Peptide-Assisted Design of Peptoid Sequences: One Small Step in Structure and Distinct Leaps in Functions

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ABSTRACT: Using peptide sequences for the design of functional peptoids is demonstrated for a peptide-based formulation additive that was specifically tailored to solubilize the photosensitizer meta-tetra(hydroxyphenyl)-chlorin. A set of peptoid-block-poly(ethylene glycol) solubilizers with systematic sequence variations are synthesized to reveal contributions of side-chain sequence and backbone functionalities on drug hosting and release properties. The drug payload sensitively depends on the side-chain patterns, and the best performing peptoid sequence reaches 3-times higher capacity than the corresponding peptide. The peptoid backbone not only acts as a neutral scaffold but also impacts the drug release kinetics compared to the analogues peptide, by reducing the capability to assist drug transfer to blood plasma protein models.

The advent of monodisperse precision polymers, which exhibit discrete monomer sequences composed from fully synthetic monomer alphabets, enables us to foresee the advance of polymer sciences toward exciting sequence-specific functions.1−3 Oligopeptides of 4−15 residues in length proved already the enormous potential of sequence control, being exploited in materials science applications over the last decades.4−12 While precision polymers are pushed toward sophisticated applications,13,14 strategies to assist sequence design of functional precision polymers are highly demanded. Some approaches are available such as, for instance, the rational sequence design strategies leading to antibacterial precision polymers15 or supported library screenings selecting precision formulation additives.16 Though combinatorial approaches that screen through precision oligomer libraries are advancing,16,17 a complementary strategy was described recently to assist the design of functional precision polymer sequences by learning from peptide sequences.18 The latter is exploited as a blueprint of a functional peptide, which was selected by combinatorial means to structure specifically host the photosensitizer meta-tetra(hydroxyphenyl)-chlorin (m-THPC) for delivery purposes.19−23 By mapping the side-chain functionality sequence of the peptide-based formulation additive toward precision platforms, functional precision polymers could be generated.18 The resulting precision segments mimic distinct aspects of the peptidic parent sequence, showed related sequence-dependent properties, and reached eventually higher drug payload capacities and more suitable release kinetics.18 Employing this strategy, peptidomimetic precision polymer segments for m-THPC hosting and release were realized based on oligo(N-substituted acrylamide),18 oligo(α-hydroxyl ester),18 and oligo(amide-urethane).16,22 It was noticeable that sequence-specific effects could be found rather independently of the backbone chemistries, reaching in the case of the oligo(amide-urethane) far off the peptide backbone structure. Nonetheless, the investigations made clear that the backbone cannot be seen simply as a neutral scaffold for presenting side-chain functionalities. Backbone polarity, conformational flexibility, and functionality contribute to the constituted function of those precision polymers. To gain more insights into the effects of backbone chemistry and site chain functionality sequences on m-THPC loadability and release properties, the class of peptoids with a closest relation to peptides was investigated. Peptoids (oligo(N-substituted glycines)) are among the first reports of peptidomimetics and provide a well-studied platform for (bio)medicinal chemistry and materials sciences.24−27

Here we present the direct translation of side-chain functionality sequences from a functional 7mer peptide toward a peptoid sequence by applying one-by-one residue mapping (Figure 1). Besides the peptoid sequence that correlates to the parent peptide, a set of peptoids with systematic sequence variations were synthesized, including a sarcosine scan, single residue substitution, and positional residue shifting to reveal structure-sequence function relationships (Figure 1). Similarly to the peptide formulation additive, the peptoid segments were synthesized as peptoid-block-
An inverse conjugation approach utilized a PAP resin automated solid-phase submonomer method (Figure 2 and (peptoid), (B) pool of used peptoid residues to mimic amino acids, Figure 2. Rebuilding peptides by peptoids. (A) Structural changes poly(ethylene glycol) (peptoid – substitution and position shifts to analyze sequence peptoids (left) and systematic sequence variations including Figure 1. Rebuilding peptide-based drug solubilizer sequences by backbone dipeptide dyads. After the peptoid segment synthesis was relationships (right).

A set of peptoid–PEG conjugates were accessed by the automated solid-phase submonomer method (Figure 2 and sarcosine scan to reveal relevant sequence positions, (iii) a systematic mutation of the central sequence position to reduce the hydrophobicity by going from NLeu to NVal to NAla, and (iv) sequence variants with positional shifts of the central NLeu residue implemented to evaluate the importance of NPehe dyads. After the peptoid segment synthesis was completed, peptoid–PEG conjugates were directly liberated from the support to be purified by dialysis. All conjugates were readily soluble in aqueous media, and mass spectrometry (MALDI-TOF-MS) confirmed the molecular identity of all set members (cf. SI).

The different peptoid–PEG conjugates were loaded with m-THPC by using the forced loading procedure as described for the peptide–PEG conjugates, and the payload capacities were determined by UV/vis spectroscopy. The m-THPC/ peptoid–PEG complexes were analyzed by dynamic light scattering (DLS), showing the formation of colloidal stable aggregates (SI Table S1). Considering the kinetically controlled loading procedure, it is noteworthy that mostly aggregates with hydrodynamic radii ($R_h$) of 80–100 nm and rather narrow polydispersities of >0.06 were found.

Comparing the capacities of both conjugates that exhibit the parent sequences indicated a significantly reduced payload of the peptoid NFFLFFN–PEG versus the peptide QFFLFFQ–PEG (1:15.1 and 1:3.9 (molar ratio drug:carrier) (Figure 3, SI Table S3). The cryo-TEM analysis of the peptoid NFFLFFN–PEG conjugate in solution showed small aggregates of ∼30 nm prior to the loading with m-THPC, which apparently involves dynamics as indicated by DLS analysis. However, after drug loading, spherical aggregates with about 76 nm were found (SI Figures S1–S3 and Table S2). In this respect the peptoid–PEG follows the QFFLFFQ–PEG behavior, showing aggregates of 37 nm without and 165 nm with m-THPC cargo. Interestingly, the differences in loadability between the peptide and peptoid platforms disappear, if conjugates were compared that exhibit only the central hydrophobic domain FFLFF in the peptide-based solubilizer proved to be relevant for m-THPC hosting. This effect seems to be even more pronounced in a peptoid–PEG conjugate, which reaches 74% higher cargo capacity compared to the peptoid conjugate having both polar flanking residues included. This could be

![Figure 2](https://pubs.acs.org/acsmacrolett/fig/20209/233-237_F02_233-237.png)

Figure 2. Rebuilding peptides by peptoids. (A) Structural changes occurring by side-chain mapping from α-carbon (peptide) to amide (peptoid), (B) pool of used peptoid residues to mimic amino acids, and (C) synthesized peptoid–PEG library.

![Figure 3](https://pubs.acs.org/acsmacrolett/fig/20209/233-237_F03_233-237.png)

Figure 3. m-THPC loading (left) and release (right) properties of peptoid–PEG and peptide–PEG conjugates having both parent sequences (payload was normalized to masses of the functional segments and values at columns give molar ratio drug:carrier).
rationalized by the fact that the peptoid structure is generally more hydrophobic than a peptide backbone, which makes incorporation of polar residues in a peptoid more dramatic, effecting the hydrophobic/hydrophilic balance and thus the ability to host a hydrophobic drug.

Besides loading, the drug release kinetics are an important parameter for drug solubilizers.19 The trans-solubilization of -THPC from the drug/solubilizer complexes to blood plasma protein models (albumins) proceeds along with regaining the sensitizer capability to produce singlet oxygen, which can be followed by fluorescence spectroscopy (Figure 3).19 The peptide—PEG solubilizers demonstrate that the polar Gln flanks contribute to rapid m-THPC release kinetics,18 which is desired for photodynamic therapy as patients might otherwise suffer from extended light sensitivities.28 Thus, it is not surprising that the peptide-PFFFLFF-PEG conjugate with a more hydrophobic backbone shows slow release properties (Figure 3). Both peptide—PEG and peptoid—PEG conjugates with the full parent sequences exhibit faster release kinetics. Taking the understanding of the peptide function into account, the polar full parent sequences exhibit faster release kinetics. Taking the high sensitivity of the peptoid loadability on removing the polar NAsn residues imposes questions about the importance of every single side-chain functionality along the peptoid backbone. To gain further insights, a sarcosine scan of the peptoid-NPFFLFN—PEG was performed that corresponds to a previously reported Ala scan of the peptide QFFLFFQ—PEG solubilizer.29 The systematic exchange of single residues by sarcosine (Figure 2) provides a set of peptide—PEG conjugates that were loaded with m-THPC to reveal relevant sequence positions (Figure 4). Taking the aromatic drug structure into account, it is expectable that the aromatic residues (Phe and NpHe) at positions 2, 3, 5, and 6 are important for drug hosting in both peptide and peptoid sequences. The exchange of only one of these residues decreases the payload capacity by up to 74% and 77% for the peptide—PEG and the peptoid—PEG solubilizers, respectively. Furthermore, the m-THPC payload capacity decreases by 59% and 81%, when the central residues Leu4 or NLeu4 were exchanged. Both the sarcosine scan and the alanine scan confirmed the segment FFLF/peptoid-PFFLF as the main hosting motif of m-THPC.

Interestingly, the peptoid mimics in several aspects accurately the peptide function as the aromatic dyads with particularly Pos2, and the central residue seems to be relevant for loading. Moreover, a consistent trend across both platforms was also obvious for substitution of the flanking residues at Pos1 and 7 (Glu/NAsn), which improves the reachable payload. However, the peptoid—PEG loadability acts way more sensitive on the residue exchange, compared to the peptide—PEGs. The exchange of the NAsn1 or NAsn7 by NAla residues improves drug loading capacity of the peptoid—PEG 3-fold or 7-fold, respectively, whereas the analogues substitution of Gln1 or Gln7 by Ala increased the loading of the peptoid—PEG conjugates only by 14—39%, respectively. While those effects on loadability are more pronounced in the peptoid compared to the peptide system, it seems to be notable that the position-specific trends are rather well met. Glu/NAsn substitution at the C-terminal Pos7 has in both platforms a stronger effect than this at Pos1.

The release kinetics data from the different m-THPC/peptoid—PEG complexes confirmed the importance of NAsn at Pos7. The exchange of NAsn7 leads to an unfavored reduction of the release kinetics (Figure 4), whereas NAsn1 at the non-PEGylated N-terminal side affects the loading but not the release kinetics compared to the conjugate having the parent sequence. This is straightforward to be understood and makes distinct sequence effects on loading/release evident. NAsn7 is located directly at the PEGylation site and is thus compared to NAsn1, well positioned to be active for interface transfer of the drug. Similar effects have previously been observed for monodisperse oligo(N-substituted acrylamides), where the addition of a small block of acrylamide residues resulted in a minor increase in polarity but dramatically enhanced the drug release kinetics from m-THPC/solubilizer complexes.18 Thus, the importance of polar functionalities to assist drug transfer to BSA appears to be generic in peptide, peptoid, and oligo(acrylamide) platforms.

The sequence—function relationship of the original QFFLFFQ—PEG indicated the central Leu4 position to be highly relevant for drug hosting. This was supported by computational simulations19 and by porphyrin binding sites of native proteins, where Leu can be frequently found to lock with the isobutyl side chain onto the bridging sections of the porphyrin.30 To prove analogies in the peptoid—PEG conjugates, the central NLeu4 residue was altered to NVal and NAla, thereby systematically decreasing the hydrophobicity of the side chain. Apparently, peptoids mimic the peptide sequences also in this aspect closely. The observed payload follows the trend of the peptide by considerably reducing the reached capacity from Leu4 → Val4 → Ala4 (Figure 4). When the NLeu4 → NAla4 exchange decreased the m-THPC loading of the peptoid—PEG conjugates by 76%, the Leu4 → Ala4 substitution reduced the capacity of peptide—PEG conjugates by 92%.18 The trend occurs even more pronounced, if the central drug hosting domain was

Figure 4. m-THPC loading (left) and release (right) properties of peptoid—PEG and peptide—PEG conjugates from the sets of alanine/sarcosine sequence scans (top) and central residue mutation (bottom) (payload was normalized to masses of the functional segment, and values at columns give molar ratio drug:carrier).
investigated, as the peptoid\textsuperscript{ffAFF–PEG} reaches only 9% of the \textit{m}-THPC capacity compared to the peptoid\textsuperscript{ffLFF–PEG} (Figure 4). The substitution shows in both platforms significant effects of loading. However, reduced impact of the central residue exchanges was evident on release properties. In fact, no conclusive behavior was found within the NLeu4 \textrightarrow{} NVa4 \textrightarrow{} NAla4 set, except that the alternation of the parent sequence leads to slower release kinetics of \textit{m}-THPC. Interestingly, the importance of the central butyl side chain for drug uptake was highlighted independently of the backbone chemistries across several platforms from peptides to peptoids (N-substituted acrylamide)s,\textsuperscript{18} oligo(\textalpha{-hydroxyl ester})s,\textsuperscript{18} to oligo(amide-urethane)s,\textsuperscript{30} showing that all of them solubilized \textit{m}-THPC most efficiently, compared to isopropyl or methyl analogues.

Obviously, the interaction capabilities of the peptoid\textsuperscript{ffLFFFN–PEG} with \textit{m}-THPC were suitable to allow drug hosting and release. The origin of these hosting/release functions in the peptoid segment might arise from the distinct sequence of functional residues but could also be only a consequence of simply the presence of appropriate residues. To distinguish between these situations, a set of sequences were investigated that have been composed of the same residues as the parent sequence but with alternative order (Figure 2). Focus was set on the central NLeu4 residue that was systematically shifted to show the relevance of the isobutyl group position within the sequence (SI Table S3). The peptoid–PEG with the parent sequence reached the highest loading within this set of NLeu-shifted sequences. The shift of NLeu4 by only one position to Pos3 or Pos5 reduced the payload capacity by about 15%. This indicates a slight sequence effect as the casting of nearby residues breaks one of the NPh e dyads, which proved to be relevant for \textit{m}-THPC hosting in the peptide system by calculations.\textsuperscript{19} Moreover, the shift of NLeu4 to the flanking positions of the central hydrophobic hosting motif changes the capacity substantially differently where the shift of NLeu4 to Pos6 (close to the PEGylation site) leads to a reduction of \approx{}10% and the shift to the opposite Pos2 reduces the capacity by \approx{}50%. Considering that in both cases a NPh e tetrad was formed, a clear positional effect on the payload capacity was visible, which suggested the importance of sequence on the loading function. A related sequence sensitivity was found in oligo(\textalpha{-hydroxyl ester}),\textsuperscript{18} where a minor shift of butyl residue to a more decentralized position led to a significant drop of drug uptake. However, release traces were less dramatic depending on the position of the NLeu residue as all peptoid–PEG conjugates with shifted sequences show rather similar release kinetics (SI Table S3). Taking the initial release rates into account, the parent sequence shows the highest values, and the sequence with NLeu2, having the poorest loadability, reached the slowest initial release to albumins (SI Table S3).

In conclusion, a set of \textit{mer} peptoids were synthesized based on the functional peptide sequence QFFLFFQ as a blueprint, on the photosensitizer \textit{meta}-tetra(hydroxyphenyl)-chlorin (\textit{m}-THPC). The peptide sequence NAsn-NPh e-NPh e-NLeu-NPh e-NAsn originated from a one-by-one residue mapping of the peptide side-chain functionalities. Similarly to the peptoid–PEG solubilizer, the peptoid–PEG conjugate proved the capability to solubilize \textit{m}-THPC. While the drug loadability sensitively responds on sequence alternations, release kinetics can be controlled by interplay of backbone and polar side chains. The sarcosine scan (analogues alanine scan) proved the residues NPh e2,5,6 and NLeu4 responsible for the \textit{m}-THPC loading and NAsn7 contribute to drug release toward blood plasma protein models. The single residue alternation of NAsn7 to NAla7 dramatically improved the loadability of the peptoid–PEG by 710\%, and exchanging the central NLeu4 by NAla4 reduced the \textit{m}-THPC loading by 76\%. Complete deletion of both hydrophilic NAsn flanks further showed that the central hydrophobic segment was mainly responsible for hosting \textit{m}-THPC, mimicking the peptidic counterpart most accurately. The shifting of the NLeu4 residue systematically toward the flanking positions revealed sequence-specific effects as the parent sequence performed best. Therefore, the direct comparison of peptides and peptoids provided interesting insights into relevance of side-chain sequences and contributions of backbone functionalities on drug hosting and release properties. Moreover, generic effects that determine loadability and release are evident across a chemically rather diverse set of precision polymers, ranging from closely related peptide mimics like peptoids to the rather diverse structures of oligo(amide-urethane).\textsuperscript{22} The understanding of sharply defined structure–sequence-property relationships in monodisperse precision polymers might pave the way to design fully synthetic polymers. Those might enable us to mimic effectively purposefully adapted peptides with their capabilities to realize complex functions like for instance drug-structure-specific barrier transporters, material-specific adhesives, or specific interface compatibilizers in composites.

### ASSOCIATED CONTENT

- **Supporting Information**
  The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmacrolett.9b00977.
  Experimental procedures and analytical data (PDF)

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#### Notes

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