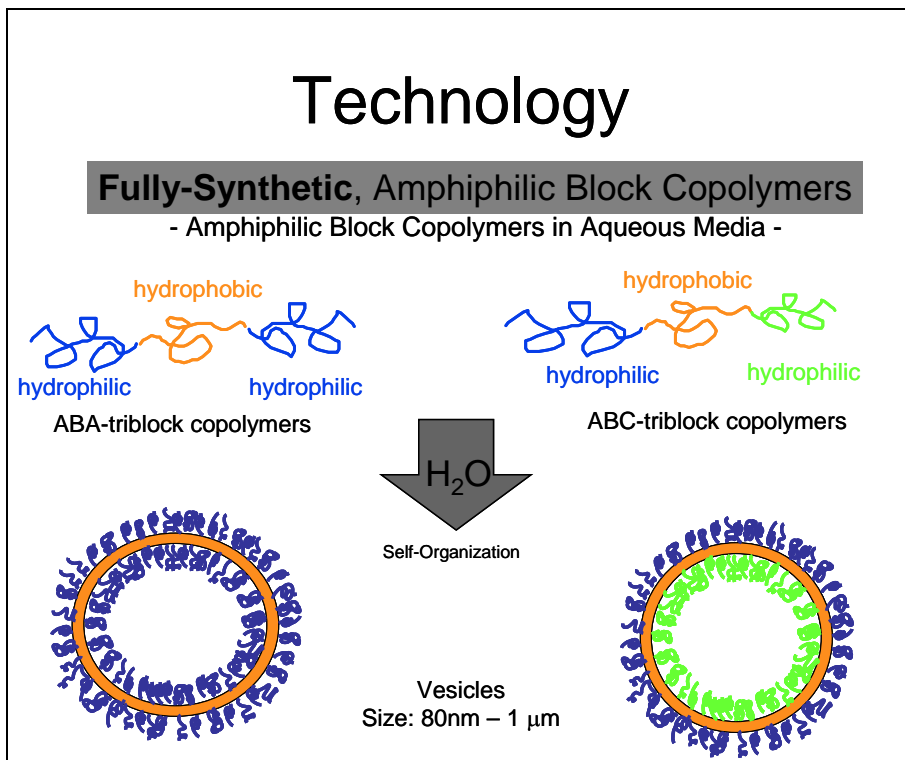


## Polymeric Vesicles

Polymeric vesicles from block copolymers that spontaneously form in water or saline have been developed since the late 90'. The development was mainly driven by academic groups in North America and Switzerland.

BioCure is looking at the commercialization of this technology in the area of drug delivery and in-vitro diagnostics. BioCure is synthesizing their own block copolymers for the formation of vesicles and nanocontainers in the range of 80-800 nm. Molecules, such as membrane proteins, can be incorporated into the wall of the vesicles in order to allow diffusion of molecules. So called nanoreactors can be used for a prodrug therapy or as amplification schemes for in-vitro diagnostics.



**Figure 1: Self-assembly of block copolymers into vesicles**

**Volume that can be encapsulated with 1 g of polymer and different vesicles sizes:**

Inner radius of vesicle [nm]	Encapsulated Volume [ml]
90	2.7
150	4.7
200	6.3
500	16.3

### Vesicles have a unique combination of properties

- Large hydrophobic and hydrophilic compartments
- Inherent stealth properties
- Flexibility in design
- Nanoreactors (Channel proteins in membrane + active enzymes inside)
- Stable vesicles compared to liposomes
- Crosslinkable shell
- Synthetic (non-biological) building blocks for the nano-vesicles
- High volume to mass ratio
- Indications that the vesicles can survive the endosome and release their content in the cytosol

### Property-Application Matrix

Property	Advantages	Application
High stability	Encapsulation of very toxic drugs; protection of the load from the environment, long shelf-life	Cancer therapy, Enzymes, Proteins, Extended release times, Gene and RNA delivery
Flexibility in Design	Versatile Technology; adjustable to needs	Many
Circulation Time	Improved efficiency for targeting, prolonged release	Targeted therapies
Triggered Release	Release when desired (possible)	Controlled release at site, release time is adjustable
Large hydrophobic and hydrophilic compartment	Multidrug therapy	Cancer
High loading	Effect	Many
Protein encapsulation	Nanoreactor concept	Enzyme deficiencies, triggered release/action, e.g. diabetes
Synthetic materials	Mild immune reaction	In vivo
Reproducibility	Manufacturing	All
Targeting	Different coupling groups	Many

### Drug Delivery

Hollow polymeric vesicles form upon self-assembly of amphiphilic block copolymers. The higher molecular weight of the block copolymers compared to lipids gives the vesicles a higher stability while allowing the encapsulation of water soluble active molecules in the lumen or hydrophobic active molecules in the shell. The high flexibility in the choice of polymer segments makes this

technology highly tunable for the different needs in drug delivery applications. 1) **Stability:** The high stability is provided by the length of the hydrophobic segment and the hydrophobicity of the segment. The middle block of the triblock copolymer has to be more hydrophobic than the outside blocks in order to produce stable vesicles. We can either use PDMS, Polybutadien or degradable segments such as PLA and PCL. The delivery device has to survive blood contact, the mucosal layer, the endosome, the lysosome and release its content only after it has been released to the cytosol. The shell should also be resistant to protons for a period of a few hours in order to protect the payload, e.g. siRNA, from the lower pH in the endosome/lysosome. *In-vitro* studies have also shown that the vesicles from non-degradable block copolymers fall apart once they have been taken up by cells. The possibility to use neutral, non-charged carriers could be an advantage with respect to stability, release and toxicity. 2) **Targeting:** Targeting is another important aspect for which linkers can be attached to the surface of the vesicles or to the block copolymer before self-assembly. 3) **Clearance:** Clearance out of the body after the release of the active is another important issue that needs to be considered. Degradable hydrophobic segments are the first logical choice here. Poly( $\gamma$ -methyl- $\epsilon$ -caprolacton) is an ideal segment, since the Tg is below room temperature and it does not crystallize. 4) **Biocompatibility:** The hydrophilic segment should preferably be composed of poly(2-methyl-2-oxazoline) due to its stability, protein repellent properties, biocompatibility and fast clearance through the kidneys. Toxicological studies with the new hydrophobic, degradable middle-block have not been conducted to this point. 5) **Shelf-life:** Shelf-life is another important point to consider for the design of a commercial product. Hydrolysable systems represent a challenge in this respect, because the final formulations will most probably be stored in a buffered solution.

### Specific applications

#### siRNA delivery - Why a good fit?

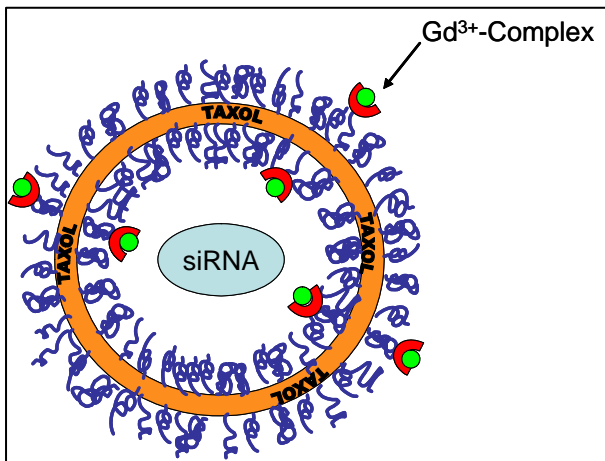
- SiRNA delivery is very complex
- The delivery vehicle needs to fulfill several requirements:
  - Stability
  - Protection from RNAase, pH, in endosome, lysosome
  - Targeting
  - Release in cytosol
  - Non-toxic
  - Cleared out of the body
  - Encapsulation efficiency
- Needs a lot of polymer design input and understanding of cell-biology
- Our system offers a lot of freedom in the design
- Indications from other experiments that most requirements can be fulfilled

### Multidrug Therapy

Hydrophilic molecules and hydrophobic molecules have been incorporated in the vesicles. Blood circulation studies by Discher et al. have shown that the half life

is at least that of stealth liposomes, and *in-vivo* and *in-vitro* results have shown that targeting can work. Discher *et. al.* have also shown that two chemotherapeutics can be delivered at the same time. Studies by Hunziker *et. al.* indicate that the payload is only delivered in the cytoplasm and that vesicles can survive the endosome, which will be important for efficient silencing with siRNA.

BioCure's proprietary PMOXA-based self-assembling nano-vesicle technology is ideal for development of multifunctional delivery vehicle, e.g. siRNA/paclitaxel.

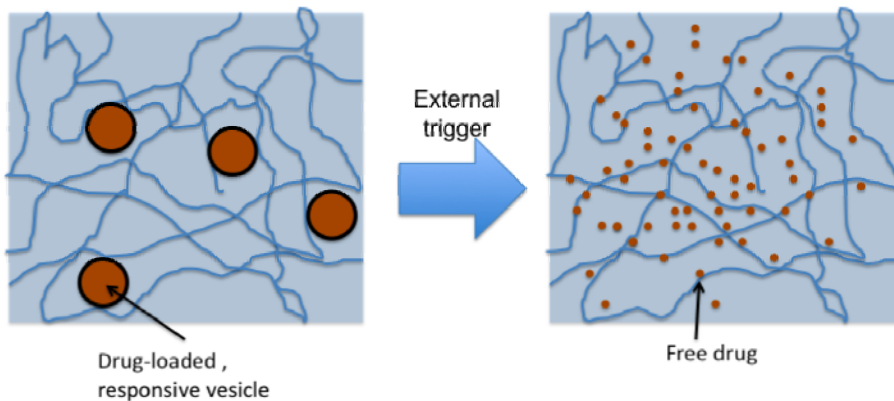


Additionally, surface modification with ligands to, e.g., chelate ( $Gd^{3+}$ ) ions for MRI contrast enhancement or ligands to target the delivery of the vesicles. The loading of the hydrophobic shell compartment provides close to 100% efficiency. The hydrophilic molecules like siRNA are encapsulated in the neutral core will be released after rupturing of the vesicles rather than electrostatic decomplexation, as seen with cationic polymers like poly(ethyleneimine) or chitosan.

**Figure 2: Scheme for multifunctional vesicle**

### Drug Delivery from hydrogels

Here we suggest to incorporate drug loaded nanosized vesicles into a hydrogel. The vesicles provide nanocompartments for storage and controlled release of drugs within the hydrogel. In addition the mechanical properties, degradation times or moisture handling properties of the hydrogel matrix can be adjusted independently of the drug release profile, because the release is only controlled by the vesicles. The release from vesicles will also be independent of the drug



properties, and can be triggered by external (physiologic) stimuli and thus make it a very versatile technology with a large market potential.

**Figure 1: Schematic representation of triggered drug release from block copolymer vesicles entrapped in a hydrogel.**

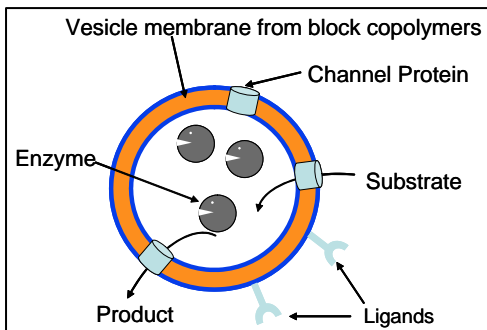
## Diagnostic

### Biosensors - Immunoassays

Hand-held biosensors and Point of Care (POC) diagnostics are emerging technologies promising many benefits for the healthcare system. An important precondition for success is the combination of an increase in sensitivity with an 'easy to use' device. This can be achieved with a system that provides signal amplification and a reduction of the background noise through the use of Nanoreactors. The detection limit should be decreased from the current mM (i.e. glucose sensor) to the pM range in order to allow diagnostics of cancer and other major diseases.

#### Examples

It is possible to develop a generic, hand-held diagnostic device for the parallel assessment of the most common markers, such as prostate specific antigen (PSA), CA15-3 (breast cancer), or CA125 (ovarian cancer) based on glucose oxidase, or other oxidoreductases, loaded nanoreactors. The specificity of the sensor is the same as for ELISA (Enzyme-linked Immunosorbant Assay) since the same combinations of antigen and antibodies can be used. The sensor design will depend on the properties of the nanoreactors with respect to amplification, non-specific binding and reaction times. In a next phase, we are interested in proving our hypothesis by addressing four key issues with respect to the nanoreactors: reaction kinetics, amplification, non-specific binding and stability. In a second phase the device has to be build and validated.



**Figure 4: Nanoreactor concept.**

The preliminary study between ETH (internally financed), University of Basel and BioCure has shown that we can make nanoreactors with glucose oxidase that bind to surfaces and generate an electrochemical signal through glucose conversion that is proportional to the concentration of a model analyte in the solution. The excellent low non-specific binding property of the nanoreactors has also been confirmed and sub pM sensitivity was already demonstrated in a model assay on a Quartz Crystal Microbalance.

Figure 4 shows a schematic nanoreactor that contains all the essential elements required for the sensing and amplification. The membrane is formed from triblock copolymers, the channels are constituted from natural channel proteins and the lumen holds active enzymes. The channel proteins allow diffusion of certain molecules (e.g. substrate, product, and mediator) in and out of the vesicle and the enzymes convert these molecules into a signal that can be detected by the sensor. The vesicle membrane protects the enzymes from the environment and holds them in one location.

Based on our feasibility results, we assume that if a high enough number of enzymes will be encapsulated in the nanoreactors a signal amplification of  $10^3 - 10^4$  can be achieved. The number of channel protein OmpF (outer membrane protein f) in the nanoreactor shell or the porosity has to be sufficient to assure that they are not the diffusion controlling moiety. According to literature, this is achievable. The reduction of non-specific binding needs to be tested against an available reference and should be reduced by a factor of at least 100. In total this will result in an improvement of the S/N of about  $10^6$  over the current hand-held glucose sensor.

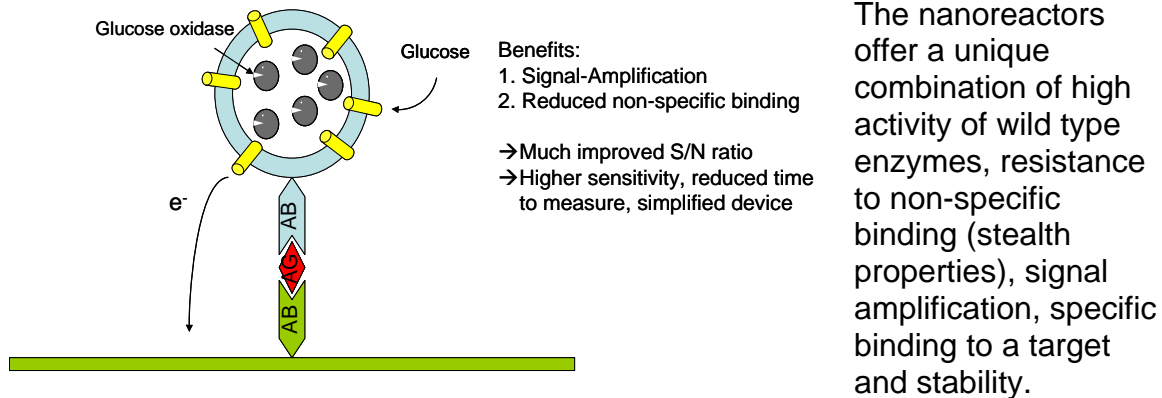


Figure 5: Schematic drawing of sensor concept: a standard sandwich assay extended with the nanoreactor amplification system.

### **Amphiphilic Self-Assembling Nanocapsules and Planar Membranes**

Amphiphilic block copolymers can also form planar membranes. The copolymers are formed into planar membranes through self-assembly methods. Planar membranes can further be stabilized by crosslinking of the copolymers to form a stabilized planar membranes.