Karlsruhe Institute of Technology
Institute of Functional Interfaces

UNIVERSITÄT HEIDELBERG

Amphiphilic Polysaccharides as Inert Surface Coatings

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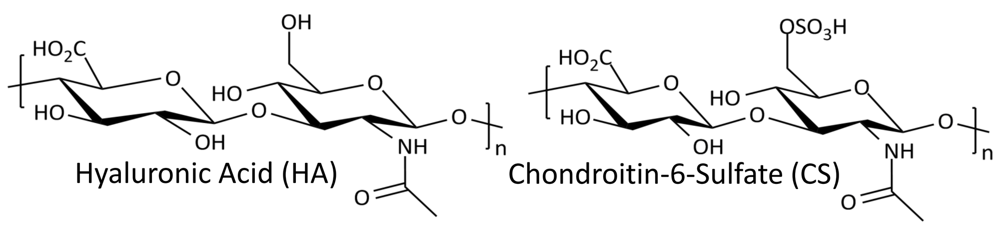
Introduction

- Polysaccharides are resistant to the adhesion of proteins, mammalian cells and bacteria[1]
- Against marine biofoulers, the coatings perform worse than expected due to the complexation of bivalent ions[2]



- Glycosaminoglycans, found in the extracellular matrix, highly hydrophilic, differ in sulfatation of CS, both have only one carboxylic-acid moiety per disaccharide unit. The use of CS is bioinspired due to its presence in fish mucus and its potential contribution to protection of the skin of fish[3]
- Amphiphilic surface-coatings have a higher anti-fouling performance than those which are only hydrophilic or hydrophobic[4]

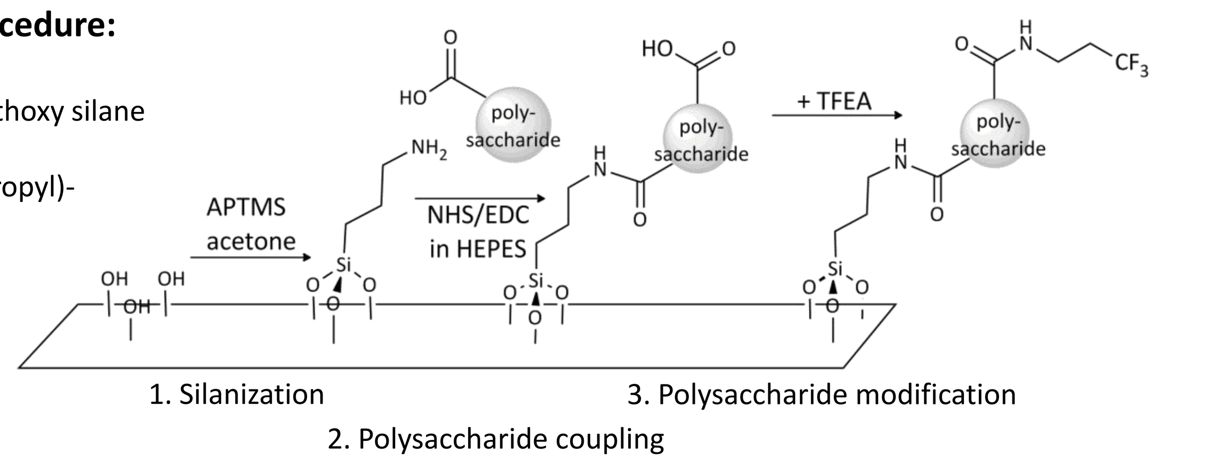
Motivation and Hypothesis

- Polysaccharides are biocompatible and non-toxic
- Capping of free carboxyl-groups prevents complexation of bivalent cations like Ca^{2+} and hence syneresis of the films
- A hydrophobic protection molecule (fluorinated amine) establishes amphiphilic properties in the highly hydrophilic polysaccharide network
- Additionally, the contact angle is shifted towards the minimum in the Baier curve
- Standard assays with a range of different foulers to correlate the change in surface chemistry with the different settlement preferences: biofilm building microorganisms, hard and soft macrofoulers

Surface Preparation

Polysaccharide coupling procedure:

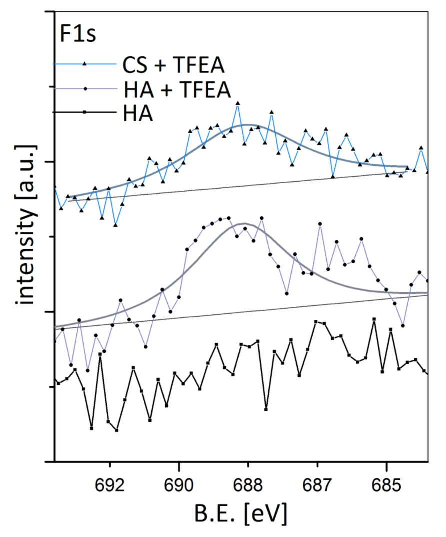
APTMS - 3-Aminopropyltrimethoxy silane
NHS – *N*-Hydroxysuccinimid
EDC – *N*-(3-Dimethyl amino propyl)-3-ethyl carbodiimid
TFEA – Trifluoroethylamine



Characterization:

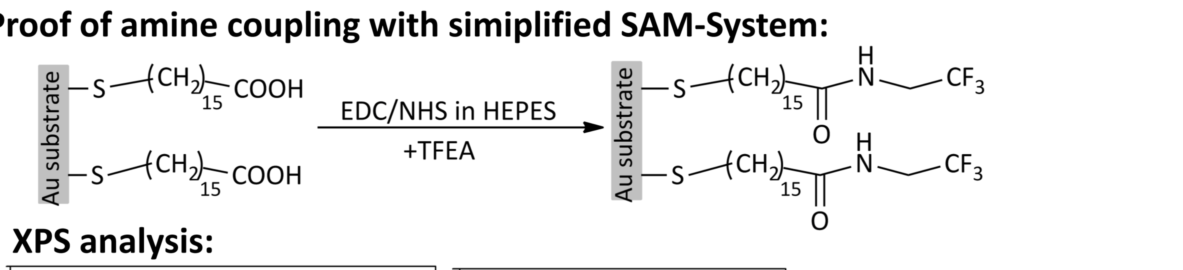
Coating	APTMS	HA	CS	HA + TFEA	CS + TFEA
Contact angle [°]	35 ± 5	< 10	11 ± 3	25 ± 5	28 ± 3
Ellipsometric thickness [Å]	11.4 ± 4.0	26.6 ± 6.6	26.5 ± 7.1	28.2 ± 5.1	28.6 ± 4.0
Thickness from XPS [Å]*	n.a.	23.3 ± 2.5	18.6 ± 5.6	24.8 ± 4.2	23.2 ± 7.7

* Thickness determined by attenuation of the Si2p signal of a representative APTMS layer

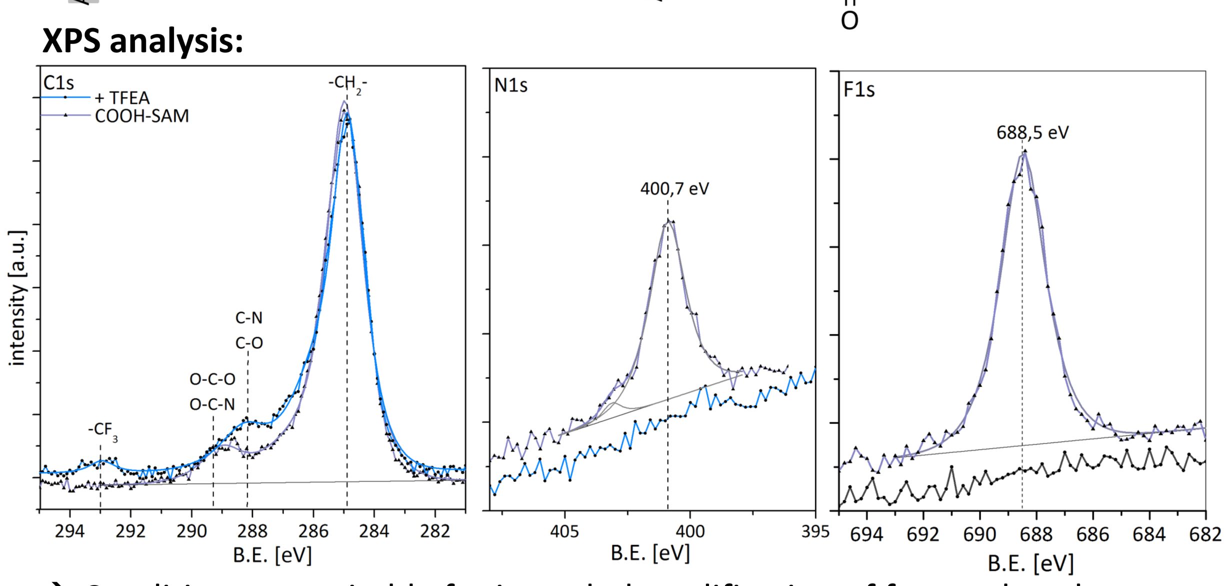


XP-detail spectrum of F1s region

Proof of amine coupling with simiplified SAM-System:



XPS analysis:



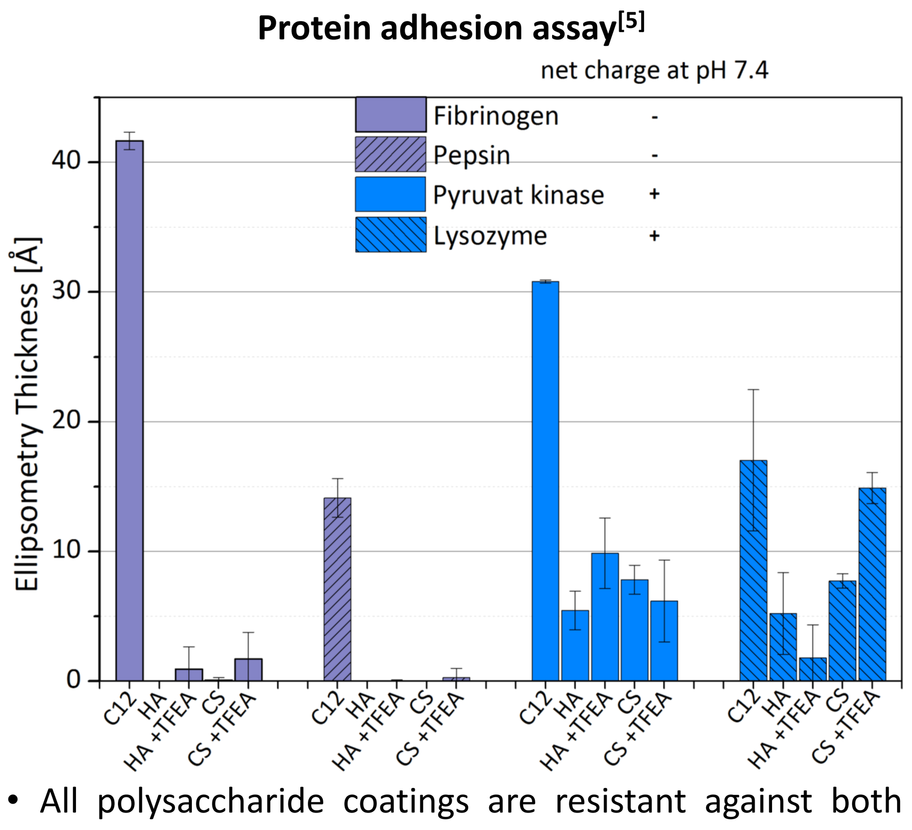
→ Conditions are suitable for intended modification of free carboxyl groups

Biological Evaluations

Lab adhesion experiments: Proteins and biofoulers

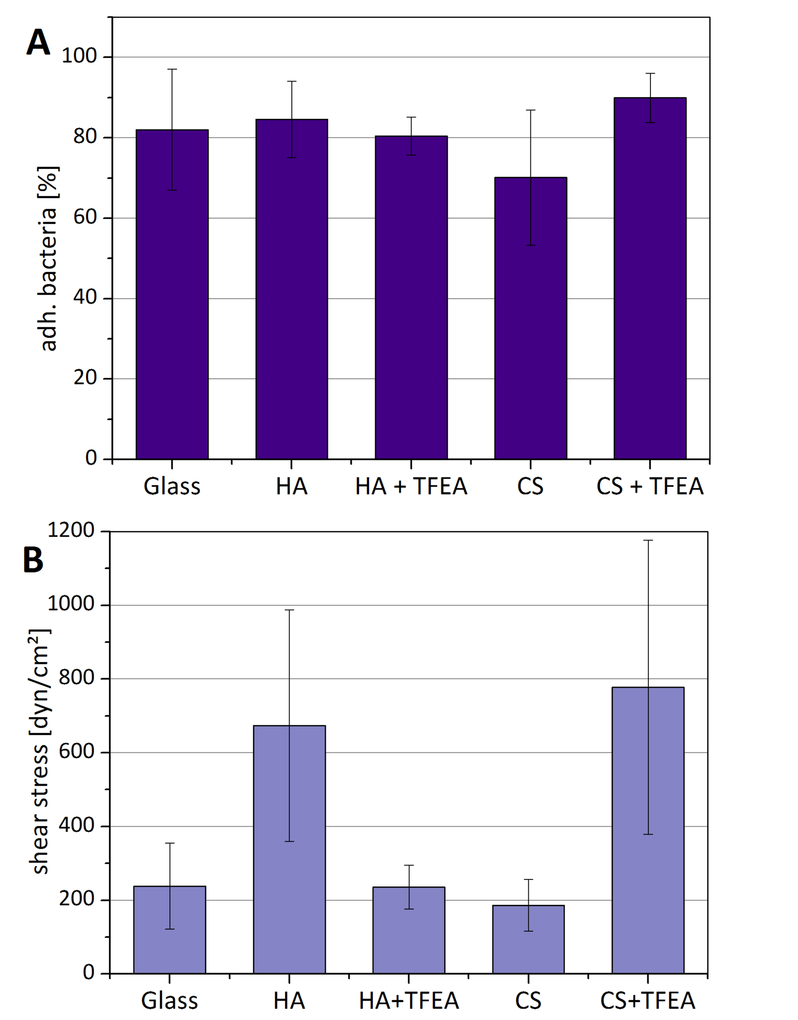
- Protein affinity test:[5]
  Determination of protein film thickness by spectral ellipsometry
- Bacteria:
  Microfluidic *Cobetia marina* assay (M.P. Arpa Sancet)
- Algea:
  Navicula perminuta settlement and detachment assay (J. Finlay, University of Birmingham)
  Ulva linza settlement and detachment assay (J. Finlay, University of Birmingham)
- Barnacles:
  Balanus amphitrite settlement assay (N.Aldred, University of Newcastle)

Protein adhesion assay[5]



- All polysaccharide coatings are resistant against both negatively charged proteins
- Positively charged proteins adhere more readily compared to the negative ones, but in most cases weakly compared to the not-resistant C_{12}–SAM standard

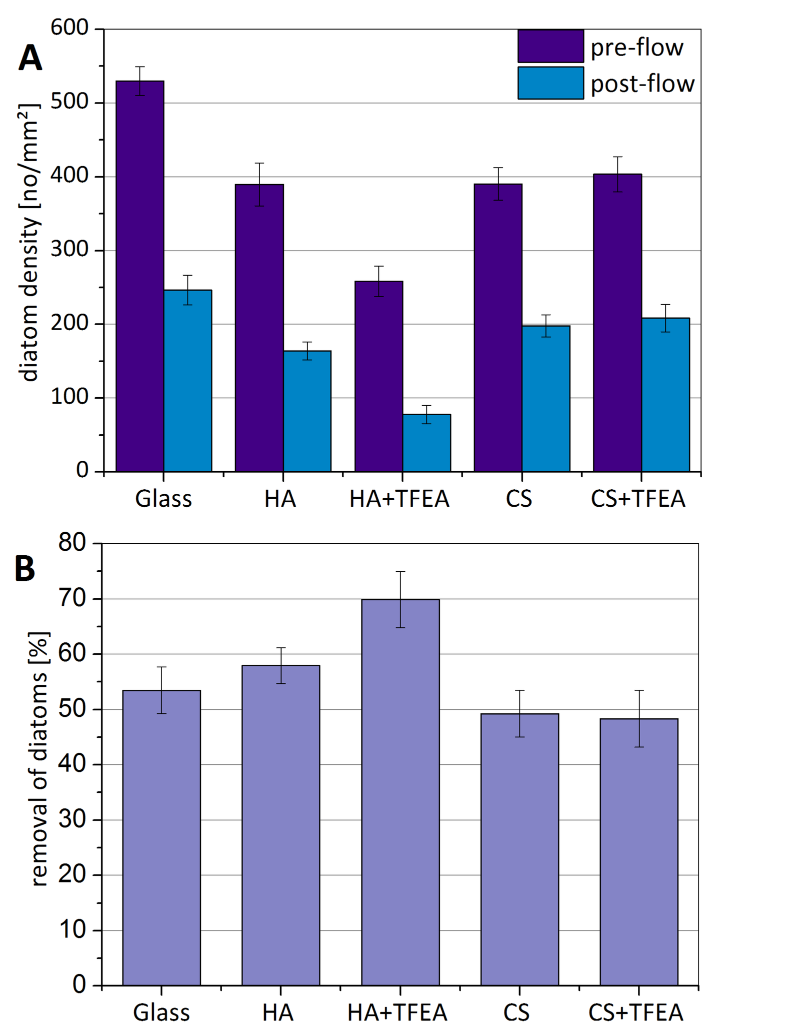
Adhesion strength of *Cobetia marina*



A - Initial settlement after incubation
B - Critical shear stress to remove 50% of adherent bacteria

- Initial settlement is not influenced by different surface chemistries
- Adhesion strength is weakened on TFEA modified HA surfaces
- Native CS performs very well, behaviour after TFEA capping is reversed

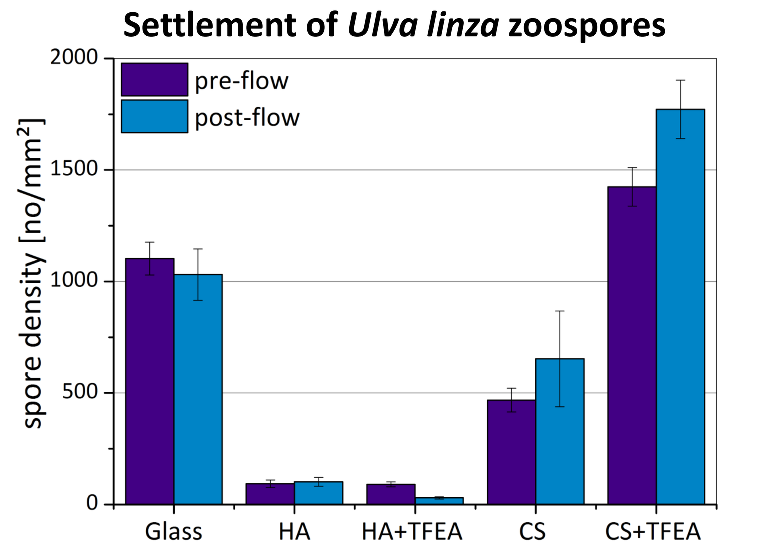
Attachment of *Navicula perminuta*



A - Comparison of diatom density before and after shear stress (32Pa)
B - Percentage removal

- TFEA capping weakens attachment for HA
- Attachment on CS surfaces is in range of glass standard, modification shows no effect

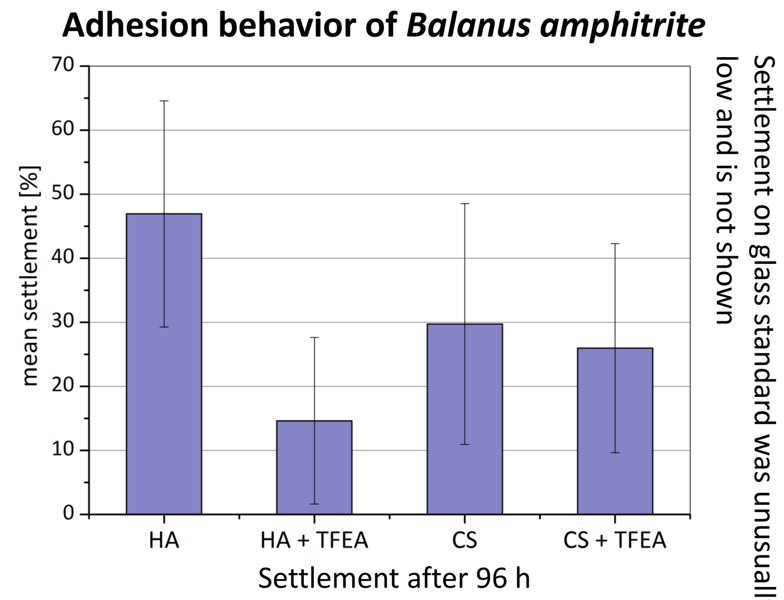
Settlement of *Ulva linza* zoospores



Spore density before and after shear stress (52Pa)

- Settlement is very low on both HA coatings
- Modification makes CS films more attractive and adhesion strength increases

Adhesion behavior of *Balanus amphitrite*



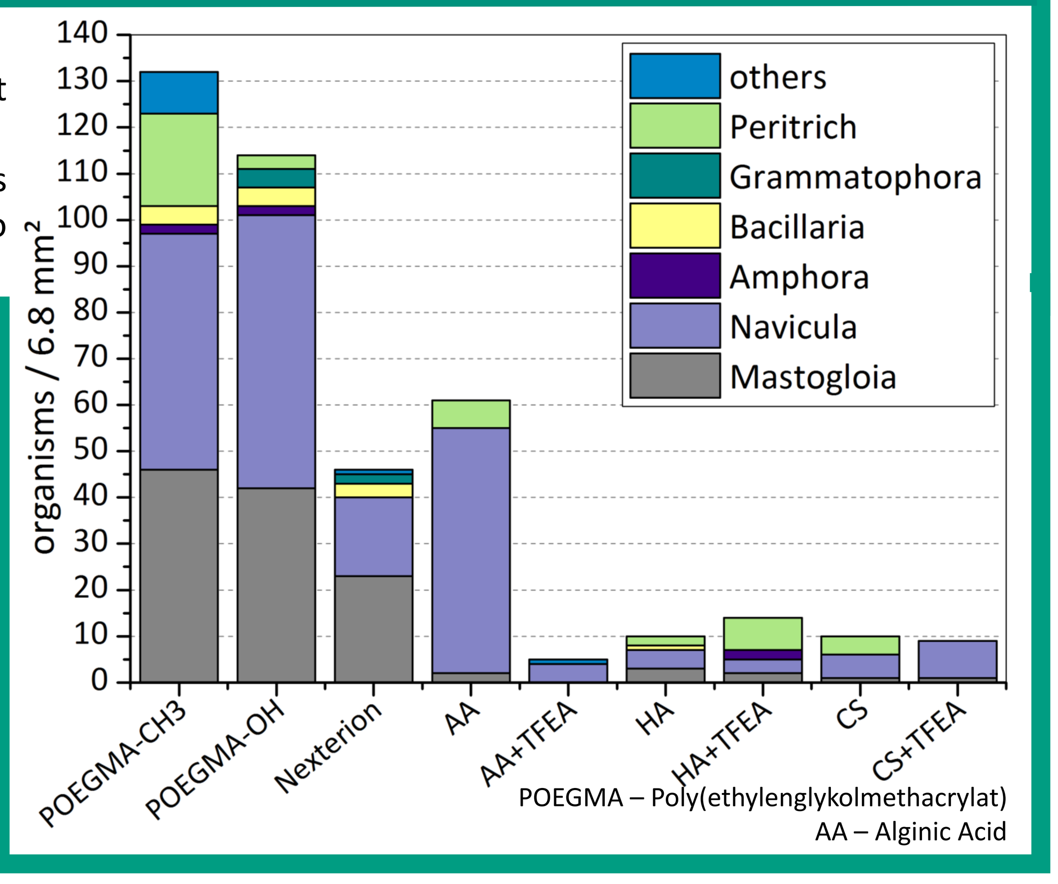
Settlement after 96 h

- Settlement is strongly reduced on modified HA
- Settlement comparable on both CS surfaces

Field tests in Melbourne, Florida (USA)

- Settlement after 48h incubation in sea water
- Quantification of organisms bigger than 10µm by optical microscopy

- Mainly diatom settlement
- All sugar coatings reduce settlement significantly
- Strongest effect of TFEA coupling is observed for AA, which contains two carboxyl-groups per disaccharide unit



Literature

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Conclusions

- Amide modification does not worsen good protein resistance
- Modification with TFEA has a positive impact on HA coatings: decreased adhesion strength of *C.marina*, lower settlement and decreased adhesion strength of *N.perminuta*, *U.linza* and *B.amphitrite*
- Opposite behavior or no change in settlement of the different species is observed for CS coatings (likely related to the negative charge carrying sulfate-group)

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