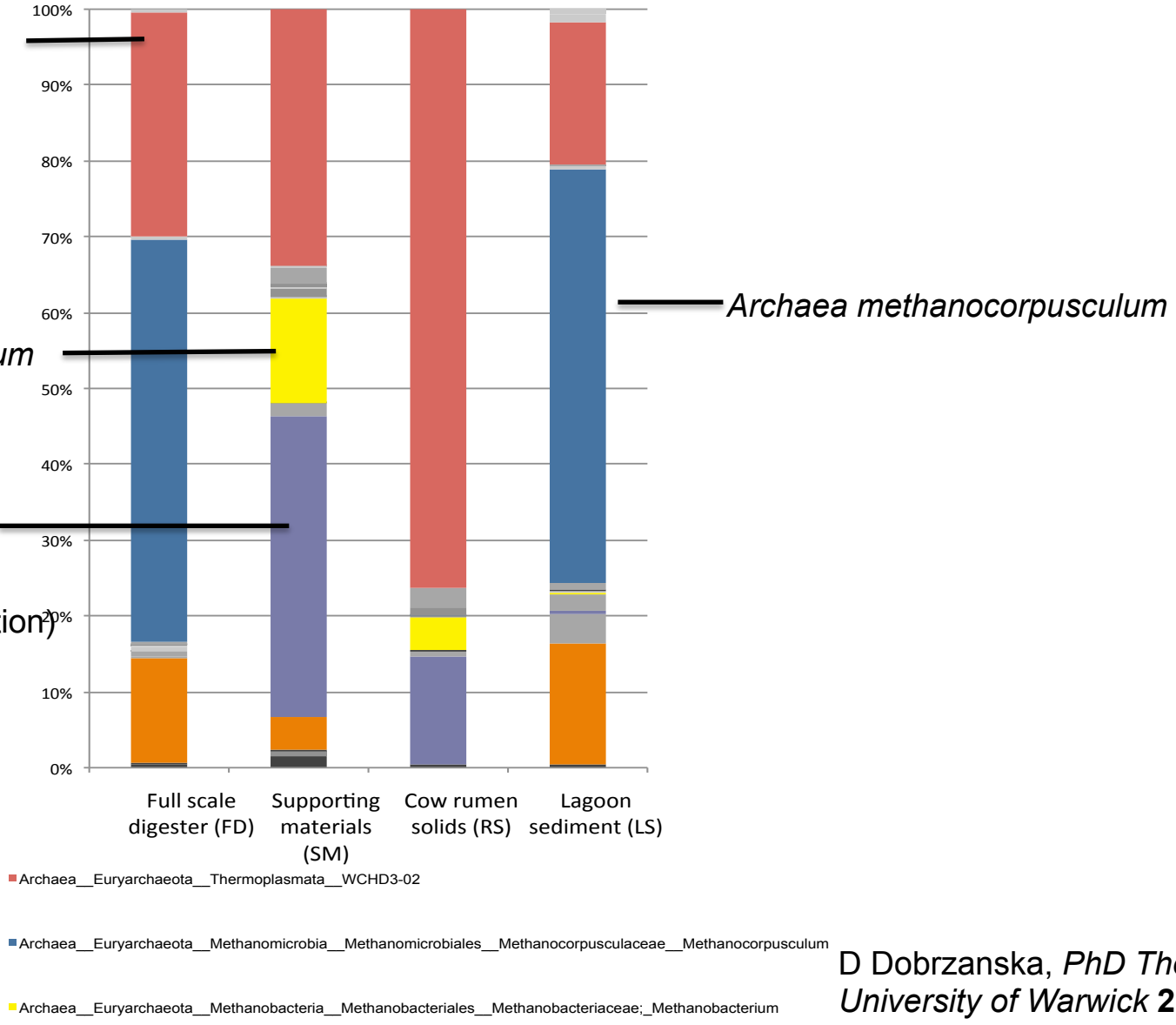
Archaea at genus level from 16S rRNA sequencing

Archaea thermoplasmata

Archaea methanobacterium

Archaea crenarchaeota thermoprotei

(contribute to carbon fixation)



Archaea methanocorpusculum

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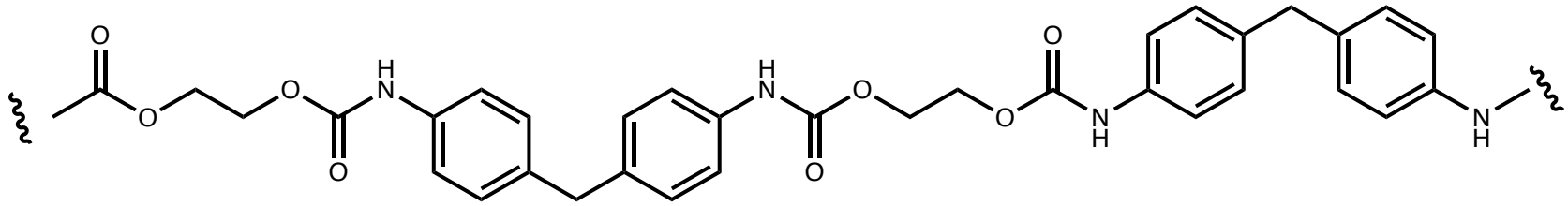
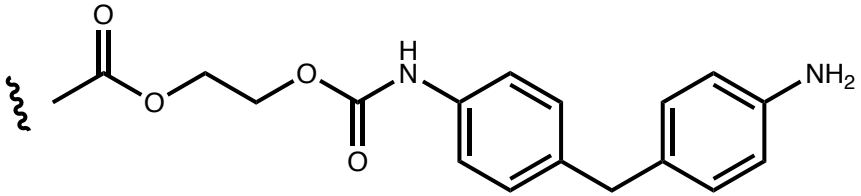
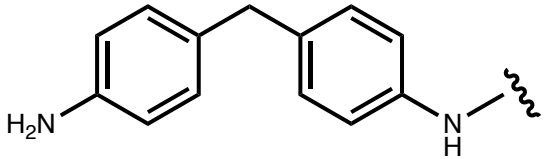
Autoclaved polyurethane surface chemistry

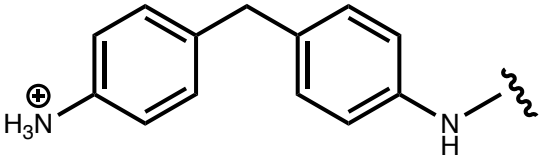


H_2O, 121°C





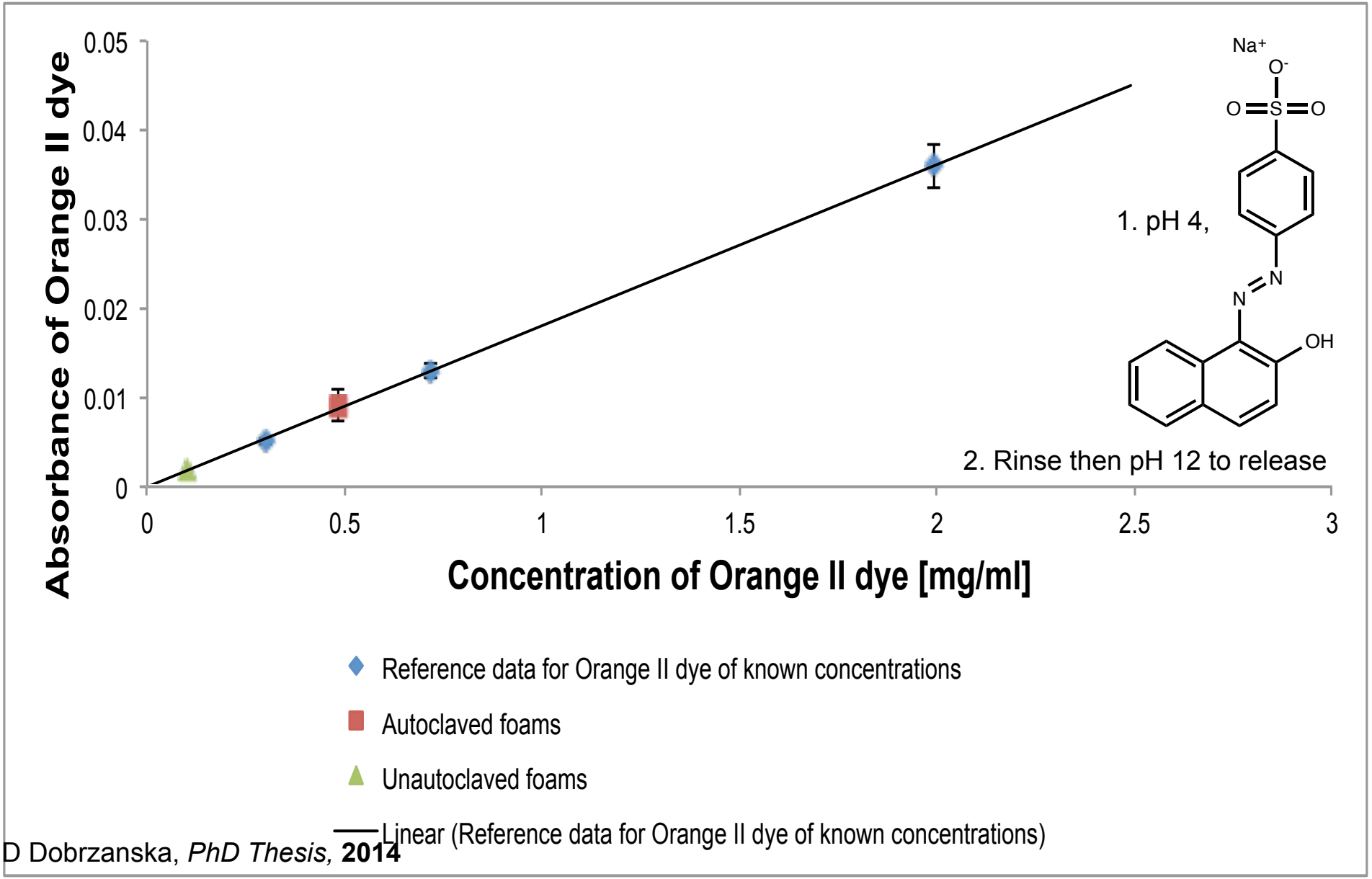
H_2O



pK_a $PhNH_3^+$ = 4.6 (H_2O)

…. its why medical devices containing PU are not sterilised with heat!

Measuring amines exposed at surface



D Dobrzanska, *PhD Thesis,* **2014**

Summary

• Studied a wide range of substrates and derivatives at interfaces and demonstrated utility of tertiary amine *N*-oxide as a protein resistant functional group

•Found uncharged surfaces preferentially attract a prototype methanogen, *M. barkeri*, possibly interacting with glycoproteins set in a hydrophobic cell envelope

• PU foam attracts a unique set of microorganisms, including methanogens from a large scale anaerobic digester

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- Christopher Dowson, Craig Griffin, Jacqueline Marshall Richard Napier (School of Life Sciences & HRI)
- John Griffin, Stephen Brown (Dept of Physics)
- Stephen Evans, Benjamin Johnson (University of Leeds)

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go.warwick.ac.uk/marshgroup