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Coassembly of Enantiomeric Amphipathic Peptides into Amyloid-Inspired Rippled β-Sheet Fibrils

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Supporting Information

ABSTRACT: Amphipathic peptides composed of alternating hydrophobic and hydrophilic amino acids self-assemble into amyloid-inspired, β-sheet nanoribbon fibrils. Herein, we report a new fibril type that is formed from equimolar mixtures of enantiomeric amphipathic peptides (L- and D-$(FKFE)_2$). Spectroscopic analysis indicates that these peptides do not self-sort and assemble into enantiomeric fibrils composed of all-L and all-D peptides, but rather coassemble into fibrils that contain alternating L- and D-peptides in a "rippled β-sheet" orientation. Isothermal titration calorimetry indicates an enthalpic advantage for rippled β-sheet coassembly compared to self-sorted β-sheet assembly of enantiomeric peptides.

Peptide self-assembly into cross-β amyloid structures is significant in the formation of both pathological and functional materials.[1,2] Amphipathic peptides, $(XZXZ)_n$, composed of alternating hydrophobic (X) and hydrophilic (Z) amino acids self-assemble into amyloid-inspired, bilayer β-sheet fibrils (Figure 1) that have been exploited as functional biomaterials including hydrogel matrices for the *ex vivo* growth of cells for regenerative medicine and multivalent scaffolds for the display of biological signal molecules.[3−5] Based on the promising utility of these self-assembled materials, the development of sophisticated assembly systems in which peptides either selectively coassemble into hybrid, multicomponent fibrils or self-sort into distinct fibrils (Figure 1) is of interest.[6,7] Herein we report the selective coassembly of enantiomeric L- and D-amphipathic Ac-$(FKFE)_2$-NH_2 peptides into two-component fibrils composed of alternating L- and D-sequences.

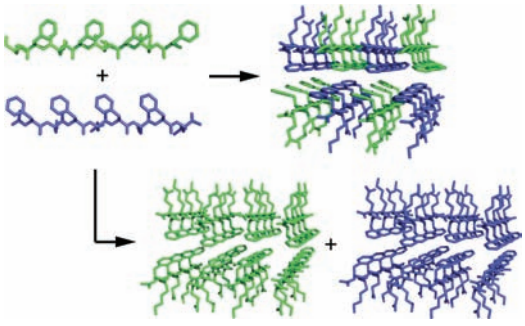

Figure 1. Schematic depiction of self-assembly of enantiomeric, amphipathic Ac-$(FKFE)_2$-NH_2 peptides (blue is L-Ac-$(FKFE)_2$-NH_2, green is D-Ac-$(FKFE)_2$-NH_2) into coassembled rippled β-sheet fibrils (top) or self-sorted enantiomeric fibrils (bottom).

There are few available methods to induce coassembly of complementary peptides into multicomponent fibrils. Coassembly of amphipathic peptides into two-component fibrils has previously been realized by exploiting complementary charged peptides (positive and negative) that fail to self-assemble due to charge repulsion, but that effectively coassemble.[8,9] In 1953, Pauling and Corey predicted that equimolar mixtures of L- and D-peptides should pack into rippled β-sheet structures that feature alternating L- and D-sequences.[10] There have been only a few examples in which these predicted rippled β-sheet structures have been exploited.[11] Conversely, it has been shown that equimolar mixtures of short, enantiomeric amyloid peptides, which form fibrils that consist of extended β-sheet laminates, do not coassemble into two-component rippled β-sheets but rather self-sort to form distinct L- and D-enantiomeric fibrils[12] or fail to form fibrils altogether.[13]

Based on these precedents, we hypothesized that mixtures of L- and D-enantiomeric Ac-$(FKFE)_2$-NH_2 peptides should undergo selective self-sorting to form L- and D-enantiomeric fibrils. L-Ac-$(FKFE)_2$-NH_2 self-assembles in water (2 mM peptide) to initially form left-handed helical ribbons[14−17] (8.3 ± 0.7 nm in diameter with a helical pitch of ∼19 nm, Figure 2A); the initial helical structures relax into flat nanoribbons (Figure S1, Supporting Information). We found that, in isolation, D-Ac-$(FKFE)_2$-NH_2 self-assembles into enantiomeric right-handed helical ribbons with diameter (8.2 ± 1.0 nm) and helical pitch (∼19 nm) values that correspond to those found with the L-peptide (Figure 2B). The CD signature (Figure 2D) of fibrils derived from L-Ac-$(FKFE)_2$-NH_2 and D-Ac-$(FKFE)_2$-NH_2 peptides are mirror images, with signals at 218 nm (corresponding to β-sheet secondary structure) and 205 nm (presumably from $\pi-\pi$ effects of neighboring Phe aromatic groups in the hydrophobic core).[16] IR spectra of the amide I region confirm antiparallel β-sheet secondary structure in the resulting fibrils (see Figure 3, below).

As indicated previously, we predicted that equimolar mixtures of L-Ac-$(FKFE)_2$-NH_2 and D-Ac-$(FKFE)_2$-NH_2 should form mixtures of self-sorted left- and right-handed helical fibrils composed exclusively of either all-L or all-D peptides. However, we observed no helical fibrils in mixtures of the L- and D-peptides by TEM analysis; instead, flat nanoribbon structures were observed that were about half the width (3.0 ± 0.4 nm) of the fibrils formed from the individual peptides in isolation

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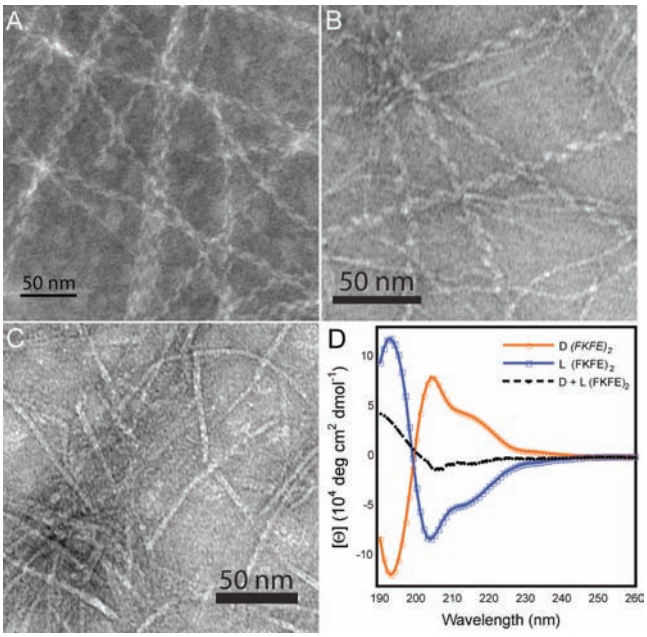

Figure 2. (A) TEM image of L-Ac-$(FKFE)_2$-NH_2 fibrils. (B) TEM image of D-Ac-$(FKFE)_2$-NH_2 fibrils. (C) TEM image of mixed L-Ac-$(FKFE)_2$-NH_2/D-Ac-$(FKFE)_2$-NH_2 fibrils. (D) CD spectra of L-Ac-$(FKFE)_2$-NH_2 fibrils (blue), D-Ac-$(FKFE)_2$-NH_2 fibrils (orange), and L-Ac-$(FKFE)_2$-NH_2/D-Ac-$(FKFE)_2$-NH_2 fibrils (black).

(Figure 2C). The CD signatures of enantiomeric fibrils at equimolar peptide concentrations should be effectively eliminated due to destructive interference. Instead, we observed a weakened signal (Figure 2D) that was hypothesized to be due to slight excess of the L-peptide in these mixtures; however, the same CD signature was observed even when the D-peptide was included in excess (1.2 mol equiv relative to the L-peptide). These unexpected results forced us to consider that these mixtures might undergo alternative assembly modes than those found for each enantiomeric peptide in isolation. Specifically, we hypothesized that these peptides may undergo coassembly to form hybrid fibrils.

We exploited isotope-edited IR (IE-IR) spectroscopy to characterize the assembly mode in fibrils of mixed L- and D-Ac-$(FKFE)_2$-NH_2 peptides. Antiparallel β-sheet structures exhibit strong amide I IR stretches at ~1620 cm^{-1} with a weaker stretch at ~1690 cm^{-1}. Incorporation of 1-^{13}C-labeled amino acids results in a shift of the amide I stretch of the labeled peak due to the lower vibrational frequency of the heavier ^{13}C carbonyl group.[18] The magnitude of the frequency shift can be used to analyze strand registry in amyloid fibrils; if ^{13}C-labeled carbonyls are in close spatial proximity cross-strand in the β-sheet, the magnitude of the shift in the IR is greater due to coupling of these signals.[18] For example, L-Ac-$(FKFE)_2$-NH_2 fibrils have an amide I stretch at 1618 cm^{-1} (Figure 3). When a 1-^{13}C label is incorporated at Phe 1, the amide I stretch splits and two signals are observed at 1620 cm^{-1} (unlabeled amide groups) and at 1603 cm^{-1} (labeled Phe 1 amide). When two 1-^{13}C amino acids are incorporated at Phe 1 and Phe 7, the labeled peak shifts an additional 10 cm^{-1} to 1593 cm^{-1} since Phe 1 and Phe 7 are in close spatial proximity (coupled) cross-strand in the resulting β-sheet fibrils.

Based on these results, we conducted an IE-IR analysis of fibrils formed from mixed L- and D-Ac-$(FKFE)_2$-NH_2 peptides in order to assess whether these peptides were coassembled

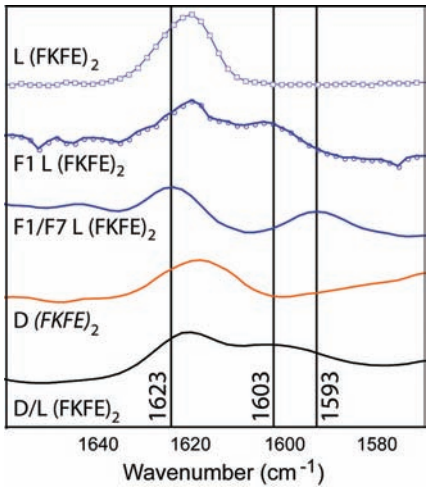

Figure 3. IR spectra of unlabeled L- and D-Ac-$(FKFE)_2$-NH_2 fibrils and various 1-^{13}C isotope-edited IR spectra of the L-Ac-$(FKFE)_2$-NH_2 and mixed L- and D-Ac-$(FKFE)_2$-NH_2 fibrils.

into fibrils reminiscent of Pauling's predicted rippled β-sheets. L-Ac-$(FKFE)_2$-NH_2 that was doubly 1-^{13}C-labeled at Phe 1/Phe 7 was assembled with D-Ac-$(FKFE)_2$-NH_2 (0.5 mM of each peptide). If these peptides self-sort into distinct enantiomeric fibrils, amide IR signals at 1620 and 1593 cm^{-1} should be observed since the coupled cross-strand relationship between the Phe 1/Phe 7 ^{13}C-labels will be preserved in the all-L fibrils. Conversely, if L-Ac-$(FKFE)_2$-NH_2 and D-Ac-$(FKFE)_2$-NH_2 coassemble with high fidelity into rippled β-sheets with alternating L- and D-peptides, the labeled stretch should shift to 1603 cm^{-1} since the coupled relationship between the Phe 1/Phe 7 ^{13}C-labels of L-Ac-$(FKFE)_2$-NH_2 will be perturbed by cross-strand pairing with the unlabeled D-peptide. We found that equimolar mixtures of L-Ac-$(FKFE)_2$-NH_2 and D-Ac-$(FKFE)_2$-NH_2 had amide I stretches at 1620 (unlabeled) and 1603 cm^{-1} (labeled) (Figure 3), consistent with an uncoupled cross-strand orientation of the Phe 1/Phe 7 labels on the L-peptide, and thus with a coassembled fibril consisting of alternating L-Ac-$(FKFE)_2$-NH_2 and D-Ac-$(FKFE)_2$-NH_2 in the extended β-sheets.

We next conducted fluorescence resonance energy transfer (FRET) experiments in order to further confirm coassembly of L-Ac-$(FKFE)_2$-NH_2 and D-Ac-$(FKFE)_2$-NH_2 into hybrid, rippled β-sheet fibrils. We prepared L-Ac-$(FKFE)_2$-NH_2 that was labeled with the EDANS fluorophore (Figure 4A) at the Glu 4 side chain (L-Ac-FKFE(EDANS)-FKFE-NH_2) and D-Ac-FKFK(Dabcyl)-FKFE-NH_2 that was labeled with Dabcyl at the Lys 4 side chain (the Glu 4 to Lys mutation was made to facilitate attachment of Dabcyl at the Lys ϵ-amine via an amide bond). EDANS/Dabcyl are a fluorophore/quencher pair with a Förster radius of 3.3 nm.[19] The described labeling pattern places EDANS and Dabcyl in cross-strand proximity on the exposed hydrophilic face of the proposed coassembled fibrils (Figure 4B). We confirmed that L-Ac-FKFE(EDANS)-FKFE-NH_2 and D-Ac-FKFK(Dabcyl)-FKFE-NH_2 independently self-assembled into β-sheet fibrils in isolation as evidenced by characteristic IR amide I signatures (Figure 4C) and that the EDANS fibrils were highly fluorescent (Figure S2). Mixing preassembled L-Ac-FKFE(EDANS)-FKFE-NH_2 and D-Ac-FKFK(Dabcyl)-FKFE-NH_2 fibrils elicited minor quenching of EDANS fluorescence at higher concentrations due to incidental contact between the surface-exposed fluorophore/quencher

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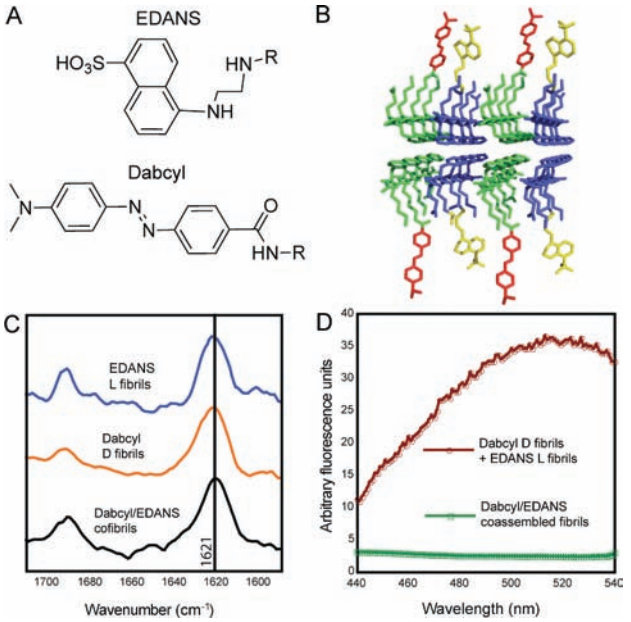

Figure 4. (A) Chemical structures of the EDANS and Dabcyl molecules. (B) Proposed architecture of L-Ac-FKFE(EDANS)-FKFE-NH_2 (blue)/D-Ac-FKFK(Dabcyl)-FKFE-NH_2 (green) rippled β-sheet fibrils indicating the relative positions of EDANS (yellow) and Dabycl (red) in the fibril assemblies. (C) IR spectra of L-Ac-FKFE(EDANS)-FKFE-NH_2 fibrils (blue), D-Ac-FKFK(Dabcyl)-FKFE-NH_2 fibrils (orange), and L-Ac-FKFE(EDANS)FKFE-NH_2/D-Ac-FKFK(Dabcyl)-FKFE-NH_2fibrils (black). (D) Fluorescence spectra of L-Ac-FKFE-(EDANS)FKFE-NH_2 fibrils and D-Ac-FKFK(Dabcyl)-FKFE-NH_2 fibrils that have been assembled in isolation and then mixed (black line) and L-Ac-FKFE(EDANS)FKFE-NH_2/D-Ac-FKFK(Dabcyl)-FKFE-NH_2 coassembled fibrils (green line).

molecules. Strikingly, fibrils derived from equimolar mixtures (0.5 mM of each peptide) of L-Ac-FKFE(EDANS)-FKFE-NH_2 and D-Ac-FKFK(Dabcyl)-FKFE-NH_2 were completely non-fluorescent (Figure 4D), confirming coassembly of these enantiomeric peptides into hybrid β-sheet fibrils with high fidelity.

We subsequently sought to understand the preference for coassembly of these enantiomeric amphipathic peptides. It is significant that amyloid peptides in which self-sorting of enantiomers has been observed are not amphipathic peptides with alternating hydrophobic/hydrophilic patterning.[10] Fibrils derived from amphipathic $(XZXZ)_n$ sequences form putative bilayer fibrils in which the nonpolar X residues define a hydrophobic core that leaves the hydrophilic side chain groups exposed to solvent (Figure 1); this bilayer structure is unique relative to amyloid formed from other self-assembling peptide classes in which lamination of β-sheets in fibrils is not limited to bilayer structures. In order to adopt the putative bilayer, neighboring strands in Ac-$(FKFE)_2$-NH_2 fibrils must orient the hydrophobic amino acid side chain groups on the same face; this structural constraint requires strands to be one residue out of register, which results in one dangling amino acid wherein hydrogen bond donors and acceptors and one side chain group are left unsatisfied/unpaired. Molecular modeling of Ac-$(FKFE)_2$-NH_2 fibrils indicates that the strand alignment with the *N*-terminal Phe residue out of register (Figure S3A) is more favorable than that with the *C*-terminal Glu out of register.[14,15] Based on molecular models (Figure S3B), it is apparent that L-Ac-$(FKFE)_2$-NH_2/D-Ac-$(FKFE)_2$-NH_2 cofibrils can adopt a packing orientation in which the neighboring peptides are in perfect cross-strand alignment with all side chain groups and hydrogen bond donors/acceptors in a paired orientation. The larger width of the all-L or all-D fibrils (about double the width of L/D-cofibrils) is consistent with β-sheets that are two peptides wide (Figure S3), which may be promoted by the dangling amino acids at the edges of the constituent β-sheets that possibly function as "sticky edge" groups. The smaller width of the L/D-cofibrils is consistent with β-sheets that are one peptide wide; the lack of "sticky edge" functionality in a perfectly aligned L/D-cofibril may account for these morphological differences between L/D-cofibrils and all-L or D-fibrils. We hypothesized that the perfectly aligned orientation of L/D-cofibrils should provide an enthalpic advantage that explains the preference for formation of coassembled vs self-sorted fibrils in equimolar L-Ac-$(FKFE)_2$-NH_2/D-Ac-$(FKFE)_2$-NH_2 mixtures.

We conducted isothermal titration calorimetry (ITC) experiments to explore a possible enthalpic advantage for coassembly vs self-sorting in L-Ac-$(FKFE)_2$-NH_2/D-Ac-$(FKFE)_2$-NH_2 mixtures. In order to conduct these experiments, we designed amphipathic peptides (L-Ac-$(FEFE)_2$-NH_2 and D-Ac-$(FKFK)_2$-NH_2) that fail to undergo self-assembly in isolation due to charge repulsion at neutral pH/low ionic strength. IR spectra for these peptides in isolation show broad amide I stretches at 1646 cm^{-1}, consistent with random structures; no evidence of self-assembled fibrils was observed by extensive TEM analysis. However, when L-Ac-$(FEFE)_2$-NH_2 and D-Ac-$(FKFK)_2$-NH_2 are mixed (0.5 mM of each peptide, adjusted to pH 7 with NaOD), coassembly into antiparallel rippled β-sheet fibrils occurs as evidenced by an IR amide I stretch at 1616 cm^{-1} with a minor stretch at 1681 cm^{-1} (Figure S5) and by the appearance of abundant fibrils in TEM images (Figure S6).

The design of enantiomeric peptides that do not self-assemble in isolation, but readily coassemble, enables ITC analysis of enthalpy of coassembly. ITC was conducted to compare the enthalpy of coassembly for L-Ac-$(FEFE)_2$-NH_2 and D-Ac-$(FKFK)_2$-NH_2 compared to coassembly of L-Ac-$(FEFE)_2$-NH_2 and L-Ac-$(FKFK)_2$-NH_2. The cationic peptides were titrated into the anionic peptides and the enthalpy for assembly was measured (measurements were corrected for dilution energies; see Supporting Information for experimental details). The titration data (Figure 5A,C) was fit using a one-site binding model (Figure 5B,D) and enthalpy values were derived. While entropy values are also commonly derived from ITC data, the changing nature of the fibrils (changes in length, diffusion, etc.) as these titration experiments proceed complicates interpretation of entropy values; we therefore only report enthalpy values here. It was found that the enthalpy of coassembly for the L-Ac-$(FEFE)_2$-NH_2/D-Ac-$(FKFK)_2$-NH_2 pair was -31.6 ± 0.5 kcal mol^{-1} and the enthalpy of assembly for the L-Ac-$(FEFE)_2$-NH_2/L-Ac-$(FKFK)_2$-NH_2 pair was -40.9 ± 0.6 kcal mol^{-1}. This indicates a clear enthalpic advantage for coassembly of enantiomeric amphipathic peptides (9.3 kcal mol^{-1}) relative to self-assembly of single stereoisomers of amphipathic peptides, as would be expected from the more perfect alignment of peptides within the rippled β-sheets of these cofibrils (as depicted in Figure S3). It should also be noted that the inflection point for these titrations is not precisely at molar ratios of 1:1 but varies between 1:1 and 1.5:1. This variance is most likely due to minor changes in peptide concentration during degassing procedures, but may also be due to imprecision in peptide packing during coassembly.

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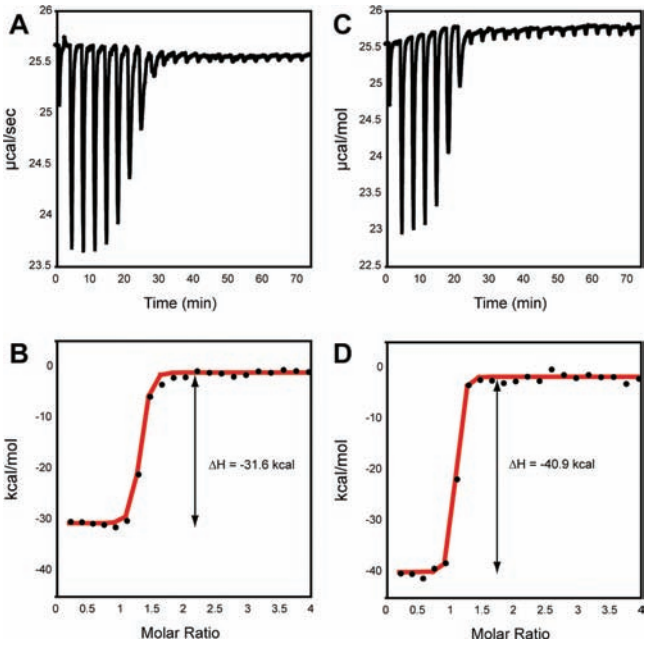

Figure 5. Representative raw (A) and fit (B) ITC data for injection of L-Ac-$(FKFK)_2$-NH_2 into L-Ac-$(FEFE)_2$-NH_2, and representative raw (C) and fit (D) ITC data for injection of D-Ac-$(FKFK)_2$-NH_2 into L-Ac-$(FEFE)_2$-NH_2.

In conclusion, we have reported herein the high-fidelity coassembly of enantiomeric peptides into rippled β-sheet L/D-cofibrils. This work complements recent work by Schneider, Pochan, and co-workers in which they observed unique emergent hydrogelation properties of mixtures of self-assembling L/D-amphipathic β-hairpin peptides.[20] Schneider and Pochan's work indicates that these mixtures form hybrid fibrils that contain both L/D-peptides, and that the resulting hydrogels exhibit significant enhancements in viscoelasticity relative to all-L or all-D fibrils. While they could not fully explain these interesting observations, the work reported herein suggests that enthalpically favorable coassembly of L/D-peptide mixtures into hybrid rippled β-sheet fibrils may be a general feature of amphipathic peptides with alternating hydrophobic/hydrophilic patterning. This fundamentally new class of material increases the complexity that can be accessed in the design of amyloid-inspired materials.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional figures, experimental procedures, and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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