Swiss Soft Days

16th Edition
Paul Scherrer Institut

Monday, May 4th 2015

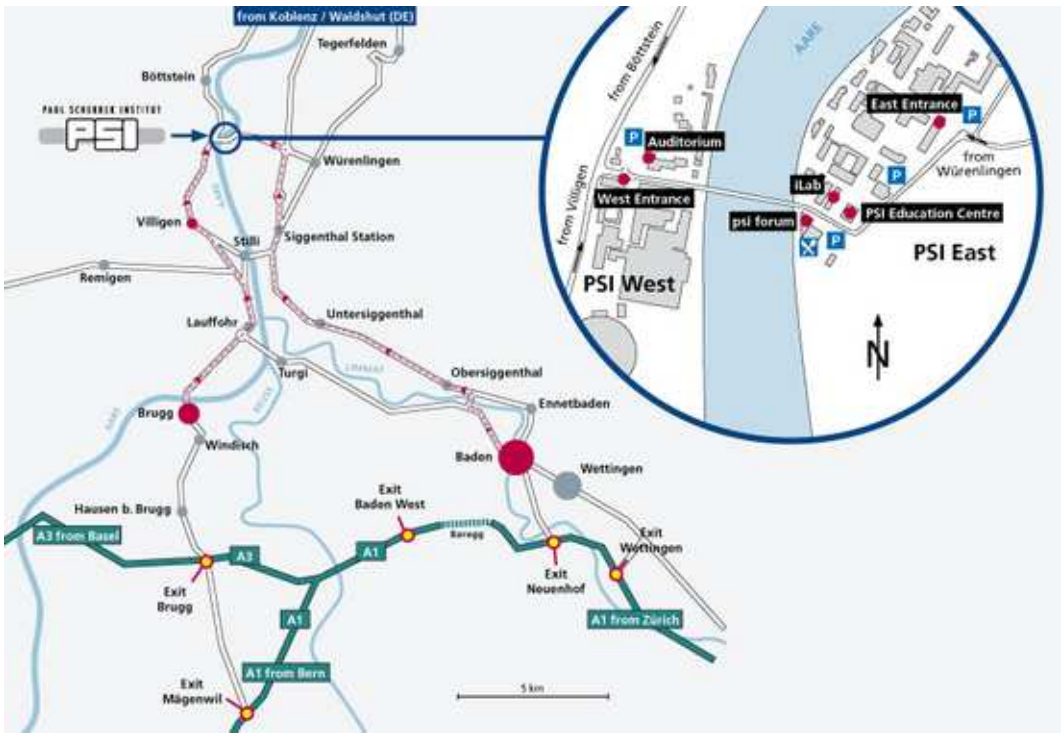
Program
&
Abstracts

Swiss Soft Days XVI

4. May 2015, 10:00 to 17:40
Paul Scherrer Institut, Villigen – PSI West, Auditorium

10:00-10:20	Registration and coffee	
10:20-10:30	*J. Kohlbrecher (PSI)*	Welcome
Session I – Crystals and Colloids		
10:30-10:50	*G. Nyström (ETHZ)*	Nanocellulose Chirality, Structure and Physical Properties Revealed Through High-Resolution Microscopy and Statistical Analysis
10:50-11:10	*C. Myers (Uni Zurich)*	Information Storage and Retrieval in a Single Levitating Colloid
11:10-11:30	*A. Lavrenova (Uni Fribourg)*	Encapsulation of Charge-Transfer Complexes for Mechanochromic Sensors Applications
11:30-11:50	*D. Calzolari (Uni Fribourg)*	Stress-induced Hardening of Colloidal Gels
11:50-12:00	Coffee	
Session II – Functionalized Surfaces		
12:00-12:40	*M. Kristiansen (INKA)*	Functionalization of Polymers by Surface or Bulk Modification - From Lab-scale Towards Industrial Applications Through Technology Development
12:40-13:00	*C. Padeste(PSI)*	Functionalization of Polymer Films Using Lithographic Radiation Grafting
13:00-14:00	Lunch (OASE)	
Session III – Controlled Release		
14:00-14:20	*E. Amstad (EPFL)*	Production of Amorphous Nanoparticles Through Microfluidic Spray Drying
14:20-14:40	*J. Duskey (Uni Basel)*	Photo-Sensitive Cationic Polymers for Non-Toxic Stimulated Release and Delivery of Small Molecules
14:40-15:00	*M. Spulber (Uni Basel)*	Ceria Encapsulating Nanoreactors for ros detoxification
15:00-15:20	Coffee	
Session IV – Methods		
15:20-15:40	*M. Liebi (PSI)*	Scanning SAXS in 2D and 3D
15:40-16:00	*M. Reufer (LS Instruments)*	Recent Advances in Light Scattering on Turbid Systems
16:00-17:30	Poster Session /Drinks/ Wrap-up/	
17:35	J. Kohlbrecher	Closing remarks

How to get to the Paul Scherrer Institute?



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PSI is located in northern Switzerland, approximately midway between Zürich and Basel. The nearest towns and railway stations are Baden and Brugg. Frequent air and train connections via Zürich or Basel are available from all major European cities.

To find PSI by car

You can reach PSI via Brugg or Baden.

Via Brugg:
Follow the Koblenz–Zurzach signs through Brugg. After passing through Lauffohr and a short hill, branch off to the left towards Remigen/Villigen; then after about 500 m turn right towards Villigen. Approximately 1 km after leaving Villigen you will reach PSI-West. You can drive to PSI-East via the bridge over the river Aare.

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Follow the Koblenz–Zurzach signs through Baden. Drive through Nussbaumen, Untersiggenthal and Station Siggenthal. Approximately 1.5 km beyond the roundabout, follow the sign left towards PSI at the crossroads and you will reach PSI-East. You can drive to PSI-West via the bridge over the river Aare.

Programming of the navigation system:
For PSI West: enter city "Villigen", street "PSI"
It is permitted to use the connecting road across the river Aare.

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Abstracts for talks

Information Storage and Retrieval in a Single Levitating Colloid

C.Myers[1], M.Celebrano[2], M.Krishnan[1]

[1] *University of Zürich, Zürich, Switzerland.* [2] *Politecnico di Milano,Milan, Italy.*

INTRODUCTION: The binary switch is a basic element in digital information processing. Most approaches are based on the solid state but photonic, logic and even rudimentary computing capabilities have been demonstrated in bulk suspension. Nonetheless experimental approaches and devices that harness the properties of isolated mesoscopic entities in solution remain elusive. Here, we present a generic methodology for precise and parallel spatiotemporal control of nanometre-scale matter in a fluid, unveiling potential for digital technologies.

METHODS: We work with a fluid-filled slit composed of two parallel charged surfaces with topographic indentations in one of the walls. This lowers the magnitude of the local electrostatic potential, creating a free energy minimum that stably traps an object in solution[1]. We have shown that the shape of the well can further dictate the orientation of the trapped object, e.g., a rod-like particle orients along the long axis of a cigar-shaped trap[2]. Here we used T-shaped traps, shown in figure 1a. Plasmonic nanorods display a strong polarization-dependent scattering response, we thus expect a bimodal optical readout of the trapped object's state in the double well. Controlled motion over the barrier, via optical or electrical manipulation, allows a state to be recorded and read.

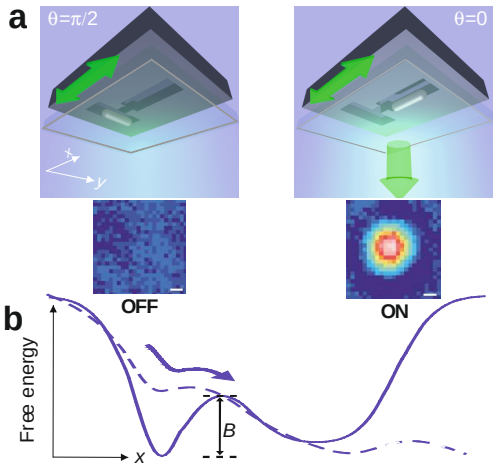

Fig. 1a: 3D illustration, and corresponding optical images (scale bar denotes 200nm), of a fluid-phase flip flop where a nanorod is trapped in either of two angular states. b: The bistable electrostatic potential along x, showing the barrier B. The dashed line shows the potential in the presence of an applied field.

RESULTS: Figure 2 shows non-volatile operation of a fluid-phase flip-flop, both with electrical and optical stimuli. In a, an alternating pulsatile electric field generates electroosmotic flows in the channel, which drag the trapped particle over the barrier, after which it rotates to align with the long axis of the trap. In b, laser light is used to impart angular momentum on the trapped nanorod, which rotates to align with the laser polarization. It then sees a smaller barrier and so can diffuse freely into the neighboring minimum. The solid and dashed lines represent orthogonal polarizations.

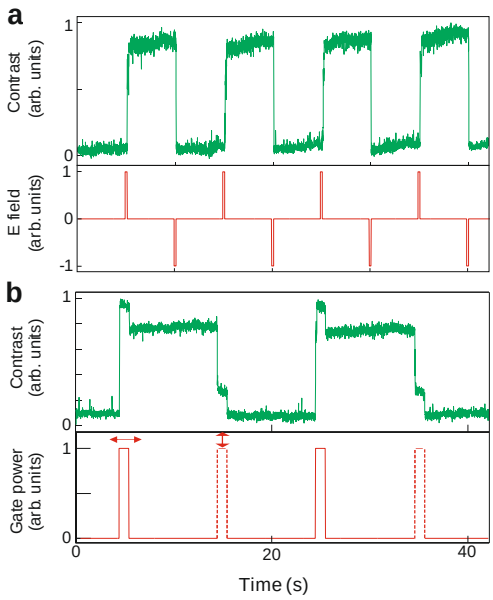

Fig. 2: Information storage in a single colloid via electrical, a, and optical, b, impulses.

DISCUSSION & CONCLUSIONS: Our experiments demonstrate for the first time the feasibility of controlled spatial manipulation of a free-standing nanoscale entity on timescales approaching 10μs in optical control and 1ms in electrical switching.

REFERENCES:[1]Krishnan, M. *et al*. Nature 467, 692-695, (2010).

[2]Celebrano, M. *et al*. Nano Letters 12, 5791-5796, (2012).

ACKNOWLEDGEMENTS: We gratefully acknowledge financial support from the SNSF and University of Zürich. Nanofabrication was carried out at FIRST Center for Micro- and Nanoscience, ETH Zürich.

Title: Encapsulation of Charge-Transfer Complexes for Mechanochromic Sensors Applications

Anna Lavrenova[1], Jacob Farkas[1], Yoan C. Simon[1] and Christoph Weder[1]

[1]Adolphe Merkle Institute, University of Fribourg

Mechanochromic polymers are an emerging class of stimuli-responsive materials that can be used in a variety of industrial applications, such as the detection of mechanical damage or pressure sensors. The encapsulation of charge-transfer complexes (CTCs) to create mechanochromic materials is reported here. Hexamethylbenzene (donor) and chloranil (acceptor) were separately encapsulated in poly(urea-formaldehyde) microcapsules. These capsules were dispersed in a poly(dimethylsiloxane) (PDMS) pre-polymer, which was subsequently cured to produce the desired mechanoresponsive elastomers. When local stresses were applied to the materials, the microcapsules broke, releasing the donor and acceptor molecules, which led to the formation of a CTC and, ultimately, to a remarkable yellow-to-red color change (Figure 1). These composite structures responded under different type of stress: shearing, stretching and compression. This work demonstrates a novel strategy to fabricate mechanochromic materials by means of a straightforward and scalable methodology, which could potentially be extended to other matrices and optical systems.

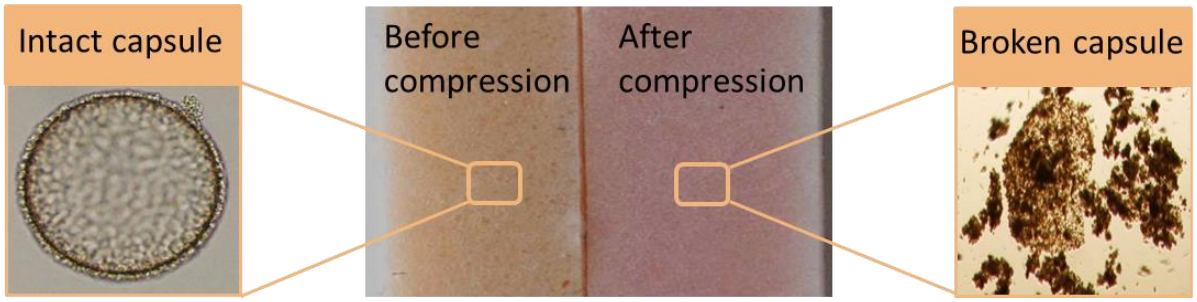

Figure 1: PDMS film containing poly(urea-formaldehyde) microcapsules filled with hexamethylbenzene and chloranil before and after exposure to compressive load. Optical microscopy images of intact and broken capsules are also shown.

Stress-induced hardening of colloidal gels

D. Calzolari[1], I. Bischofberger[2], V. Trappe[1]

[1] *University of Fribourg, Department of Physics*

[2] *University of Chicago, Department of Physics*

When an external stress is applied to a solid material, it deforms: first elastically (reversibly) and then plastically (irreversibly) when strained beyond the yield point. Such material response function has been intensively studied for crystalline materials such as metals, where it has been found that plastic deformation can also lead to hardening. Both plastic deformation and work-hardening are here related to dislocations within the crystalline structure.

However, many amorphous systems display response functions to an applied stress that are strikingly similar to those of crystalline materials. Understanding the mechanisms of deformation in such systems remains a topic of intense research.

In this contribution we present our investigations of the mechanical properties of a gel composed of colloidal particles, for which the attractive interactions are conveniently tuned by temperature. This temperature sensitivity enables us to trigger gelation by increasing the temperature. The gel exhibits aging evidenced by both an increase in the relaxation time and the elastic modulus with the gel age.

We explore how these aging effects impact the creep behavior of the gel subjected to stresses that are just below the yield stress. Our findings reveal that both aging and hardening define the material response function, where hardening under stress is distinct from that obtained by thermal aging.

Functionalization of polymers by surface or bulk modification - from lab-scale towards industrial applications through technology development

P.M. Kristiansen[1,2], C. Rytka[1,2], S. Neuhaus[1], H. Schift[3], C. Padeste[3], J. Gobrecht[1,3]

[1] *Institute of Polymer Nanotechnology, Fachhochschule Nordwestschweiz, Windisch, Switzerland.*
[2] *Institute of Polymer Engineering, Fachhochschule Nordwestschweiz, Windisch, Switzerland.*
[3] *Laboratory for Micro- and Nanotechnology, Paul Scherrer Institute, Villigen, Switzerland.*

INTRODUCTION: Functionalization of polymer surfaces can be achieved by 3 principal pathways: Topographical patterning on the micro- and nanoscale finds use in optics and security applications as well as for creation of lab-on-chip devices for modern diagnostics. Chemical modification by grafting allows introduction of chemical functionalities, which change the wetting behaviour or render the substrate antimicrobial, to only name a few. Finally, polymers may be modified in the bulk by addition of functional additives. This presentation gives an overview on these functionalization strategies and sheds some light on the underlying physics and chemistry.

MICRO-/NANOSTRUCTURED SURFACES: The generation of functional surface topographies is enabled by advanced lithography techniques employing different types of radiation. For instance, complex surface topographies are achieved by the combination of grayscale e-beam lithography and subsequent thermal reflow, a process referred to as "TASTE" in the recent literature [1]. And large-scale replication by nano-imprint lithography (NIL) has meanwhile found its way to industry serving vastly different markets.

Industrial replication processes, particularly injection (compression) molding have meanwhile been well developed and understood to such an extent that industrial applications have started to become increasingly more feasible [2]. Examples include microfluidic devices with a multitude of structural features for diagnostic purposes as well as micro-optical components for applications in light management and the automotive industry.

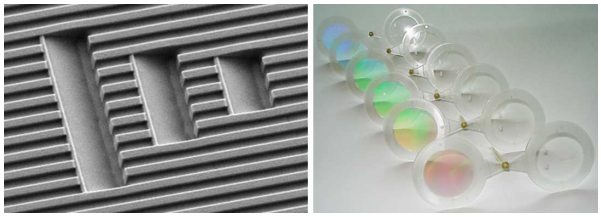

Fig. 1: Light out-coupling structure prepared by NIL+TASTE (left), and injection compression molded samples of a large area array (Ø=45mm) of 200 nm pillars (period 600 nm) (right)

NOVEL APPROACHES TO GRAFTING: So-called grafting reactions, i.e. the controlled polymerization of functional polymers onto polymer substrates, have received tremendous attention in the scientific literature [3]. Despite the large variety of chemical functionalities introduced by the common academic grafting-from approach, industrial applicability is still hampered by inconvenient boundary conditions (long reaction times, need for O_2 exclusion, high exothermic).

The use of low-energy electron beams for triggering the coupling reactions between the substrate to be modified and readily synthesized functional polymers (e-grafting) offers a nearly unexhausted wealth of possibilities for polymer functionalization on an industrial scale.

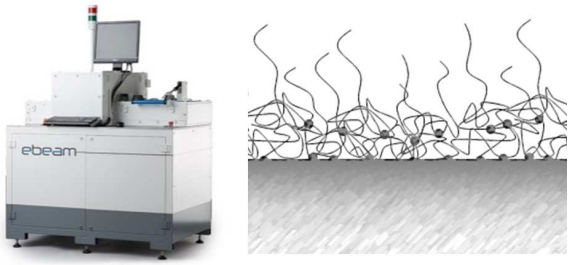

Fig. 2: Laboratory low-energy e-beam equipment (left) and schematic of modified polymer surface prepared by the e-grafting approach (right)

BULK MODIFICATION OF POLYMERS: Incorporation of functional additives into polymer systems by means of melt compounding allows tailoring of specific macroscopic properties. Frequently, substantial changes are achieved at surprisingly low loading levels, which will be illustrated by some examples.

REFERENCES:

[1] A. Schleunitz, V.A. Guzenko, M. Messerschmidt, H. Atasoy, R. Kirchner, H. Schift; **2014**; *Nano Convergence;* 1: 7.

[2] C. Rytka, P.M. Kristiansen, A. Neyer; **2015**; *J. Micromech. Microeng.;* 25(5): accepted.

[3] C. Padeste, S. Neuhaus; **2015**; *Polymer Micro- and Nanografting;* Elsevier.

Functionalization of polymer films using lithographic radiation grafting

Celestino Padeste[1], Matthias Dübner[1,2], Katarzyna Gajos[3], Sonja Neuhaus[4]

[1]Paul Scherrer Institut, Villigen PSI, Switzerland. [2]ETHZ, Zürich, Switzerland.
[3]Jagiellonian University, Krakow, Poland, [4]Univ. of Applied Sciences, Windisch, Siwtzerland.

INTRODUCTION: Radiation grafting is an elegant method to adapt the properties of polymeric substrates via introducing functionalities at their surface or in the bulk. Beams of electrons, photons or ions of sufficient energy may be used to break bonds in a polymer substrate and to create radicals, which act as initiators in a subsequent graft polymerization. In this work we are combining radiation grafting with structuring technologies in order to define specific areas on the substrate to be chemically modified. While micro- to millimeter sized structures are achieved with large-area activation tools and masking techniques, structures with sub-micrometer resolution can be obtained using EUV-interference lithography or e-beam lithography.

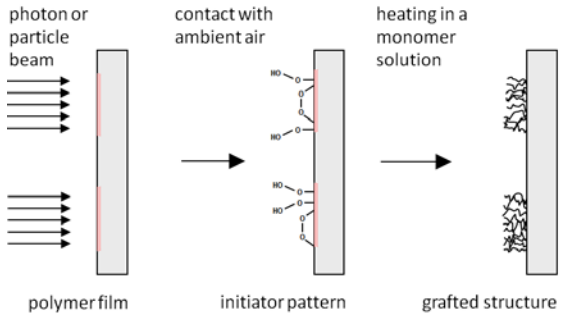

Fig.1: Scheme of lithographic radiation grafting.

METHODS: The grafting process is schematically shown in Fig. 1. Films of commercially available fluoropolymers such as ETFE or Teflon or of polyolefins such as polyethylene can selectively be activated with photons or electrons to create defined radical patterns. Subsequent exposure to ambient air leads to the transformation of the radicals into hydroperoxide and peroxide species, which are stable over weeks to months when stored at low temperatures (-80 °C). Samples are immersed into degassed solutions of monomers (typically methacrylates or other vinyl monomers), which are then heated to 50-80 °C to start the grafting reactions by cleaving of the (hydro)peroxides. This re-creates the radicals which then initiate chain growth. The selection of the monomer allows defining the properties of the grafted areas in a wide range. Furthermore, chemical modification of the grafted chains enables further adaptation of properties and the introduction of specific functionalities.

RESULTS: This presentation will provide a summary on structure types and functionalities that were achieved using lithographic radiation grafting. For instance, exposure to electrons with an e-beam lithography tool is used for the creation of high resolution surface structures, while selective bulk-modification was accessed using large-area exposure to 150 kV electrons through a stencil mask (Fig. 2). Surface properties such as the wettability of intrinsically hydrophobic fluoropolymers may be adapted in a very large range[2]. Functionalities introduced by post-polymerization modification of grafted structures include photo-responsiveness[3] and bio-functionality such as specific protein binding and enzymatic activity.

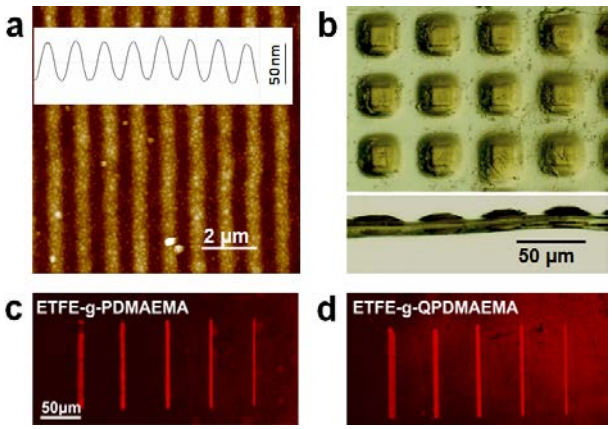

Fig. 2: Structures grafted after e-beam activation. a) High resolution polymer brush structure. b) Microstructure grafted in the bulk. c/d) Binding of fluorescence labelled protein to polymer brush line structures.

DISCUSSION & CONCLUSIONS: Lithographic radiation grafting has proven to be a very versatile functionalization technique for polymer films with resolution capabilities in the sub-micrometer range. After in-depth exploration of the parameters defining the structure formation and the grafting and post-functionalization steps in the process, research is currently directed more towards the formation of adaptive ("smart") surfaces and bio-functional structures.

REFERENCES: [1] C. Padeste and S. Neuhaus, Polymer Micro- and Nanografting. Elsevier, **2015**.
[2] S. Neuhaus et al. **2011**, Plasma Proc. Polym.; 8, 512.
[3] M. Dübner *et al.*; **2014**; *Langmuir;* 30, 14971.

Production of amorphous nanoparticles through microfluidic spray drying

Esther Amstad

Soft Materials Laboratory, Department of Materials, EPFL, Switzerland

INTRODUCTION

More than half of the newly developed drugs are hydrophobic. The poor water solubility limits their bioavailability and thus their effectiveness as medication. Their bioavailability can be increased if these drugs are formulated as nanoparticles as the dissolution kinetics scales with the surface-to-volume ratio. Their bioavailability increases even more if these drugs are formulated as amorphous nanoparticles as the solubility of the amorphous phase is much higher than that of their crystalline counterpart. However, many of these drugs have a high propensity to crystallize; it is thus difficult to formulate them as amorphous nanoparticles. Certain amorphous drug nanoparticles can be produced if precipitated in the presence of crystallization-inhibiting additives. However, crystallization is only inhibited if sufficient concentrations of an appropriate crystallization-inhibiting polymer are used. This makes the formulation of amorphous drugs laborious. Moreover, the drug content inside the resulting particles is often low.

I will present a microfluidic spray drier, a so-called microfluidic nebulator, which enables the precipitation of amorphous nanoparticles without the need of any crystallization-inhibiting additives. The nebulator produces sub-µm sized drops in a stream of air that flows at supersonic speeds. These drops dry sufficiently fast to kinetically suppress the formation of crystal nuclei. Hence, the resulting nanoparticles are amorphous.

METHODS

The microfluidic nebulator is made from poly(dimethyl siloxane) (PDMS) using soft lithography. It contains two inlets for liquids to enable performing chemical reactions on-chip before the solvent is evaporated. It also contains six pairs of air inlets, as shown in Figure 1. We dissolve clotrimazole, a hydrophobic model drug, in ethanol and inject this solution at 1 ml/h into the nebulator using pumps. We set the pressure at all the air inlets to 0.28 MPa and collect the dry nanoparticles on a polished Si wafer or a carbon coated TEM grid for analysis.

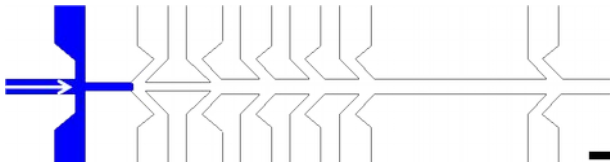

Figure 1: Schematic illustration of the microfluidic nebulator. The blue inlets are for liquids, the white ones for air. The arrow indicates the flow direction. Scale bar = 100 µm.

RESULTS

Ethanol wets the channel walls of the microfluidic nebulator, forming thin films that flow along the walls whereas the air flows through the center of the channel. The last junction is three dimensional as air is injected from the sides as well as from the top and bottom. This type of junction enables simultaneous detachment of all the thin film from the channel walls. The films break into drops with an average diameter of 300 nm. The high air velocities achieved in the final parts of the main channel result in a fast evaporation of the solvent; indeed these drops dry within a few µs. This time is insufficient for crystal nuclei of a variety of drugs but also inorganics and even salts to form. As a result the spray dried nanoparticles are amorphous. Indeed, amorphous drug nanoparticles remain amorphous for up to 6 months if stored at ambient conditions and even if stored for 2 months at temperatures above their glass transition temperature, these nanoparticles do not show any sign of crystallinity. We assign this excellent stability of the nanoparticles against crystallization to their small size.

CONCLUSION

The microfluidic nebulator enables the production of sub-µm sized drops in a stream of air that flows at supersonic speeds. These drops dry so fast that the formation of crystal nuclei is kinetically suppressed. As a result, these spray dried nanoparticles are amorphous.

ACKNOWLEDGEMENTS

I thank David A. Weitz, Frans Spaepen, and Michael P. Brenner for helpful discussions, and SNSF (PBEZP2_137304) and BASF for financial support.

Photo-Sensitive Cationic Polymers for Non-Toxic Stimulated Release and Delivery of Small Molecules

J. Duskey[1], I. A Dinu[1], A. Car[1], C. Palivan[1]

[1] *University of Basel, Basel, Switzerland.*

INTRODUCTION: Cationic polymeric nanoassemblies, such as those formed from PDMS-*b*-PDMAEMA,[1] are good candidates to protect and deliver proteins, enzymes, nucleic acids,[2] and imaging agents.[3] However, the positive charge that allows these systems to encapsulate and deliver molecules is also the factor that causes them to have poor release and be toxic to cells. By adding a photo responsive moiety that converts the cationic species into its respective zwitterion,[4] it is possible to create a delivery system for binding, protection, and controlled release of negatively charged molecules.

METHODS: PDMS-*b*-PDMAEMA cationic block copolymers were synthesized with either 5 or 27 repeating cationic PDMAEMA units.[1] The terminal amine was quaternized with a pendant photo-labile o-nitrobenzyl-carboxymethyl moiety. NMR, DLS, TEM, and ζ-potential were used for characterization. Photo-cleavage kinetics were analyzed by NMR and UV-vis. The toxicity and cell uptake of self-assemblies formed from the pristine polymer, quaternized polymer, and irradiated polymer were tested in Hela cells. Finally, small molecule entrapment and release was monitored by UV-vis over 72 hours.

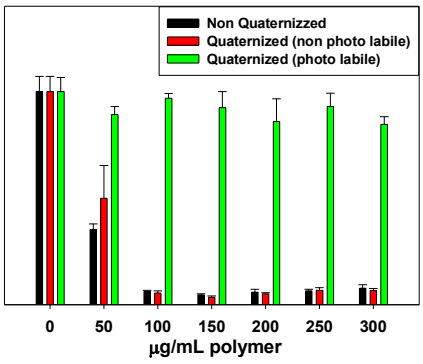

Fig. 1: Cell toxicity of nanoparticles (27 repeating PDMAEMA) units after 24 h.

RESULTS: Quaternized PDMS-*b*-PDMAEMA polymers self-assembled in aqueous solutions forming nanoparticles between 100-200 nm with a charge between 11-16 mV. When irradiated, the size did not change; however, the surface charge decreased to neutral. Cell uptake and toxicity studies demonstrated that quaternized PDMS-*b*-PDMAEMA nanoparticles were readily uptaken by cells, but they were less toxic than non-quaternized particles (Fig 1). Also, upon irradiation, quaternized nanoparticles became neutral and slowly released anionic molecules over 72 hours (Fig 2).

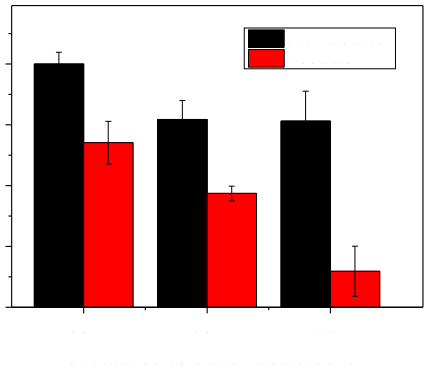

Fig. 2: Release of entrapped dye after photo cleavage

DISCUSSION & CONCLUSIONS: Photo-labile cationic PDMS-*b*-PDMAEMA nanoparticles were able to bind and encapsulate small anionic molecules and be uptaken into cells. However, unlike previous PDMS-*b*-PDMAEMA nanoparticles, quaternized particles showed less cell toxicity. Also, these particles demonstrated that upon stimulation by photo-irradiation, a slow release of anionic cargo could be induced that was not previously observed. This data represents a major advancement in the safe delivery and controlled release mechanism that is needed to produce more efficacious delivery systems.

REFERENCES: [1]A. Car, et al. *J. Controlled Release* 2014, 190, 465.
[2]H. Cabral, K. Kataoka, *J. Controlled Release* 2014, 190, 465.
[3]R. Srikar, et al., *Nanomed. Nanobiotechnol.* 2014, 6, 245.
[4]P. Sobolciak, et al. *Macromol. Rapid Commun.* 2013, 34, 635.

ACKNOWLEDGEMENTS: The Swiss National Science Foundation (SNSF), the National Center of Competence in Research Nanoscale Science, the Marie Curie Actions-Intra European fellowship (IEF) (p.n. 301398) and University of Basel.

CERIA ENCAPSULATING NANOREACTORS FOR ROS DETOXIFICATION

M.Spulber[1], P. Baumann[1], J. Liu[1], C.G. Palivan[1]

[1] *University of Basel, Basel, Switzerland*

INTRODUCTION: Oxidative stress induces an imbalance of reactive oxygen species (ROS), which may overwhelm cellular defences and thus cause toxicity effects. Due to the impact of oxidative stress in a variety of pathologic conditions from cancer to Parkinson's disease, Alzheimer's disease, or neurodegeneration, finding new efficient solutions to fight against oxidative stress is extremely important [1]. Ceria nanoparticles (CeNP) have been used as superantioxidant systems to reduce oxidative stress [2]. However, recent reports have indicated that CeNP lead to oxidative stress and DNA damage, and in high doses promote radical formation, via a possible Fenton-like reaction [3]. In order to reduce CeNP inherent toxicity, while preserving their unique catalytic regenerative qualities we introduce a strategy based on CeNP encapsulation in polymer nanoreactors. By encapsulation the superantioxidant activity of CeNP is preserved, whilst preventing their aggregation and interaction with H_2O_2, and decreasing their toxicity without further particle stabilization.

METHODS: Dynamic and static light scattering (DLS, SLS) were used to determine the sizes of the free CeNP and CeNP containing nanoreactors. Transmission electron microscopy (TEM) was used for characterization of the morphology of free CeNP and CeNP nanoreactors. UV-Vis spectroscopy was used to determine the conce Electron paramagnetic resonance (EPR) measurements were performed on a Bruker CW EPR Elexsys-500 spectrometer equipped with a variable temperature unit.

RESULTS: CeNP-containing nanoreactors were engineered by encapsulation of CeNP inside vesicles generated by self-assembly of PDMS-PNVP triblock copolymers under physiological pH conditions. Spin trapping EPR established that CeNP have a dual activity, involving both scavenging and generation of reactive oxygen species in the presence of hydrogen peroxide. In contrast, CeNP loaded nanoreactors benefit from polymer membrane protection, which blocks access of hydrogen peroxide to the inner cavity where CeNP are located, and therefore exhibit only an efficient scavenging activity for both hydroxyl and superoxide radicals. Upon encapsulation, the nanorectors prevent the aggregation of CeNP, and the Fenton-like reaction with hydrogen peroxide, which are known to be the main reasons for CeNP toxicity. CeNP nanoreactors were taken up by HeLa cells, and showed almost no cytotoxicity, even after long incubation times. In addition, inside nanoreactors CeNP preserved their superantioxidant activity, for both hydroxyl and superoxide radicals. Indeed, inside cells exposed to oxidative stress CeNP-containing nanoreactors were effective in ROS scavenging because of the regenerative redox chemistry of loaded CeNP. Compared to free CeNP, which induces significant cytotoxicity, CeNP-containing nanoreactors possess high superantioxidant activity, long term stability, and almost no toxicity.

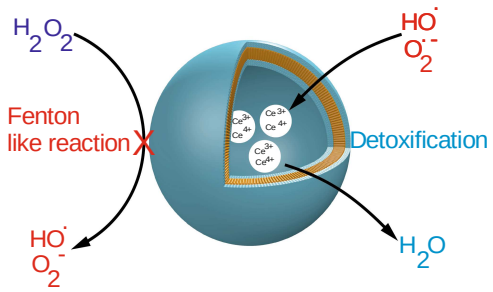

Fig. 1: Schematic representation of CeNP nanoreactors based on CeNP encapsulation inside polymer vesicles for detoxification of ROS

DISCUSSION & CONCLUSIONS: We engineered nanoreactors by encapsulation of CeNP inside polymer vesicles in order to solve their toxicity issues, by avoiding aggregation in solution and shielding them from H_2O_2, while preserving their ability to scavenge free radicals.Our strategy for engineering CeNP-containing nanoreactors represents a straightforward solution to reducing CeNP toxicity, and serving to offer the nanoreactors as an efficient solution to fighting oxidative stress.

REFERENCES: [1] M. S. Wason; **2013**; *Am. J. Transl. Res;* 5:126-131.

[2] R. C. Merrifield; **2013**; *Environ. Sci. Technol;* 47:12426-12433.

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Recent advances in light scattering on turbid systems

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INTRODUCTION: Many interesting and industrial relevant colloidal systems are turbid and thus difficult to study using conventional light scattering techniques. For example, particle sizing using Dynamic Light Scattering (DLS) underestimates the hydrodynamic radius if multiple scattering light is present.

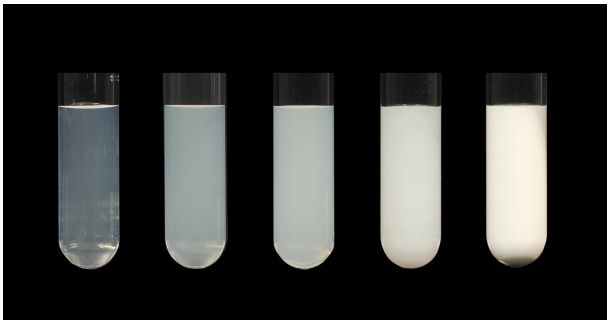

Fig. 1: Samples with different turbidity.

In order to measure highly turbid systems two approaches can be taken. Either one reduces the optical path and thus minimizing the possibility of multiple scattering, or one suppresses the multiple scattered light (e.g. 3D-DLS). The combination of both approaches yield the best results. Moreover, for extremely turbid samples, where those approaches do not work anymore, one can employ Diffusing Wave Spectroscopy (DWS). In DWS, only multiple scattering light is detected.

In this presentation, I will briefly summarize the different approaches that help us deal with turbid samples. Then, I will present recent advances such as the significantly improved modulation technique for the 3D-DLS and DWS particle sizing at very high particle concentrations.

3D-Suppression techniques:

The principle of the 3D technique is the setup of two simultaneous light scattering experiments probing the same volume. The two measured photon streams are then cross-correlated using a hardware correlator. As a result, only the single scattered photons contribute to the measured correlation function.

A standard 3D -DLS setup unfortunately does not account for cross-talk of the two experiments, which reduces the signal-to-noise ratio. To avoid eliminate cross-talk— we developed a system of nearly simultaneous scattering experiments using very fast modulation technologies. This system counteracts the negative effects of a pure 3D system. The newest advances in this technology include a highly increased efficiency as an extended range of particle sizes.

Finally, we developed a special set-up that allows simultaneously neutron-scattering and 3D-light scattering (see Fig 2).

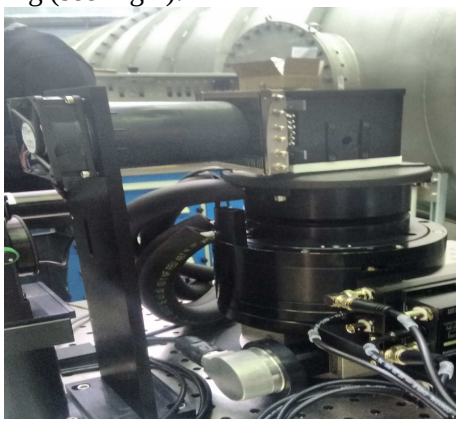

Fig 2: Combined 3D-DLS/SLS and neutron scattering installed at Helmholtz-Zentrum Berlin.

DWS-Particle Sizing:

DWS applies to highly turbid media such as concentrated particle suspensions or emulsions. In such opaque mixtures, the light is scattered multiple times by solid or liquid particles. These particles perform Brownian motion, which makes the intensity of scattered light fluctuate over time. Depending on the used analysis method, one can use DWS for microrheology or particle sizing.

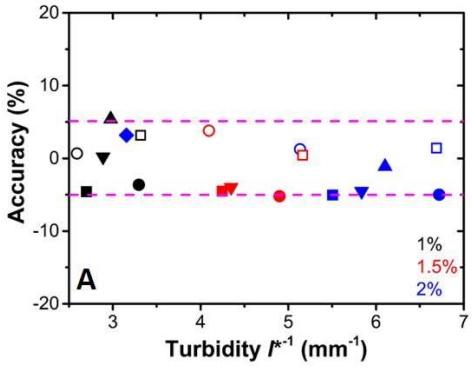

Fig 3: Accuracy of DWS sizing as a function of turbidity, characterized by the inverse of the transport mean free path, l. Measurements were carried out on aqueous suspensions of particles with radii in the range of 100 to 1000 nm and different particle concentration.*

Abstracts for posters

Self-Healing Colloidal Crystals: Why Soft Particles Feel the Squeeze

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INTRODUCTION: Point defects in crystalline materials disturb the crystal structure and often prevent crystallization. In particular, this is the case for too big particles that are put into a crystal. In metal melts, a size mismatch of 15% of the atoms in the melt suppresses crystallization. Furthermore, hard spheres with a polydispersity greater than 12% do not form crystals, and the polydispersity in the crystal state does not exceed 5.7%, as local segregation occurs. These restrictions do not necessarily apply for soft microgels. Lyon et al.[1] find bigger microgels to shrink and fit into the lattice formed by smaller ones

METHODS: We study the spontaneous deswelling of big particles in a crowed environment of small particles. Fully swollen poly(N-isopropylacrylamide) (pNIPAM) microgel particles have been used. The thermodynamic quantity that rules the phase behavior is the generalized volume fraction ζ. Phase behavior is studied by using series of 10 - 15 samples, which cover the ζ range of interest. Small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS) and dynamic light scattering are used to characterize the particles and their phase behavior. We observe crystallization in samples with a fraction of big particles n_{big} as high as 0.286.

RESULTS: We find that charged groups in the microgel and their counter-ions are the key to explain this remarkable spontaneous deswelling of microgels. These groups come from the starter used during the synthesis. The overlap of counter-ion clouds build up an osmotic pressure that deswells the large microgels. Using small-angle neutron and X-ray scattering, we directly observe the deswelling of bigger particles with increasing volume fraction and the effect of the bigger particles on the phase behavior of the suspension. Furthermore, we determine the osmotic pressure using osmometry and present a model for the deswelling of the big particles.

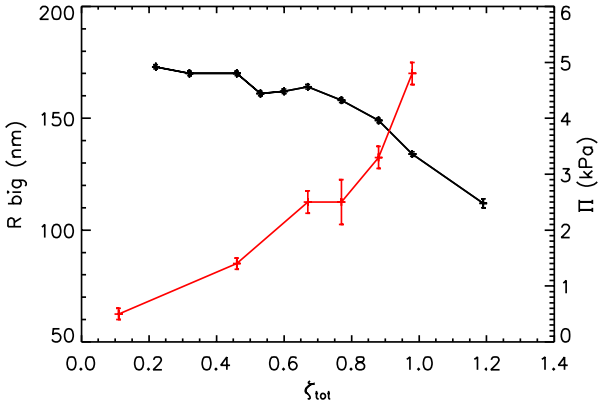

Fig. 1 Variation of the radius of the big particles with ζ is shown. The osmotic pressure is in red.

DISCUSSION & CONCLUSIONS: Our results explain the deswelling mechanism in suspensions of soft and deformable microgels and justify the observed phase behavior.

REFERENCES: [1] A. St. John Iyer et al.; **2009**; *Angew. Chem.*; 48: 4562-4566

CORRELATION BETWEEN NANO-STRUCTURE AND PERFORMANCE-RELATED PROPERTIES OF RADIATION-GRAFTED PROTON-CONDUCTING MEMBRANES

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INTRODUCTION: Polymer electrolyte fuel cells using hydrogen can serve as clean and efficient energy converters. One of the crucial components of these electrochemical cells is the proton conducting membrane. The proton conductivity, mechanical stability and durability of these membranes are strongly affected by the phase segregation found on the nanoscale, a topic extensively studied with various methods[1,2] such as small-angle neutron scattering, an ideal technique for this class of materials[3].

METHODS: We combined the structural characterization tools of small-angle neutron (SANS – Fig. 1.) and X-ray scattering (SAXS), the energy dispersive X-ray analysis (EDX) and the dynamic experimental method of nuclear magnetic resonance (NMR) spectroscopy[4] to gain insight into the fuel-cell relevant properties of membranes synthesized via radiation grafting. For the preparation of the membranes we used a commercially available base film (ETFE, poly(ethylene-*alt*-tetrafluoroethylene)) grafted with polystyrene, sulfonated subsequently.

RESULTS: We found structural and/or dynamic variations of the membranes upon changing the following parameters: basefilm manufacturer (DuPont/Saint-Gobain), level of grafting, radiation grafting protocol and sulfonation. We also revealed how the structural and dynamic parameters vary along different directions in the membrane (Fig. 2.). Structural studies were facilitated by the development of a sample environment allowing measurements at different membrane orientations and humidity conditions. The obtained results were correlated with membrane performance relevant measurements, such as membrane conductivity, and with macroscopic characteristics, such as dimensional changes.

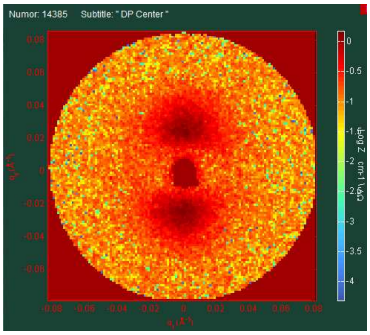

Fig. 1: SANS signal of ETFE basefilms.

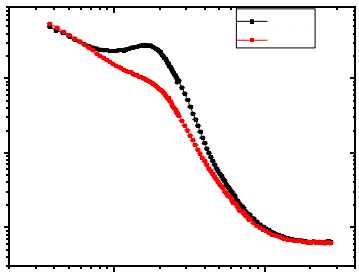

Fig. 2: Anisotropy of 25 % grafted ETFE films as revealed by SANS experiments.

DISCUSSION & CONCLUSIONS: These results allow us to better understand the correlation between synthesis, structure, (proton) dynamics, and functionality of radiation grafted membranes, which will lead to the development of membranes with improved performance.

REFERENCES: [1] Balog, S. et al. (2013) Polymer 54; [2] Balog, S. et al. (2011) J. Membr. Sci. 383; [3]Balog, S. et al. (2010) Macromol. Chem. Phys. 211; [4] Sproll, V. et al. (2015) Radiat. Phys. Chem. (in press)

ACKNOWLEDGEMENTS: The authors gratefully acknowledge financial support from the Paul Scherrer Institute within the framework of the "CROSS Departmental Initiative" and from the European Commission under the Seventh Framework Program by means of the grant agreement for the Integrated Infrastructure Initiative N. 262348 European Soft Matter Infrastructure (ESMI).

MICROFLUIDICS FOR DRUG-ASSAYS AND DIAGNOSTICS ON *TRYPANOSOMA BRUCEI*

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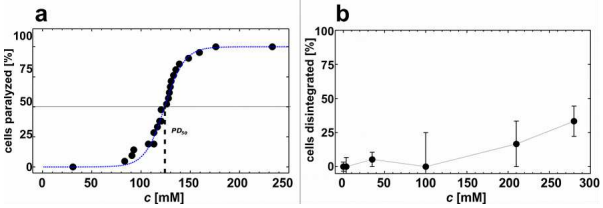
INTRODUCTION: Microfluidics is a scientific field, where small quantities of fluids are studied in a microscopic setting. Emerging from the interface between chemistry, physics and engineering, microfluidic applications for biological problems allow for high-speed analysis on the single cell level.

Exploiting the laminar flows in this microscopic regime (at low Reynolds numbers) we present a rapid drug-assay based on chemical gradient microfluidics and optical micromanipulation.

METHODS: Here, we used this combination to in situ monitor the effects of drugs and chemicals on the motility of the flagellated unicellular parasite *Trypanosoma brucei*; specifically, the local cell velocity and the mean squared displacements (MSD) of the cell trajectories.

RESULTS: With our method, we are able to record in situ cell fixation by glutaraldehyde, and to quantify the critical concentration of 2-deoxy-D-glucose required to completely paralyzing trypanosomes. In addition, we detected and quantified the impact on cell propulsion and energy generation at much lower 2-deoxy-D-glucose concentrations. Our microfluidics-based approach advances fast cell-based drug testing in a way that allows us to distinguish cytocidal from cytostatic drug effects, screen effective dosages, and investigate the impact on cell motility of drugs and chemicals. Using suramin, we could reveal the impact of the widely used drug on trypanosomes: suramin lowers trypanosome motility and induces cell-lysis after endocytosis.[1]

Additionally, we used our setup to characterize the force generation and energy consumption of the trypanosomal flagellar motor[2] and test designs to modify the displacement of trypanosomes based on their own motility.



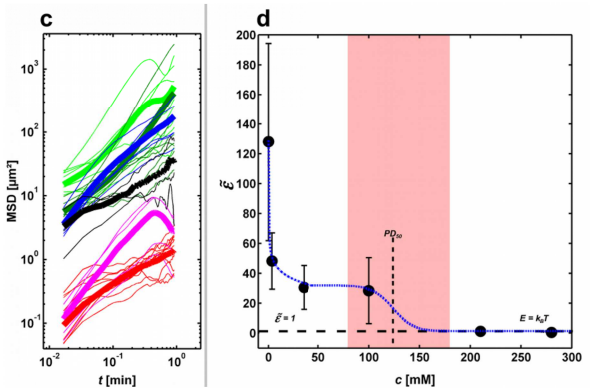


Fig. 1: Quantitative analysis of the impact of suramin on trypanosome propulsion and disintegration.
(a) Concentration dependent paralysis by suramin.
(b) Disintegration of trypanosomes in response to different suramin concentrations.
(c) MSD of confined trypanosomes over time.
(d) The motility factor $\tilde{\epsilon}$ at different concentrations of suramin in the microchamber.. [1]

DISCUSSION & CONCLUSIONS: Our straightforward microfluidic-based method allows not only for an in situ analysis of single cells in defined, gradually changeable environments but also for a rapid evaluation of the impact of the environment by analyzing cell motility.

REFERENCES: [1] A. Hochstetter, E.Stellamanns, S.Deshpande, S. Uppaluri, M. Engstler and T. Pfohl; **2015**; *Lab Chip;* 15: 1961-1968.
[2] E.Stellamanns, S. Uppaluri, A. Hochstetter, N. Heddergott, M. Engstler and T. Pfohl; **2014**; *Sci Rep;* 4: 6515.

ACKNOWLEDGEMENTS: We gratefully acknowledge the DFG and the SNF for funding.

Stable and ion selective polymer nanocompartments

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[1] *University of Basel, Basel, Switzerland*

INTRODUCTION: Intracellular processes are mediated by the presence of ions, which should be capable of crossing the plasma membrane. The direct exchange of ions across membranes is possible due to the presence of ion channels, biopores, or ion-carriers. Similarly, bio–compartments and mimics of cell membranes are important for designing applications in theranostics.[1] Therefore, we introduce biomimetic nanocompartments (polymersomes) with gramicidin (gA)[2] or ionomycin (Ion)[3] permeabilized membranes (Figure 1), as stable and ion selective nanodevices.

METHODS: Polymersomes were obtained by film rehydration technique, simultaneously encapsulating fluorescent dyes, sensitive to pH, Na^+, K^+, or Ca^{2+}. The self-assembly process, as well as the entrapment efficiency of the dyes inside polymersomes, were verified by a combination of dynamic and static light scattering (DLS/SLS), transmission electron microscopy (TEM), and fluorescence correlation spectroscopy (FCS). Fluorescence and stopped-flow spectroscopy were used to prove the influx of ions across the membranes.

RESULTS & DISCUSSIONS: The formation of polymersomes (~100 nm) was confirmed by TEM and DLS/SLS. The same methods were used to show that the insertion of the biopores or ion carriers into the membrane preserved the architecture of the polymersomes, in specific ranges of gA, Ion, and ions concentrations. The diffusion times of the free dyes (30-40 μs) were much lower than the ones corresponding to the polymersomes in which the dyes were entrapped (4000-7000 μs), offering information about the attachement and entrapment of the dye to or inside the nanostructures, respectively. The differences in fluorescence intensities of the permeabilized versus the non-permeabilized polymersomes gave insights into the successful exchange of ions through the membranes, either by passive passage through the imbedded biopores (gA), or transported by ion-carriers (Ion).

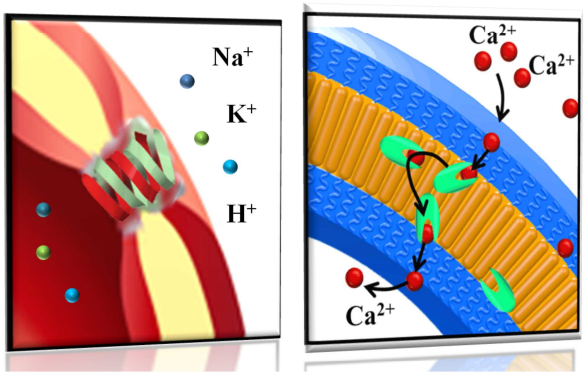

Fig. 1: Representations of polymer membranes with ion selectivity and stability through insertion of gA (left) or ionomycin (right).

CONCLUSIONS: By successfully permeabilizing the membrane of polymersomes, ions can pass through the membrane in a selective fashion leading to the development of biomimetic nanostructures with ion selectivity and preserved architecture. This enables the further design of nanoreactors or biosensors with sensitivity and stimuli-responsiveness.

REFERENCES:

[1]C.G. Palivan; **2012**; *Chemical Society Reviews*; 7:2800-23. [2]M. Lomora, et al; **2015**; *Biomaterials;* 51:406-414. [3]M. Lomora, F. Itel, et. al.; **2015**; *PCCP*; DOI: 10.1039/C4CP05879H.

ACKNOWLEDGEMENTS: Financial support was provided by the Swiss National Science Foundation, and the National Center of Competence in Research "Molecular Systems Engineering, which are gratefully acknowledged. We thank Prof. W. Meier (University of Basel) for providing the block copolymers, Samuel Lörcher, and Sven Kasper for polymer synthesis. We are grateful to G. Persy and A. Najer (University of Basel) for TEM measurements.

Preparation of novel composite materials via CO-Coagulation of NPs

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Since the term 'nanocomposites' has been proposed for the first time by Theng in 1970, the science dealing with these materials has arisen at the border of different areas of knowledge.

Numerous procedures for the preparation of nanocomposite materials have been investigated in order to generate materials with controlled features, which is still one of the great challenges in nanotechnology.

The purpose of this work is to use the extended and large knowledge of self-assembly behavior of spherical nanoparticles and its dependence on interparticle interactions for the preparation of various polymeric nanoparticles and the investigation of their self-assembly, to generate hybrid nanostructured composites.

The nanocomposite comprises different concentrations of hard silica nanoparticles (20 nm diameter) blended with a colloidal dispersion of soft poly(butyl acrylate/methyl methacrylate) copolymer particles (80 nm diameter). In order to investigate the specific role of the interparticles interaction on the final structure and mechanical properties of the materials, the nanocomposites have been synthetized following three different assembly stategies: mixing of stable particle suspensions with same surface charge, aggregation/gelation of the filler particles inside the matrix particle stable suspantion, hetero-aggregation of particle suspensions with opposite surface charge.

The different hetero particle suspensions have then been precipitated and subsequently annealed, giving rise to a nanostructured polymeric composite, which has been characterized in term of structure and mechanical properties using different techniques (SAXS, AFM, SEM, tensile test).

Cryo-electron microscopy and tomography for soft materials research

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INTRODUCTION: Cryo-electron microscopy (cryo-EM) has been a potential method to analyse 3D structure of biological macromolecules for decades. Recent progress allowed visualization of proteins and nucleic acids at subnanometer resolution. In most successful cases, ~3A resolution can be achieved, which enables protein biologists to identify amino acids in the 3D density maps and thus to build atomic models. Due to poor contrast between proteins and background water as well as high sensitivity of proteins, image averaging is necessary to extract structural information from noisy cryo-electron micrographs. These approaches should be applicable for non-biological soft materials in water. Indeed there are studies on polymers using cryo-EM. However until now it is limited to morphological description: potential of this method for soft materials has not been exploited. In this presentation, I will discuss how cryo-EM can contribute to soft materials research based on our recent studies.

METHODS: Materials of interest in water is mounted on EM grids. Excess of solution is removed by filter paper and the grid is plunged into liquid ethane at liquid nitrogen temperature for quick (~10micro seconds) freezing to embed the objects in vitreous ice. Cryo-EM images are formed by transmission electron microscopy based on phase contrast imaging.

One challenge of cryo-EM is to reconstruct 3D information from 2D micrographs, which are, if simplified, projection of 3D the density map along the direction of electron beam. There are two approaches to reconstruct 3D structure. In single particle analysis, each object is illuminated once. Micrographs of many objects are merged into 3D, under the assumption that they share the identical 3D structure and show random orientations. The other method is electron tomography: the object is illuminated multiple times with the specimen stage tilted at various angles. This method allows relatively straightforward 3D reconstruction, although the spatial resolution is limited by radiation damage.

RESULTS: Tomographic approaches provided us various information, which cannot be obtained by microscopy from one direction: curvature of bicelles (Fig. 1) and encapsulation of metal clusters in the hollow of zeolite (Fig. 2). With cryo-electron tomography, we could achieve 3D analysis at several nanometers resolution.

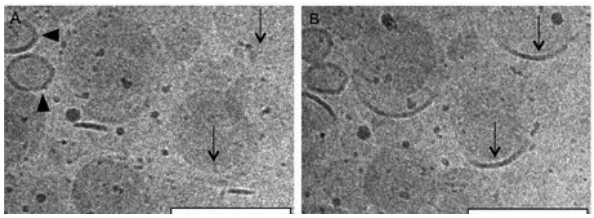

Fig. 1: Cryo-EM of acqueous mixture of DPPC/ Cholesterol/DPPE-DTPA/Tm^{3+}. A: projection. B:30 deg tilt. Vesicles (allowheads) and bicelles are observed. Edge of the bicelles is bent as revealed by tilting (allows). Modified from ref.1

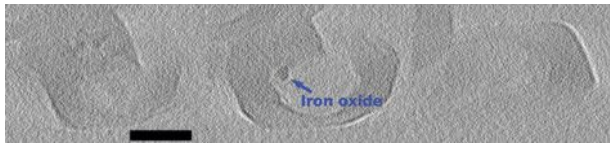

Fig. 2: Three horizontal sections from cryo-electron tomography of ZSM-5 hollow crystal. Tomography enabled us to recognize iron oxides inside the hollow. Modified from ref.2.

For further improvement of resolution, we need to employ averaging technique. However, averaging is valid only when objects share the identical structure. To avoid artefact from averaging heterogeneous structures, we classify density maps of objects using multivariate statistical analysis (ref. 3).

DISCUSSION & CONCLUSIONS: Another remaining challenge is to measure the thickness of membranes or fibres. Due to phase contrast imaging, density appearing in the micrograph is not proportional to the real projection. We need to develop optics or image analysis to locate the boundary between organic materials and water.

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ACKNOWLEDGEMENTS: I appreciate our collaborator groups for opportunities of soft materials research: Prof. P. Walde (ETHZ), Prof. P. Fischer (ETHZ), Dr. J. Kohlbrecher (PSI), Dr. M. Liebi (PSI), Prof. J. van Bokhoven (PSI/ETHZ), Prof. Y. Yamakoshi (ETHZ), and Prof. A. Zumbuehl (Uni Freiburg).

Thiol-ene 'Clickable' Copolymer-Brush Nanostructures on Polymeric Substrates via Extreme Ultraviolet Interference Lithography

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INTRODUCTION: Patterned functional polymeric surfaces with reactive groups that can be modified under mild, metal-catalyst-free reaction conditions open a broad field of applications and are of particular interest for the bioconjugation of polymeric materials. Such patterns need to be fabricated with properties and characteristics that enable them to interact with their environment in a desired manner. Polymer brushes are ideal candidates for such applications, since polymers can be readily synthesized to be multifunctional by the incorporation of several monomers with different properties and functions. Brushes of functionalizable copolymers were obtained by copolymerization of a furan-protected maleimide monomer (FuMaMA) with different ethylene glycol-containing monomers, which provide anti-biofouling properties to these surfaces (Fig. 1). After deprotection, the reactive maleimide methacrylate (MaMA) units were utilized to conjugate thiol-containing molecules using the nucleophilic thiol-ene reaction, which proceeds at room temperature without the need for any metal-based catalyst.

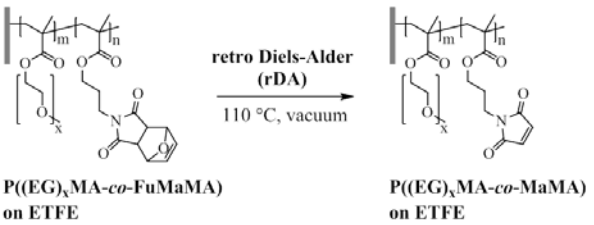

Fig. 1: Strategy for the synthesis of maleimide-containing copolymer brushes.

METHODS: In order to obtain patterned micro- and nanostructured copolymer brushes, patterns of radicals serving as polymerization initiators were created by exposure to EUV-light using the X-ray interference lithography (XIL-II) beamline at the Swiss Light Source (SLS).

RESULTS: Micro- and nanostructured polymer brushes are covalently grafted from polymer substrate surfaces (Fig. 2). Exposures through a typical mask with diffraction gratings lead to line arrays with a period down to 100 nm for two interfering beams, to dot arrays with a period down to 140 nm, and to hexagonal structures of 700 nm, respectively.[1-3] A variety of functionalities was introduced to yield polyelectrolytes, as well as fluorescent and light-responsive polymer-brush structures by coupling SH-terminated fluorescent dyes or spiropyrans, respectively (Fig. 3).

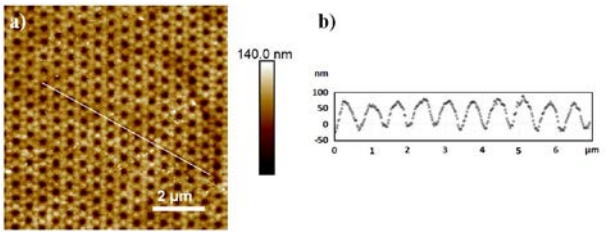

Fig. 2: AFM image of copolymer nanostructures grafted from ETFE. (a) Structure with hexagonal symmetry and (b) line profile along the line indicated in (a).

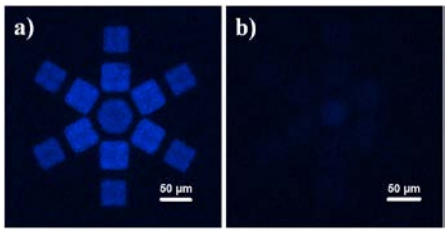

Fig. 3: (a) Fluorescence emission of copolymer brush structures after binding coumarin using the specific thiol-ene reaction. For the negative control (b) the deprotection step was omitted.

DISCUSSION & CONCLUSIONS: The presented examples of functionalization of nanopatterned maleimide-containing copolymer brushes on polymeric substrates demonstrate the versatility of this approach. With the future application potential of multifunctional "smart surfaces" in mind, a detailed understanding of the parameters that determine the different functionalities of these engineered brush structures is valuable for the development of further, more complex responsive systems.

REFERENCES: [1] M.Dübner *et al.*; **2015**; *ACS Appl. Mater. Interfaces;* submitted. [2] M.Dübner *et al.*; **2014**; *Langmuir;* 30, 14971-14981.

ACKNOWLEDGMENTS: EUV-interference lithography exposures were performed at the SLS at PSI. Financial support from the SNF is gratefully acknowledged.

Crystallization in binary mixtures of charged microgels

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[2] *Metropolitan Autonomous University, Mexico City, Mexico.*
[3] *National Polytechnic Institute, Mexico City, Mexico.*

INTRODUCTION: At large enough packing fractions monodisperse colloids interacting by repulsive interactions generally crystallize. To suppress or delay crystallization one generally introduces some polydispersity into the system; this can be achieved by producing binary mixtures. In this contribution we discuss crystallization in binary mixtures of charged microgels at quasi-deionized conditions.

METHODS: We use two Poly-N-isopropyl acryl-amide microgel systems that slightly differ in size and quite significantly differ in their effective charge. For both systems we set the number concentration to $N \sim 8.65\ \mu m^{-3}$, which corresponds in both cases to a particle volume fraction below 10%. To maximize the screened Coulomb interactions among the microgels we put the systems in contact with an ion-exchange resin, thereby achieving quasi-deionized conditions. At these conditions the more highly charged system S1 crystallizes, while the less charged system S2 remains in an amorphous liquid state even after long equilibration times. Binary mixtures are produced by mixing the two systems at different ratios, which we indicate as

$$X = \alpha\ \mathrm{S2} / [(1-\alpha)\ \mathrm{S1} + \alpha\ \mathrm{S2}].$$

The structural and dynamical properties of the mixtures at T=20°C are characterized by static and dynamic light scattering.

RESULTS: With increasing X the degree of correlation between the particles decreases, as evidenced by the decrease of the magnitude of the nearest neighbour peak of the liquid structure factor $S(q)$ obtained just after shear-rejuvenating the systems (Fig. 1 left and Fig. 2 right). After a week of equilibration we find that all systems with $X < 0.25$ contain crystals, exhibiting Bragg peaks typical of BCC-crystals. A preliminary analysis suggests that these crystals are formed by S1 only, which entails that the mixtures phase separate. However, the nucleation times τ_{nucl} remain very fast as compared to the time τ_{rm} it takes for the particles to move by the mean distance among them. The limit to crystal formation and thus presumably phase separation at $X \sim 2.2$ is thus not obviously related to the difficulty to form a stable crystalline nucleus within a binary mixture. Instead, we find that the boundary to crystal formation corresponds to the point at which the magnitude of the nearest neighbour peak of the liquid states $S(q_{max})$ drops below the Hansen-Verlet criterion for freezing ($S(q_{max})$=2.8) [1].

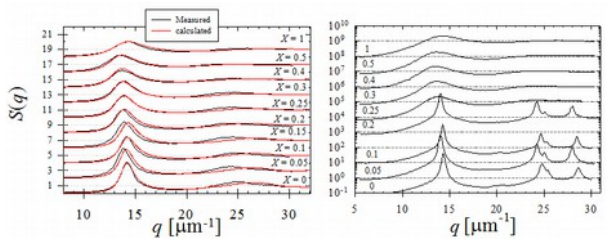

Fig. 1: S(q) after shear-rejuvenation (left) and after a week of equilibration (right). For more clarity the data have been vertically shifted by applying an arbitrary factor.

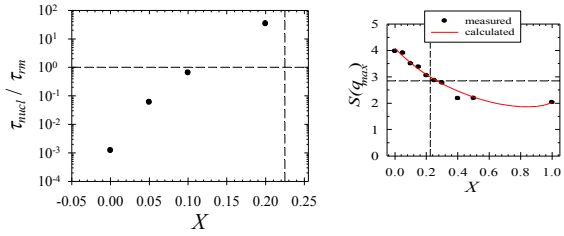

Fig. 2: (Left) Nucleation time normalized by the time needed to move over a mean particle distance. (Right) X-dependence of $S(q_{max})$ as obtained for the shear-rejuvenated systems. Horizontal line denotes the limit to freezing according to the Hansen-Verlet criterion. In both graphs the vertical lines indicate the limit to crystallization.

DISCUSSION & CONCLUSIONS: This seems to indicate that the limit to phase separation, i.e. crystal formation is set by the degree of correlation within the liquid state rather than by the constrains imposed by the binary nature of the system.

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pH-triggered reversible multiple protein-polymer conjugation based on molecular recognition

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INTRODUCTION: Polymer conjugation for protein based therapeutics has been developed extensively for over 40 years. The conjugation of polymers to proteins improves their stability, half-life, solubility, and reduces immunogenicity. Site specific non-covalent polymer conjugation allows the modulation of binding affinity and stability, and affects the pH dependent binding of protein-polymer conjugates without disrupting protein function.

In this work, we present a robust and efficient method to site-specifically and reversibly bind multiple His-tagged proteins per polymer chain using trisNTA-Me^{2+}-His_6 molecular recognition. His-tagged enhanced yellow fluorescent protein (His_6-eYFP) was used as a model protein for binding poly(N-isopropylacrylamide-co-tris-nitrilotriacetic acid acrylamide) (PNTn) copolymers bearing multiple tris-nitrilotriacetic acids (trisNTA) and they were complexed with different metal cations (Me^{n+}).[1] We employed these PNTn- Me^{n+}-His_6-eYFP conjugates to analyze the effect on the stability, pH responsiveness and toxicity when variables such as the nature of the metal, the distance between trisNTA sites, and the addition of inter-protein interactions were varied.

METHODS: The binding affinity of PNTn-Me^{n+} for His_6-eYFP with different distances between binding sites was determined by isothermal titration calorimetry (ITC). The stability and the pH responsiveness of polymer-protein conjugates were analyzed by fast protein liquid chromatography (FPLC). The stability of proteins before and after release from the polymer conjugates was investigated by circular dichroism (CD) and fluorescence spectroscopy. The cell viability was assessed by MTS assay.

RESULTS: ITC results showed that the dissociation constant of PNT-Me^{n+}-His_6-eYFP conjugates varied from 1.35±0.12 µM to 0.09±0.03 µM depending on the loaded metals in trisNTA pockets and the average distance between trisNTA binding sites. PNT1 and PNT4 copolymers with average distances between trisNTA binding sites of 31.5 nm and 5.2 nm, respectively, were employed for the following studies. PNT4-Cu^{2+}-His_6-eYFP showed the highest stability but had no significant pH-triggered release of His_6-eYFP until the pH was dropped to 5. PNT4-Zn^{2+}-His_6-eYFP exhibited a significant pH-triggered release of His_6-eYFP at both pH 6 and 5 (Fig. 1). The decrease of average distance between trisNTA binding sites significantly enhanced the stability of the conjugates, for the case of PNT4-Zn^{2+}-His_6-eYFP and PNT1-Zn^{2+}-His_6-eYFP, which was attributed to inter-protein interactions. No influences of the structure and the function of His_6-eYFP were observed by CD and fluorescence spectroscopies before and after release from the PNTn-Me^{2+} copolymers at selective pH. Moreover, PNTn-Zn^{2+} didn't exhibit any cell toxicity assessed by MTS assay.

*Fig. 1: The release of His_6-eYFP was analysed by FPLC. Stars indicate significance in two-tailed Student's t-test; *P<0.05, **P<0.005.*

DISCUSSION & CONCLUSIONS: We have demonstrated that the nature of the Me^{n+}, and the number and distance between metal binding pockets of trisNTA, enable great selectivity for the binding affinity. This led to control of the stability and pH triggered release of the protein from the polymer chain due to the lack of, or presence of, inter-protein interactions. The presented system serves as a platform for the combination of various active agents into one nanosystem to potentially fulfill multiple tasks such as therapy, diagnosis, and targeting in a combined manner.

REFERENCES: [1] J. Liu et al.; **2014**; *J. Am. Chem. Soc.;* 136:12607-12614.

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