Topics for project & master thesis works academic year 2016/2017

Biological polymers: Mesoscale structure formation and interactions.

Supervisors: Bjørn Torger Stokke and co-workers,

Webpage: http://home.phys.ntnu.no/brukdef/prosjekter/biopolymerphysics/

Please visit the webpage for links to publications, additional information. The webpage provide information on topics that we so far have published within. We are currently also working within additionally topical areas that can be suitable for project / master thesis topics, please contact me to learn about these.

Symmetry breaking in responsive hydrogel membranes

Supervisors: Bjørn Torger Stokke (bjorn.stokke@ntnu.no), Jonas Myren Ribe (jonas.ribe@ntnu.no), Eleonora Jonasova (eleonora.jonasova@ntnu.no)

Hydrogels are three-dimensional networks consisting of polymers. They can absorb large quantities of water and swell to several times their size in dry state. The combination of polymer strands and a large volume of water gives them properties of both solids and liquids.

A subgroup of hydrogels, called responsive or smart hydrogels, has the ability to change their shape and/or structure in response to external stimuli (pH, temperature, a specific molecule). This change is often the change in swelling equilibrium, meaning the gel swells or shrinks as a result of being exposed to a trigger. This makes hydrogels particularly interesting for fabrication of label-free biosensors, as they translate a change in their environment into easily observable swelling. Other applications include preparation of drug-delivery agents. In this case, the gel particles would change their shape or other properties and release the drug at the desired location in the body.

Hydrogels can be prepared in various geometries and our activities include preparing semi-hemispherical responsive hydrogels. The proposed project would consist of preparation and characterization of patterned hydrogel membranes, similar to those described by Wu et al [1], which can be seen (fig. 1)

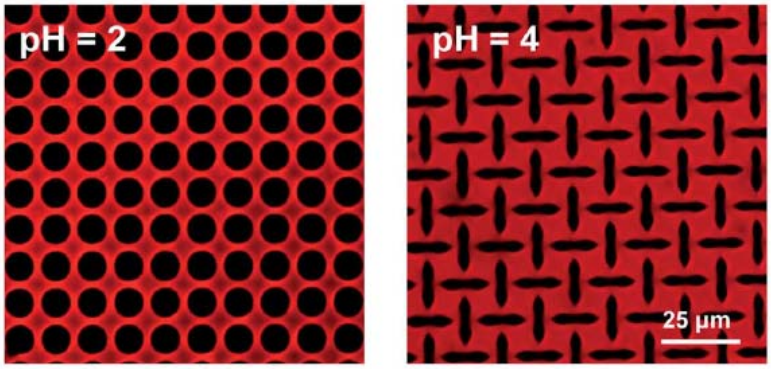

Figure 1 Hydrogel membranes equilibrated at two different pH values. The round holes at pH 2, due to swelling and subsequent buckling, have changed their shape at pH 4. Image taken from Wu [1].

The swelling of these gels creates stresses and strains and consequently changes in geometry and symmetry of the pattern. The layers can be prepared with holes of various sizes and shapes. During the swelling the size and shape of these holes is changing and buckling and breaking of symmetry is observed. The patterned surfaces can be used to study the mechanical aspects of hydrogel

swelling and (in case the stimulus is a molecule) relate these mechanical aspects to the migration and binding of the trigger molecule within the gel. The patterned gels could also be eventually used as biosensors.

The project will require preparation of patterned hydrogels using soft lithography and studying the influence of various geometric factors, such as the ratio of the hole size to the distance between the holes, the shape of the holes, thickness of the gel layer, etc. The visualization of the gels will be carried out using various imaging techniques, including e.g. quantitative phase contrast microscopy. Parts of the soft litography process, such as preparation of PDMS molds, may be carried out in NTNU NanoLab.

Dissecting the impact of cellulose production on the elastic modulus of epidermal cells in Arabidopsis seedling roots

Supervisors: Bjørn Stokke and Thorsten Hamann
Thorsten.hamann@ntnu.no Bjorn.Stokke@ntnu.no

Plant cell walls represent the first line of defense against environmental stress as well as form key elements during plant cell morphogenesis and plant growth. During these different biological processes the walls have to maintain their functional integrity to perform their activities. The available evidence shows that plant cells have evolved a mechanism to monitor the functional integrity of their cell walls by modifying cell wall composition and structure as well as cellular metabolism in order to compensate for cell wall damage (CWD) impairing the integrity. Plant cell wall composition/structure influence the quality of plant biomass and the yield of food crops. Understanding the mode of action of the cell wall integrity maintenance mechanism could therefore generate novel strategies to facilitate bioenergy and food production.

The Hamann lab has established a model system to study the mode of action of the cell wall integrity maintenance mechanism. Arabidopsis seedling roots are being used as biological material while cellulose biosynthesis inhibition is used to simulate CWD. Cellulose was chosen because cellulose microfibrills are the main load bearing elements in plant cell walls. The Hamann lab has previously shown that different signaling molecules are required to mediate the response to CWD and several genes have been implicated. However, it is not known how quickly and in what way the inhibition of cellulose production changes the mechanical characteristics of the cell wall.

The Stokke lab is in the process of establishing nanomechanical mapping of soft materials (Peak Force Quantitative Nanomechanical Mapping) as mode for using atomic force microscopy. This mode, different from more conventional known ones such as contact, non-contact or tapping mode, potentially allows correlations between the morphological features (height variations) and the resulting mechanical (elasticity) properties of cells. The elastic properties are obtained by using the maximum indentation force as the feedback signal for each pixel in the scanning process. Elastic properties are deduced using a model for the deformation geometry induced by the tip of the cantilever. Elastic maps can potentially be obtained with lateral resolution in the order of 10 nm.

The Hamann and Stokke labs have teamed up to offer a project with the aim to apply atomic force microscopy to determine changes in the mechanical characteristics of plant cell walls induced by manipulation of cellulose production. This will provide the foundation to understand how plant cells perceive CWD and translate a physical stimulus into quantitative biochemical signals regulating adaptive downstream responses.

Below preliminary results are shown where the mechanical characteristics of a seedling root tip have been examined with AFM.

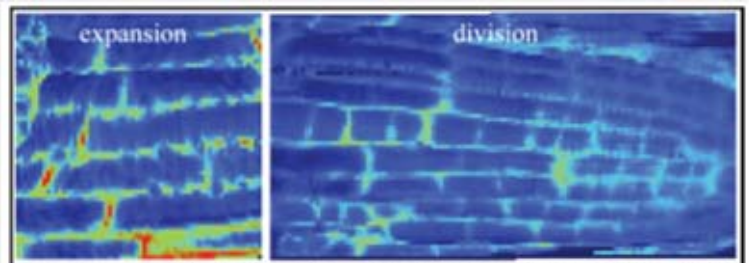

Figure : Map of cell wall elasticity in the root derived from AFM indentation experiments. Blue, more elastic. Red, less eslatic. (Braybrook and Peaucelle, unpublished)

Fig .2
Hamann et al., Plant Journal 2009
Denness and Hamann, Plant Signaling and Behavior 2011
Milani et al., Plant journal 2011
Peaucelle et al., Current Biology 2011

Bioresponsive hydrogels as signal transducers

Supervisors: Bjørn T. Stokke (bjorn.stokke@ntnu.no), Eleonora Jonasova (eleonora.jonasova@ntnu.no)

Hydrogels adopt an equilibrium swelling state based on thermodynamic principles, where changes in ionic environment, pH, temperature, pressure etc., can induce various swelling states depending on the molecular properties of the polymers constituting the network. Within this project, we have recently developed a line of research for technological utilization of molecular interactions integrated in hydrogels to be applicable for biosensors. In addition to tailor-making of hydrogel materials to act as biological signal transducers, this line of research takes advantage of a high resolution (2 nanometer) interferometric technique for the characterisation of optical length of responsive gels. This technology providing a 100 fold improved resolution compared to diffraction limited optical imaging, is currently being applied for the characterisation of developed, bioresponsive (various components) hydrogel materials aiming at sensor development. The 50-60 µm radius, hemispherical hydrogel manufactured at the end of an optical fiber constituting the environmental sensing element makes up a Fabry-Perot cavity for high resolution interferometric detection of the optical length. The interference of light guided by the optical fiber and reflected at the fiber-gel and gel-solution interfaces enables detection of the optical pathlength within the gel and thus the swelling degree of the gel (Figure 3a).

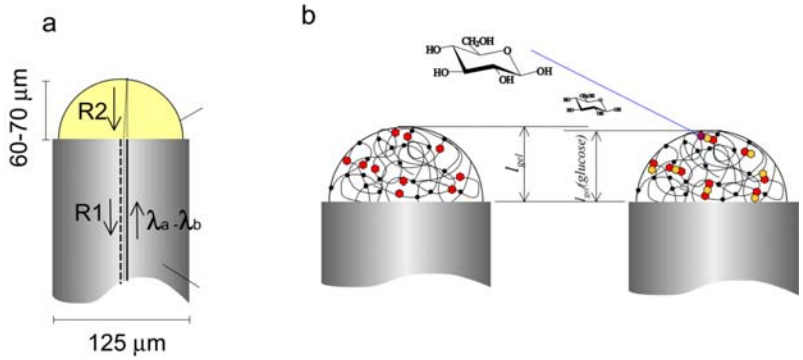

Figure 3. (a) Shematic illustration of optical detection of changes of a hemispherical hydrogel. (b) Schematic illustration of glucose induced reduction of equilibrium swelling volume of a glucose-selective hydrogel.

Both the amplitude and phase of the interference wave reflected back through the optical fiber contains signatures that can be used to deduce changes of the optical properties of the responsive hydrogel material, but the phase represents the highest resolution information. The function of the miniaturized bioresponsive hydrogel material is both to embed specific biological recognition event and to transduce this to changes of the hydrogel that readily can be read-out by the interferometric platform.

The technique has been applied to characterize various developed hydrogel matrices to explore the potential resolution of the technique and for demonstration of biospecific sensing transduction. A glucose sensor is realized on this platform by utilizing glucose sensing functionality incorporated into the hydrogel matrix (Figure 3b). The interaction between glucose and a recognition element, changes the driving forces for gel swelling thus inducing a glucose sensitive hydrogel swelling. The properties of the responsive hydrogel as a glucose sensor were determined in more detail with respect to swelling kinetics and equilibrium swelling degree for the physiological relevant range of glucose. Results showed there was a good degree of reversibility, both for equilibrium swelling and swelling kinetics.

Within this topical area, the aim for a project work/thesis will be to design a recognition element for a selected biological (macro) molecule, prepare such sensor and to characterise it. Part of the work can be developed within NTNU NanoLab infrastructure.

Macromolecular interactions at the single-molecule level

Supervisor: Bjørn T. Stokke (bjorn.stokke@ntnu.no)

Macromolecular interactions are underpinning signal-recognition, biocatalysis, hybridization and soft materials assembly. Such interactions can be characterized at different level of detail, from thermodynamics, to identification of specific atomic groups involved in recognition, binding and eventual catalysis. In the present project we will develop a strategy for the determining characteristic properties of selected macromolecular interactions using equilibrium fluctuation analysis of single binding events. This will be conducted by applying total internal reflection fluorescence microscopy, combined with immobilization one of the components and implement image processing strategies for the fluctuation analysis.

In total internal reflection, there is generated an evanescent wave on the low-refractive index side that decays with the distance from the interface as:

$$I(z) = I_0 e^{-z/d} \qquad (1)$$

where I_0 is the intensity at the interface, and d the characteristic distance for penetration depth of the evanescent wave. The parameter d depends on the optical properties of the materials and the angle of incidence. Typically d is of the order of 100 nm, but can be tuned by varying the incident angle of the light. The exponentially decaying evanescent field at the sample side in TIRF will be exploited for selective observation of fluorescently labelled molecules in this thin section. The fluctuation characteristics will be affected by binding to the surface. Analysis of the time dependent trajectory of individually fluorescent macromolecules that is bound specifically to its binding partner (immobilized on the surface) forms the basis for characterization of the interaction at the single molecule level.

The project include various subtopics for the particular macromolecular pair to investigate (to be decided among those in the laboratory: e.g: competitive displacement in dsDNA,):

- Implement immobilization protocol on glass surface and characterize the functionalized surface (AFM, fluoresecence)

- Spatiotemporal observation of fluorescent labelled ligand by TIRF; specificity and characteristics. (Observations on TIRF will be carried out using an EMCCD camera supporting up to 24 frames/second in full frame (1024x1024 pixels) or faster for selected / binned area. Sensitivity: photon counting in each pixel)
- Analysis of spatiotemporal behavior at the single-molecule level to extract fluctuation parameters

The goal of the project is to establish procedures for a single-molecule sensitive interaction assay and its application of a particular macromolecular pair. The principles of the assay is expected to be transferable to numerous applications.

NTNU Microfluidics Group (http://www.ntnu.edu/microfluidics/)

Within microfluidics, we explore application of droplet-based and continuous flow microfluidics for molecular assemblies, separation. We have recently established platforms that enable production of various polysaccharide microstructures (single and multi layered microparticles, microfibers, egg-shaped particles etc). Some applications include:

- Cell encapsulation for bone mimicking materials
- Biocompatible drug delivery systems (we are currently working on producing sub-micron particles)
- Characterization of mechanical properties of particles using force probing techniques (optical tweezers and atomic force microscope)
- Encapsulation of bacteria to study colony growth in 3D scaffolds

In continuous microfluidics, our focus is on lab-on-a-chip devices for applications in diagnostics. We are currently developing a platform for isolating exosomes from blood samples. Exosomes are nanoparticles secreted from many cell lines, including tumor cells. The information enclosed in exosomes may be used for early diagnosis of various diseases, including tumor metastasis. We believe a lab-on-a-chip device can revolutionize this field.

Current projects builds on a fundament including the following stragegy:

- AutoCAD for design of microfluidic devices (masks for fabrication)
- Working in the NanoLab for fabrication of devices
- Microfluidics experimental setup. This includes using an inverted microscope with mounted high speed camera and applying new high persistency syringe pumps

Some projects will include:

- Modeling in COMSOL Multiphysics
- Nanoscale patterning using electron beam lithography
- Characterization using scanning electron microscopy (SEM)
- Optical tweezers and/or AFM
- Confocal microscopy to characterize produced particles
- Cell work

Note that training will be given on all activities. There are no requirements on previous lab/cleanroom experiences. We have recently established collaboration with the SMaL group at

EPFL (Lausanne, Switzerland). A short-time stay at EPFL will be possible and considered on demand. http://smal.epfl.ch/

Ongoing research activity

Micron sized polysaccharide particle production
Supervisors: *Bjørn T. Stokke*

Immobilization of cells in gel beads have been widely explored using various gel bead generation technologies. This include e.g., electrostatic bead generators, core-shell particles, and microfluidic. All of these strategies explore only one type of polymer constituents at the time. In the present project, we aim at designing janus gel bead particles that can be exploited for subsequent self-organization of the particles. This will be based on a recently developed process to include Ca-induced polysaccharide gelation in microfluidics.

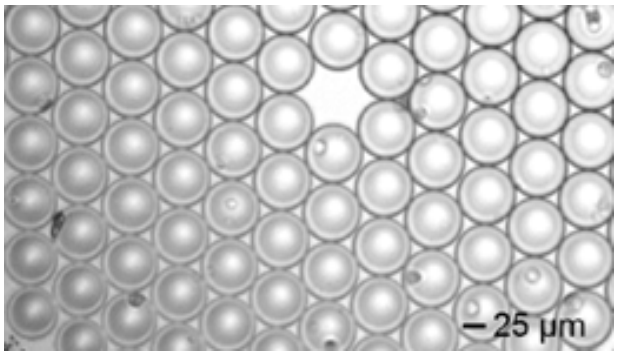

Figure 4. Polysaccharide gel beads synthesized using a microfluidic device

The project will include fabrication of microfluidic devices and on-chip polymer gel bead assembly, their characterization

Inexpensive lab-on-a-chip for exosome isolation
Supervisors: *Jonas M. Ribe and Bjørn T. Stokke*

Using the state-of-the-art electron beam lithography (EBL) system in NTNU NanoLab we pattern submicron structures with nanoscale precision for filtering exosomes. The pattern is replicated in an inexpensive polymer (PDMS) creating a transparent microfluidic device with submicron channels. As this process is optimized for speed, we can go from idea to device testing within hours.

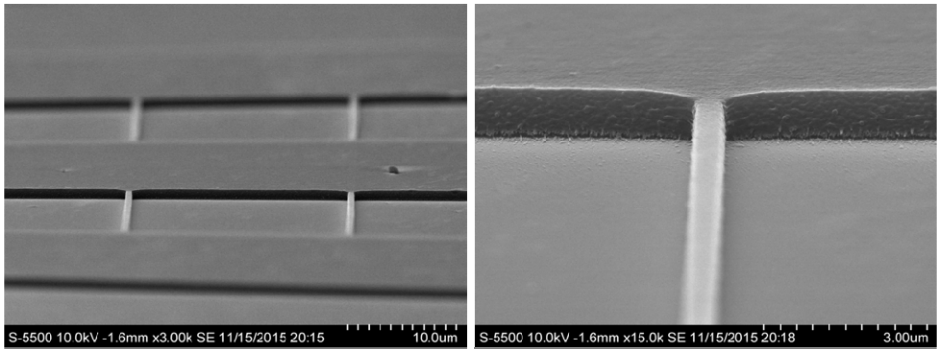

Figure 5: Submicron filtration mold made by electron beam lithography in NTNU NanoLab.

The project is focused on optimizing the device design for efficiently isolating exosomes from biological fluids. The design can be optimized for minimal hydrodynamic resistance using

CFD. The project will involves advanced fabrication and/or FEM simulation together with sample preparation and testing on our microfluidic setup.

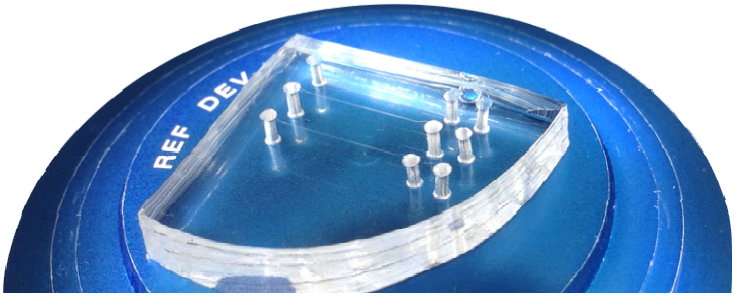

Figure 6: *Submicron filtration device replicated in transparent polymer.*

Acoustic purification of exosome samples

Supervisors: *Jonas M. Ribe and Bjørn T. Stokke*

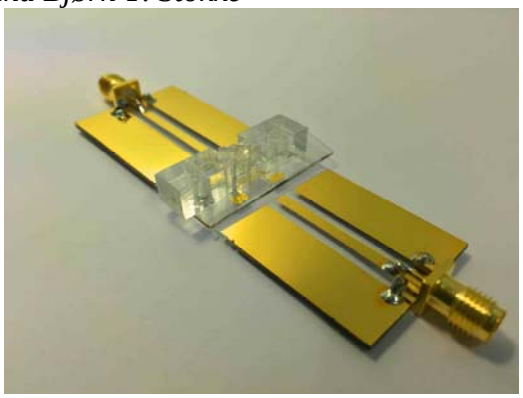
Figure 7: *Acoustic filtration device.*

As a complementary technique to passive devices we are developing devices with active components for isolating exosomes. Our ultrasound on-chip device can manipulate particles flowing in a microchannel using acoustic forces. The susceptibility of a particle to an acoustic field is determined by its size, compressibility and density. The project is focused on optimizing the system to utilize the size differences between exosomes and other extracellular vesicles for efficient isolation of exosomes by controlling the acoustic field and sample flow rates.

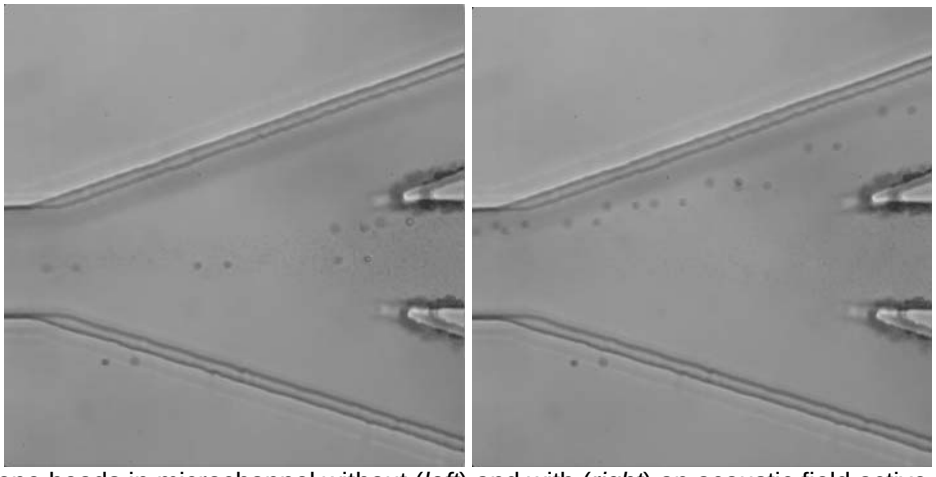
Figure 8: Polystyrene beads in microchannel without (*left*) and with (*right*) an acoustic field active – pushing the larger particles to another outlet.