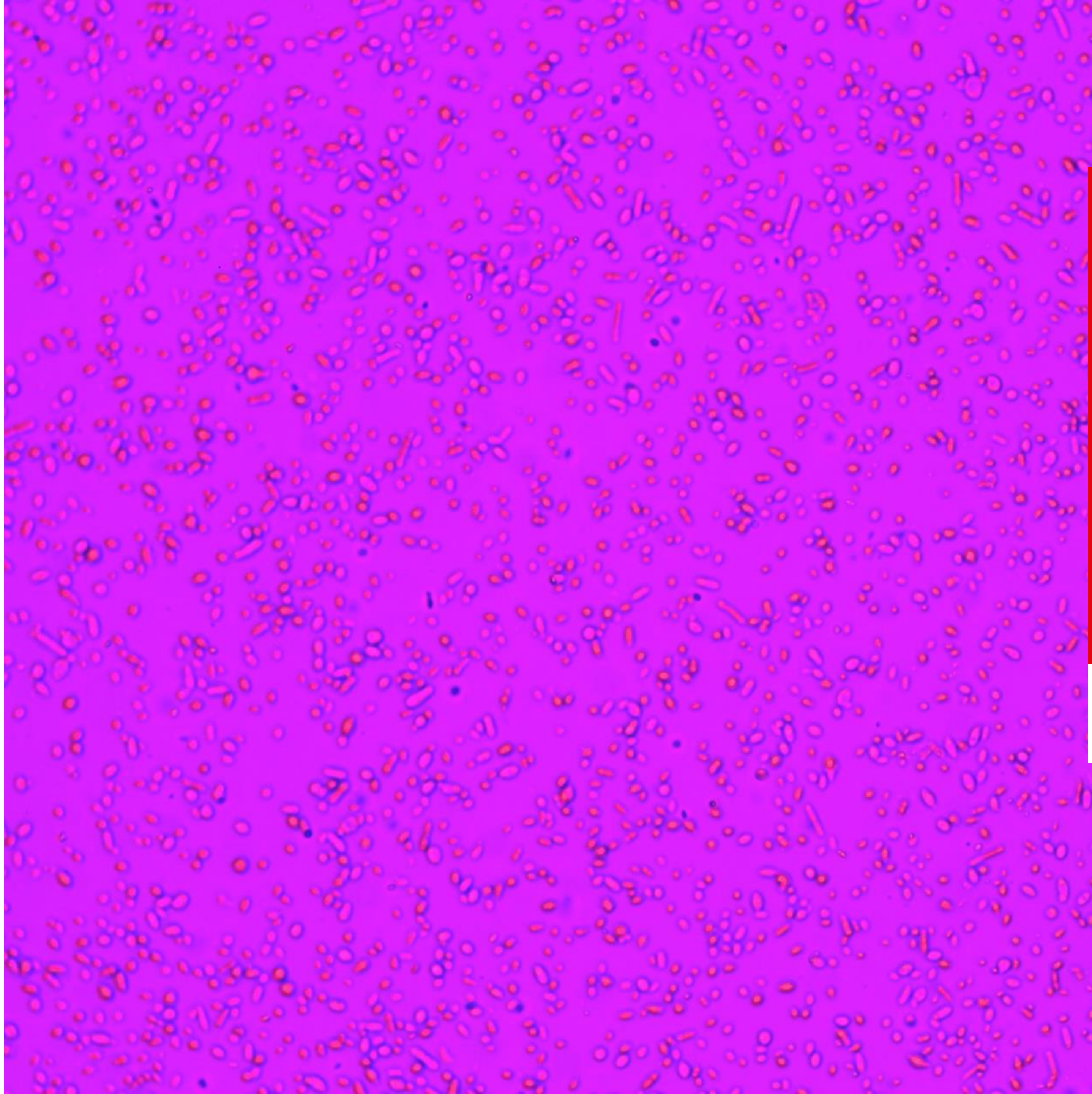


Feedback and amino acid coatings

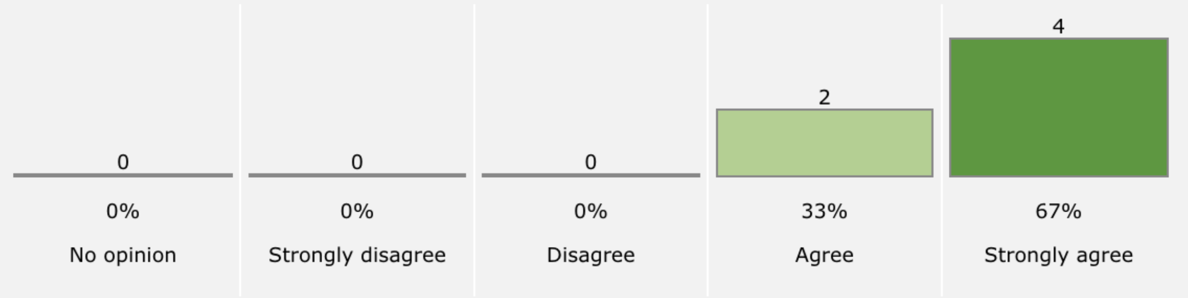
Prof. Tiffany Abitbol

2025

Mid-term feedback

Year	2025-2026
Course	Engineered living materials
Questionnaire	Indicative feedback of teaching (since 2022-2023)
Nb Registered	20
Nb Answered	6

The running of the course enables my learning and an appropriate class climate



Remarks
[2 remarque(s)]

- I like that we work with real papers and discuss them. I also like the course atmosphere. Besides that the lab was very cool!
- Very interesting topics and it is good to analyse together some scientific papers. It might be interesting to learn more about searching for scientific papers (using Google Scholar, etc.), as well as learning and writing. It could also be interesting to have more time to speak about the poster.

Thanks for the feedback!

- 1/3 of the class
- Final feedback will be in class during poster
- Today we will discuss the poster extensively in the second half

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ChemComm

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

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Received 12th May 2014,
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DOI: 10.1039/c4cc03578j

www.rsc.org/chemcomm

Self-assembly of a tripeptide into a functional coating that resists fouling†

Sibaprasad Maity,‡ Sivan Nir,‡ Tal Zada and Meital Reches*

2014

What if you want surfaces to be inhospitable to microorganisms?

- IF = 4.3
- Time to 1st decision = 24 days
- Urgent communications of outstanding significance from across the chemical sciences. Opt in for double-anonymised peer review.
- Cited 85 times

Biofouling

https://en.wikipedia.org/wiki/Biofouling

- Unwanted accumulation of microorganisms (plants, algae, small animals) and their biproducts on surfaces

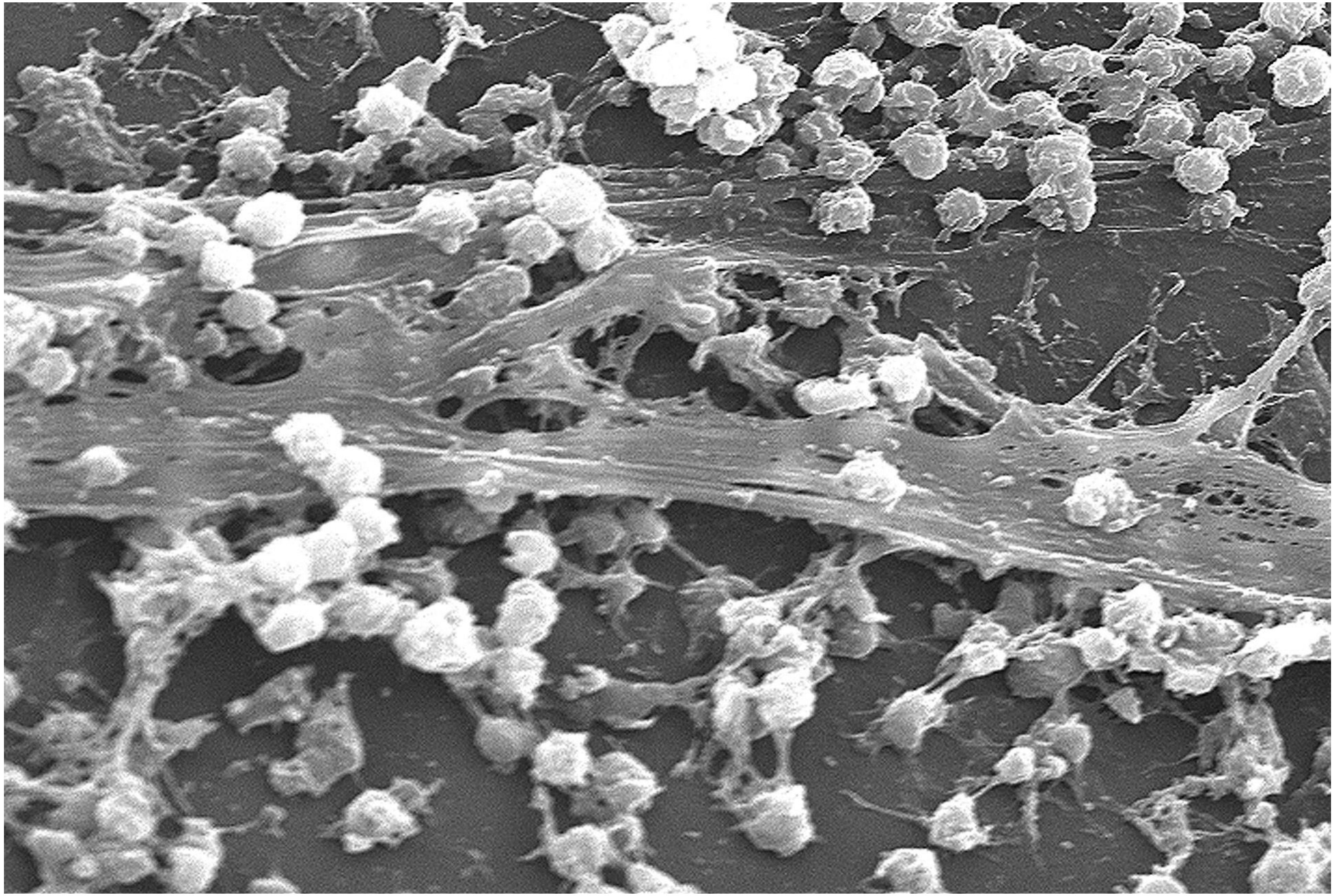
- Starts by the adsorption of bioorganic molecules and then continues with the attachment of organisms to this bioorganic layer

- In the case of bacteria, this leads to a well-defined bacterial network called a biofilm

Current measurement instrument encrusted with zebra mussels

Bacterial biofilm

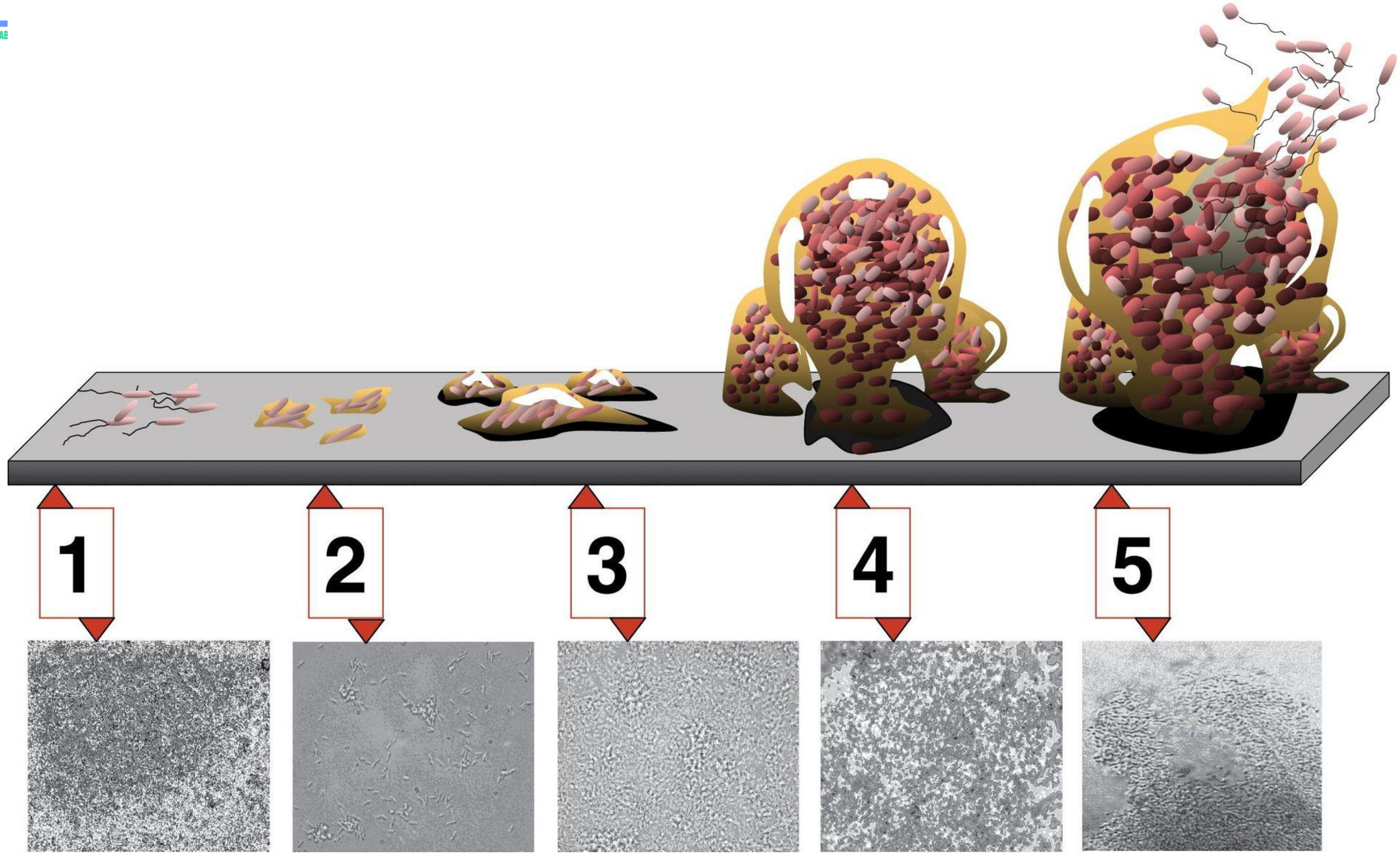
https://en.wikipedia.org/wiki/Biofilm



Staphylococcus aureus biofilm on an indwelling catheter

Bacterial biofilm

https://en.wikipedia.org/wiki/Biofilm



(1) Initial attachment, (2) Irreversible attachment, (3) Maturation I, (4) Maturation II, and (5) Dispersion. Each stage of development in the diagram is paired with a photomicrograph of a developing *P. aeruginosa* biofilm.

Bacterial biofilm

- Bacteria in biofilm can have different properties from free-floating bacteria of same species – the biofilm can protect the bacteria from antibiotics and other threats, like detergents

- Biofilm in medical devices and implants can lead to severe infection – termed hospital acquired infections (very worrisome!)

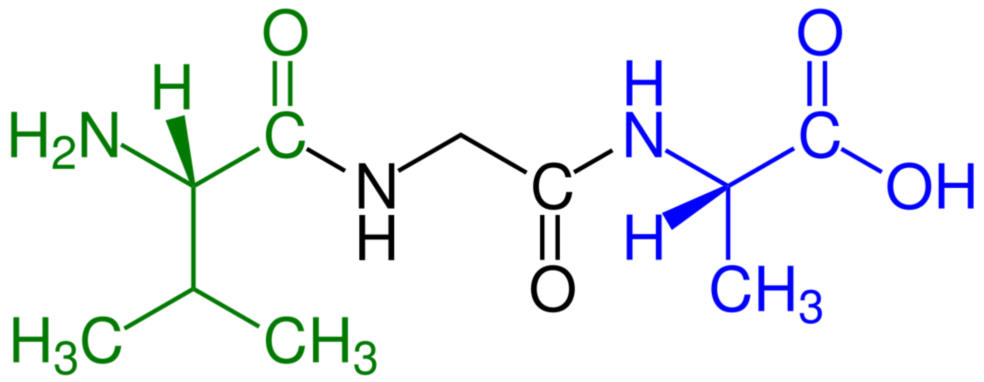
- Also, a big impact on marine industry due to attachment or marine organisms like barnacles and mussels to ships and devices – heavy and thick layer can lead to delays and higher fuel consumption, also linked to gas emissions and the incursion of invasive species into marine habitats

Antifouling approach

- Antifouling materials change the physical and/or chemical properties of a surface in such a way as to prevent the accumulation of microorganisms
- Strategies for non-toxic antifouling can include enzymatic degradation, sonication, and chemical modification (pluses and minuses)
- This paper proposes an antifouling material based on **the self-assembly of a low molecular weight peptide**
- Synthetic tripeptide that interferes with the first step of biofouling
- Numerous reports in the literature on antibacterial peptides but none on antifouling peptides

Tripeptide

- Peptide made of 3 amino acids, joined by 2 or 3 peptide bonds

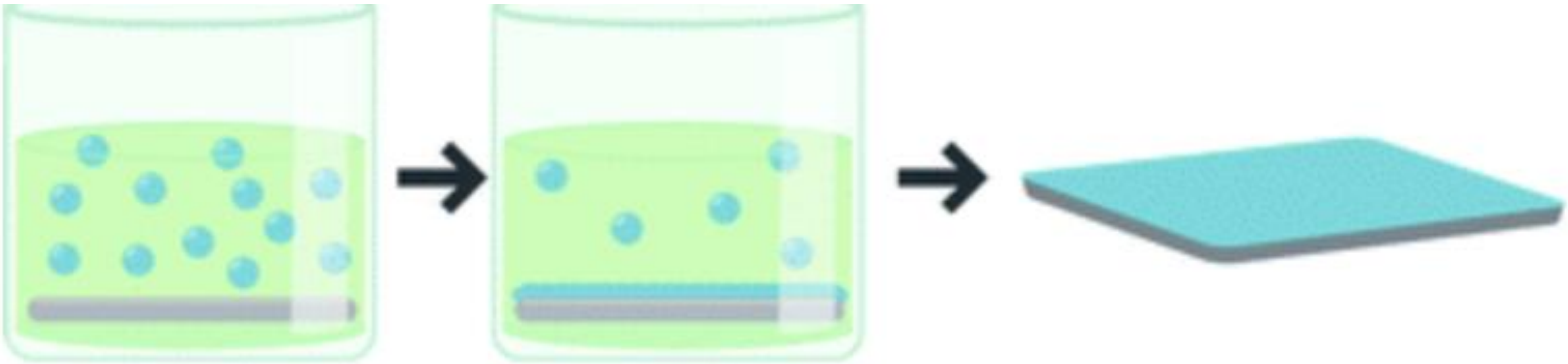


An example of a tripeptide: Val-Gly-Ala

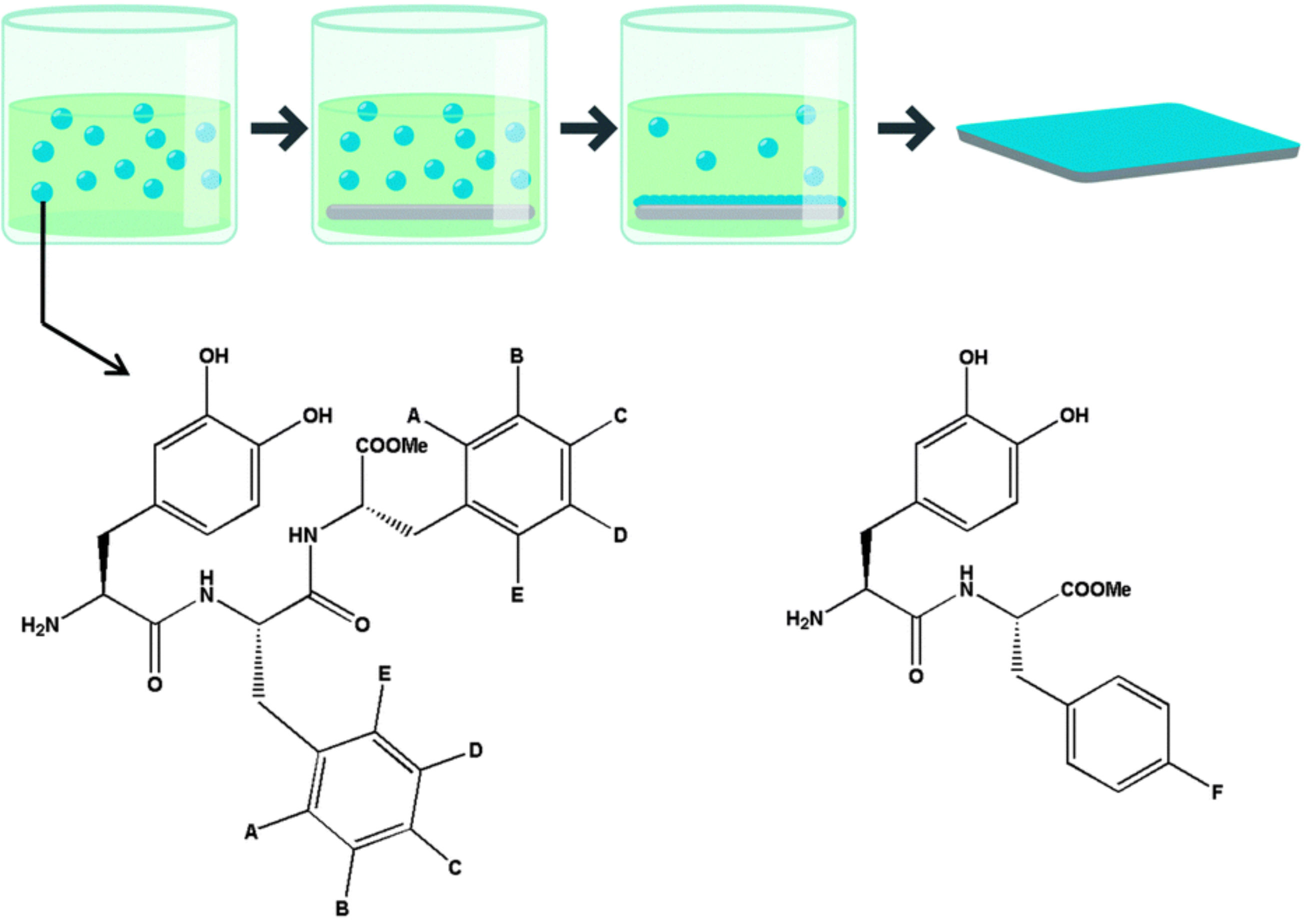
https://en.wikipedia.org/wiki/Tripeptide

Abstract

- This communication describes the self-assembly of a **tripeptide into a functional coating that resists biofouling**. Using this peptide-based coating we were able to prevent protein adsorption and interrupt biofilm formation. This coating can be applied on **numerous substrates** and therefore can serve in applications related to **health care**, **marine** and **water treatment**.



Antifouling with tripeptides

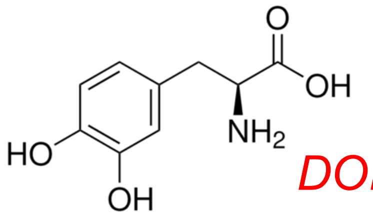


Peptide 1: A=B=D=E=-H, C= -F
Peptide 2: A=B=C=D=E=-F
Peptide 3: A=B=C=D=E=-H

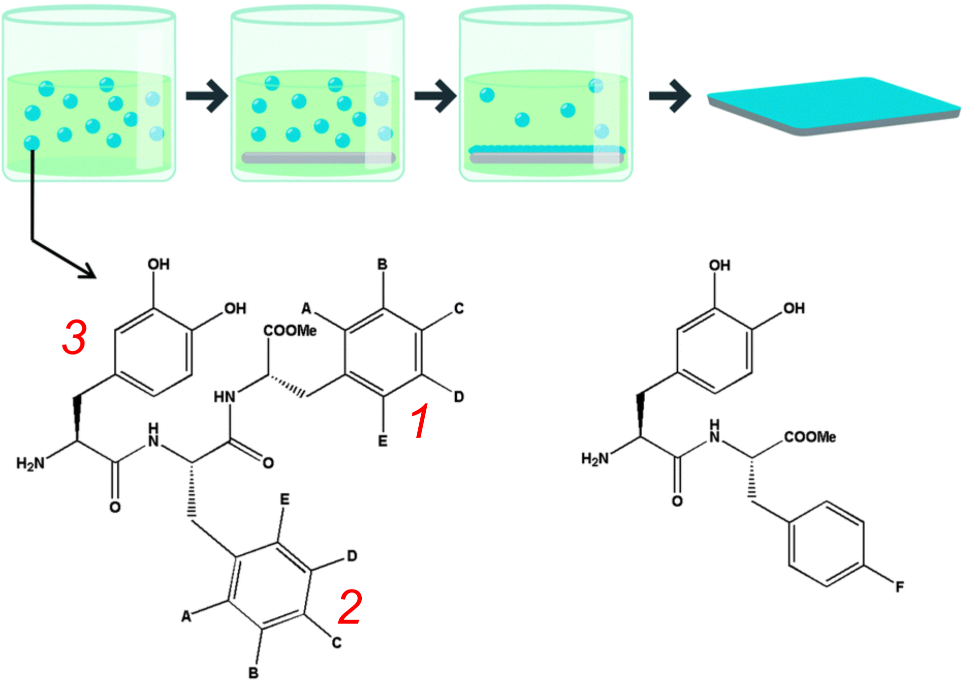
Peptide 4

- Dip coating
- Peptide sequence contains 3 elements that enable: (1) self-assembly into a coating, (2) adsorption onto any substrate, and (3) antifouling activity

Antifouling with tripeptides

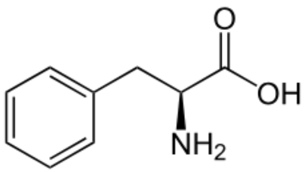


DOPA



Peptide 1: A=B=D=E=-H, C= -F
Peptide 2: A=B=C=D=E=-F
Peptide 3: A=B=C=D=E=-H

Peptide 4



phenylalanine

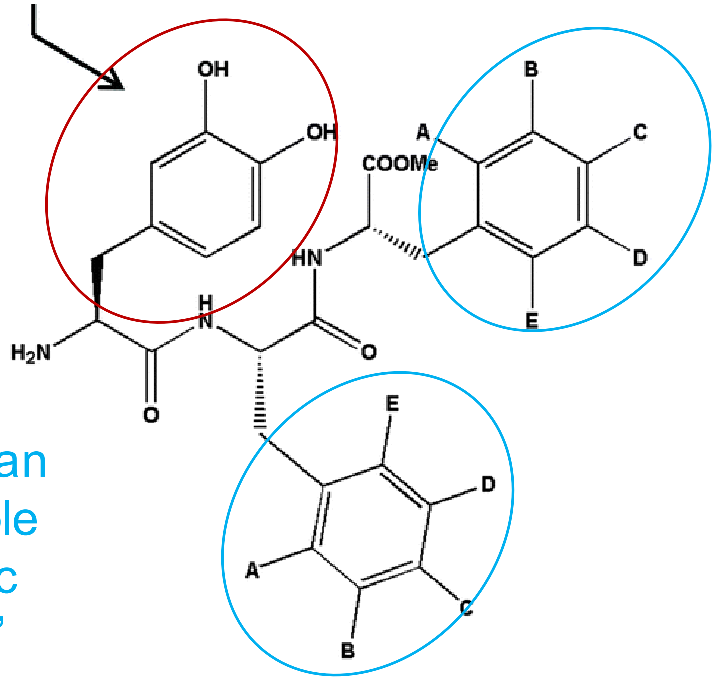
- 2 adjacent **fluorinated** phenylalanine residues (1, 2)
- Self-assembly **via aromatic interactions to** give films
- Carbon-fluorine bonds expected to form a “Teflon-like” non-stick surface to prevent the attachment of proteins, acting as an antifouling motif
- *peptide 1* with only 1 fluorine atom on each benzene ring, and *peptide 2* with 5 fluorine atoms
- 3rd amino acid is DOPA, the main constituent of mussel adhesive proteins (MAPs) – glue of marine mussels – act as a glue in tripeptide
- MAPs can adhere to almost any substrate and under harsh conditions, so can DOPA

Breaking it down

The trifecta:

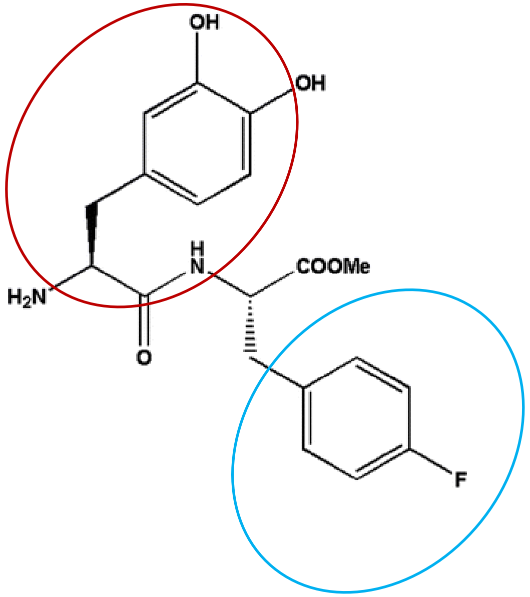
- Nonstick
- Super sticky
- Self assembling

Sticky part/binds to everything



Benzenes can self-assemble via “aromatic interactions”

Peptide 1: A=B=D=E=-H, C= -F
Peptide 2: A=B=C=D=E=-F
Peptide 3: A=B=C=D=E=-H



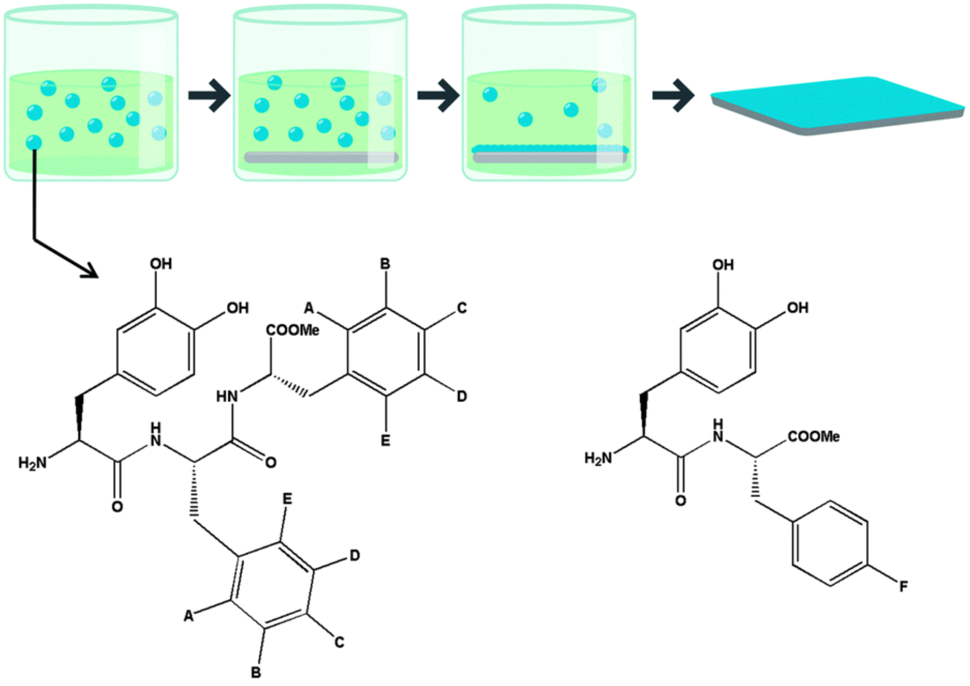
Peptide 4

Benzene with a fluorine atom = non-stick, antifouling part

Questions:

- How many C-F bonds are needed for antifouling?
- What’s the difference between a tri- and a dipeptide in terms of antifouling?

Antifouling with tripeptides



Peptide 1: A=B=D=E=-H, C= -F
Peptide 2: A=B=C=D=E=-F
Peptide 3: A=B=C=D=E=-H

Peptide 4

- Peptides 1 and 2 with fluorine
- Peptide 3 with no fluorine
- Peptide 4: 1 DOPA and 1 fluorinated phenylalanine residue

- Coating was done by dipping (gold, silicon, titanium, glass or stainless steel) in peptide solution

Peptide 1 – contact angle increases

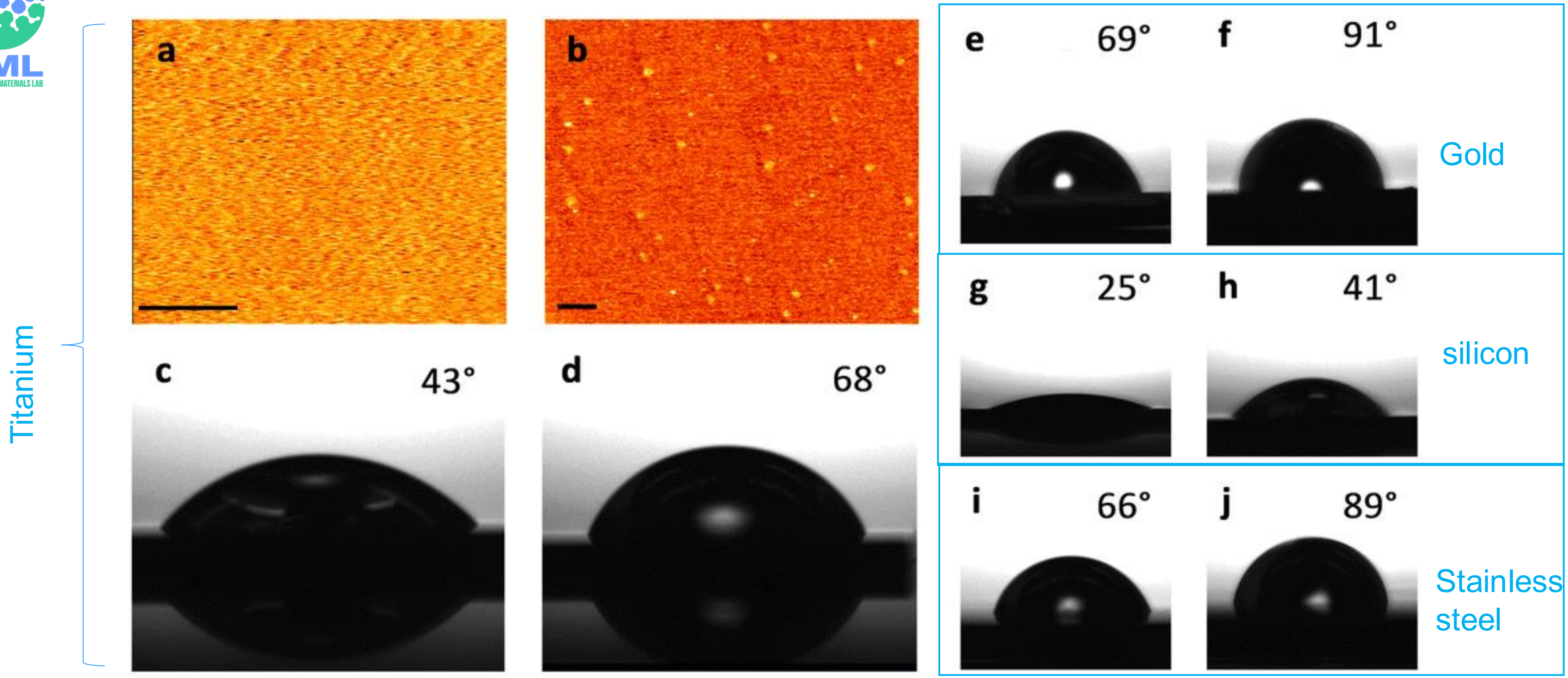


Fig. 2 Surface characterization of bare substrates versus substrates coated with peptide 1. AFM topography images of (a) bare mica, (b) coated mica, the scale bars represent 500 nm. Contact angle measurements of (c) bare titanium, (d) coated titanium (e) bare gold (f) coated gold, (g) bare silicon, (h) coated silicon, (i) bare stainless steel, (j) coated stainless steel.

Peptide 1 – ATR-FTIR

a

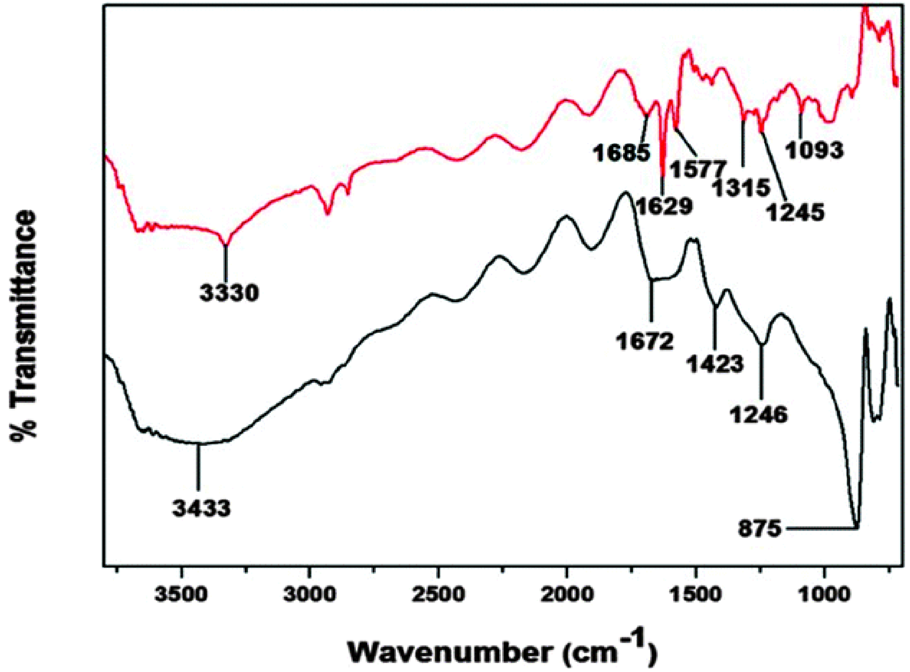
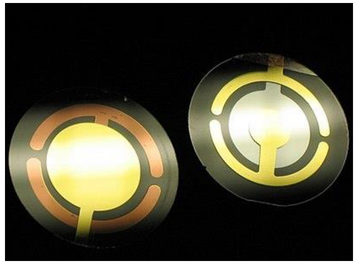


Fig. 3 (a) ATR-FTIR spectra of bare titanium (black) and titanium coated with peptide 1 (red).

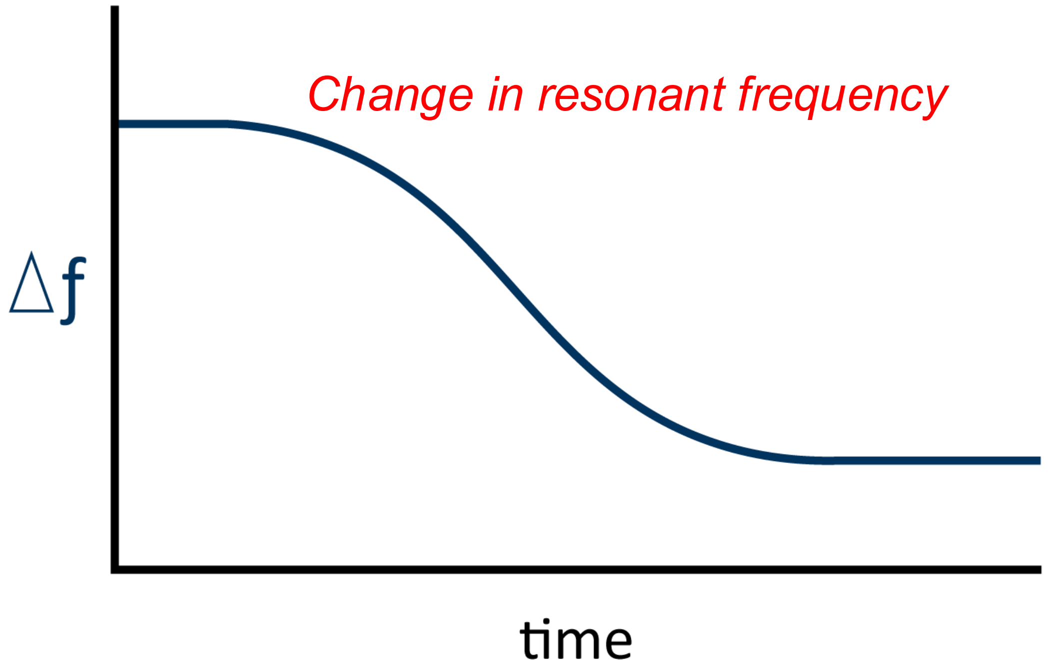
- Compare bare titanium to coated titanium
- Peak at 875 cm^{-1} related to bare titanium disappears; taken as evidence of coating
- Narrow peak in 3500–3200 cm^{-1} range corresponds to the N–H stretching vibrations of the peptide; appeared at 3300 cm^{-1}
- Additional peaks in the region 1310–1000 cm^{-1} appeared in the spectra of all peptides

QCM-D – What is it?

- **Q**uartz **C**rystal **M**icrobalance with **D**issipation (duh!)



Mass adsorbed on an oscillating sensor (doesn't distinguish between solvent and solute mass):



QCM basics

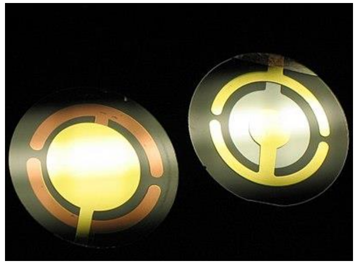
For rigid, non-dissipative layers, Sauerbray equation applies:

$$\Delta m = -C\Delta f/n$$

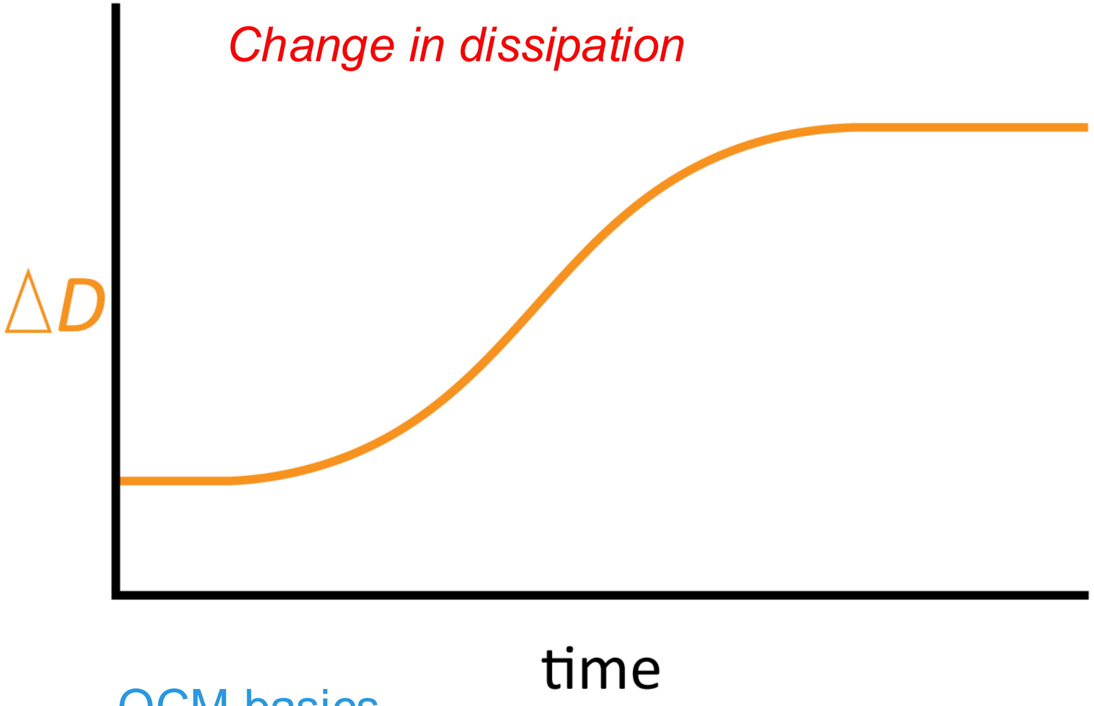
Where:
Δm is the change in mass per unit area (ng/cm²),
C is the sensitivity constant of the quartz crystal (typically 17.7 ng/(Hz·cm²) for a 5 MHz crystal in air)
Δf is the change in frequency (Hz)
n is the harmonics number (usually 1, 3, 5, etc.).

QCM-D – What is it?

- **Q**uartz **C**rystal **M**icrobalance with **D**issipation (duh!)



Dissipation or energy loss to the system is also measured:



QCM basics

Provides info on viscoelastic properties of adsorbed layer:

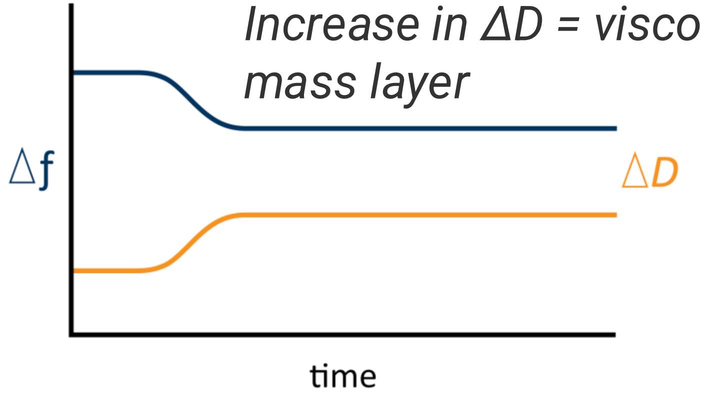
$$D = E_{lost}/2\pi E_{stored}$$

Where:
E_{lost} is the energy lost per oscillation cycle,
E_{stored} is the energy stored in the oscillating system

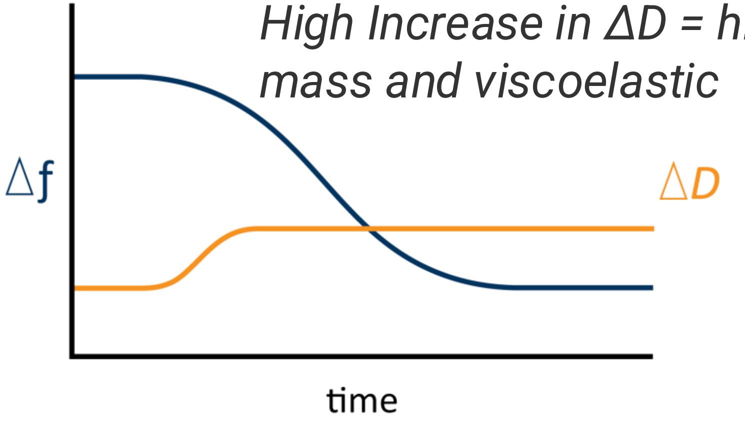
High dissipation suggests a viscoelastic (soft + hydrated) layer = more lost energy, whereas low dissipation suggests a rigid layer

Making sense of it all

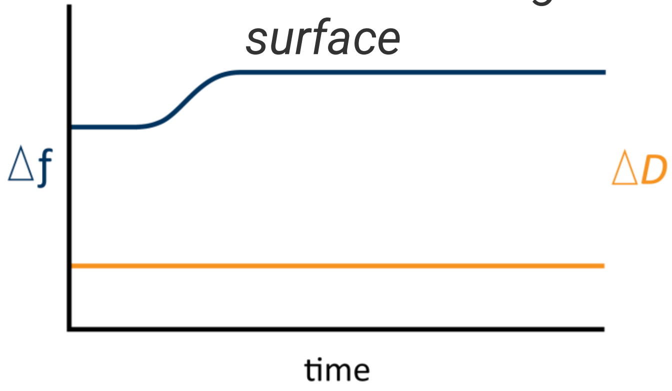
Low Decrease in Δf and High Increase in ΔD = viscoelastic low mass layer

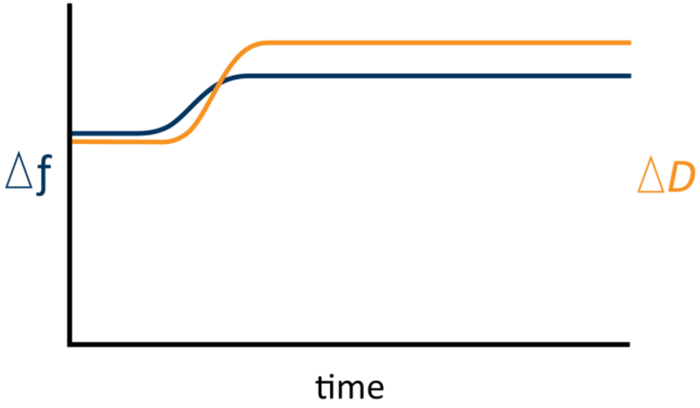


High Decrease in Δf and High Increase in ΔD = high mass and viscoelastic



Increase in Δf and No Change in ΔD = etching of a rigid surface





Increase in Δf with Increase in ΔD = mass removal associated with a softening of the layer

[QCM basics]

Peptide 1 – QCM-D

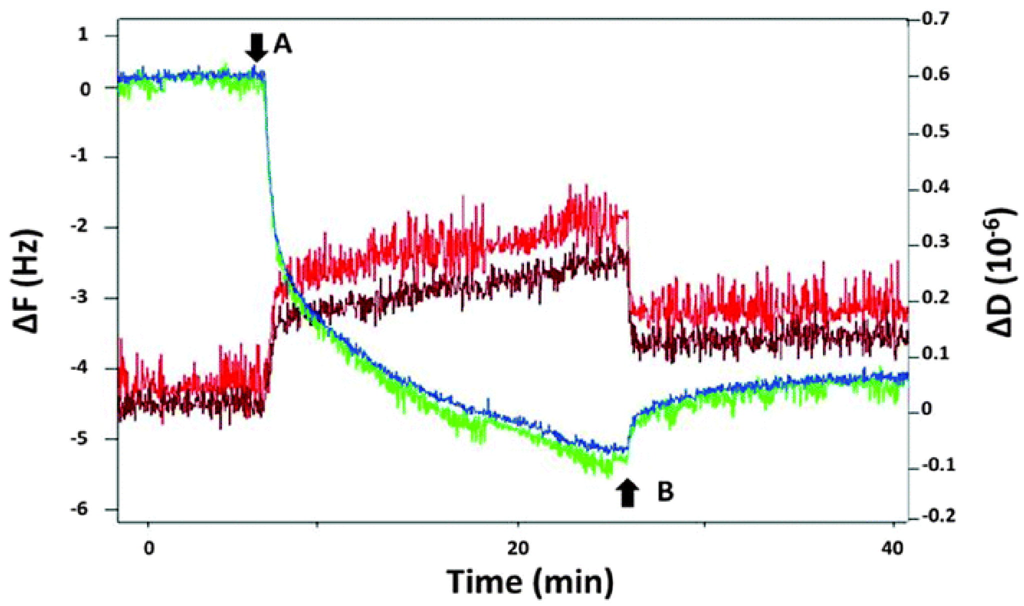
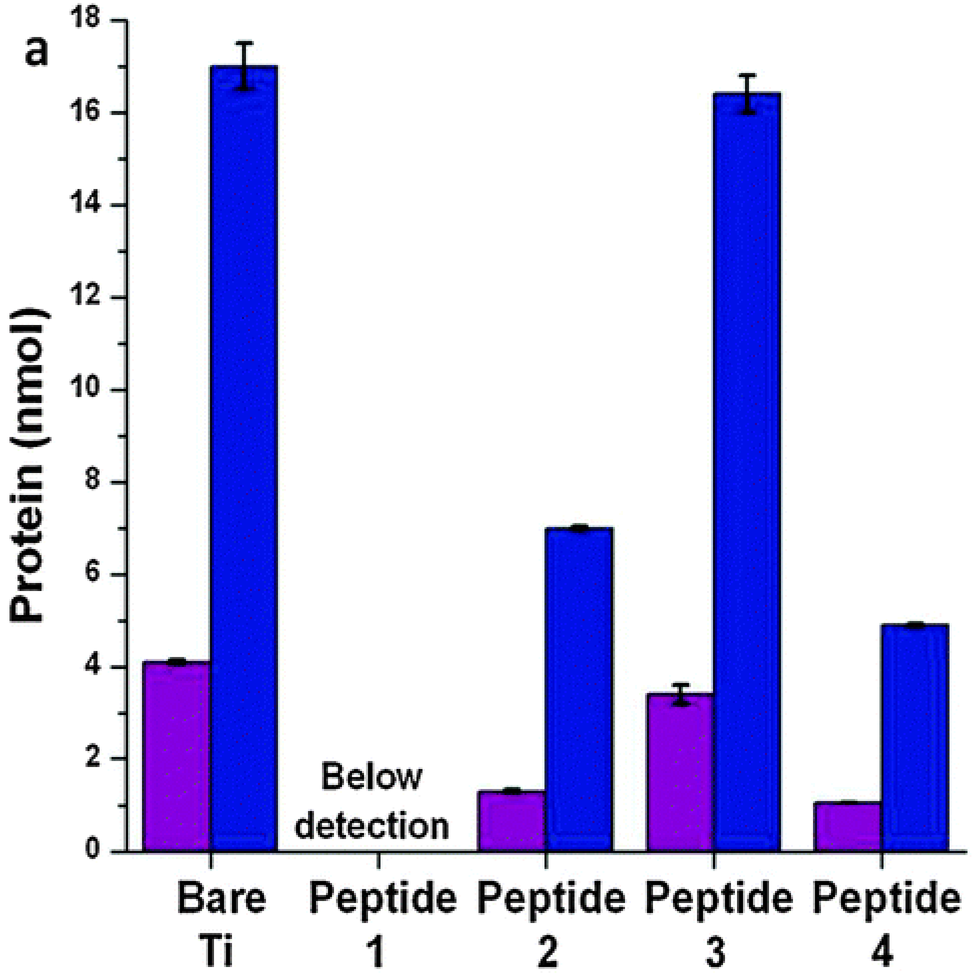


Fig. 3 (b) Real-time QCM-D measurements of peptide 1. Frequency overtones 5 and 7 are presented in green and blue respectively. Dissipation overtones 5 and 7 are presented in red and maroon respectively. Arrow A, indicates peptide addition, arrow B, washing with methanol.

- What do we see?
- Decrease in Δf when peptide is added (A), which means mass is absorbed, even after washing (B)
- Small increase in ΔD , indicating a rigid film, so Sauerbray equation can be used to estimate the adsorbed mass
- Here is what they got: 72.1 ± 0.4 ng cm^{-2}, 56 ± 2 ng cm^{-2}, 14 ± 3 ng cm^{-2} and 13 ± 2 ng cm^{-2} for peptides **1–4**, respectively
- They used another technique (XPS) to model the thickness of these layers, all were about 4 nm btw

Antifouling activity – protein adsorption

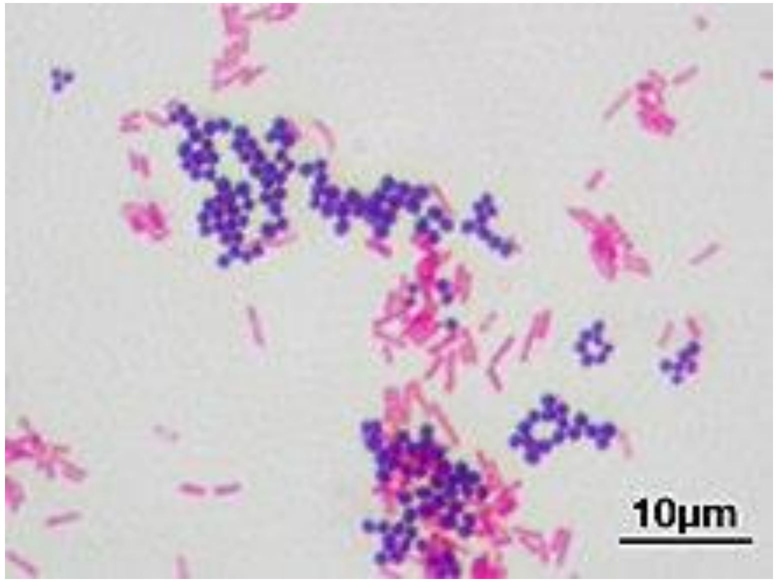


- Looked at the adsorption of protein on peptide-coated titanium after incubation; violet = BSA and blue = lysozyme
- A "non-interfering protein assay kit™" was used to quantify the adsorbed protein
- Peptide 1 with 1 F atom shows the best performance (having 5 F atoms does not seem to help)
- Somehow the "configuration" of peptide 1 is best

Remember Gram Staining?

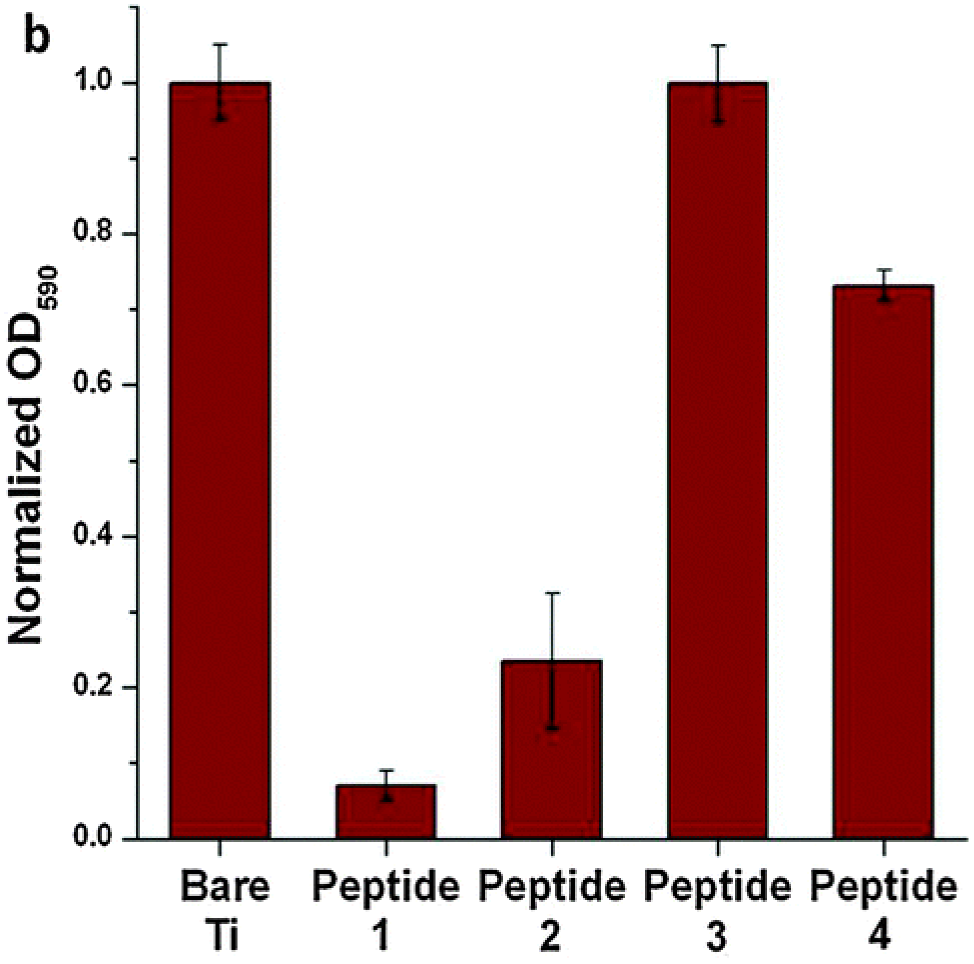
Gram stain

- Used to classify bacteria into 2 groups: gram-positive and gram-negative
- Gram-positive have a thick layer of peptidoglycan in the cell wall that retain the primary stain (Crystal Violet), whereas the thinner peptidoglycan layer is washed away with rinsing in gram-negative bacteria



A Gram stain of mixed *Staphylococcus aureus* (*S. aureus* ATCC 25923, gram-positive cocci, in purple) and *Escherichia coli* (*E. coli* ATCC 11775, gram-negative bacilli, in red), the most common Gram stain reference bacteria

Antifouling activity – interactions with bacteria



- Bare and peptide-coated substrates were incubated in inoculums of *Pseudomonas aeruginosa* and *Escherichia coli* (P. *aeruginosa results showed on left)*
- Staining with 2%(w/w) Crystal Violet after incubation; microscopy showed a thick and dense purple layer for bare titanium, more sparse for peptide-coated substrates
- Quantified by extracting the CV and measuring its absorbance
- End result: peptide 1 shows a 93% reduction in absorbance for *P. aeruginosa* and 74% for *E. coli*, again best performing

Conclusions

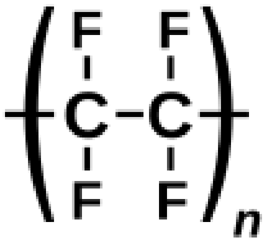
- New antifouling material based on the self-assembly of peptides
- Peptide design includes: (1) DOPA as the adsorptive/sticky motif and (2) diphenylalanine to direct the self-assembly
- For antifouling, an F atom (non-stick part in C-F bond) is needed
- Seems to work – attaches to different surfaces, etc. (what about plastics?)
- Suggested for coatings on medical devices and hospital equipment to reduce hospital-acquired infections, maybe also useful in aquatic environments, OK…

How do you imagine this will be used? Anything missing here?

Remind you of anything else you know that's not sticky?

Remind you of anything else you know that's not sticky?

Yes! You got it! It's the miracle and curse of Teflon!



Brought to you by Dupont..
It's the Forever Chemical!

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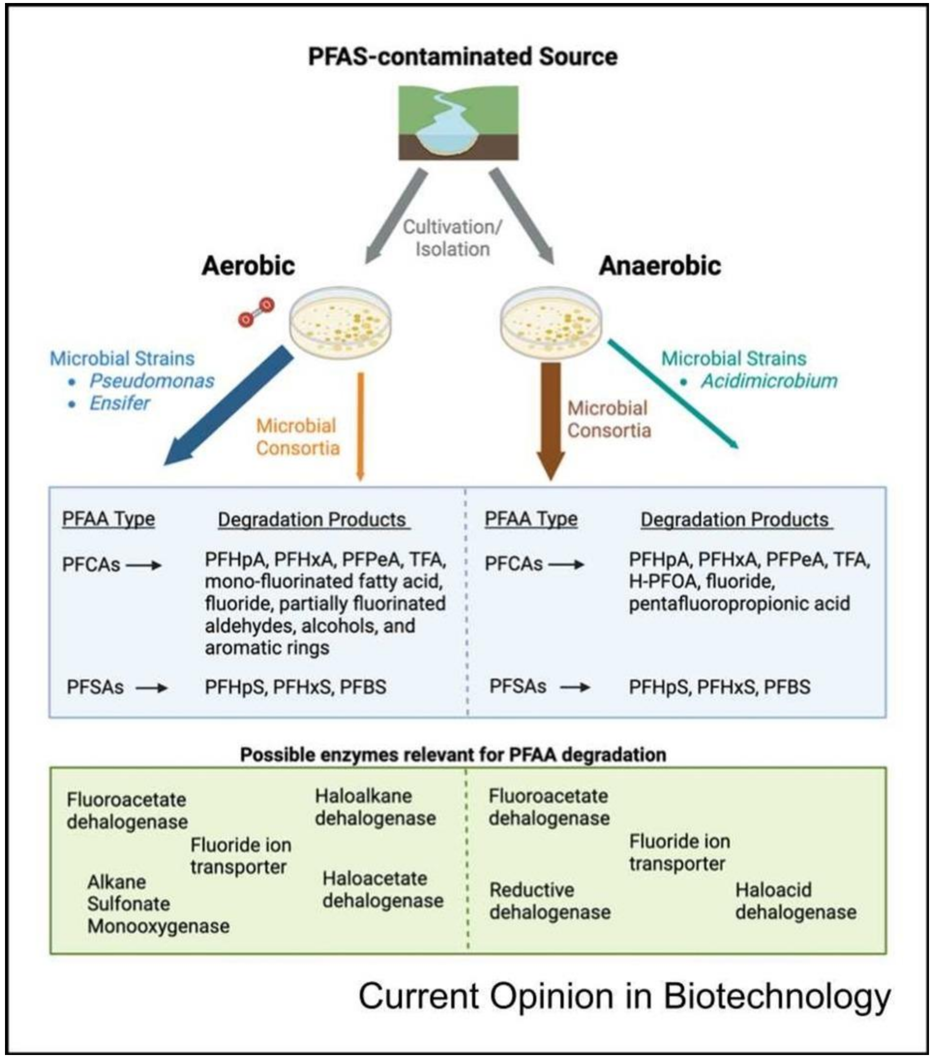
What are “Forever Chemicals”?

- Per- and polyfluoroalkyl substances, included in consumer groups since the 1940s
- Used everywhere – textiles, industrial equipment, cosmetics, jet engines, medical devises, refrigeration systems, electrical devices, rain gear, etc.,
- They are incredibly chemically inert (think about the bond) – do they do not break down and accumulate in our soil and water (and us!)
- Tied to health implications
- Remediation approaches exist (many examples in literature)

2024 Article in the Guardian

ELM relationship? Bacterial remediation?



Highlights:

•PFAA biodegradation with detection of degradation products has been reported.

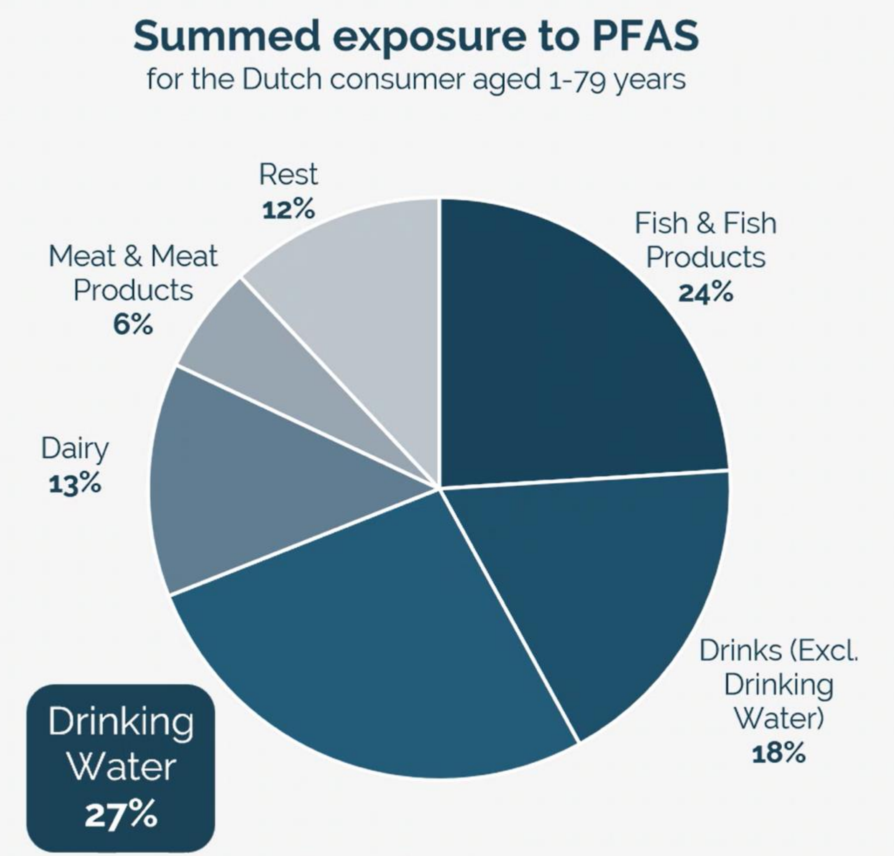
•Biodegradation of PFAAs has been linked to specific genes.

•Biodegradation of PFAAs was observed under both aerobic and anaerobic conditions.

•A majority of PFAA biodegradation studies were reported for microbial consortia.

https://doi.org/10.1016/j.copbio.2024.103170

Drinking water is one of the primary exposure routes for humans to PFAS



Source: RIVM report 2023-0011

You can still use these polymers for coatings?

Teflon™ Fluoropolymers Maintain Purity and Resist Biofilm Buildup

Contact Us With Your Questions

Connect with experts to learn more about Teflon™ products and to find the right solution for your needs.

CONTACT US ›

Safety Data Sheets

Use our database to access product SDS.

FIND PRODUCT SDS ›

A Shield Against Chemical Attack and Biofilm Buildup

Teflon™ fluoropolymers offer a nearly universal chemical resistance. They produce a smooth, low-surface energy layer that resists biofilm buildup and facilitates their use in many applications within the food/beverage, chemical processing, and semiconductor manufacturing industries.

How Teflon™ Fluoropolymers Help

With extremely low extractables and reactivity, plus high purity, Teflon™ fluoropolymers meet a wide range of regulatory and industry requirements.

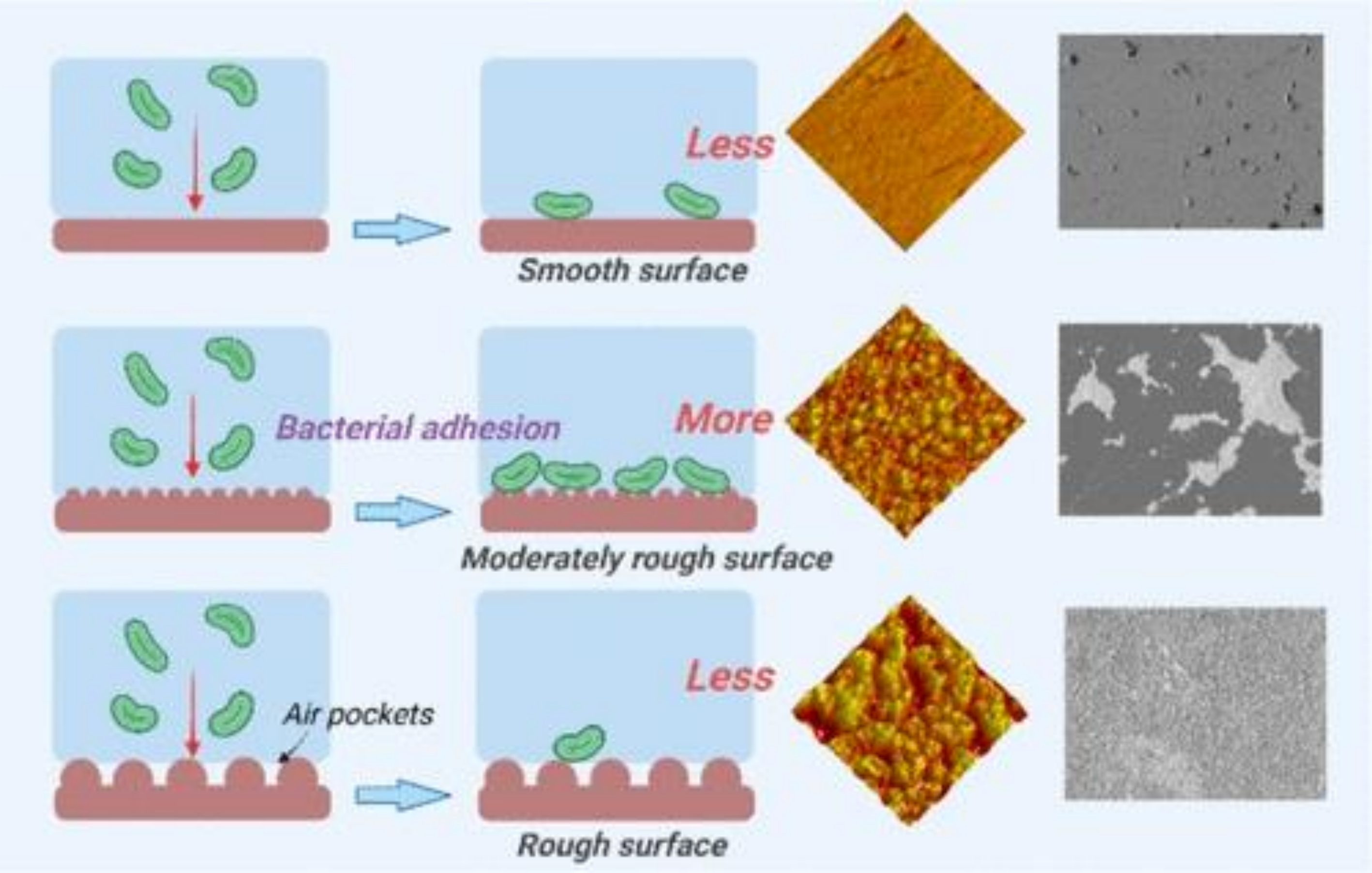
RESOURCE LINKS

- Industrial Uses for Teflon™ Solutions
- Teflon™ Products
- Solutions from Teflon™ Fluoropolymers
- Teflon™ Brand Sales and Support

Commercial Teflon polymers marketed to the food industry

Still, the problem of biofilms is major!

Influence of Surface Roughness, Nanostructure, and Wetting on Bacterial Adhesion



https://doi.org/10.1021/acs.langmuir.3c00091

Takeaways

- Hydrophobic coatings can be useful to for antifouling
- Somehow, it's not only "hydrophobic" it's also about the structure of the layer itself
- This is a good learning, even if adding more C-F bond-containing molecules to the environment is maybe not
- PFAS are problematic – remediation strategies and legislation!
- Recent paper from this group with non-fluorinated amino acids – maybe to cover next?

ELSEVIER
Chemical Engineering Journal
Volume 504, 15 January 2025, 158658

A fluorine-free superhydrophobic coating fabricated by amino acids on soft electroadhesives