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Complete List of Authors:	Mikhalevich, Viktoria; University of Basel, Department of Chemistry Craciun, Ioana; Universitat Basel Departement Chemie Kyropoulou, Myrto; Universitat Basel Departement Chemie Palivan, Cornelia; University of Basel, Chemistry Department Meier, Wolfgang; University of Basel, Department of Chemistry

Amphiphilic peptide self-assembly: Expansion to hybrid materials

*Viktoria Mikhalevich †, Ioana Craciun †, Myrto Kyropoulou †, Cornelia G. Palivan †, Wolfgang Meier †**

† University of Basel, Department of Chemistry, Klingelbergstrasse 80, 4056, Basel, Switzerland

Abstract

The design of functional systems with sizes in the nanometer range is a key challenge in fields such as biomedicine, nanotechnology or engineering. Some of the most promising materials nowadays consist of self-assembling peptides or peptide-polymer hybrid materials because of the versatility, and the resulting properties, which can be achieved with these structures. Self-assembly of pure amphiphilic peptides or in combination with block copolymers results in a large variety of nanostructures (micelles, nanoparticles (NPs), compartments, planar membranes) each with different characteristics and tunable properties. Here, we describe such novel peptide- or peptide-polymer-based supramolecular nanostructures, and emphasize their functionality and various promising applications.

Keywords: vesicles, membranes, peptide-polymer conjugates, peptide nanostructure

1. Introduction

Peptides possess unique properties, such as bioactivity and good biocompatibility, and serve as the major molecular assemblies and scaffolds in nature at the nano-scale, micro-scale, and macro-scale.[1] There, the peptide building blocks serve as scaffolds for different bio-assemblies including collagen, keratin, pearl and actin.[2] Although there are only 20 natural amino acids, the variety of combinations results in an amazing diversity in material properties and functionalities. The self-assembly process is mediated through non-covalent interactions including hydrogen bonding, van der Waals, electrostatic, and stacking interactions. [3] Certain thermodynamic and kinetic conditions are leading to the self-assembly of peptides. These mechanisms can be explained, for example by multiscale molecular simulations, showing dynamics of hierarchical self-assembly from the molecular to mesoscale level.[4] The ability of the peptides to form specific secondary structures helps to design nanomaterials with controllable structural features. Taking advantage of intrinsic properties of natural peptides, a huge number of peptide-based building blocks, such as cyclic peptides (CPs), amphiphilic peptides, copolypeptides, surfactant-like oligopeptides, dendritic peptides, and aromatic dipeptides, have been developed for different supramolecular structures, and their possible applications in biology and nanotechnology are explored.[5,6-7] Their key advantages include biocompatibility, biodegradability, and flexibility. Particularly appealing are the amphiphilic peptides because they can self-assemble into supramolecular structures, such as nanotubes (NTs), NPs, vesicles, and micelles, depending on their chemical and physical properties (sequence, charge, size etc).[8-11] Such supramolecular

assemblies have been proposed for carrier-mediated drug delivery, tissue engineering, biomineralization, molecular imaging and membrane protein stabilization.[12, 8, 13-14]

The sequence of amphiphilic peptides is organized in two regions-the hydrophobic and the hydrophilic part. The hydrophilic part is usually composed of amino acids with hydrophilic side chains, such as lysine or glutamic acid, whilst the hydrophobic part can consist of amino acids with non-polar side chains, such as tryptophan or valine.[15] A special case are the so called "peptide amphiphiles", in which the hydrophobic part consists of an alkyl chain attached to a biofunctional peptide epitope.[16] These conjugates are able to self-assemble into high-aspect-ratio cylindrical nanofibers upon triggering via light, pH, ions, and enzymes.[17]

Another way to expand on the potential of peptide-based structures is by using a combination of peptides and synthetic polymers to obtain functional hybrid materials. The combination between these two families of compounds is a useful tool, where the selective and yet diverse nature of peptides is enhanced by the mechanical stability and robustness of synthetic polymers. In order for these novel hybrid materials to reach their full potential, the functionality of the peptides should remain unaltered during the polymer-peptide assembly. If this requirement is fulfilled, then new opportunities for applications in various fields open up. In this review, we will focus on some important nanostructures of self-assembled peptides and peptide/polymer hybrid systems. We will also emphasize different properties and applications, such as biomedical or filtration membranes.

2. Self-assembled amphiphilic peptide nanostructures: NPs, micelles and vesicles

Self-assembling peptides are synthesized via solid-phase peptide synthesis, or recombinantly produced in bacteria. The sequence of amino acids that make up the peptide, as well as the

choice of self-assembling process, all together determine the types of nanostructures that can be obtained.[11, 18-19] Peptides rely on non-covalent binding interactions between amino acid sequences to form ordered secondary structures, such as β-sheets, where the peptide chains are aligned laterally and held in place by back bone hydrogen bonding interactions between the chains, and α-helices, where the peptide chain adopts a spiral conformation again held in place by back bone hydrogen bonding interactions along the peptide sequence. Self-assembly of peptides into various nanostructures is promoted by designing amphiphilic peptides or by incorporating aromatic amino acids such as tryptophan and phenylalanine that can form non-covalent binding interaction via π-stacking.[20-21] These aromatic amino acid sequences can be further coupled to hydrophilic amino acids to afford self-assembling amphiphilic peptides. In this section, we focus on recent developments and applications of self-assembled amphiphilic peptide NPs, micelles and vesicles.

Self-assembled peptide NPs

Self-assembled spherical peptide NPs, if smaller than 100 nm in diameter, are particularly amenable for biomedical applications because they are readily uptaken by cells. NPs can load hydrophobic drugs within their inner core during the self-assembly process thus making them ideal candidates for drug delivery systems.[22-23] One such self-assembling peptide is EAK16-II (amino acid sequence: n-AEAEAKAKAEAEAKAK-c) that formed NPs of approximately 100 nm in diameter and successfully encapsulated a hydrophobic anticancer agent ellipticine.[24] These small NPs were readily uptaken by A549 lung carcinoma cells and exhibited high cytotoxicity. Also, by changing the peptide to hydrophobic drug ratio, NPs in the micron range were obtained, however due to their large size they had a significantly lower uptake and related cytotoxicity.

Such NP systems can be designed to be responsive to various biologically relevant stimuli such as pH, redox potential, metal ions or even specific enzymes resulting in release of the encapsulated cargo. [25-27]

Peptide sequences that form helical coiled-coil motifs can also be self-assembled into peptide NPs. In one such example, a peptide that forms two helical coiled-coil motifs, with a short linker region in between the two helices, was meticulously studied to determine the effect of point mutations in the linker region on the self-assembling process.[28] Studies that use *in silico* modeling of peptide sequences combined with *in vitro* testing to optimize peptide length, and the overall self-assembly into small peptide NPs, aid in the global understanding of the peptide self-assembling process.

Antimicrobial peptides, most often containing sequences of positively charged arginine and lysine residues, have been self-assembled into peptide NPs as well.[29-30] Antimicrobial peptides can be highly toxic towards not only bacteria, but also normal cells; however, self-assembling into various nanostructures such as NPs, can result in more selective peptides because the self-assembling process alters the charge distribution and secondary structure of the peptide.[31] A major issue still surrounding such peptides is their susceptibility to inactivation by proteases. One way of overcoming protease degradation can be achieved by tethering the antimicrobial peptides to a substrate such as gold NPs.[32] It was shown that the antimicrobial peptides maintain their activity and have increased stability towards trypsin digestion. Depending on the self-assembling properties of the peptide tethered to the gold NP, they can further assemble into NP superstructures.[33]

Self-assembled peptide micelles

Amphiphilic peptides can also self-assemble to form micelles that contain a hydrophobic inner core and hydrophilic exterior corona. Peptide amphiphiles that contain a charged head group conjugated to a long hydrophobic tail were self-assembled to form micelles.[30, 34] Such micelles, similarly to NPs, have been explored for applications as drug delivery systems for hydrophobic drugs or imaging agents, and have been shown to have low toxicity *in vivo* and can be cleared rapidly through the renal system.[35]

Apart from longer peptide amphiphiles, peptides containing aromatic residues, such as truncated gramicidin A (gA) sequence composed of L-tryptophan-D-leucine repeating units, self-assemble into micellar structures. These peptides can be coupled via a reduction sensitive linker to a hydrophilic histidine rich domain to obtain stimuli-responsive NPs (Figure 1).[36] The amphiphilic peptides first assembled into micelles, entrapping doxorubicin (DOX), an anticancer drug, within the hydrophobic domain and antisense oligonucleotides along their hydrophilic outer core. Secondly, the micelles self-assembled into spherical NPs, termed peptide beads, of 100-200 nm.[33, 37] These peptide beads can not only deliver both hydrophobic and hydrophilic payloads but can also selectively release them upon a physiological trigger, such as the reductive environment surrounding tumors.

Peptide micelles can also be decorated with functional peptide motifs, targeting ligands as well as epitopes to the ends of the peptide thus providing selectivity for specific cell types.[38] By incorporating these motifs onto self-assembling peptides, their affinity for select targets is enhanced and their application can be expanded. Thermally responsive elastin-like peptides have been conjugated to tumor targeting recombinant Llama heavy-chain antibody fragments (VHHs) against epidermal growth factor receptor (EGFR).[39] Alongside the targeting VHHs', a photosensitizer was also linked to the elastin-like peptide. When heated, the elastin-like peptides

assembled into micelles exposing both the targeting VHHs and the photosensitizer. Upon illumination, cells expressing EGFR are selectively killed. This example illustrates the versatility that can be obtained through the use of self-assembling peptides to obtain both stimuli-responsive as well as target selective nanostructures.

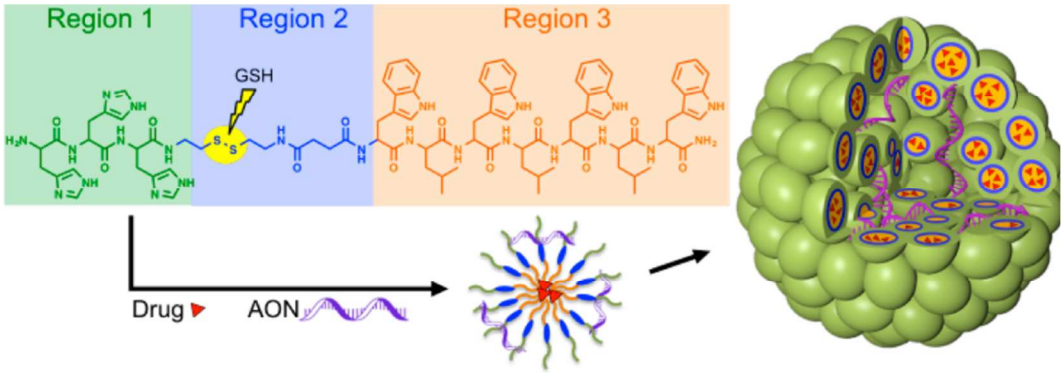

Figure 1. Self-assembly of amphiphilic peptides containing a histidine rich hydrophilic domain (region 1) connected by reduction sensitive linker (region 2) to a tryptophan rich hydrophobic domain (region 3). A two-step self-assembly can occur: first, the peptides assemble into micelles loaded with DOX within the inner hydrophobic core and a nucleotide in the hydrophilic exterior; secondly, the micelles cluster together to form multicompartment micelles. Reprinted from ref. 36. Copyright 2016 American Chemical Society.[29] Originally adapted from ref. 40 with permission of The Royal Society of Chemistry. http://dx.doi.org/10.1039/c4bm00230j [40]

Self-assembled peptide vesicles

Apart from NPs and micelles, amphiphilic peptides can self-assemble into vesicular structures as well. Peptide vesicles are versatile candidates for designing drug delivery systems, as active molecules can be encapsulated within the inner core of the vesicles or inserted in their membrane.[41] One approach to generate peptide vesicles is by using lipid-like peptides where the peptide chain contains a charged head group connected to a long hydrophobic tail. The charged

head group can be either positively charged via incorporation of positively charged lysine residues or negatively charged via the incorporation of negatively charged aspartic acid residues. The hydrophobic tail contains non-polar residues such as repeating alanine residues. In a recently published study, such peptides were employed as sustained release drug delivery systems.[42]

Another approach to form vesicular assemblies is based on short water-soluble dipeptides. An interesting architecture obtained consists of a multicompartment architecture in which small peptide vesicles are encapsulated within a larger one.[43] These multicompartment structures were shown to be pH stable but could disassemble in the presence of calcium ions releasing both an encapsulated dye and cyclic adenosine monophosphate. While these mutivesicular structures were pH stable, other peptide vesicles are designed to assemble and disassemble based on changes in pH. For instance, recombinant peptides containing glutamic acid residues that form a hydrophilic domain followed by a sequence of alanine, valine and leucine residues that comprise the hydrophobic domain of the oligopeptide, were shown to self-assemble into vesicles at pH values above 5.[44] At pH 4 these vesicles disassembled and formed aggregates, however neutralizing the pH reversed this process and the peptides once again reassembled into vesicles.

Peptide-based drugs have gained increased momentum in the past couple of years, with more and more hitting the market and even more undergoing clinical trials. While still in the developmental stage, self-assembled peptide NPs, micelles and vesicles have shown great promise for applications as drug delivery systems, nanoscaffolds for tissue engineering, and diagnostic probes.[9, 45] Preparing large quantities of peptides can be quite expensive and obtaining the desired final nanostructure is not always guaranteed when working with synthetic peptides. One way to improve upon these systems is to incorporate self-assembling peptides into polymeric structures, thus obtaining more stable and multifunctional hybrid materials.

3. Peptide-Polymer conjugation: synthesis and applications

Hybrid materials based on the combination of peptides with copolymers takes advantage of the polymer science that is able to provide well-controlled structures, down to the molecular level, and multiple chemical functionalities.[46] Polymers have exceptional properties regarding stability, they are able to exhibit stimuli responsiveness and some of them, amphiphilic copolymers, are able to self-assemble in different nano-structures and mimic biological materials. One of the most important features of such amphiphilic block copolymers is the ability to self-assemble into mono- and bilayers. The bilayer membranes can appear in different shapes, such as planar or three-dimensional vesicular structures.[47-48] Diblock copolymers (AB), symmetric triblock copolymers (ABA) or asymmetric triblock copolymers (ABC) are used to self-assemble into a large variety of supramolecular assemblies. Note that usually when we discuss about amphiphilic polymers A and C stand for the hydrophilic domains, and B for the hydrophobic domain.

The chemical conjugation of peptides to polymers, or as commonly called the functionalization of polymers with peptides, leading to the self-assembly of various nanostructures takes place mainly via a specific type of chemical reaction.[49] The so-called "click" reaction is the most common way to achieve the conjugation of a peptide to a synthetic polymer.[50] The most important parameters for these reactions are: i) a high yield ii) mild beginning conditions (aqueous solution, room temperature, physiologic pH), and iii) small amounts or byproducts. Apart from the classic approach, which is the use of Copper-catalyzed azide-alkyne cycloaddition, many other types of "click" reactions have been reported, as for example strain-promoted azide-alkyne cycloaddition, thiol-ene reaction, Diels-Alder reaction, oxime ligation.[51] Depending on specific "click" chemistry, the reaction conditions for peptide-polymer conjugates

vary accordingly. The main bio-applications of peptide-polymer conjugates that self-assemble into supramolecular assemblies, are drug and gene delivery systems, and tissue regeneration.[52]

A recent example of a nano-sized drug-delivery system is based on the conjugation of angiopep2 peptide with poly(dimethylsiloxane)-poly(2-methyloxazoline) (PDMS-PMOXA) diblock copolymer *via* 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide/N-hydroxysuccinimide coupling chemistry (Figure 2).[53] Angiopep2, is a targeting peptide with 19 amino acids with high binding affinity to the low density lipoprotein receptor-related protein 1 and therefore with the ability to pass the Blood Brain Barrier and target glioma cells. The angiopeg2 functionalized polymer formed polymer vesicles (polymersomes), which served as drug nanocarriers, when loaded with DOX.[54] The presence of angiopep2 peptide triggered the cellar uptake of the DOX-loaded polymersomes, which selectively released DOX into glioblastoma U87MG cells *in vitro*. The polymer membrane (thickness of 16nm) protected the drug, allowing it to diffuse slowly through.

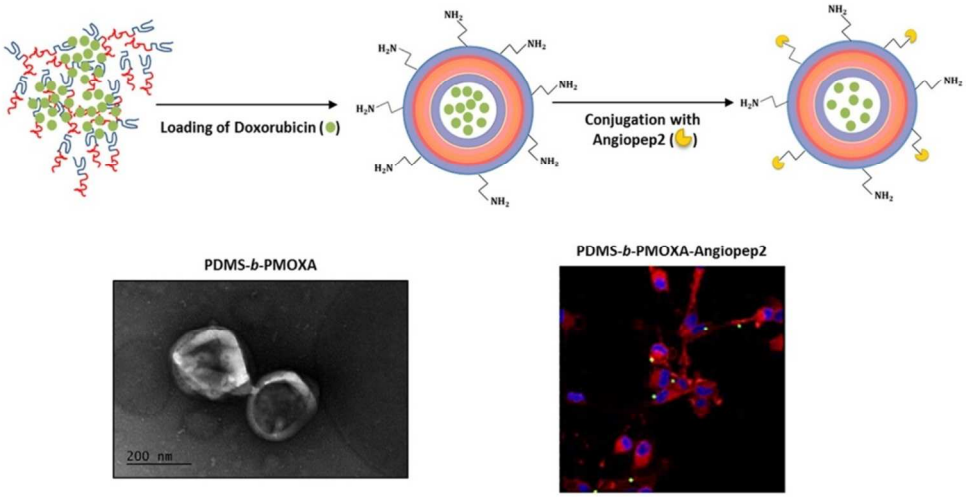

Figure 2. Encapsulation of DOX into peptide-polymer vesicles, functionalized with peptide Angiopep2. Reprinted from ref. 53, with permission from Elsevier.

In another example, polymersomes have been functionalized with peptides for high binding specificity to colon cancer cells.[55] Polymersomes based on block copolymer poly(ethylene oxide)-b-poly(1,2-butadiene) were externally functionalized, via an azide-alkyne ‘‘click’’ reaction with a fibronectin mimetic peptide PR_b (with sequence KSSPHSRN$(SG)_5$RGDSP). This conjugation not only promoted the cell uptake, but also led to a controlled delivery of an antitumor drug into colon cancer cells in vitro. A similar concept has been exploited when polystyrene-*block*-poly[L-isocyanoalanine(2-thiophen-3-yl-ethyl) amide] based polymersomes were externally functionalized with cell penetrating peptides (CCPs).[56] CCPs-functionalized polymersomes loaded with either green fluorescent protein or horseradish peroxidase were up taken by HeLa cells, and served to release *in vitro* the encapsulated proteins.

Apart from the conjugation of polymers and peptides, the insertion of peptide-based ion-channels into polymer supramolecular structures represents another strategy to combine, in a functional manner, peptides and polymers. Alamethicin was used to control the ion concentration within copolymer giant vesicles during mineralization of calcium phosphate,[57] whilst the insertion of α-HL into PB-PEO vesicles induced calcein leakage.[58] The functional insertion of gA inside the membrane of poly(2-methyloxazoline)-block-poly-(dimethysiloxane)-block-poly(2-methyloxazoline) PMOXA-PDMS-PMOXA polymersomes served to obtain stimuli-responsive polymersomes that preserve their architecture, whilst their membrane has stimuli-responsive permeability. gA is a short helical polypeptide from Bacillus brevis that forms a transmembrane channel when inserted in lipidic or polymer membranes, and allows the passage of small cations (including H^+).[59] Indeed, successful insertion of gA did not affect the stability and architecture of the polymersomes, and resulted in a rapid influx of protons and monovalent ions through the

membrane (Figure 3 A-C).[60] The change of the fluorescence intensity of 5(6)-carboxyfluorescein encapsulated inside polymersomes after gA insertion, served to prove the functional insertion of these biopores in synthetic membranes that are significantly thicker (thicknesses of the membrane ranging from 9.2 to 16.2 nm) than the size of the gA (Figure 3 D,E). gA has been inserted and remained functional in membranes with a thicknesses of ≤ 13.1 nm, which is more than 4 times higher than the size of gA. This unexpected result is due to the high flexibility of the synthetic membranes based on PMOXA-PDMS-PMOXA, which overcome the mismatch between the pore length and the membrane thickness.

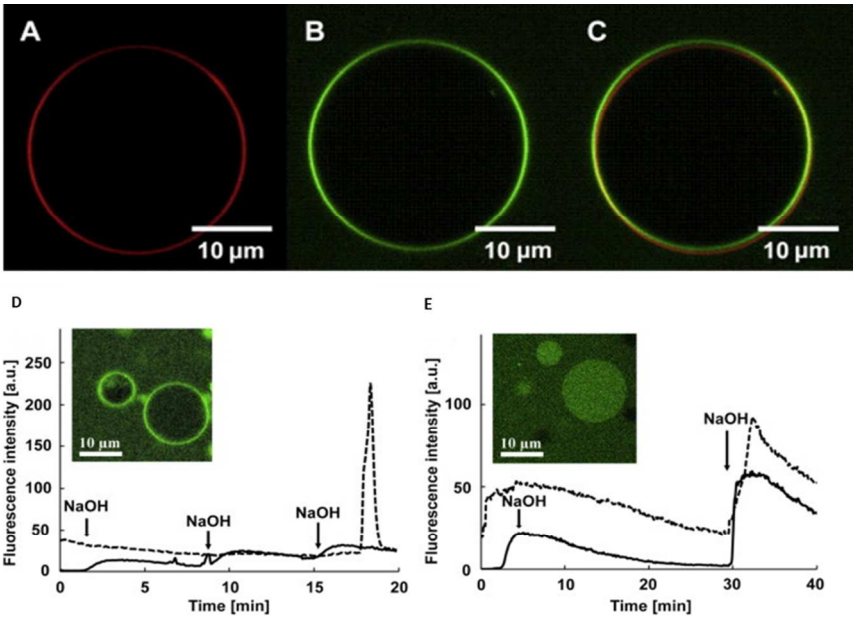

Figure 3. CLSM images of a giant unilamellar vesicle (GUV) composed of $A_7B_{49}A_7$ triblock copolymer in the presence of: (A) Bodipy (red), (B) labeled gA-OG488 (green), (C) Overlay of A and B (red and green). Fluorescent intensity change over time of 5(6)-carboxyfluorescein inside and outside of polymer GUVs (D) in absence and (E) after insertion of gA. Reprinted from ref. 60, with permission from Elsevier.

Another type of polymer-peptide hybrid system concerns NPs based on amphiphilic block copolymers which are grafted with peptides.[61] In particular, proline-valine-glycine-leucine-isoleucine-glycine peptides were conjugated to a biodegradable polymer, poly(trimethylene carbonate) via UV activated "click" thiol-ene chemistry. Such NPs have potential for biomedical applications in targeted cancer therapy because the peptides can be cleaved selectively by the well-known tumor-associated enzyme matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) that appear in very high concentration in aggressive tumors. Similarly, degradable and biocompatible poly(benzyl malate) NPs decorated with biotinylated cyclic RGD peptides, and loaded with the anticancer drug DOX, were efficiently up taken by HepaRG cells. [62] In another example, PMOXA was combined with a cleavable peptide block poly(aspartic acid) (PASP), and finally modified with diethylenetriamine (DET). PMOXA-b-PASP(DET) self-assembled into biodegradable and biocompatible NPs after complexation with plasmid DNA (pDNA), and served to efficiently transfect HEK293 and HeLa cells with green fluorescent protein (GFP) pDNA in vitro.[63]

A different approach to form polymer-peptide materials is based on the integration of CPs within polymer NTs.[64] Eight- amino acid based CPs act as precursors for the polymerization of the polymer N-isopropylacrylamide. The peptide-polymer material self-assembles into hybrid NTs, constructed by a peptide core and a covalently attached polymeric coating. This creates a novel class of non-toxic hybrid NTs with promising application in biomedical technology. In another example, CPs were used in combination with several polymers including poly(butyl acrylate), poly(dimethyl amino ethyl acrylate), poly(acrylic acid), poly(styrene) and poly(hydroxyl ethyl acrylate) for efficient and controlled preparation of peptide-polymer NTs.[65] Later on, a formation of a peptide- polymer NT was reported (Figure 4).[66] In this work, both thiol-terminated polymers

and alkyne-terminated polymers where conjugated with cyclic oligopeptides generating polymer-peptide NTs with potential application as synthetic transmembrane protein channel mimics. The novelty in these particular NTs is that they offer the possibility to self-assemble into either Janus or mixed corona form. They also performed a Calcein dye leakage experiment using large unilamellar vesicles (LUVs) where no leakage of the dye was observed for the conjugates.

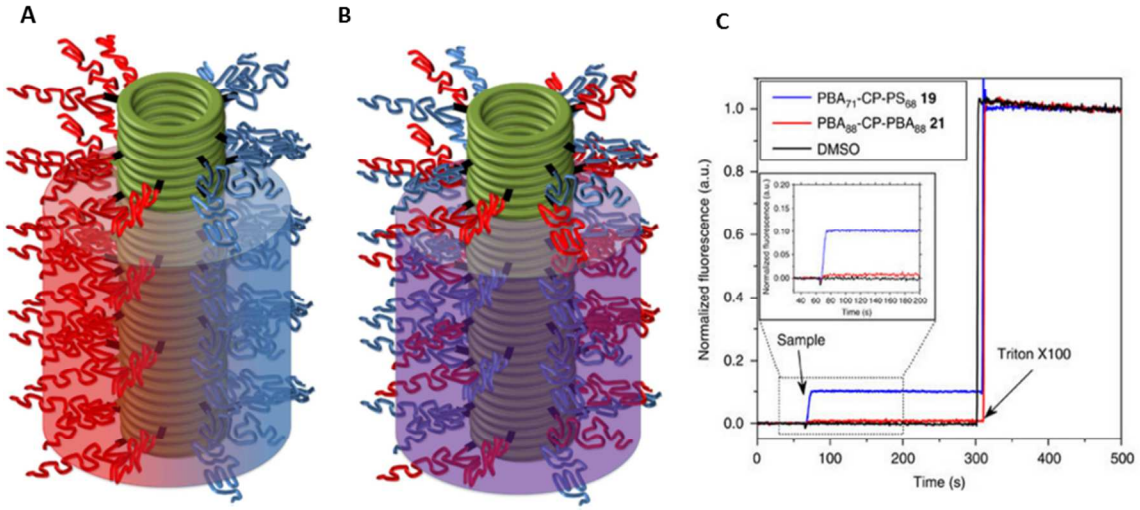

Figure 4. CP–polymer NTs with two corona configurations: (A) Janus assembly with 'demixed' corona. (B) Hybrid assembly with 'mixed' corona (C) Calcein dye leakage experiment using LUVs with 2 μM Janus conjugate. No dye leakage was observed for non-Janus conjugate. Reprinted by permission from Macmillan Publishers Ltd: *Nature Communications*, ref. 66, Copyright 2013. https://www.nature.com/articles/ncomms3780

The exploitation of polymer-peptide self-assembly processes which leads to self-organized supramolecular structures nanostructures (polymersomes, NTs, NPs) opens fascinating and

promising routes in biotechnology, nanotechnology and nanomedicine. At the same time, polymer-peptide conjugates dilate upon more complex structures, such as polymer films, but the functional reconstitution of peptide structures into planar polymer membranes remains highly challenging and there are many parameters yet to be investigated and defined, as the peptides do not appear any more in their natural environment.

4. Reconstitution of peptides in planar polymer membranes

Another strategy to generate peptide-polymer hybrid materials is to integrate peptides into solid supported or free standing planar polymer membranes, which have high potential for applications as bio-sensing platforms. In this respect, pore formation in polymer membranes is in focus by now, due to the advantage of the membrane technology for separation, and more precisely for the transport selectivity of the membrane.[67] Various peptides, such as gA, alamethicin, or melittin are able to form pores in cell membranes, lipid membranes, but also in copolymer membranes.[68-70] The phase behavior of alamethicin, an antimicrobiotic peptide, in planar Langmuir polymer films on the air-water interface was extensively studied in the group of Meier, to gain a deep insight into their physicochemical properties.[71] Films of PMOXA-PDMS-PMOXA triblock copolymers with different lengths were used to insert alamethicin. The mixing properties of this copolymer with peptides resulting on mixed monolayers and the phase behavior were dependent on the length of the copolymer. The monolayer at the air-water interface was compressed and showed progression of the domain growth and their changes in shape (Figure 5). The reason for the changes was that the larger polymer had a greater flexibility and ability to adopt more conformations, and therefore more possibilities to host the peptide.

Additionally, at high surface pressures, the polymer matrix stabilized the peptide and preserved it from collapse.

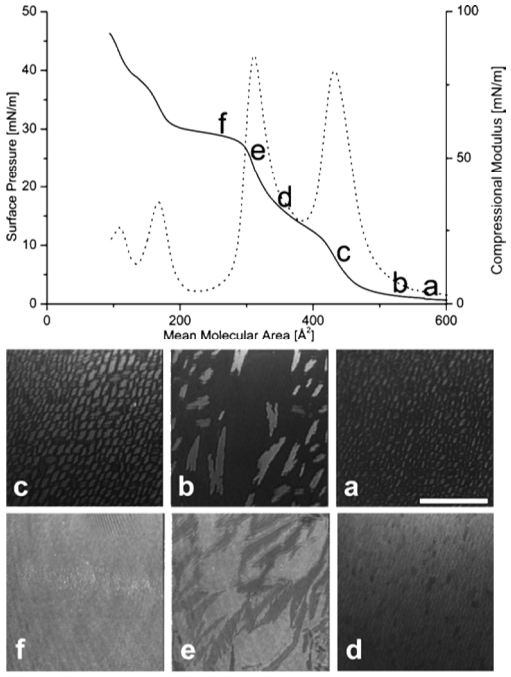

Figure 5. BAM images of a $PMOXA_{13}PDMS_{23}PMOXA_{13}$−alamethicin (molar ratio 0.3:0.7) monolayer at different surface pressures. Reprinted by permission from ref. 71. Copyright 2006 American Chemical Society.

The effect of the polymer environment on the function of incorporated peptides (for example α-hemolysin (α-HL) and alamethicin) was first described using a single molecule transport pore in free-standing membranes (9 and 5.7 nm lengths).[72] Unfortunately, in contrast to alamethicin, αHL was not able to be incorporated into the thick polymer membrane. Interesting, the measured functionality of the incorporated peptides (e.g. conductance) was very similar to their behavior in conventional lipid membranes. When gA peptide was added to polymer membranes, it was able to adopt distinct conformations in different environments: inside membrane arrays made of

PMOXA-PDMS-PMOXA it preserved its functionality indicating a high potential for high-throughput screening applications.[70] In another approach, planar membranes with synthetic AB and ABA block copolymers of PMOXA and PDMS were used to study the reconstitution of alamethicin and gA and compared with their reconstitution in lipid bilayers.[73] Alamethicin was successfully inserted and showed gating properties similar to those found in diphytanoyl phosphocholine lipid bilayers, whilst gA behavior in polymer membranes was different then in lipid bilayers: the conductance was significantly lower, and there was no evidence of conductance-state switching.

An amazing breakthrough in the field was the functional pore formation of peptides in solid-supported planar membranes showed by the Meier group. The functionality of a peptide pore in a solid-supported polymer membrane has been established with α-HL as model.[74] α-HL was functionally incorporated in an artificial polymer, tethered, solid-supported bilayer membrane (TSSBM) based on poly(butadiene) -*block*-poly(ethylene oxide) (Figure 6 A).[75] The copolymer was transferred to a surface by using the so called Langmuir-Blodgett and Langmuir-Schaefer transfer technique, which allows control of layer density by the surface pressure of the molecules. After a surfactant-free insertion of α-HL, the electrical conductance across the membrane indicated that the peptide was functionally inserted and the ion flow through was modeled with a Donan potential (Figure 6 B). Such peptide-TSSBMs can be used for drug screening, trace analyzing, and biosensing.

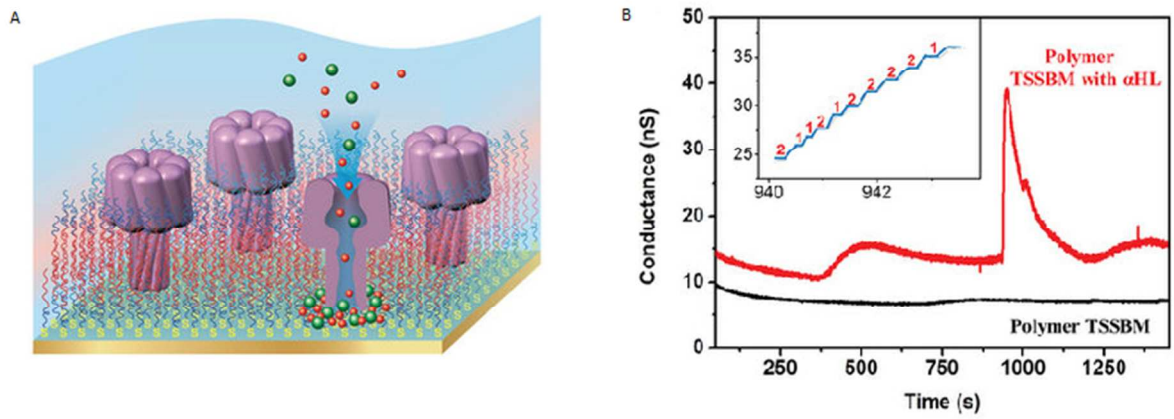

Figure 6. Schematic representation of the TSSBM (A) and conductance measurement across it (B). Reprinted by permission from Macmillan Publishers Ltd: *Scientific Reports*, ref. 75, Copyright 2013. https://www.nature.com/articles/srep02196

The usage of peptides for pore formation in polymer membranes becomes more and more important, because in contrast to the insertion of membrane proteins, no difficult expression and no surfactants are needed for peptides. The field of self-assembled polymer structures with peptides incorporated hold promising applications in the area of highly selective separations, high-throughput screenings or biosensing.

5. Conclusions and future perspectives

Peptidic supramolecular assemblies with sizes in the nanometer range have gained traction in recent years as biomaterials due to the versatility of peptides themselves to self-assemble into various architectures for the design of complex systems, as well as recent advancements in peptide synthesis which enables further research and design of non-naturally occurring peptides. Peptides that can self-assemble into supramolecular architectures such as micelles, nanofibrils,

NTs or vesicles, are of particular interest firstly due to the innate properties of the peptide building blocks regarding biocompatibility and roles in multiple biological systems and secondly, the gained advantages such supramolecular architectures possess, for example stability with retained functionality.

Peptide vesicles and NPs have been shown to be of use in medical applications as drug delivery or imaging tools as they can be loaded with active compounds (drugs, proteins, DNA) or fluorescent probes and selectively accumulate at specific bio-locations, as for example at tumor sites through the enhanced permeability and retention effect. In addition, these peptide-based supramolecular structures can be functionalized with targeting ligands for direct delivery of the payload to the site of interest, where they can be released. A promising new perspective appears to be the fabrication of photosensitive and photo-thermal peptide based hybrid biomaterials with antitumor activity.[76-79] Although self-assembled architectures have improved stability as compared to the peptide building blocks, they lack the robustness that polymeric nanostructures offer. Therefore, peptide-polymer hybrid materials have emerged to address such problems. Peptide-polymer hybrid supramolecular assemblies can offer efficient and targeted drug and gene delivery, while stimuli-responsiveness of the polymeric component makes them interesting candidates for tissue engineering. Furthermore, spherical polymersomes with inserted peptide nanopores allow for *in situ* reactions within the compartments for local production and release of active molecules. Planar polymer membranes can also be used to insert peptides, and understand their functionality in synthetic templates or develop active surfaces for biosensing or ion transport. Depending on the polymer and self-assembly method chosen, each hybrid material can possess unique properties and fulfill numerous functions thus truly expanding on the versatility.

Even though the different materials are showing promising properties, there are still some challenges to be overcome, such as control of the self-assembly process and stability of both the structure of the self-assembled peptides and stability towards degradation by proteases *in vivo*. Another key challenge is still the knowledge of how to obtain full and rational control of the final spatial organization of aggregating peptides at the nanoscale.[80] *In silico* molecular modeling of self-assembling peptides can aid in overcoming this challenge but more studies are needed along with a more cost effective route to obtain large quantities of tailor made peptides. The field of peptide based biomaterials is rapidly expanding and further research is warranted for the development of such novel systems and successful translation into clinical applications.

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Author Information

* Wolfgang Meier, Wolfgang.Meier@unibas.ch, Klingelbergstrasse 80, 4056 Basel, Switzerland

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Abbreviations

α-HL	α-hemolysin
CCPs	cell penetrating peptides
CPs	cyclic peptides
DET	diethylenetriamine
GFP	green fluorescent protein
GUVs	giant unilamellar vesicle
DOX	doxorubicin
EGFR	epidermal growth factor receptor
gA	gramicidin A
LUVs	large unilamellar vesicles
MMP	metalloproteinase
NPs	nanoparticles
NTs	nanotubes
PASP	poly(aspartic acid)
PDMS	poly(dimethylsiloxane)
pDNA	plasmid DNA
PMOXA	poly(2-methyloxazoline)

TSSBM tethered solid-supported bilayer membrane

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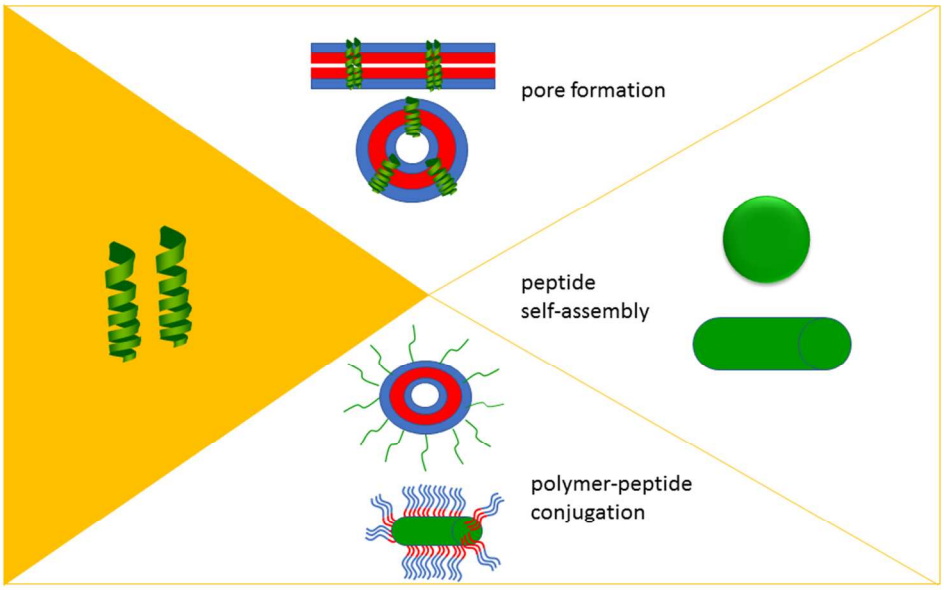
TOC Image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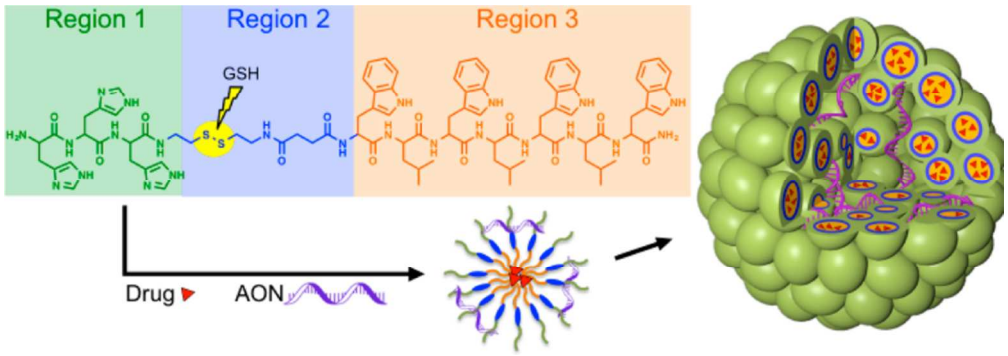

Self-assembly of amphiphilic peptides containing a histidine rich hydrophilic domain (region 1) connected by reduction sensitive linker (region 2) to a tryptophan rich hydrophobic domain (region 3). A two-step self-assembly can occur: first, the peptides assemble into micelles loaded with DOX within the inner hydrophobic core and a nucleotide in the hydrophilic exterior; secondly, the micelles cluster together to form multicompartment micelles

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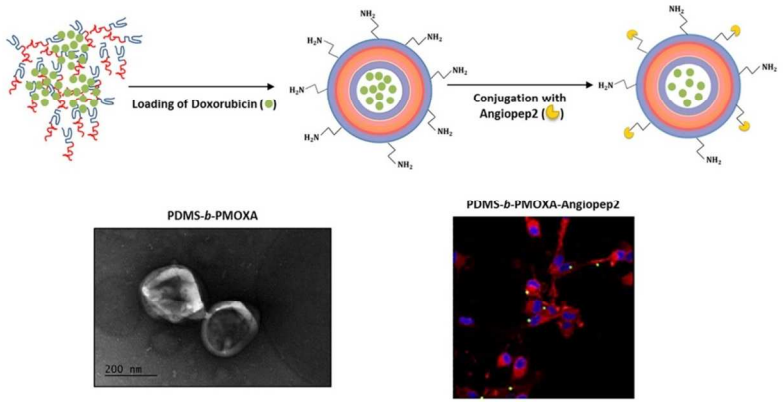

Encapsulation of DOX into peptide-polymer vesicles, functionalized with peptide Angiopep2. Reprinted from "Angiopep2-functionalized polymersomes for targeted DOX delivery to glioblastoma cells"

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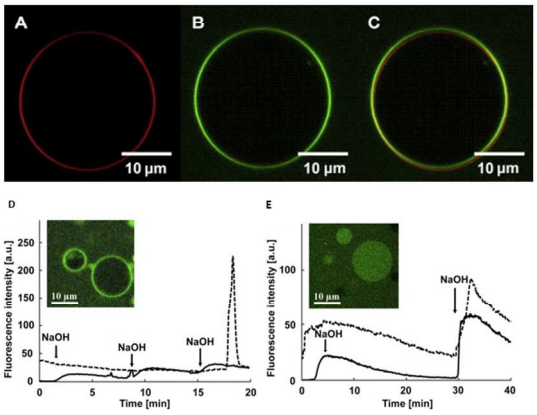

CLSM images of a giant unilamellar vesicle (GUV) composed of A7B49A7 triblock copolymer in the presence of: (A) Bodipy (red), (B) labeled gA-OG488 (green), (C) Overlay of A and B (red and green). Fluorescent intensity change over time of 5(6)-carboxyfluorescein inside and outside of polymer GUVs (D) in absence and (E) after insertion of gA

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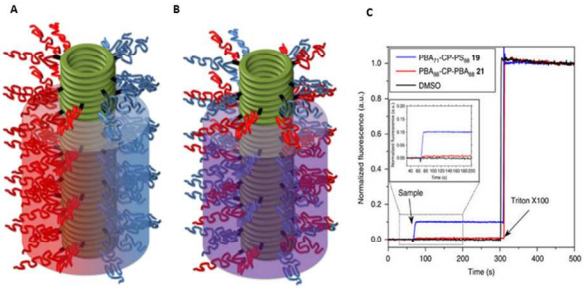

. CP–polymer NTs with two corona configurations: (A) Janus assembly with 'demixed' corona. (B) Hybrid assembly with 'mixed' corona (C) Calcein dye leakage experiment using LUVs with 2 μM Janus conjugate. No dye leakage was observed for non-Janus conjugate

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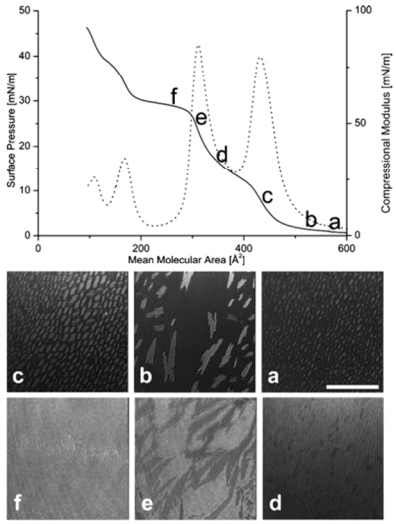

BAM images of a PMOXA13PDMS23PMOXA13–alamethicin (molar ratio 0.3:0.7) monolayer at different surface pressures.

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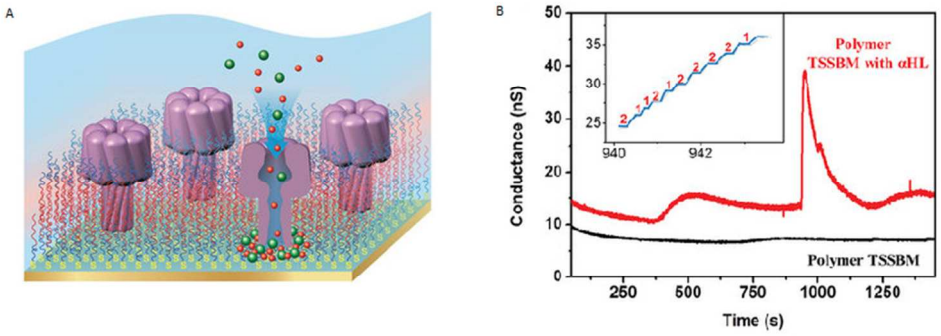

Schematic representation of the TSSBM (A) and conductance measurement across it (B).

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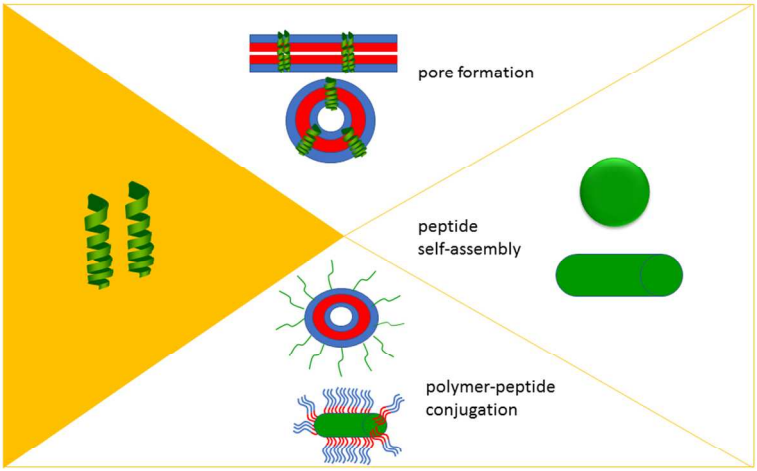

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