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Stable porous silicon membranes for fast bacterial detection

micromachines MDPI

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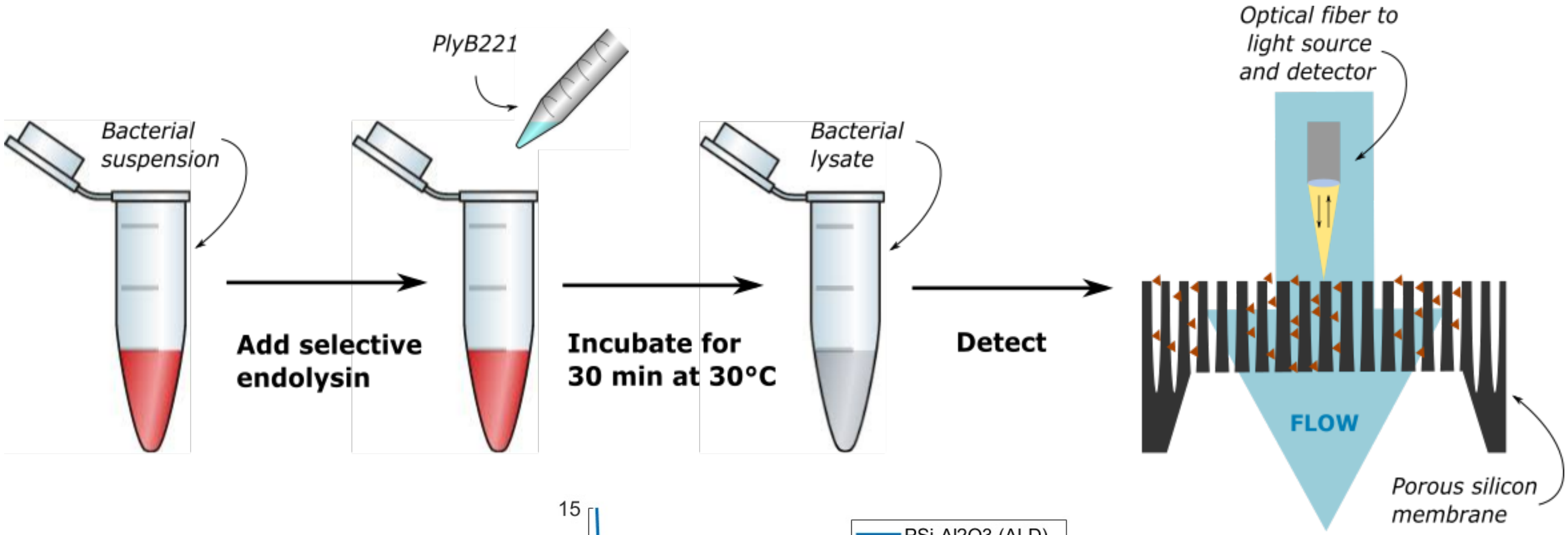
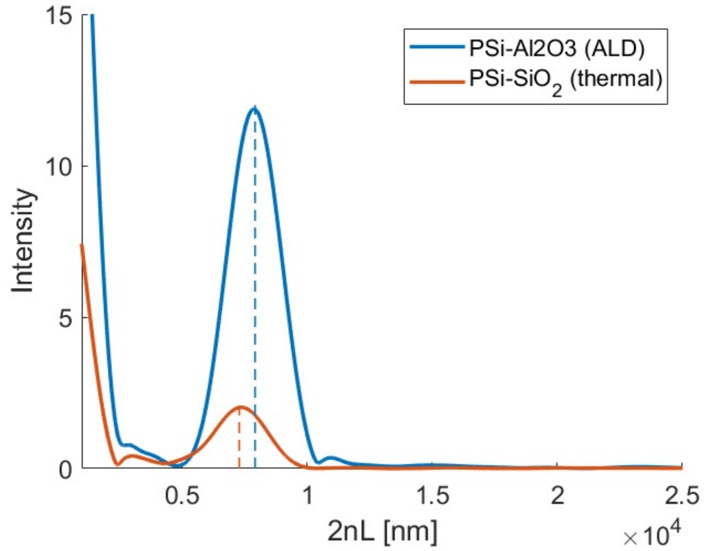
Stable porous silicon membranes for fast bacterial detection





Graphical Abstract

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Stable porous silicon membranes for fast bacterial detection

Optical sensing using porous silicon (PSi) substrates can be used for detecting hazardous bacteria and to reduce the usage of broad-spectrum antibiotics.

In most configurations, PSi optical biosensors are close-ended porous layers that limits their sensitivity and responsiveness due to diffusion-limited infiltration of the analytes in the porous layer. Also, PSi is a reactive material, its oxidation in buffer solutions results in undesirable time-varying shifts.

Three main improvement points are investigated:

- the **bacteria lysis** prior to its exposure to the sensor, such that the selective detection is based upon the percolation of bacterial residues inside the pores rather than the bacteria themselves;
- the **flow-through in a PSi membrane** that enhances the interactions between the lysate and the sensor's surface and reduces detection time;
- the stability over time in saline solutions helped by **atomic layer deposition** of metal oxides inside the pores, that is also helping with an increase of the optical signal to noise ratio thus reducing the limit of detection.

We tested the selective detection of *Bacillus cereus* lysate with concentrations between 10^4 to 10^6 CFU/mL. Future works are dedicated to further improvements, including optical signal enhancement techniques and dielectrophoretic assisted percolation in the porous silicon membrane.

Keywords: optical biosensor; porous silicon membrane; atomic layer deposition; bacteria.

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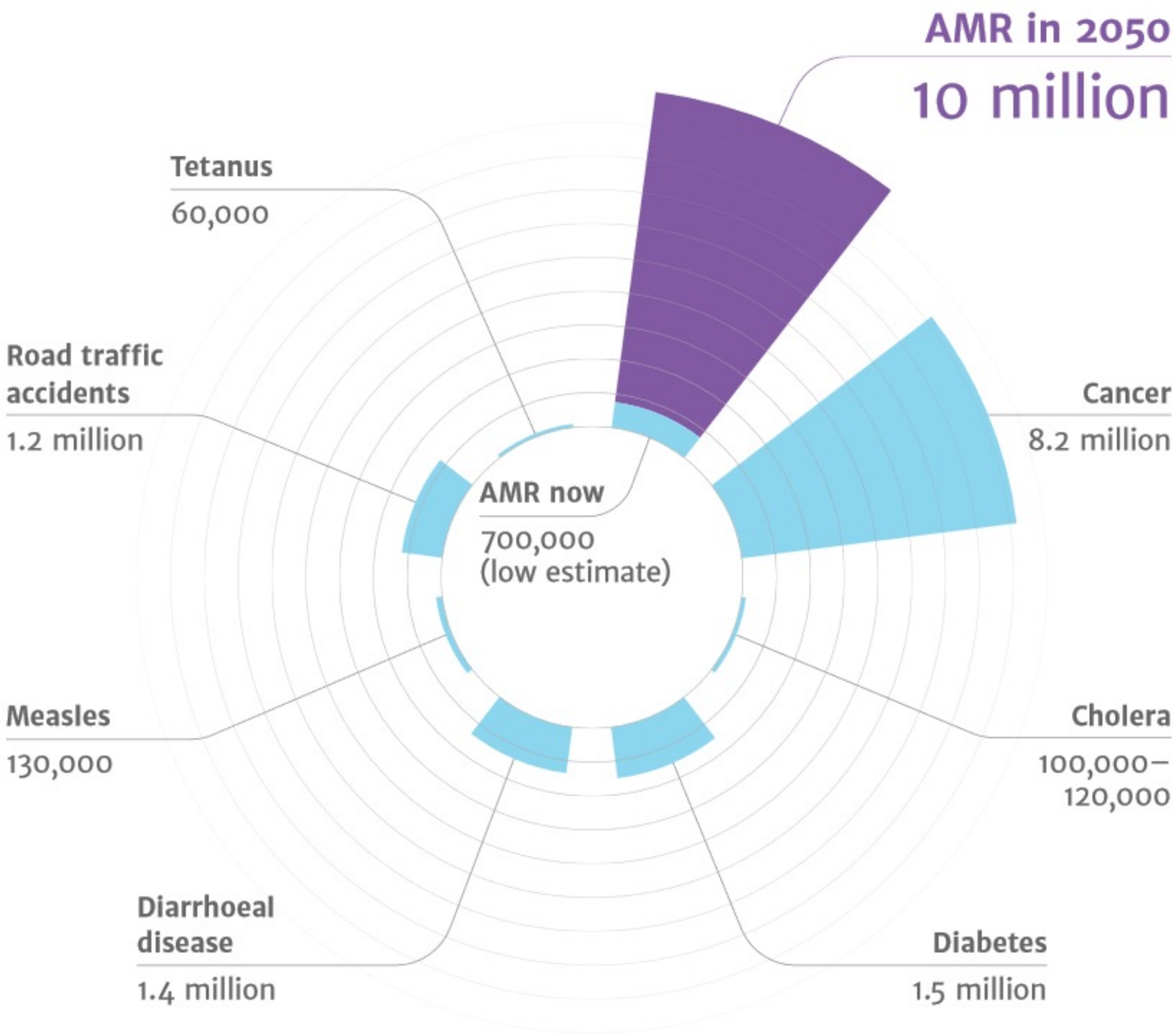
Context: Antibiotic Microbial Resistance



Source: Jim O'Neill. Tackling drug-resistant infections globally: final report and recommendations - the review on antimicrobial resistance, 2016.

Early detection of pathogens

↓

Biosensors

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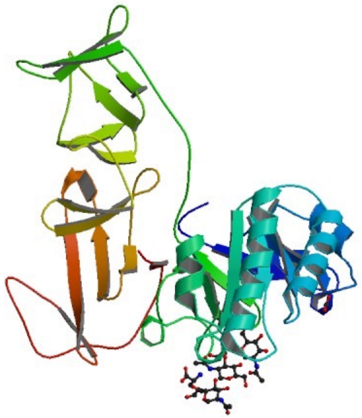
Biosensing

Biorecognition Element

Selective endolysin

✓Can be added to sample volume

✓No need for functionalization



Optical Transducer

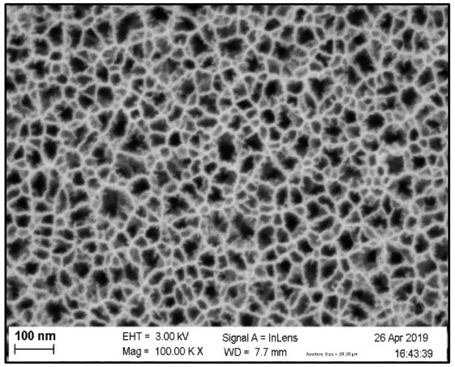
Porous Silicon

✓Large surface area

✓Very sensitive

Optical detection

✓Easy to set up



Source endolysin illustration: Perez-Dorado, I., Campillo, N.E., Monterroso, B., Hesek, D., Lee, M., Paez, J.A., Garcia, P., Martinez-Ripoll, M., Garcia, J.L., Mobashery, S., Menendez, M., Hermoso, J.A.
http://www.ebi.ac.uk/pdbe-srv/view/entry/2j8g/summary

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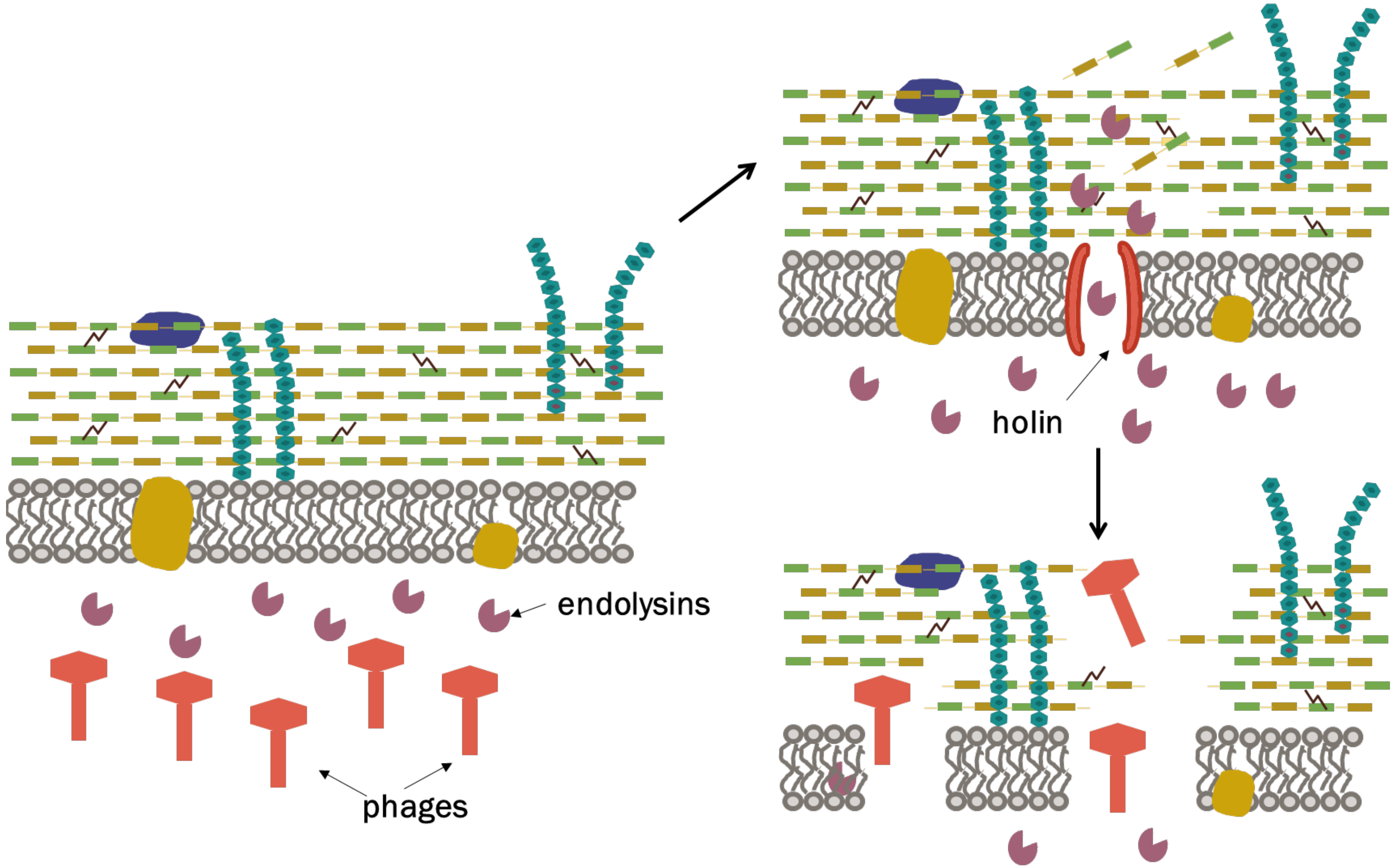
Endolysin as a Biorecognition Element



Illustrations from A. Leprince, 2019

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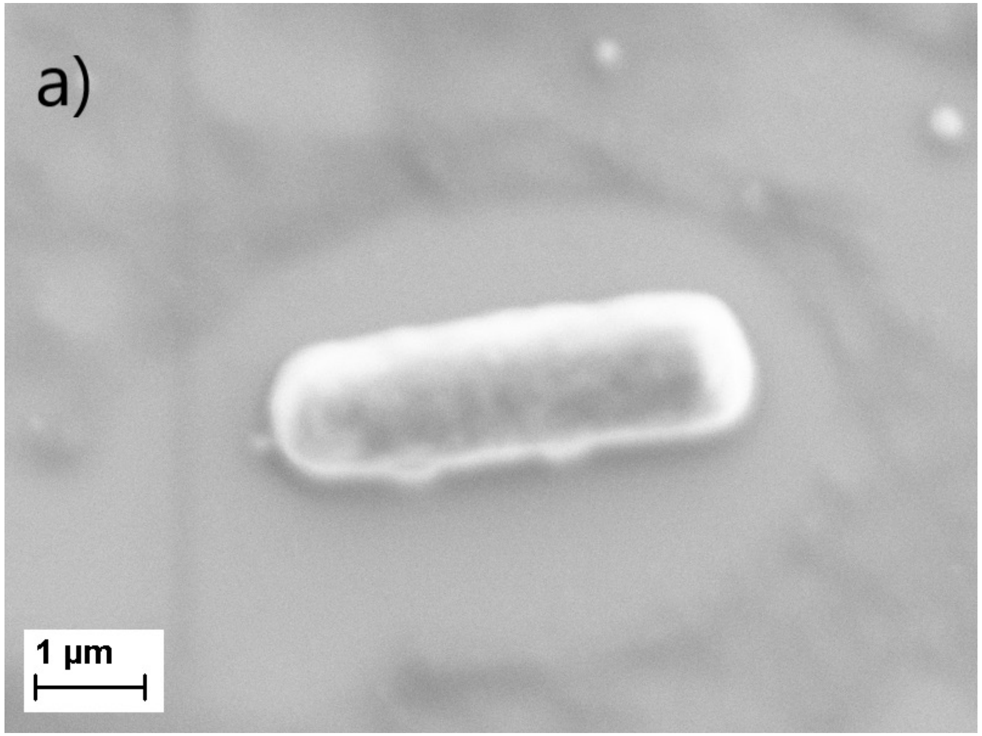
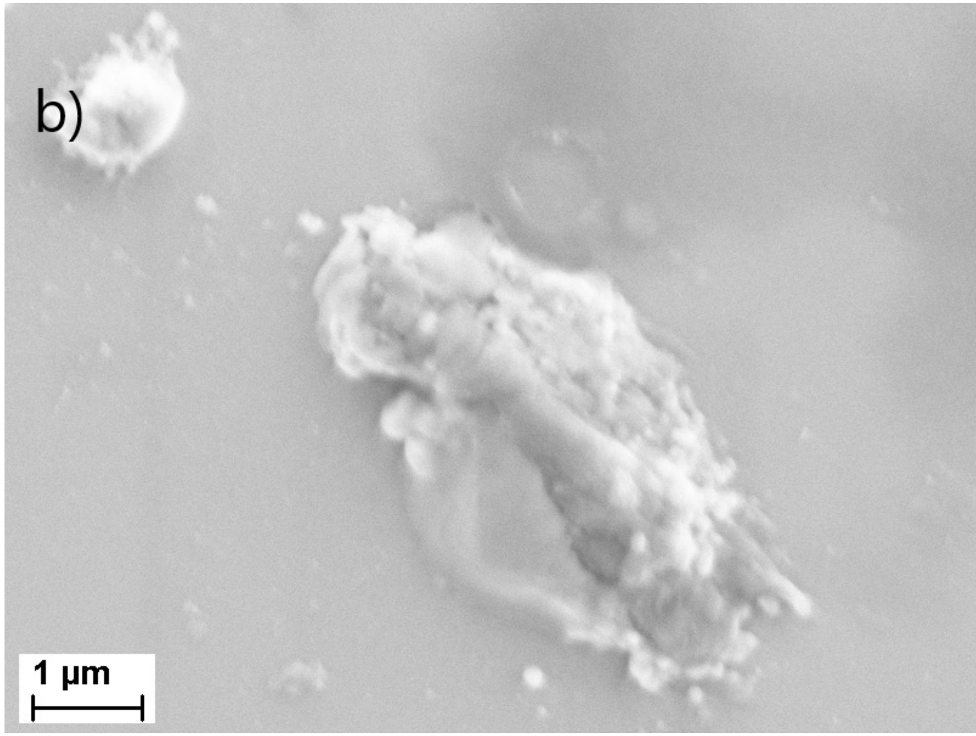


Example of targeted bacteria:
B. cereus (ATCC10987)

Bacteria found *e.g.* in food poisoning

(a) Before lysis

(b) After lysis with PlyB221



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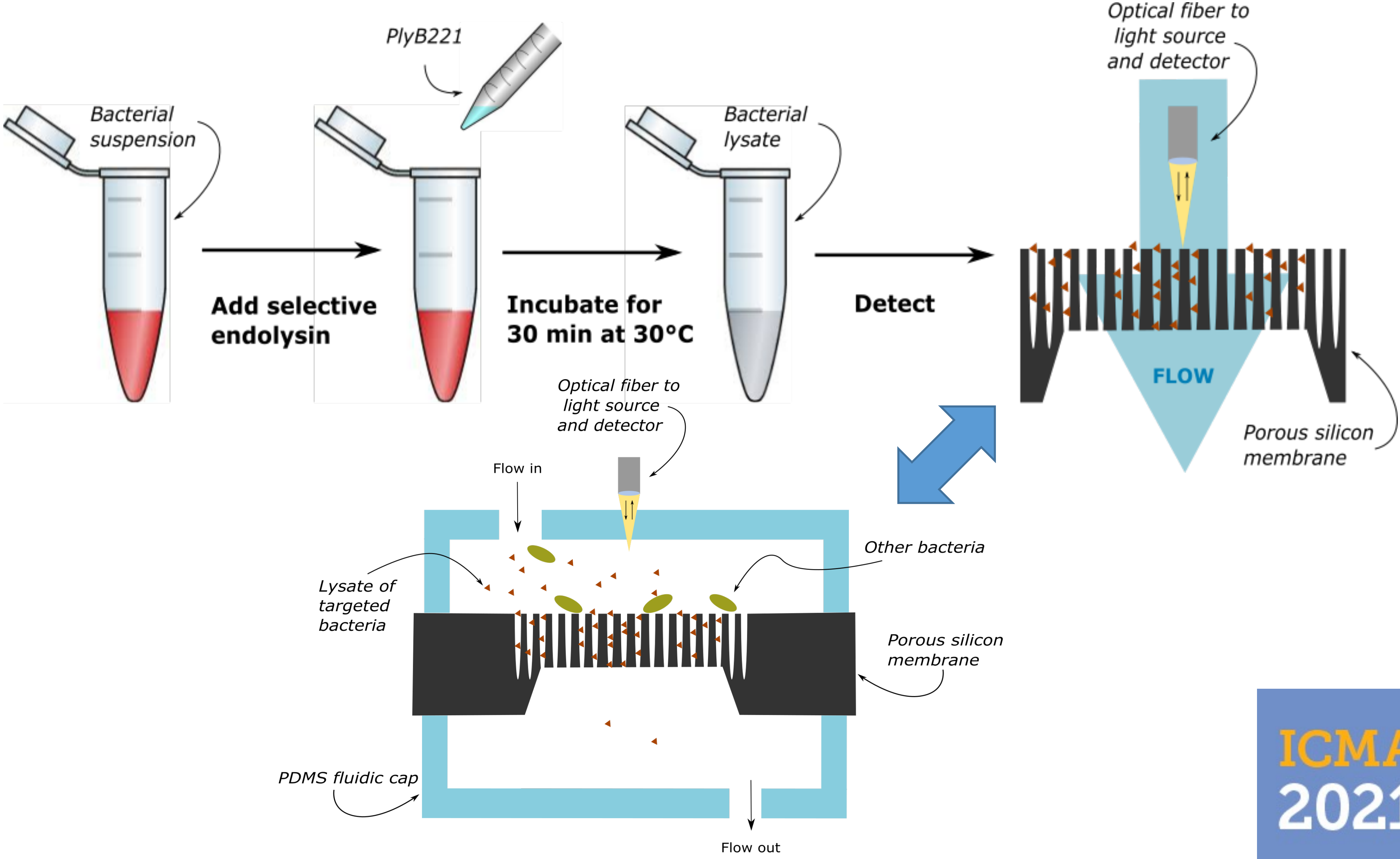
Porous Silicon Membrane Biosensor



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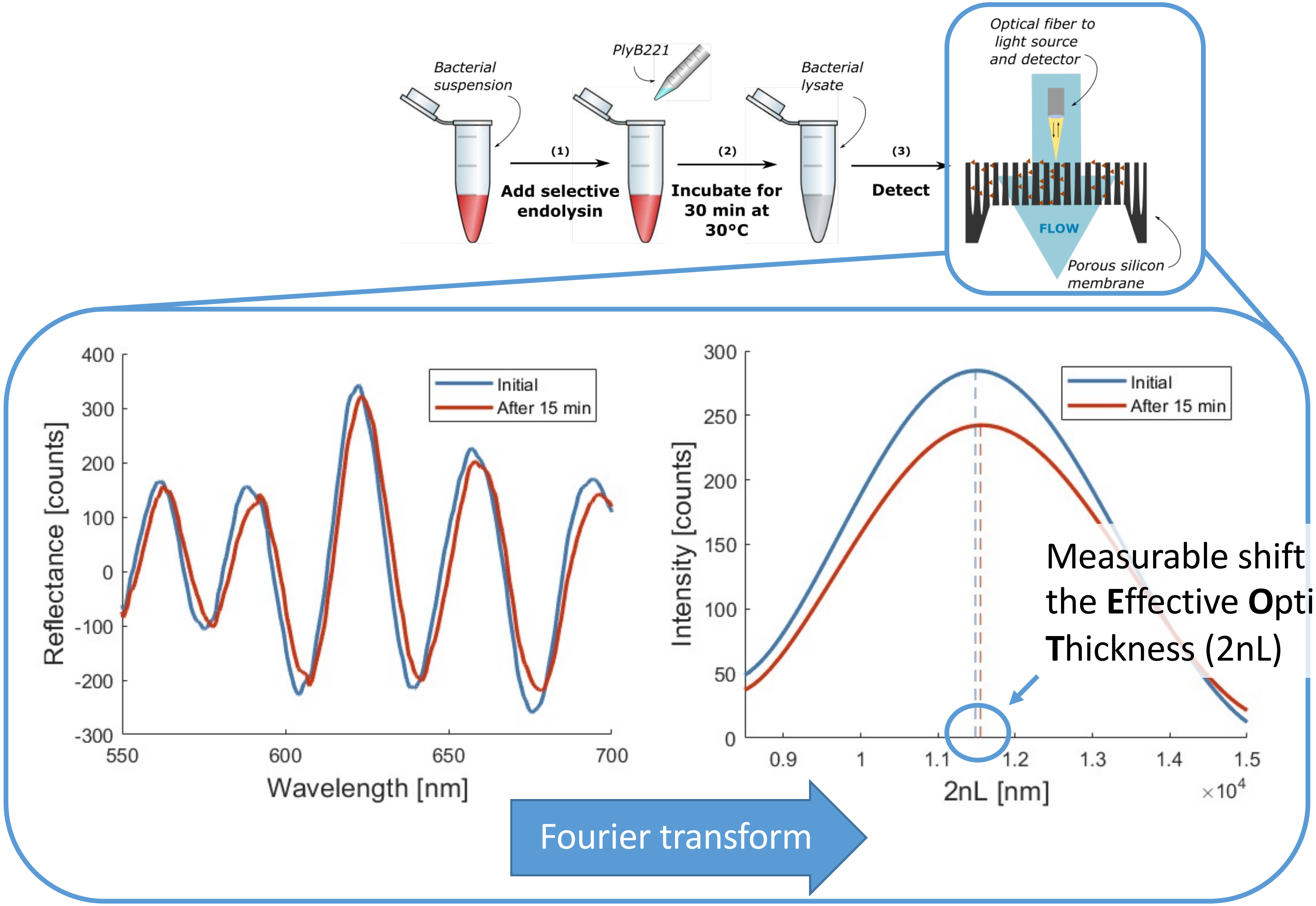
Optical Detection Method

Reflective Interferometric Fourier Transform Spectroscopy



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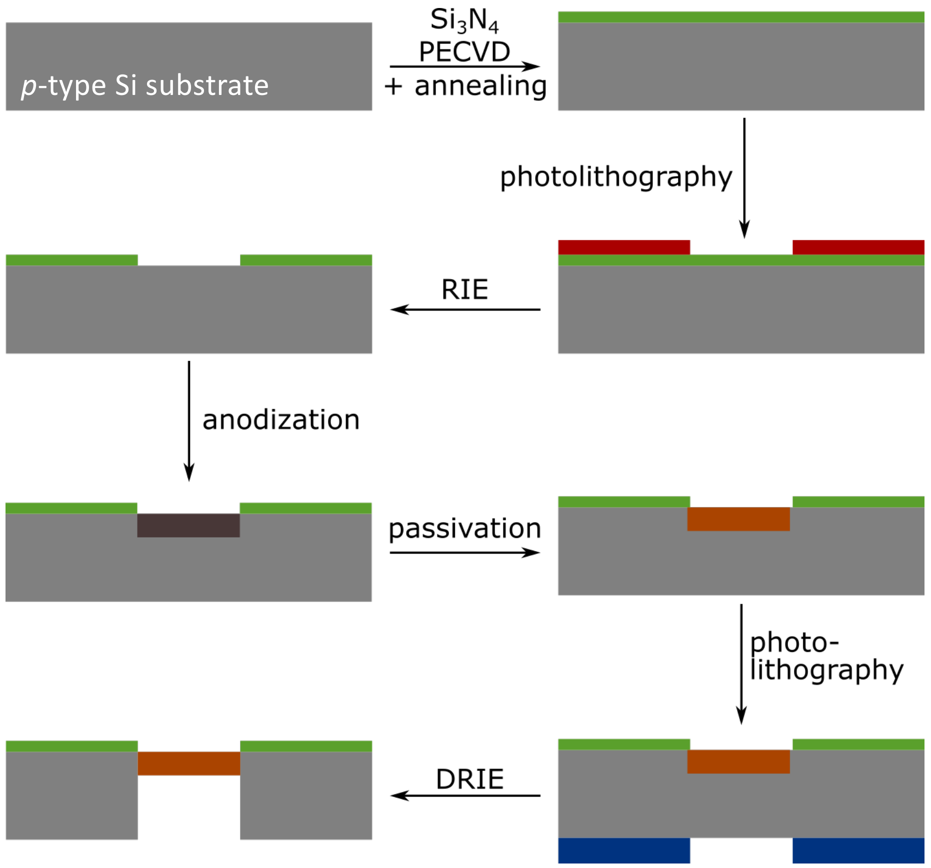
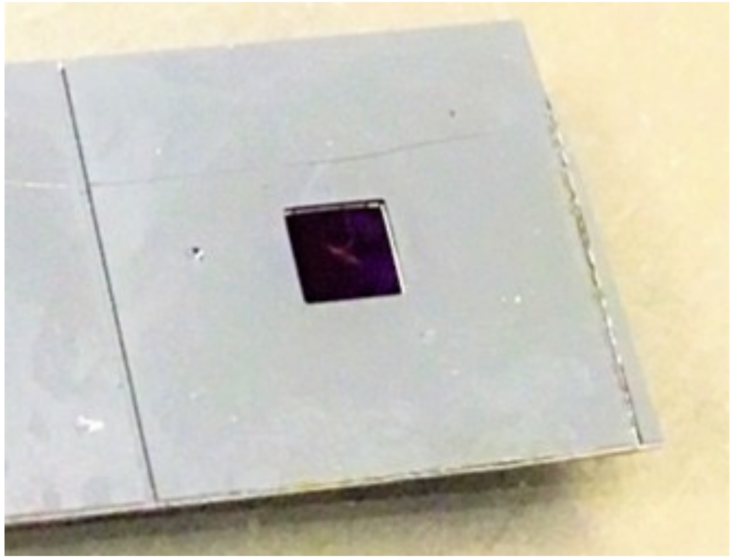
Sensor Microfabrication Process





Porous Silicon Anodization

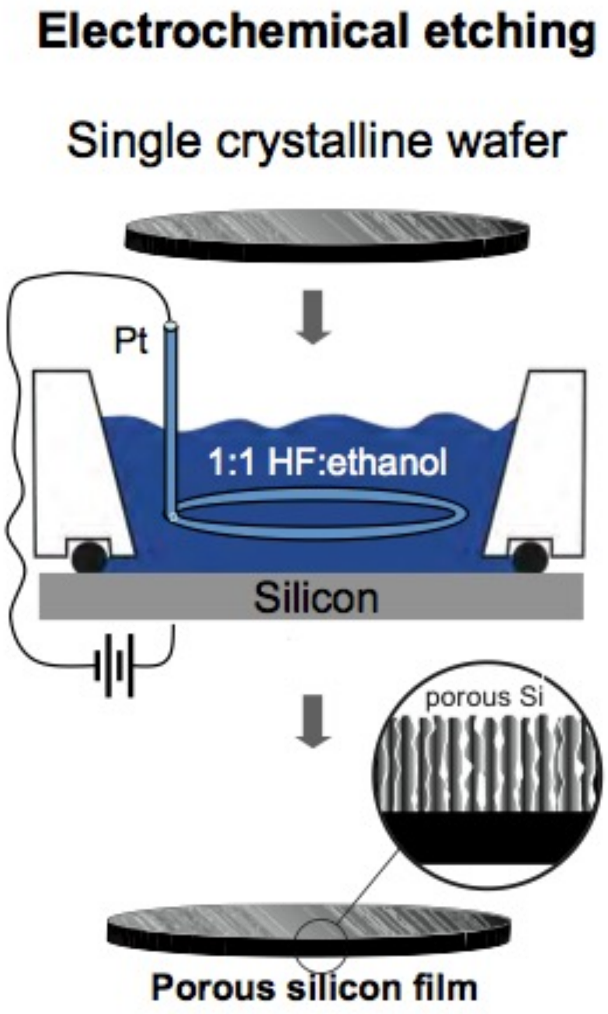
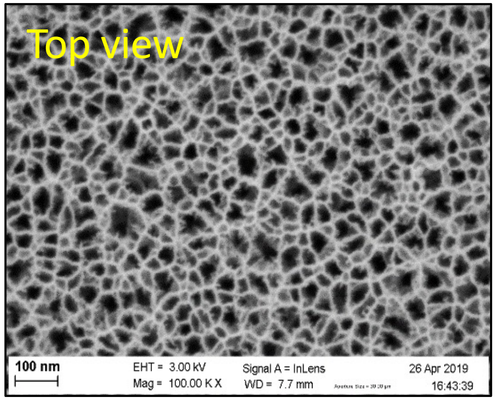


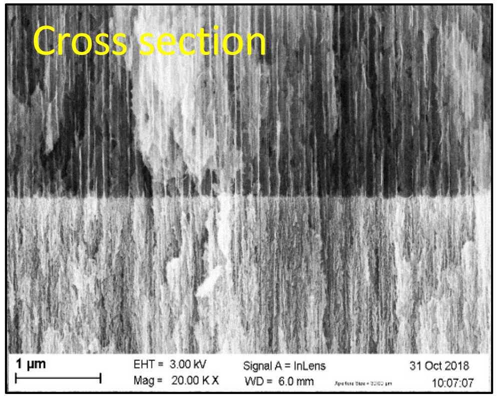
Illustration from Summer School for Nanotechnology 2019 presentation by Michael Sailor, UCSD

$$Si + 2h^+ + 6HF \rightarrow SiF_6^{2-} + H_2 + 4H^+$$

Use of an **HF**-based electrolyte

Based on **hole conduction** through the Si bulk





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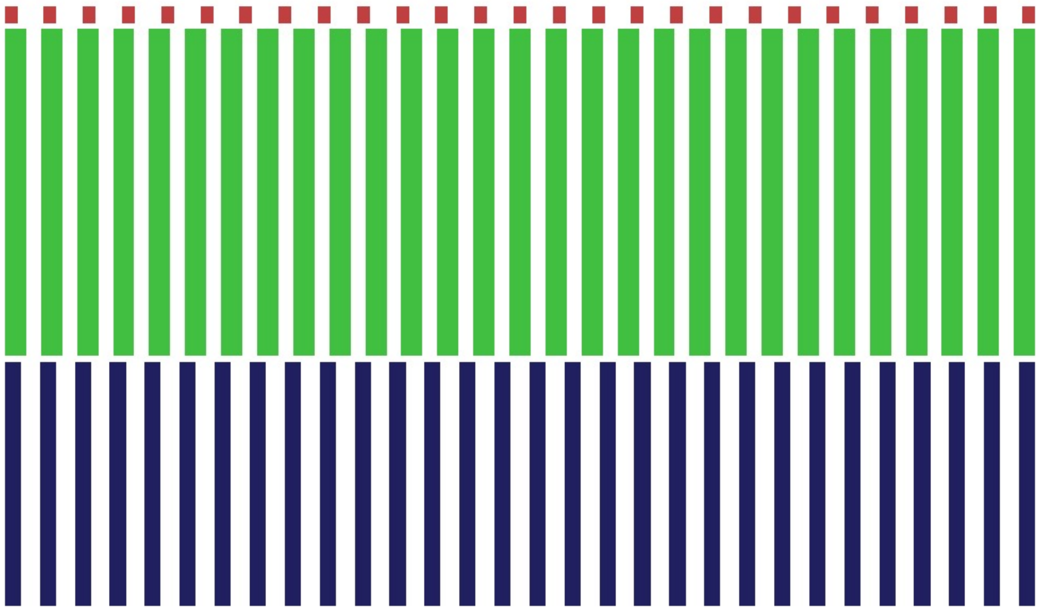
Porous Structure Layers



Sensing layer

Contrast layer → enhance optical signal

Mechanical support layer → robustness

Layer	Current density [mA/cm^2]	Time [s]	Pore diameter [nm]	Thickness [µm]	Porosity [%]
Sensing layer	200	50	41.1 ± 20.4	4.1 ± 0.7	75.4
Contrast layer	50	1500	14.6 ± 7.8	22.8 ± 6.8	48.5
Support layer	1000	2000	25.5 ± 10.4	- *	- *

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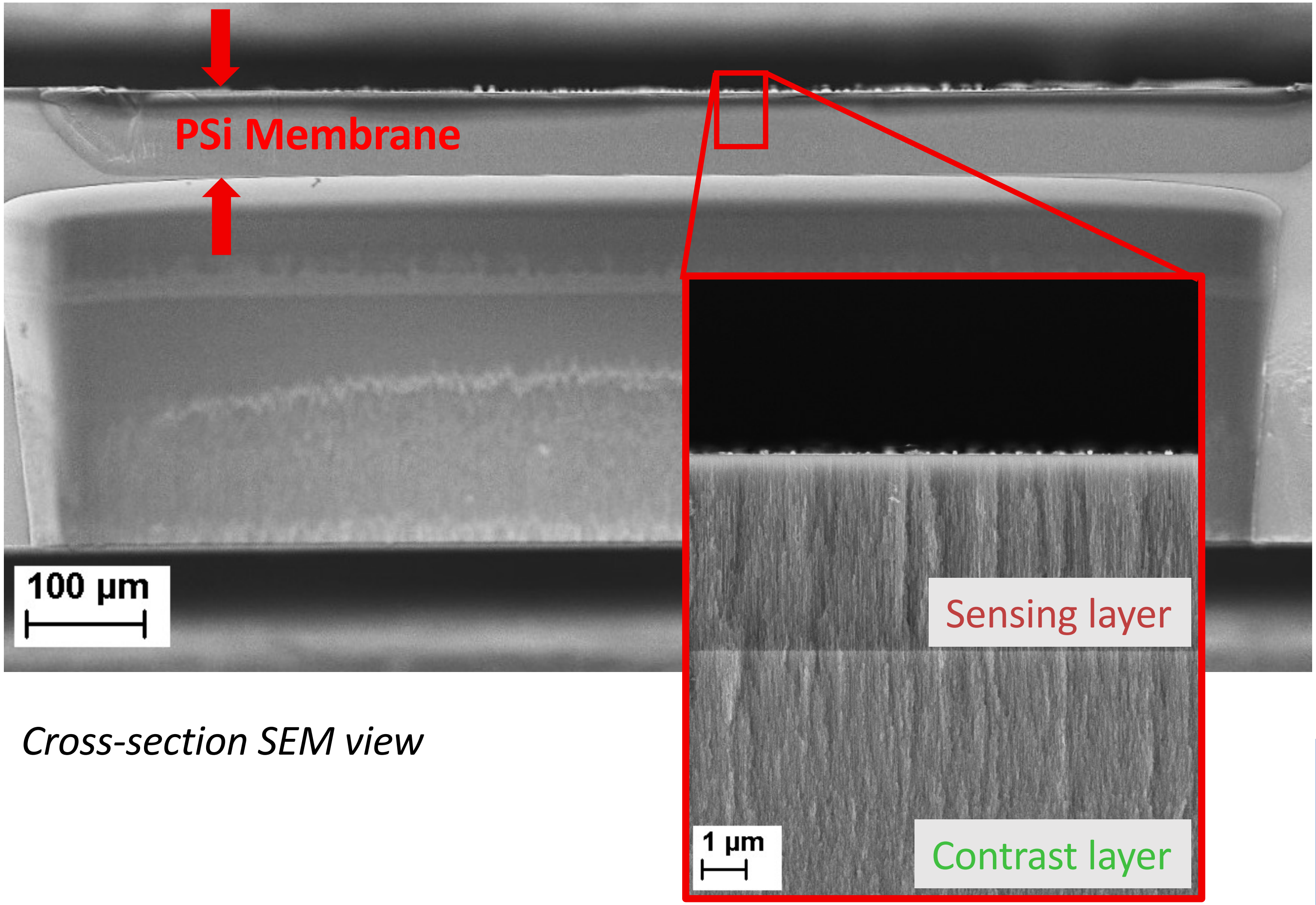
Porous Structure Layers



Cross-section SEM view

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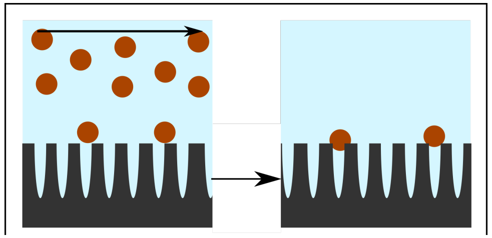
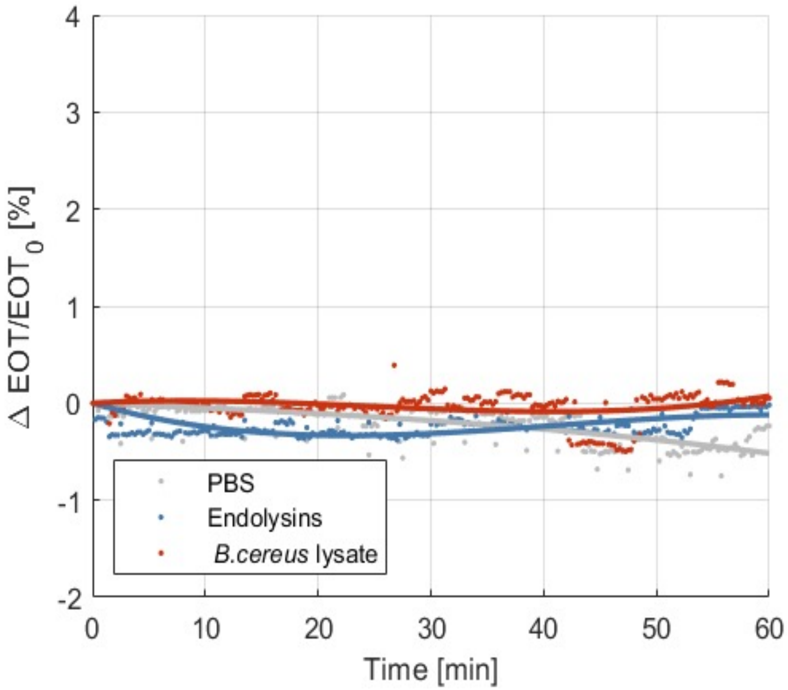
Benefits of the Membrane

Targeted bacteria: 10^6 CFU/mL of *B. cereus*, lysed by PlyB221

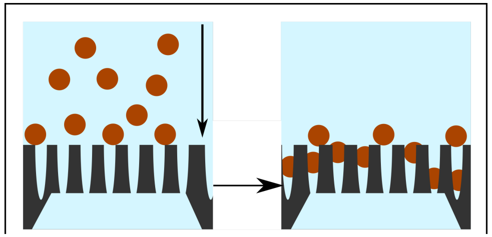
PSi layer: current studies on biosensing



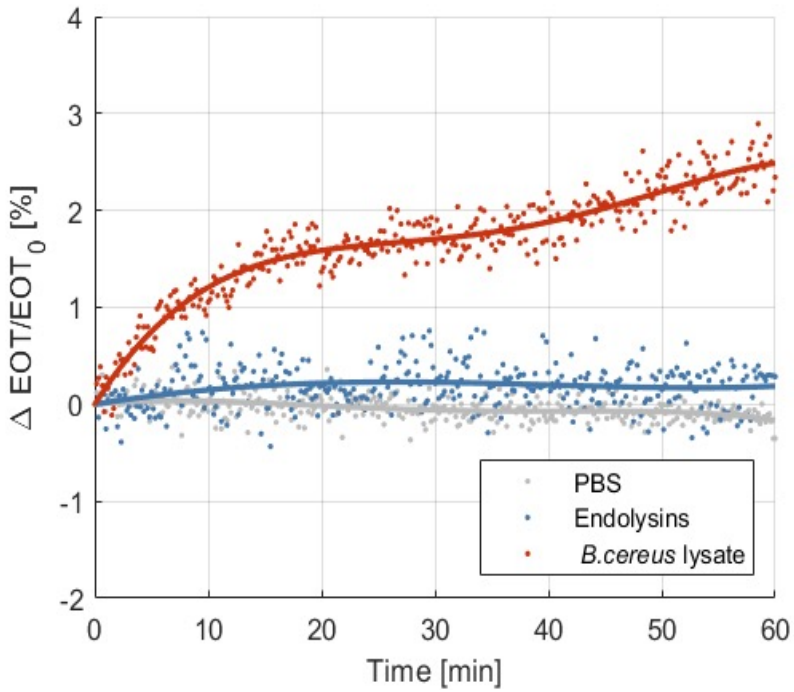
FLOW OVER



PSi membranes: emerging biosensor



FLOW THROUGH



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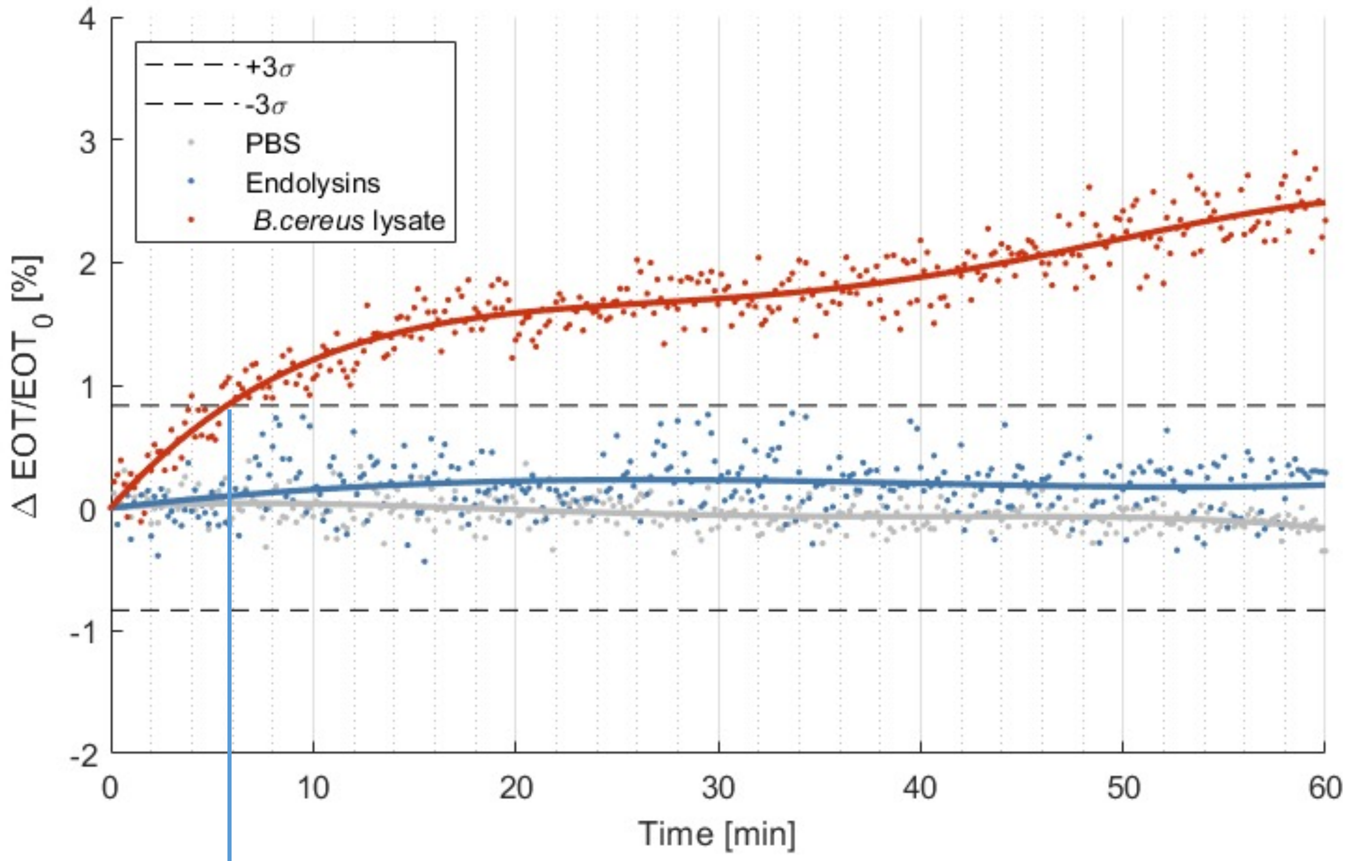
Fast Detection

Targeted bacteria: 10^6 CFU/mL of *B. cereus*, lysed by PlyB221



Significant increase after 6 min !

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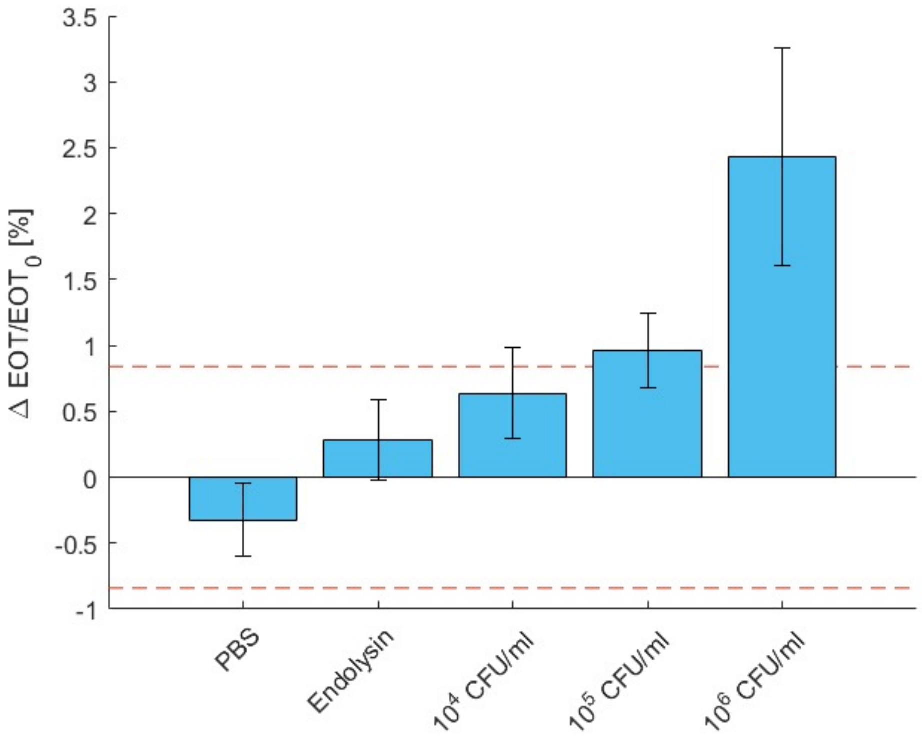
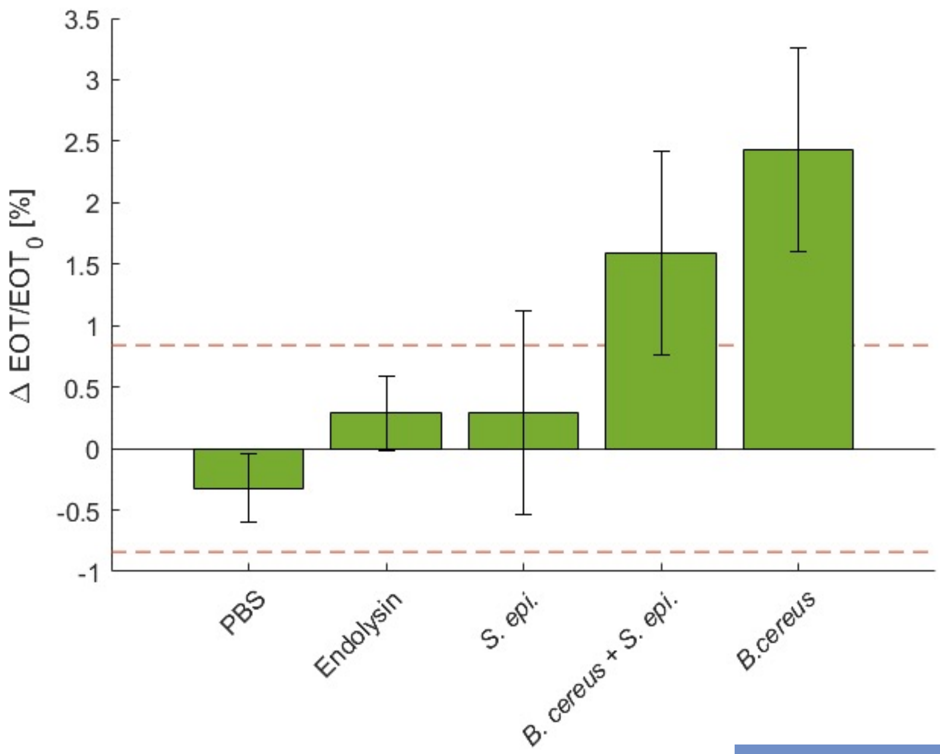
Limit of Detection and Selectivity

Targeted bacteria *B. cereus* lysed by PlyB221, *vs. Staph. epidermidis*

LoD < 10^5 CFU/ml



Selectivity in mixed samples



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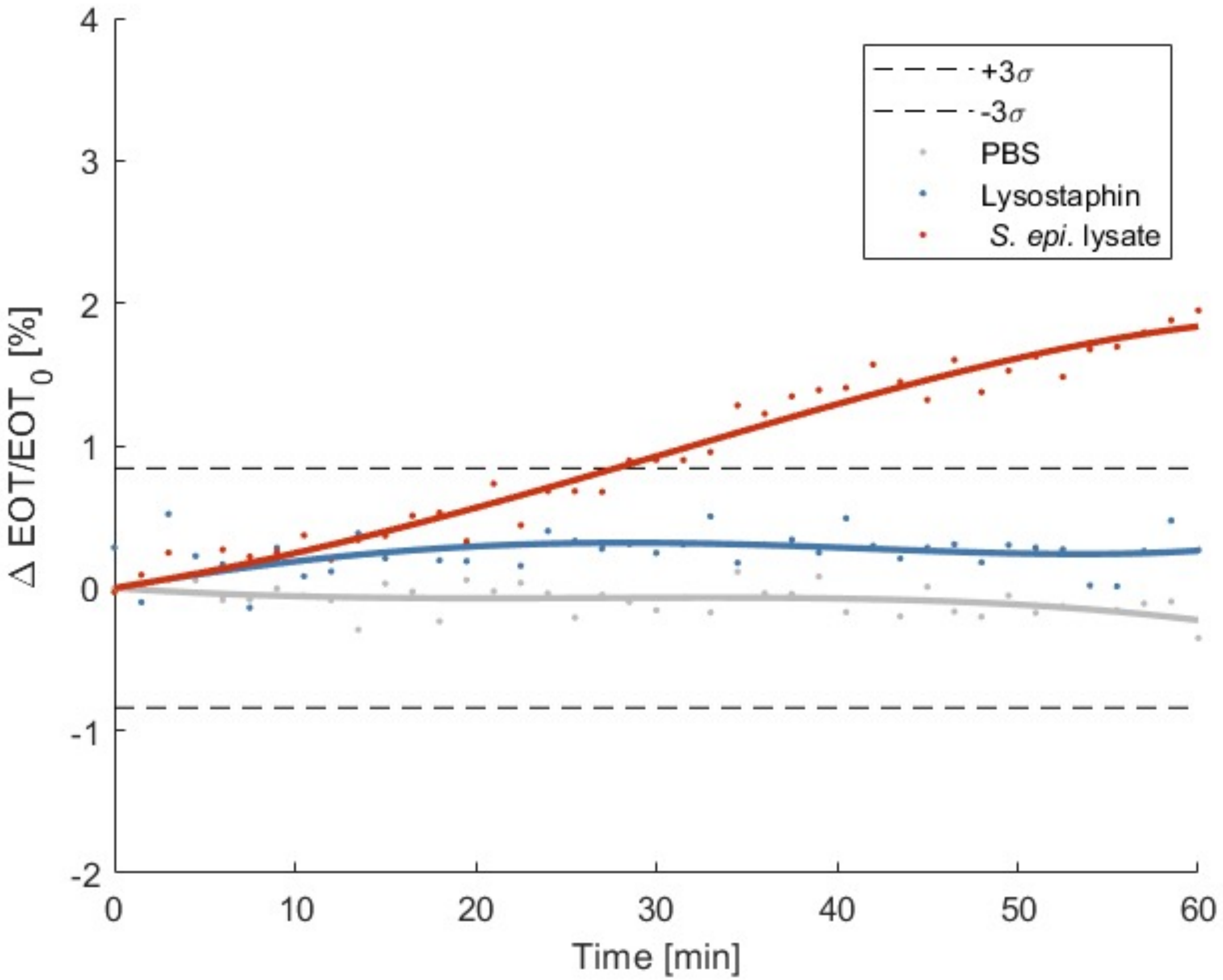
A Versatile Biosensor

Targeted bacteria: *Staph. epidermidis* (ATCC35984), lysed by lysostaphin



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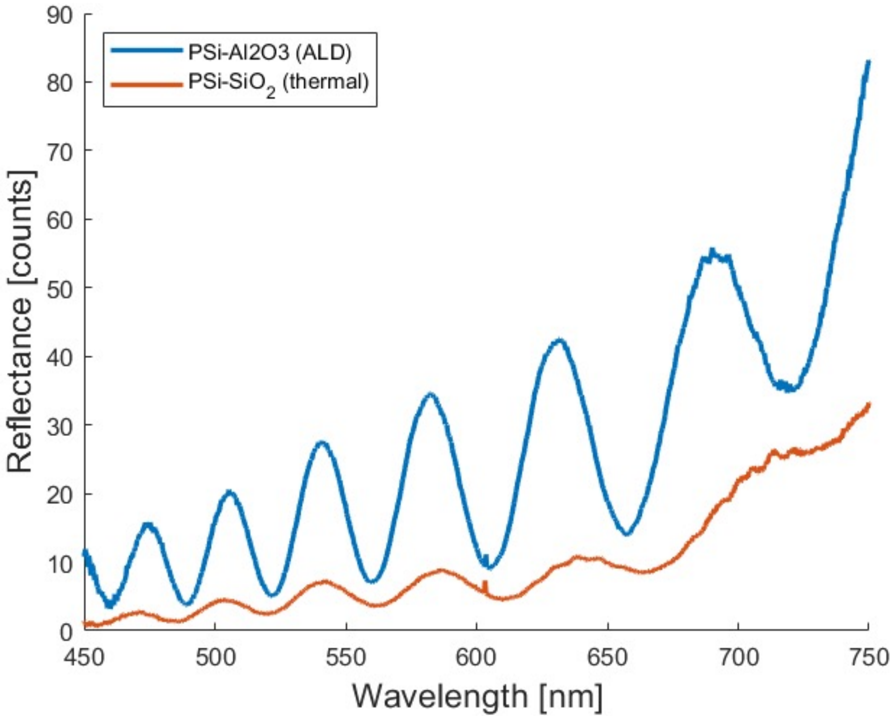
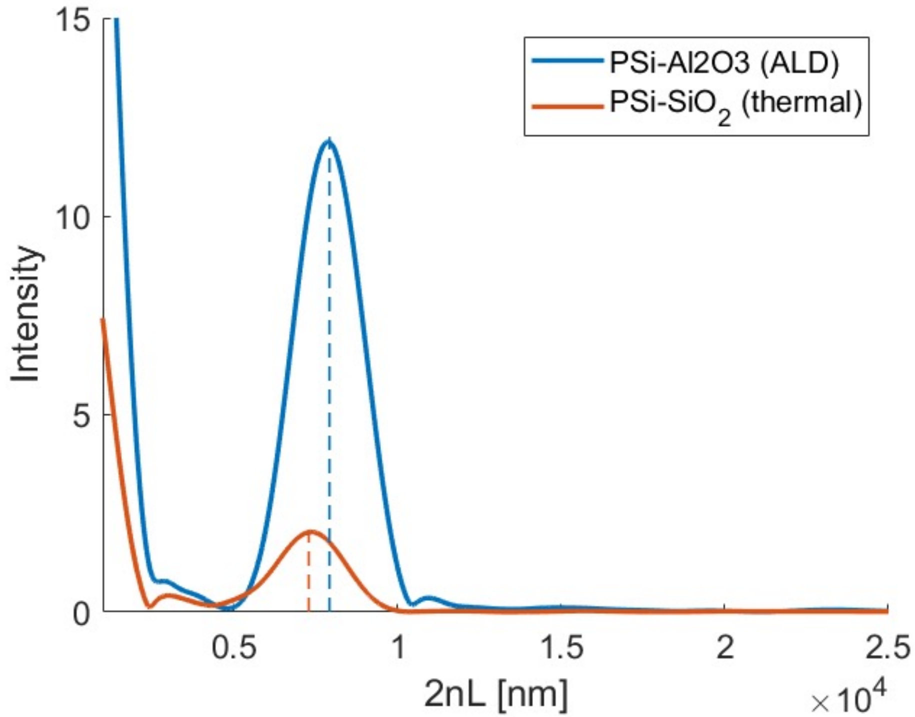
Optical Signal Enhancement with ALD





Atomic **L**ayer **D**eposition of *ca.* 6 nm aluminum oxide on top of thermal oxide

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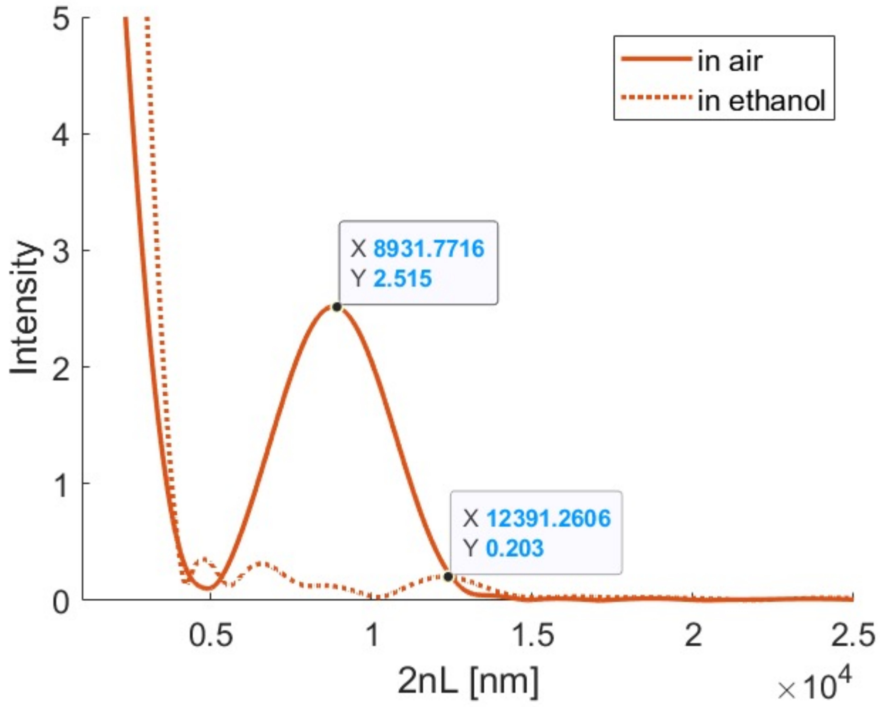
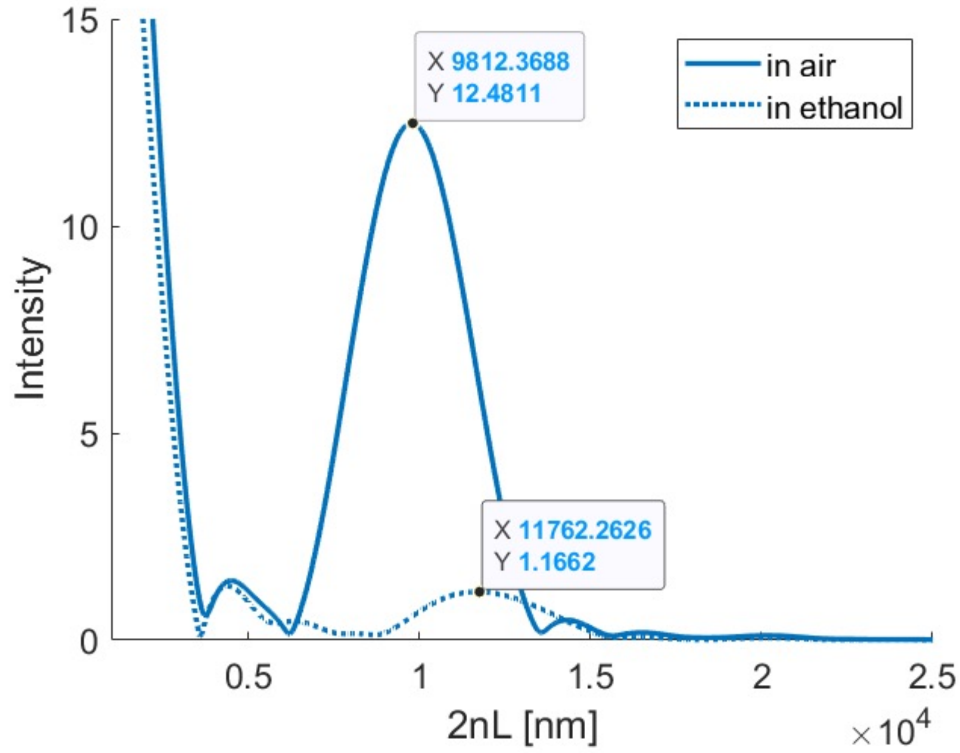
Refractive Index Sensitivity

PSi – SiO_2 (thermal)



Sensitivity ~40%

PSi – SiO_2–Al_2O_3 (ALD)



Sensitivity ~20%

Signal intensity: 5 x increased → decrease noise level
Sensitivity: 2 x decreased → **LoD theoretically lower with ALD coating**

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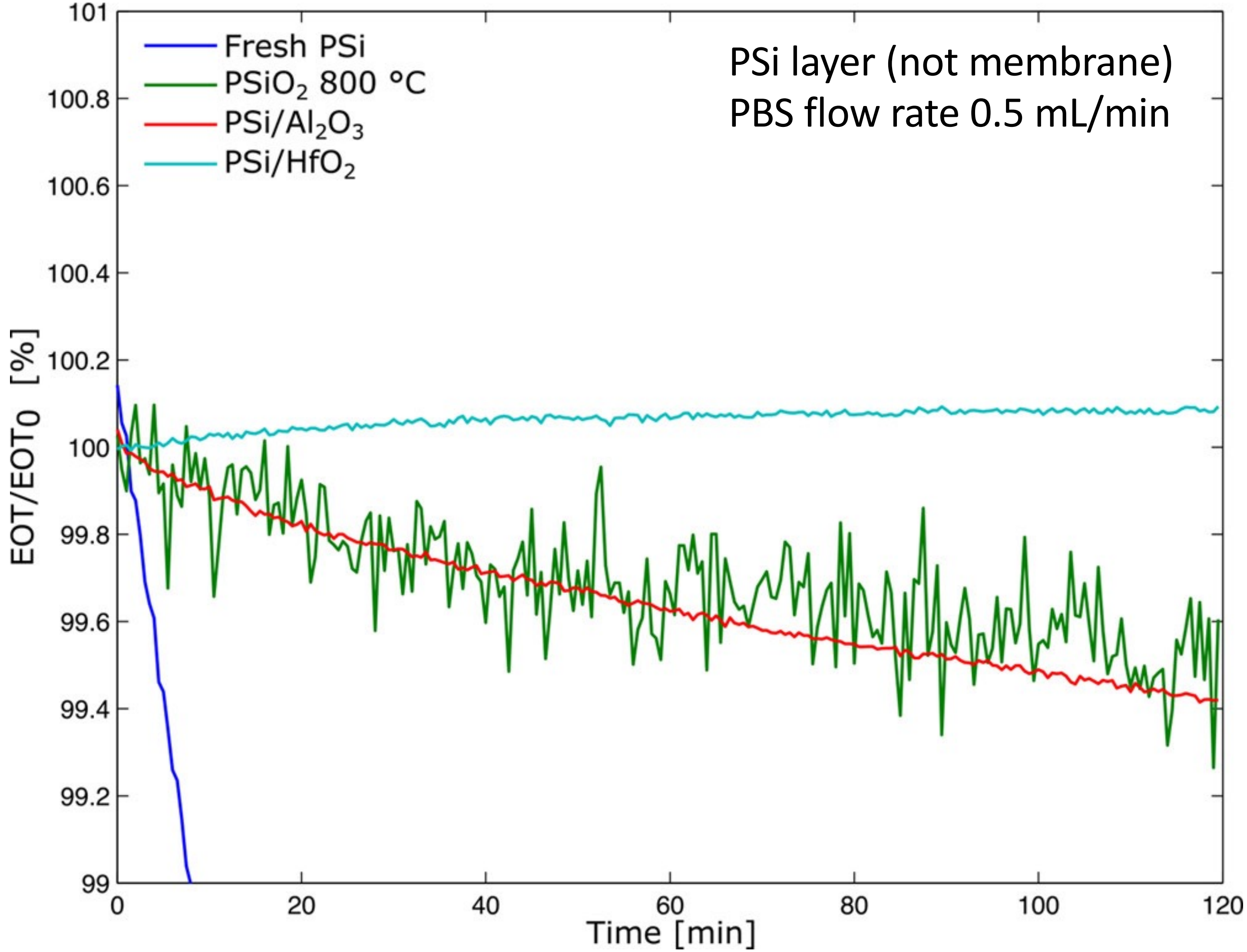
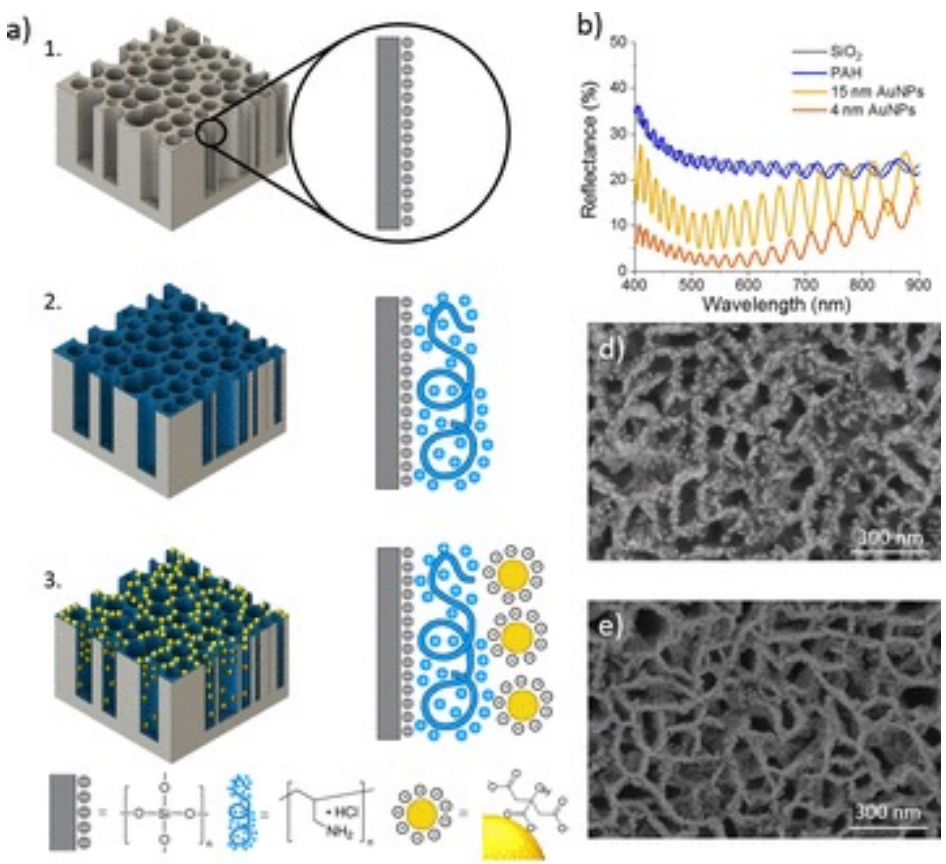
Stable Porous Si with ALD Coatings

Demonstrated over 3 hours in PBS



Rasson, J., and Francis, L.A., J. Phys. Chem. C 2018, 122, 1, 331-338

Future work and perspectives

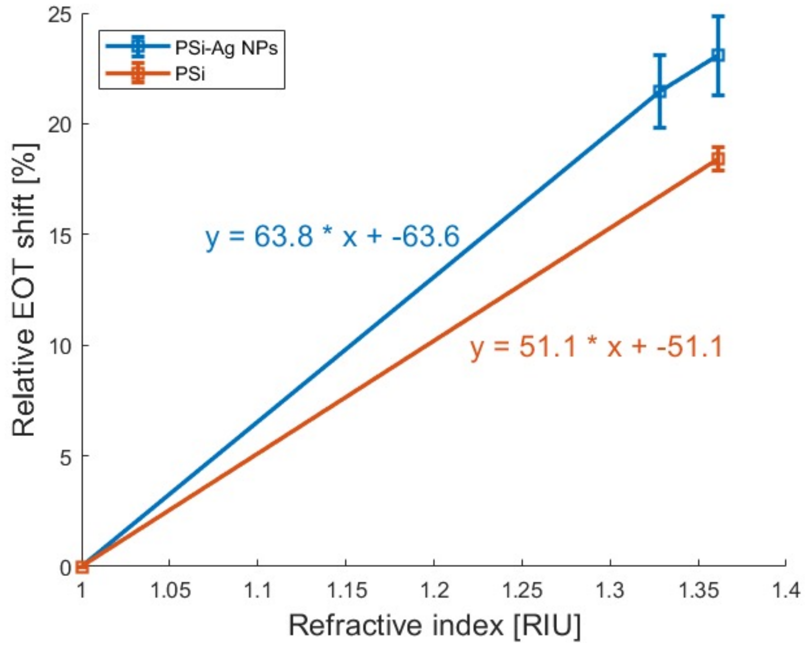


Decoration of Porous Silicon with Gold Nanoparticles via Layer-by-Layer Nanoassembly for Interferometric and Hybrid Photonic/Plasmonic (Bio)sensing

Stefano Mariani, Alessandro Paghi, Antonino A. La Mattina, Aline Debrassi, Lars Dähne, and Giuseppe Barillaro

ACS Applied Materials & Interfaces 2019 11 (46), 43731-43740
DOI: 10.1021/acsami.9b15737

- Decoration with **Ag nanoparticles** for even more signal



- **Dielectrophoresis** to assist particles flow through the membrane

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Conclusions

- **Bacterial lysis** is a selective, efficient and versatile preparation of the analyte prior to its actual exposure to the sensor

- **Flow-through in a PSi membrane** offers an emerging label-free optical biosensing with low detection time (< 30 min) compared to single-ended PSi

- **Atomic Layer Deposition** of metal oxides in the porous material improves its stability over time in saline solutions, and may decrease further the Limit of Detection thanks to a strong signal enhancement

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Acknowledgments

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Thanks to Gilles Scheen, Numa Couniot and Jonathan Rasson for their PhD researches related to the presented topic

Thanks to Clara Whyte Ferreira for her ongoing master thesis investigation on the membrane mechanical and chemical stabilisations