

1 **A structure-based model for the electrostatic interaction of the N-terminus of**
2 **protein tau with the fibril core of Alzheimer's Disease filaments**

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7
8 **Abstract:**

9 Although portions of tau protein within the microtubule binding region have been
10 shown to form the ordered core of tau filaments, the structural details of how other
11 regions of tau participate in filament formation are so far unknown. In an attempt to
12 understand how the N-terminus of tau may interact with fibril core, we crystallized and
13 determined the structure of the N-terminal segment ₅RQEFEV₁₀ of tau. Several lines of
14 evidence have shown the importance of this segment for fibril formation. The crystal
15 structure reveals an out-of-register Class 5 steric zipper with a wet and a dry interface.
16 To examine the possible interaction of ₅RQEFEV₁₀ with the tau fibril core, we modeled
17 the binding of the wet interface of the ₅RQEFEV₁₀ structure with the ₃₁₃VDLSKVTSKC₃₂₂
18 region of the Alzheimer's Disease tau filament structures. This model is consistent with,
19 and helps to explain previous findings on the possible interaction of these two
20 segments, distant in sequence. In addition, we discuss the possible conservation of this
21 interaction across multiple polymorphs of tau.

22
23 **Introduction:**

24 The aggregation of tau into amyloid fibrils is associated with some 25
25 neurological diseases, collectively termed tauopathies. Although scientists have for
26 decades associated fibrous tau aggregates with disease for decades, the molecular
27 events driving aggregation of tau into amyloid fibrils remain unknown. It is generally
28 thought that tau remains in three pools in the cell: attached to microtubules to promote
29 their stability(1,2), bound to molecular chaperones to protect nucleating sequences of
30 tau from enabling aggregation(3), or in a fibrous state where each fiber contains
31 hundreds to many thousands of tau molecules(4–6). Under what conditions the fibril
32 state begins to dominate is unclear.

33 Previous studies have shown that soluble, monomeric tau largely lacks a defined
34 3-dimensional shape(7); however, other studies posit that tau adopts a “paper clip”
35 conformation in solution(8) or a seed-competent conformation where amyloid nucleating
36 sequences are exposed and able to seed fibril formation(9). In addition, the binding of
37 different tau constructs to microtubules has been visualized by cryo-EM(2). Despite
38 these findings, information on the structure of soluble, monomeric form of tau is limited
39 due to its largely disordered nature; therefore, most structural studies have focused on
40 the aggregated state of tau(10–14). Our laboratory first focused on the segments of tau
41 shown to be essential for *in vitro* aggregation, the primary nucleating sequences
42 VQIINK and VQIVYK, located at the beginning of tau microtubule binding repeats 2 and
43 3, respectively(15). The crystal structures of these segments revealed classical “steric
44 zipper” structural features(10,11). Mutations to these segments inhibit full-length tau
45 aggregation, and we have shown that inhibitors designed to “cap” the crystal structures

46 of VQIINK and VQIVYK segments also inhibit full-length tau aggregation, further
47 demonstrating the importance of these segments(11,16).

48 Recently, cryo-EM studies of extracted tau filaments from Alzheimer's Disease
49 and Pick's Disease patients have revealed several tau fibril polymorphs in near-atomic
50 detail(12–14). In all of these structures, residues 306-378 spanning the length of
51 Repeats 3 and 4 plus an additional six residues to the C-terminus of Repeat 4, are
52 ordered in the fibril core, and in Pick's Disease, Repeat 1 residues 254-274 are also
53 ordered(14). Although these landmark discoveries help illuminate the fold adopted by
54 the microtubule binding region of tau, it is still unknown to what degree other parts of tau
55 participate in the aggregation process.

56 In the AD fibril structures, there is additional density consistently seen near
57 residues K317 and K321 that may indicate another region of tau is interacting with the
58 fibril core(12,13). Fitzpatrick *et al.* hypothesize that this extra density belongs to the
59 residues γ EFE₉, an N-terminal sequence of tau that is part of the Alz50/MC-1 antibody
60 binding epitope(12,17). To better understand the potential interaction of the N-terminus
61 and the AD fibril core, we sought to determine the structure of this N-terminal segment.

62

63 **Results:**

64 We first searched for segments containing γ EFE₉ that are likely to crystallize.
65 Although no segment containing γ EFE₉ scored well on the structure-based ZipperDB
66 server(18), the ability to form fibrils from segment δ RQEFEV₁₀ was previously predicted
67 by a sequence-based method and demonstrated biochemically(19). Therefore, we

68 crystallized and determined the structure of the hexameric segment $_5\text{RQEFEV}_{10}$ (Figure
69 1 A-C).

70 The crystal structure of $_5\text{RQEFEV}_{10}$ revealed a Class 5 homozipper where beta-
71 strands assemble in antiparallel sheets and these sheets mate together in distinct face-
72 to-face and back-to-back interfaces. Notably, the sheets are out-of-register and are
73 related to each other by a 2_1 “fibril axis” (20) (Figure 2 A, B). This combination of
74 symmetry elements produces an $\sim 80^\circ$ crossing angle between strands of one sheet and
75 its mated sheet (Figure 2 A)(21). The alternating sequence of charged and
76 hydrophobic/uncharged residues leads to wet and dry interfaces in the crystal structure.

77 The wet interface features electrostatic interactions among polar, charged
78 residues and water molecules. In particular, glutamates form an extensive hydrogen
79 bond network with water molecules and arginines originating from the same sheet and
80 from the opposing sheet (Figure 2 B). The dry interface features hydrophobic packing of
81 phenylalanine, glutamine, and valine leading to the exclusion of water (Figure 2 A, B).
82 Also, glutamine side chains clasp each other through a pair of hydrogen bonds, further
83 stabilizing connections between neighboring strands in a sheet (Figure 2 A). This
84 interaction is similar to the polar clasp described by Gallagher-Jones, *et al.*, with the
85 distinction that glutamines in that study originated within the same strand(22). Similar to
86 that polar clasp, neighboring aromatic residues restrict the glutamines to a conformation
87 in which they bond to each other within a hydrophobic pocket (Figure 2 A). As stated by
88 Gallagher-Jones, *et al.* the shielding of glutamines by neighboring aromatic residues
89 may be essential for the formation of this polar clasp.

90 The crystal structure of $_5$ RQEFEV $_{10}$ can account for the low resolution density
91 found in the cryo-EM reconstructions of Alzheimer's Disease (AD) tau filaments near
92 residues K317 and K321, much as suggested by Fitzpatrick, *et al* (12). The positioning
93 of $_5$ RQEFEV $_{10}$ near these residues in the tau filament conformation is supported by the
94 binding of the MC-1 and Alz50 antibodies to a discontinuous epitope consisting of both
95 $_7$ EFE $_9$ and $_{313}$ VDLSKVTSKC $_{322}$ (17).

96 In order to examine the potential interaction of the N-terminal $_7$ EFE $_9$ segment with
97 the AD fibril core, we first computationally docked the $_6$ QEFEV $_{10}$ segment seen in the
98 crystal structure into the low-resolution density shown to be adjacent to residues K317
99 and K321 in the AD Paired Helical Filament (PHF) (Figure 3 A-B)(12). In this model, the
100 wet interface glutamates found in the crystal structure form electrostatic interactions with
101 the exposed lysines in the PHF fibril, while the dry interface faces away from the PHF
102 surface (Figure 3 A, B). Notably, we omitted Arg5 in this model due to steric clashes
103 with Leu315 on the PHF. We speculate that Arg5 would have to adopt a different
104 conformation in the fibril structure than in the crystal structure in order to maintain the
105 interaction of Glu7 and Glu9 with Lys317 and Lys321.

106 To examine further the relevance of the $_7$ EFE $_9$ and $_{313}$ VDLSKVTSKC $_{322}$
107 interaction in tau fibrils, we searched the literature for other evidence that implicates the
108 N-terminus of tau in fibril formation. Poorkaj, P. *et al*. described a missense mutation
109 found in a Progressive Supranuclear Palsy (PSP) patient that changes R5 to a
110 leucine(23). In addition, it has been shown that deletion of residues 2-18 produces less
111 aggregated tau than the wild-type sequence whereas the inclusion of the R5L mutation
112 increases the amount of aggregated tau in the presence of arachidonic acid(24). This is

113 consistent with our model of τ EF_{E9} binding to ₃₁₃VDLSKVTSKC₃₂₂ in the AD PHF; in that
114 the deletion of residues 2-18 would abrogate the interaction of τ EF_{E9} with
115 ₃₁₃VDLSKVTSKC₃₂₂. In addition, in our model the R5L mutation would result in a more
116 stable interaction with Leu315 as discussed below.

117 To analyze if the R5L mutation might affect the binding of ₅RQEF_{E9}V₁₀ to the
118 ₃₁₃VDLSKVTSKC₃₂₂ region in the AD filaments, we modeled the putative interaction of
119 the sequence ₅LQEF_{E9}V₁₀ with the AD PHF. To accomplish this, we mutated the R5 that
120 was omitted in the wild-type model due a potential steric clash with L315 on the PHF, to
121 a rotamer of leucine that would maximize its buried surface area and shape
122 complementarity to L315 on the PHF (Figure 3 B). The model demonstrates that the
123 mutation R5L would result in a more favorable interaction with the PHF than the native
124 sequence, providing an explanation for R5L's ability to increase tau aggregation.

125 Our attempts to dock the ₅RQEF_{E9}V₁₀ crystal structure into the
126 ₃₁₃VDLSKVTSKC₃₂₂ region on the cryoEM structure of the straight filaments (SFs) were
127 hindered due to the tight packing protofilaments that occurs in this region. By truncating
128 the residues present in the crystal structure to only τ EF_{E9} it is possible to place these
129 residues within hydrogen bonding distance of K317 on one protofilament and K321 on
130 the other protofilament. This results in a binding site comprised of residues from two
131 different tau monomers, as opposed to a binding site comprised of only one monomer
132 as in the PHF (Figure 3D). However, this two-tau monomer model of τ EF_{E9} bound to the
133 SF would result in steric clashes if any other residues were added to the τ EF_{E9}
134 sequence (Figure 3D), particularly with L315, making it harder to assess whether there
135 is enough space in the SF inter-protofilament interface for the N-terminal τ EF_{E9}

136 sequence. Likewise, it was difficult to examine the effect of the R5L mutation on this
137 interaction due to the resulting steric clashes.

138

139 **Discussion:**

140 The initial proposal that τ 7EFE₉ interacts with τ 313VDLSKVTSKC₃₂₂ came from
141 biochemical studies in which Jicha, *et al.* confirmed that two antibodies, MC-1 and
142 Alz50, most likely bind a single epitope of tau comprised of discontinuous segments
143 τ 7EFE₉ and τ 313VDLSKVTSKC₃₂₂. The idea of a single epitope comprising these two distal
144 sequences was supported by antibody binding assays using a series of tau constructs
145 containing truncations or mutations in these regions(19). Tau constructs missing either
146 τ 7EFE₉ or τ 313VDLSKVTSKC₃₂₂ did not exhibit antibody binding, demonstrating that both
147 sequences need to be present for antibody reactivity. In addition, a series of mutations
148 to the τ 7EFE₉ segment (Glu7,9 -> Ala7,9; Phe8 -> Ser8) abrogated antibody binding to
149 tau. Importantly, Jicha, *et al* showed that tau constructs missing τ 7EFE₉ or
150 τ 313VDLSKVTSKC₃₂₂ could not be mixed in solution to recover the MC-1/Alz50 epitope,
151 indicating that this epitope is formed intramolecularly.

152 In an attempt to examine which sequences might interact with the primary
153 nucleating sequences of tau τ 275VQIINK₂₈₀ and τ 306VQIVYK₃₁₁, Moore *et al.* tested the
154 ability of different tau sequences to accelerate and increase τ 275VQIINK₂₈₀ and
155 τ 306VQIVYK₃₁₁ aggregation(19). Through these experiments, the authors predicted the
156 heterozipper interaction formed between τ 306VQIVYK₃₁₁ and τ 375KLTFR₃₇₉. This predicted
157 interaction was later confirmed by the AD tau filament structure(12). In addition, Moore,
158 *et al* showed that τ 5RQEFEV₁₀ can form fibrils *in vitro*(19), although it did not affect the

159 aggregation of either ²⁷⁵VQIINK₂₈₀ or ³⁰⁶VQIVYK₃₁₁. This supports the idea that
160 ⁵RQEFEV₁₀ instead interacts with ³¹³VDLSKVTSKC₃₂₂ in a different region of the fibril
161 core. Further experiments similar to those performed by Moore, *et al*, including
162 aggregation kinetics and circular dichroism of the individual peptides and a mixture of
163 both peptides, could help strengthen evidence for the interaction of ⁵RQEFEV₁₀ and
164 ³¹³VDLSKVTSKC₃₂₂ in the fibril state.

165 The results obtained by Jicha, *et al* and Moore, *et al* are consistent with the
166 model proposed here where ⁵RQEFEV₁₀ occupies the un-modeled density that flanks
167 residues K317 and K321 in the Fitzpatrick, *et al*. PHF cryo-em reconstruction(12). In
168 particular, the abrogation of antibody binding by Glu7,9 -> Ala7,9 mutations performed
169 by Jicha, *et al* can be explained by the disruption of the charge-charge interaction of
170 glutamate and lysine residues in the proposed model (Figure 3 B)(17). The loss of this
171 interaction would most likely greatly reduce the affinity of ⁷EFE₉ for the
172 ³¹³VDLSKVTSKC₃₂₂ segment, leading to the loss of the MC-1 and Alz50 epitope. The
173 loss of antibody binding from the Phe8 -> Ser8 can be explained in the proposed model
174 given that Phe8 is facing away from the fibril, allowing it to remain exposed for antibody
175 binding. Therefore, mutation of Phe8 may not prevent the far N-terminal segment from
176 binding to the exposed lysines on the fibril core, but may still eliminate antibody
177 reactivity. This suggests that the ⁷EFE₉ segment needs to be not only in a stacked
178 conformation bound to K317 and K321 on the fibril core, but also needs F8 to be facing
179 away from the fibril core and presented for antibody binding. A loss of either of these
180 conditions would result in a loss of MC-1 reactivity.

181 The model of τ EFE₉ interacting with K317 and K321 in the SF (Figure 3 C, D)
182 suggests that either the τ EFE₉ sequence binds in a different manner to the
183 ₃₁₃VDL SKVT SKC₃₂₂ region on the SF or that the un-modeled density present in the
184 Fitzpatrick, *et al* SF reconstruction does not result from the binding of the τ EFE₉ motif,
185 but perhaps some other anion. Tau AD filament structures from 3 additional cases seem
186 to recapitulate the extra density seen at the SF inter-protofilament interface(13). This
187 indicates that this density may be a common feature of the SF fold and necessary to
188 interact with the four lysines resulting from K317 and K321 of each protofilament coming
189 together at the SF inter-protofilament interface.

190 It is worth noting that the cryo-EM structures of AD tau fibrils display parallel, in-
191 register beta-strands, whereas the RQEFEV crystal structure forms out-of-register,
192 antiparallel beta-sheets. Because residues N-terminal to Val306 are not resolved in the
193 cryo-EM structure, we cannot determine whether ₅RQEFEV₁₀ stacks into parallel or
194 antiparallel sheets in the fibril. Our model used two strands of ₅RQEFEV₁₀ stacked in an
195 anti-parallel beta-sheet as seen in the crystal structure. Although different from the
196 crystal structure, parallel, in-register beta-sheets of ₅RQEFEV₁₀ would still form a wet
197 and dry interface due to the alternating sequence of hydrophilic, charged residues and
198 uncharged, mostly hydrophobic residues. Therefore, a parallel, in-register conformation
199 of ₅RQEFEV₁₀ would still allow Glu7 and Glu9 to form electrostatic interactions with
200 Lys317 and Lys321 in a manner similar to the model proposed in Figure 3 A-B.

201 Recently, a new polymorph of tau from the brain of a Pick's Disease case has
202 been visualized by cryo-EM(14). This structure adopts a drastically different fold from
203 the AD filaments; however, the Pick's Disease filaments are still MC-1 reactive,

204 indicating the preservation of the ${}_{7}\text{EFE}_9$ and ${}_{313}\text{VDLSKVTSKC}_{322}$ epitope(14). In this
205 structure, K317 and K321 are exposed to the solvent in a beta-sheet conformation,
206 which would allow the N-terminal ${}_{7}\text{EFE}_9$ segment to bind K317 and K321 through
207 electrostatic interactions between the glutamates and lysines similar to the AD PHF
208 model (Figure 3 A, B). This electrostatic interaction would preserve the MC-1 epitope
209 and provide an explanation for why MC-1 recognizes both tau fibril polymorphs.

210 In addition, the potential strengthening of the N-terminal interaction with the fibril
211 core through the R5L mutation and its discovery in a PSP patient, suggests that this
212 interaction may also occur in the PSP tau fibril. Although there is evidence that so-called
213 4R tauopathies, where the dominant species found in aggregated tau are the 4R
214 isoforms, PSP and Corticobasal Degeneration (CBD) form different tau polymorphs,
215 their structures have not yet been determined(25). However, as long as the
216 ${}_{313}\text{VDLSKVTSKC}_{322}$ region adopts a beta-sheet like fold, and K317 and K321 remain
217 solvent-exposed, the long-range charge-charge interaction with ${}_{7}\text{EFE}_9$ could be
218 preserved. In short, there may be a common interaction among the disparate folds of
219 tau polymorphs.

220 In the past, our lab has developed inhibitors of tau aggregation by structure-
221 based drug design(11,16). This requires detailed structural knowledge of a site of the
222 tau protein in the aggregated state obtained by X-ray crystallography or MicroED. These
223 inhibitors target segments of the tau protein in the microtubule binding region that is
224 thought to participate in the fibril core of all tau filaments. However, given the structural
225 evidence thus far that the microtubule binding region can adopt different folds in
226 different diseases, it is likely that a spectrum of inhibitors will be necessary to most

227 effectively block aggregation or spreading of specific tau polymorphs. Immuno-labeling
228 with MC-1 seems to indicate that the N-terminal interaction with the fibril core modeled
229 here is preserved in both AD and Pick's Disease tau filaments. Therefore, an inhibitor
230 targeted towards this interaction may be general to all tau filaments, providing another
231 target for treating tauopathies.

232

233 **Methods:**

234 **Crystallization and Data Collection:** Synthetic peptide RQEFEV was ordered from
235 GenScript. RQEFEV was crystallized using the hanging drop method with a 2:1 mixture
236 of 60 mg/mL RQEFEV and 0.2 M Ammonium Citrate Dibasic, 30% PEG 3350.

237 Diffraction data was collected at APS Beamline 24-ID-E using an Eiger detector.

238 **Data Processing and Structure Determination:** Diffraction data were indexed and
239 integrated using XDS and scaled using XSCALE(26). Molecular replacement was
240 performed using Phaser and an idealized beta-strand as a molecular replacement
241 probe(27). Model-building and manual real-space refinement was performed in
242 COOT(28). Automated reciprocal-space and real-space refinement was performed
243 using Refmac and Phenix(29,30).

244 **Modeling:** Modeling was performed in COOT using the RQEFEV crystal structure and
245 the cryo-em structures for the AD PHF (5o3l.pdb) and SF (5o3t.pdb) downloaded from
246 the PDB. Cryo-em maps for the PHF (EMD-3741) and SF (EMD-3743) were also used
247 for modeling and generating figures. All figures were made in Pymol (Schrodinger).

248

249

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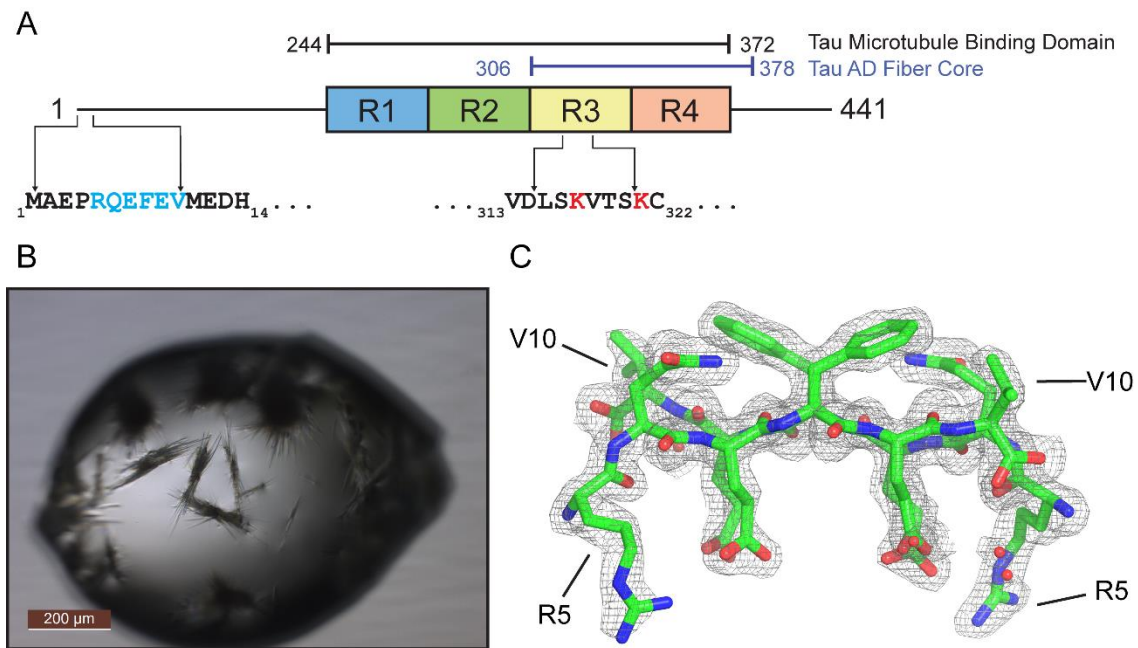
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365 **Figures:**



367 **Figure 1: Crystal Structure of tau N-terminal segment 5 RQEFEV₁₀**

368 A) Schematic of tau primary structure. B) Crystals of 5 RQEFEV₁₀ grown using the

369 hanging drop method. C) Atomic model and electron density of 5 RQEFEV₁₀

370 demonstrating the quality of fit. The view is down the fibril axis, showing two anti-parallel

371 strands.

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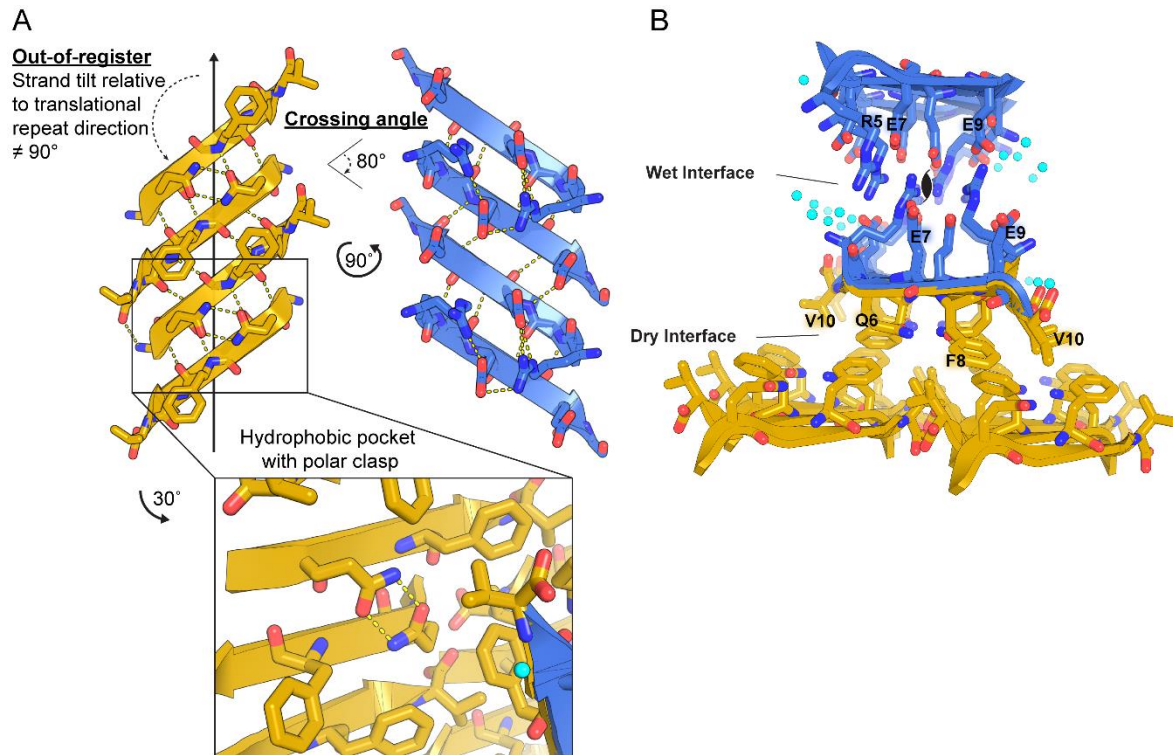
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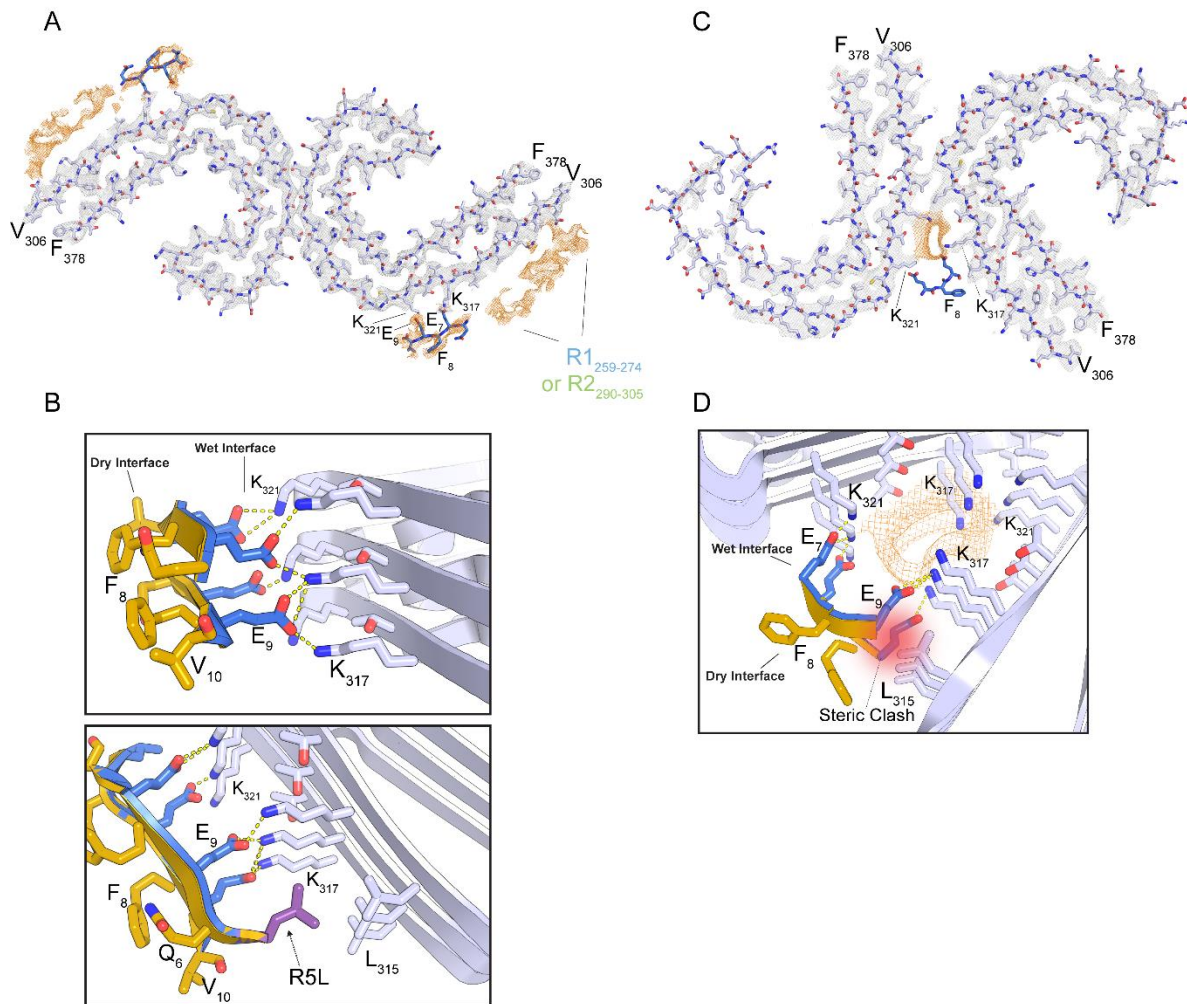
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379 **Figure 2: Crystal structure of $5RQEFEV_{10}$ reveals a wet and a dry interface**

380 A) $5RQEFEV_{10}$ forms amyloid-like out-of-register protofilaments with wet and dry
381 interfaces. Inset shows formation of a polar clasp with neighboring glutamines in the
382 hydrophobic pocket of the dry interface. B) View down the fibril axis of $5RQEFEV_{10}$
383 highlighting the interactions between residues within the wet and dry interfaces. Water
384 molecules are shown by aqua spheres.



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386 **Figure 3: Speculative model for 5RQEFEV₁₀ interaction with Alzheimer's Disease**
387 **paired helical and straight filaments fibril cores**

388 A) Atomic model of Alzheimer's disease paired helical filaments (PHF) (5o3l.pdb) shown
389 with electron density of modeled (grey) and un-modeled (orange) regions(12).

390 5RQEFEV₁₀ is docked into un-modeled density flanking the solvent-exposed K317 and

391 K321 residues of the PHF. B) Detail (top) highlighting the interaction of the glutamates

392 in the wet interface with K317 and K321 of the PHF. Detail (bottom) demonstrating the

393 possible interaction of the R5L mutation with L315 of the PHF. C) Overview of potential

394 interaction of γ EFE₉ with straight filaments (SF) (5o3t.pdb) at the inter-protofilament
395 interface. D) Detail of the potential hydrogen bonding of wet interface glutamates with
396 K317 and K321 and potential steric clash with L315 of the SF.

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418 **Table 1. Data collection and refinement statistics.**

	RQFEV (PDB ID: 6N4P)
Wavelength	0.9792
Resolution range	16.08 - 1.851 (1.918 - 1.851)
Space group	P2 ₁
Unit cell	16.59 11.45 25.42 90 104.236 90
Total reflections	2416 (226)
Unique reflections	842 (80)
Multiplicity	2.9 (2.8)
Completeness (%)	97.2 (96.4)
Mean I/sigma(I)	4.2 (1.6)
Wilson B-factor	14.8
R-merge	0.16 (0.60)
R-meas	0.20 (0.72)
R-pim	0.11 (0.40)
CC1/2	0.97 (0.83)
CC*	0.99 (0.95)
Reflections used in refinement	838 (80)
Reflections used for R-free	85 (8)
R-work	0.19 (0.30)
R-free	0.27 (0.47)
CC(work)	0.96 (0.81)
CC(free)	0.91 (0.83)
Number of non-hydrogen atoms	118
macromolecules	114
solvent	4
Protein residues	12
RMS(bonds)	0.013
RMS(angles)	1.48
Ramachandran favored (%)	100.00
Ramachandran allowed (%)	0.00
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.00
Clashscore	4.55
Average B-factor	21.9
macromolecules	21.5
solvent	32.5

419 Statistics for the highest-resolution shell are shown in parentheses