

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 <sub>5</sub>RQEFEV<sub>10</sub> 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 <sub>5</sub>RQEFEV<sub>10</sub> with the tau fibril core, we modeled  
17 the binding of the wet interface of the <sub>5</sub>RQEFEV<sub>10</sub> structure with the <sub>313</sub>VDLSKVTSKC<sub>322</sub>  
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  $\tau$ EFE<sub>9</sub>, 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  $\tau$ EFE<sub>9</sub> that are likely to crystallize.  
65 Although no segment containing  $\tau$ EFE<sub>9</sub> scored well on the structure-based ZipperDB  
66 server(18), the ability to form fibrils from segment  $\tau$ RQEFEV<sub>10</sub> 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  $\tau$ EF<sub>9</sub> binding to <sub>313</sub>VDLSKVTSKC<sub>322</sub> in the AD PHF; in that  
114 the deletion of residues 2-18 would abrogate the interaction of  $\tau$ EF<sub>9</sub> with  
115 <sub>313</sub>VDLSKVTSKC<sub>322</sub>. 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 <sub>5</sub>RQEF<sub>10</sub> to the  
118 <sub>313</sub>VDLSKVTSKC<sub>322</sub> region in the AD filaments, we modeled the putative interaction of  
119 the sequence <sub>5</sub>LQEF<sub>10</sub> 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 <sub>5</sub>RQEF<sub>10</sub> crystal structure into the  
126 <sub>313</sub>VDLSKVTSKC<sub>322</sub> 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  $\tau$ EF<sub>9</sub> 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  $\tau$ EF<sub>9</sub> bound to the  
133 SF would result in steric clashes if any other residues were added to the  $\tau$ EF<sub>9</sub>  
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  $\tau$ EF<sub>9</sub>

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  $\tau$ 7EFE<sub>9</sub> interacts with  $\tau$ 313VDLSKVTSKC<sub>322</sub> 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  $\tau$ 7EFE<sub>9</sub> and  $\tau$ 313VDLSKVTSKC<sub>322</sub>. 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  $\tau$ 7EFE<sub>9</sub> or  $\tau$ 313VDLSKVTSKC<sub>322</sub> 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  $\tau$ 7EFE<sub>9</sub> segment (Glu7,9 -> Ala7,9; Phe8 -> Ser8) abrogated antibody binding to  
149 tau. Importantly, Jicha, *et al* showed that tau constructs missing  $\tau$ 7EFE<sub>9</sub> or  
150  $\tau$ 313VDLSKVTSKC<sub>322</sub> 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  $\tau$ 275VQIINK<sub>280</sub> and  $\tau$ 306VQIVYK<sub>311</sub>, Moore *et al.* tested the  
154 ability of different tau sequences to accelerate and increase  $\tau$ 275VQIINK<sub>280</sub> and  
155  $\tau$ 306VQIVYK<sub>311</sub> aggregation(19). Through these experiments, the authors predicted the  
156 heter zipper interaction formed between  $\tau$ 306VQIVYK<sub>311</sub> and  $\tau$ 375KLTFR<sub>379</sub>. This predicted  
157 interaction was later confirmed by the AD tau filament structure(12). In addition, Moore,  
158 *et al* showed that  $\tau$ 5RQEFEV<sub>10</sub> can form fibrils *in vitro*(19), although it did not affect the

159 aggregation of either <sup>275</sup>VQIINK<sub>280</sub> or <sup>306</sup>VQIVYK<sub>311</sub>. This supports the idea that  
160 <sup>5</sup>RQEFEV<sub>10</sub> instead interacts with <sup>313</sup>VDLSKVTSKC<sub>322</sub> 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 <sup>5</sup>RQEFEV<sub>10</sub> and  
164 <sup>313</sup>VDLSKVTSKC<sub>322</sub> 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 <sup>5</sup>RQEFEV<sub>10</sub> 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 <sup>7</sup>EFE<sub>9</sub> for the  
172 <sup>313</sup>VDLSKVTSKC<sub>322</sub> 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 <sup>7</sup>EFE<sub>9</sub> 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  $\tau$ EFE<sub>9</sub> interacting with K317 and K321 in the SF (Figure 3 C, D)  
182 suggests that either the  $\tau$ EFE<sub>9</sub> sequence binds in a different manner to the  
183 <sub>313</sub>V<sub>D</sub>L<sub>S</sub>K<sub>V</sub>T<sub>S</sub>K<sub>C</sub><sub>322</sub> 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  $\tau$ EFE<sub>9</sub> 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 <sub>5</sub>RQEFEV<sub>10</sub> stacks into parallel or  
194 antiparallel sheets in the fibril. Our model used two strands of <sub>5</sub>RQEFEV<sub>10</sub> 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 <sub>5</sub>RQEFEV<sub>10</sub> 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 <sub>5</sub>RQEFEV<sub>10</sub> 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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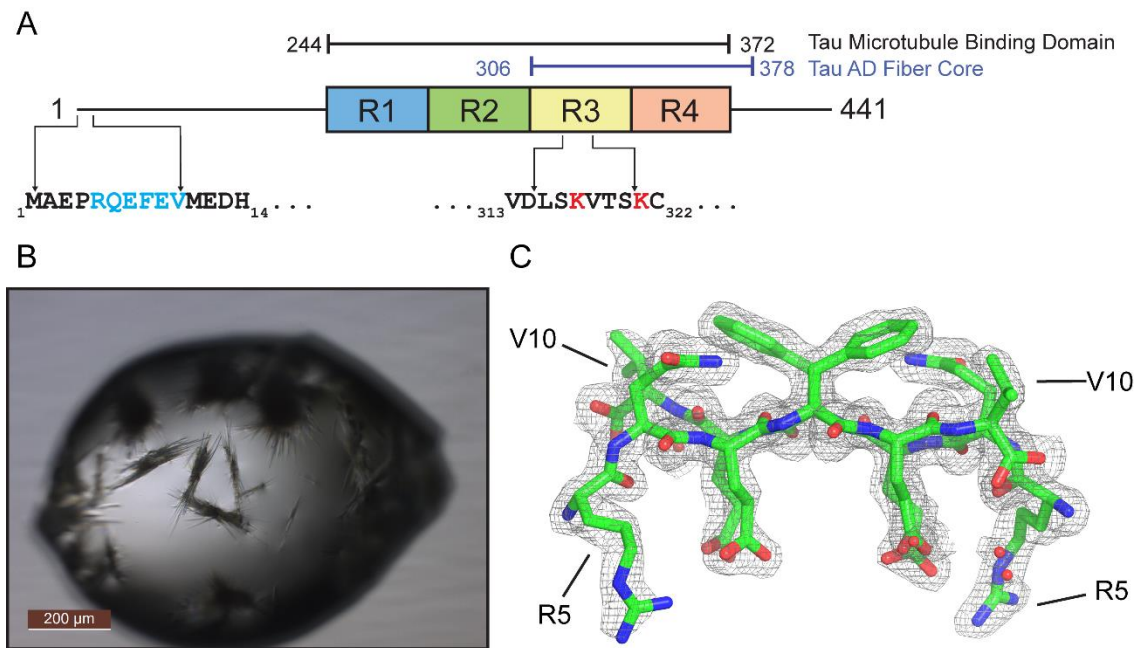
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365 **Figures:**



367 **Figure 1: Crystal Structure of tau N-terminal segment 5RQEFEV<sub>10</sub>**

368 A) Schematic of tau primary structure. B) Crystals of 5RQEFEV<sub>10</sub> grown using the

369 hanging drop method. C) Atomic model and electron density of 5RQEFEV<sub>10</sub>

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

371 strands.

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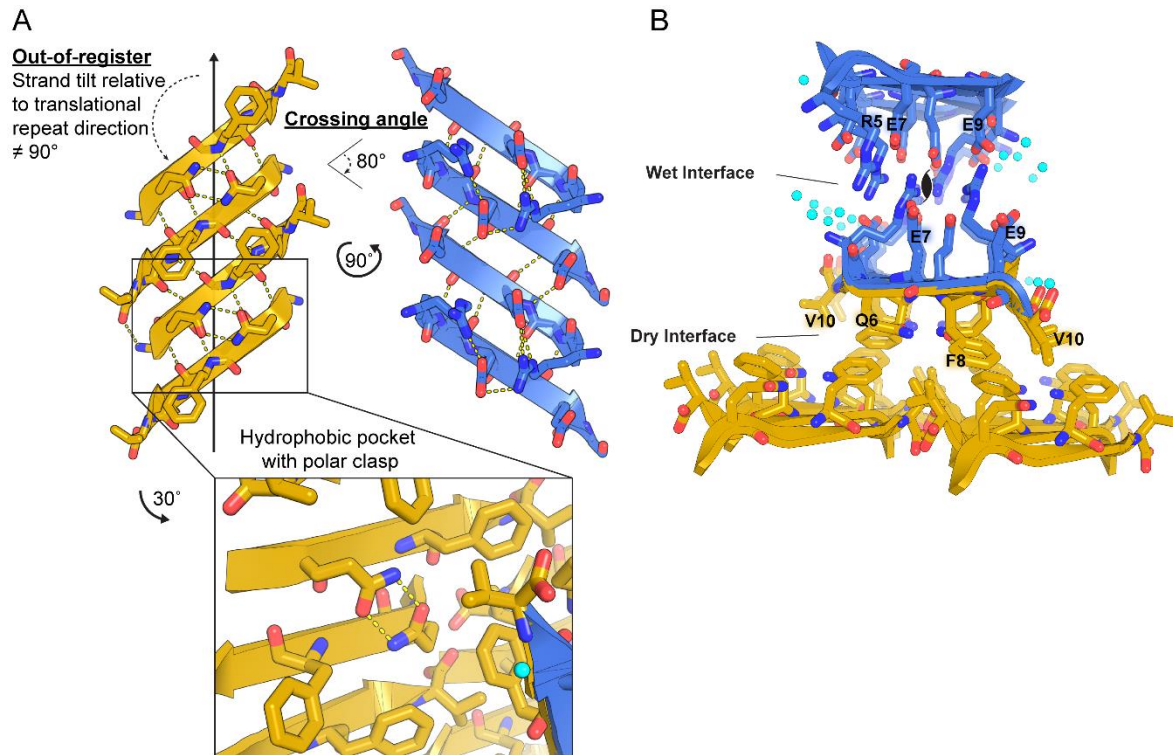
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379 **Figure 2: Crystal structure of  $5RQEFEV_{10}$  reveals a wet and a dry interface**

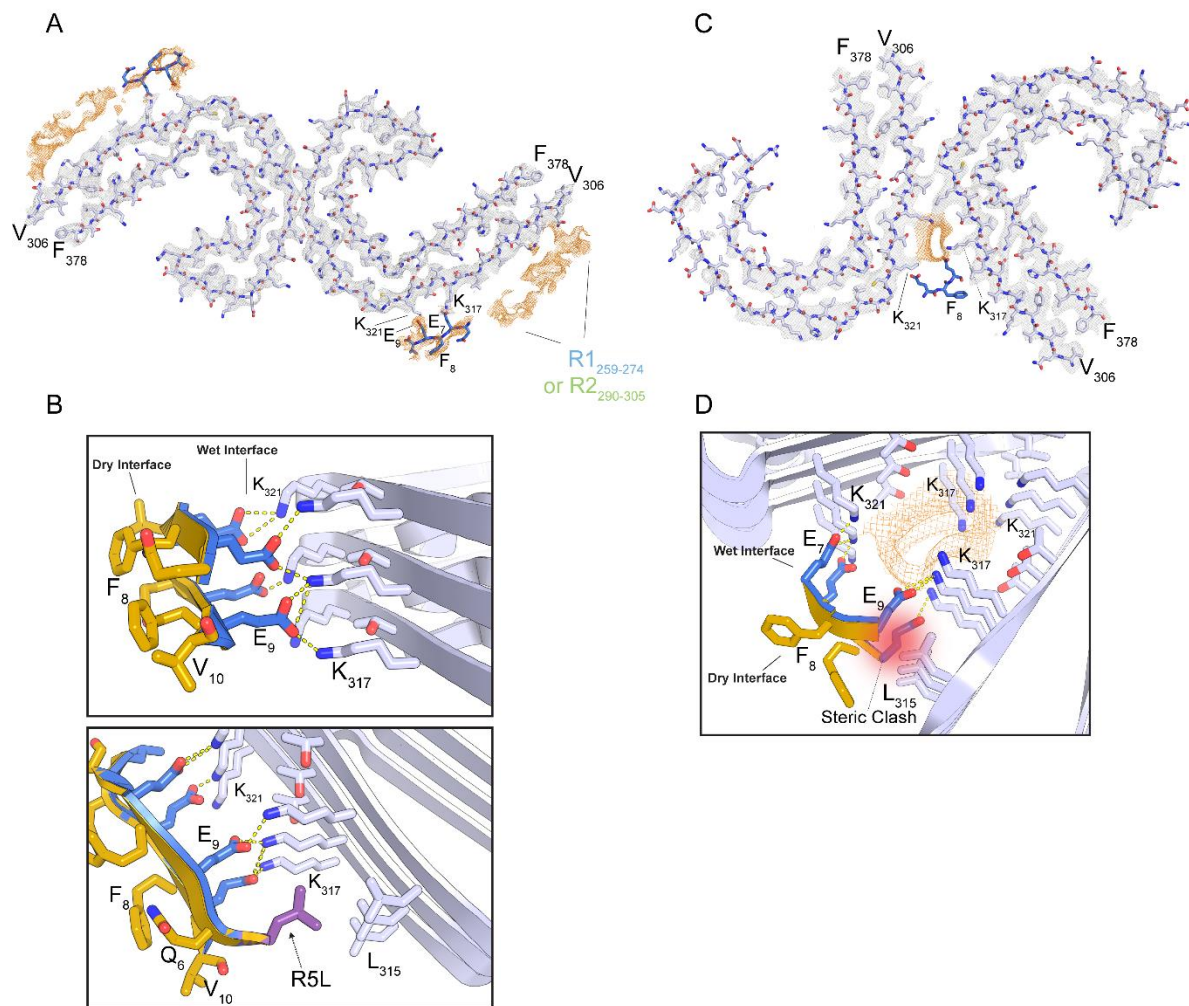
380 A)  $5RQEFEV_{10}$  forms amyloid-like out-of-register protofilaments with wet and dry

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 5RQEFEV<sub>10</sub> 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 5RQEFEV<sub>10</sub> 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  $\gamma$ EFE<sub>9</sub> 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)
<b>Wavelength</b>	0.9792
<b>Resolution range</b>	16.08 - 1.851 (1.918 - 1.851)
<b>Space group</b>	P2 <sub>1</sub>
<b>Unit cell</b>	16.59 11.45 25.42 90 104.236 90
<b>Total reflections</b>	2416 (226)
<b>Unique reflections</b>	842 (80)
<b>Multiplicity</b>	2.9 (2.8)
<b>Completeness (%)</b>	97.2 (96.4)
<b>Mean I/sigma(I)</b>	4.2 (1.6)
<b>Wilson B-factor</b>	14.8
<b>R-merge</b>	0.16 (0.60)
<b>R-meas</b>	0.20 (0.72)
<b>R-pim</b>	0.11 (0.40)
<b>CC1/2</b>	0.97 (0.83)
<b>CC*</b>	0.99 (0.95)
<b>Reflections used in refinement</b>	838 (80)
<b>Reflections used for R-free</b>	85 (8)
<b>R-work</b>	0.19 (0.30)
<b>R-free</b>	0.27 (0.47)
<b>CC(work)</b>	0.96 (0.81)
<b>CC(free)</b>	0.91 (0.83)
<b>Number of non-hydrogen atoms</b>	118
<b>macromolecules</b>	114
<b>solvent</b>	4
<b>Protein residues</b>	12
<b>RMS(bonds)</b>	0.013
<b>RMS(angles)</b>	1.48
<b>Ramachandran favored (%)</b>	100.00
<b>Ramachandran allowed (%)</b>	0.00
<b>Ramachandran outliers (%)</b>	0.00
<b>Rotamer outliers (%)</b>	0.00
<b>Clashscore</b>	4.55
<b>Average B-factor</b>	21.9
<b>macromolecules</b>	21.5
<b>solvent</b>	32.5

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