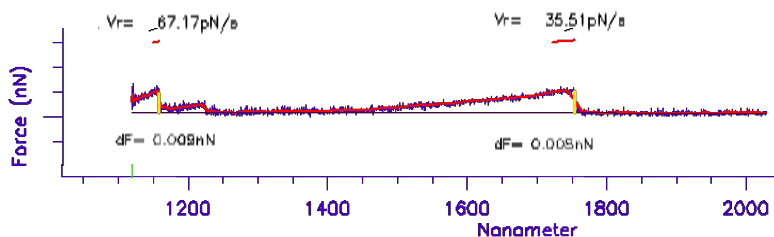


Single molecule study of enzyme-substrate interaction

Considerable research interests has focussed on the ability of atomic force microscopy (AFM) and related techniques to record forces on or between single biological molecules.

This project aims at quantifying the interaction between AlgE enzymes, central in the biosynthesis of the biopolymer alginate, and its polymer substrate. Such interactions have previously been studied for certain AlgE enzymes. However, due to intensive research efforts aimed at revealing the structure – function relationship of these biologically and industrially important enzymes, many mutants are now available, displaying a variety of different behaviour when interacting with the substrate. Some of these enzymes are shown to display a processive mode of action, meaning that it slides along the polymer and catalyses several conversion reactions before detaching. Detailed information concerning the energy landscape of the interaction between different structurally related yet functionally different enzymes, may open for increased insight into the factors governing the behaviour of enzymes. Recently, we initiated studies of some of these enzymes using the sensitive force probe optical tweezers, studies which have provided interesting results and which will be continued also in the spring semester 2011. However, this work will benefit from additional information obtainable using AFM. Combined, the results obtained by AFM and optical tweezers allow access to a broad range of force loading rates, and thus allow access to a complete description of the energy landscape of the interaction occurring between different mutants of these enzymes and their substrate.



Preliminary data obtained using optical tweezers, revealing the force needed to rupture one AlgE – substrate bond.

Requirements: Basic knowledge of AFM.

Supervisor: Marit Sletmoen, Department of Physics. email: marit.sletmoen@ntnu.no

Collaborators: Gudmund Skjåk-Bræk and Finn Aachmann, Department of Biotechnology, NTNU.

Biophysical aspects of bacterial adhesion

In this project, bacterial glycan mediated adhesion will be investigated at the single molecule or single bacteria level by sensitive force probes. Intermolecular bonds, such as the specific interactions between molecules on the surface of the bacteria and receptor molecules on the adhesive surface, have a given strength and can be characterized based on its energy landscape. This energy landscape is used to describe the transition between the different states of the bond, i.e. open and closed, as a stochastic process that is strongly affected by the properties of the landscape, such as activation energy and bond length. The average time after which it opens is termed the lifetime and serves as a measure of its strength. In this project, force spectroscopy of individual bacterial adhesin – glycan bonds will be performed by pulling on the system under scrutiny with controlled forces. Force spectroscopy will be realised by the use of both AFM and optical tweezers, both present at the Dept. of Physics, NTNU. In addition, the interaction between bacterial pili and protein receptors will be quantified.

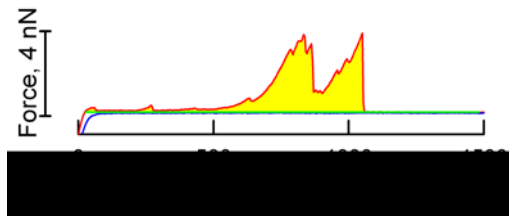
Requirements: Basic knowledge of AFM.

Supervisor: Marit Sletmoen, Department of Physics. email: marit.sletmoen@ntnu.no

Collaborators: John Michael Koomey, Department of Molecular Biosciences, University of Oslo.

Direct determination of forced unbinding of macromolecules in a model biological barrier

This project aims at the forced unbinding of mucus macromolecular components in a model system relevant for cystic fibrosis lung disease, realised by atomic force microscopy. Cystic fibrosis (CF) is a potentially lethal, genetic disease where mutations in a chloride channel, the Cystic Fibrosis Transmembrane Regulator (CFTR), lead to hyperviscous mucus secretions. Hyperviscous mucus leads to a failure of mucociliary clearance, mucus stasis, and bacterial colonization. Most patients eventually succumb to infections with *Pseudomonas aeruginosa*, which secretes a highly viscous polysaccharide, alginate, exacerbating the problem of hyperviscous mucus through its innate viscosity and its interaction with the mucin polymers that comprise mucus. The aim of the present study is to design and implement a CF patient relevant model system that supports direct determination of mucin-alginate interactions applying single-molecule techniques. The main focus is on the dose-dependent perturbation of the interaction profiles in the presence of oligoguluronates that are under development for treatment of mucus hyperviscosity.



The work performed on the mucin-alginate system will be determined as shown in the figure (yellow area):

$$W = \int_{z_a}^{z_b} F(z) dz$$

where $F(z)$ is the force vs z -piezo translation

Requirements: Basic knowledge of AFM.

Supervisor: Marit Sletmoen and Bjørn Torger Stokke, Department of Physics.
email: marit.sletmoen@ntnu.no, bjorn.stokke@ntnu.no

Collaborators: Kurt Draget, Department of Biotechnology, NTNU.