J Membrane Biol (2014) 247:861–881
DOI 10.1007/s00232-014-9679-3

Amphiphilic Macromolecules on Cell Membranes: From Protective Layers to Controlled Permeabilization

E. Marie · S. Sagan · S. Cribier · C. Tribet

Received: 1 February 2014 / Accepted: 9 May 2014 / Published online: 6 June 2014

Abstract Antimicrobial and cell-penetrating peptides have inspired developments of abiotic membrane-active polymers that can coat, penetrate, or break lipid bilayers in model systems. Application to cell cultures is more recent, but remarkable bioactivities are already reported. Synthetic polymer chains were tailored to achieve (i) high biocide efficiencies, and selectivity for bacteria (Gram-positive/Gram-negative or bacterial/mammalian membranes), (ii) stable and mild encapsulation of viable isolated cells to escape immune systems, (iii) pH-, temperature-, or light-triggered interaction with cells. This review illustrates these recent achievements highlighting the use of abiotic polymers, and compares the major structural determinants that control efficiency of polymers and peptides. Charge density, sp. of cationic and guanidinium side groups, and hydrophobicity (including polarity of stimuli-responsive moieties) guide the design of new copolymers for the handling of cell membranes. While polycationic chains are generally used as biocidal or hemolytic agents, anionic amphiphilic polymers, including Amphipols, are particularly prone to mild permeabilization and/or intracell delivery.

Keywords Amphiphilic polymers · Amphipols · Antimicrobial and cell penetrating peptides · Cell membrane permeabilization

E. Marie · C. Tribet (✉)
Département de Chimie, Ecole Normale Supérieure, UMR 8640 CNRS-ENS-UPMC, 24, rue Lhomond, 75005 Paris, France
e-mail: christophe.tribet@ens.fr

S. Sagan · S. Cribier
Département de Chimie, Sorbonne Universités - UPMC University Paris 06, École Normale Supérieure-PSL University, CNRS, LBM, 4 Place Jussieu, 75005 Paris, France

Introduction

Controlled perturbation of lipid membranes upon interaction with macromolecules is of enormous importance, both to fundamental studies in membrane biophysics and to practical applications including developments of cost-effective antimicrobial compounds (Munoz-Bonilla and Fernandez-Garcia 2012), design of drug-loaded particles (Hu and Jing 2009; Liechty et al. 2010; Nicolas et al. 2013), or advanced biofunctional capsules (Allen and Cullis 2013; Matile et al. 2011; Torchilin 2012; Yessine and Leroux 2004) that prevail in the currently approved drug-delivery systems. Various water-soluble compounds can be used to affect lipid membranes and cell membranes properties. They generally belong to the class of amphiphilic molecules, having a significant affinity for both aqueous and apolar environments. For instance, detergent molecules partition into lipid bilayers and can break membranes, or solubilize membrane proteins, when their concentration reaches critical values (le Maire et al. 2000). Amphiphilic copolymers, and specifically Amphipols, share many similarities with detergents (self-assemblies into micelle-like globules, hydrophobic binding, binding to interfaces, solubilization of lipids and membrane proteins) (Popot et al. 2011; Giusti et al. 2012) and form mixed assemblies with detergents and lipids (Ladaviere et al. 2002; Popot et al. 2011). It is thus not surprising that they could similarly be used as a tool to control cell membranes. Amphiphilic block copolymers may also affect cell membranes, (Huin et al. 2011; Yang et al. 2008) but to date most studies on this latter class of macromolecules were done in vitro on model lipid bilayers.

In vitro, model systems based on mixtures of polymer and liposomes have been extensively investigated and their properties are summarized in recent reviews (Tribet and

Vial 2008; Binder et al. 2003; Schulz et al. 2012). Hydrophilic polymers attached to lipid bilayers were shown to form a protective (repulsive) corona that enhanced the circulation time of liposomal formulations in vivo, and may substitute for glycolipids and glycoproteins. Interactions between lipid-anchored macromolecules confer also to the layer above the membrane high viscosity and/or visco-elastic properties, and affect budding or invagination. Non-covalent attractions between lipids and segments in macromolecules can locally perturb the composition of bilayers (formation of domains), lipid organization (scrambling, translocations), or stabilize local curvatures (e.g., formation of pores). Poly(propylene oxide)-b-poly(ethylene oxide) block copolymer, usually noted PPO-PEO, can be incorporated in DPPC membranes and lead to a transition from a fluid lipid phase to a more rigid liquid-condensed (LC) one. Infrared Reflection Absorption Spectroscopy and Brewster Angle Microscopy allowed to get insight into the molecular organization of the lipid membrane in the presence of artificial block copolymers (Amado et al. 2008, 2009) or diblock peptides (Blume and Kerth 2013; Travkova et al. 2013). Mixed lipid/polymer membranes are generally not homogeneous and demix into lipid-rich clusters and polymer-rich domains. The effect of polymer to lipid ratio, and bilayer fluidity or rigidity on completion of the phase separation has been documented (Chemin 2012; Le Meins 2013; Olubummo 2013). In contrast, the mixing with lipids is more easily made homogeneous with Amphipols and a variety of other amphiphilic macromolecules having short hydrophobic side groups (and no long hydrophobic block), including natural and non-natural amphiphilic peptides and anti-bacterial peptides (Epand et al. 2011).

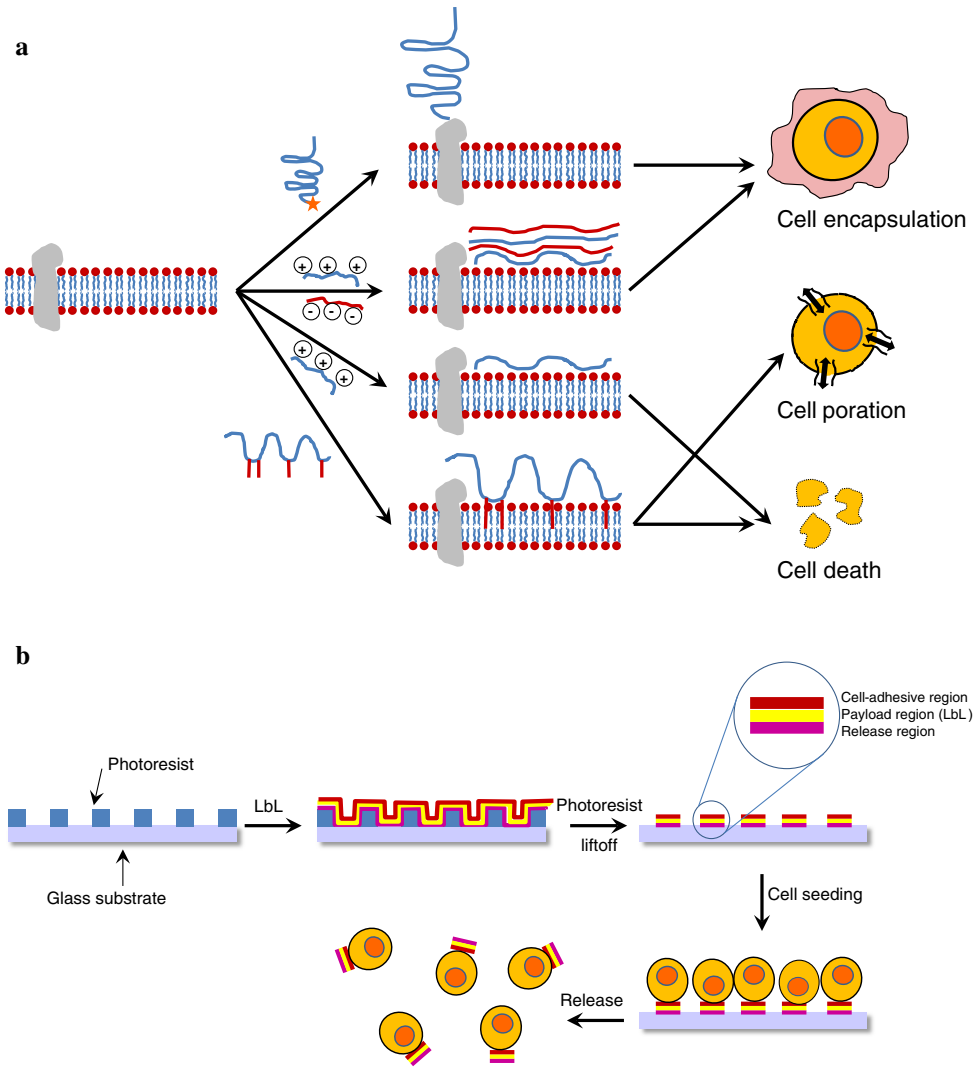
In general, interaction between polymers and lipid membranes proceed from complex interplay between hydrophobic binding, coulombic contributions, self-assemblies, and possible additional effects (e.g., effect of structural constraints) that are specific to the case of peptides (e.g., due to secondary/tertiary folding, (Khandelia et al. 2008)) or block copolymers. Amphiphilic peptides represent the class of macromolecules that could be design with the highest degree of control (chain length, sequence, folding) (Bechinger and Aisenbrey 2012a). In comparison, the synthetic amphiphilic copolymers are devoid of secondary structure, and present higher polydispersity in length and poor control on their sequence. Nevertheless, when they are used to permeabilize or break model liposome membranes, it is difficult to point to any clear advantage of peptide-based agents over synthetic copolymers. Subtle selectivity may emerge from application of these compounds onto more complex membranes, and specifically on cell membranes. The present mini-review is focused on reporting recent works that implemented polymer-controlled perturbation on the membranes of living cells, which is mostly achieved with non-blocky, amphiphilic copolymers including peptides and amphipol's relatives. Here, we review recent and still emerging works, on synthetic polymers (i.e., abiotic) with brief parallel summaries of their commonalities with peptide tools that are actively developed nowadays. The first section illustrates promising applications of polymer-controlled functions of interest for biomedical purposes. The second section lists the variety of responses achieved on cell membranes, with emphasize on the parameters enabling to optimize their properties, specifically the molecular determinants of polymer translocation and formation of polymer-stabilized pores. Though it is certainly oversimplification, recent articles proposed interesting classification of polymers of various chemical natures on the basis of their hydrophobic/hydrophilic balance. This point of view guided development of stimuli-controlled modulation of the polarity or degree of ionization of polymer chains as a general route to achieve remote control on cell penetration or on toxicity, which is described in the last section of this review.

Representative Uses of Copolymers to Manipulate Cells

One recognizes nowadays three main domains of application involving polymers as disruptive agents, or modifiers of the cell membranes properties: encapsulation, biocides, and cell penetration. Coating of cells with macromolecules (that may reach condition of complete encapsulation) is generally sought to avoid contacts with immune systems or with deleterious interfaces. Biocidal and cell-penetrating agents are both macromolecules that bind membranes tightly, which in turn contributes to their internalization and/or formation of pores (Scheme 1). Below are summarized works that look promising for applied developments and that have in particular reached the stage of studies or implementation in vivo.

Coating the Cell Periphery with Polymers

The various methods proposed in literature to attach polymers on the outer surface of living cells can be classified according to the nature of polymer interaction with the cell membrane, including: (i) attachment of macromolecules, typically fluorescent ones for in vivo imaging of membrane proteins upon recognition of a polymer end-function (see Relogio et al. 2013), or orthogonal covalent chemistry on recombinant substrates (see Devaraj et al. 2012); (ii) adsorption of copolymer (adsorption often proceeds from hydrophobic anchoring of one or several hydrophobic moieties of the chain into lipid bilayers) (Guo et al. 1995), or with mammalian cells (Teramura et al. 2007; Yook et al. 2012); or (iii) electrostatic binding and



Scheme 1 Drawing of different modes of cell-surface modification and the corresponding applications. **a** From *top* to *bottom*, covalent attachment, coulombic binding (monolayer or LbL multilayers), and hydrophobic anchoring of polymers to obtain protective encapsulation (e.g., PEGylation), poration or apoptosis upon binding of either polycations or highly hydrophobic polyamphiphiles; **b** preparation of polyelectrolyte multilayers under the form of micrometer-large patches, "backpacks," attached to macrophage cell carriers: a polymer multilayer film is deposited on glass patterned with photoresist, dissolution of the photoresist leaves intact the fragments of the film that have been deposited on glass. After cell seeding, the adhesion between glass and LbL patches is released by a temperature shift leaving in solution cells attached to one LbL patch (redrawn from Swiston et al. 2008, 2010)

assembly of polyelectrolytes multilayers onto the cell surface (Fakhrullin et al. 2012; Teramura and Iwata 2010). In the latter case, composite polymer layers are usually obtained on the basis of successive deposition of polymers of unlike ionic charges onto the cell surface (a method called "layer by layer" deposition, LbL). Non-toxic capsules made by similar techniques, and loaded with drugs, are promising carriers in pharmaceutical applications (De Koker et al. 2012). As regard cell encapsulation, the earliest attempts used immobilized (and dead) human red blood cells (RBC) as well as Escherichia coli bacteria as sacrificial template for the elaboration of polymer hollow capsules (Neu et al. 2001). Then living yeast cells were successfully encapsulated (into alternated layers of poly(allylamine hydrochloride) and poly(styrene sulfonate sodium salt)) (Diaspro et al. 2002). The polyelectrolyte multilayers can be further functionalized with biomolecules for instance to improve Langerhans islets transplantation (Totani et al. 2008) or doped by nanoparticles thereby enabling membrane labeling (application on fungi is reported (Fakhrullin et al. 2009)). An interesting application, showing that mild adhesion of LbL assemblies could be achieved, was developed on macrophages by Rubner et al. (Swiston et al. 2008, 2010; Doshi et al. 2011). Macrophages were safely loaded with micrometer-large polyelectrolyte patches that form sorts of "backpacks" possibly containing drugs (Scheme 1). Cells bear such patches for days, which makes the cells an active "partners" for carrying and targeting therapeutics.

In general, polymer coats have been used to isolate cells from interaction with the external medium and specifically to escape the immune system, mostly for applications in cell or organ transplantation. Other yet marginal applications relate to tight adhesion onto tissue, for instance, in intranasal delivery of vaccines (cationic nanogels carrying protein antibodies can stick to the nasal epithelium and are effectively taken up by mucosal dendritic cells) (Nochi et al. 2010), encapsulation of biotechnologically relevant

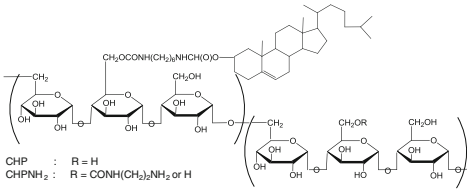
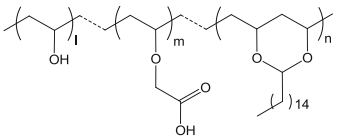
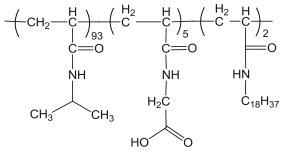
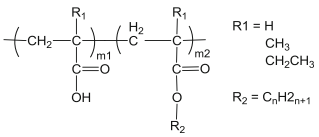
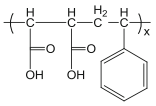
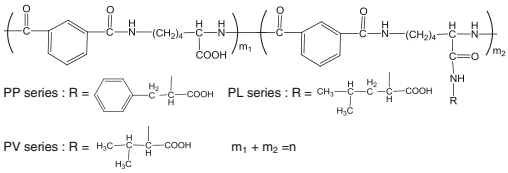
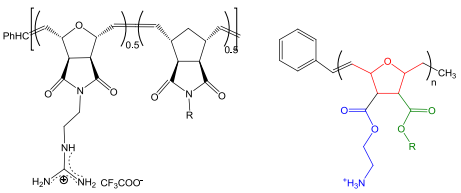
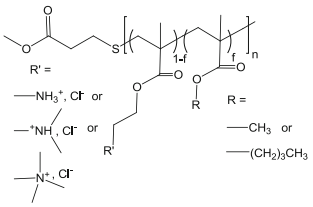
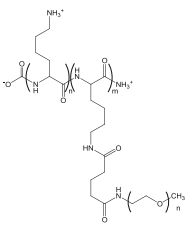
microorganisms such as bacteria (Franz et al. 2010), or protection of mammalian cells in biosensors (e.g., MELN cell line used for estrogen detection (Germain et al. 2006)). Lots of efforts were put on encapsulation, or surface modification, of pancreatic islets. To this aim, diffusion of small molecules, ions, and water must be preserved in the polymer layer(s), while the layer must represent a strong barrier against proteins with diameters above a few nanometers in diameter. Encapsulation can preserve cell viability, activity and in particular the capacity to release insulin upon glucose stimulation. The strategies listed below and illustrated in Scheme 1 have reached a significant degree of achievement: conjugation of PEG on the cell surface (Teramura et al. 2013), adsorption of amphiphilic polymer bearing alkyl side chains (Totani et al. 2008), adsorption of lipid-conjugated poly(ethylene glycol) (Teramura et al. 2007; Yook et al. 2012), LbL encapsulation (Krol et al. 2006; Wilson et al. 2011) or a combination of hydrophobic anchoring and LbL encapsulation (Miura et al. 2006). Similarly, polymers were attached to red blood cells with the aim of shielding interaction with plasma protein and producing a "universal" blood. PEG coupling (Scott et al. 1997) or LbL self-assembly (alginate:lipid-modified chitosan (Mansouri et al. 2011)) were successfully employed to mask antigens at the red blood cell surface and escape immunological rejection, while preserving the ability to carry oxygen. Finally, mesenchymal stem cells could also be encapsulated in a polyelectrolyte multilayer based on hyaluronic acid and poly(L-Lysine) that maintain cell viability (Veerabadran et al. 2007; Garg et al. 2012).

Permeabilization of Cell Membranes, Biocide Activity

In a context of persistence of incurable nosocomial infections by multi-resistant bacteria, it is desirable to search for new antibiotics, disinfectants, and antibacterial materials for usage in household, healthcare, functional textiles, and food packaging. A vast variety of polymers bearing cationic moieties, such as ammonium, guanidinium, sulfonium, or phosphonium, are tailored to this end (for recent reviews on polymer biocides, see (Munoz-Bonilla et al. 2012; Siedenbiedel and Tiller 2012), and on peptides (Futaki et al. 2002; Fillon et al. 2005). Molecular and spatial structures of polymeric biocides were initially inspired from cationic amphiphilic antimicrobial peptides (AMP, cf *infra*) that are known since decades to facilitate pore opening in bacterial membranes. Not surprisingly, most synthetic copolymers that are lacking the well-defined sequence of AMPs, and do not fold into stable secondary structures, are typically less specific and of poorer efficiency than peptide biocides. Successful optimizations (cf *infra*) of these abiotic compounds were motivated by opportunities to produce them more economically and at larger scale compared to peptides. It is not yet possible to predict the exact properties of such molecules from their mere chemical structure. However, the general trend is a gradual variation of properties across a polymer series (e.g., upon increasing the density of hydrophobic groups in polymer chains), enabling one to maximize the bacterial killing activity, while avoiding toxicity on mammalian cells (in practice, author's checked either the absence of permeability of red blood cells, or day-long viability of mammalian cells in 2D cultures). Among parameters affecting biocide activity, recent studies illustrate the role of charge density/hydrophobicity ratio in random copolymers (e.g., statistic distribution of cationic and hydrophobic/philic units in polyacrylic derivative (Paslay et al. 2012), or poly(ethyleneimine) (He et al. 2012), or poly(oxetanes) (Chakrabarty et al. 2011)) (cf Table 1 for the general structure of the poly(acrylic) backbones). The length of an hydrophobic spacer introduced between the cationic charge and the polymer backbone also affects efficiency (Palermo et al. 2012). The minimum inhibitory concentration (MIC) reached with optimal compounds, as low as 1–5 μg mL^{-1}, compares with 0.5–10 μg mL^{-1} determined with AMPs (Chakrabarty et al. 2011; Meng et al. 2012). There are even a few examples of antibacterial synthetic copolymers that display unexpected selectivity against Gram-negative versus Gram-positive bacteria, as for example poly(norbonene)-based compounds (Lienkamp et al. 2009) and methacrylate copolymer with a pendant dodecyl-quaternized ammonium moiety (Dizman et al. 2004).

In comparison, peptides biocides are generally not more selective than non-peptidic copolymers. Among membrane-interacting peptides, different classes have been described according to their intrinsic biological activity that include antimicrobial, anti-cancer or cell penetration. In this paragraph, we consider antimicrobial peptides. Such peptides are known since 70 years, and are generally based on sequences of less than 30 amino acids with a dominant cationic amphipathic pattern. Although examples of negatively charged peptides were reported (Paulmann et al. 2012), membrane-active peptides are generally cationic (containing Lys and Arg residues) and also contain hydrophobic (Ala, Val, Leu, Ileu, Trp, Phe) amino acids, leading to the general assumption that electrostatic interactions represent a key step in the binding process of these peptides to biological membranes. A well-studied membrane-active peptide is melittin (NH_2-GIGAVLKVLTTG LPALISWIKRKRQQ-$CONH_2$), isolated from the bee venom. This peptide is active against Gram-positive and Gram-negative bacteria but it has also strong hemolytic activity against red blood cells. Melittin is an amphiphilic peptide with a hydrophobic amino-terminal domain [1–20]

Table 1 Characteristic structures of amphiphilic polymers tailored by adjustment of hydrophobicity to either coat, translocate, permeabilize cells, or kill bacteria

Neutral chains	
	Cholesterol-modified Pullulans for cell coating (neutral form) or internalization (cationic form) predominantly by endocytosis. (Ayame et al. 2008)
	Hydrophobically modified poly (vinylalcohol) for cell coating, slow endocytosis (Teramura et al. 2008)
Alkyl-PEO or lipid-PEG	Poly(ethyleneglycol) with one or two alkyl end-group(s) for cell coating or aggregation (Miura et al. 2006; Rao et al. 2013; Teramura et al. 2013)
Anionic (weak acid) chains	
	Poly(acrylate), or methacrylate, or ethylacrylate derivatives with random hydrophobization: endocytosis, then endosomal escape at low pH (Chen et al. 2004; Yessine and Leroux 2004; Yessine et al. 2007)
	Studies of bilayer permeability, and poration in (Vial et al. 2005, 2007, 2009)
	Poly(styrene)-co-(maleic anhydride) for controlled endosomal escape (Henry et al. 2006)
	Carboxylate-containing polyamides. Cell penetration, mechanism still debated (Ho et al. 2011; Khormaee et al. 2013)
Cationic chains	
	Poly(oxanorbornene) and poly(norbornene). Antimicrobial activity with selectivity modulated by hydrophobicity/cationicity ratio (Lienkamp et al. 2008, 2009; Som et al. 2012; Tezgel et al. 2011)
	Amino-modified poly(acrylate). Anti-microbial agents with selectivity modulated by side groups (Kuroda et al. 2009; Palermo and Kuroda 2009; Palermo et al. 2009, 2011)
	PEGylated poly(lysine) (or not shown poly(ethylene imine)) for mild cell adhesion, and LbL coating (Mansouri et al. 2011; Wilson et al. 2011)

The generic names refer to the main chain, parent polymer without hydrophobic or other functional pendant groups

while the carboxy-terminal [21–26] region is hydrophilic and positively charged at biological pH. Interestingly, taken separately, the two domains do not show any hemolytic activity, a result that highlights the requirement for a favorable hydrophobic:hydrophilic balance (>1) in the amino acid content of the peptide, to induce membrane perturbation (DeGrado et al. 1982). Being water-soluble, melittin binds to negatively charged bacteria (Mollay et al. 1976) and also zwiterionic eucaryote cell membrane phospholipids (Mollay and Kreil 1973; Georghiou et al. 1982). The positively charged melittin targets the membrane from the aqueous phase and partitions into zwitterionic phosphatidylcholine bilayers. It was shown that formation of nonpolar hydrophobic interactions between melittin and phospholipids represents a key step for the stabilization of the peptide/phospholipid complex. Magainin-2 (NH_2-GIGKFLKKAKKFGKAFVKILKK-$CONH_2$) was isolated from amphibian skin (Zasloff 2002) and is also a cationic and amphiphilic antimicrobial peptide, but with much less hemolytic activity than melittin (Unger et al. 2001). By contrast with melittin, the two types of amino acids are not clustered within identified domains but are spread over the whole sequence of the peptide. In the case of magainin-2, binding and insertion of the peptide into the lipid bilayer is predominantly driven by electrostatic interactions (Wieprecht et al. 1999). Thus, the two peptides, melittin and magainin-2 are characterized by an interfacial model of interaction with membranes that are representative of most of AMPs (Wimley 2010). Despite their similarity in terms of charges and non-polar amino acid content, the interfacial differences in the mode of action of melittin and magainin-2, clearly show that subtle interactions, conformational motion and kinetics should be accounted for to characterize the formation and the stabilization of peptide/phospholipid complexes. Sequence adjustment, variation of charge, hydrophobicity and amphiphilicity, and/or propensity to fold into helices have been studied on several antimicrobial peptides, and led to significant selectivity between Gram-negative and Gram-positive bacteria (Giangaspero et al. 2001). Some antimicrobial peptides have also killing activity on cancer cells, in addition to their biocide action (Hoskin and Ramamoorthy 2008) [http://aps.unmc.edu/AP/main.php]. Their killing activity to bacteria or cancer cells may result from irreversible perturbation of the cell membrane integrity, or from intracellular targets of the peptides.(Riedl et al. 2011) For instance the host defense-like lytic peptide $_{D}$-K6L9 (NH_2-LK**l**LK**k**L**l**KLL**k**LL-$CONH_2$, where bold lowercase letters are D-amino acids) induces necrosis of tumor cells via a membrane depolarizing lytic process.(Papo et al. 2006) Besides, buforin IIb (RAGLQFPVGRLLRRLL RRLLR) has been described as an anticancer histone H2A-derived peptide. Buforin IIb crosses without damage cancer cell membranes and induces mitochondria-dependent apoptosis (caspase 9 activation and cytochrome c release into the cytosol) (Lee et al. 2008).

Cell-Penetrating Polymers

Some water-soluble polymer chains are capable to bind to lipid membranes, and to turn after binding into a form that becomes solubilized in the hydrophobic interior of the lipid bilayers, and eventually translocates and penetrates into the cytosol. Cell-penetrating peptides, CPP, are known since 20 years, and can cross membranes within any cell type, without causing irreversible damage to the cell membrane (Milletti 2012). On the other hand, completely abiotic macromolecules are also developed and have reached now penetration efficiencies that compare with CPP. Most of these CPP-mimics are cationic and contain both guanidine and hydrophobic side groups (Tsogas et al. 2007; Tezgel et al. 2011). But amphiphilic polyanions of various chemical structures have also been identified as cell-penetrating agents (Ho et al. 2011; Torchilin 2012; Yessine and Leroux 2004). CPPs and abiotic penetrating polymers usually have no deleterious action inside cells, although some CPP can interact with cellular proteins such as actin (Delaroche et al. 2010). An intriguing increase of phosphorylation (by IκB kinases) upon penetration of abiotic polypropylene oxide together with specific sequences of DNA was, however, reported (Yang et al. 2008). Innovative drug-delivery systems were based on cell-penetrating peptides (Koren and Torchilin 2012). The general therapeutic strategies implement CPPs or other polymers under the form of conjugated drug molecules that hopefully carry their load (oligonucleotides, DNA, SiRNA, peptide, protein, contrast agents, drugs) into the cytosol. Covalent attachment to CPPs includes disulfide, amide, thiazolidin bonds (Zorko and Langel 2005). Alternatively, non-covalent complex assemblies are formulated to contain both CPPs and the drug or polynucleotides of interest (Deshayes et al. 2012; Crombez et al. 2009; Andaloussi et al. 2011). With abiotic polymers, applications of macromolecular agents are at an earlier stage of development, although amphiphilic polyanions are promising pH-triggered systems (Yessine et al. 2007). We refer here to molecular penetration, and not to the vast field of nanoparticles formulation that has reached a remarkable importance in pharmaceutical sciences and in studies of cell transfection. Those drug, or DNA, cargoes (often polymer micelles, capsules, or colloid particles) are basically tailored to optimize drug loading, enhance the blood circulation time, and to protect drugs from degradation in endosomes. Cell penetration of such polymeric particles may, however, significantly differ from a molecular translocation, and for instance could proceed from active endocytosis and natural permeability of the endosomal membrane to the drug of

interest (Hu et al. 2009; Nicolas et al. 2013). In the case of diblock copolymers containing one cationic and one neutral (typically poly(ethyleneoxide)) block, stabilization of pores in lipid membrane by the polymer chain may, however, contribute to the efficiency of transfection (Huin et al. 2011).

Although numerous CPPs are positively charged, the amino acid composition, and sequence polarity, or hydrophobicity of these peptides is broadly defined (http://crdd.osdd.net/raghava/cppsite/index.php) and a general rule for identifying efficient sequences is lacking. In addition, complex sensitivity to environment is at play. For instance, the anionic (and amphiphilic) peptides called pHLIP enables tumor targeting thanks to their abrupt solubility transition near physiological pH that triggers penetration into mammalian cells upon a local pH variation of less than 0.5 pH units (Andreev et al. 2010; Weerakkody et al. 2013). The most used cell-penetrating peptides are Tat (GRKKRR QRRRPQ, derived from the transcription transactivator of (TAT) the human immunodeficiency virus, (Vives et al. 1997)), Penetratin (RQIKIWFQNRRMKWKK, derived from the Antennapedia homeodomain of drosophila (Derossi et al. 1994)), and oligoarginine (Rn, designed peptides, (Mitchell et al. 2000; Futaki et al. 2001)). These peptides deliver different types of cargoes into cells or in vivo (Koren et al. 2012; Nakase et al. 2012a). For instance, doxorubicin conjugated to Tat or Penetratin induces apoptosis of human breast cancer cells (Aroui et al. 2009), or leads to tumor growth suppression (Nakase et al. 2012b). Topical uptake of Cyclosporin A is enhanced when the molecule is conjugated to R7 peptide, and results in the inhibition of dermatitis inflammation process (Rothbard et al. 2000).

Controlling the Interaction with Cell Membranes, from Mild Attachment to Poration and Permeabilization

To identify the relevant molecular determinants, i.e., parameters that play a role in polymer:membrane interaction, a common strategy consists in optimization of the chemical structure of polymers. For example, varying the composition of polymer chains that contain two or more different functional groups affords gradual variations of properties, and is readily achieved by controlled synthesis. Typical studies investigated the relationship between cell-binding propensity and the density of a particular side group in linear segments of the chains (e.g., ammonium, guanidinium, aromatic, n-alkyl side groups, etc.). Alternatively, the cell responses (including cell death) to variation of the composition of polymers provided indirect indications of the importance of hydrophobicity, charge, or hydrogen bonds. Additional and experimentally accessible determinants include (i) steric repulsion that hampers binding (e.g., introduction of large repulsive polyethyleneglycol, PEG, Stratton et al. 2011), (ii) spatial distribution of functional groups (e.g., variation of the architecture of branched chains, or dendrimers), and (iii) chain length. The main conclusions drawn from these studies are summarized for abiotic polymers in the section "Synthetic Copolymers." Obviously in the case of peptides, similar approaches can focus on specific effects of sequences, and stereochemistry of amino acids. This case is briefly discussed in section "Structure-Properties Relationship for Peptides." Finally, and not surprisingly, polarity (or hydrophobicity) of segments in the polymers is a recognized criterion that deserves attention. In section "Controlling the Degree of Polymer Insertion in Membranes: A Basic Principle of Stimuli-Responsive Systems," we present both experimental studies and modelization of hydrophobically driven penetration of chains inside bilayers. This last section illustrates also how the simplicity of a rational tailoring of hydrophobic/hydrophilic balance has in practice been exploited to achieve on/off control on perturbations of cell membranes upon stimuli-triggered switch of responsive chains.

Synthetic Copolymers

Due to the exploration with synthetic polymers of a variety of monomers having very different chemical natures, results obtained with synthetic chains help to identify the main determinants of the function of interest. In practice, adsorption, penetration or biocide activities were obtained with either neutral, anionic, or cationic polymers, and with polymer chains containing different amount of hydrophobic groups, either aliphatic or aromatic. There is accordingly no specific chemical group associated with one class of behavior. The essential feature in all optimization strategies of copolymers is rather the combination of typically two monomer "units" of unlike polarity/hydrophilicity, namely a water-soluble unit and one unit prone to associate with lipids (e.g., anionic with hydrophobic, or cationic with neutral/hydrophilic). Within a set of homologous macromolecules, the gradual variation of the fraction of the two "units" modulates gradually the interaction with membranes, which makes properties of polymers to evolve from weak and reversible adsorption, toward tight and long-lasting adsorption (as used in coating application), penetration, selective-biocide, and finally non-selective biocide activity. Although exploration of the full window of properties listed above was usually not assessed for each set of copolymers, the correlation between higher density of cationic, or hydrophobic moieties, and tighter association is generally obeyed. It has been validated for the somewhat heterogeneous list of compounds commented in the following paragraphs (see illustrations of structures in Table 1).

Hydrophobic/Hydrophilic Balance

As regards hydrophobically-modified neutral chains, pullulan adhesion (Guo et al. 1995), or endocytosis of pullulan cationic derivatives (Ayame et al. 2008) were strengthened by increased degree of hydrophobic modification. Similarly, tissue adhesion could be enhanced upon increasing the degree of hydrophobic side groups in gelatin-based glues (Matsuda et al. 2012). End-functionalized PEGs containing lipid(s) at their chain end(s) also showed obvious strengthening of binding, while turning from mono- to difunctional chains. Monofunctional chains are rapidly removed/desorbed from the cell surface, with no uptake in the cytoplasm, when cells are subjected to dilution or washing steps (Teramura et al. 2008). But PEG with two oleyl ends may act somewhat cooperatively to stably agglomerate dilution-resistant cell-spheroids (Ito and Taguchi 2009; Rao et al. 2013). Moreover, if the polymers carry several hydrophobic anchors, the binding appeared significantly more stable: Teramura and coll. (Teramura et al. 2008) randomly grafted (neutral) poly(vinyl alcohol) copolymer carrying multiple *n*-alkyl side groups (PVA-alkyl) that tightly adsorb on the whole surface of cells, at short time after supplementation of the cell culture medium with the polymer. Despite removal of the excess unbound chains by washing, PVA-alkyl was slowly gathered into patches on cell surface, or was taken up into the cytoplasm (Teramura et al. 2008). Anionic copolymers obey similar trends as the neutral ones. Yessine and Leroux (2004) showed the increase of the efficacy toward membrane breakage, and sp. endosome escape, with increasing hydrophobicity of poly(glycolic acid-co-octadecylacrylate), or alkyl-modified poly(ethylacrylate) (Chen et al. 2004) (Table 1). Stayton et al. did similar studies on poly(styrene-co-maleic acid) derivatives (Henry et al. 2006). Studies in Ronjung Chen's group showed that poly(acrylamide) derivatives (namely poly(Lysine)-phtalamide) bearing amino-acide pendant groups obeyed the same rule in that hydrophobicity (due to phenylalanine grafts) brought better penetration in cell spheroids (Ho et al. 2011; Khormaee et al. 2013), or more pronounced perturbation of model lipid layers (Zhang et al. 2011). Ishihara and coll. similarly tailored the hydrophobicity of polymeric derivatives of phospholipids to achieve control on cell penetration, here for fluorescent labeling (Goda et al. 2010; Ukawa et al. 2010). Finally, the larger range of hydrophobicity studied on homologous model systems was presented in the work by Ladaviere, Vial et al. that increased the degree of random n-alkyl grafting (octyl or dodecyl groups) on poly(acrylic) chains. These copolymers added to liposome solutions affected properties of the liposomes by (i) dilution-resistant attachment of chains containing a few mol% hydrophobes, (ii) permeabilization and stabilization of nanometer large pores with more hydrophobic chains, (iii) membrane disruption upon increasing further their hydrophobicity by either decreasing pH, or decreasing the charge density of the copolymers. In practice, the authors achieved controlled formation of lipid lateral domains in egg-PC bilayers (Ladaviere et al. 2002), membrane buddings, poration (Vial et al. 2007), and lipid solubilization into polymer mixed micelles (Vial et al. 2005, 2009).

Effect of Cationicity

We turn now to cationic chains that adhere to negatively charged cell surfaces by coulombic attraction, irrespective of the presence of other functional groups such as hydrophobic ones. Here, due to a lack of clear identification of the mechanism of binding, the term coulombic attraction gathers all observed trends toward association of polyelectrolytes with membranes of unlike ionic charge, that may include electrostatic effects and entropic ones (release of counter-ions) Exposure of cell surface to polycations raises problems of toxicity. The cytotoxicity of polycationic macromolecules is influenced by different properties of the polymer such as molecular weight, charge density, chemical nature, and macromolecular chain flexibility (Munoz-Bonilla et al. 2012; Fischer et al. 2003; Chanana et al. 2005). With synthetic polycations commonly used in transfection studies (poly(ethylenimine), poly(L-lysine), poly(diallyl-dimethyl-ammonium chloride), diethylaminoethyl-dextran, poly (vinyl pyridinium bromide)) higher molecular weight and higher charge density induce higher toxicity on mammalian cells. Similar effect exists with natural chains, as longer aminoethyl-modified chitosan show enhanced porogenicity and biocide activity compared to its shorter relatives (Meng et al. 2012). But this simple trend may not be valid for antimicrobial activity. It has been suggested that molecular weight affects selectivity of antimicrobial amphiphilic polycations for Gram-positive versus Gram-negative bacteria (Lienkamp et al. 2008), and that increasing chain length may decrease activity against *S.aureus* because long macromolecules are better trapped in the negatively charged murein layer of this bacteria. Thus generalization from toxicity studies must be considered with caution. Higher rigidity decreases the cytotoxicity (presumably because rigid macromolecules encounter difficulties to fully adsorb into the cell membrane) (Chanana et al. 2005). Among the most studied cationic polymers, Poly(ethylene imine) (PEI) and poly(L-Lysine) (PLL) display the highest charge density and thus are the most toxic polycations (N.B. dendrimers may also reach higher charge densities than PEI). But the toxicity of PLL can be modulated by the composition of the buffer, during the cell-coating process. Cell viability was accordingly preserved in the presence of K^+ in the buffer, which enabled reduced

interaction between polyelectrolytes in LbL capsule preparation. In this case, incomplete encapsulation could be the origin of the higher cell survival rate (Germain et al. 2006). With the aim of full encapsulation of cells in LbL (which requires preparation of a first homogenous cationic layer around the cells), one way to reduce toxicity is to reduce accessibility of cations upon grafting cell-repellant poly(ethylene glycol) (PEG) chains on the amine groups, or elsewhere in the polycation chain (Mansouri et al. 2011; Wilson et al. 2011). In model liposomes, sterically controlled formation of lateral domains of (anionic) lipids were convincing proof of possible balance of coulombic attraction by PEG–PEG repulsion (Pashkovskaya et al. 2006). In cell cultures, however, the exact role of steric hindrance is not understood. Variation of the fraction of primary amines (vs secondary and tertiary ones) should be considered, and is clearly involved in biocide activity (Paslay et al. 2012). It has been shown that primary amines enhance complexation with the phosphate groups of lipids and increase bilayer's permeability (Palermo et al. 2011). Molecular modeling highlighted in addition a possible change in conformation of polycations upon increasing the degree of grafting of PEG. Starting from a extended coil, the chain containing higher PEG density turns into a more compact globular structure(Wilson et al. 2011) which may explain a lower accessibility of its charges, and thus lower toxicity (Fischer et al. 2003; Hong et al. 2006). In practice, the grafting ratio of PEG can be optimized to decrease markedly toxicity while preserving the ability to bind on cells, thus such chains are good precursor for encapsulation by LbL methods. The remarks above apply also to guanidinium-containing polymers, but peculiar properties of this cationic group are now clearly identified (Cooley et al. 2009; Hennig et al. 2008; Holowka et al. 2007; Mattheis et al. 2013; Tezgel et al. 2011). Guanidinylation of polymer chains speeds up cytotoxicity, and affords better penetration of highly hydrophilic polymers in cells by energy-independent pathways. Enhanced penetration compared to macromolecules bearing amine groups presumably comes from the ability of guanidine group to form hydrogen bonds with phosphate ions, stabilizing complexes with phospholipids (Rothbard et al. 2004; Rothbard et al. 2005), and references in (Tribet and Vial 2008). Accordingly, guanidinium-containing copolymers are promising carriers for cytosol delivery of small molecules with high water solubility (due to their high charge density), and high efficiencies modulated by competitive interaction with anionic counterions, such as ATP or heparin (Hennig et al. 2008).

Amphiphilic Polycations

Finally as for neutral or anionic chains, the introduction of hydrophobicity in polycations reinforces their propensity to bind and penetrate lipid membranes. In its fruitful quest for synthetic mimics of antimicrobial peptides, the group of Kenichi Kuroda has published systematic studies of the variation of structural parameters of polycations (Palermo et al. 2009; Kuroda et al. 2009). Although the mechanism of antimicrobial activity is debated, and may differ significantly from a simple permeabilization (Sovadinova et al. 2011b), exponential decrease with linear incorporation of hydrophobic monomers in the chains of both minimum inhibitory concentration and HC50 (concentration of half hemolysis of red blood cells) clearly confirmed the importance of hydrophobic moieties. It is interesting to note that the distribution of hydrophobes in the polymer chains affected hemolysis more than antimicrobial activity. Dibloc copolymers (one hydrophobic and one hydrophilic bloc) had similar biocide activity as random ones (containing the same monomers in similar amount per chain), but essentially no hemolytic activity (Oda et al. 2011). Authors suggest that because dibloc copolymers come into solution under the form of stable micelles, with all cationic groups pointing in the periphery of the hydrophobic core, they may essentially bind to cell by coulombic interaction (they can trigger hemagglutination). In contrast, random copolymers were bound by both coulombic and hydrophobic interaction, which renders these polymers more hemolytic. A different family of chains has been developed by Tew and coll. Based on original poly(oxanorbornene) derivatives, with one or two pendant group(s) per repeat unit to modulate both hydrophobic and cationic densities (Hennig et al. 2008; Lienkamp et al. 2008, 2009; Som et al. 2012; Tezgel et al. 2011). These polymers obeyed the same rules as the acrylic derivatives discussed above, in that increasing the density of a given hydrophobic side group improved biocide activity, and finally turned the polymers into hemolytic (non-specific cell permeabilizers). These trends were correlated with permeabilization of model lipid vesicles, confirming that the affinity for lipids plays a major role (Gabriel et al. 2008). Interestingly, the role of the aliphatic or aryl nature of hydrophobic side groups were compared on the basis of their biocide efficiency, and apparent hydrophobicities (as determined by reverse phase chromatography) (Som et al. 2012). It appeared somewhat surprisingly that translocation was not solely controlled by hydrophobicity, but that aromaticity played a crucial role. Despite a markedly lower retention of the corresponding monomer on reverse-phase chromatography, phenyl-modified polymers showed significantly higher activity than aliphatic-modified macromolecules of comparable size (i.e., when both monomers were carrying hydrophobic side groups with the same number of carbon atoms), and inverse correlation with hydrophobicity and biocide activity were observed in a set of polymers modified with aromatic side groups (Som et al. 2012). Tew and colleagues report

observation of an optimal length for *n*-alkyl side groups that presumably betrayed a contribution of intra-chain hydrophobic collapse (polymers containing high densities of long alkyl groups may prefer to form micelle-like globules remaining in water) (Lienkamp et al. 2008).

Structure-Properties Relationship for Peptides

The Predominant Role of Composition

Linear membrane-active peptides are generally unstructured in water solution, and adopt secondary structure when they bind to membranes. Various secondary structures (α-helical, β-sheet, cyclic, globular, random coil) have been described for both antimicrobial and cell-penetrating peptides. Studies of antimicrobial and cell-penetrating peptides show that there are no preferred secondary structures that correlate with their membrane activity. It is also important to note that the folding state adopted on the membrane depends on peptide concentration or peptide/lipid ratio, a property that confers to some membrane-active peptides a chameleon-like behavior. For example, Penetratin adopts an α-helical structure at low peptide:lipid ratio and shifts to a β-sheet with increasing density (Magzoub et al. 2002). These different conformations of penetratin could be observed also in cells (Ye et al. 2010).

The nature of amino-acid (ionic, hydrophobic, polar) in the primary sequence appears more important than secondary structure to explain the effect of peptides on membrane organization. Whatever the class of peptides with antimicrobial, anticancer or cell-penetration activity, their sequence typically contains cationic and hydrophobic residues (with a few exceptions such as Glu- and Asp-rich, pHLIP (Andreev et al. 2010; Weerakkody et al. 2013) or Dermcidin peptides that are anionic). Positively charged peptides interact with bacteria or mammalian cells by electrostatic and hydrophobic association, at least at the onset of binding. For mammalian cells, peptides have to diffuse through the glycocalyx in the extracellular matrix. In this context, multiple routes of entry of CPPs have been described, that include active endocytic pathways (clathrin-dependent, caveolin-dependent, macropinocytosis) and temperature-independent translocation. The question of the peptide release from intracellular endocytic vesicles has been extensively studied within the last years and endosomolytic activity (escape from endosomes at acidic pH) of cell-penetrating peptides have been correlated with hydrophobicity (Madani et al. 2013). Coulombic effects are also involved in targeting lethal activity of peptides against cancer compared to non-cancer mammalian cells. As a result of the multiple modifications of cancer cells, the outer membrane leaflet of cancer cells displays excess anionic phosphatidylserine and/or of glycosylated species such as sialic acid or heparan sulfate, and have a more negative membrane potential, a more acidic pH in the cell environment (Riedl et al. 2011; Harris et al. 2013). It appears that cell killing by AMP through necrosis (cell membrane lysis) or apoptosis (lysis of mitochondria) both depend on the presence of anionic lipids in the outer leaflet of the membrane bilayer. A similar importance of surface charge is valid for cell-penetrating peptides (CPP). At the cell-surface, these peptides interact first with negatively charged glycosaminoglycans. These proteoglycans are possible portals for entry into cells. The influence of GAGs on the entry was shown qualitatively, (Suzuki et al. 2002; Console et al. 2003; Poon and Gariepy 2007) and could be measured quantitatively (Jiao et al. 2009; Alves et al. 2011a; Walrant et al. 2011; Bode et al. 2012; Bechara et al. 2013). In the absence of glycocalix, simulations show that hydrogen-bonds and anion-cation pairing between the bilayer and arginines or lysines are the key determinants of the association of penetratin (Pourmousa and Karttunen 2013). In addition, tryptophans but not phenylalanine makes hydrogen-bonds with the phosphate group of lipids (Pourmousa et al. 2013). These results are in good agreement with experimental observation of [W48 → F] Penetratin mutant entering less in cells than their parent peptide (Derossi et al. 1994). Charge–pair interactions and hydrogen bonds are also crucial in the cell-penetrating properties of oligoarginine sequences. In this case, guanidinium moieties form bidentate hydrogen-bonds with the phosphate groups of phospholipids, and makes the peptide capable to partition in the membrane bilayer and migrate to the inner leaflet, along with the membrane potential (Rothbard et al. 2004). Of major interest is a recent work by Nakase and collaborators (Nakase et al. 2012c), who report the transformation of an antimicrobial peptide into a plasma-membrane permeable one. In this study, all lysyl residues of the KLA antimicrobial peptide (KLAKLAKKLAKLAK) were replaced by arginine, and the peptide shows no longer antimicrobial activity but gains cell-penetration properties and can accumulate into mitochondria. This result highlights the importance and the contribution of guanidinium side chains in the fine tuning of membrane-active peptide activity.

Role of Hydrophobicity on Peptide Penetration in the Membrane

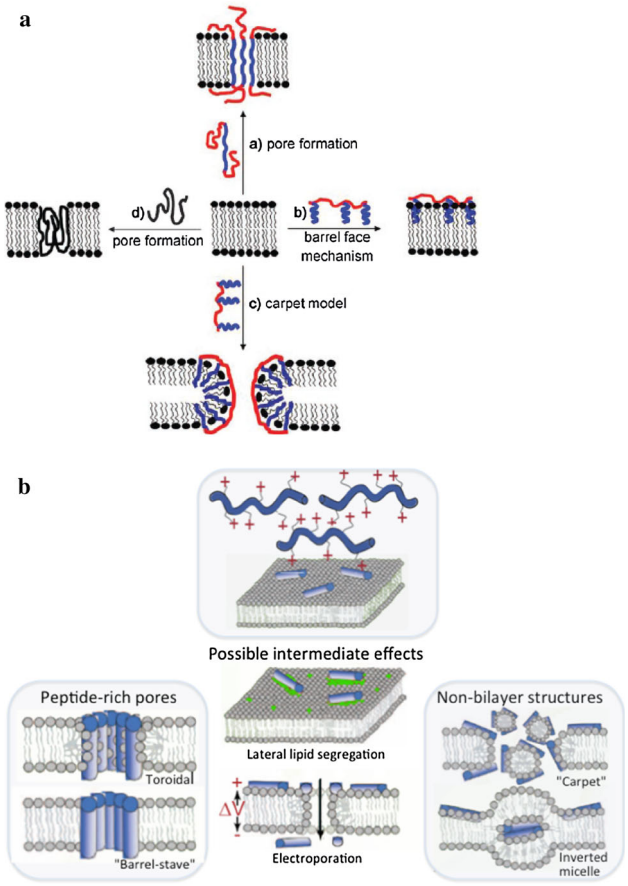
Kinetics and thermodynamics of binding of all membrane-active peptides are crucial parameters to understand how peptides reversibly or irreversibly perturb the organization of biological membranes. In this regard, lessons from the antimicrobial peptides, are important guides for the whole field of membrane-active peptides. A striking example is provided by a kinetic and thermodynamic study on Melittin, a peptide that is known to form pores in zwitterionic

membranes and on Magainin that does not, although the two peptide sequences are similar in their amino acid composition (Papo and Shai 2003). Electrostatic interactions may or not govern the initial step of the peptide binding to the membrane. For instance, the rate of association of Magainin is increased ($\approx$10-fold) while the rate of dissociation is decreased ($\approx$10-fold) when the peptide binds anionic lipids compared to zwitterionic ones. Once bound, insertion kinetics of Magainin within the hydrophobic core or into the inner surface of the bilayer is similar with anionic and zwitterionic bilayers, indicating that this second step is no longer driven by electrostatics (Papo and Shai 2003). In addition, Magainin has little preference for anionic bilayers compared to monolayers, a point that has been interpreted as a hint of predominant parallel adsorption on the membrane surface (Bechinger et al. 1993). Uncomplete insertion of Magainin into anionic bilayers advocates for a translocation requiring the presence of pores rather than the direct crossing of individual, membrane-soluble peptides (Matsuzaki et al. 1995). Formation of pores is also in line with antibacterial activity. By contrast, Melittin binds with similar association and dissociation rates onto anionic and zwiterrionic bilayers (Papo and Shai 2003). In the case of zwitterionic lipids, the peptide has much higher affinity for bilayers than monolayers, suggesting a deep insertion. These results suggest that hydrophobic interactions are involved in Melittin/membrane binding (Vogel and Jahnig 1986). Melittin induces pore formation in zwitterionic membranes and has detergent-like properties in anionic membranes and hemolytic and bactericide effects (Ladokhin and White 2001).

As for antimicrobial peptides, membrane models have been proposed to understand how cell-penetrating peptides interact with the lipid bilayer and the main ones are illustrated below. Penetratin (RQIKIWFQNRRMKWKK) does not bind to specific phospholipids, since the same partition constant was measured, irrespective of anionic/zwitterionic lipid ratio (Persson et al. 2004). Penetratin interacts and lies parallel with the surface of the lipid bilayer (Magzoub et al. 2002), while tryptophans are not well buried into the bilayer core (Berlose et al. 1996; Christiaens et al. 2002; Brattwall et al. 2003). Similarly Tat peptide (GRKKRRQRRRPQ) cannot insert into the hydrophobic bilayer, and the peptide binds at the membrane surface via electrostatic interactions, just tight enough to change the conformation of anionic phospholipid polar heads (Ziegler et al. 2003). Other cell-penetrating peptides, including R9 (RRRRRRRRR), RW9 (RRWWRRWRR) and RL9 (RRLLRRLRR), behave differently. RW9 and R9 have high efficiency to enter into cells, while RL9 is poorly internalized, a rather striking observation since the replacement of Trp with Leu led to a peptide of higher hydrophobicity (Walrant et al. 2011). RW9 and R9 have similar interactions with anionic phospholipids. They destabilize the gel phase state of the lipid bilayer, affect the packing of the fatty acid chains and insert loosely into the hydrophobic core of anionic membranes, while RL9 inserts deeper but does not affect the packing of the acyl chains (Walrant et al. 2011, 2012). In addition, R9 and RW9 can influence the membrane curvature while RL9 does not. Thus, it appears from these few examples of cell-penetrating peptides, that a deep insertion/hydrophobic binding in the membrane bilayer is not always required for translocation of the peptides. Although all these membrane-active peptides look similar in terms of their physico-chemical properties, they clearly have a finely tuned mode of interaction with, and action on the membrane.

Controlling the Degree of Polymer Insertion in Membranes: A Basic Principle of Stimuli-Responsive Systems

At supramolecular scales, the effect produced by polymers on properties of lipid membranes can be described by variation of membrane's equilibrium curvature, bending moduli, partition or binding constants of macromolecules, lipid ordering or in other terms shift in lipid phase transition. Irrespective of the chemical class of membrane-active polymers, these parameters govern the stability of membranes. Except for cases involving peptide self-organization into well-defined assemblies, it is thus not surprising that the models of perturbation by either peptides or abiotic amphiphilic chains share many similarities. The reader is referred to reviews that recall well-known models of polymer insertion in bilayers and porogenicity (Binder 2008; Alves et al. 2011b) (Scheme 2). As regard permeabilization, or translocation mechanisms, peptides have been the purpose of significantly more studies than abiotic macromolecules. The recognized effects of peptides on membrane properties are briefly recalled in the following. Citations of available results on abiotic chains are also inserted in this paragraph, when tentative mechanisms could be proposed in conditions similar to that established with peptides. A well-accepted view is that at low concentration, an antimicrobial peptide binds to the membrane, modifies lipid organization and alters membrane structure. Upon increasing AMP concentration and lipophilicity, a breakpoint is reached, that enables translocation. Above a threshold concentration in the bilayer (peptide:lipid of ca. 1:500–1:50 mol/mol), AMPs become membrane-disruptive (Nguyen et al. 2011). Similar concentration and affinity threshold are reported for cationic abiotic biocides (see above). Several structures of pores were suggested for AMPs (Scheme 2): the barrel-stave model proposes that a pore is formed with peptides standing parallel with one another to form the inner "wall" of the pore. In toroidal-pores, no specific peptide–peptide alignment is



Scheme 2 Drawing of polymer assemblies with lipids that are typically proposed in the literature to illustrate the origin of permeabilization and/or membrane ruptures occurring upon polymer adsorption **a** synthetic (abiotic) copolymers, reprinted with permission from (Binder 2008) Copyright 2008 Wiley-VCH Verlag GmbH&Co.KGaA, Weinheim, **b** models of peptide insertion in lipid membranes

required. Instead, peptides modify the local curvature in a cooperative manner, which locally stabilizes highly curved, peptide-rich, toroidal shapes. Other models suggest that peptides become antimicrobial when they can "carpet" the lipid surface, which results as for detergents in gradual enhancement of the propensity to form highly curved structures. These three models have obviously inspired the mechanisms (not yet fully validated) proposed in the case of abiotic chains, devoid of secondary structures (Binder 2008; Tribet and Vial 2008). Note that an individual AMP may "utilize" different mechanisms depending on the experimental conditions. Studies suggested that Melittin forms transmembrane pores in zwitterionic lipid bilayers via a barrel-stave mechanism (Vogel et al. 1986) but acts as a detergent in negatively charged membranes (Ladokhin and White 2001). AMPs were splitted into two classes depending on their mechanism of action being pore-dependent or pore-independent. As recently pointed out, all membrane-active peptides should, however, be seen as a single family (Bechinger and Aisenbrey 2012b), since the early classification was essentially based upon the biological functions, and did not consider gradual variation of interaction with biological membranes. One attractive model is that all peptides may be ranked on a common scale depending on the perturbation energy achieved on membrane bilayers (Last et al. 2013). First, upon simple adsorption, this perturbation comes from the lateral tension induced on the membrane, due for instance to the fact that peptide binding causes changes in the packing of polar head groups and acyl chains of lipids. Lateral tension increases the frequency of occurrence of membrane thinning, and/or formation of defects decreasing the energy of pores (N.B.: tension is released by opening a pore). Second, membrane-active peptides could recruit and cluster specific membrane lipids (Scheme 2), and thus locally evokes membrane thinning and defects. The intrinsic kinetics to partition between surface and defect regions would represent fundamental physico-chemical mechanism controlling the biological activity (cytotoxicity, translocation).

Presumably because abiotic chains generally lack the capacity to form well-organized, rigid structures, a more direct correlation is expected between the propensity of polymer segments to associate with lipids and membrane penetration, or translocation. This point of view found recent experimental validation in vitro, on model liposomes with non-ionic chains (Yaroslavov et al. 2006; Demina et al. 2005), or cationic polymers on monolayers studied by X-ray scattering (Hu et al. 2013). Recent Monte Carlo simulation of homopolymer:bilayer association show that there is an adsorption transition at a threshold polarity of the chain. Close to this threshold both the translocation probability and the permeability of the membrane with respect to solvent are enhanced (Werner et al. 2012). In another molecular dynamic simulation of a cationic poly(amphiphile), Palermo et al. (2012) showed that the chain backbone can be anchored flat in the subsurface of lipid bilayers. Flat conformation of adsorbed chains and orthogonal penetration of *n*-akyl side groups in the lipids was experimentally validated on lipid monolayers by advanced interfacial spectrophotometry (Avery et al. 2011). Finally, comparison of the membrane curvature induction, and variation of phase transition diagrams of lipids upon adsorption of abiotic or peptidic antimicrobial compounds (Ishitsuka et al. 2006) also suggests that these chemically remote macromolecules may be closer than expected from the point of view of membrane energetics. To finally present an homogenous sketch of the field, it is interesting to compare polymers of different chemical structures on an hydrophobicity scale (Fig. 1 (Hu et al. 2013) and refs therein). Hu et al. averaged water–octanol partition, LogP, of the constitutive monomers of

antimicrobial lysine-rich peptides, or cationic derivatives of poly(acrylate), in order to plot resulting values (N.B. sequence-independant averages) as the x-axis in a diagram ranking efficient biocides. Synthetic random copolymers appear in Fig. 1 to be of similar or slightly higher hydrophobicity in general than macromolecules in the peptide family. In contrast, poly(acrylate) chains were found significantly more cationic, with an average charge densities up to four time that of peptides of similar efficiency. Obviously, charge–charge repulsion occurred in flexible random copolymers has a marked impact on chain extension, that in turn may hamper hydrophobic collapse and penetration in lipid layers. Suitable pre-orientation of charged moieties, e.g., facial distribution, due to folding of peptides may be responsible of this large apparent shift. It is important to note, however, that the averaging, not only masks details about important contribution of sequences, but even for random copolymers, does not properly reflect the accessibility of side groups of the polymers. For example, increasing hydrophobicity should favor self-assemblies of amphiphilic chains into micelle-like globules, as for Amphipols. Though systematic studies are missing, it seems that self-assemblies represent another threshold for polymer:lipid interaction: in the case of cationic derivatives, non-monotonous variation of biocide (and hemolytic) efficiencies with increasing hydrophobicity was tentatively ascribed to formation of highly hydrophilic globules with cationic groups in their outer shell (Kuroda et al. 2009; Sovadinova et al. 2011a). In a somehow opposite observation, micellar assemblies of polyanionic amphiphiles, belonging to the family of Amphipols, were shown to solubilize lipid vesicles, whereas relatives of lower hydrophobicity could not and were only adsorbed in the bilayers (Ladaviere et al. 2002; Vial et al. 2009).

Of practical interest, the recognition of critical switch by the charge/hydrophobicity ratio enabled the design of polymers affording the remote control of membrane destabilization (or permeabilization) with stimuli-responsive macromolecules. These macromolecules contain hydrophobic anchors to bind to the membranes and most importantly are close to poor solvent conditions. A wide variety of stimuli-responsive macromolecular structures have been tested in liposome formulations, under the form of amphiphilic copolymers (Hoffman et al. 2002; Pack et al. 2005; Yessine and Leroux 2004). The membrane breakage is generally obtained near a threshold pH or temperature conditions that make the polymer to abruptly undergo a transition from water-soluble into water-insoluble coil–globule conformation (Roux et al. 2003; Yessine and Leroux 2004). Typically, polymers containing carboxylic acids side groups are hydrophilic at high pH (>7) under their polyanionic form (cf Table 2), but turn into water-insoluble globules when pH is decreased below the

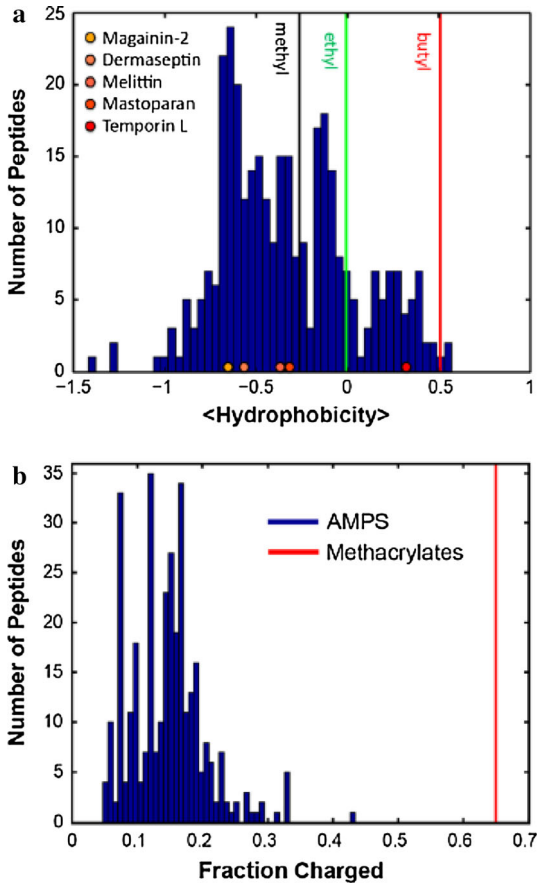
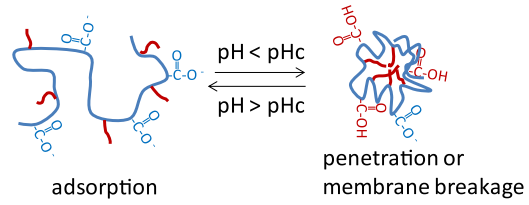
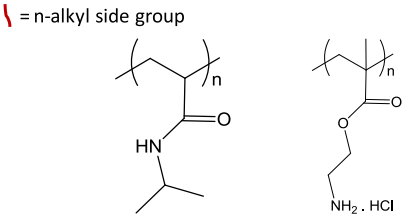
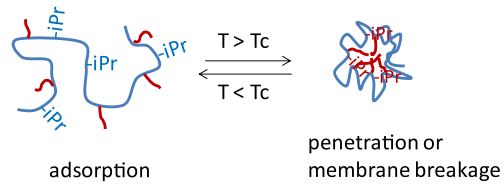
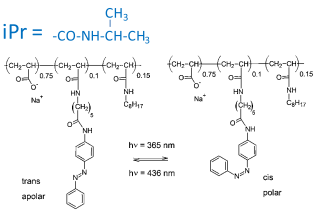


Fig. 1 Average hydrophobicity (based on logP, octanol:water partitioning) and cationic density in biocides polymers belonging to the class of either peptides or poly(acrylate) derivatives. Reprinted with permission from (Hu et al. 2013) Copyright 2013 American Chemical Society

pKa of the monomer units (typically 5.5–6.5, or in vivo at the pH of late endosomes). These polymers become abruptly insoluble upon neutralization (Chen et al. 2004; Thomas et al. 1996; Yessine and Leroux 2004). Amphipol A8-35 and A8-75 belong to the family of pH-responsive polyanions. Our results indicate that A8-75 is typically a pH-dependant cell permeabilizer, whereas the more hydrophobic A8-35 slowly solubilizes lipids and breaks membranes at acidic pH (Vial et al. 2005, 2007, 2009). On the other hand, temperature-responsive polymers have been tailored to trigger bilayer permeability above a threshold temperature. Such systems are based on solubility transition of *N*-isopropylacrylamide units (Ringsdorf et al. 1993; Kim and Kim 2002), propylene oxide units (Chandaroy et al. 2002; Firestone and Seifert 2005), or organophosphazene (Couffin-Hoarau and Leroux 2004). In all cases, the monomers become less hydrated at temperatures above 35–40 °C (i.e., the lower critical solubility temperature, LCST, of the chains). Finally, light is a clean, versatile trigger that enables spatial and temporal control.

Table 2 Characteristic structural determinants of stimuli-responsive polymers

Stimuli-responsive polymers	
	pH-Triggered penetration in mammalian cells (detailed structures in Table 1, polystyrene-co-maleic anhydride from Ho et al. 2011) or pH-responsive endosomal escape in mammalian cells (poly(acrylic acid) derivatives from (Henry et al. 2006; Yessine and Leroux 2004)), or poly(lysylphtalate) derivatives with pH-sensitive hemolytic activity (Chen et al. 2009)
	Temperature-triggered biocide, (Iwasaki et al. 2013; Mattheis et al. 2012, 2013)
	
	Light-triggered cytosolic penetration of additives in mammalian cells (Sebai et al. 2010)

To date only one example of biocompatible light-responsive polymer permeabilizer has been published (Sebai et al. 2010, 2012) (Fig. 2; Table 2). But other photosystems exist (they are yet toxic or not efficient in cell culture condition). Biocompatibility issues have motivated the development of stimuli-responsive polymers of diverse chemical structures, in order to optimize responses in serum (Francis et al. 2001) for cell agglomeration (Iwasaki et al. 2013) permeabilization of mammalian cells, or biocide activity (see Fig. 2 for illustrations on T-responsive and light-responsive polymers, or for pH-triggered ones: (Henry et al. 2006; Lackey et al. 1999; Eccleston et al. 2000; Kusonwiriyawong et al. 2003). The exact origin of stimuli-controlled membrane disruption is to date largely conjectural. It is likely that insoluble segments of the chains penetrate inside the bilayers and introduce defects in their organization by a deep inclusion of ethoxy or carboxy groups in the apolar lipid core (Ferri et al. 2005; Ringsdorf et al. 1993). In addition, polymers that can form micelles in water are presumably capable of stabilizing the rim of fragments of lipid bilayers with local curvatures below 3–4 nm.

Conclusion

Recent experiments implemented synthetic copolymers in cell cultures and evidenced that abiotic macromolecules are interesting substitutes of peptides for controlled cell permeabilization, or biocide activity. On the other hand, mild polymer coats were tailored to prepare "decorated" cells that escape recognition by the immune systems, or are imparted with better resistance to external stress. There are several motivations to use macromolecules as tools to manipulate the membrane of cell (Teramura et al. 2010). First, macromolecular systems facilitate a combinatorial approach, connecting several functions and independent regions in polymer chains (or assemblies) in order to target, carry, and deliver activation or contrast agents at specific location (Shokeen et al. 2011; Relogio et al. 2013). Second,

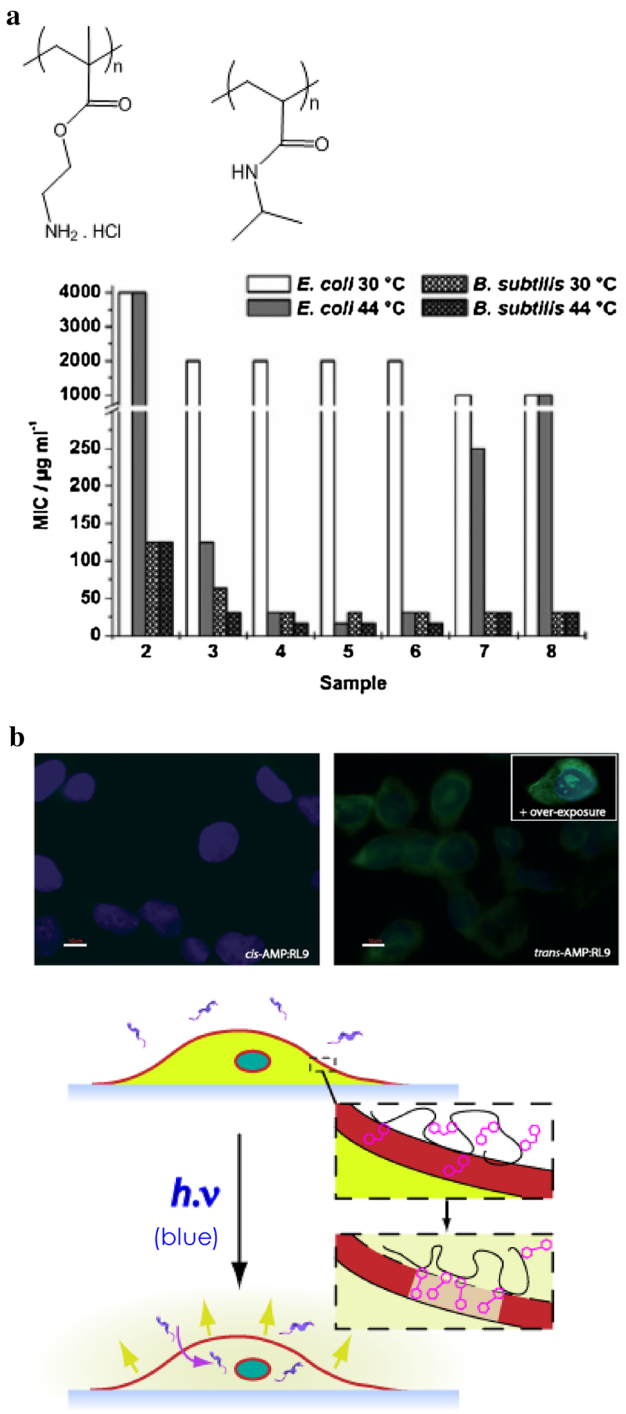


Fig. 2 Stimuli-responsive amphiphilicity of polymers controlling cell membranes. **a** Temperature-triggered biocide activity, samples from 2 to 7 correspond to increasing fraction (from 10 to 54 mol%) of the amino-monomer in the copolymer also shown in Table 2 with transition temperature being in the range 32–36 °C;(Mattheis et al. 2012). **b** Light-triggered penetration of peptides in cell. Other possible stimuli include temperature or pH (Sebai et al. 2010, 2012). Reprinted with permission from (Binder 2008), and (Mattheis et al. 2012) Copyright 2012 Wiley-VCH Verlag GmbH&Co.KGaA, Weinheim

the properties of one macromolecule can gradually be tailored by variation of the density of (re)active moities per chain, and of the chain architecture, enabling one to control its interaction (attractive or repulsive) at nanometer distances (e.g., to achieve for instance stealthiness, or stimuli-triggered responses). The range of molecular backbones that are studied to this purpose is becoming large (poly(acrylate) or acrylamide, polynorbornene, poly(styrene), polyamides, modified natural polysaccharides, etc.) and simple chemistry affords in most family of macromolecules large variations of structural parameters (e.g., chain length, charge density, hydrophobicity of aliphatic or aromatic side groups). Because this chemical variety has not yet been fully explored, one may consider that developments of synthetic polymers for cell manipulation are in their infancy. Basic guiding rules on structure–properties relationships have, however, emerged from the data available, and point to the importance of amphiphilicity of polymers.

Researches aiming at optimization of biocides, which are by far the most developed field among researches on polymer-controlled cells, clearly point to critical roles of both cationic charge density, and hydrophobicity. It appeared possible to compensate the lower specificity of synthetic macromolecules (compared to peptides) by a subtle balance between fraction of ammonium and alkyl side groups, in order to achieve low hemolytic activities but high antibacterial (MIC < 10 µg/L) activities. Surprisingly the hydrophobic density of efficient copolymers does not differ significantly from the one of antimicrobial cationic peptides. As for peptides, peculiar enhancement of membrane penetration can be found upon introduction in the chains of guanidinium side group, or phenyl ones, likely because of formation of complexes with lipids. The recognized importance of the amphiphilic nature of cationic artificial biocides does not, however, suffice to conclude that hydrophobic attachment of polymers in lipid membranes is required. Based on years of investigation of the rich complexity of cell-penetrating peptides and antimicrobial peptides, it is known that multiple steps and several mechanisms are involved in the penetration of macromolecules into cells, including contributions of association in the glycocalix, or with specific lipids. It is, however, clearly established with model liposomes that amphiphilic polycations (showing biocide activity) bind tightly to lipid bilayers and may translocate when their structure reaches a critical hydrophobicity. On the other hand, upon hydrophobic self-assemblies into micelle-like globules, synthetic cationic polymers become markedly less toxic and loss their hemolytic activity. High hydrophobicity, such as the

one reached in Amphipol, favors a full collapse/sequestration of the hydrophobic segments into the core of globules, which in turn markedly modify interaction between polymer and cells. In the case of anionic Amphipol, hydrophobic collapse makes the chain capable of solubilizing lipids, which accelerates membrane breakage in model liposomes. The hydrophobic:hydrophilic balance at the level of either the monomers, or in the whole polymer chain is thus an essential criterion, though monotonous correlation with activity on membranes shall not be expected.

Neutral or anionic chains, and preferably amphiphilic ones, are essentially used as drug-delivery agents or for mild cell coating, suggesting that they are markedly less toxic than cationic ones. Additional functions may be brought by side groups that respond to external stimuli (light, pH or temperature shift) and enables one to trigger solubility transition. This was successfully exploited to control endosomal escape, or to target cell penetration upon exposure to light or to low pH. Stimuli-controlled polymers include poly(acrylic acid) derivatives belonging to the family of Amphipols and undergoing a transition from water-soluble chains to cell-penetrating ones with decreasing degree of ionization (typically upon decreasing pH below 5.5–6.5). Membrane breakage and lipid solubilization occurs when they self-associate into micelles. Peptides are also developed as pH-responsive. Future developments and sequence adjustments will certainly enable subtle sensitivities to specific biomembranes and environment conditions. In comparison, the chemistry of stimuli-responsive abiotic polymers is not yet suited to design highly specific systems, but its larger toolbox affords new modes of targeting. The adjustment of abrupt transition upon stimulations, specifically using light, can reach high spatial and temporal resolution. It makes no doubt that ongoing progress in the design of bioactive, stimuli-responsive chains for cell manipulation will be actively pursued with the aim to develop abiotic tools for cell therapies, cultures of stem cells (immuno-protection, controlled differentiation), and for studies requiring high spatial control on cell perturbations.

Acknowledgments EM and CT were supported by "programme Investissement d'Avenir ANR-11-LABX-0011-01."

References

Allen TM, Cullis PR (2013) Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev 65(1):36–48

Alves ID, Bechara C, Walrant A, Zaltsman Y, Jiao CY, Sagan S (2011a) Relationships between membrane binding, affinity and cell internalization efficacy of a cell-penetrating peptide: penetratin as a case study. PLoS ONE 6(9):e24096

Alves ID, Rodriguez N, Cribier S, Sagan S (2011b) Membrane crossover by cell-penetrating peptides: kinetics and mechanisms—from model to cell membrane perturbation by permeant peptides. Fundam Biomed Technol 5:179–196

Amado E, Kerth A, Blume A, Kressler J (2008) Infrared reflection absorption spectroscopy coupled with Brewster angle microscopy for studying interactions of amphiphilic triblock copolymers with phospholipid monolayers. Langmuir 24(18):10041–10053

Amado E, Kerth A, Blume A, Kressler J (2009) Phospholipid crystalline clusters induced by adsorption of novel amphiphilic triblock copolymers to monolayers. Soft Matter 5(3):669–675

Andaloussi SE, Lehto T, Lundin P, Langel U (2011) Application of PepFect peptides for the delivery of splice-correcting oligonucleotides. Methods Mol Biol 683:361–373

Andreev OA, Karabadzhak AG, Weerakkody D, Andreev GO, Engelman DM, Reshetnyak YK (2010) pH (low) insertion peptide (pHLIP) inserts across a lipid bilayer as a helix and exits by a different path. Proc Natl Acad Sci USA 107(9):4081–4086

Aroui S, Brahim S, De Waard M, Bréard J, Kenani A (2009) Efficient induction of apoptosis by doxorubicin coupled to cell-penetrating peptides compared to unconjugated doxorubicin in the human breast cancer cell line MDA-MB 231. Cancer Lett 285:28–38

Avery CW, Palermo EF, McLaughin A, Kuroda K, Chen Z (2011) Investigations of the interactions between synthetic antimicrobial polymers and substrate-supported lipid bilayers using sum frequency generation vibrational spectroscopy. Anal Chem 83(4):1342–1349

Ayame H, Morimoto N, Akiyoshi K (2008) Self-assembled cationic nanogels for intracellular protein delivery. Bioconj Chem 19(4):882–890

Bechara C, Pallerla M, Zaltsman Y, Burlina F, Alves ID, Lequin O, Sagan S (2013) Tryptophan within basic peptide sequences triggers glycosaminoglycan-dependent endocytosis. FASEB J 27(2):738–749

Bechinger B, Aisenbrey C (2012a) The polymorphic nature of membrane-active peptides from biophysical and structural investigations. Curr Protein Pept Sci 13(7):602–610

Bechinger B, Aisenbrey C (2012b) The polymorphic nature of membrane-active peptides from biophysical and structural investigations. Curr Prot Peptide Sci 13(7):602–610

Bechinger B, Zasloff M, Opella SJ (1993) Structure and orientation of the antibiotic peptide magainin in membranes by solid-state nuclear magnetic resonance spectroscopy. Protein Sci 2:2077–2084

Berlose JP, Convert O, Derossi D, Brunissen A, Chassaing G (1996) Conformational and associative behaviours of the third helic of antennapedia homeodomain in membrane-mimetic environments. Eur J Biochem 242:372–386

Binder WH (2008) Polymer-induced transient pores in lipid membranes. Angew Chem-Int Edn 47(17):3092–3095

Binder WH, Barragan V, Menger FM (2003) Domains and rafts in lipid membranes. Angew Chem-Int Edn 42(47):5802–5827

Blume A, Kerth A (2013) Peptide and protein binding to lipid monolayers studied by FT-IRRA spectroscopy. Biochimica et Biophysica Acta-Biomembranes 1828(10):2294–2305

Bode SA, Thévenin M, Bechara C, Sagan S, Bregant S, Lavielle S, Chassaing G, Burlina F (2012) Self-assembling mini cell-penetrating peptides enter by both direct translocation and glycosaminoglycan-dependent endocytosis. Chem Commun 48:7179–7181

Brattwall CE, Lincoln P, Nordén B (2003) Orientation and conformation of cell-penetrating peptide penetratin in phospholipid vesicle membranes determined by polarized-light spectroscopy. JACS 125:14214–14215

Chakrabarty S, King A, Kurt P, Zhang W, Ohman DE, Wood LF, Lovelace C, Rao R, Wynne KJ (2011) Highly effective, water-

soluble, hemocompatible 1,3-propylene oxide-based antimicrobials: poly (3,3-quaternary/PEG)-copolyoxetanes. Biomacromolecules 12(3):757–769

Chanana M, Gliozzi A, Diaspro A, Chodnevskaja I, Huewel S, Moskalenko V, Ulrichs K, Galla HJ, Krol S (2005) Interaction of polyelectrolytes and their composites with living cells. Nano Lett 5(12):2605–2612

Chandaroy P, Sen A, Alexandridis P, Hui SW (2002) Utilizing temperature-sensitive association of Pluronic F-127 with lipid bilayers to control liposome-cell adhesion. Biochim Biophys Acta-Biomembr 1559(1):32–42

Chen T, McIntosh D, He Y, Kim J, Tirrell DA, Scherrer P, Fenske DB, Sandhu AP, Cullis PR (2004) Alkylated derivatives of poly(ethylacrylic acid) can be inserted into preformed liposomes and trigger pH-dependent intracellular delivery of liposomal contents. Mol Membr Biol 21:385–393

Chen R, Khormaee S, Eccleston ME, Slater NKH (2009) The role of hydrophobic amino acid grafts in the enhancement of membrane-disruptive activity of pH-responsive pseudo-peptides. Biomaterials 30(10):1954–1961

Christiaens B, Symoens S, Verheyden S, Engelborghs Y, Joliot A, Prochiantz A, Vandekerckhove J, Rosseneu M, Vanloo B (2002) Tryptophan fluorescence study of the interaction of penetratin peptides with model membranes. Eur J Biochem 269:2918–2926

Console S, Marty C, Garcia-Echeverria C, Schwendener R, Ballmer-Hofer K (2003) Antennapedia and HIV transactivator of transcription (TAT) "protein transduction domains" promote endocytosis of high molecular weight cargo upon binding to cell surface glycosaminoglycans. J Biol Chem 278(37):35109–35114

Cooley CB, Trantow BM, Nederberg F, Kiesewetter MK, Hedrick JL, Waymouth RM, Wender PA (2009) Oligocarbonate molecular transporters: oligomerization-based syntheses and cell-penetrating studies. J Am Chem Soc 131(45):16401

Couffin-Hoarau AC, Leroux JC (2004) Report on the use of poly(organophosphazenes) for the design of stimuli-responsive vesicles. Biomacromolecules 5(6):2082–2087

Crombez L, Morris MC, Dufort S, Aldrian-Herrada G, Nguyen Q, Mc Master G, Coll JL, Heitz F, Divita G (2009) Targeting cyclin B1 through peptide-based delivery of siRNA prevents tumour growth. Nucleic Acids Res 37:4559–4569

De Koker S, Hoogenboom R, De Geest BG (2012) Polymeric multilayer capsules for drug delivery. Chem Soc Rev 41(7): 2867–2884

DeGrado WF, Musso GF, Lieber M, Kaiser ET, Kézdy FJ (1982) Kinetics and mechanism of hemolysis induced by melittin and by a synthetic melittin analogue. Biophys J 37:329–338

Delaroche D, Cantrelle FX, Subra F, Van Heijenoort C, Guittet E, Jiao CY, Blanchoin L, Chassaing G, Lavielle S, Auclair C, Sagan S (2010) Cell-penetrating peptides with intracellular actin-remodeling activity in malignant fibroblasts. J Biol Chem 285(10):7712–7721

Demina T, Grozdova I, Krylova O, Zhirnov A, Istratov V, Frey H, Kautz H, Melik-Nubarov N (2005) Relationship between the structure of amphiphilic copolymers and their ability to disturb lipid bilayers. Biochemistry 44(10):4042–4054

Derossi D, Joliot AH, Chassaing G, Prochiantz A (1994) the 3rd helix of the antennapedia homeodomain translocates through biological-membranes. J Biol Chem 269(14):10444–10450

Deshayes S, Konate K, Rydstrom A, Crombez L, Godefroy C, Milhiet PE, Thomas A, Brasseur R, Aldrian G, Heitz F, Munoz-Morris MA, Devoisselle JM, Divita G (2012) Self-assembling peptide-based nanoparticles for siRNA delivery in primary cell lines. Small 8(14):2184–2188

Devaraj NK, Thurber GM, Keliher EJ, Marinelli B, Weissleder R (2012) Reactive polymer enables efficient in vivo bioorthogonal chemistry. Proc Natl Acad Sci USA 109(13):4762–4767

Diaspro A, Silvano D, Krol S, Cavalleri O, Gliozzi A (2002) Single living cell encapsulation in nano-organized polyelectrolyte shells. Langmuir 18(13):5047–5050

Dizman B, Elasri MO, Mathias LJ (2004) Synthesis and antimicrobial activities of new water-soluble bis-quaternary ammonium methacrylate polymers. J Appl Polym Sci 94(2):635–642

Doshi N, Swiston AJ, Gilbert JB, Alcaraz ML, Cohen RE, Rubner MF, Mitragotri S (2011) Cell-based drug delivery devices using phagocytosis-resistant backpacks. Adv Mater 23(12):H105–H109

Eccleston ME, Kuiper M, Gilchrist FM, Slater NKH (2000) pH-responsive pseudo-peptides for cell membrane disruption. J Controlled Release 69(2):297–307

Epand RF, Mor A, Epand RM (2011) Lipid complexes with cationic peptides and OAKs; their role in antimicrobial action and in the delivery of antimicrobial agents. Cell Mol Life Sci 68(13): 2177–2188

Fakhrullin RF, Zamaleeva AI, Morozov MV, Tazetdinova DI, Alimova FK, Hilmutdinov AK, Zhdanov RI, Kahraman M, Culha M (2009) Living fungi cells encapsulated in polyelectrolyte shells doped with metal nanoparticles. Langmuir 25(8):4628–4634

Fakhrullin RF, Zamaleeva AI, Minullina RT, Konnova SA, Paunov VN (2012) Cyborg cells: functionalisation of living cells with polymers and nanomaterials. Chem Soc Rev 41(11):4189–4206

Ferri JK, Miller R, Makievski AV (2005) Equilibrium and dynamics of PEO/PPO/PEO penetration into DPPC monolayers. Colloids Surfaces A 261(1–3):39–48

Fillon YA, Anderson JP, Chmielewski J (2005) Cell penetrating agents based on a polyproline helix scaffold. J Am Chem Soc 127(33):11798–11803

Firestone MA, Seifert S (2005) Interaction of nonionic PEO-PPO diblock copolymers with lipid bilayers. Biomacromolecules 6(5):2678–2687

Fischer D, Li YX, Ahlemeyer B, Krieglstein J, Kissel T (2003) In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials 24(7):1121–1131

Francis MF, Dhara G, Winnik FM, Leroux JC (2001) In vitro evaluation of pH-sensitive polymer/niosome complexes. Biomacromolecules 2(3):741–749

Franz B, Balkundi SS, Dahl C, Lvov YM, Prange A (2010) Layer-by-layer nano-encapsulation of microbes: controlled cell surface modification and investigation of substrate uptake in bacteria. Macromol Biosci 10(2):164–172

Futaki S, Suzuki T, Ohashi W, Yagami T, Tanaka S, Ueda K, Sugiura Y (2001) Arginine-rich peptides—an abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. J Biol Chem 276(8):5836–5840

Futaki S, Nakase I, Suzuki T, Zhang YJ, Sugiura Y (2002) Translocation of branched-chain arginine peptides through cell membranes: flexibility in the spatial disposition of positive charges in membrane-permeable peptides. Biochemistry 41(25): 7925–7930

Gabriel GJ, Pool JG, Som A, Dabkowski JM, Coughlin EB, Muthukurnar M, Tew GN (2008) Interactions between antimicrobial polynorbornenes and phospholipid vesicles monitored by light scattering and microcalorimetry. Langmuir 24(21):12489–12495

Garg P, Debnath T, Chelluri LK, Hebalkar N (2012) Feasibility of polymer based cell encapsulation using electrostatic layer by layer assembly. J Biomater Tissue Eng 2(3):215–219

Georghiou S, Thompson M, Mukhopadhyay AK (1982) melittin-phospholipid interaction studied by employing the single tryptophan residue as an intrinsic fluorescent-probe. Biochim Biophys Acta 688(2):441–452

Germain M, Balaguer P, Nicolas JC, Lopez F, Esteve JP, Sukhorukov GB, Winterhalter M, Richard-Foy H, Fournier D (2006) Protection of mammalian cell used in biosensors by coating with a polyelectrolyte shell. Biosens Bioelectron 21(8):1566–1573

Giangaspero A, Sandri L, Tossi A (2001) Amphipathic alpha helical antimicrobial peptides—a systematic study of the effects of structural and physical properties on biological activity. Eur J Biochem 268(21):5589–5600

Giusti F, Popot JL, Tribet C (2012) Well-defined critical association concentration and rapid adsorption at the air/water interface of a short amphiphilic polymer, amphipol a8-35: a study by forster resonance energy transfer and dynamic surface tension measurements. Langmuir 28(28):10372–10380

Goda T, Goto Y, Ishihara K (2010) Cell-penetrating macromolecules: direct penetration of amphipathic phospholipid polymers across plasma membrane of living cells. Biomaterials 31(8):2380–2387

Guo ZJ, Kallus S, Akiyoshi K, Sunamoto J (1995) Artificial cell-wall for plant protoplast—coating of plasma-membrane with hydrophobized polysaccharides. Chem Lett 6:415–416

Harris F, Dennison SR, Singh J, Phoenix DA (2013) On the selectivity and efficacy of defense peptides with respect to cancer cells. Med Res Rev 33(1):190–234

He YC, Heine E, Keusgen N, Keul H, Moller M (2012) Synthesis and characterization of amphiphilic monodisperse compounds and poly(ethylene imine)s: influence of their microstructures on the antimicrobial properties. Biomacromolecules 13(3):612–623

Hennig A, Gabriel GJ, Tew GN, Matile S (2008) Stimuli-responsive polyguanidino-oxanorbornene membrane transporters as multicomponent sensors in complex matrices. J Am Chem Soc 130(31):10338–10344

Henry SM, El-Sayed MEH, Pirie CM, Hoffman AS, Stayton PS (2006) pH-responsive poly(styrene-alt-maleic anhydride) alkylamide copolymers for intracellular drug delivery. Biomacromolecules 7(8):2407–2414

Ho VHB, Slater NKH, Chen RJ (2011) pH-responsive endosomolytic pseudo-peptides for drug delivery to multicellular spheroids tumour models. Biomaterials 32(11):2953–2958

Hoffman AS, Stayton PS, Press O, Murthy N, Lackey CA, Cheung C, Black F, Campbell J, Fausto N, Kyriakides TR, Bornstein P (2002) Design of "smart" polymers that can direct intracellular drug delivery. Polym Adv Technol 13(10–12):992–999

Holowka EP, Sun VZ, Kamei DT, Deming TJ (2007) Polyarginine segments in block copolypeptides drive both vesicular assembly and intracellular delivery. Nat Mater 6(1):52–57

Hong SP, Leroueil PR, Janus EK, Peters JL, Kober MM, Islam MT, Orr BG, Baker JR, Holl MMB (2006) Interaction of polycationic polymers with supported lipid bilayers and cells: nanoscale hole formation and enhanced membrane permeability. Bioconj Chem 17(3):728–734

Hoskin DW, Ramamoorthy A (2008) Studies on anticancer activities of antimicrobial peptides. Biochim Biophy Acta Biomembr 1778(2):357–375

Hu XL, Jing XB (2009) Biodegradable amphiphilic polymer-drug conjugate micelles. Expert Opin Drug Deliv 6(10):1079–1090

Hu K, Schmidt NW, Zhu R, Jiang YJ, Lai GH, Wei G, Palermo EF, Kuroda K, Wong GCL, Yang LH (2013) A critical evaluation of random copolymer mimesis of homogeneous antimicrobial peptides. Macromolecules 46(5):1908–1915

Huin C, Gall TL, Barteau B, Pitard B, Montier T, Lehn P, Cheradame H, Guégan P (2011) Evidence of DNA transfer across a model membrane by a neutral amphiphilic block copolymer. J Gene Med 13:538–548

Ishitsuka Y, Arnt L, Majewski J, Frey S, Ratajczek M, Kjaer K, Tew GN, Lee KYC (2006) Amphiphilic poly(phenyleneethynylene)s can mimic antimicrobial peptide membrane disordering effect by membrane insertion. J Am Chem Soc 128(40):13123–13129

Ito M, Taguchi T (2009) Enhanced insulin secretion of physically crosslinked pancreatic beta-cells by using a poly(ethylene glycol) derivative with oleyl groups. Acta Biomater 5(8):2945–2952

Iwasaki Y, Sakiyama M, Fujii S, Yusa S (2013) Surface modification of mammalian cells with stimuli-responsive polymers. Chem Commun 49(71):7824–7826

Jiao CY, Delaroche D, Burlina F, Alves ID, Chassaing G, Sagan S (2009) Translocation and endocytosis for cell-penetrating peptide internalization. J Biol Chem 284(49):33957–33965

Khandelia H, Ipsen JH, Mouritsen OG (2008) The impact of peptides on lipid membranes. Biochim Biophys Acta Biomembr 1778(7–8):1528–1536

Khormaee S, Choi Y, Shen MJ, Xu BY, Wu HT, Griffiths GL, Chen RJ, Slater NKH, Park JK (2013) Endosomolytic anionic polymer for the cytoplasmic delivery of sirnas in localized in vivo applications. Adv Funct Mater 23(5):565–574

Kim JC, Kim JD (2002) Release property of temperature-sensitive liposome containing poly(*N*-isopropylacrylamide). Colloids Surfaces B 24(1):45–52

Koren E, Torchilin VP (2012) Cell-penetrating peptides: breaking through to the other side. Trends Mol Med 18(7):385–393

Krol S, del Guerra S, Grupillo M, Diaspro A, Gliozzi A, Marchetti P (2006) Multilayer nanoencapsulation. New approach for immune protection of human pancreatic islets. Nano Lett 6(9):1933–1939

Kuroda K, Caputo GA, DeGrado WF (2009) The role of hydrophobicity in the antimicrobial and hemolytic activities of polymethacrylate derivatives. Chem Eur J 15(5):1123–1133

Kusonwiriyawong C, van de Wetering P, Hubbell JA, Merkle HP, Walter E (2003) Evaluation of pH-dependent membrane-disruptive properties of poly(acrylic acid) derived polymers. Eur J Pharm Biopharm 56(2):237–246

Lackey CA, Murthy N, Press OW, Tirrell DA, Hoffman AS, Stayton PS (1999) Hemolytic activity of pH-responsive polymer-streptavidin bioconjugates. Bioconj Chem 10(3):401–405

Ladaviere C, Tribet C, Cribier S (2002) Lateral organization of lipid membranes induced by amphiphilic polymer inclusions. Langmuir 18(20):7320–7327

Ladokhin AS, White SH (2001) 'Detergent-like' permeabilization of anionic lipid vesicles by melittin. Biochim Biophys Acta Biomembr 1514(2):253–260

Last NB, Schlamadinger DE, Miranker AD (2013) A common landscape for membrane-active peptides. Protein Sci 22(7):870–882

le Maire M, Champeil P, Moller JV (2000) Interaction of membrane proteins and lipids with solubilizing detergents. Biochim Biophys Acta Biomembr 1508(1–2):86–111

Lee HS, Park CB, Kim JM, Jang SA, Park IY, Kim MS, Cho JH, Kim SC (2008) Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. Cancer Lett 271(1):47–55

Liechty WB, Kryscio DR, Slaughter BV, Peppas NA (2010) Polymers for drug delivery systems. Annu Rev Chem Biomol Eng 1:149–173

Lienkamp K, Madkour A, Musante A, Nelson C, Nusslein K, Tew GN (2008) Antimicrobial polymers prepared by ROMP with unprecedented selectivity: a molecular construction kit approach. JACS 130:9836–9843

Lienkamp K, Kumar KN, Som A, Nusslein K, Tew GN (2009) "Doubly selective" antimicrobial polymers: how do they differentiate between bacteria? Chem Eur J 15(43):11710–11714

Madani F, Abdo R, Lindberg S, Hirose H, Futaki S, Langel U, Graslund A (2013) Modeling the endosomal escape of cell-penetrating peptides using a transmembrane pH gradient. Biochim Biophys Acta Biomembr 1828(4):1198–1204

Magzoub M, Eriksson LEG, Graslund A (2002) Conformational states of the cell-penetrating peptide penetratin when interacting with phospholipid vesicles: effects of surface charge and peptide concentration. Biochim Biophys Acta Biomembr 1563(1–2):53–63

Mansouri S, Merhi Y, Winnik FM, Tabrizian M (2011) Investigation of layer-by-layer assembly of polyelectrolytes on fully functional human red blood cells in suspension for attenuated immune response. Biomacromolecules 12(3):585–592

Matile S, Jentzsch AV, Montenegro J, Fin A (2011) Recent synthetic transport systems. Chem Soc Rev 40(5):2453–2474

Matsuda M, Ueno M, Endo Y, Inoue M, Sasaki M, Taguchi T (2012) Enhanced tissue penetration-induced high bonding strength of a novel tissue adhesive composed of cholesteryl group-modified gelatin and disuccinimidyl tartarate. Colloids Surfaces B 91:48–56

Matsuzaki K, Murase O, Miyajima K (1995) kinetics of pore formation by an antimicrobial peptide, magainin-2 in phospholipid-bilayers. Biochemistry 34(39):12553–12559

Mattheis C, Zhang Y, Agarwal S (2012) Thermo-switchable antibacterial activity. Macromol Biosci 12(10):1401–1412

Mattheis C, Wang H, Meister C, Agarwal S (2013) Effect of guanidinylation on the properties of poly(2-aminoethylmethacrylate)-based antibacterial materials. Macromol Biosci 13(2): 242–255

Meng XT, Xing RG, Liu S, Yu HH, Li KC, Qin YK, Li PC (2012) Molecular weight and pH effects of aminoethyl modified chitosan on antibacterial activity in vitro. Int J Biol Macromol 50(4):918–924

Milletti F (2012) Cell-penetrating peptides: classes, origin, and current landscape. Drug Discovery Today 17(15–16):850–860

Mitchell DJ, Kim DT, Steinman L, Fathman CG, Rothbard JB (2000) Polyarginine enters cells more efficiently than other polycationic homopolymers. J Pept Res 56(5):318–325

Miura S, Teramura Y, Iwata H (2006) Encapsulation of islets with ultra-thin polyion complex membrane through poly(ethylene glycol)-phospholipids anchored to cell membrane. Biomaterials 27(34):5828–5835

Mollay C, Kreil G (1973) Fluorometric measurements on interaction of melittin with lecithin. Biochim Biophys Acta 316(2):196–203

Mollay C, Kreil G, Berger H (1976) Action of phospholipases on cytoplasmic membrane of *Escherichia coli*—stimulation by melittin. Biochim Biophys Acta 426(2):317–324

Munoz-Bonilla A, Fernandez-Garcia M (2012) Polymeric materials with antimicrobial activity. Prog Polym Sci 37(2):281–339

Nakase I, Akita H, Kogure K, Graslund A, Langel U, Harashima H, Futaki S (2012a) Efficient intracellular delivery of nucleic acid pharmaceuticals using cell-penetrating peptides. Acc Chem Res 45(7):1132–1139

Nakase I, Konishi Y, Ueda M, Saji H, Futaki S (2012b) Accumulation of arginine-rich cell-penetrating peptides in tumors and the potential for anticancer drug delivery in vivo. J Controll Release 159(2):181–188

Nakase I, Okumura S, Katayama S, Hirose H, Pujals S, Yamaguchi H, Arakawa S, Shimizu S, Futaki S (2012c) Transformation of an antimicrobial peptide into a plasma membrane-permeable, mitochondria-targeted peptide via the substitution of lysine with arginine. Chem Commun 48(90):11097–11099

Neu B, Voigt A, Mitlohner R, Leporatti S, Gao CY, Donath E, Kiesewetter H, Mohwald H, Meiselman HJ, Baumler H (2001) Biological cells as templates for hollow microcapsules. J Microencapsul 18(3):385–395

Nguyen LT, Haney EF, Vogel HJ (2011) The expanding scope of antimicrobial peptide structures and their modes of action. Trends Biotechnol 29(9):464–472

Nicolas J, Mura S, Brambilla D, Mackiewicz N, Couvreur P (2013) Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery. Chem Soc Rev 42(3):1147–1235

Nochi T, Yuki Y, Takahashi H, Sawada S, Mejima M, Kohda T, Harada N, Kong IG, Sato A, Kataoka N, Tokuhara D, Kurokawa S, Takahashi Y, Tsukada H, Kozaki S, Akiyoshi K, Kiyono H (2010) Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. Nat Mater 9(7):572–578

Oda Y, Kanaoka S, Sato T, Aoshima S, Kuroda K (2011) Block versus random amphiphilic copolymers as antibacterial agents. Biomacromolecules 12(10):3581–3591

Pack DW, Hoffman AS, Pun S, Stayton PS (2005) Design and development of polymers for gene delivery. Nat Rev Drug Discov 4(7):581–593

Palermo EF, Kuroda K (2009) Chemical structure of cationic groups in amphiphilic polymethacrylates modulates the antimicrobial and hemolytic activities. Biomacromolecules 10(6):1416–1428

Palermo EF, Sovadinova I, Kuroda K (2009) Structural determinants of antimicrobial activity and biocompatibility in membrane-disrupting methacrylamide random copolymers. Biomacromolecules 10(11):3098–3107

Palermo EF, Lee DK, Ramamoorthy A, Kuroda K (2011) Role of cationic group structure in membrane binding and disruption by amphiphilic copolymers. J Phys Chem B 115(2):366–375

Palermo EF, Vemparala S, Kuroda K (2012) Cationic spacer arm design strategy for control of antimicrobial activity and conformation of amphiphilic methacrylate random copolymers. Biomacromolecules 13(5):1632–1641

Papo N, Shai Y (2003) Exploring peptide membrane interaction using surface plasmon resonance: differentiation between pore formation versus membrane disruption by lytic peptides. Biochemistry 42(2):458–466

Papo N, Seger D, Makovitzki A, Kalchenko V, Eshhar Z, Degani H, Shai Y (2006) Inhibition of tumor growth and elimination of multiple metastases in human prostate and breast xenografts by systemic inoculation of a host defense-like lytic peptide. Cancer Res 66(10):5371–5378

Pashkovskaya AA, Lukashev EP, Antonov PE, Finogenova OA, Ermakov YA, Melik-Nubarov NS, Antonenko YN (2006) Grafting of polylysine with polyethylenoxide prevents demixing of *O*-pyromellitylgramicidin in lipid membranes. Biochim Biophys Acta 1758:1685–1695

Paslay LC, Abel BA, Brown TD, Koul V, Choudhary V, McCormick CL, Morgan SE (2012) Antimicrobial poly(methacrylamide) derivatives prepared via aqueous RAFT polymerization exhibit biocidal efficiency dependent upon cation structure. Biomacromolecules 13(8):2472–2482

Paulmann M, Arnold T, Linke D, Oezdirekcan S, Kopp A, Gutsmann T, Kalbacher H, Wanke I, Schuenemann VJ, Habeck M, Buerck J, Ulrich AS, Schittek B (2012) Structure-activity analysis of the dermcidin-derived peptide DCD-1L, an anionic antimicrobial peptide present in human sweat. J Biol Chem 287(11):8434–8443

Persson D, Thoren PEG, Lincoln P, Norden B (2004) Vesicle membrane interactions of penetratin analogues. Biochemistry 43(34):11045–11055

Poon GMK, Gariepy J (2007) Cell-surface proteoglycans as molecular portals for cationic peptide and polymer entry into cells. Biochem Soc Trans 35:788–793

Popot JL, Althoff T, Bagnard D, Baneres JL, Bazzacco P, Billon-Denis E, Catoire LJ, Champeil P, Charvolin D, Cocco MJ, Cremel G, Dahmane T, de la Maza LM, Ebel C, Gabel F, Giusti F, Gohon Y, Goormaghtigh E, Guittet E, Kleinschmidt JH, Kuhlbrandt W, Le Bon C, Martinez KL, Picard M, Pucci B, Sachs JN, Tribet C, van Heijenoort C, Wien F, Zito F, Zoonens M (2011) Amphipols from a to z. Annu Rev Biophys 40(40): 379–408

Pourmousa M, Karttunen M (2013) Early stages of interactions of cell-penetrating peptide penetratin with a DPPC bilayer. Chem Phys Lipids 169:85–94

Rao Z, Sasaki M, Taguchi T (2013) Development of amphiphilic, enzymatically-degradable PEG-peptide conjugate as cell cross-linker for spheroid formation. Colloids Surfaces B 101:223–227

Relogio P, Bathfield M, Haftek-Terreau Z, Beija M, Favier A, Giraud-Panis MJ, D'Agosto F, Mandrand B, Farinha JPS, Charreyre MT, Martinho JMG (2013) Biotin-end-functionalized highly fluorescent water-soluble polymers. Polym Chem 4(10):2968–2981

Riedl S, Zweytick D, Lohner K (2011) Membrane-active host defense peptides—challenges and perspectives for the development of novel anticancer drugs. Chem Phys Lipids 164(8):766–781

Ringsdorf H, Sackmann E, Simon J, Winnik FM (1993) Interactions of liposomes and hydrophobically-modified poly-(*N*-isopropylacrylamides)—an attempt to model the cytoskeleton. Biochim Biophys Acta 1153(2):335–344

Rothbard JB, Garlington S, Lin Q, Kirschberg T, Kreider E, McGrane PL, Wender PA, Khavari PA (2000) Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. Nat Med 6(11):1253–1257

Rothbard JB, Jessop TC, Lewis RS, Murray BA, Wender PA (2004) Role of membrane potential and hydrogen bonding in the mechanism of translocation of guanidinium-rich peptides into cells. J Am Chem Soc 126(31):9506–9507

Rothbard JB, Jessop TC, Wender PA (2005) Adaptive translocation: the role of hydrogen bonding and membrane potential in the uptake of guanidinium-rich transporters into cells. Adv Drug Deliv Rev 57(4):495–504

Roux E, Lafleur M, Lataste E, Moreau P, Leroux JC (2003) On the characterization of pH-sensitive liposome/polymer complexes. Biomacromolecules 4(2):240–248

Schulz M, Olubummo A, Binder WH (2012) Beyond the lipid-bilayer: interaction of polymers and nanoparticles with membranes. Soft Matter 8(18):4849–4864

Scott MD, Murad KL, Koumpouras F, Talbot M, Eaton JW (1997) Chemical camouflage of antigenic determinants: stealth erythrocytes. Proc Natl Acad Sci USA 94(14):7566–7571

Sebai S, Cribier S, Karimi A, Massotte D, Tribet C (2010) Permeabilization of lipid membranes and cells by a light-responsive copolymer. Langmuir 26(17):14135–14141

Sebai SC, Milioni D, Walrant A, Alves ID, Sagan S, Huin C, Auvray L, Massotte D, Cribier S, Tribet C (2012) Photocontrol of the translocation of molecules, peptides, and quantum dots through cell and lipid membranes doped with azobenzene copolymers. Angew Chem Int Edn 51(9):2132–2136

Shokeen M, Pressly ED, Hagooly A, Zheleznyak A, Ramos N, Fiamengo AL, Welch MJ, Hawker CJ, Anderson CJ (2011) Evaluation of multivalent, functional polymeric nanoparticles for imaging applications. ACS Nano 5(2):738–747

Siedenbiedel F, Tiller JC (2012) Antimicrobial polymers in solution and on surfaces: overview and functional principles. Polymers 4(1):46–71

Som A, Reuter A, Tew GN (2012) Protein transduction domain mimics: the role of aromatic functionality. Angew Chem Int Edn 51(4):980–983

Sovadinova I, Palermo EF, Huang R, Thoma LM, Kuroda K (2011a) Mechanism of polymer-induced hemolysis: nanosized pore formation and osmotic lysis. Biomacromolecules 12(1):260–268

Sovadinova I, Palermo EF, Urban M, Mpiga P, Caputo GA, Kuroda K (2011b) Activity and mechanism of antimicrobial peptide-mimetic amphiphilic polymethacrylate derivatives. Polymers 3(3):1512–1532

Stratton TR, Applegate BM, Youngblood JP (2011) Effect of steric hindrance on the properties of antibacterial and biocompatible copolymers. Biomacromolecules 12(1):50–56

Suzuki T, Futaki S, Niwa M, Tanaka S, Ueda K, Sugiura Y (2002) Possible existence of common internalization mechanisms among arginine-rich peptides. J Biol Chem 277(4):2437–2443

Swiston AJ, Cheng C, Um SH, Irvine DJ, Cohen RE, Rubner MF (2008) Surface functionalization of living cells with multilayer patches. Nano Lett 8(12):4446–4453

Swiston AJ, Gilbert JB, Irvine DJ, Cohen RE, Rubner MF (2010) Freely suspended cellular "backpacks" lead to cell aggregate self-assembly. Biomacromolecules 11(7):1826–1832

Teramura Y, Iwata H (2010) Cell surface modification with polymers for biomedical studies. Soft Matter 6(6):1081–1091

Teramura Y, Kaneda Y, Iwata H (2007) Islet-encapsulation in ultrathin layer-by-layer membranes of poly(vinyl alcohol) anchored to poly(ethylene glycol)-lipids in the cell membrane. Biomaterials 28(32):4818–4825

Teramura Y, Kaneda Y, Totani T, Iwata H (2008) Behavior of synthetic polymers immobilized on a cell membrane. Biomaterials 29(10):1345–1355

Teramura Y, Oommen OP, Olerud J, Hilborn J, Nilsson B (2013) Microencapsulation of cells, including islets, within stable ultrathin membranes of maleimide-conjugated PEG-lipid with multifunctional crosslinkers. Biomaterials 34(11):2683–2693

Tezgel AO, Telfer JC, Tew GN (2011) De Novo designed protein transduction domain mimics from simple synthetic polymers. Biomacromolecules 12(8):3078–3083

Thomas JL, Borden KA, Tirrell DA (1996) Modulation of mobilities of fluorescent membrane probes by adsorption of a hydrophobic polyelectrolyte. Macromolecules 29(7):2570–2576

Torchilin VP (2012) Multifunctional nanocarriers. Adv Drug Deliv Rev 64:302–315

Totani T, Teramura Y, Iwata H (2008) Immobilization of urokinase on the islet surface by amphiphilic poly(vinyl alcohol) that carries alkyl side chains. Biomaterials 29(19):2878–2883

Travkova OG, Andra J, Mohwald H, Brezesinski G (2013) Influence of Arenicin on Phase Transitions and Ordering of Lipids in 2D Model Membranes. Langmuir 29(39):12203–12211

Tribet C, Vial F (2008) Flexible macromolecules attached to lipid bilayers: impact on fluidity, curvature, permeability and stability of the membranes. Soft Matter 4(1):68–81

Tsogas I, Sideratou Z, Tsiourvas D, Theodossiou TA, Paleos CM (2007) Interactive transport of guanidinylated poly(propylene imine)-based dendrimers through liposomal and cellular membranes. ChemBioChem 8(15):1865–1876

Ukawa M, Akita H, Masuda T, Hayashi Y, Konno T, Ishihara K, Harashima H (2010) 2-Methacryloyloxyethyl phosphorylcholine polymer (MPC)-coating improves the transfection activity of GALA-modified lipid nanoparticles by assisting the cellular uptake and intracellular dissociation of plasmid DNA in primary hepatocytes. Biomaterials 31(24):6355–6362

Unger T, Oren Z, Shai Y (2001) The effect of cyclization of magainin 2 and melittin analogues on structure, function, and model membrane interactions: implication to their mode of action. Biochemistry 40(21):6388–6397

Veerabadran NG, Goli PL, Stewart-Clark SS, Lvov YM, Mills DK (2007) Nanoencapsulation of stem cells within polyelectrolyte multilayer shells. Macromol Biosci 7(7):877–882

Vial F, Rabhi S, Tribet C (2005) Association of octyl-modified poly(acrylic acid) onto unilamellar vesicles of lipids and kinetics of vesicle disruption. Langmuir 21(3):853–862

Vial F, Oukhaled AG, Auvray L, Tribet C (2007) Long-living channels of well defined radius opened in lipid bilayers by polydisperse, hydrophobically-modified polyacrylic acids. Soft Matter 3(1):75–78

Vial F, Cousin F, Bouteiller L, Tribet C (2009) Rate of permeabilization of giant vesicles by amphiphilic polyacrylates compared to the adsorption of these polymers onto large vesicles and tethered lipid bilayers. Langmuir 25(13):7506–7513

Vives E, Brodin P, Lebleu B (1997) A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. J Biol Chem 272(25): 16010–16017

Vogel H, Jahnig F (1986) The structure of melittin in membranes. Biophys J 50(4):573–582

Walrant A, Correia I, Jiao C-Y, Lequin O, Bent EH, Goasdoue N, Lacombe C, Chassaing G, Sagan S, Alves ID (2011) Different membrane behaviour and cellular uptake of three basic arginine-rich peptides. Biochim Biophys Acta Biomembr 1808(1):382–393

Walrant A, Vogel A, Correia I, Lequin O, Olausson BES, Desbat B, Sagan S, Alves ID (2012) Membrane interactions of two arginine-rich peptides with different cell internalization capacities. Biochim Biophys Acta Biomembr 1818(7):1755–1763

Weerakkody D, Moshnikova A, Thakur MS, Moshnikova V, Daniels J, Engelman DM, Andreev OA, Reshetnyak YK (2013) Family of pH (low) insertion peptides for tumor targeting. Proc Natl Acad Sci USA 110(15):5834–5839

Werner M, Sommer JU, Baulin VA (2012) Homo-polymers with balanced hydrophobicity translocate through lipid bilayers and enhance local solvent permeability. Soft Matter 8(46): 11714–11722

Wieprecht T, Beyermann M, Seelig J (1999) Binding of antibacterial magainin peptides to electrically neutral membranes: thermodynamics and structure. Biochemistry 38(32):10377–10387

Wilson JT, Cui WX, Kozovskaya V, Kharlampieva E, Pan D, Qu Z, Krishnamurthy VR, Mets J, Kumar V, Wen J, Song YH, Tsukruk VV, Chaikof EL (2011) Cell surface engineering with polyelectrolyte multilayer thin films. J Am Chem Soc 133(18):7054–7064

Wimley WC (2010) Describing the mechanism of antimicrobial peptide action with the interfacial activity model. ACS Chem Biol 5(10):905–917

Yang Z, Sahay G, Sriadibhatla S, Kabanov AV (2008) Amphiphilic block copolymers enhance cellular uptake and nuclear entry of polyplex-delivered DNA. Bioconj Chem 19(10):1987–1994

Yaroslavov AA, Melik-Nubarov NS, Menger FM (2006) Polymer-induced flip–flop in biomembranes. Acc Chem Res 39(10): 702–710

Ye J, Fox SA, Cudic M, Rezler EM, Lauer JL, Fields GB, Terentis AC (2010) Determination of penetratin secondary structure in live cells with raman microscopy. J Am Chem Soc 132(3): 980–988

Yessine MA, Leroux JC (2004) Membrane-destabilizing polyanions: interaction with lipid bilayers and endosomal escape of biomacromolecules. Adv Drug Deliv Rev 56(7):999–1021

Yessine MA, Dufresne MH, Meier C, Petereit HU, Leroux JC (2007) Proton-actuated membrane-destabilizing polyion complex micelles. Bioconj Chem 18(3):1010–1014

Yook S, Jeong JH, Jung YS, Hong SW, Im BH, Seo JW, Park JB, Lee M, Ahn CH, Lee H, Lee DY, Byun Y (2012) Molecularly engineered islet cell clusters for diabetes mellitus treatment. Cell Transpl 21(8):1775–1789

Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415(6870):389–395

Zhang SW, Nelson A, Coldrick Z, Chen RJ (2011) The effects of substituent grafting on the interaction of pH-responsive polymers with phospholipid monolayers. Langmuir 27(13):8530–8539

Ziegler A, Blatter XL, Seelig A, Seelig J (2003) Protein transduction domains of HIV-1 and SIV TAT interact with charged lipid vesicles. Binding mechanism and thermodynamic analysis. Biochemistry 42(30):9185–9194

Zorko M, Langel U (2005) Cell-penetrating peptides: mechanism and kinetics of cargo delivery. Adv Drug Deliv Rev 57(4):529–545