Marine diatoms and their networks: an AFM study

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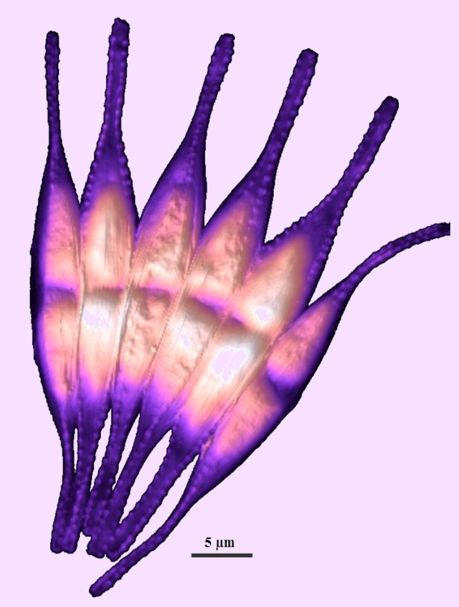
OKOLIŠ

Marine Diatoms

Diatoms are unicellular microalgae responsible for about 20% of the world's net primary production. They are also known for the species-specific design and ornamentation of their silica-based cell walls.

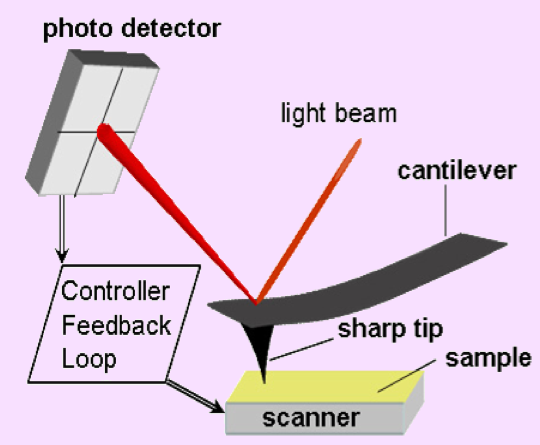
As a model organism we have selected *Cylindrotheca closterium*, isolated from the Northern Adriatic seawater. The unique characteristics of *Cylindrotheca spp*. cell wall are regions which are believed to be completely unsilicified. This makes them ideal candidates for studying structure and organization of organic and inorganic domains of cell wall. *C. closterium* is also considered as a main producer of long chain polysaccharide molecules in the northern Adriatic Sea and is associated with the formation of an enigmatic gel phase. Extracellular polysaccharide production by marine diatoms is a significant route by which photosynthetically produced organic carbon enters the trophic web and may influence the physical environment in the sea.

Diatom *Cylindrotheca closterium*



Atomic Force Microscopy (AFM)

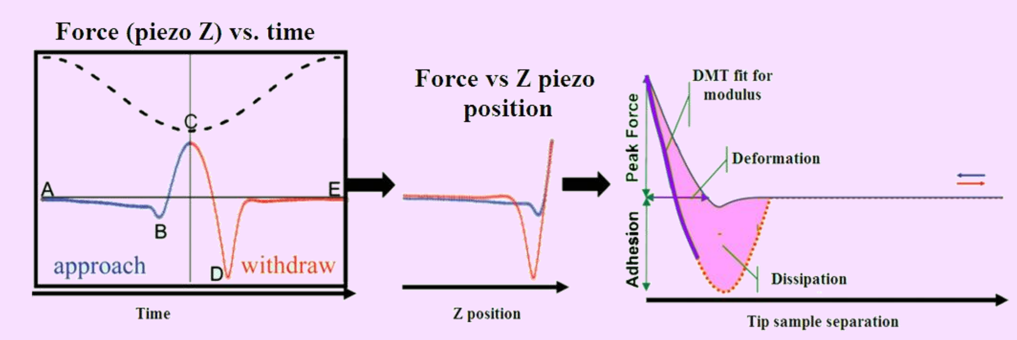
Scheme of the AFM principle



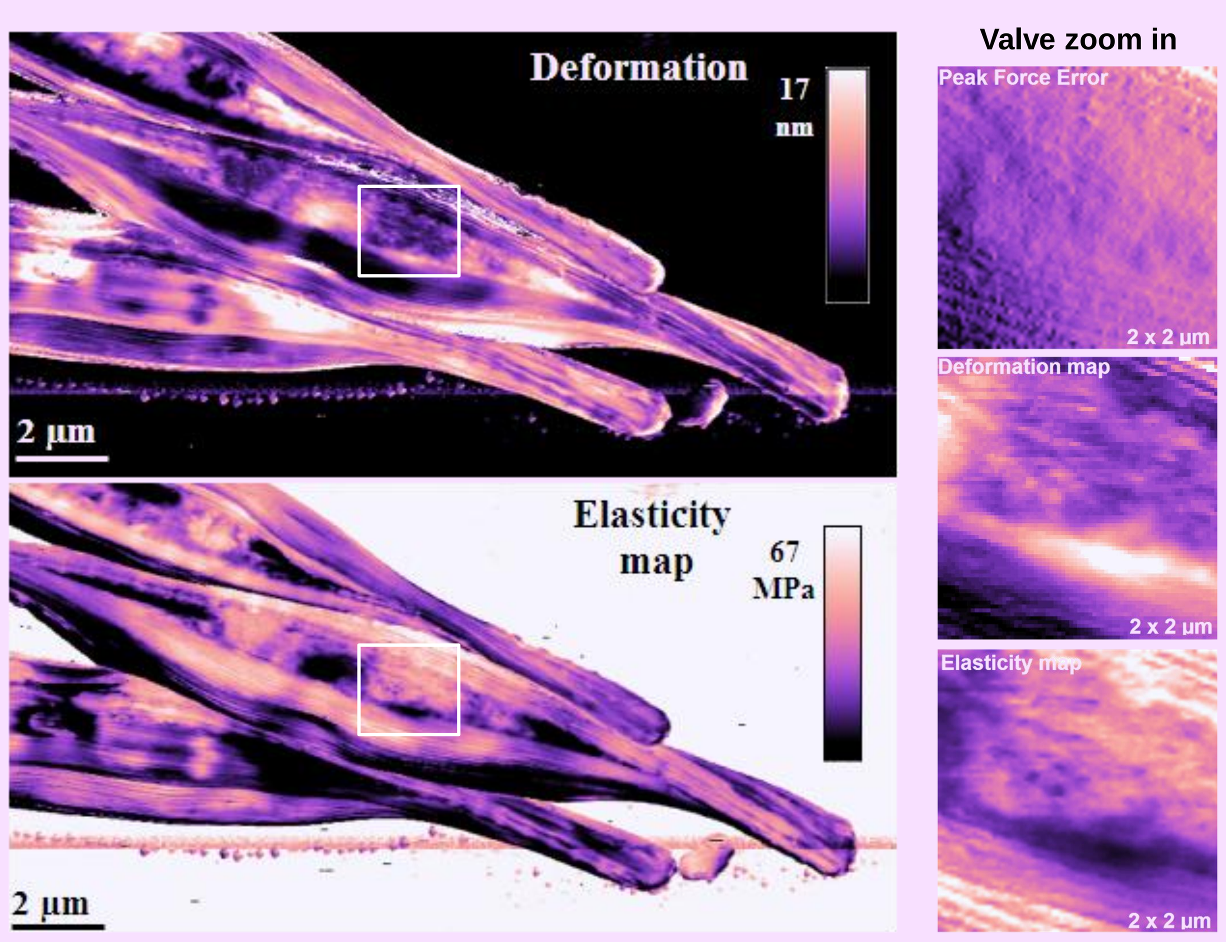
AFM belongs to the broad family of scanning probe microscopies in which a proximal probe is exploited for properties of surfaces with subnanometer resolution.

Besides high resolution imaging AFM is often used for nanomechanical characterization of biological molecules and living cells. In this study for nanomechanical mesurements we used a novel Peak Force Tapping AFM mode which simultaneously generates height (topography), Young's modulus, deformation, adhesion and dissipation data.

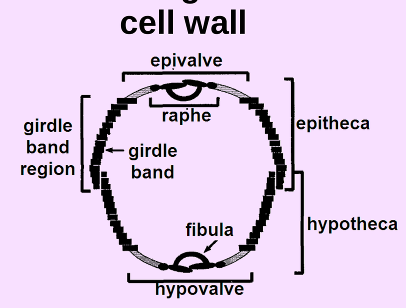
Schematics of Peak Force Tapping mode



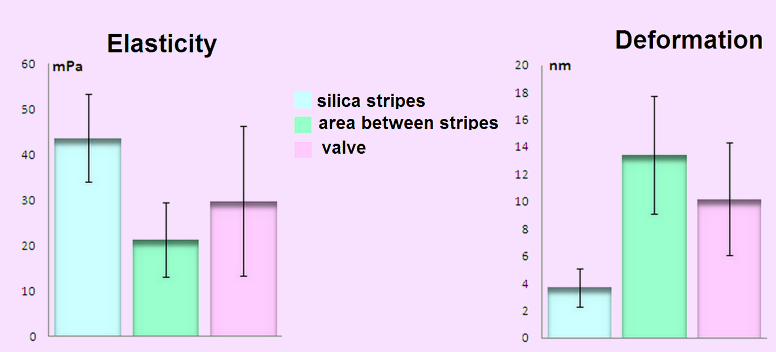
Quantitative Nanomechanical Maps at high resolution obtained by Peak Force Tapping AFM in seawater



Schematic drawing of ***C. closterium*** **cell wall**

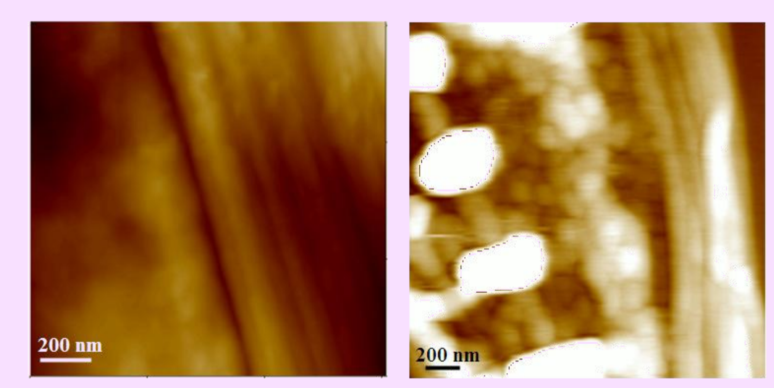


Histograms of nanomechanical properties



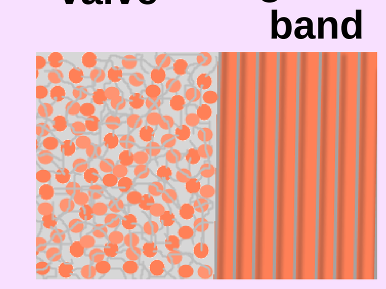
Silica nanoparticles revelead using contact mode AFM in air

Diatom cell, before and after treatment with sulfuric acid after deposition on mica.



Cell wall model

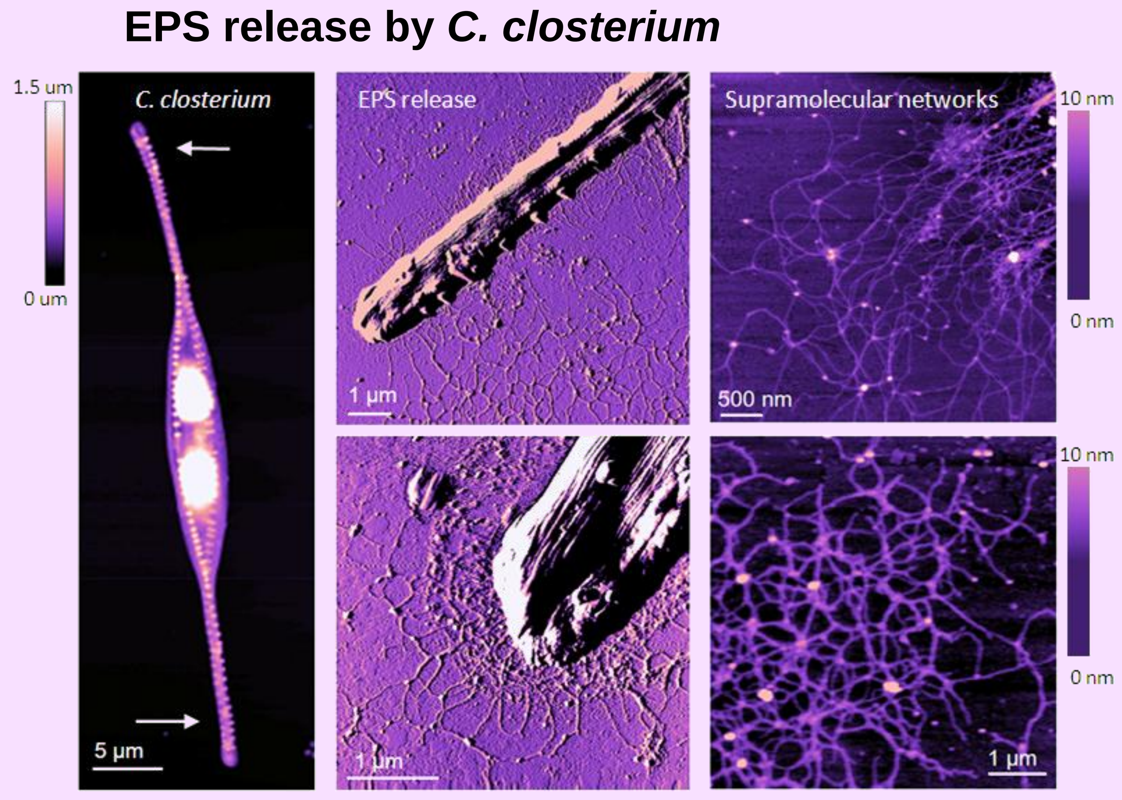
valve **girdle band**



Based on nanomechanical mapping of diatom cell wall, we discovered silica nanoparticles in the valve region that has been reported to be unsilicified and purely organic. A cell wall model is proposed with individual silica nanoparticles incorporated in an organic matrix forming organo-silica composit. Such organization of girdle band and valve regions enables the high flexibility needed for movement and adaptation to different environments while maintaining the integrity of the cell.

Extracellular polymers (EPS) imaged using AFM contact mode in air

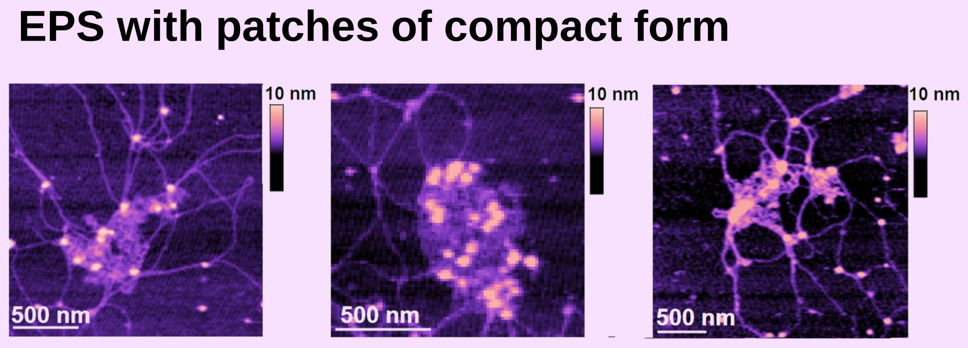
EPS release by ***C. closterium***



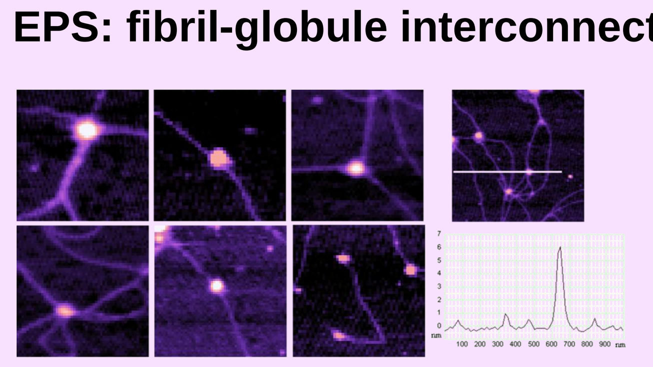
AFM imaging revealed the process of marine gel formation at the nanoscale, starting from the extracellular production of polysaccharide chains by this diatom, to gradual to multiplet entaglement of polysaccharide molecules into gel networks.

The *C. closterium* EPS represents a web of polysaccharide fibrils with two types of cross-linking: fibrils association forming junction zones and fibril–globule interconnections with globules connecting two or more fibrils. The globules are positevely charged proteins whose function is intracellular packing of negatively charged polysaccharide fibrils.

EPS with patches of compact form

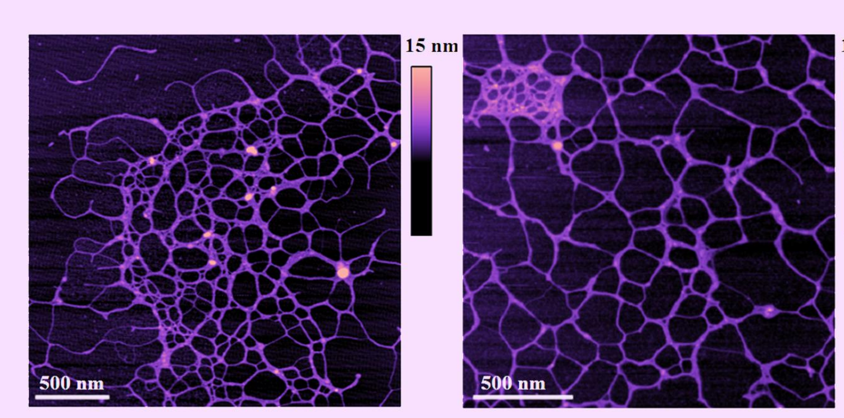


EPS: fibril-globule interconnections

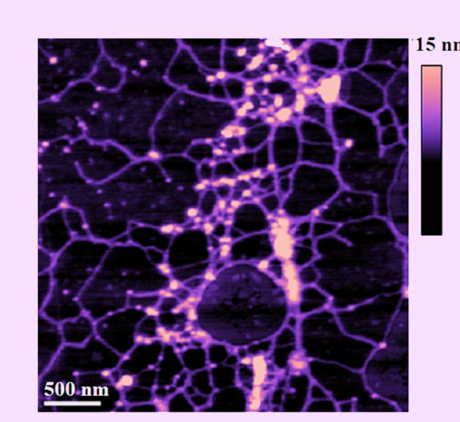


Polymer networks imaged using AFM contact mode in air

Self assembly of purified ***C. closterium*** **polysaccharides into gel network**

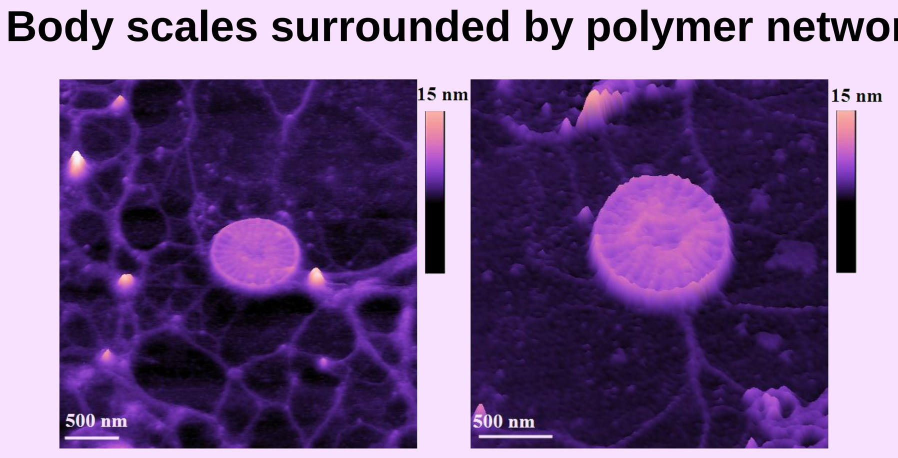


Polymer network produced by ***C. Closterium*** **with incorporated nanoparticles**

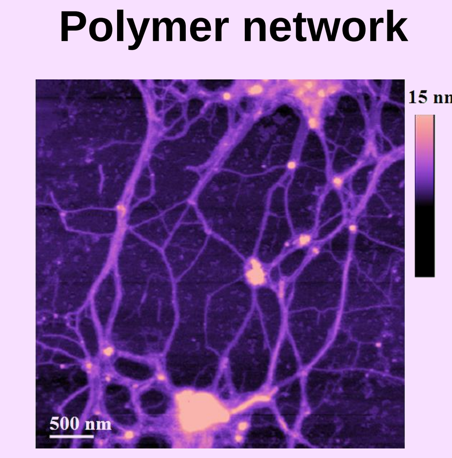


Field data (Northern Adriatic seawater samples)

Body scales surrounded by polymer network



Polymer network



CONCLUSION

This study highlights the capacity of atomic force microscopy (AFM) for quantitative nanomechanical mapping of diatom cell wall using a novel Peak Force Tapping AFM mode [1] and investigating diatom extracellular polysaccharides with a subnanometer resolution [2, 3].

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