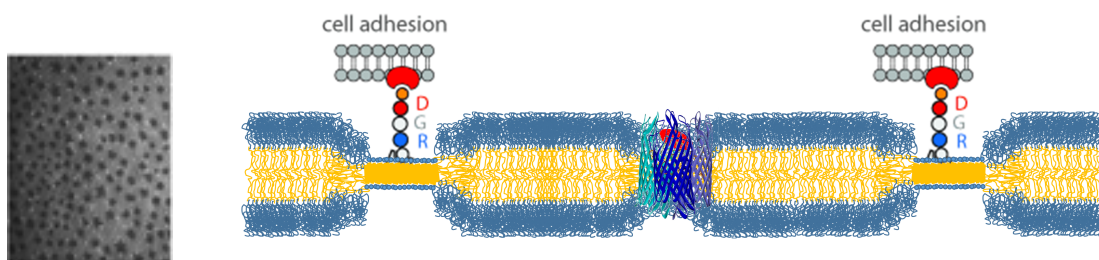


## Selective Reconstitution of Biomolecules in Polymer-Lipid Membranes

Wolfgang Meier, Department of Chemistry, University of Basel (UniBas), Klingelbergstrasse 80, 4056 Basel.  
Uwe Piele, School of Life Sciences, Institute for Chemistry and Bioanalytics, University of Applied Sciences  
Northwestern Switzerland (FHNW), Grundenstrasse 40, 4132 Muttenz

Understanding of the structural organization of cell membranes is highly relevant for the development of pharmaceutical drugs and functional combination with biomolecules (e.g. proteins, enzymes, etc.). Amphiphilic block copolymers are interesting as model systems that can self-assemble in aqueous media to membranes with high stability, and properties that can be optimized by varying their molar mass, and hydrophilic-to-hydrophobic block length ratio.<sup>1</sup> In previous studies we have shown that mixtures of amphiphilic block copolymers and lipids form membranes of distinct domains: lipid- (3-5 nm thickness), and copolymer- (5-20 nm thickness) phases, with enhanced stability of the polymer domains and enhanced mobility in the lipid domains<sup>2</sup>. Interestingly the phase behavior of the polymer and lipid domains significantly triggers the insertion of membrane proteins. For example, below the glass transition temperature of the lipid domains membrane proteins selectively insert only into the polymer domains.<sup>3</sup>

In this proposal, we plan to synthesize functionalized amphiphilic block copolymers (and lipids) to further modulate the polymer-lipid hybrid membrane for a spontaneous, selective combination with more than one type of biomolecules. We selected poly(dimethylsiloxane)-*block*-poly(2-methyl-2-oxazoline) (PDMS-*b*-PMOXA) copolymers and saturated- (e.g. similar to DPPC or DPPE) or unsaturated- (e.g. similar to DOPC) lipids to form model membranes for selective insertion/attachment of biomolecules. A selective (partial) crosslinking of functional lipid- or polymer- domains will locally fix the biomolecules<sup>1</sup>, so that in a second step, another type of biomolecule will decorate the second type of membrane domain. As model membrane protein we will use a mutant of OmpF (with stimuli-triggered property),<sup>4</sup> which allows diffusion of molecules through the membrane under pH trigger. As a second biomolecule type we plan to insert arginyglycylaspartic acid (RGD peptides) for adhesion of cells into the second domain (Figure). We are interested to evaluate the desired selective distribution and function of the two types of biomolecules in the polymer-lipid membranes. This approach will serve as a model for further insertion of different types of membrane proteins with high industrial and medical interest.



**Figure. BAM of a polymer-lipid hybrid membrane and a scheme for selective and spontaneous reconstitution of pH triggered OmpF in the block copolymer domains and RGD peptide attached to lipid domains for cell adhesion.**

The project is divided into three milestones.

**In the first milestone** we will synthesize the functionalized building blocks for the membrane assembly. Chemically functionalized amphiphilic copolymers and lipids will be synthesized with different functional groups, e.g.  $-\text{NH}_2$ ,  $-\text{SH}$ ,  $-\text{N}_3$ . The modified polymers and lipids will be characterized by NMR, GPC and IR. We will study three polymer-lipid hybrid systems: i) copolymer with functional end groups and non-functionalized lipids, ii) lipid with functional groups and non-functionalized copolymers, and iii) both polymer and lipid with functional end groups. The resulting

membranes will be characterized by a combination of Langmuir isotherms, BAM, AFM, XPS, and CLSM.

**In the second milestone** we will establish where pH triggered OmpF will be inserted into the hybrid membranes, and control a desired position as function of the properties of the membrane. By combining biochemical, physical, and spectroscopic techniques (i.e. AFM, CLSM, XPS, ATR-FTIR, EIS, conductance measurements), the distribution and function of the pH triggered OmpF will be investigated. Protein insertion will be optimized by varying block copolymer parameters, lipid chemical nature, polymer to lipid ratio, protein to polymer-lipid ratio, and the reconstitution strategy. Furthermore, crosslinkers will stabilize the domains containing the reconstituted proteins.

**In the third milestone** we will bind a second biomolecule, RGD peptides to the polymer-lipid hybrid membrane. The distribution and function of the RGD will be evaluated with similar techniques as OmpF (see the second milestone). We will investigate the triggered transport efficiency of OmpF, and the cell adhesion properties of RGD. The different properties of the lipid and polymer domains of the hybrid membrane will induce a selective interaction with the biomolecules. Systematic investigation of the effect of different domains on simultaneous biomolecules functionality will allow the optimization of the hybrid membranes.

**Time schedule for the interdisciplinary project.** Each milestone will require a hard working PhD student about 12 months. Altogether, the project will take about 36 months for a PhD student.

**Necessity of interdisciplinary collaboration.** This challenging project requires combining expertise in polymer chemistry, nanotechnology, nanobiology, and nanochemistry. W. Meier (UniBas) is the co-inventor of block copolymer membranes and successfully reconstituted various membrane proteins (OmpF, AqpZ) in nanoscopic copolymer membranes. The main research activities of U. Pieleles (FHNW) are on surface chemistry/functionalization, bioconjugation of materials and cell biology imaging, and biosensors which are complementary aspects for hosting a joint PhD thesis with W. Meier.

**Organization of the interdisciplinary PhD.** The student requires the input and know-how of both research groups located in Basel. Therefore, the student will be formally inscribed at UniBas and co-inscribed at FHNW. In addition, the PhD will be a full member of the Meier and Pieleles research groups. We estimate that, during his PhD, the student will spend half time at UniBas and half-time at FHNW.

## Literature

1. W. Meier, C. Nardin and M. Winterhalter, *Angew Chem Int Edit*, 2000, **39**, 4599; C.G. Palivan, R. Goers, A. Najer, X. Zhang, A. Car and W. Meier, *Chem. Soc. Rev.* 2016, **45**, 377
2. F. Itel, A. Najer, C. G. Palivan, W. Meier, *Nano Letters* 2015, **15(6)**, 3871
3. J. Kowal, D. L. Wu, V. Mikhalevich, C. G. Palivan and W. Meier, *Langmuir*, 2015, **31**, 4868; J. Thoma, S. Belegriou, P. Rossbach, M. Grzelakowski, K. Kita-Tokarczyk and W. Meier, *Chem Commun*, 2012, **48**, 8811.
4. T. Einfalt, R. Goers, I. A. Dinu, A. Najer, M. Spulber, O. Onaca-Fischer and C. G. Palivan, *Nano Lett*, 2015, **16**, 823.

## Wolfgang Meier

Professor of Chemistry, University Basel, Department Chemistry, Switzerland.

Born: 09.11.1964, in Biberach, Germany. Citizenship: German

### Education and Theses

1998            Habilitation (Chemistry), University of Basel, Switzerland.  
1992            Ph.D. (Chemistry), University of Freiburg, Germany.  
1989            Diploma (Chemistry), University of Freiburg, Freiburg, Germany

### Employment

2003 – pres.    Full Professor in Chemistry, University of Basel, Department of Chemistry  
Switzerland.  
2008 – 2011    Head of the Department of Chemistry, University of Basel, Switzerland  
2001 – 2002    Professor, International University of Bremen, Bremen, Germany.  
1996 - 2000    Lecturer, Physical Chemistry, Department of Chemistry, University of Basel,  
Switzerland  
1993 - 1995    Postdoctoral Scientist at the University of Basel, Switzerland

### Honors

2006            Hermann-Staudinger Price  
2001            Ruzicka Prize  
1990-1992    Chemical Industry Scholarship

### Publications/Patent

Currently ca. 220 publications in scientific journals and ca. 24 patents

### Examples:

Natural channel protein inserts and functions in a completely artificial, solid-supported bilayer membrane;  
X. Zhang, W. Fu, C. Palivan, W. Meier; *Scientific Reports*, 3, 2196, (2013)

Molecular Organization and Dynamics in Polymersome Membranes: A Lateral Diffusion Study  
F. Itel, M. Chami, A. Najer, S. Lörcher, D. Wu, I. A. Dinu and W. Meier; *Macromolecules* 47, 7588–7596  
(2014)

Hybrid Polymer–Lipid Films as Platforms for Directed Membrane Protein Insertion  
J. Kowal, D. Wu, V. Mikhalevich, C. G. Palivan, W. Meier *Langmuir*, 31, 4868–4877, (2015)

Dynamics of membrane proteins within synthetic polymer membranes with large hydrophobic mismatch  
F. Itel, A. Najer, C. G. Palivan, W. Meier, *Nano Letters*, 15(6), 3871-3878(2015)

Bioinspired polymer vesicles and membranes for biological and medical applications; C.G. Palivan, R. Goers, A.  
Najer, X. Zhang, A. Car and W. Meier, *Chem. Soc. Rev.* 45, 377 – 411 (2016)

## Curriculum Vitae

### Uwe Pieleles

Born in Germany 21.07.59 in Warburg/Germany. Studies in Chemistry at the Universities Bielefeld and Göttingen (1981-1985). Diplomathesis (1985) and PhD-Thesis (1988) at the MaxPlanck Institute for experimental medicine in the field of chemically modified oligonucleotides under the supervision of Prof.Dr. F.Cramer. 1989-1991 Postdoc (EMBL-Fellow) at the EMBL (European molecular biology laboratory). 1991-1996 Labhead Central research laboratories Ciba/Novartis Switzerland (Basel). 1997-2000 Groupleader pharmaceutical research Altana AG Germany (Konstanz). Since 2000 Head of nanotechnology/materials sciences and Professor at the FHNW (University of applied sciences North West Switzerland) School of life Sciences in Muttenz (Switzerland)

Main research activities of the group: Surface science, surface chemistry, supra molecular chemistry, material sciences with strong focus on biomaterials and implants. Nanomaterials for biomedical applications .

### Awards

International Collano Recognition Award 2007 Figgemeier, Chabreck, Pieleles

### Publications – Patents

Author and Coauthor of more than 60 peer reviewed Publications patents and patent applications

### Selected - Publications

- 1 Self-Sealing and Puncture Resistant Breathable Membranes for Water-Evaporation Applications** Rother M Barmettler J, Reichmuth A, Araujo JV, Rytka C, Glaied O, Pieleles U, Bruns N *Adv Mater.* 2015; 6620-4
- 2 Indocyanine green loaded biocompatible nanoparticles: Stabilization of indocyanine green (ICG) using biocompatible silica-poly( $\epsilon$ -caprolactone) grafted nanocomposites** Schönbachler, Andrea; Glaied, Olfa; Huwyler, Jorg; Frenz, Martin; Pieleles, Uwe *Journal of Photochemistry and Photobiology, A: Chemistry* (2013), 261, 12-19.
- 3 Photoreaction of a Hydroxyalkyphenone with the Membrane of Polymersomes: A Versatile Method To Generate Semipermeable Nanoreactors** Spulber, Mariana; Najer, Adrian; Winkelbach, Katharina; Glaied, Olfa; Waser, Marcus; Pieleles, Uwe; Meier, Wolfgang; Bruns, Nico *Journal of the American Chemical Society* (2013), 135(24), 9204-9212
- 4 Micro- and nanostructured polymer substrates for biomedical applications** Althaus, Jasmin; Urwyler, Prabhitha; Padeste, Celestino; Heuberger, Roman; Deyhle, Hans; Schiff, Helmut; Gobrecht, Jens; Pieleles, Uwe; Scharnweber, Dieter; Peters, Kirsten; et al *Proceedings of SPIE* (2012), 8339(Bioinspiration, Biomimetics, and Bioreplication 2012),
- 5 J. Althaus, C. Padeste, J. Köser, U. Pieleles, K. Peters, and B. Müller** Nanostructuring polyetheretherketone for medical implants *European Journal of Nanomedicine* (2012) 2011-001
- 6 Design of a high-throughput device for screening surface modification protocols** Saxer, Sina; Pieleles, Uwe; Elsener, Martin; Horisberger, Michael; Tosatti, Samuele; Textor, Marcus; Gademann, Karl; Zuercher, Stefan *Progress in Organic Coatings* (2010), 67(1), 20-27.
- 7 CVD of silanes on silicon stamp for nanoimprint lithography** Schiff, Helmut; Saxer, Sina; Park, Sunggook; Padeste, Celestino; Pieleles, Uwe; Gobrecht, Jens *Nanotechnology* (2005), 16(5), S171-S175.
- 8 Uptake and fate of surface modified silica nanoparticles in head and neck squamous cell carcinoma** Gyenge, Emina Besic; Darphin, Xenia; Wirth, Amina; Pieleles, Uwe; Walt, Heinrich; Bredell, Marius; Maake, Caroline *Journal of Nanobiotechnology* (2011), 9, 32.