March 6, 2016

Revised

Peptides Comprised of Alternating L- and D- Amino Acids Inhibit Amyloidogenesis in Three Distinct Amyloid Systems Independent of Sequence

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Abstract

There is now substantial evidence that soluble oligomers are primary toxic agents in amyloid diseases. Development of an antibody recognizing the toxic soluble oligomeric forms of different and unrelated amyloid species suggests a common conformational intermediate during amyloidogenesis. We previously observed common occurrence of a novel secondary structure element, which we call α -sheet, in molecular dynamics simulations of various amyloidogenic proteins, and we hypothesized that the toxic conformer is comprised of α -sheet structure. As such, α -sheet may represent a conformational signature of the misfolded intermediates of amyloidogenesis and a potential unique binding target for peptide inhibitors. Recently, we reported the design and characterization of a novel hairpin peptide (α 1 or AP90) that adopts stable α -sheet structure and inhibits the aggregation of the β -Amyloid Peptide A β 42, and transthyretin. AP90 is a 23-residue hairpin peptide featuring alternating D- and L- amino acids with favorable conformational propensities for α -sheet formation, and a designed turn. For this study, we reverse engineered AP90 to identify which of its design features is most responsible for conferring α -sheet stability and inhibitory activity. We present experimental characterization (CD and FTIR) of 7 peptides designed to accomplish this. In addition, we measured their ability to inhibit aggregation in three unrelated amyloid species: A β 42, transthyretin, and human islet amylin polypeptide. We found that a hairpin peptide featuring alternating L- and D-amino acids, independent of sequence, is sufficient for conferring α -sheet structure and inhibition of aggregation. Additionally, we show a correlation between α -sheet structural stability and inhibitory activity.

KEYWORDS: Beta Amyloid, Amyloid, Toxic Oligomer, Inhibitor, α -sheet

Abbreviations: AD, Alzheimer's disease; MD, molecular dynamics; CD, circular dichroism spectroscopy; NOESY, Nuclear Overhauser effect spectroscopy; A β 42, Amyloid Beta (1-42); IAPP, Islet Amyloid Polypeptide; TTR, Transthyretin; CR, Congo Red; ThT, Thioflavin T; HFIP, hexafluoro – 1, 1, 1, 3, 3, 3 – isopropanol

Introduction

Over 40 distinct human amyloid diseases have been identified, many of which contribute to severe neurodegeneration and organ dysfunction [1]. Amyloid diseases are associated with the aggregation of normally-soluble peptides or proteins to insoluble, β -rich amyloid deposits [2,3]. Multiple aggregation pathways are thought to be available to amyloidogenic species, with different pathways furnishing multiple distinct conformational intermediates [4–6]. The mechanism of amyloid assembly and its associated pathology are not well understood; however, there is substantial experimental evidence that the soluble oligomeric intermediates populated during amyloidosis are the primary species responsible for cellular toxicity [7–10].

The development of conformation-specific antibodies recognizing the toxic oligomeric intermediates of many different amyloid peptides and proteins suggests a common backbone structure associated with amyloidogenic intermediates [11,12]; this is supported by the cross-reactivity of different, unrelated amyloid proteins and peptides [13–17]. Proposed amyloid oligomer models in the literature are widely variant, including helix-containing assemblies [18,19], extended β -sheets resembling small fibrillar structures [20], β -barrels [21–24], loosely-structured assemblies [25], and spiraling sheets [26]; the apparent availability of multiple aggregation pathways allows for structurally and compositionally diverse intermediates, each associated with one of many aggregation pathways. Notably, NMR experiments do not detect β -sheet or α -helix structure in toxic A β 42 (Amyloid β -peptide residues 1-42) pentamers and hexamers [25], a finding corroborated by the absence of β -sheet or α -helix fingerprints in CD spectra taken during the lag (or nucleation) phases of polyglutamine, A β 42, islet amylin polypeptide (IAPP), and α -synuclein aggregation [27–32].

Previous analysis of low-pH molecular dynamics (MD) simulations of the misfolding of various amyloidogenic proteins showed common occurrence of a novel secondary structure called α -sheet [33–36]. α -sheet secondary structure is defined by the alternation of α_L and α_R conformations through sequential amino acid residues, resulting in the parallel alignment of all carbonyl groups and all amide protons on opposite faces of the peptide strand (Fig. 1). This alignment of identical functional groups promotes cross-strand hydrogen bonding for sheet-type structure. Based on the shared presence of α -sheet structure across these simulations, we proposed that α -sheet is a structural signature of the misfolded intermediates of amyloidogenesis, and that α -sheet secondary structure might provide a binding target for potential inhibitors of amyloid formation [36]. Our recent studies have demonstrated that designed α -sheet peptides (~20 residues) effectively inhibit amyloid formation by binding to toxic oligomeric intermediates [37]. We hypothesize that the mechanism of binding is a backbone-backbone intermolecular interaction between α -strand segments. Among these α -sheet inhibitors was a 23-residue designed α -hairpin named αI , which we refer to here as AP90 (Fig. 1). The AP90 design exploits alternating D- and L- C α chirality through its α -strand segments to stabilize the alternating α_L and α_R amino acid conformations necessary for α -sheet structure. The spectroscopic properties of AP90 have been characterized, and they provide evidence for stable α -sheet structure [37]. The CD spectrum of AP90 is relatively featureless, as is expected for a peptide with alternating D- and L- amino acids. Its FTIR spectrum features a strong absorbance peak centered near 1675 cm⁻¹ and a shoulder centered at 1640 cm⁻¹, both of which match theoretical predictions for α -sheet FTIR absorbance [38]. AP90 also gives NH-NH NOESY couplings among amide protons of sequential (*i*, *i*+1) residues, indicating the alignment of

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4 successive NH and C=O groups, characteristic of α -sheet. Furthermore, AP90 lacks the
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7 characteristic signals of α -helix and β -structure.
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10 In this study, we explored the relative importance of the design features of AP90 in
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12 conferring α -sheet structure and inhibitory activity against amyloid formation by reverse
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14 engineering the peptide. Specifically, we designed peptides that successively eliminate features
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16 presumed to stabilize the structure, and we experimentally characterized the spectroscopic
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18 properties and inhibitory activity of these resulting peptides. Three amyloidogenic species were
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20 chosen to examine general inhibitory activity of the designs: the A β 42 peptide associated with
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22 Alzheimer's disease [9], amylin or IAPP associated with type 2 diabetes [39], and transthyretin
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24 (TTR), which is associated with peripheral polyneuropathy and systemic senile amyloidosis [40].
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26 Aggregation for each of these amyloid species can be monitored by dye-binding assays [41,42],
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28 allowing quantitation of inhibition against aggregation.
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36 Our study of the AP90 parent and its derivatives revealed that all of the peptides
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38 templated to have alternating L- and D-chirality possess inhibitory activity against amyloid
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40 formation independent of sequence, although the sequence can modulate the strength of the
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42 inhibition. In addition, there is a correlation between α -sheet structural stability and activity that
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44 supports our hypothesis that α -sheet is a structural signature of intermediates during amyloid
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46 formation.
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52 **Results and Discussion**

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55 **Deconstructing determinants of inhibition**

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Our initial interest was in determining which of AP90's design features were most responsible for both stabilizing α -sheet secondary structure and conferring inhibitory activity in amyloidogenic systems. The AP90 design was constructed from a 23-residue hairpin template featuring two α -strands joined by a central turn. The template contains D chirality at the C α of strand residues 4, 6, 8, 16, 18, and 20 (Fig. 1). This sequence template is referred to henceforth as the "L/D template." In an attempt to optimize stability, AP90's strand segments are composed of amino acids with favorable propensities for either the α_L or α_R conformations [43,44]. Additionally, AP90 features a 5-residue designed turn (-NEYSG-) [34] to promote cross-strand hydrogen bonding. The N- and C-termini of AP90 are capped with Ac-RG- or -GR-NH₂, respectively, for improved solubility [45]. Solubility is a particularly important concern for side chain selection as well, because aggregation is critical to toxicity and we want to ensure that our α -sheet designs are nontoxic.

The peptides reported here were designed to study the contributions of these various sequence elements of the AP90 parent design to α -hairpin structure and inhibitory activity. The particular elements selected for study include: the presence of a designed turn; amino acid conformational propensity; and, the L/D template. For our design process, we proceeded from the assumption that the AP90 peptide represents an optimized design. Our sequential design modifications to this parent sequence used sequence randomization through the L/D template of AP90 to successively eliminate the peptide's design features and, presumably, destabilize α -sheet structure. Our expectation was that increasing destabilization would be confirmed and monitored by reduced inhibitory activity and changes in predicted spectroscopic hallmarks. Modifications gradually decreased sequence similarity to the AP90 parent, an effect we represented using sequence identity relative to AP90. The designs are listed in Table 1. They are

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4 assigned to one of two groups: peptides that follow the L/D template (“templated” designs) and
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6 peptides that do not (“non-templated” designs). They are ordered according to sequence identity
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8 relative to AP90.
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10 11 12 **Main chain structure responsible for inhibition independent of sequence** 13 14

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16 Inhibition experiments were used to study which of AP90’s design features confer
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18 general inhibitory activity. Inhibitory activity was measured by co-incubating peptide designs
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20 with each of the amyloid species A β 42, IAPP, and TTR at suitable conditions for *in vitro*
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22 aggregation; aggregation was then quantitated by a dye-binding assay using either Thioflavin T
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24 (ThT) fluorescence (A β 42 and IAPP) or Congo Red (CR) absorbance (TTR). Unfortunately, the
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26 inability to apply a single dye-binding assay to several different amyloidogenic species is a
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28 recurring problem in the relevant literature [45,46].
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34 We have previously shown that the AP90 lead compound is an inhibitor of A β 42 and
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36 TTR aggregation at conditions similar to those reported here. Under the assay conditions used
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38 for this study, AP90 nearly completely abolished A β 42 aggregation when co-incubated at 4-fold
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40 excess (Fig. 2a, Table 2). Co-incubation of AP90 with IAPP at an 8-fold excess resulted in 83%
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42 inhibition of aggregation (Table 2). Co-incubation of AP90 with TTR at a 10-fold excess
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44 resulted in 65% inhibition of aggregation (Table 2). All modified designs were evaluated relative
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46 to the parent AP90. Representative traces for inhibition experiments involving all designs are
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48 included in Figure 2 and the average inhibition values and associated error over 3 independent
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50 trials are provided in Table 2.
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57 For our first design modification, we scrambled the sequence (retaining the amino acid
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59 composition) through AP90’s α -strand segments (residues 3-9 and 15-21) while maintaining the
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4 L/D template, RG tails, and designed turn (Fig.1). The resulting derivative (AP5) had 100%
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6 retention of sequence composition and chirality but only 65% sequence identity relative to AP90,
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8 and it demonstrated nearly identical inhibitory activity against A β 42 and improved inhibition
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10 against IAPP, with activity against TTR reduced to 37% inhibition (Table 2). However, because
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12 AP90's α -strand residues all possess favorable conformational propensities, scrambling only
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14 through strand segments likely produced little destabilization. That scrambling the strand
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16 sequences did not affect inhibitory potency demonstrates that the sequence *per se* is not
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18 determining activity – the same inhibitory effect is achieved with an alternate amino acid order.
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25 To both remove any stabilizing effect of the designed turn and introduce amino acids
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27 lacking high α -sheet propensity into the strand segments, we next scrambled the sequence
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29 through both α -strands and the turn (residues 3-21), while again maintaining the L/D template
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31 and RG tails. This yielded a peptide (AP6) with only 25% sequence identity to the parent AP90.
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33 Co-incubation of AP6 with amyloid species gave 35% inhibition against A β 42 and 75%
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35 inhibition of IAPP, thereby displaying significantly reduced activity due to the loss of the
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37 designed turn relative to both AP90 and AP5 (Table 2). AP6 interfered with Congo Red binding
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39 to TTR, preventing quantitation of its inhibition of TTR amyloidosis using the CR-binding assay.
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41 Unfortunately, several subsequent designs were similarly unsuitable for the Congo Red binding
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43 assay, and they had to be evaluated by an alternative method, as discussed further below.
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50 To further test the apparent resilience of the L/D template in conferring inhibitory
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52 activity, we randomly selected amino acids for all 23 residues while maintaining the L/D
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54 template. This design choice was intended to decouple effects due to the L/D template from any
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56 composition-specific effects. Random selection yielded two designs (AP3 and AP4) with less
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4 than 20% sequence identity to AP90. Neither design showed uniformly high inhibition across all
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6 systems studied: AP3 inhibited 36% of the IAPP aggregation but only 11% of the A β 42
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8 aggregation (Table 2). AP4 inhibited 42% of the A β 42 aggregation and 11% of IAPP
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10 aggregation (Table 2). Like AP6, both AP3 and AP4 were unsuitable for the CR-binding assay
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12 due to interference with the dye.
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17 Two additional peptides were designed without the L/D template: P2 is a scramble of the
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19 AP90 parent sequence that does not enforce alternating L- and D- chirality through strand
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21 segments, while P90 is an all-L-amino acid analog of AP90 (Table 1). The non-templated,
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23 scrambled AP90 sequence, P2, was inactive against both A β 42 and IAPP aggregation (Table 2).
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25 Like several of the other designs, P2 bound Congo Red so that the TTR assay could not be
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27 performed and only inhibition of A β 42 and IAPP aggregation could be measured. The inactivity
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29 of P2 demonstrates the necessity of the L/D template for conferring inhibitory activity.
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31 Additionally, it shows that the absolute amino acid composition of AP90, AP5, AP6, and P2 (all
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33 100%) is not responsible for activity, as all of the L/D template peptides inhibit amyloid
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35 formation to some degree while a peptide utilizing the same amino acids as AP90, AP5 and AP6
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37 but lacking the L/D template does not. This also shows that it is not the D-amino acids *per se* that
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39 are responsible for inhibition in the templated designs.
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48 Eliminating D-chirality from the AP90 sequence resulted in a completely insoluble
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50 peptide (P90) despite the unchanged isoelectric point and side chains (relative to AP90). Due to
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52 this lack of solubility, inhibition experiments could not be performed with P90. Note that the
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54 AP90 and P90 peptides contain exactly the same chemical sequence but 6 of the residues in
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4 AP90 have D-chirality to impose templating of the α -sheet structure (Table 1). The difference in
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7 the behavior of AP90 and P90 is striking.
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10 Unfortunately the inhibition (or lack thereof) of TTR could not be evaluated for many of
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12 the designs due to their binding to Congo Red. Consequently we also evaluated the compounds
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14 in a more qualitative binding assay involving immobilization of the designs to agarose beads and
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16 then determining whether they preferentially bind pre-incubated toxic TTR samples, as described
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18 earlier [37]. We have previously shown that AP90 preferentially binds toxic but not nontoxic
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20 solutions of A β 42 and TTR [37], consistent with our α -sheet hypothesis. In line with the A β 42
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22 and IAPP inhibition results, the L/D templated designs all bind species from the pre-incubated
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24 toxic TTR solutions while the non-templated designs do not, and in this case P90 could also be
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26 evaluated because its self-aggregation is controlled through immobilization on the beads (Fig. 3,
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28 Table 2).
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35 Successive elimination of design features resulted in a decrease in binding to the toxic
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37 oligomeric species as probed by the agarose bead binding experiment with TTR or as reflected in
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39 the inhibition of fibril formation by binding of the oligomeric precursor to the fibrillar state. This
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41 decrease correlated with decreasing sequence identity relative to AP90 (Fig. 4), confirming the
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43 predicted effects of our design modifications. Despite our sequence modifications, however, we
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45 were unable to fully quench inhibition by the L/D templated peptides, as both the completely
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47 randomized designs, AP3 and AP4, inhibited amyloid aggregation and/or preferentially bound
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49 the TTR toxic oligomers. Our inability to abolish inhibition suggests that the L/D chirality
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51 template is predominantly responsible for conferring inhibitory activity to our designs, rather
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53 than the nature of the side chains, the absolute composition, or the designed turn. Even without a
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55 well-designed turn or residues with high propensity to form α -strands, the L/D template confers
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4 meaningful inhibitory activity and the ability to distinguish between nontoxic and toxic
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6 conformers. Consequently, the L/D template appears to be a novel structural template for design
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8 of general inhibitors of amyloidosis whose mechanism of inhibition appears to be distinct from
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10 any previously reported peptides or organic molecules.
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14 15 **Destabilization of α -sheet structure lowers inhibition** 16

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18 In order to assess the structural effects of our design modifications, CD spectra were
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20 recorded for all peptides (Fig. 4a). Due to the variable solubility and to ensure solubility of all
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22 designs and uniformity of solution conditions, all CD spectra were taken in phosphate buffer
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24 with 50% acetonitrile (v/v). The presence of acetonitrile did not significantly affect the CD
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26 spectra of the very soluble compounds and allowed us to record the spectra of non-templated
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28 designs that are not as soluble under aqueous conditions.
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34 Featureless spectra were anticipated for peptides including alternating L- and D-amino
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36 acids due to the opposite absorption of circularly polarized light [37]. A CD signal near 195 nm
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38 is typically attributed to unstructured conformers, often being referred to as the ‘random coil’
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40 signal, and if it is due to the L-amino acids the signal is negative and positive for D-amino acids.
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42 We have therefore interpreted larger negative signals near 195 nm as being due to distortions of
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44 the turn and fraying of the ends of the hairpin upon modification, as both of these regions are
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46 comprised of L-amino acids. Furthermore, the coupling of a single chiral C α and N-terminal
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48 peptide bond is sufficient for production of a CD minimum near 200 nm [48]. So, while a
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50 negative signal in this region is typically taken to represent “random coil”, it is potentially more
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52 complicated involving dynamic exchange between many non-random conformers, particularly
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54 after loss of the turn. As such, we take a more negative minimum near 195-200 nm to reflect a
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4 change in the dynamics and structure away from the AP90 state without being able to precisely
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6 describe the detailed nature of the changes.
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10 In any case, the first of our design modifications involving shuffling of the residues in the
11 strands (AP5) resulted in a CD spectrum very similar to that of AP90, each with a relatively
12 featureless, flat region between 210-260 nm and a minimum near 195 nm (Fig. 4a). In the
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14 spectrum of AP6, which is due to shuffling of the strands and the turn residues, there is a broad
15 shoulder centered at roughly 215 nm (extending to 225 nm) that suggests the possibility of some
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17 β -character among the L-amino acids. The randomly selected sequences (AP3, AP4) both gave a
18 relatively strong CD minimum shifted from 195 to 200 nm. AP4's spectrum features an
19 additional band at 224 nm that resembles that characteristic of L-Trp absorbance. Notably, a
20 similar minimum was not observed in the CD spectra of the L-Trp-bearing designs AP90, AP5,
21 AP6, or AP3, all of which follow the L/D template.
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35 Among the five templated peptides, the CD minimum ($[\Theta]_{\min}$) near 195 nm shifted to 200
36 nm and increased in magnitude with each of our sequential design modifications (Table 3),
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38 indicating increasing random coil character among their L-amino acids. $[\Theta]_{\min}$ is strongest in the
39 spectra of AP3 and AP4, the two peptides expected to possess the lowest α -sheet stability. $[\Theta]_{\min}$
40 also red-shifted among templated peptides with sequentially destabilizing modifications, possibly
41 due to contributions of α -helix and β -sheet conformations transiently populated by the
42 destabilized peptide in the L-amino acid stretch [37].
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54 Surprisingly, the CD spectrum for the scrambled design P2 is very similar to that of
55 AP90. We expected a stronger minimum near 195 nm. Regardless, P2's inactivity as an inhibitor
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4 suggests an absence of any structural feature – such as α - or β -sheet – that could confer activity.
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7 The CD spectrum of the all-L peptide P90 reflects weak β -sheet structure (Fig. 4a).
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10 To further characterize sequence effects on structure, we recorded solid-state FTIR
11 absorbance spectra for all AP90 derivatives (Fig. 4b). α -sheet secondary structure produces
12 FTIR bands that are distinct from characteristic β -sheet and α -helix bands [38]. The FTIR
13 absorbance spectrum for AP90 features a dominant peak at 1675 cm^{-1} , with a shoulder centered
14 at roughly 1640 cm^{-1} ; notably, these are the predicted features for α -sheet absorbance spectra
15 [38]. AP5, AP3, and AP4 gave dominant peaks at either 1640 or 1675 cm^{-1} , suggesting that there
16 are subtle differences in the α -sheet structures. The FTIR absorbance spectra of AP4 and AP5
17 feature a broad band centered at 1640 cm^{-1} . AP6 stands out with a dominant peak centered at
18 1620 cm^{-1} in the spectral region typically associated with β -sheet, and a broad shoulder near
19 1660 cm^{-1} . In the past, we have found that the drying process required for the solid-state FTIR
20 measurements can sometimes induce β -structure, an effect that might be at play here in the 7-
21 residue segment of L-amino acids in AP6.
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41 The non-templated sequence P2 gave a narrow absorbance band centered near 1690 cm^{-1} ,
42 with a shoulder near 1710 cm^{-1} . The all-L-amino acid P90 showed a dominant FTIR band near
43 1620 cm^{-1} in the region associated with β -sheet, with a broad, weak shoulder at 1660 cm^{-1} . P90's
44 sequence includes a designed turn and several residues with high β -propensity, supporting FTIR
45 identification of β -hairpin structure, consistent with its CD.
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55 We have previously hypothesized that α -sheet secondary structure represents a
56 conformational signature of toxic intermediates of amyloidosis [33–36]. Low-pH MD
57 simulations provided the initial basis for this hypothesis, and subsequent design and
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experimental characterization of α -sheet inhibitors of amyloidosis have supported our assertion [37]. Under the assumption that α -sheet is a structural feature common to oligomeric intermediates, stable α -hairpins were expected to inhibit amyloid formation in all three amyloid systems studied (A β 42, IAPP, and TTR) through backbone/backbone interactions between α -sheet/strand segments. Because of the nature of this proposed inhibitory mechanism, a correlation between α -hairpin structural stability and general inhibitory activity is expected.

As discussed above, increasing randomization of the sequences among the L/D templated designs (represented quantitatively by sequence identity to AP90) correlated with an increase in magnitude and a shift of the CD minimum near 195 to 200 nm (Fig. 5, Table 3). Direct comparison of the CD and inhibition results (Fig. 5) illustrates the link between changes in the structure and decreasing inhibitory activity, a result consistent with an inhibitory mechanism featuring backbone/backbone intermolecular interactions. However, the side chains modulate the behavior of these templated L/D peptides in two main ways: (1) by affecting their structural stability, which when compromised leads to a drop in inhibition; and (2) by affecting the complementarity of the binding with the target amyloid α -sheet species, as displayed by the different inhibition rankings. For example, for A β 42 the compounds ranked as follows, from best to worst: AP90 ~ AP5 > AP4 > AP6 > AP3. For IAPP the ranking is: AP5 > AP90 > AP6 > AP3 > AP4.

Although the non-templated design P90 was expected to be an inactive, unstructured control, it contained β -character as evidenced by CD and FTIR (Fig. 4b). Inhibition results could not be obtained for P90 because it is insoluble in our aqueous assay conditions; however, it does not bind the toxic oligomer species of TTR, and instead preferentially binds native tetrameric

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4 TTR (Fig. 3). We have shown that a designed trpzip β -hairpin can inhibit TTR aggregation by
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6 binding to the native tetramer, while other β -hairpins do not [37,52]. β -hairpins are also known
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8 inhibitors of A β by binding the monomer, so it is possible P90 could inhibit aggregation, but it is
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10 far too insoluble and it would do so by a different mechanism than the templated L/D designs
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12 Nevertheless, the complete loss of solubility caused solely by the design's elimination of D-
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14 chirality further reflects a substantial structural change. This structural change associated with
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16 elimination of D-chirality demonstrates the remarkable effect of the L/D template.
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22 23 **Conclusions**

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26 Our results identify a new sequence template for α -sheet peptide inhibitors of
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28 amyloidosis. Additionally, the apparent correlation between α -sheet stability and inhibitory
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30 potency in our templated peptides supports an inhibitory mechanism featuring
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32 backbone/backbone interactions among α -strand segments. The growing evidence for the
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34 association of α -sheet with amyloidogenic intermediates identifies a new target for inhibitors of
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36 amyloidosis. Considering that we are only now characterizing the spectroscopic properties and
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38 inhibitory activity of α -sheet peptides, we have much room for further optimization of both
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40 stability and activity. Future designs are expected to possess even higher inhibitory potency as
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42 we further inform our design process. Looking forward, potential therapeutic peptides following
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44 an L/D template could have an advantage in *in vivo* studies, as they should not be metabolized as
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46 rapidly as their all-L analogs. In addition, to further optimize our designs for inhibitory activity,
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48 we are now exploiting our proposed inhibitory mechanism to develop diagnostic assays for
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50 binding toxic oligomeric species from solution, and which may also aid in structural
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52 characterization of the toxic oligomers.
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Materials and Methods

Materials

Designed peptides were purchased from CPC Scientific or American Peptide Company (APC) and supplied as $\geq 95\%$ purity as assessed by RP-HPLC and mass spectrometry. A β 42 and IAPP were also purchased from APC. Lyophilized A β 42 and IAPP were treated with 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) purchased from Sigma (St. Louis, Missouri) upon receipt and aliquotted from stock solutions made in HFIP. TTR purchased from Lee Biosolutions (St. Louis, Missouri) was dissolved to 10 mg/mL in 20 mM ammonium carbonate buffer (pH 8.0) and aliquotted from this stock solution. Amyloid peptide/protein aliquots were dried under vacuum and stored at -18°C until use.

Solution Aggregation Assays

A β 42 was thawed from -18°C and re-treated in HFIP for 30 min. The HFIP was then removed under gentle air, and the resulting dry peptide was further exsiccated 30 min in a Thermo Scientific Savant SC110A SpeedVac. HFIP-treated A β 42 was dissolved to 0.75 mg/mL in 6 mM NaOH by sonication (5 min). The 0.75 mg/mL stock was filtered through a 0.22 μm Costar Spin-X cellulose centrifuge filter, and its concentration was verified by UV/Vis absorbance at 220 nm ($\epsilon_{220}=50,000\text{ M}^{-1}\text{ cm}^{-1}$) using a Thermo Scientific NanoDrop 2000 spectrometer [49]. The stock was allowed to rest 4 hr at room temperature (RT) for NaOH treatment; aliquots were then portioned into wells of a Nunc 96-well fluorescence plate and diluted to 10 μM A β 42 with PBS (11 mM phos, 152 mM NaCl, 3 mM KCl, 22 μM ThT, pH 7.4) containing inhibitor peptide (inhibitor concentrations verified by Trp/Tyr UV/Vis abs at 280

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4 nm). In the case of the less soluble AP6 and P2 peptides, 0.9% DMSO was added. Plates were
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6 sealed and incubated away from light at RT. RT was chosen over 37° C because high
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8 temperatures are thought to favor fibril elongation over oligomerization for this system, and the
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10 oligomer is our intended binding target [50]. AFM imaging confirmed the presence of relatively
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12 short (rather than long) fibrils following incubation of Aβ42 at the conditions detailed above (not
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14 shown).

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20 Dry IAPP was thawed from -18° C and treated with HFIP, as described above. The HFIP-
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22 treated IAPP was dissolved to 1 mg/mL in DMSO. 1 mg/mL stock aliquots were portioned into
23
24 wells of a 96-well fluorescence plate and diluted to 10 μM nominal IAPP concentration with
25
26 PBS (11 mM phos, 152 mM NaCl, 3 mM KCl, 22 μM ThT, pH 7.4) containing inhibitor peptide
27
28 (inhibitor concentrations verified by Trp/Tyr UV/Vis abs at 280 nm). Plates were then sealed and
29
30 incubated at 37° C. The ThT fluorescence assay was carried out as described above.
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36 Dry TTR was thawed from -18° C and dissolved directly to 10 μM TTR (40 μM
37
38 monomer) in either low-pH acetate buffer (50 mM NaOAc, 100 mM KCl, 1 mM EDTA, pH 4.5)
39
40 or neutral-pH PBS (11 mM phos, 152 mM NaCl, 3 mM KCl, pH 7.4) containing inhibitor
41
42 peptide. Samples were incubated at either 4° (neutral pH) or 37° C.
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46 47 **ThT Fluorescence Assay**

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49
50 For the ThT fluorescence assay, plates were removed from incubation and read once per
51
52 timepoint in a Tecan Safire² plate reader (λ_{ex} =450 nm, λ_{em} =480 nm). Plates were shaken prior to
53
54 the initial (“t = 0”) timepoint, but not prior to any subsequent timepoints.
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58 59 **Congo Red Binding Assay**

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4 For the CR-binding assay, three 10 μ L sample aliquots were each combined with 190 μ L
5
6 of a 10 μ M CR solution made in PBS (11 mM phos, 152 mM NaCl, 3 mM KCl, pH 7.4) in wells
7
8 of a 96-well clear micro-plate (Falcon). The plate was then read for absorbance at 477 nm and
9
10 540 nm, and CR binding was determined according to a previously published equation [37].
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15 **Transthyretin Column Binding Assay**

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18 Peptide designs were immobilized to the Pierce Amino Link resin following the manufacturer's
19
20 instructions. Coupling and all further steps and analyses have been described by Hopping *et al.*
21
22 [37]. Fresh, tetrameric TTR at neutral pH and pre-incubated toxic solutions of TTR at low pH
23
24 (toxicity confirmed through cell-based assays using neuroblastoma cells) were prepared and
25
26 applied to the column. Bound material was eluted with guanidine hydrochloride and analyzed by
27
28 dot blot analysis. The difference between the binding of the toxic and nontoxic solutions was
29
30 determined for each peptide, except AP3, which lacks a lysine and the N-terminus is acetylated
31
32 preventing its coupling to the resin.
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39 **Circular Dichroism Spectroscopy**

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42 Dry peptide was dissolved to 90 μ M in 50/50 - AcCN/PB (10 mM phosphate, pH 7.4).
43
44 Concentrations were verified by Trp/Tyr UV/Vis absorbance. CD spectra were taken at 25° C on
45
46 an Aviv model 420 spectrometer (Aviv Biomedical). The dynode voltage was monitored to
47
48 ensure it was below 500 volts. Average values from 3 scans were plotted using the Origin 8
49
50 software (Originlab, Northhampton, MA). All spectra were smoothed using the Savitsky-Golay
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52 method with 5-12 points/window and polynomial order 2. The smoothed data were subtracted
53
54 from the raw data and the differences were minimal and evenly distributed around zero.
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Fourier Transform Infrared Spectroscopy

FTIR spectroscopy assays were carried out according to the method described by Hopping *et al.* without any deviations [37].

Acknowledgements

We thank Drs. James Bryers, David Baker, Cecilia Giachelli, Buddy Ratner, and Pat Stayton for generous use of equipment. We thank Dr. Tom Schmidlin for his advice on figure design. This study was funded by the W.H. Coulter Foundation Translational Research Partnership Program (to V.D.), grants from the National Science Foundation (CBET-0966977 to V.D.), the National Institute of Health (GM 95808 to V.D.), the Coins for Alzheimer's Research Trust (to V.D.), and the Intramural Research Program of the NIAID, NIH (to B.C.).

Author Contributions: J.K., G.H., B.C., and V.D. designed research; J.K., G.H., and B.C. performed experiments; J.K., G.H., B.C., and V.D. analyzed results; and all authors contributed to the writing of the paper.

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Table Legends

Table 1. Modifications to deconstruct AP90 design features

^aRegions of the sequence modified are colored to correspond to the portion of the structure affected, as highlighted in Figure 1. ^bPercentage sequence identity relative to AP90 factoring in chirality. ^cPercent absolute composition of amino acid side chains (disregarding placement and C α chirality) relative to AP90 (i.e. scrambled sequences produce 100% sequence composition). ^dMeasure of the extent (as a percentage) to which C α chirality matches L/D template (i.e. D-amino acids at residues 4, 6, 8, 16, 18, and 20 produce 100% chirality identity). Note, D-amino acids are distinguished by lower case, underlined letters.

Table 2. Summary of inhibition and binding assays

% Inhibition values reported as endpoint fluorescence relative to that of the uninhibited control, with the endpoints defined as the endpoint of the lines through the plateau regions, as illustrated in Figure 2. Inhibition and error values are reported over 3 independent aggregation reactions. ^aValues apply to co-incubation of 10 μ M A β 42 with 40 μ M inhibitor. ^bValues apply to co-incubation of 10 μ M IAPP with 80 μ M inhibitor. ^cValues apply to co-incubation of 40 μ M TTR monomer with 400 μ M inhibitor. ^dTally of relative binding of toxic versus nontoxic TTR samples by peptide designs taken from Fig. 3. AP3 does not contain a lysine residue, which is required for coupling to the beads.

Table 3. Summary of CD spectroscopy results for “templated” peptides

^aSequence identity relative to □□□□. ^bAbsolute composition of amino acid sidechains (disregarding placement and C α chirality) relative to AP90 (i.e. scrambled sequences produce 100% sequence composition). ^cValue of the CD minimum in the 195-200 nm ($[\Theta]_{\min}$) region. Negative CD in this region is typically considered a reflection of lack of structure, often referred to as random coil. Sequence identity and composition relative to AP90 are reiterated to compare decreasing $[\Theta]_{\min}$ with increasing randomness-of-design.

Figure Legends

Fig. 1. Structural model of AP90. α -sheet region is highlighted in yellow. Each of the regions highlighted in either yellow, blue, or red were subject to modification for design of specific peptides. The highlighting on the structure illustrates which sequence segments were modified for each design, and they are color matched with Table 1. Also see Table 1 for specific sequences. The image was generated using UCSF Chimera [51].

Fig. 2. α -sheet designs inhibit aggregation in solution. (a) ThT fluorescence time courses obtained from 10 μ M A β 42 samples incubated with (red triangles) or without (black squares) a 4-fold excess of inhibitor, respectively. Fluorescence units are normalized to the endpoint of the negative control (black squares), with the endpoint defined as the time at which fluorescence reaches the first local maximum of the curve's plateau region. Samples were incubated at room temperature in PBS (pH 7.4) containing 20 μ M ThT. (b) ThT fluorescence time courses obtained from 10 μ M IAPP samples incubated with (blue triangles) or without (black squares) a 8-fold excess of inhibitor. Fluorescence units are normalized to the negative control (black squares) in the same way as for A β 42 data. Samples were incubated at 37° C in PBS (pH 7.4) containing 20 μ M ThT and 4% DMSO vehicle. (c) CR-binding time courses obtained from 40 μ M TTR monomer samples incubated with 400 μ M inhibitor (colored triangles) and without inhibitor (black squares). Additionally, CR-binding time courses for 10 μ M TTR samples (40 μ M monomeric TTR) incubated at neutral pH (open squares) are shown as a positive inhibition control. Binding units are normalized to the negative control in the same way as for the A β 42 data.

Fig. 3. Difference between binding of toxic versus nontoxic solutions of TTR. Peptides were immobilized on agarose beads and either fresh TTR at neutral pH was applied or TTR incubated at low pH. Toxicity was assayed using SH-SY5Y neuroblastoma cells, as described earlier [37].

Fig. 4. Structural properties of the peptide designs by CD and FTIR. (a) CD spectra of 90 μ M peptide solutions taken in 50/50 – AcCN/PB (10 mM phosphate, pH 7.4). Units of molar ellipticity ($10^4 \text{ deg cm}^2 \text{ dmol}^{-1}$) are plotted due to the identical length (23 residues) of all reported peptides. (b) FTIR absorbance spectra (blue). The 1640 cm^{-1} and 1675 cm^{-1} absorbance bands associated with α -sheet structure are indicated by dashed vertical lines, as is the 1620 cm^{-1} β -sheet band.

Fig. 5. Summary of results from solution inhibition assays. The percentage inhibition values are reported as endpoint fluorescence relative to that of the uninhibited control, with the endpoint defined as the time at which fluorescence reaches its first local maximum within the curve's plateau. Sequence identity to AP90 is given beneath the x-axis to correlate changes in the designs with their inhibitory activity; a black to white gradient is included to illustrate decreasing sequence identity (dark to light). Percentage inhibition values are reported as the average of three independent experiments along with reproducibility errors (Table 2). Correlation of CD spectroscopy and inhibition results for “templated” peptide designs. Top curve: Plot of ellipticity minimum at 195-200 nm ($[\Theta]_{\text{min}}$) against sequence identity relative to AP90 for all designs. The specific design associated with a sequence identity value is indicated. Bottom curves: Plot of the percentage inhibition against sequence identity relative to AP90.

Table

Table 1

Design ^a	Sequence	Sequence identity ^b	Sequence composition. ^c	Chirality identity ^d	Description
<i>templated</i>					
	Ac-tail-L/ <u>D</u> strand- turn -L/ <u>D</u> strand-tail-NH ₂				
AP90	Ac-RGE <u>m</u> <u>N</u> <u>I</u> <u>S</u> <u>w</u> <u>M</u> NE Y S G W <u>t</u> <u>M</u> <u>n</u> <u>L</u> <u>k</u> MGR-NH ₂	100	100	100	Parent design
AP5	Ac-RGN <u>w</u> <u>N</u> <u>e</u> <u>S</u> <u>k</u> <u>M</u> NE Y S G W <u>m</u> <u>L</u> <u>m</u> <u>L</u> <u>t</u> MGR-NH ₂	65	100	100	Scrambled α -strand sequences of AP90 (residues 3-9 and 15-21)
AP6	Ac-RGE <u>a</u> <u>W</u> <u>m</u> <u>Y</u> <u>I</u> KNNLSE <u>T</u> <u>m</u> <u>M</u> <u>s</u> <u>N</u> <u>m</u> WGR-NH ₂	25	100	100	Scrambled sequences through α -strands and turn of AP90 (residues 3-21)
AP3	Ac-AQ <u>Q</u> <u>i</u> <u>E</u> <u>c</u> <u>l</u> <u>t</u> NVWDKE <u>I</u> <u>t</u> <u>M</u> <u>y</u> <u>F</u> <u>n</u> VSE-NH ₂	16	43	100	Random sequence selection, maintained L/D template
AP4	Ac-WRP <u>w</u> <u>A</u> <u>i</u> <u>D</u> <u>g</u> MNTVKQR <u>k</u> <u>A</u> <u>s</u> <u>V</u> <u>y</u> LQP-NH ₂	9	52	100	Random sequence selection, maintained L/D template
<i>non-templated</i>					
P2	Ac-MNWES <u>k</u> GE <u>w</u> IRMRYG <u>t</u> mLSNG <u>n</u> M-NH ₂	24	100	65	Scrambled sequence and chirality of AP90
P90	Ac-RGEMNLSWM NE Y S G WTMNLKMGR-NH ₂	100	100	74	All L-amino acid analog of AP90

Table 2

Design	Aβ42 % inhibition ^a	IAPP % inhibition ^b	TTR % inhibition ^c	Toxic TTR Binding ^d
<i>templated</i>				
AP90	95 ± 3	83 ± 3	65 ± 15	✓
AP5	93 ± 3	92 ± 3	37 ± 15	✓
AP6	35 ± 8	75 ± 7	ND-CR	✓
AP3	11 ± 4	36 ± 10	ND-CR	ND-No Lys
AP4	42 ± 4	11 ± 13	ND-CR	✓
<i>non-templated</i>				
P2	9 ± 11	13 ± 20	ND-CR	×
P90	ND-Insoluble	ND-Insoluble	ND-Insoluble	×

Table 3

Design	%sequence identity ^a	%sequence composition ^b	$[\Theta]_{\text{min}}$ ($10^4 \text{ deg cm}^2 \text{ dmol}^{-1}$) ^c
AP90	100	100	-7.79
AP5	65	100	-10.35
AP6	25	100	-13.41
AP3	16	43	-16.73
AP4	9	52	-14.48

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5