Topics for project & master thesis works academic year 2013/2014

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.

Nanomechanical mapping of polyelectrolyte multilayers
Supervisors: Bjørn Torger Stokke

Polyelectrolyte multilayers have developed to an active research area due to ease of preparation, versatility in the selection of polyanion and polycation components, and possibility of integration of additional components, e.g. particles of different sizes and functionalities.[1] Polyelectrolyte multilayers are prepared through sequential adsorption of oppositely charged polyelectrolytes from aqueous solutions where experimental parameters such polymer type, molecular properties of the polymers (chain length, total charge, charge density, chain flexibility), solvent conditions (concentration of polymer, salt type and ionic strength, pH, temperature) and duration of each deposition cycle can be selected to influence the properties of the final composite thin film. A prerequisite for sustained growth in such processes is the reversal of the charge in the layer following each deposition step. The thickness and elastic properties of such multilayer films represents fundamental properties of the resulting materials. The main objective of the present project, is to apply nanomechanical mapping tools for determination of topographical distributions of elasticity of multilayers prepared from different biopolymers and assembly strategies. Figure 1 shows AFM topographs where topographical variation of height is the primary observable.

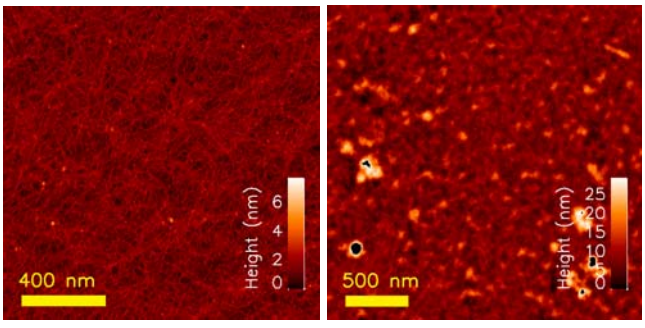

Figure 1. Tapping mode AFM height topographs[2,3] of biopolymer multilayers showing different structural morphologies.

The main tool in the project will be atomic force microscopy applied in a novel way. While standard imaging modes realized in AFM, e.g. contact mode and tapping mode, are implemented to obtain structural information, we will in this project novel modes that also provide mechanical

properties. The project focuses on establishing the correlation between the morphological features (e.g. Fig 1) and the resulting mechanical (elasticity) properties of biopolymer multilayers, and and how these eventually can be controlled by implementing various assembly modes (electrostatic, covalent, number of layers) as well as selection of biopolymers with different properties. The approach involve various tasks:

- Preparation of multilayers
- Application of Multimode 8 Peak QNM for ultrastructural and nanomechanical characterization
- Analyses of nanomechanical maps

The project is mainly of experimental nature, but development of image analysis tools related to the nanomechanical mapping can also be included. The project involves preparation of multilayers with various numbers of layers and from different biopolymers to address how the thickness and dynamic (elastic) properties depends on the molecular properties of the polymers. This includes chitosan (polycation) and alginates and xanthan (polyanions).

Lab-on-a-Chip for Isolation of Exosomes from Blood

Supervisors: Bjørn T. Stokke (bjorn.stokke@ntnu.no), Jonas M. Ribe (jonas.ribe@ntnu.no)

Exosomes are nano-sized vesicles (30-100 nm) secreted by many types of cells, including tumor cells. These cell membrane enclosed structures contain proteins and RNA-molecules that can provide vital diagnostic information. Exosomes can be obtained from body fluids such as blood, saliva, urine, semen, synovial fluid and breast milk. The biological significance of exosomes has yet to be fully elucidated, but they have been suggested to be involved in multiple cellular functions, including intercellular communication, antigen cross-presentation, and transfer of oncogenic proteins as well as mRNA and miRNA, the contents depending on their cell of origin.

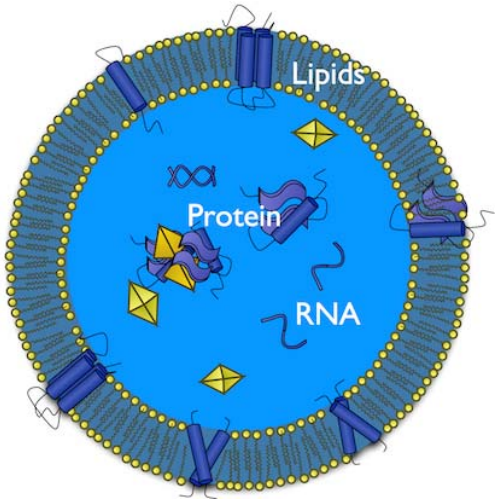

Figure 2: Exosome – an extracellular vesicle containing diagnostic information in the form of proteins and RNA.

Isolation and proper molecular profiling of membrane-enclosed vesicles are expected to provide a large potential in screening the population. Exosomes can yield up to 60 times greater concentrations of high integrity RNA compared to that extracted directly from blood. Proper screening programs can potentially discover abnormal states before malignant cancer is developed. The current protocols for isolation of plasma membrane derived vesicles and exosomes are based on either ultracentrifugation, polymer assisted precipitation or immunoaffinity capture using specific antibody-coated magnetic bead separation. These are time consuming and demanding in terms of personnel and reagents,

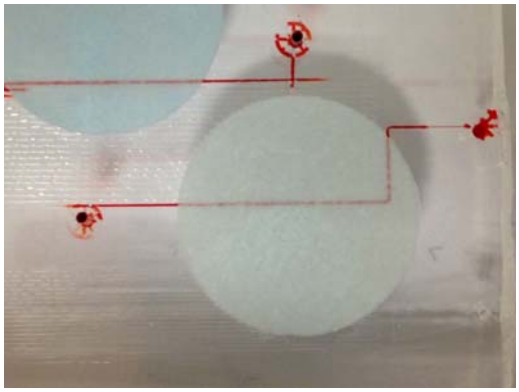

Figure 1: Nanostructured membranes embedded in a microfluidic PDMS device

making them unfit for low resource settings. However, emerging micro/nano fabrication techniques can enable development of new filtration platforms with great potential for point-of-care diagnostics.

The project will mainly consist of micro-/nanofabrication and characterization in NTNU NanoLab. Exosome-samples will be supplied by a collaborating research group St.Olavs hospital. The project-student will design and fabricate microfluidic devices using soft-lithography or other rapid-prototyping techniques. For efficient mechanical filtration of exosomes sub-micron structures will be pursued. An inexpensive nanofiltration platform can be made by embedding nanostructured membranes inside a microfluidic PDMS device. The efficiency of the system can be characterized using exosomes tagged with fluorescent labels (e.g. GFP). If time allows it, the project will include blood separation on-chip allowing for a fully automated lab-on-a-chip system.

Ultramicroscopy of amyloid formation

Supervisors: Mikael Lindgren (Mikael.lindgren@ntnu.no), Bjørn Torger Stokke (bjorn.stokke@ntnu.no)

Processes leading to insoluble protein fibrils, socalled amyloids, eventually damaging tissue and leads to diseases such as systemic amyloidosis, maturity onset diabetes, and the prion-related transmissible spongiform encephalopathies. A molecular understanding of these processes is therefore important in generating knowledge leading to these type of diseases. Amyloid fibrils form when proteins that are normally soluble in aqueous environments misfold and thereby lead to self-association beyond the normal.

Combination of fluorescence and topography imaging employing total internal reflection microscopy and atomic force microscopy, respectively, supports determination of ultrastructure of amyloid fibrils and filaments of the fluorescent labelled entities. While conventional diffraction limited confocal optical microscopy offer lateral resolution of 200 nm upwards, and less in the optical sectioning, TIRF mode recently established provide more depth-selective fluorescence exitation. Combined with recently developed surfaces and immobilization procedures (Figure 2), high resolution TIRF micrographs and topographical ultrastructure using atomic force microscopy of identical sample regions are accessible.

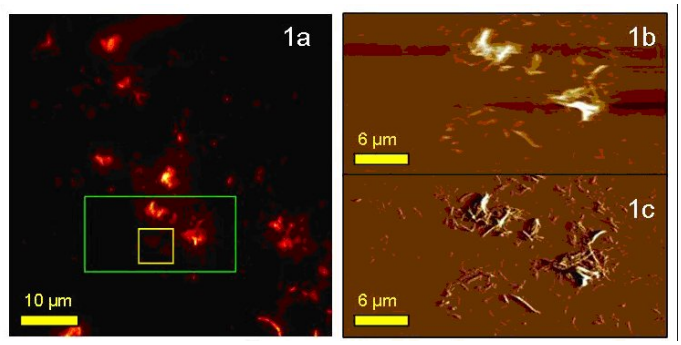

Figure 2: TIRF and AFM images of single insulin amyloid fibrils: TIRFM image (1a) and colocalized structural topography as marked with green frame: contact mode AFM images (b – height, c – deflection error

This approach requires that surfaces simultanously fullfill requirements with respect to immobilization and optical transparency. AFM imaging of dry/native state amyloids provide a quick and reliable test of sample/sufrace preparation protocols (Figure 2). This technique support determination of ultrastructure (with nanometer resolution) of single fibrils/filaments without the necessity of attaching a fluorescent probe . Preliminary results of TIRFM/AFM simultaneous observation of insulin amyloids (Figure 2) have been obtained.

The project here will focus on determination of ultrastructure of such oligomeric aggregates are of vast interest to understand amyloid diseases and amyloid formation.
The objectives of the project are:

- Visualization of individual amyloid structures revealing the detailed ultrastructure at nanoscale (AFM) colocalized with the fluorescent signal originated from the bound luminescent selective fluoroprobes (LCO).
- Time-lapse AFM imaging of kinetics of amyloid formation.
- Assessment of LCO perturbation of amyloid formation.
- Further development of custom designed image analysis platform and application of this (implemented on the IDL platform),

Part of the surface preparation needed for the ultramicroscopy include application of techniques provided in NTNU NanoLab.

Microfluidic based fabrication of Sub-micrometer Sized Biopolymer Particle and their application

Supervisors: Bjørn Torger Stokke, Armend Håti

NTNU has a long standing tradition of biopolymer research that is focused on finding applications for biopolymers originating from the sea (alginates, chitosans, etc.). Biopolymer particles are attractive materials because of their properties (size, mechanical, surface, loading) can be finely tuned using a variety of parameters. The control of particle size is critical for many applications. A few techniques exist to limit the polydispersity of particles but flow focusing in microfluidic devices stands out.

Microfluidic is the study of fluid properties in micrometer sized channels. Microfluidic devices are fabricated using techniques borrowed from the semiconductor industry. Intricate channel patterns can be fabricated both in hard and soft materials. Microfluidic has been used in a variety of research fields ranging from theoretical fluid mechanics to sensors. In flow focusing devices, droplets of liquids are ejected from an aperture between two sheath of another fluid. The size of the droplet varies as a function of the aperture

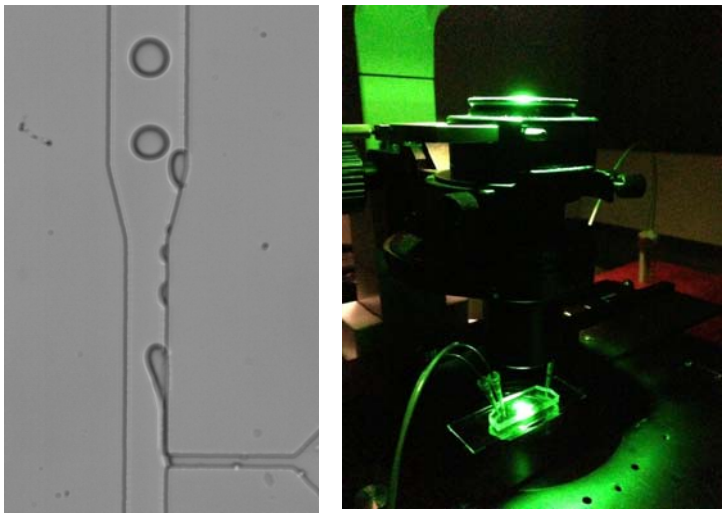

Figure 3: (Left) Droplet formation in a microfluidic channel with the dimensions 10x50 μm. (Right) Picture of the microfluidic chip mounted on an inverted microscope.

size, the dimensions of the channels, the overall geometry, the materials (and their surface treatments), the characteristics of the liquid as well as the ratio of flow rates between the liquid being focused and the surrounding liquid. The literature on flow focusing for the production of biopolymer particles is very limited especially for sub-micrometer particles. We want to establish the technology and work out the different parameters to be able to produce particles routinely. The master student will be involved in the fabrication of the microfluidic devices, its testing, as well as their use in diverse applications

Some applications of biopolymer particles will be:

- Study interactions between particles and between surfaces and particles.
- Deposition on surfaces to make biocompatible surfaces (cell growth, differentiation).
- Drug delivery

The project will include use of NTNU NanoLab in the design and preparation of the flow-focusing microfluidic device. Moreover, the obtained particles in the target size range less than 10 micrometers, will be integrated in hydrogels and multilayers to perturb the properties of these materials group.

Numerical simulation of droplet dynamics in a microfluidic channels

To control and optimize the creation of the biopolymer particles, the master student is encouraged to develop numerical analysis routines where a concrete goal will be to improve the geometrical design of the microfluidic devices based on numerical simulations. This will include droplet dynamics, droplet breakup and coalescence, the interfacial tension at the droplet-liquid interface and the shear forces generated by the carrier flow around the droplets. Moreover, the effect of the biopolymer properties (e.g. molecular weight and structural composition) on droplet formation is of great interest. The analysis should focus on parameters which are controllable in the experimental approach such as the flow rates and viscosity of the different liquid components, widths and depths of the channels as well as channel configuration. A starting point will be to analyse droplet formation in a T-junction as illustrated in Figure 4 and to further investigate the possibility of introducing new geometries to enable production of sub-micron particles.

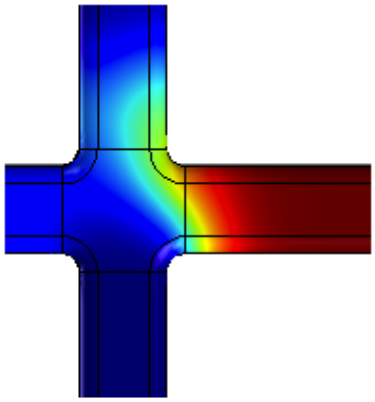

Figure 4: Cross-sectional plug formation in a microfluidic device (COMSOL model).

Bioresponsive hydrogels as signal transducers

Supervisors: Bjørn T. Stokke (bjorn.stokke@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).

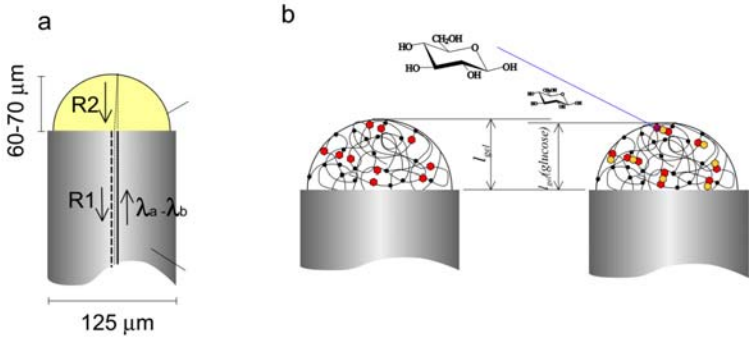

Figure 3. (a) Shematic illustration of optical detection of changes of a hemispherical hydrogel. (b) Schematic illustration of glucose induced reduction of equilibrium swelling volume of a glucose-selective hydrogel.

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.

Finite element modelling of nanoindentation of responsive hydrogel materials

Supervisors: Victorien Prot (victorien.prot@ntnu.no) Bjørn Skallerud (bjorn.skallerud@ntnu.no) and Bjørn T. Stokke (bjorn.stokke@ntnu.no)

Hydrogel consist of a crosslinked polymer phase immersed in a solution – here we limit ourselves to aqueous solution. These materials possess both solid- and liquid like properties. 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. 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. This technique is currently being applied for the determination of swelling properties of hydrogels that is coated with a polymer that through its mechanical coupling with the hydrogel core, affect the ionic strength dependence of the equilibrium swelling.

Such a mechanial effect of the hydrogel coating is similar to what is expected when applying coating steps for immobilization of living cells in alginate gel beads. In the present project we plan to establish quantitative tools for determination of mechanical properties of polymer impregnated hydrogels. Hydrogels will be prepared using the same polysaccharides as used for immobilization of living cells, alginates, that subsequently will be impregnated with various polycations. Nanoindentation, e.g. determination of force displacement profiles using an AFM tip with well defined geometry, will be performed at various locations over the surface of the specimens. Partnered with quantitative modelling of indentation profiles using a finite element approach, this addresses fundamental issues related to application of contact mechanics approaches in their analysis. The focus can be on either the experimental, the numerical or a combination of these facets.

The topic involves establishing routines within commercial software for finite element analysis of deformation fields of thin hydrogel films subjected to nanointendation. This includes implementing hydrogel swelling theory in the material functions needed for the modelling. Key features to address in the the finite element modelling include

- Deformation field as function of parameters of the nanointentation
- Effect of possible hydrogel design parameters on deformation characteristics
- Assessment of numerical results relative to experimental results

(1) Decher, G.; Schlenoff, J. B. *Multilayer thin films. Sequential assembly of nanocomposite materials*; Wiley-VCH Verlag Gmbh: Weinheim, 2003.

(2) Maurstad, G.; Morch, Y. A.; Bausch, A. R.; Stokke, B. T. *Carbohydrate Polymers* **2008**, *71*, 672.

(3) Marken, E.; Maurstad, G.; Stokke, B. T. *Thin Solid Films* **2008**, *516*, 7770.