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VIEWPOINT

Antibacterial Activity of Polymers – Discussions on the Nature of Amphiphilic Balance

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The purpose of this viewpoint is to discuss the molecular design principles that guide development of synthetic antimicrobial polymers, especially those intended to mimic the structure of host defense peptides (HDPs). In particular, we focus on the principle of "amphiphilic balance" as it relates to some recently developed polyphosphoniums with somewhat atypical structure. We find that the fundamental concept of amphiphilic balance is still applicable to these new polymers, but that the method to achieve such balance is somewhat unique. We then briefly outline the future challenges and opportunities in this field.

In the recent paper "Surprising Antibacterial Activity and Selectivity of Hydrophilic Polyphosphoniums Featuring Sugar and Hydroxyl Substituents",[1] a set of novel polyphosponium-based polymers with alkyl, hydroxyl and sugar substituents was presented. This is a set of macromolecules that was synthesized through a new chemical approach and complements the existing classes of antimicrobial polymers. In particular, the mannose epitopes incorporated into these polymers for potential biorecognition via protein-carbohydrate interactions provides them with molecular features that could enable a different interaction mechanism for polymer-bacterial interaction compared to conventional polycationic antimicrobial polymers. Moreover, this library of phosphonium polymers yielded one member with very high antibacterial potency combined with very little toxicity to human red blood cells, and thus with very high selectivity: the hallmark of host defense peptide (HDP) and HDP mimics efficacy. This structure is a promising candidate for further study. Upon reflection, we felt the need to offer some additional discussion regarding the nature of the so-called "amphiphilic balance" design principle and how it might be applied to the structures in this recent paper.

In numerous studies over the past few decades, several groups have established the relationships between the structure and activity of antimicrobial polymers.[2] Universally, it has been found that some "balance" of hydrophobicity and cationic charge is an essential ingredient

for optimization of antimicrobial activity combined with low toxicity to human cells.[3] Many efforts have been directed towards mimicking the structures and activities of HDPs, which are composed of various sequences of amino acids that incorporate cationic, hydrophobic, and neutral hydrophilic pendant groups. It has been proposed from a mechanistic standpoint that HDPs first bind to the bacterial cell membrane via electrostatic attraction between their cationic residues and the anionic lipid headgroups, followed by insertion of their hydrophobic residues into the non-polar membrane core, resulting in membrane permeabilization.[4] Cationic and hydrophobic synthetic polymers behave in much the same manner. While neutral, hydrophilic residues (*e.g.* PEG, zwitterions, hydroxyls, and sugars) have been incorporated to fine-tune the amphiphilic balance to reduce toxicity, most antimicrobial polymers to date have involved hydrophobic alkyl chains, either directly conjugated to cationic centers or randomly alternating with cationic pendant groups (Figure 1a). This is particularly true of antimicrobial phosphonium polymers. Many structural variants have been investigated including different alkyl chains conjugated to phosphonium centers, different lengths of spacers to the polymer backbone, and different backbones.[5] These studies demonstrated structure-dependent antimicrobial activity.[5] In this context, the potent antibacterial activity of **HydroxyP^{P}**, a tris(hydroxypropyl)phosphinum-functionalized polystyrene derivative containing hydrophilic pendant groups, was not predicted *a priori* but rather it was originally intended as a negative control sample. The excellent hemocompatibility of this polymer was also serendipitous, as it was not the product of targeted structure-based design. That being said, these serendipitous results do not overturn the idea of amphiphilic balance as a molecular design principle.

Recently, a number of examples of active antibacterial polymers that do not contain linear alkyl side chains have been reported. For example, Gellman and coworkers were able to obtain good antibacterial activity and low hemolytic activity towards red blood cells using cyclic alkyl

groups as the hydrophobic residues on a polyamide backbone.[6] Haldar similarly showed that cyclization and unsaturation of the hydrophobic groups in amphiphilic polymers are important determinants of bioactivity.[7] Removing pendant hydrophobic groups altogether, Lienkamp and coworkers recently reported polyoxanorbornenes with pendant zwitterionic groups where the backbone imparted hydrophobicity instead.[8] Similarly, Palermo and coworkers reported self-immolative poly(benzyl ether)s with pendant oligo(ethylene glycol) and thioether-linked primary amines, which are both nominally hydrophilic, whereas the backbone is intensely hydrophobic.[9] Like **HydroxyP^{P}**, while these polymers do lack pendant linear alkyl side chains, they do not lack amphiphilicity. Thus, we do not want the final sentence of reference [1]: "This intriguing result challenges the hydrophilic/lipophilic balance previously thought to be required for antibacterial activity." to be misinterpreted. Indeed, all of the above molecules exhibit amphiphilicity. For example, as noted in reference [1] the hydrophobic polystyrene backbone and even the terminal reversible addition-fragmentation chain transfer (RAFT) group impart amphiphilicity to **HydroxyP^{P}**. Thus, we wish to emphasize that the observations described in reference [1], and other recent examples, do not require us to abandon the idea of amphiphilic balance altogether. Rather, we think it is important to highlight that amphiphilic balance is *not restricted to linear alkyl side chains* as the source of hydrophobicity.

Previous polyphosphoniums reported by Endo and coworkers featured long, linear alkyl side chains that increased the hydrophobicity of the polymer chains to such an extent that the polymers indiscriminately lysed biomembranes by a surfactant-like mode of biocidal action. By replacing the hydrophobic alkyl chains with neutral, modestly hydrophilic $-(CH_2)_3OH$ groups, we believe that the overall hydrophobicity has been dialed down to enable cell-type selectivity. In other words, a combination of cationic and neutral, hydrophilic groups provided the requisite

"balance" to counter the very hydrophobic polymer backbones. In **HydroxyP^{P}**, this balance just so happens to provide high antibacterial activity and low haemolytic activity.

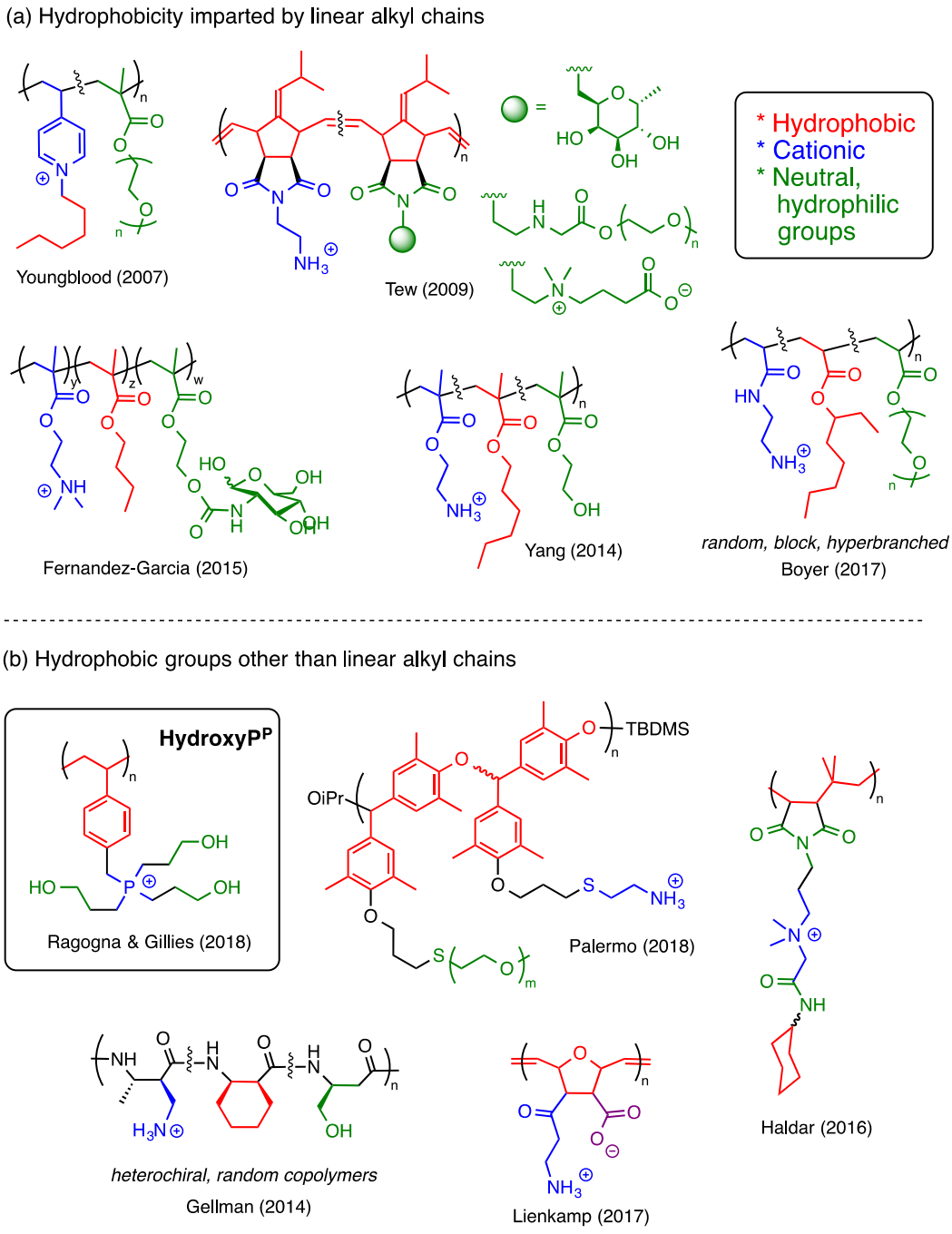

Figure 1. The broad diversity of polymer structures that employ a balance of hydrophobic, cationic, and neutral/hydrophilic groups to optimize biological activity. The image was reproduced, in part, from reference [3].

At this stage, the mechanism of action of **HydroxyP^{P}** has not yet been investigated. As suggested in reference [1], it is possible that **HydroxyP^{P}** acts by membrane disruption, or by an alternative mechanism. This can only be resolved through future experiments. For example, it will be important to quantify the hydrophobicity of the polymer directly using measurements such as HPLC retention times[10] and water-octanol partition coefficients,[2q, 9a] to probe the mechanism through experiments such as dye leakage from liposomes[2l, 2q-t, 2w, 10-11] and through electron microscopy.[12] It is possible that **HydroxyP^{P}** will turn out to be amphiphilic and membrane disrupting, in line with the other selective antimicrobial polymers. It will also be interesting to tune further the structural features of **HydroxyP^{P}** including its backbone and substituents to understand and optimize its activity.

As the field of antimicrobial polymers continues to expand, there are many key challenges and opportunities to deepen our understanding of structure-activity relationships as well as the mechanism(s) of action. In terms of structural optimization of polymer chains, increasingly precise control of comonomer sequence, chain length distributions, tacticity, and chain architecture are currently considered high value targets (Figure 2). Molecules with new structural features are being introduced with the aim of affording both high antibacterial activity and selectivity, a key challenge in this field.

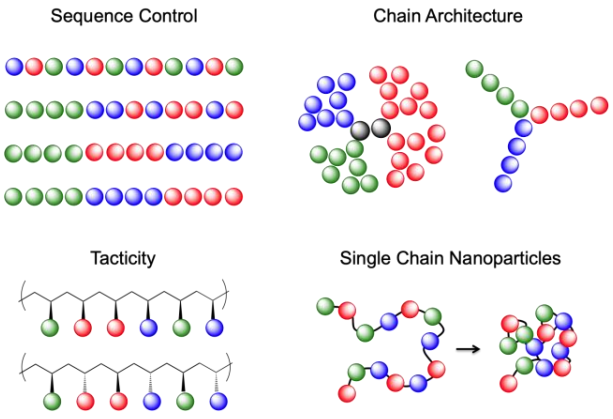

Figure 2. Precision control of polymer synthesis for next-generation antimicrobial polymers.

While **HydroxyP^{P}** incorporates novel structural features, it is nevertheless possible that it acts by a membrane-disruption mechanism with selectivity afforded through its optimal hydrophilic-hydrophobic balance.

Whether new classes of cationic antimicrobial polymers that act by alternative mechanisms can or have been developed remains an open question.

Growing concerns over the increasing emergence of antibiotic-resistant bacterial strains, combined with new synthetic polymer techniques, have led to a surge of recent interest in antibacterial polymers. Since the discovery of HDPs over 30 years ago, antimicrobial peptides and their synthetic mimics are just beginning to show promise in clinical trials. For example, the AMP-mimetic oligomer Brilacidin,[13] developed by Polymedix and later acquired by Cellceutix, has successfully completed a phase 2b clinical trial for skin infections, showing efficacy similar to daptomycin. It is also in phase 2 trials for oral mucositis. Considering that Brilacidin was specifically designed and synthesized to mimic the cationic and hydrophobic properties of HDPs, the clinical success provides strong motivation for further development in this field. Once AMP-mimetic polymer structures have been optimized for activity *in vitro*, it is crucial to translate prime candidates to *in vivo* studies that include biodistribution, pharamacokinetics and pharamacodynamics (PK/PD), as well as any side effects. Although the drug development process is exceedingly difficult, the benefits of a hit are potentially enormous. We look forward to witnessing the continued development of these vital polymers, ranging from the fundamentals of design and synthesis, to structure-activity relationships, mechanism of action, development of *in vivo* models, and ultimately clinical application.

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