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Algal Biophysics: *Euglena Gracilis* Investigated by Atomic Force Microscopy

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Abstract. Matter produced by organisms is remarkable. Evolutionary optimized properties, e.g. regarding hydrodynamic, aerodynamic, wetting and adhesive behavior, can already be found in the "simplest" forms of organisms. *Euglena gracilis*, a single-celled algal species, performs tasks as diverse as sensing the environment and reacting to it, converting and storing energy and metabolizing nutrients, living as a plant or an animal, depending on the environmental constraints. We developed a preparation method for atomic force microscopy investigation of dried whole *Euglena* cells in air and obtained data on whole cells as well as cell parts. Our studies corroborate TEM, SEM and optical microscopy results. Furthermore, we found new features on the pellicle, and set the ground for AFM force spectroscopy and viscoelastic studies on the nanoscale.

Introduction

There is a rising interest of materials scientists in biology. The hydrodynamic, aerodynamic, wetting and adhesive properties of natural materials are remarkable. The results of evolution often converge on limited constituents or principles. For example, the same material component will be found just slightly but effectively varied to obey different functions in the same organism (e.g. collagen occurs in bones, skin, tendons and cornea). One smart feature of natural materials concerns their beautiful organization in which structure and function are optimized at different length scales. Natural systems also show a high level of integration: miniaturization whose objective is to accommodate a maximum of elementary functions in a small volume, hybridization between inorganic and organic components optimizing complementary possibilities and functions and hierarchy [1].

The organism we chose for our experiments is the single-celled algal species *Euglena gracilis* with a typical length between 20 and 100 μm (Fig. 1). *E. gracilis* is a very compact organism and uses efficient functional organelles for optimized tasks so diverse as sensing the environment and reacting to it, converting and storing energy and metabolizing nutrients, living as a plant or an animal, depending on the environmental constraints. Furthermore, *E. gracilis* is easy to keep in culture and is used by research groups around the world. Interesting material properties of *E. gracilis* comprise a tough and yet flexible cell wall (pellicle), dense semicrystalline deposits serving for energy storage [2] and other various components with highly ordered molecular structures such as its monocrystalline photoreceptor that is used for light sensing.

The striated pellicle covering the whole cell is a distinct exoskeletal feature of the *Euglena* species [3-6, Fig. 2]. The pellicle is a proteinaceous structure that provides mechanical stability to the cell, yet it is flexible. Its single strips are connected via interlocking ridges that can slide against each other and are lubricated via biogenic lubricants excreted from pellicle pores (Fig. 2). These features and the concepts they are based on stimulated our interest in the *Euglena gracilis*

organism. There are already many TEM and SEM as well as optical microscopy images available of this algal species, but only sparsely atomic force microscopy (AFM) data [7].

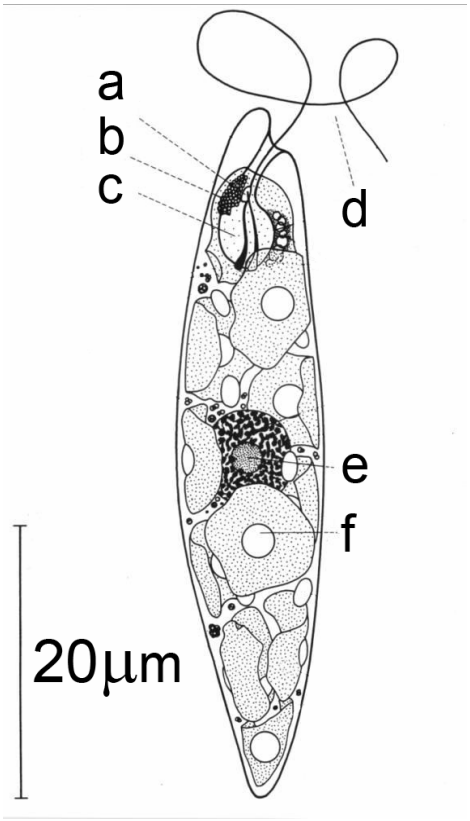

Fig. 1 Sketch of an *Euglena gracilis* cell, a photoreceptor, b eyespot, c reservoir, d flagellum, e cell nucleus, f chloroplast with paramylon sheath of pyrenoid. Image adapted from Fig. 11 [8]. Reproduced with permission from [8]. © 1967 Englewood Cliffs.

Atomic force microscopy is a type of scanning probe microscopy that allows for investigation of conducting as well as insulating samples with unprecedented resolution. Therefore, AFM is regarded an excellent tool for investigation of biological materials. Furthermore, AFM yields not only information on topography but also micro- and nanomechanical properties such as viscoelasticity. For an introduction to scanning probe microscopy see [9].

Some biomolecules such as amino acids and thereby also proteins are defined in their structure down to the atomic level. They are materials built with molecular precision. In principle, each and every cell, plant, animal as well as human individual can be seen as nanotechnological wonder.

Nowadays, materials scientists have just started to make man-made materials of such precision.

The ultimate goal of our AFM studies of *Euglena gracilis* is imaging the crystalline photoreceptor (Fig. 3) at molecular resolution. Among the naturally occurring crystalline light-detecting organelles [10], the *Euglena gracilis* photoreceptor is unique because it is the only known crystal of a photodetecting protein consisting of about a hundred layers [11]. This rhodopsin-like protein shows optical bistability, with negligible thermal deactivation [12]. This simple two state photocycle makes it a promising building block for biocomputers.

Furthermore, rhodopsin has single photon sensitivity at room temperature: Hecht showed, in 1942, the exquisite sensitivity of human rod cells (where the active substance is rhodopsin) by demonstrating that already a single photon can initiate a response [13]. Hecht *et al.* found that about 90 photons had to enter the eye for a 60% success rate in responding. Since only about 10% of photons which arrive at the eye actually reach the retina, it means that about 9 photons were actually required at the receptors. Since the photons would have been spread over about 350 rods they were able to conclude statistically that the rods must be responding to single photons even if the subjects were not able to see them when they arrived too infrequently.

In 1979 Baylor, Lamb and Yau were able to use rods from toads placed into electrodes to show directly that they respond to single photons [14].

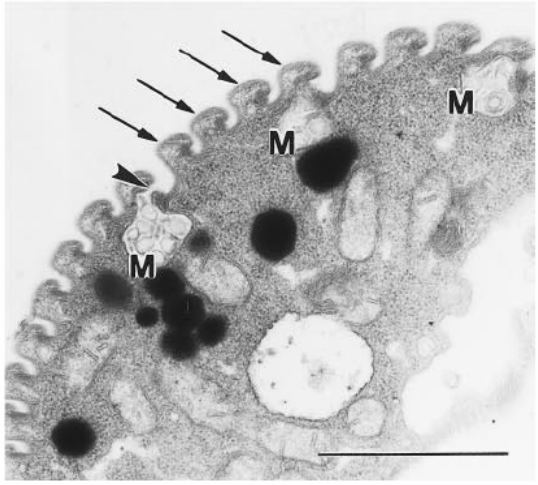

Fig. 2 TEM image of *Euglena terricola* showing the pellicle strips in cross-section (arrows). The arrowhead points toward a pellicle pore, where the biogenic lubricant, the mucus (M), is excreted. Image reproduced with permission from [5]. © 2000 by the Society of Protozoologists.

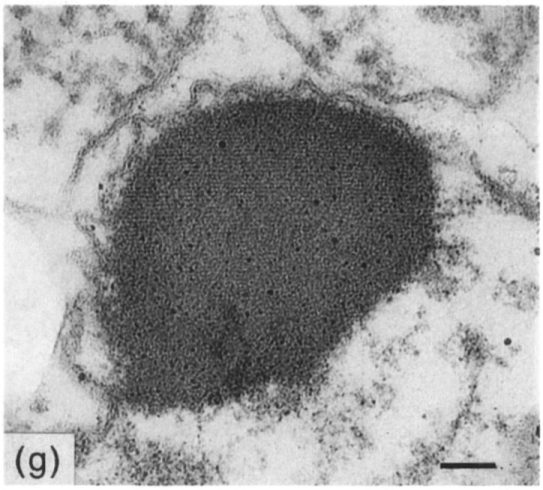

Fig. 3 TEM image of a thin section of an isolated *Euglena gracilis* photoreceptor, shows about 100 component layers. Scale bar 300nm. Image reproduced with permission from [11]. © 1998 by CRC press LLC.

Materials and Methods

The algae material for our investigation was kindly provided by Paolo Gualtieri and Laura Barsanti and coworkers from the CNR Istituto di Biofisica in Pisa. Pure cultures of *E. gracilis* cells were grown in Cramers-Myers medium. Dried whole cells for AFM investigation were prepared according to the following protocol: 100 μl of cell suspension were pipetted onto a glass slide and covered with a cover slip. Finger-tight force was applied onto the coverslip, removing excess solution and air bubbles. Slow evaporation of the solvent at room temperature resulted in a concentration gradient of nutrient embedding whole unburst cells especially at the edges. After 5 minutes the coverslip was carefully removed by dragging it horizontally, and the samples were investigated with AFM. Such preparations were good AFM specimens for several days.

Crystalline cell parts for AFM investigation were prepared by the group of Paolo Gualtieri and Laura Barsanti and coworkers from the CNR Istituto di Biofisica in Pisa according to the preparation methods described in [15]. Before AFM imaging, one millilitre of the solution was centrifuged with a home-made centrifuge at 3300 RPM (with a mean distance between center of rotation and liquid of 6.5 cm, equivalent to ca. 800 g) for 10 minutes in order to remove small suspended particles. Then, the fraction of the solid precipitate containing the heaviest particles was resuspended in one milliliter of HEPES solution, spread on a glass slide and dried.

Cells and cell parts were investigated with an MFP-3D atomic force microscope (Asylum Research, Santa Barbara, CA, USA) prototype equipped with top-view optics. The AFM head was mounted on an inverted optical microscope (Axiovert MAT-200, Zeiss, Jena) allowing for investigation of transparent as well as opaque samples. Olympus AC 240 TS-E cantilevers with a resonant frequency between 61 and 77 kHz in air and a spring constant of 1.2 to 2.5 N/m were used for the experiments described below. The data acquisition was performed at a rate of 0.1Hz.

Results and Discussion

The AFM images of the whole cells show the pellicle, flagellum and some cameo and intaglio shapes of cell parts and features (see Fig. 4 for 20×20μm image). More detailed imaging (Fig. 5, 5×5μm) of the cell wall reveals pellicle strips, arranged diagonally (the pellicle usually grows as a left-handed spiral) and the articulations between the strips showing lubricant material features.

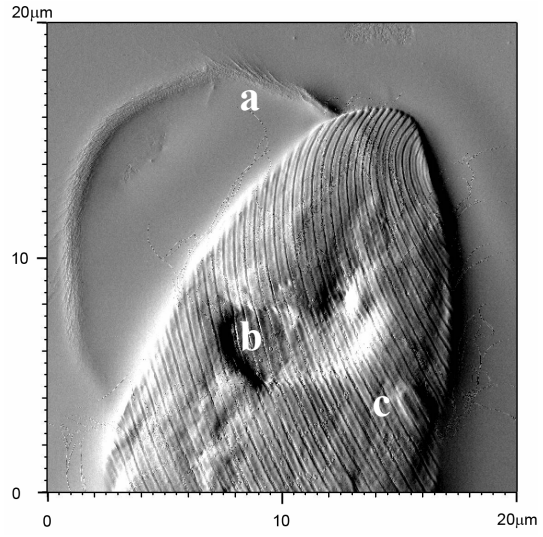

Fig. 4 Intermittent contact mode AFM image of the apical part of an *E. gracilis* cell. a flagellum, b reservoir, c paramylon grain. Image size 20×20µm, imaging parameter amplitude.

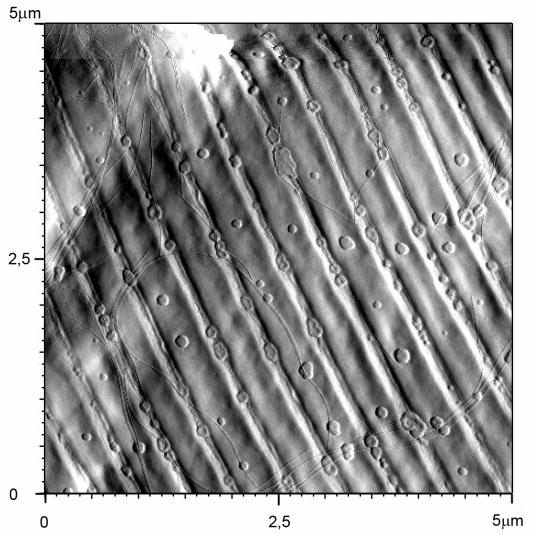

Fig. 5 Intermittent contact mode AFM image of the pellicle of an *E. gracilis* cell showing details of the pellicle including the mucus excretion pores. Image size 5×5µm, imaging parameter amplitude.

In three-dimensional view of some of the AFM images new surface features that have not been seen before with SEM preparations are visible. A 3-D view of the middle part of a dried *E. gracilis* cell is shown in Fig. 6.

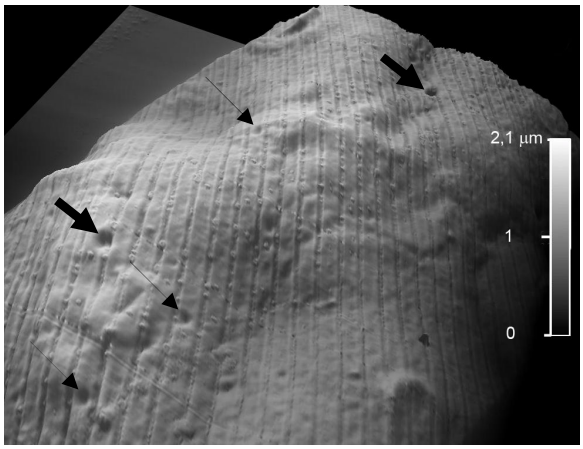

Fig. 6 3-D view of the middle part of a dried *E. gracilis* cell. The bold arrows point towards pellicle pores, the thin arrows indicate new surface features not yet seen before with any other imaging mode on *E. gracilis*. The fact that they can be imaged with the AFM might be due to preparation methods that differ from preparation methods for SEM.

The bold arrows point towards pellicle pores as they have been known from SEM investigations since the late 1960s [4]. Thin arrows indicate new surface features. Furthermore, some cameo and intaglio shapes of cell parts and features can be seen.

Paramylon grains are energy storage deposits in *Euglena* [2]. Our preparation method for crystalline inclusions allows for AFM investigation of the circumventral stripes of the crystallised carbohydrates that the paramylon mainly consists of (Fig. 7). The preparation process removes most of the membrane remnants.

The crystalline cell parts preparation also contained features not yet determined. These features were visible by optical microscopy and AFM (Fig. 8).

They might be crystalloid bodies. There are around 20 of these structures in a living *Euglena gracilis* cell. They are mostly lipid in nature and consist primarily of crystalline wax esters [16].

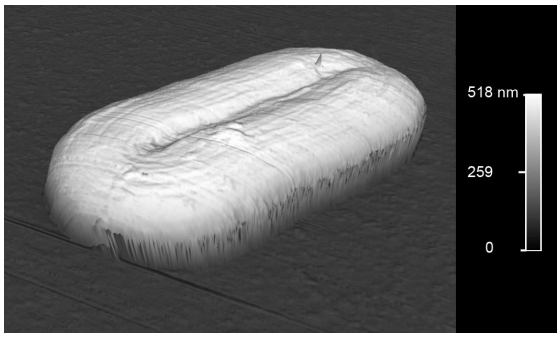

Fig. 7 Intermittent contact mode AFM image of a paramylon grain. This cell inclusion is made of crystalline carbohydrates and serves as energy storage deposit. The circumventral stripes are a topic of ongoing scientific discussion. Image size 2.5×2.5μm, height scale 353 nm

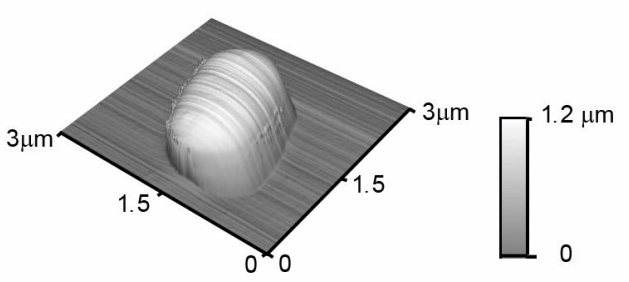

Fig. 8 Intermittent contact mode AFM image of a feature repeatedly found in the crystalline parts preparation.

Our AFM results corroborate SEM and TEM images of the *Euglena* pellicle and the paramylon grain. Furthermore, some pellicle surface features were observed that have no correspondence in *Euglena* preparations for SEM investigation.

Future Directions

This paper presents the development of a preparation method for AFM imaging of dried whole cell walls of *Euglena gracilis* and some of its cell constituents. AFM data of mucus excretion pellicle pores as well as AFM data of new surface features and crystalline cell inclusions are presented.

Since AFM does not only yield information about the topography, but also allows for manipulation on the micro and nanoscale and the measurement of viscoelastic properties of the surfaces investigated, future research attempts along these lines seem encouraging.
The ultimate goal of our AFM studies concerning *Euglena gracili*s is acquiring information on the photoreceptor at atomic resolution.

However, the fraction of crystalline parts contains only few photoreceptors, since crystalloid bodies, paramylon grains and not completely removed cell material amount for a large portion of the fraction. Possible solutions to this challenge comprise the development of a preparation technique for a highly purified photoreceptor fraction. It might also be possible that combination with fluorescence microscopy simplifies observational search for the location of the photoreceptor.

As a more general outlook concerning biomaterials in materials science, it is stressed that relating structure to function in biomaterials can only be the beginning of promising developments. The thermal and hydrolytic sensitivities of biological materials limit their applicability in many important synthetic material applications [17].

A real breakthrough requires an understanding of the basic building principles of living organisms and a study of the chemical and physical properties at the interfaces, to control the form, size and compaction of objects [1].

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