SEM for E.coli-Peptoid Interaction

Morphology and Membrane Damage Characterization

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SEM for *E.coli*-Peptoid Interaction: Morphology and Membrane Damage Characterization

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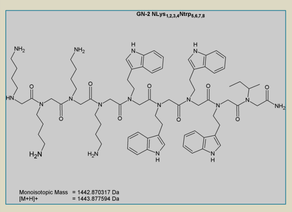
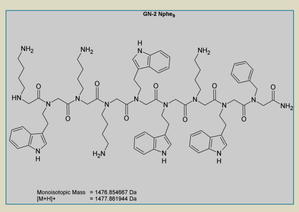
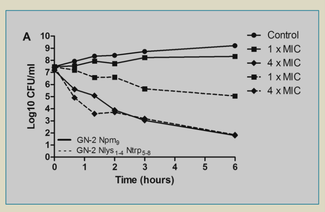
Introduction

A scanning electron micrograph is a true representation of the surface of the sample, which is imaged. For most biological samples by the time the sample is imaged, several preparation steps have been performed, and the rendered surface might be far from that of the pristine sample. Besides the sample preparation the correct operation of the SEM has a great influence on the quality and ultimately the information that can be deducted from the micrographs.
We present here a best practice for obtaining *E.coli*-peptoid micrographs *worth thousand words*.
These micrographs corroborate that the antimicrobial peptoids studied target the *E.coli* membrane.

Materials and Methods







Chemical structure of tested peptoids GN-2NLys$_{1,2,3,4}$Ntrp$_{5,6,7,8}$ and GN-2 Nphe$_9$ and the corresponding growth inhibition curves. The data are log values of viable bacteria in log phase at 2-5x10^7 CFU/ml with the corresponding peptoids, removed at various time points during 6 h incubation.

Chemical fixation of *E.coli* and *E.coli* challenged with peptoids:

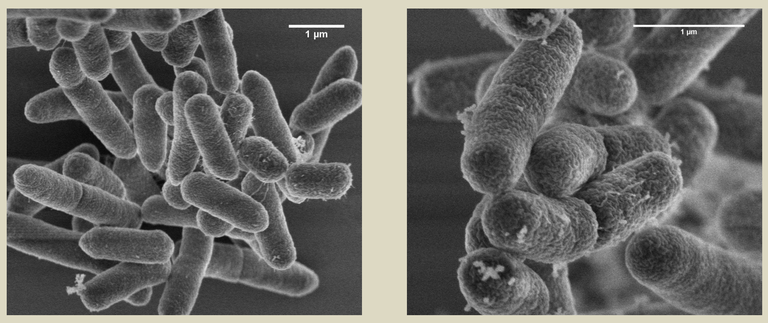
E.Coli grown in Mueller Hinton media is fixed in 3% glutaraldehyde at 4°C for 16h, stained with 1% OsO_4 at 4°C for 16h and dried in solvents (ethanol 30%, 50%, 70%, 80%, 90% and 100%; acetone 30%, 50% and 100%).

50 µL sample is pipetted onto a square piece of Si wafer and further dried in the Leica EM CPD300. The dry sample is attached onto an Al stub with a double sided C tape and grounded with silver paint. Next, the sample is coated with 2 nm Pt in a HR Cressington Sputterer and imaged in an FEI Helios dual beam electron microscope by monitoring the SE signal with the through the lens detector using a 2keV, 43 pA primary electron beam.

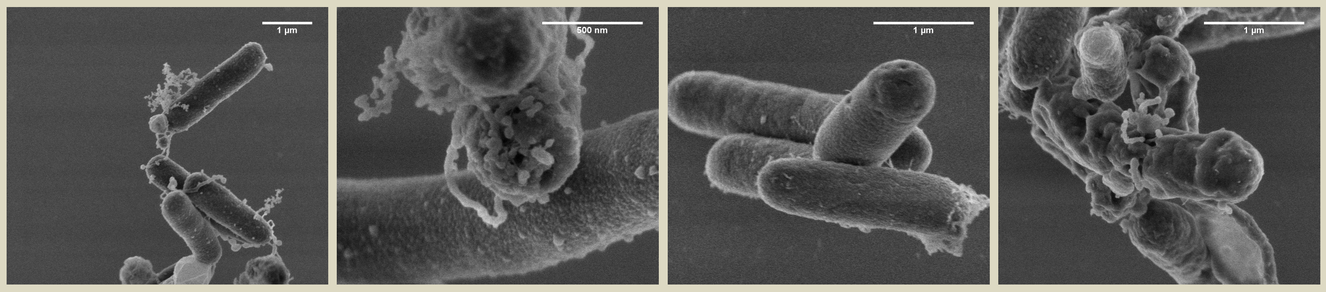
Further information ramona.mateiu@cen.dtu.dk

Results

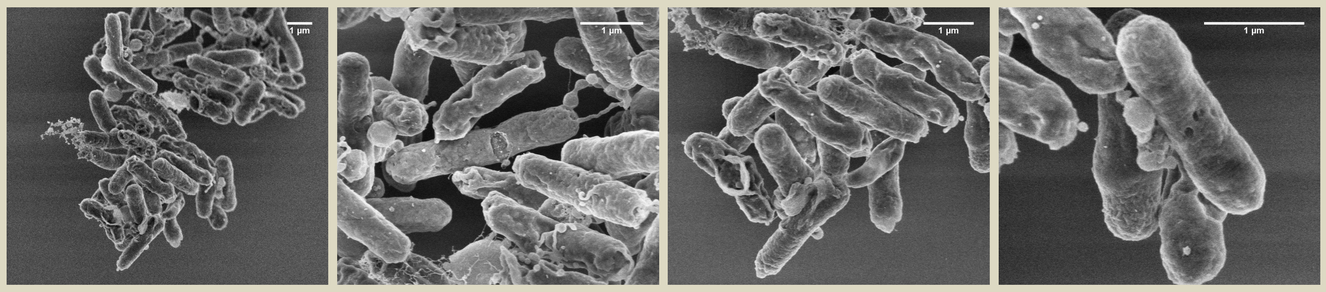
Control sample: untreated *E.coli*



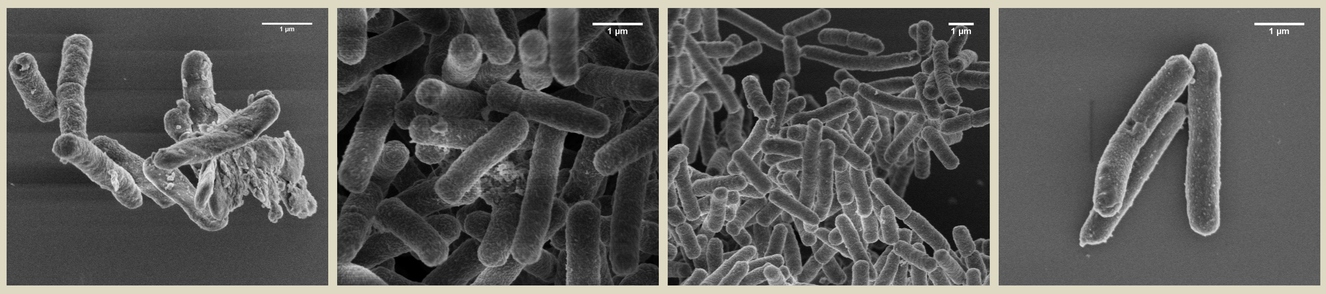
Cell modification of *E.coli* challenged with GN-2 Nphe$_9$ at 1 MIC for 1h



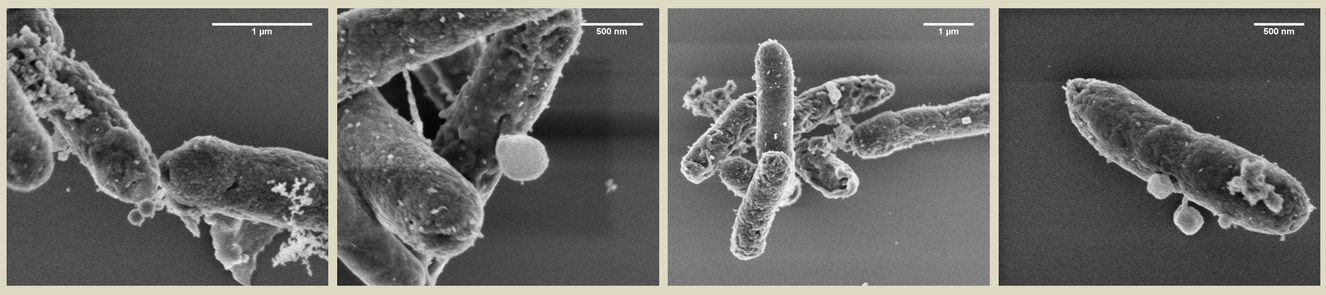
Cell modification of E.coli challenged with GN-2 Nphe$_9$ at 4 MIC for 1h



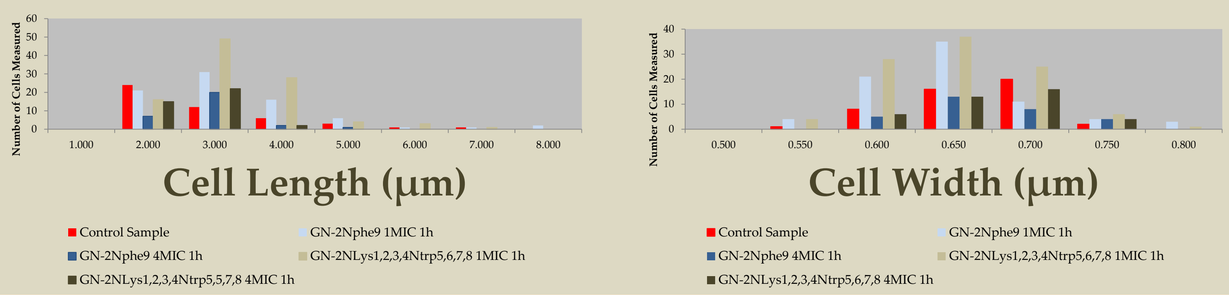
Cell modification of *E.coli* challenged with GN-2NLys$_{1,2,3,4}$Ntrp$_{5,6,7,8}$ at 1 MIC for 1h



Cell modification of *E.coli* challenged with GN-2NLys$_{1,2,3,4}$Ntrp$_{5,6,7,8}$ at 4MIC for 1h



E.coli Morphology: control and peptoid challenged:



Conclusion

The SEM can is successfully used for the study of *E.coli*-peptoid interaction with the presented protocol. Vizalization of the membrane damage and information on cell morphology changes is recorded. The changes in cell morphology indicate that the studied peptoids exhibit not only membrane permeabilization activity but also inhibition of metabolic process by targeting intacellurar structures.

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