

**POLYMER NANOCONTAINERS FOR BIOMEDICAL APPLICATIONS**S.Benito<sup>1</sup>, A.Graff<sup>1</sup>, R.Stoenescu<sup>1</sup>, P.Broz<sup>2</sup>, C.Saw<sup>2</sup>, H.Heider<sup>3</sup>, S.Marsch<sup>2</sup>, P.Hunziker<sup>2</sup> & W.Meier<sup>1</sup><sup>1</sup>Dept. of Chemistry, University of Basel, Switzerland. <sup>2</sup>Medical Intensive Care Unit, University Hospital Basel. <sup>3</sup>Institute for Biochemistry and Genetics, University of Basel.

**INTRODUCTION:** During the last decade self-organization of soft materials has shown to be valuable for the creation of a wide variety of nanostructures that could be used for applications in fields ranging from materials science to biology.

In this context amphiphilic block copolymers are of particular interest due to their ability to self-assemble in aqueous media and their broad accessibility to different length and time scales and levels of interaction. Similar to conventional low molar mass surfactants they may form micelles, vesicles or lyotropic mesophases. These aggregates can be significantly more stable than those formed by low molar mass amphiphiles and additionally they can be further stabilized by a subsequent crosslinking polymerization<sup>1</sup>. The long-term stability of these structures makes them well suited for applications and guarantees a constant nonchanging environment for embedded therapeutic or analytic molecules. Moreover, block copolymer chemistry allows introducing easily additional design criteria, like targeting moieties, temperature- or pH sensitivity<sup>1</sup>.

**RESULTS & DISCUSSION:** Here we designed functional block copolymer nanocontainers and tested their potential as generic carriers that can be addressed to specific targets. In particular, we were interested in their receptor- and cell- specific binding and uptake by cells. The nanocontainers were labeled with fluorescent dyes and functionalized on their outer surface with polyguanylic acid (Poly-G) a specific ligand to the scavenger receptor A1 that is found on macrophages, a cell line that plays a major role in infection, autoimmune diseases and cancer.

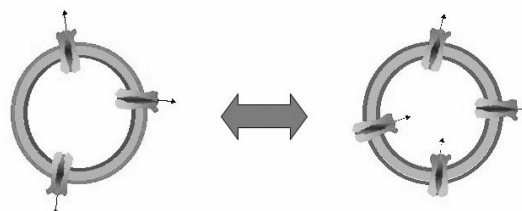


Fig. 1: Scanning Electron Microscopy analysis of endocytosis-inhibited COS-7 cells showing surface bound nanocontainers with diameters of approx. 200 nm.

In the experiments, macrophages derived from the human monocytic THP-1 cell line and the simian tumor cell line COS-7 were used as target cells expressing SRA1. The ligand-bearing nanocontainers showed strong receptor specific binding. Surface-bound nanocontainers were rapidly taken up by the cells through an active process, most probably the endocytotic pathway, without leading to discernible cytotoxicity. In strong contrast, no binding and uptake is observed in cells devoid of the target receptor. Macrophages are unable to bind and to take up those nanocontainers nonspecifically targeted against them, thereby reducing the common problem of conventional targeting approaches, namely, unspecific elimination of carriers by the macrophage system. Thus synthetic polymer nanocontainers appear very promising as novel, target-specific carriers for diagnostic or therapeutic agents.

The walls of the block copolymer containers are formed by membrane-like superstructures that can be regarded as mimetics of biological membranes. Recently, we were able to show that despite their enormous thickness and stability such block copolymer membranes can be used as a matrix for functional reconstitution of membrane proteins<sup>2,3</sup>. This allows an additional control of the exchange of material and provides new interactions between nanocontainers and biological structures.

Generally membrane proteins are vectorial molecules with distinct extracellular and a cytoplasmic parts. In biological membranes these proteins have a well-defined orientation that is also a basic requirement for their function. However, during their isolation from cells and subsequent incorporation into a new artificial membrane system usually the information about their orientation is lost, and the membrane proteins are inserted randomly without any preferred direction. Unfortunately many potential technical applications (e.g., biosensors) of such reconstituted systems depend on a correct and uniform orientation of the membrane proteins.



Asymmetric membrane - directed insertion

Symmetric membrane - random insertion

Fig. 2: Directed vs. random protein insertion in ABC and ABA triblock copolymer vesicles.

Amphiphilic block copolymers offer here a particularly interesting approach to overcome this problem by breaking the symmetry of the membranes. For that purpose we recently synthesized a new type of an amphiphilic ABC-triblock copolymer with a water-soluble poly(ethylene glycol) block A, a hydrophobic poly(dimethylsiloxane) block B and again a water-soluble poly(2-methyloxazoline) block C<sup>4</sup>. For certain hydrophilic-to-hydrophobic block length ratio these polymers also form membrane-like superstructures and nanometer-sized vesicles in aqueous media. It is well known that different water-soluble polymers are inherently incompatible and undergo phase separation in aqueous media. Hence, membranes and walls of nanocontainers formed by ABC triblock copolymers are asymmetric: one side is predominantly covered by the blocks A and the other by the blocks C<sup>4</sup>.

As a model system to investigate the insertion of proteins into such ABC type membranes we reconstituted Aquaporin 0 labeled with a His-Tag unit on its cytoplasmic side into the walls of ABC

block copolymer nanocontainers. Binding studies with fluorescently (Alexa Fluor 555) labeled monoclonal Anti-His antibodies clearly showed that in contrast to 'symmetric' block copolymer and lipid membranes where the insertion occurs randomly (i.e., 50:50 'physiological' to 'non-physiological orientation'), in asymmetric ABC block copolymer membranes 80% of the proteins have a 'physiological' orientation with the His-Tag toward the inside the nanocontainers.

**REFERENCES:** <sup>1</sup>C. Nardin and W. Meier (2002) *Rev. Mol. Biotechnol.* **90**: 17-26. <sup>2</sup>C. Nardin, M. Winterhalter and W. Meier (2002) *Angew. Chem. Int. Ed.* **39**: 4599-4602. <sup>3</sup>A. Graff, M. Sauer, P. van Gelder and W. Meier (2002) *Proc. Acad. Sci. (USA)* **99**: 5064-5068. <sup>4</sup>R. Stoenescu and W. Meier (2002) *Chem. Commun.* 3016-3017.

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