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Network-based Analysis of Transthyretin Variants

Lorenza Pacini, Claire Lesieur, Laurent Vuillon

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Introduction

Transthyretin (TTR) is a protein involved in neurological and cardiac genetic diseases, due to structural damages, such as formation of amyloid fibrils associated with mutations.[1, 2, 3] Mutations (protein sequence variants) can be pathogenic for several reasons: the original protein structure may not be reproduced by the variant or the variant may present different stability and/or dynamics. Since the X-ray structures of TTR and of some pathogenic and non-pathogenic variants are known, TTR is a good model to infer relations between mutations and structures and subsequently investigate their functional consequences. Table 1 reports the Protein Data Bank (PDB) codes and characteristics of the human wild-type (WT) TTR and of four TTR variants that have been analyzed in this study, using a network-based approach. All structures contain a TTR dimer. Amino Acids Networks, that have been proven successful in investigating protein dynamics and robustness to mutations [4, 5], are employed to infer candidate interaction interfaces to be used as a starting point for the construction of fiber models through a tiling procedure.

Table 1: Analyzed TTR variants.

PDB id	Mutation	Characteristics
1f41	WT	
3djz	L55P	Pathogenic
3kgs	V30M	Pathogenic
4tne	T119Y	Non pathogenic
1bze	T119M	Compensates V30M

Methods

Amino Acids Network Each protein structure is analyzed thanks to the construction of its Amino Acids Network (AAN), represented by a weighted graph $G = (V, E)$ with nodes $V = \{i \mid i \text{ is an amino-acid}\}$, links

$$E = \{(i, j) \mid i, j \in V \text{ and } \exists (\text{atom}_i \in i, \text{atom}_j \in j) \text{ with } \text{dist}(\text{atom}_i, \text{atom}_j) \leq 5\text{\AA}\}$$

and link weights w_{ij} defined as the the number of atomic pairs $(\text{atom}_i \in i, \text{atom}_j \in j)$ that satisfy $\text{dist}(\text{atom}_i, \text{atom}_j) \leq 5\text{\AA}$. An example of AAN is reported in Fig. 1.

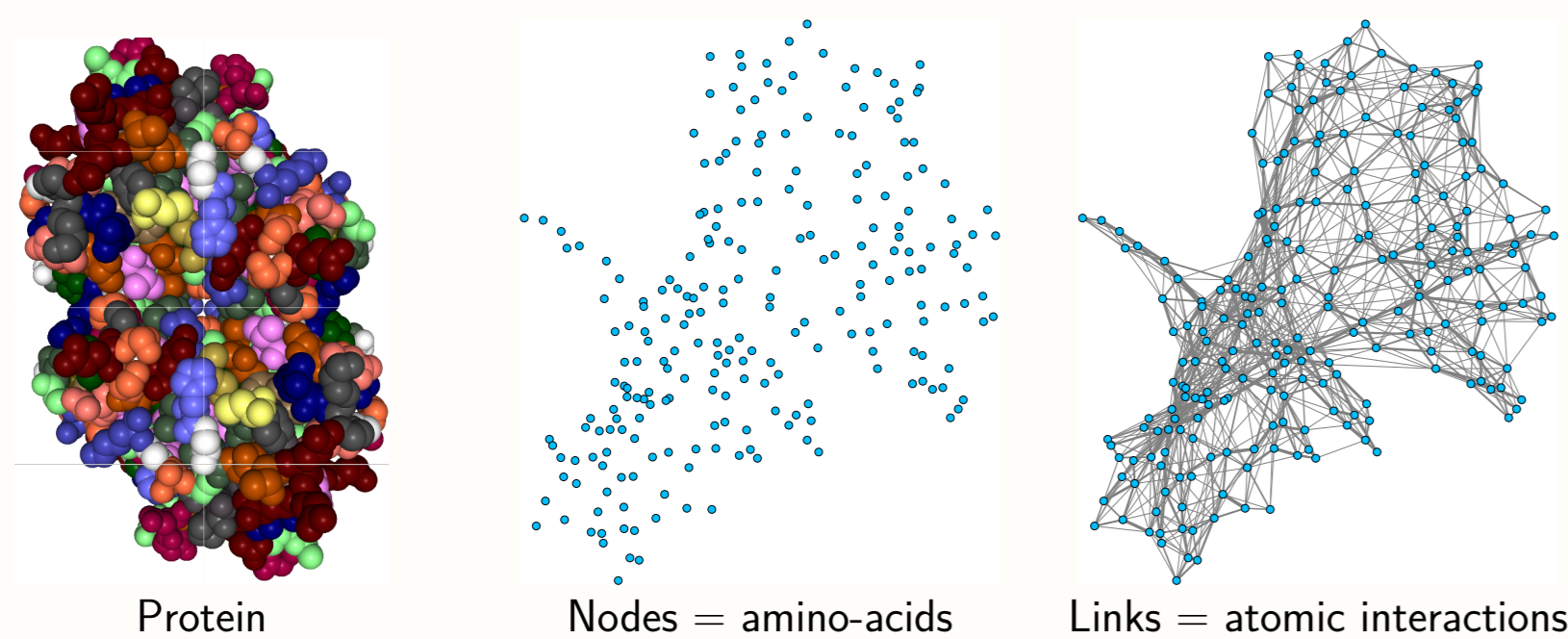


Figure 1: Human wild-type Transthyretin Amino Acids Network (PDB id: 1f41)

Perturbation Network The amino acids interactions within a protein variant (var) and the wild-type (WT) are compared through the analysis of the Perturbation Network (PN) of threshold \bar{w} , represented by a graph $G_p = (V_p, E_p)$ with links $E_p = \{(i, j) \in E_{WT} \cup E_{var} \text{ s.t. } |w_{var}(i, j) - w_{WT}(i, j)| > \bar{w}\}$, link weights $w_p(i, j) = |\Delta w(i, j)| = |w_{var}(i, j) - w_{WT}(i, j)|$ and link color

$$\text{color}(i, j) = \begin{cases} \text{red} & \text{if } w_{mut}(i, j) - w_{ref}(i, j) < -\bar{w} \\ \text{green} & \text{if } w_{mut}(i, j) - w_{ref}(i, j) > \bar{w} \end{cases}$$

In the present study, $\bar{w} = 4$ is employed.

Induced Perturbation Network The Induced Perturbation Network (IPN) of a variant is the connected component of the PN that contains the mutation site. The IPNs of TTR variants are used to infer candidate new interaction interfaces.

Tiling In order to construct a fiber from an oligomer, a new interface needs to be made accessible and the resulting repetitive unit needs to be compatible with a one- or two-dimensional tiling model, as summarized in Fig. 2. The candidate interfaces resulting from the IPNs analysis are subject to analysis to verify the above-mentioned condition.

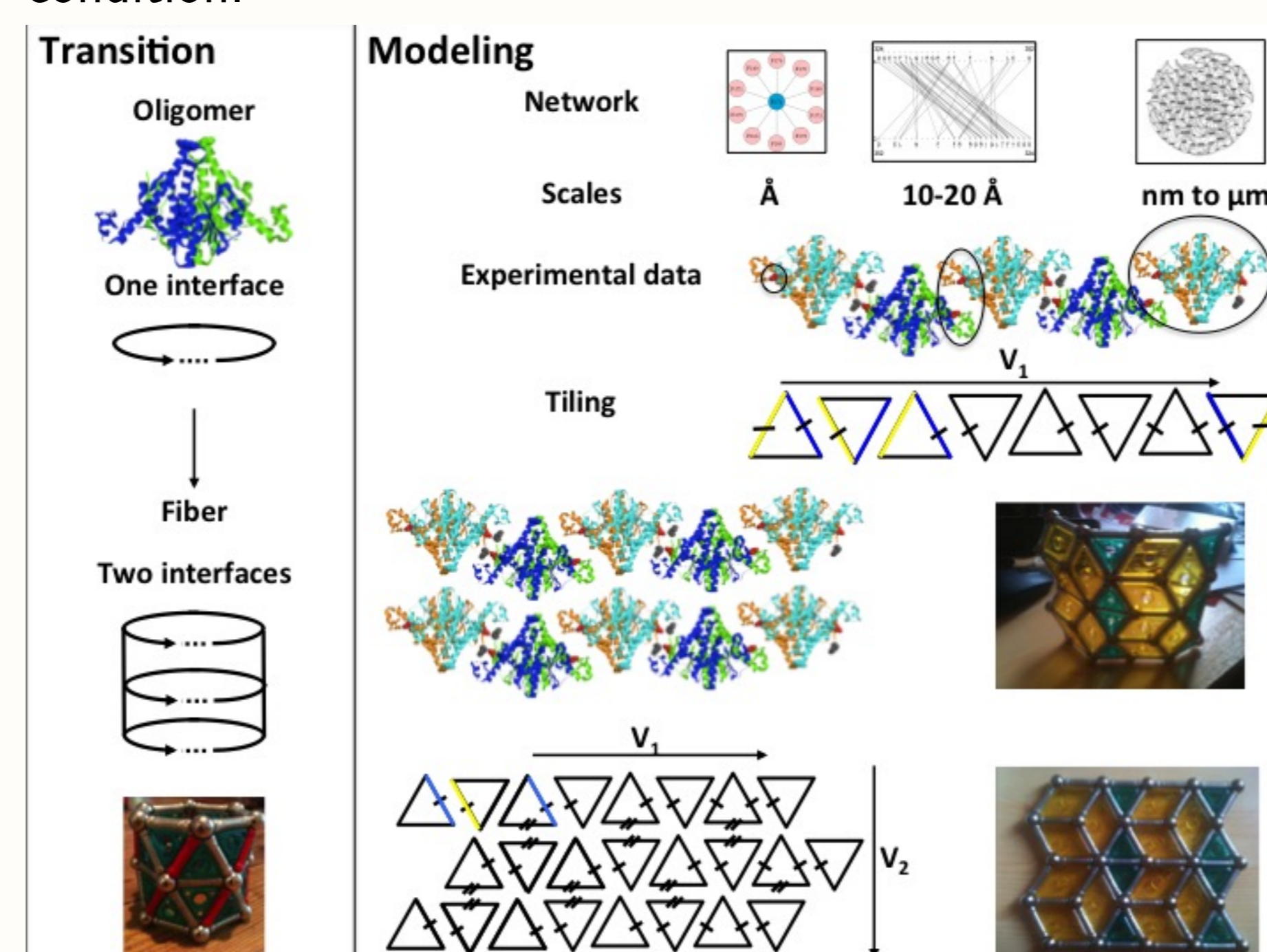


Figure 2: Schematics of possible tiling models underlying the process of fiber formation starting from an oligomeric unit: the creation of a new interface is required, followed by aggregation according to one- or two-dimensional tiling of a plane.

Results and discussion

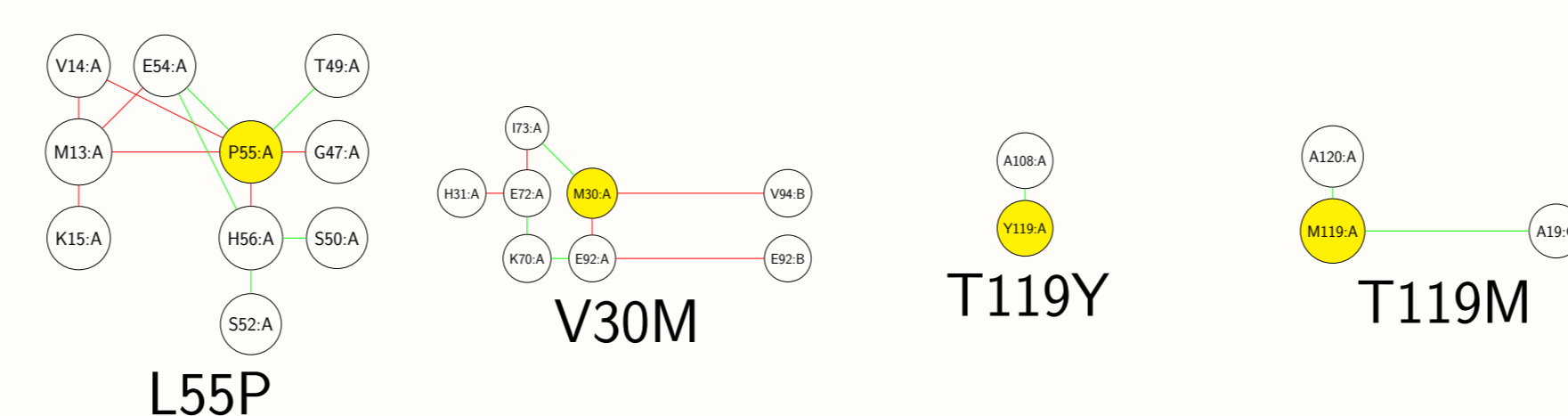


Figure 3: Induced Perturbation Networks of some TTR variants.

IPNs The analysis of the IPNs of the TTR variants (Fig. 3) shows dissimilarities among pathogenic and non-pathogenic ones and within the pathogenic ones, suggesting that the L55P and V30M mutations favour the formation of TTR amyloids according to different mechanisms. Red links in the IPN point out residues that are further in the variant structure compared to the WT: the V30M variant presents a weakening of the interactions between chains A and B, while in the L55P variant the loop containing the mutation point is further from the β -sheet containing the M13, V14 and K15 residues. No red links are present on the T119Y and T119M IPNs. This suggests that the two latter mutations are non-pathogenic because they stabilize the interactions among the TTR chains. Moreover, the IPNs of re-constructed tetramers of the variants showed an increase in proximity between the chains A and C for the non pathogenic variants compared to the WT structure.

TTR L55P: candidate interface It is proposed that the loop containing P55, freed from the interaction with the β -strand containing M13-V14-K15, approaches the β -strand containing T49 and S50 and becomes part of the β -sheet (see Fig. 4). The new-formed β -strand would be then available to perform self-interactions with its equivalent belonging to another tetramer, establishing a one-dimensional fiber growth mechanism. The analysis of the propensity of amino acids pairs to perform backbone or side-chains interactions in β interfaces, as de-

scribed in [9], allows the identification of the best candidate interacting segment. For illustration, Table 2 reports the propensities for pairs interactions resulting for some choices of interfaces. The G57-L58-T59-T60 segment has high propensity of interacting with itself forming a parallel interface using both backbone and side-chain interactions. Moreover, the presence of parallel in-register arrangements of β -strands has been reported to be frequent in amyloids cores [10]. As a result, the 57-60 parallel interface is employed to construct the tiling model representing the TTR L55P fiber formation.

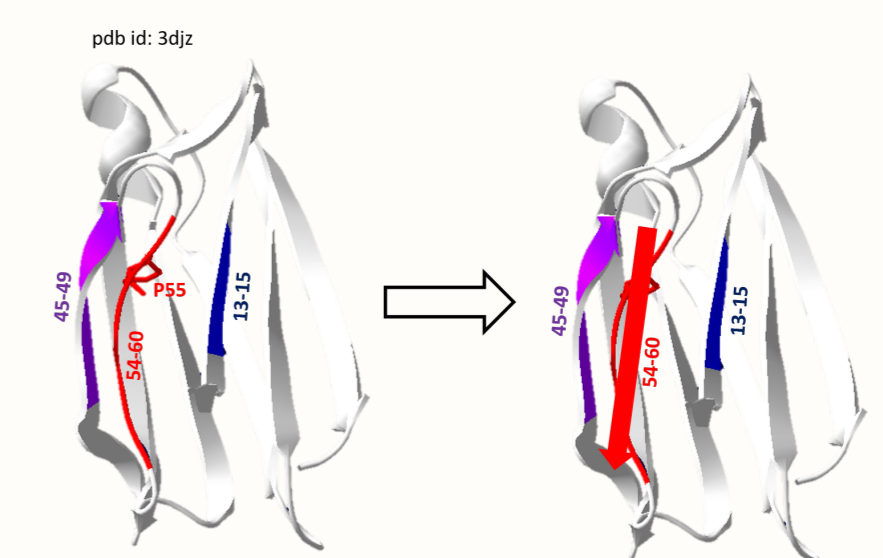


Figure 4: Transition of the loop in TTR L55P to become part of the β -sheet

Table 2: Pairwise interaction propensities for some candidate fiber-forming interfaces for TTR L55P. Data from [9].

		G53 E54 P55		
		AP	P55	E54
		BB	0	0
		SC	0	0
	BB = backbone, SC = side-chain			
	+ = favoured, - = unfavoured, 0 = neutral			
	P = parallel, AP = anti-parallel			
AP	S50 E51 S52 G53 E54 P55	S50 E51 S52 G53 E54 P55		
BB	- 0 + + 0 -	BB	0	0
SC	0 0 0 0 0 0	SC	+	+
AP	G53 E54 P55 H56 G57 L58 T59 T60	AP	G53 E54 P55 H56 G57 L58 T59 T60	
BB	+ 0 0 0 0 0 0 +	BB	+	+
SC	0 + + - - + + 0	SC	+	+
P	G53 E54 P55 H56 G57 L58 T59 T60	P	G53 E54 P55 H56 G57 L58 T59 T60	
BB	+ 0 - - + + + +	BB	+	+
SC	- 0 0 - - + + + +	SC	-	+

TTR L55P Fiber model

Fig. 5 shows the proposed fiber model for the L55P variant. It would lead to a fiber diameter equivalent to the size of a tetrameric unit, in accordance with experimental results reported in [6] (Fig. 5, right). Moreover, our model does not require the dissociation of the tetrameric unit (contrary to previously proposed models, see [7] for review), that could explain the earlier onset of amyloidogenesis in patients carrying this mutation compared to patients with WT TTR.

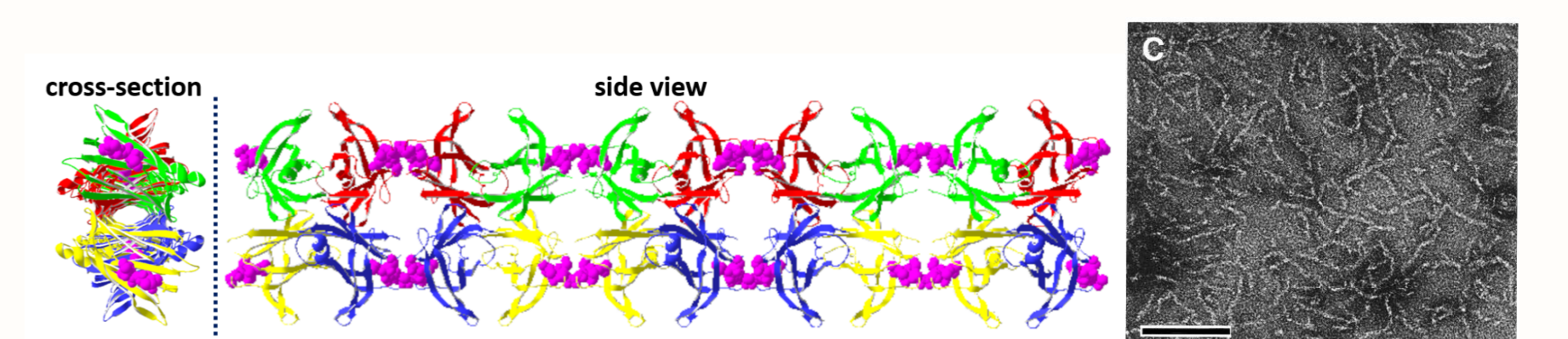


Figure 5: Left: model for the L55P fiber. In pink, the proposed interaction interfaces. Right: EM image of L55P TTR amyloids. The scale bar represents 1000 Å. From [6]

Conclusion and future work

The presented procedure allows the construction of a model for the TTR L55P fiber. Future work involves the application of the same protocol to construct a model for the TTR V30M fiber. Due to the difference in IPNs and fiber size ([8]) compared to L55P, different interaction interfaces and tiling model must be defined.

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