Cell-penetrating poly(disulfide)s: focus on substrate-initiated co-polymerization

Bang, Eun Kyoung; Ward, Sandra Marie; Gasparini, Giulio; Sakai, Naomi; Matile, Stefan

How to cite

BANG, Eun Kyoung et al. Cell-penetrating poly(disulfide)s: focus on substrate-initiated co-polymerization. In: Polymer chemistry, 2014, vol. 5, n° 7, p. 2433–2441. doi: 10.1039/c3py01570j

This publication URL: https://archive-ouverte.unige.ch/unige:34700
Publication DOI: 10.1039/c3py01570j

Cell-penetrating poly(disulfide)s: focus on substrate-initiated co-polymerization

Eun-Kyoung Bang,[a] Sandra Ward,[a] Giulio Gasparini,[a] Naomi Sakai[a] and Stefan Matile*[a]

Outperforming cell-penetrating peptides, cell-penetrating poly(disulfide)s are attracting increasing attention. Recently we have shown that cell-penetrating poly(disulfide)s can be grown directly on substrates of free choice before delivery and depolymerized right afterwards. These unique characteristics are compatible with the general, non-toxic, traceless yet covalent delivery of substrates in unmodified form. The objective of this study was to elaborate on substrate-initiated co-polymerization. The original propagators contain a strained disulfide for ring-opening disulfide-exchange polymerization and a guanidinium cation to assure cell-penetrating activity. Here, we report individually optimized conditions to polymerize these original propagators together with several other propagators. The nature of these new propagators significantly affected polymerization efficiency and conditions as well as size, polydispersity and transport activity of the final co-polymers. According to gel permeation chromatography, the length of co-polymers increases with hydrophobicity, bulk and valency of the co-propagators, whereas ion pairing with boronates gives shorter co-polymers and branching increases polydispersity. The activity of co-polymers increases with length, π-acidity, superhydrophobicity and boronate counterions. Hydrophobicity, π-basicity, bulk and branching appear less important for activity in fluorogenic vesicles. The here reported design, synthesis and evaluation of substrate-initiated co-polymers will be essential to find the best cell-penetrating poly(disulfide)s.

Introduction

By mimicking cell-penetrating peptides (CPPs), cationic polymers have been widely studied for the delivery of broad variety substrates, including oligonucleotides. CPPs are small peptides with the ability to penetrate cellular membranes. Since the report of the first CPP, the *TAT* protein transduction domain,[1,2] numerous CPPs have been discovered and modified.[3] Most CPPs contain a large number of cationic lysine and arginine residues, which are essential for their cell penetrating ability. Their translocation across the membrane occurs presumably by counterion exchange with lipophilic anions, including lipids, either during, after or instead of endocytosis.[4-6] There is an abundance of synthetic cationic carriers covering the field of drug delivery, gene delivery, and tissue engineering that share structural motifs with CPPs.[7-18] Among cations, ammonium, amidinum, guanidinium and phosphonium cations were reported as the most effective to improve cellular uptake.[19-20]

Many cationic polymers that resemble CPPs but have a different backbone have been reported.[21-49] This includes β-peptides[29-33] and oligocarbamates.[34-35] Recently, the Kiessling group reported the synthesis of cell-penetrating polymers by ring-opening metathesis polymerization (ROMP) of norbornene,[36-37] and the Tew group studied poly(oxanorbornene)s.[38-42] Methacrylate,[43-44] L-proline and its derivatives[45-47] or cyclic carbonates[48-49] have also been used as monomers to construct CPP-like polymers. Some of these polymers were conjugated with drugs or oligonucleotides to facilitate their cellular uptake, and were used as antibacterial agents due to their high affinity to their anionic membranes. However, longer polymers with high cationic charges are toxic. Moreover, because of their exceptional affinity, they fail to release polyanionic substrates such as RNA or DNA. Biodegradable transporters are increasingly considered to minimize toxicity and maximize release.[50-58] Cell-penetrating poly(disulfide)s have been introduced as transporters of the future for this reason. However, until today, they are mostly prepared by polymerization method unrelated to disulfide chemistry and used exclusively for the non-covalent delivery of RNA and DNA in

biodegradable polyplexes. Inspired by surface-initiated polymerization of multicomponent photosystems,[59-63] we have reported this year that cell-penetrating poly(disulfide)s can be grown directly on substrates of free choice, in situ, under mild conditions, right before delivery (Fig. 1).[64] This substrate-initiated synthesis of cell-penetrating poly(disulfide)s by ring-opening disulfide-exchange polymerization is interesting because it promises access to the covalent delivery of unmodified substrates of free choice, far beyond RNA and DNA, including, hopefully, also proteins, antibodies, quantum dots, and other probes and drugs (Fig. 1).

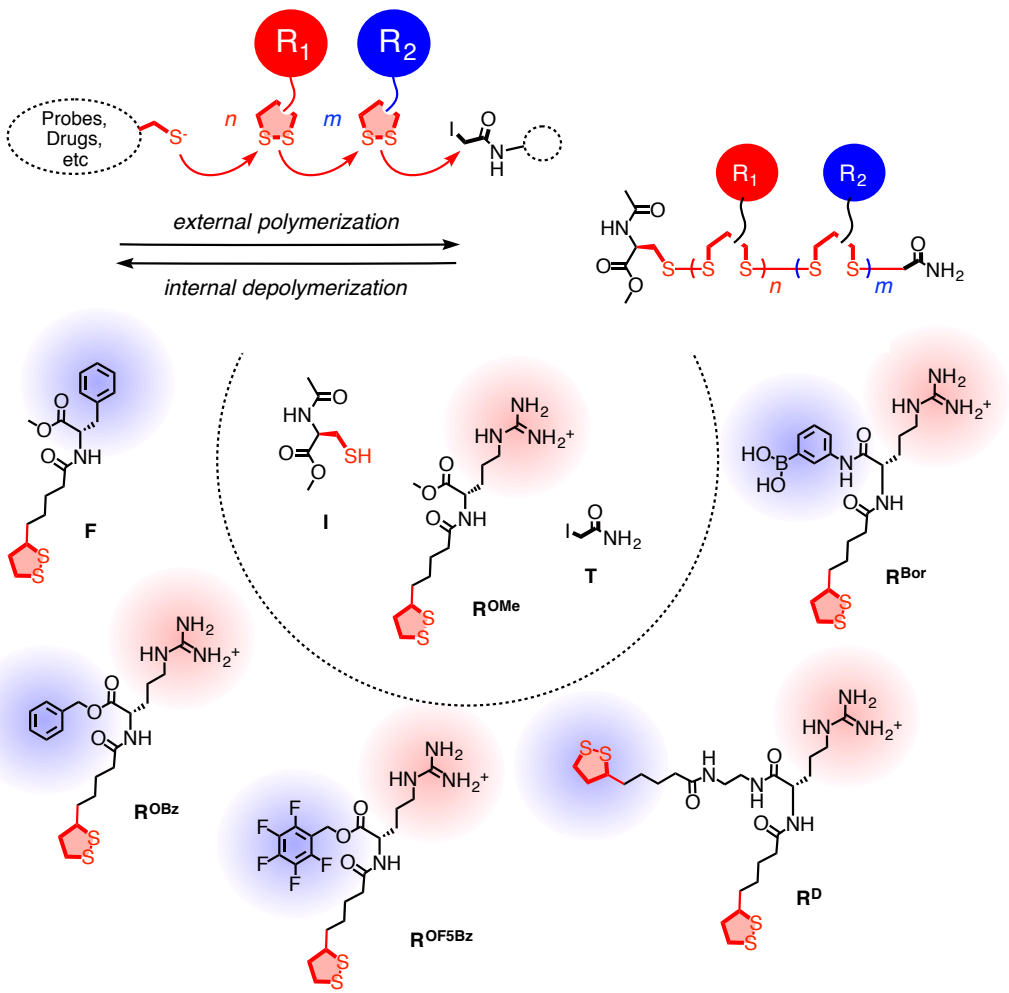


Fig. 1. Substrate-initiated co-polymerization is expected to yield bifunctional cell-penetrating pooly(disulfide)s, in situ, under mild conditions. Their depolymerisation right after uptake is expected to minimize toxicity and maximize substrate release. Based on the operational system

composed of initiator **I**, propagator $\mathbf{R}^{\mathbf{OMe}}$ and terminator **T**, substrate-initiated co-polymerization is realized for the new propagators **F**, $\mathbf{R}^{\mathbf{OBz}}$, $\mathbf{R}^{\mathbf{OF5Bz}}$, $\mathbf{R}^{\mathbf{D}}$ and $\mathbf{R}^{\mathbf{Bor}}$.

As original initiators and propagators of ring-opening disulfide-exchange polymerization, the cysteine derivative **I** and the arginine derivative $\mathbf{R}^{\mathbf{OMe}}$ were used.[64] In propagator $\mathbf{R}^{\mathbf{OMe}}$, the arginine is coupled with lipoic acid to introduce the strained disulfide needed for ring-opening disulfide polymerization. Racemic lipoic acid is used because the enantiopurity of cell-penetrating poly(disulfide)s is presumably irrelevant for activity. Alternative strained disulfides from asparagusic acid, perfect for surface-initiated polymerization, are too reactive for substrate-initiated polymerization.[64,65]

To grow cell-penetrating poly(disulfide)s on the model substrate **I**, the thiolate attacks the strained disulfide in propagator $\mathbf{R}^{\mathbf{OMe}}$, forms a disulfide bond between **I** and $\mathbf{R}^{\mathbf{OMe}}$ and regenerates a free thiolate for continuing disulfide-exchange polymerization. For termination, simple iodoacetates such as **T** have been used most often. This process occurs within minutes in neutral water at room temperature. It affords cell-penetrating poly(disulfide)s that grow in situ on their substrates but depolymerize within minutes in the presence of thiols at cytosolic glutathione concentrations. Compatibility with fluorescent initiators and terminators has been demonstrated,[64] and preliminary results from uptake into HeLa cells support highest expectations.[66] These exciting results with poly($\mathbf{R}^{\mathbf{OMe}}$) called for synthetic access to more sophisticated cell-penetrating poly(disulfide)s. Here, we introduce co-polymerization as a simple and general method to produce bifunctional cell-penetrating poly(disulfide)s, and demonstrate that the realized structural diversity is relevant for function.

Results and Discussion

Design

Five new propagators were synthesized to explore synthetic routes to refined cell-penetrating poly(disulfide)s by substrate-initiated co-polymerization (Fig. 1). All are based on racemic lipoic acid to assure unchanged properties with regard to disulfide-exchange polymerization. In **F**, lipoic acid is coupled with phenylalanine. Co-polymerized with the cationic propagator $\mathbf{R^{OMe}}$, increasing incorporation of propagators **F** should thus afford increasingly hydrophobic cell-penetrating poly(disulfide)s. Aromatic groups were preferred over aliphatic groups to increase hydrophobicity because aromatic groups are present at the outer surface of membrane proteins to both assure positioning at the interface and accelerate translocation.[67-69] Increased activity of CPP mimics with aromatic rather than aliphatic components or counterions has been observed repeatedly.[42,70-72] The preferential location of π-basic aromatics at the membrane interface has been attributed to cation-π interactions with cations in lipid head groups such as the most abundant phosphatidylcholine.[67]

Propagator $\mathbf{R^{OBz}}$ contains a π-basic phenyl group like propagator **F** but also a guanidinium cation like the original propagator $\mathbf{R^{OMe}}$. Co-polymerization of $\mathbf{R^{OMe}}$ with $\mathbf{R^{OBz}}$ will thus increase the hydrophobicity of poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$) without losses in positive charges. This is complementary to poly($\mathbf{R^{OMe}}$/**F**), in which increasing hydrophobicity comes with a decrease in charge. At the same time, $\mathbf{R^{OBz}}$ increases the steric demand of the side chains along the poly(disulfide) backbone of poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$), whereas poly($\mathbf{R^{OMe}}$/**F**) increases hydrophobicity without significant increase in steric bulk.

In propagator $\mathbf{R^{OF5Bz}}$, the π-basic phenyl group in $\mathbf{R^{OBz}}$ is replaced by a π-acidic[73-79] and superhydrophobic[80-85] pentafluorophenyl group. Comparison of co-polymers poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$) and poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OF5Bz}}$) should thus provide insights on the importance of anion-π interactions[73-79] and superhydrophobicity for transport activity in lipid bilayer membranes. Superhydrophobicity has been reported to improve transport in lipid bilayers, including gene delivery.[80-85]

In propagator $\mathbf{R^{Bor}}$, the phenyl group in $\mathbf{R^{OBz}}$ is equipped with a boronic acid. This group is mainly introduced because orthogonal dynamic covalent bonds with glycosaminoglycans at the cell

surface have been suspected to improve cellular uptake.[58,86] Proximity effects[3-5,87] are further expected to decrease the acidity of the conjugate base ($pK_a \sim 8.8$)[88-92] and stabilize the boronate anions by intramolecular ion pairing with the guanidinium cations in co-polymers poly($\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$).

Propagator $\mathbf{R^D}$, finally, contains two strained disulfides besides the guanidinium cation. Co-polymers poly($\mathbf{R^{OMe}}$/$\mathbf{R^D}$) should thus inform on the importance of branching for transport activity in lipid bilayer membranes. Branched oligoguanidiniums have attracted much recent attention because of their promise to provide access to functional materials with unique properties.[93-94]

Synthesis

The synthesis of all new propagators was very straightforward based on the protocols developed for the original propagator $\mathbf{R^{OMe}}$ (Scheme S2).[64] In brief, propagator **F** was obtained by amide coupling between an activated lipoic acid and L-phenylalanine amide. $\mathbf{R^{OBz}}$ was prepared correspondingly by amide formation between L-arginine benzyl ester and lipoic acid. Propagator $\mathbf{R^{OF5Bz}}$ was synthesized following the procedures of $\mathbf{R^{OBz}}$ using pentafluorobenzyl bromide.

For the synthesis of propagator $\mathbf{R^{Bor}}$, 3-aminophenyl-boronic acid was coupled to a Pbf- and Boc-protected L-arginine. Full deprotection followed by coupling with lipoic acid readily afforded $\mathbf{R^{Bor}}$. The divalent propagator $\mathbf{R^D}$ was prepared from Boc-protected L-arginine. *N*-Boc-ethylenediamine was reacted first with the carboxylic acid. Deprotection of the diamine and reaction with two lipoic acids readily gave $\mathbf{R^D}$. Detailed synthetic procedures and analytical data of all new compounds are reported in the Supporting Information.

Co-Polymerization

The conditions for substrate-initiated polymerization of $\mathbf{R^{OMe}}$ have been carefully optimized.[64] The reaction proceeds within minutes in degassed water at room temperature, pH 7.5, in the presence of 5 mM initiator **I**, 200 mM propagator and 800 mM triethanolamine (TEOA). Termination is best with 500 mM iodoacetate **T**. Under these conditions, little random polymerization is observed in

the absence of the initiator. The use of other initiators did not influence this behavior significantly.[64,66]

For co-polymerization, these conditions had to be slightly adjusted to reach optimal performance in the presence of both propagators. With $\mathbf{R^{OMe}}$ alone, the presence of up to 10% MeOH was tolerated without significant change (Table S1). The presence of 20% DMF generally increased solubility but decelerated the reaction. With 800 mM TEOA at pH 7.0, polymerization of $\mathbf{R^{OMe}}$ alone remained substrate-initiated (i.e., proceeded poorly without 5 mM initiator) but took 30 minutes to reach completion (Fig. 2●, Table S1).

In all co-polymerization studies, the operational propagator $\mathbf{R^{OMe}}$ was used to begin with, and the presence of the new propagators was gradually increased under constant readjustment of the conditions. Slight variations of the conditions could afford polymers with quite different properties, i.e., different length, polydispersity and transport activity. For most propagators, slow polymerization in water/DMF 4:1 at pH 7.0 was required to assure good solubility (i.e., **F**, $\mathbf{R^{OBz}}$, $\mathbf{R^{OF5Bz}}$ and $\mathbf{R^{Bor}}$, Table S1). With 200 mM total propagator concentration, 5 mM initiator and 800 mM TEOA at room temperature, the reactions were completed after 30 minutes. Only poly($\mathbf{R^{OMe}}$/$\mathbf{R^{D}}$) could be grown in less than a minute in water/MeOH 9:1 at pH 7.5. The structural and functional characteristics of each series of co-polymers will be described one-by-one in the following.

For each co-polymer prepared, the incorporation of the new propagator was confirmed quantitatively. For this purpose, they were first purified by gel-permeation chromatography (GPC). The pure co-polymers were depolymerized with DTT, and the products were characterized and quantified by LC-MS and HPLC (Fig. S1, Table S2 and S3). Incorporation of the new propagators into the co-polymers was confirmed consistently, some slightly exceeded expectations.

Attempts to polymerize the new propagators in the absence of $\mathbf{R^{OMe}}$ did not yet give satisfactory results. These difficulties confirmed observations with multicomponent architectures on surfaces

that ring-opening disulfide-exchange polymerization depends strongly on the structure of the propagators and requires always careful optimization.[59-63,65] For the substrate-initiated synthesis of cell-penetrating poly(disulfide)s, this possibility to optimize the system was explored and confirmed only for the divalent propagator $\mathbf{R^D}$ (see below).

π-Basic, Hydrophobic Cell-Penetrating Poly(disulfide)s

Co-polymers poly($\mathbf{R^{OMe}}$/**F**) with increasing content of **F** were prepared in water/DMF 8:1 at pH 7.0 in 30 minutes at room temperature. GPC profiles revealed that with increasing **F** content, the length of poly($\mathbf{R^{OMe}}$/**F**) increased. For example, a number average molecular weight M_n = 11.1 kD was found for $\mathbf{R^{OMe}}$/**F** = 40:1, whereas poly($\mathbf{R^{OMe}}$/**F**)$_{8:1}$ had M_n = 16.2 kD (Fig. 2a, Table S5). The polydispersity index PDI = 1.13, in contrast, was independent of length and hydrophobicity of poly($\mathbf{R^{OMe}}$/**F**) (Table S5). These different length' with constant PDI values were obtained although the total concentration of propagators was kept constant. This suggested that hydrophobic interactions could possibly increase the local propagator concentration during co-polymerization.

The transport activity of co-polymers was tested in fluorogenic vesicles under routine conditions. Fluorogenic vesicles are convenient analytical tools to follow reactions with minimal effort and maximal speed.[95-97] In brief, large unilamellar vesicles (LUVs) composed of egg yolk phosphatidylcholine (EYPC) were loaded with 5(6)-carboxyfluorescein (CF) at concentrations high enough to assure self-quenching. In this assay, CF export from EYPC-LUVs⊃CF is reported as fluorescence recovery because local dilution reduces self-quenching. This assay was convenient to follow the synthesis of cell-penetrating poly(disulfide)s by ring-opening disulfide-exchange polymerization because the transport activity of the polymers exceeds that of all monomers by far. To quantify transport activity of cell-penetrating poly(disulfide)s, the dependence of the activities to the monomer concentration was recorded. Analysis of the resulting curves by the Hill equation

afforded the EC_{50}'s, that is the effective concentration needed to reach 50% of the maximal accessible activity Y_{MAX}.

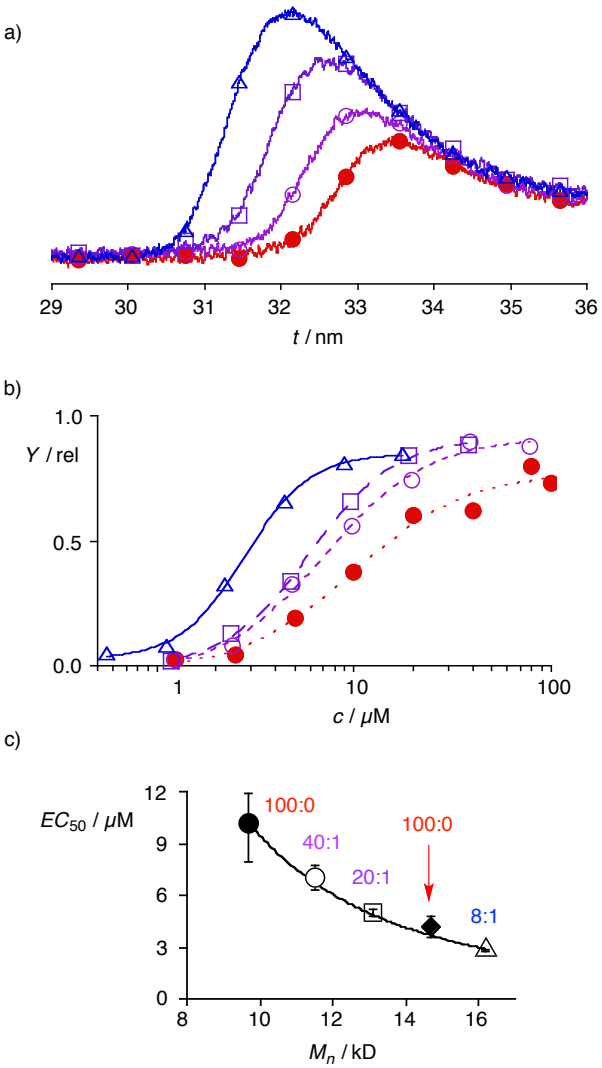


Fig. 2 The characteristics of poly($\mathbf{R^{OMe}}$/**F**): a) GPC profiles and b) transport activity Y in EYPC-LUVs⊃CF for $\mathbf{R^{OMe}}$/**F** = 8:1 (△), 20:1 (□), 40:1 (○), 100:0 (●). c) The dependence of EC_{50} for activity in fluorogenic vesicles on M_n of the (co-)polymers in a and b compared to a longer poly($\mathbf{R^{OMe}}$) (◆) and equation (1) (solid line). Concentrations were calculated based on the number of guanidinium ions, M_n indicates the number average molecular weight.

When the content of **F** in poly(**R**$^{\mathbf{OMe}}$/**F**), was increased, higher transport activity was observed in fluorogenic vesicles (Fig. 2b). To differentiate between contributions from hydrophobicity and length to this increase in activity, a longer homopolymer poly(**R**$^{\mathbf{OMe}}$) was prepared under different conditions. This longer poly(**R**$^{\mathbf{OMe}}$) had an EC_{50} value (4.2 ± 0.6 μM, M_n = 14.7 kD, Fig. 2c◆) that was similar to one of co-polymers poly(**R**$^{\mathbf{OMe}}$/**F**) of similar length (5.9 ± 0.2 μM, M_n = 13.1 kD). Independent of its composition, the dependence of activity EC_{50} on polymer size M_n showed excellent agreement with equation (1)[98]

$$EC_{50} \propto M_n^{-m} \qquad (1)$$

A cooperativity coefficient m = 2.46 was found (Fig. 2c, solid line). This value is comparably high. Other guanidinium-rich transporters had m = 1.76,[98] and highly organized anion-π slides were with m = 2.13 still less cooperative than poly(**R**$^{\mathbf{OMe}}$/**F**).[99] Only transmembrane halogen bonding cascades were clearly better, their m = 3.37 remains extraordinary.[99]

Taken together, these findings suggested that the activity of poly(**R**$^{\mathbf{OMe}}$/**F**) in fluorogenic vesicles is determined exclusively by the length of the linear polymer, whereas hydrophobicity and the presence of π-basic aromatics is irrelevant. This conclusion was surprising considering the importance of π-basic aromatics in cell-penetrating peptide mimics as well as in membrane proteins.[67-72] An appropriate quantity of lipophilic groups in cationic polymers has consistently been reported to enhance their cellular uptake.[70-72] Several amphiphilic CPPs contain hydrophobic amino acids such as phenylalanine, tryptophane, valine, leucine and isoleucine, and the balance between lipophilicity and positive charge is usually considered as essential to maximize uptake.[1-49,67-72,100-103]

The propagator **R**$^{\mathbf{OBz}}$ is both hydrophobic and cationic. Contrary to poly(**R**$^{\mathbf{OMe}}$/**F**), poly(**R**$^{\mathbf{OMe}}$/**R**$^{\mathbf{OBz}}$) allows thus to add π-basic aromatics without losses in charge density.

Interestingly, these bulky propagators $\mathbf{R^{OBz}}$ exhibited completely different behavior compared to the more compact propagators $\mathbf{R^{OMe}}$. Polymerized in the absence of $\mathbf{R^{OMe}}$, $\mathbf{R^{OBz}}$ gave sticky insoluble materials. The same was found in the presence of up to 80% $\mathbf{R^{OMe}}$. These sticky materials were inactive in fluorogenic vesicles and increasingly undetectable with increasing content of $\mathbf{R^{OBz}}$. With less than 20% $\mathbf{R^{OBz}}$, however, perfectly soluble poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$) could be obtained in 30 minutes with 5 mM initiator (**I** or equivalent), 800 mM TEOA and 200 mM propagator in water/DMF 4:1 at pH 7.0.

GPC profiles of co-polymers poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$) revealed best results at $\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$ = 8:1 (Fig. 3a□). Compared to poly($\mathbf{R^{OMe}}$/**F**)$_{8:1}$ (Fig. 2a△), the linear poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$)$_{8:1}$ was with M_n = 17.9 kD and PDI = 1.31 both longer and less homogenous. Like poly($\mathbf{R^{OMe}}$/**F**), co-polymers with decreasing content of π-basic propagators $\mathbf{R^{OBz}}$ were shorter (Fig. 3a○). Unlike poly($\mathbf{R^{OMe}}$/**F**), polymers with more $\mathbf{R^{OBz}}$, i.e., $\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$ < 8:1, were also shorter (Fig. 3a△, Table S6). This bell-shaped dependence of polymer length on polymer composition originated presumably from the unusual physical properties of $\mathbf{R^{OBz}}$-rich co-polymers.

Compared to poly($\mathbf{R^{OMe}}$/**F**)$_{8:1}$, the longest poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$)$_{8:1}$ showed comparable activity in fluorogenic vesicles (EC_{50} = 2.8 ± 0.1 μM). As with poly($\mathbf{R^{OMe}}$/**F**), the activity of poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$) decreased with decreasing content of π-basic propagators (Fig. 3cA). However, the more detailed analysis with poly($\mathbf{R^{OMe}}$/**F**) (Fig. 2c) suggested that decreasing co-polymer length rather than decreasing hydrophobicity account for the loss in activity of poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$)$_{16:1}$ compared to poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$)$_{8:1}$.

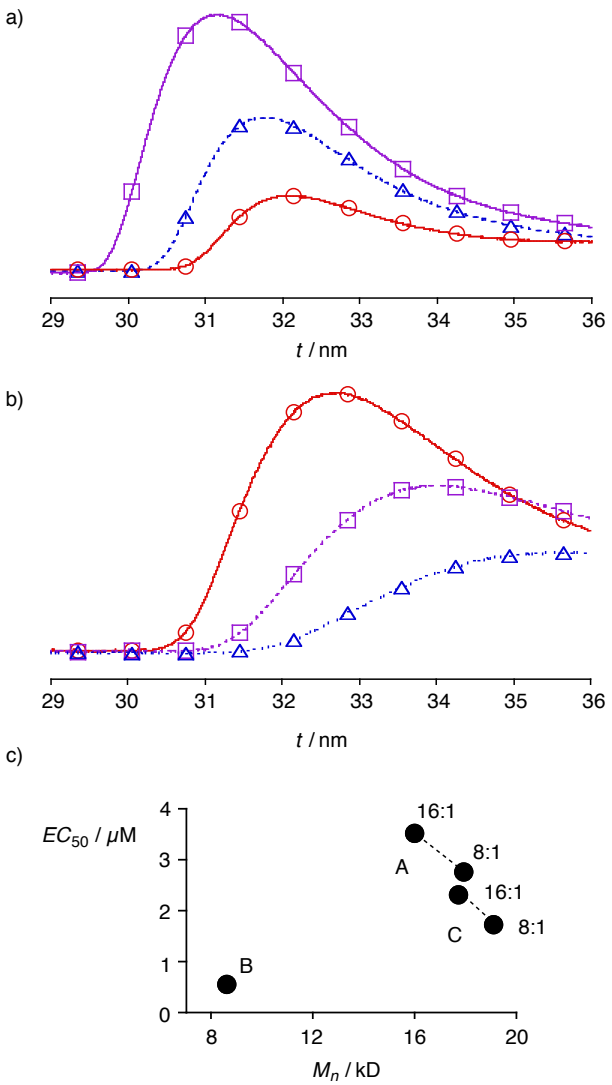


Fig. 3 The characteristics of poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$), poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$) and poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{Bor}}$): GPC profiles for a) poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$) and b) poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{Bor}}$) at $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{X}}$ = 4:1 (△), 8:1 (□) and 20:1 (○) ($\mathbf{R}^{\mathbf{X}}$ = $\mathbf{R}^{\mathbf{OBz}}$ or $\mathbf{R}^{\mathbf{Bor}}$. c) The EC_{50} for activity in EYPC-LUVs⊃CF as a function of the M_n of poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$) (A), poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{Bor}}$) (B) and poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$) (C).

π-Acidic, Superhydrophobic Cell-Penetrating Poly(disulfide)s

In propagator $\mathbf{R}^{\mathbf{OF5Bz}}$, the π-basic, hydrophobic phenyl group of $\mathbf{R}^{\mathbf{OBz}}$ is replaced by a π-acidic, superhydrophobic pentafluorophenyl group (Fig. 1). This change did not much influence conditions and outcome of co-polymerization. As with poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$), the best co-polymers were obtained at $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$ = 8:1, and further increase in $\mathbf{R}^{\mathbf{OF5Bz}}$ gave increasingly intractable, inactive material. According to GPC analysis, the π-acidic poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$) were consistently longer than the π-basic poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$) obtained under identical conditions (Fig. 3cC vs 3cA, Table S6).

Interestingly, analysis of the propagators incorporated into co-polymers revealed preferential incorporation of $\mathbf{R}^{\mathbf{OF5Bz}}$, particularly at high content. A quite remarkable $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$ = 4.6:1 was found for poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)$_{8:1}$ (Table S3). Poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)$_{16:1}$ still contained $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$ = 13.0:1. This preferential incorporation was unique for π-acidic, superhydrophobic propagators $\mathbf{R}^{\mathbf{OF5Bz}}$, their π-basic analogs $\mathbf{R}^{\mathbf{OBz}}$ did not deviate that much from expectations and were rather rejected than preferred, $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$ = 9.4:1 was found for poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)$_{8:1}$, $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$ = 20.4:1 for poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)$_{16:1}$ (Table S3). This intriguing accumulation of $\mathbf{R}^{\mathbf{OF5Bz}}$ in poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$) originates presumably from hydrophobic clustering. In the light of the recent discovery of "anion-π catalysis",[79] it is tempting to consider additional contributions from the interaction of thiolate anions on the π-acidic pentafluorophenyl surfaces during substrate-initiated ring-opening disulfide-exchange polymerization.

The π-acidic poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$) were clearly more active than the π-basic poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$) (Fig. 3cC). At identical length, the activity of poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)$_{16:1}$ exceeded that of poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)$_{8:1}$ slightly. The poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)$_{8:1}$ was both longer and more active than the complementary poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)$_{8:1}$. Preferred incorporation could account for much of the high activity of π-acidic poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$). As discussed above, the composition of poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$)$_{8:1}$ and poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$)$_{16:1}$ is not as different as expected ($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OBz}}$ = 9.4:1 against $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{OF5Bz}}$ = 13.0:1). However, at identical length, co-polymers with less π-acidic propagators $\mathbf{R}^{\mathbf{OF5Bz}}$ (1 out

of 13) remain more active than propagators with more π-basic propagators $\mathbf{R^{OBz}}$ (1 out of 9.4). The origin of this intrinsically better performance of π-acidic propagators is unclear at this point. Superhydrophobic effects and intramolecular π-stacking remain as attractive possibilities to be explored.[80-85] Most interesting would be contributions from possible, cooperative anion-π interactions[73-79] with phosphodiesters at the membrane surface, the anionic CF probes or inorganic anions in the buffer.

Cell-Penetrating Poly(disulfide)s with Boronic Acids

In propagator $\mathbf{R^{Bor}}$, the phenyl group of $\mathbf{R^{OBz}}$ is equipped with a boronic acid in *meta* position (Fig. 1). Whereas poly($\mathbf{R^{OMe}}$/**F**) grew longer with more **F** and poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OBz}}$) showed a bell-shaped length dependence, the poly($\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$) obtained with increasing amounts of $\mathbf{R^{Bor}}$ were shorter (Fig. 3b). This co-polymer shortening with increasing content of $\mathbf{R^{Bor}}$ was best explained with intramolecular ion pairing of the conjugate boronate bases with the guanidinium cations of $\mathbf{R^{OMe}}$ and $\mathbf{R^{Bor}}$. This would minimize intramolecular charge repulsion in cationic polymers and make them collapse and ultimately precipitate. The poor properties of poly($\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$) with $\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$ < 8:1, worse than with $\mathbf{R^{OBz}}$ and $\mathbf{R^{OF5Bz}}$, were in agreement with this interpretation. Despite these significant changes and contrary to the π-acidic $\mathbf{R^{OF5Bz}}$ in poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OF5Bz}}$), $\mathbf{R^{Bor}}$ was neither accumulated nor avoided in poly($\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$). After reductive depolymerization, $\mathbf{R^{OMe}}$/$\mathbf{R^{OF5Bz}}$ = 8.5:1 was found for poly($\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$)$_{8:1}$ (Table S3).

The comparably short and polydisperse poly($\mathbf{R^{OMe}}$/$\mathbf{R^{Bor}}$)$_{8:1}$ was remarkably active (M_n = 8.6 kD, PDI = 1.60, Fig. 3cB, Table S6). The EC_{50} = 551 ± 27 nM was among best in the entire series, about 3-times better than the long and π-acidic poly($\mathbf{R^{OMe}}$/$\mathbf{R^{OF5Bz}}$)$_{8:1}$ at EC_{50} = 1.73 ± 0.01 μM (Fig. 3cC). This high activity with short co-polymers suggested that intramolecular ion pairing between boronates and guanidiniums improves transport, presumably by increasing the overall hydrophobicity of the transporter. However, in anionic membranes and in the presence of glycosaminoglycans (GAGs), ion pairing to the membrane[3-5] and the formation of transient

boronate esters[86] are expected to dominate the properties of cell-penetrating poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{Bor}}$-disulfide)s.

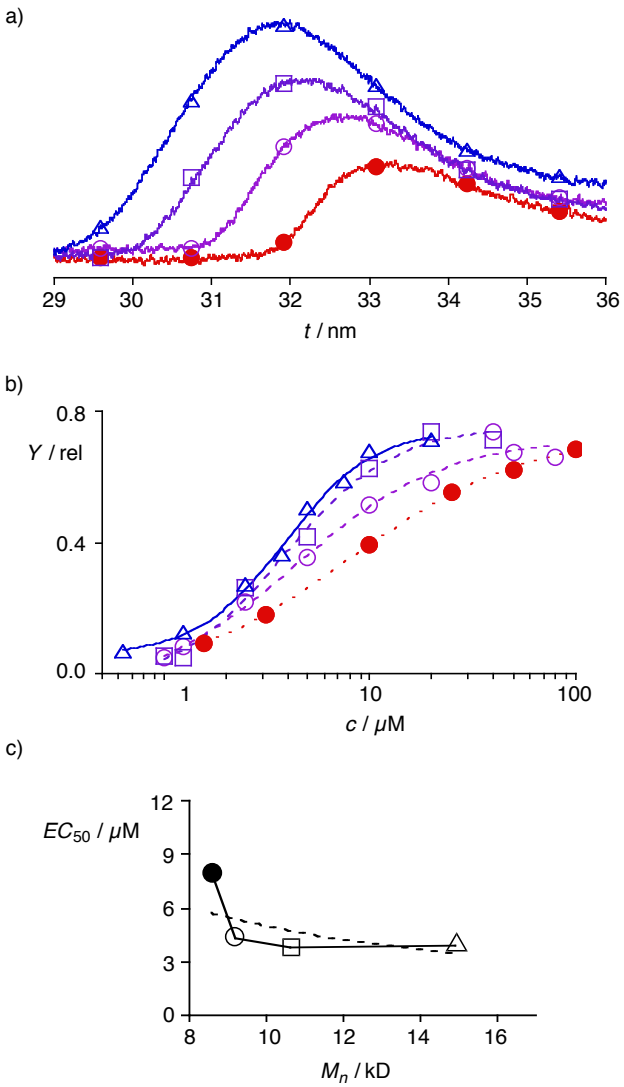


Fig. 4 The characteristics of poly($\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{D}}$): a) GPC profiles and b) transport activity *Y* in EYPC-LUVs⊃CF for $\mathbf{R}^{\mathbf{OMe}}$/$\mathbf{R}^{\mathbf{D}}$ = 4:1 (△), 8:1 (□), 20:1 (○), 100:0 (●). c) The dependence of EC_{50} for activity in fluorogenic vesicles on M_n of the (co-)polymers in a) and b) with fit to equation (1) (dashed line).

Branched Cell-Penetrating Poly(disulfide)s

Propagator $\mathbf{R^D}$ is special because of the presence of two strained disulfide together with the guanidinium cation needed for activity (Fig. 1). This divalent propagator should thus provide access to branched cell-penetrating poly(disulfide)s. Co-polymers poly($\mathbf{R^{OMe}/R^D}$) with increasing $\mathbf{R^D}$ content could be prepared in water/MeOH 9:1 at pH 7.5 in less than one minute at room temperature. Contrary to all other propagators, the presence of DMF was not required. GPC profiles revealed that with increasing content of $\mathbf{R^D}$, the size of poly($\mathbf{R^{OMe}/R^D}$) increased (Fig. 4a, Table S7). However, the M_n = 10.6 kD found for poly($\mathbf{R^{OMe}/R^D}$)$_{8:1}$ was clearly below the M_n = 16.2 kD of poly($\mathbf{R^{OMe}/F}$)$_{8:1}$ (Fig. 4a vs 2a, Table S5). Moreover, the PDI increased dramatically from 1.34 for poly($\mathbf{R^{OMe}/R^D}$)$_{20:1}$ to 1.54 for poly($\mathbf{R^{OMe}/R^D}$)$_{8:1}$. Such change was not observed with linear co-polymers and thus characteristic for branched co-polymers. For example, the PDI of poly($\mathbf{R^{OMe}/F}$)$_{40:1}$ and poly($\mathbf{R^{OMe}/F}$)$_{8:1}$ was identical and with 1.13 clearly below the 1.54 of poly($\mathbf{R^{OMe}/R^D}$)$_{8:1}$. The incorporation of divalent propagators $\mathbf{R^D}$ was preferred as expected from statistical consideration. The composition $\mathbf{R^{OMe}/R^D}$ = 4.0:1 was found for poly($\mathbf{R^{OMe}/R^D}$)$_{8:1}$ (Table S3).

The transport activity of poly($\mathbf{R^{OMe}/R^D}$) in fluorogenic vesicles increased with increasing incorporation of $\mathbf{R^D}$ (Fig. 4b, c). Interestingly, this increase did not follow equation (1) (Fig. 4c, dashed line). This finding suggested that the activity of branched poly($\mathbf{R^{OMe}/R^D}$) is not exclusively determined by size. This is contrary to the situation with linear poly($\mathbf{R^{OMe}/F}$) which was fully dominated by equation (1). We concluded that the branching of polyguanidiniums does indeed provide access to unusual properties.[93-94] However, the final activity of poly($\mathbf{R^{OMe}/R^D}$) at saturation was with an EC_{50} ~ 3.5 μM clearly, about 8-times weaker than the best activities found with linear polymers.

Propagator $\mathbf{R^D}$ was selected as an example to elaborate on the conditions for homopolymerization of new propagators (Fig. S3, Table S8). Best results were obtained with 50 mM $\mathbf{R^D}$ in water/DMF

1:1 at pH 8.0. Slowed down by the high DMF content, the reaction was complete in 60 minutes at room temperature. The presence of increasing concentrations of initiator **I** produced polymers with increasing transport activity, reaching from EC_{50} = 30.9 ± 8.8 μM without initiator to EC_{50} = 6.9 ± 1.0 μM with 5 mM initiator and remarkable EC_{50} = 870 ± 120 nM with 100 mM initiator. Clearly, the synthesis of branched homopolymer poly($\mathbf{R^D}$) occurred also by substrate-initiated disulfide-exchange polymerization. Under the same conditions, $\mathbf{R^{OMe}}$ was unable to polymerize even when the propagator concentration was doubled.

Conclusions

Earlier this year, we introduced the concept of cell-penetrating poly(disulfide)s that can grow directly on substrates of free choice right before and depolymerize right after their entry into cells.[64] This concept is attractive because it promises access to a general method for the covalent yet traceless and non-toxic delivery of native substrates, including drugs, probes, proteins, antibodies, quantum dots, RNA or DNA. This study focuses on co-polymerization. Substrate-initiated disulfide-exchange polymerization is realized in the presence of two propagators with different properties. The original propagator provides the guanidinium cation needed for transport activity in lipid bilayer membranes. The newly introduced propagators contain π-basic, hydrophobic or π-acidic, superhydrophobic aromatics, boronic acids or two strained disulfides to generate branched polymers.

Conditions were developed to incorporate all new propagators into the original cell-penetrating poly(disulfide)s. The incorporation of all new propagators was found to influence the properties of all new co-polymers. π-Basic, hydrophobic side chains besides guanidinium cations give longer polymer with increased activity. However, activity is shown to depend only on polymer length and, surprisingly, not on hydrophobicity. π-Acidic, superhydrophobic propagators are preferentially incorporated and yield co-polymers that are longer and more active than the complementary π-basic,

hydrophobic co-polymers. This finding is particularly interesting because of possible contributions of anion-π interactions to disulfide-exchange polymerization and transport across lipid bilayer membranes. The presence of boronic acids gives short co-polymers with high activity, intramolecular ion pairing between guanidinium cations and boronate anions is considered to account for these interesting properties. Branched cell-penetrating poly(disulfide)s have higher polydispersity than linear ones and their activity depends not only on size.

These interesting differences in activity were observed in fluorogenic vesicles. The question whether or not cellular uptake of the different co-polymers will be characterized by similarly attractive differences is currently being addressed. Preliminary results are highly encouraging, final results will be reported in due course.[66]

Acknowledgements

We thank C. Freyre for contributions to synthesis, D. Jeannerat, A. Pinto and S. Grass for NMR spectroscopy measurements, the Sciences Mass Spectrometry (SMS) platform for mass spectrometry services, and the University of Geneva, the European Research Council (ERC Advanced Investigator), the National Centre of Competence in Research (NCCR) Chemical Biology and the Swiss NSF for financial support.

Notes and references

[a] Department of Organic Chemistry, University of Geneva, Geneva, Switzerland Fax: (+41) 22 379 3215; E-mail: stefan.matile@unige.ch.

† Electronic Supplementary Information (ESI) available: Experimental details. See DOI: 10.1039/b000000x/

1 M. Green and P. M. Lowenstein, *Cell*, 1988, **55**, 1179-1188.

2 A. O. Frankel and C. O. Pabo, *Cell*, 1988, **55**, 1189-1193.

3 M. Ôkuyama, H. Laman, S. R. Kingsbury, C. Visintin, E. Leo, K. L. Eward, K. Stoeber, C. Boshoff, G. H. Williams and D. L. Selwood, *Nat. Methods*, 2007, **4**, 153-159.

4 N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2003, **125**, 14348-14356.

5 T. Takeuchi, M. Kosuge, A. Tadokoro, Y. Sugiura, M. Nishi, M. Kawata, N. Sakai, S. Matile and S. Futaki, *ACS Chem. Biol.*, 2006, **1**, 299-303.

6 S. Katayama, I. Nakase, Y. Yano, T. Murayama, Y. Nakata, K. Matsuzaki and S. Futaki, *Biochim. Biophys. Acta*, 2013, **1828**, 2134-2142.

7 S. Keshari Samal, M. Dash, S. Van Vlierberghe, D. L. Kaplan, E. Chiellini, C. can Bilstterswijk, L. Moroni and P. Dubruel, *Chem. Soc. Rev.*, 2012, **41**, 7147-7194.

8 J. He, K. Hristova and W. C. Wimley, *Angew. Chem. Int. Ed.*, 2012, **51**, 7150-7153.

9 K. M. Stewart, K. L. Horton and S. O. Kelley, *Org. Biomol. Chem*., 2008, **6**, 2242-2255.

10 K. L. Horton, M. P. Pereira, K. M. Stewart, S. B. Fonseca and S. O. Kelley, *ChemBioChem*, 2012, **13**, 476-485.

11 E. K. Lei, M. P. Pereira and S. O. Kelley, *Angew. Chem. Int. Ed*., 2013, **52**, 9660-9663.

12 B. A. Smith, D. S. Daniels, E. Abigail, G. E., Jordan, L. M. McGregor and A. Schepartz, *J. Am. Chem. Soc*., 2008, **130**, 2948-2949.

13 J. Fernandez-Carneado, M. Van Gool, V. Martos, S. Castel, P. Prados, J. De Mendoza and E. Giralt, *J. Am. Chem. Soc*., 2005, **127***,* 869-874.

14 M. Lnoue, W. Tong, J. D. Esko and Y. Tor, *ACS Chem. Biol*., 2013, **8**, 1383-1388.

15 A. V. Dix; L. Fischer, S. Sarrazin, C. P. H. Redgate, J. D. Esko and Y. Tor, *ChemBioChem*, 2010, **11**, 2302-2310.

16 N. W. Luedtke, P. Carmichael and Y. Tor, *J. Am. Chem. Soc*., 2003, **125***,* 12374-12375.

17 E. K. Esbjorner, P. Lincoln and B. Norden, *Biochem. Biophys. Acta*, 2007, **1768**, 1550-1558.

18 T. Jiang, E. S. Olson, Q. T. Nguyen, M. Roy, P. A. Jennings and R. Y. Tsien, *Proc. Natl. Acad. Sci. U.S.A*., 2004, **101**, 17867−17872.

19 D. Zhi, S. Zhang, S. Cui, Y. Zhao, Y. Wang and D. Zhao, *Bioconjugate Chem*., 2013, **24**, 487-519, and references therein.

20 C. Ornelas-Megiatto, P. R. Wich and J. M. Fréchet, *J. Am. Chem. Soc*., 2012, **134**, 1902-1905.

21 F. Sgolastra, B. M. Deronde, J. M. Sarapas, A. Som and G. N. Tew, *Acc. Chem. Res*., Doi:10.1021/ar400066v.

22 G. N. Tew, R. W. Scott, M. L. Klein and W. F. Degrado, *Acc. Chem. Res*., 2010, **43**, 30-39.

23 E. G. Stanzl, B. M. Trantow, J. R. Vargas and P. A. Wender, *Acc. Chem. Res*., Doi:10.1021/ar4000554.

24 P. A. Wender, C. B. Cooley and E. I. Geihe, *Drug Discov. Today: Technol*., 2012, **9**, e49-e55.

25 P. A. Wender, W. C. Galliher, E. A. Goun, L. R. Jones and T. H. Pillow, *Adv. Drug Delivery Rev*., 2008, **60**, 452−472.

26 A. D. Bautista, C. J. Craig, E. A. Harker and A. Shepartz, *Curr. Opin. Chem. Biol*., 2007, **11**, 685-692.

27 B. Layek and J. Singh, *Macromolecules*, 2013, **14**, 485-494.

28 N. Sakai, B. Baumeister and S. Matile., *ChemBioChem*, 2000, **1**, 123-125.

29 M. Rueping, Y. Mahajan, M. Sauer and D. Seebach, *ChemBioChem*, 2002, **3**, 257-259.

30 N. Umezawa, M. A. Gelman, M. C. Haigis, R. T. Raines and S. H. Gellman, *J. Am. Chem. Soc*., 2002, **124**, 368-369.

31 A. J. Karlsson, W. C. Pomerantz, K. J. Neilsen, S. H. Gellman and S. P. Palecek, *ACS Chem. Biol*., 2009, **4**, 567-679.

32 A. D. Bautista, J. S. Appelbaum, C. J. Craig, J. Michel and A. Schepartz, *J. Am. Chem. Soc*., 2010, **132**, 2904-2906.

33 E. A. Harker and A. Schepartz, *ChemBioChem*, 2009, **10**, 990-993.

34 P. A. Wender, J. B. Rothbard, T. C. Jessop, E. L. Kreider and B. L. Wylie, *J. Am. Chem. Soc*., 2002, **124**, 13382–13383.

35 Y. L. Chiu, A. Ali, C. Chu, H. Cao and T. M. Rana, *Chem. Biol*., 2004, **11**, 1165-1175.

36 E. M. Kolonko and L. L. Kiessling, *J. Am. Chem. Soc*., 2008, **130**, 5626-5627.

37 E. M. Kolonko, J. K. Pontrello, S. L. Mangold and L. L. Kiessling, *J. Am. Chem. Soc.,* 2009, **131**, 7327-7333.

38 G. J. Gabriel, A. E. Madkour, J. M. Dabkowski, C. F. Nelson, K. Nüsslein and G. N. Tew, *Biomacromolecules*, 2008, **9**, 2980–2983.

39 K. Lienkamp and G. N. Tew, *Chem. Eur. J*., 2009, **15**, 11784-11800.

40 A. O. Tezgel, J. C. Telfer and G. N. Tew, *Biomacromolecules*, 2011, **12**, 3078-3083.

41 A. O. Tezgel, G. Gonzalez-Perez, J. C. Telfer, B. A. Osborne, L. M. Minter and G. N. Tew, *Mol. Ther*., 2013, **21**, 201-209.

42 A. Som, A. O. Tezgel, G. J. Gabriel and G. N. Tew, *Angew. Chem. Int. Ed*., 2011, **50**, 6147-6150.

43 Y. Kim, S. Binauld and M. H. Stenzel, *Biomacromolecules*, 2012, **13**, 3418-3426.

44 N. J. Treat, D. Smith, C. Teng, J. D. Flores, B. A. Abel, A. W. York, F. Huang and C. L. McCormick, *ACS Macro Lett*., 2012**, 1***,* 100−104.

45 Y. Fillon, J. Anderson and J. Chmielewski, *J. Am. Chem. Soc*., 2005, **127**, 11798-11803.

46 J. Farrera-Sinfreu, E. Giralt, S. Castel, F. Albericio and M. Royo, *J. Am. Chem. Soc*., 2005, **127**, 9459-9468.

47 S. Pujals, J. Fernandez-Carneado, M. J. Kogan, J. Martinez, F. Cavelier and E. Giralt, *J. Am. Chem. Soc*., 2006, **128**, 8479-8483.

48 B. M. Trantow, C. B. Cooley, F. Nederberg, M. K. Kiesewetter, J. L. Hedrick, R. M. Waymouth and P. A. Wender, *J. Am. Chem. Soc*., 2009*,* **131***,* 16401-16403.

49 E. I. Geihe, C. B. Cooley, J. R. Simon, M. K. Kiesewetter, J. A. Edward, R. P. Hickerson, R. L. Kaspar, J. L. Hedrick, R. M. Waymouth and P. A. Wender, *Proc. Natl. Acad. Sci. USA*, 2012, **109**, 13171-13176.

50 F. H. Meng, W. E. Hennink and Z. Y. Zhong, *Biomaterials*, 2009, **30**, 2180-2198.

51 T.-I. Kim and S. W. Kim, *React. Funct. Polym*., 2011, **71**, 344-349.

52 E.-K. Bang, M. Lista, G. Sforazzini, N. Sakai and S. Matile, *Chem. Sci*., 2012, **3**, 1752-1763.

53 T. Kim, T. Rothmund, T. Kissel and S. W. Kim, *J. Control. Release*, 2011, **152**, 110-119.

54 C. A. Drake, A. Aissaoui, O. Argyros, M. Thanou, J. H. G. Steinke and A. D. Miller, *J. Control. Release*, 2013, **171**, 81-90.

55 C. Lin and J. F. J. Engbersen, *Expert Opin. Drug Deliv*., 2009, **6**, 421-439.

56 S. Son, R. Namgung, J. Kim, K. Singha and W. J. Kim, *Acc. Chem. Res*., 2011, **152**, 110-119.

57 H. Zeng, H. C. Little, T. N. Tiambeng, G. A. Williams and Z. Guan**,** *J. Am. Chem. Soc.*, 2013, **135**, 4962-4965.

58 M. Piest and J. F.J. Engbersen *J. Control. Release*, 2011, **155**, 331-340.

59 N. Sakai, M. Lista, O. Kel, S. Sakurai, D. Emery, J. Mareda, E. Vauthey and S. Matile, *J. Am. Chem. Soc*., 2011, **133**, 15224-15227.

60 M. Lista, J. Areephong, N. Sakai and S. Matile, *J. Am. Chem. Soc*., 2011, **133**, 15228-15230.

61 N. Sakai and S. Matile, *J. Am. Chem*. *Soc*., 2011, **133**, 18542-18545.

62 E. Orentas, M. Lista, N.-T. Lin, N. Sakai and S. Matile, *Nat. Chem*., 2012, **4**, 746-750.

63 G. Sforazzini, E. Orentas, A. Bolag, N. Sakai and S. Matile, *J. Am. Chem*. *Soc*., 2013, **135**, 12082-12090.

64 E.-K. Bang, G. Gasparini, G. Molinard, A. Roux, N. Sakai and S. Matile, *J. Am. Chem. Soc*., 2013, **135**, 2088-2091.

65. A. Carmine, Y. Domoto, N. Sakai and S. Matile, *Chem. Eur. J*., 2013, **19**, 11558-11563.

66 G. Gasparini, E.-K. Bang, G. Molinard, D. Tulumello, S. Ward, A. Roux, N. Sakai and S. Matile, in preparation.

67 G. Blaser, J. M. Sanderson and M. R. Wilson, *Org. Biomol. Chem.*, 2009, **7**, 5119-5128.

68 T. Hessa, N. M. Meindl-Beinker, A. Bernsel, H. Kim, Y. Sato, M. Lerch-Bader, M. Nilsson, S. H. White and G. von Heijne, *Nature*, 2007, **450**, 1026-1030.

69 M. Fernández-Vidal, S. H. White and A.S. Ladokhin, *J. Membr. Biol*., 2011, **239**, 5-14.

70 F. Perret, M. Nishihara, T. Takeuchi, S. Futaki, A. N. Lazar, A. W. Coleman, N. Sakai and S. Matile, *J. Am. Chem. Soc*., 2005, **127**, 1114-1115.

71 G. Creusat, A.-S. Rinaldi, E. Weiss, R. Elbaghdadi, J.-S. Remy, R. Mulherkar and G. Zuber, *Bioconjugate Chem*., 2010, **21**, 994-1002.

72 A. Som, A. Reuter and G. N. Tew, *Angew. Chem. Int. Ed*., 2012, **51**, 980-983.

73 A. Frontera, P. Gamez, M. Mascal, T. J. Mooibroek and J. Reedijk, *Angew. Chem. Int. Ed*., 2011, **50**, 9564-9583.

74 H. T. Chifotides and K. R. Dunbar, *Acc. Chem. Res*., 2013*,* **46**, 894-906.

75 L. M. Salonen, M. Ellermann and F. Diederich, *Angew. Chem. Int. Ed*., 2011, **50**, 4808-4842.

76 M. Giese, M. Albrecht, T. Krappitz, M. Peter, V. Gossen, G. Raabe, A. Valkonen and K. Rissanen, *Chem. Commun.*, 2012, **48**, 9983-9985.

77 D.-X. Wang and M.-X. Wang, *J. Am. Chem. Soc*., 2013, **135**, 892-897.

78 L. Adriaenssens, C. Estarellas, A. Vargas Jentzsch, M. Martinez Belmonte, S. Matile and P. Ballester, *J. Am. Chem*. *Soc*., 2013, **135**, 8324-8330.

79 Y. Zhao, Y. Domoto, E. Orentas, C. Beuchat, D. Emery, J. Mareda, N. Sakai and S. Matile *Angew. Chem. Int. Ed*., 2013, **52**, 9940-9943.

80 B. E. Smart, *J. Fluorine. Chem*., 2001, **109***,* 3-11.

81 C. J. Pace and J. Gao, *Acc. Chem. Res*., 2013, **46**, 907–915.

82 A. Vargas Jentzsch, D. Emery, J. Mareda, P. Metrangolo, G. Resnati and S. Matile, *Angew. Chem. Int. Ed*., 2011, **50**, 11675-11678.

83 A. Vargas Jentzsch, D. Emery, J. Mareda, S. K. Nayak, P. Metrangolo, G. Resnati, N. Sakai and S. Matile, *Nat. Commun*., 2012, **3**, 905.

84 M. C. Z. Kasuya, R. Cusi, O. Ishihara, A. Miyagawa, K. Hashimoto, T. Sato and K. Hatanaka*, Biochem. Biophys. Res. Commun*., 2004, **316***,* 599-604.

85 N. Dupuy, A. Pasc, S. Parant, S. Fontanay, R. E. Duval and C. Gérardin, *J. Fluorine Chem*., 2012, **135***,* 330-338.

86 G. A. Ellis, M. J. Palte and R. T. Raines, *J. Am. Chem. Soc*., 2012, **134**, 3631-3634.

87 B. Baumeister, A. Som, G. Das, N. Sakai, F. Vilbois, D. Gerard, S. P. Shahi and S. Matile, *Helv. Chim. Acta*, 2002, **85**, 2740-2753.

88 G. Springsteen and B. Wang, *Tetrahedron*, 2002, **58**, 5291-5300.

89 T. D. James, *Top Curr. Chem*., 2007, **277**, 107-152.

90 P. J. Duggan, T. A. Houston, M. J. Kiefel, S. M. Levonis, B. D. Smith and M. L. Szydzik, *Tetrahedron*, 2008, **64**, 7122-7126.

91 S. L. Wiskur and E. V. Anslyn, *J. Am. Chem. Soc*., 2001, **123**, 10109-10110.

92 S. Hagihara, H. Tanaka and S. Matile, *J. Am. Chem. Soc*., 2008, **130**, 5656-5657.

93 Y. Suzuki, K. Okuro, T. Takeuchi and T. Aida, *J. Am. Chem. Soc*., 2012, **134**, 15273−15276.

94 Q. Wang, J. L. Mynar, M. Yoshida, M.; E. Lee, M. Lee, K. Okuro, K. Kinbara and T. Aida, *Nature*, 2010, **463**, 339-343.

95 G. Das, P. Talukdar and S. Matile, *Science*, 2002, **298**, 1600-1602.

96 N. Sordé, G. Das and S. Matile, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 11964-11969.

97 N. Sakai, J. Mareda and S. Matile, *Acc. Chem. Res*., 2008, **41**, 1354-1365.

98 A. Hennig, G. J. Gabriel, G. N. Tew and S. Matile, *J. Am. Chem. Soc*., 2008, **130**, 10338-10344.

99 A. Vargas Jentzsch and S. Matile, *J. Am. Chem. Soc*., 2013, **135**, 5302-5303.

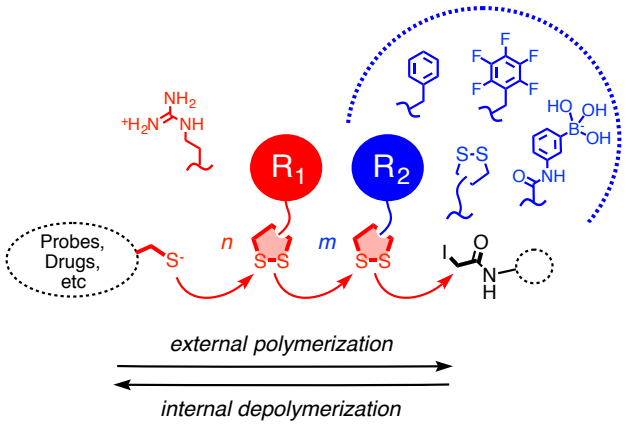
100 T. Takeuchi, J. Montenegro, A. Hennig and S. Matile, *Chem. Sci*., 2011, **2**, 303-307.

101 J. Montenegro, A. Fin and S. Matile, *Org. Biomol. Chem*., 2011, **9**, 2641-2647.

102 J. Montenegro, E.-K. Bang, N. Sakai and S. Matile, *Chem. Eur. J*., 2012, **18**, 10436-10443.

103 C. Gehin, J. Montenegro, E.-K. Bang, S. Takayama, H. Hirose, S. Futaki, A. Cajaraville, S. Matile and H. Riezman, *J. Am. Chem. Soc*., 2013, **135**, 9295-9298.

TOC



Substrate-initiated disulfide-exchange co-polymerization is introduced as the method of choice to systematically vary length, polydispersity, branching, charge, (super)hydrophobicity, intramolecular ion pairing and π-acidity of cell-penetrating poly(disulfide)s.