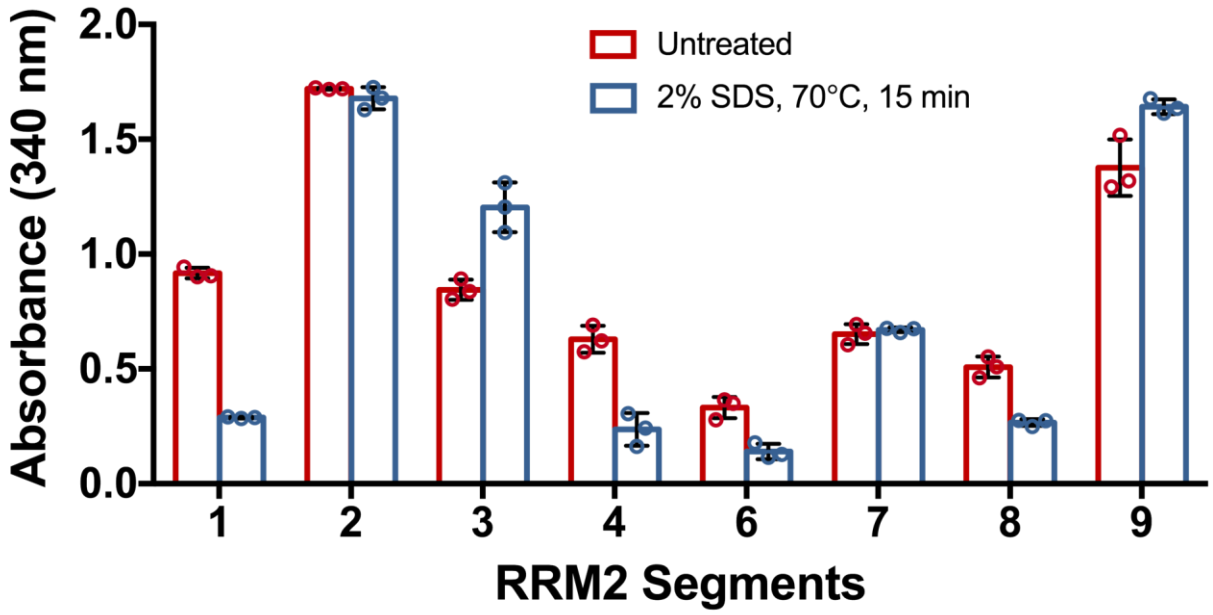
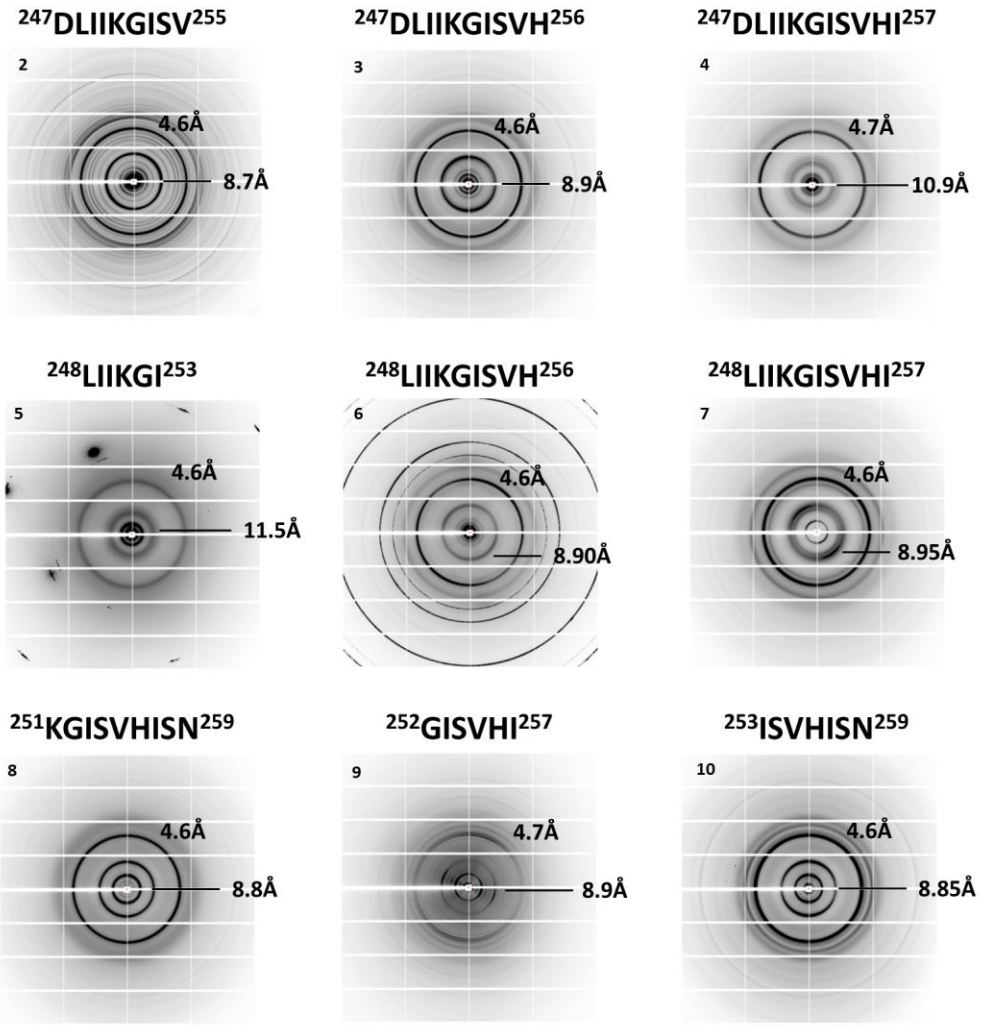


Supplementary Figure 1

RRM2 segments demonstrate high to moderate stability against treatment with SDS and heat

Fibril samples were first grown by shaking at 37°C at 20mM concentration in PBS, pH 7.5. Following fibril formation, samples were treated with 2% SDS and heated to 70°C for 15 minutes. The bar graph shows the mean raw absorbance values as well as individual data points as monitored by the Spectramax. Experiments were done in triplicate and error bars represent standard deviation.

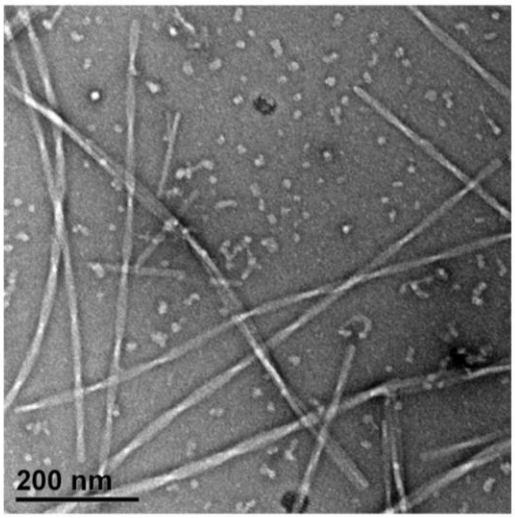


Supplementary Figure 2

Fibril diffraction of the RRM2 fibril segments shows classic amyloid diffraction

Fibril diffraction was collected at the 5um beam at the Advanced Photon Source. All nine samples exhibit the classic 4.6-4.8Å ring indicative of the stacking of β-strands and the 8-12Å ring indicative of mating sheets. We were unable to collect fibril diffraction patterns for 2 samples (#1: 247DLIIKG252 and #11: 254SVHISN259) Fibril diffraction experiments were completed once at APS. Peptide numbering corresponds with the code provided in Figure 1.

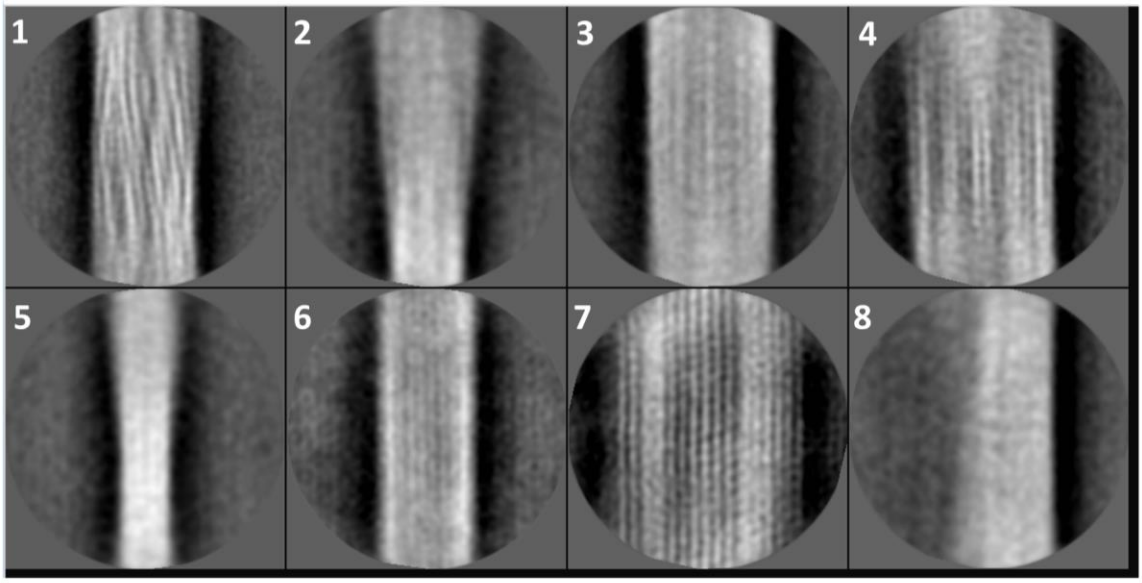
A



B

	Morphology	Abundance (%)
1	3-Start Helix	59.7
2	Wide Twist	5.3
3	Cylinder #1	8.4
4	Cylinder #2	4.4
5	Narrow Twist	2.9
6	Cylinder #3	0.6
7	Cylinder #4	0.5
8	Sheet	1.0

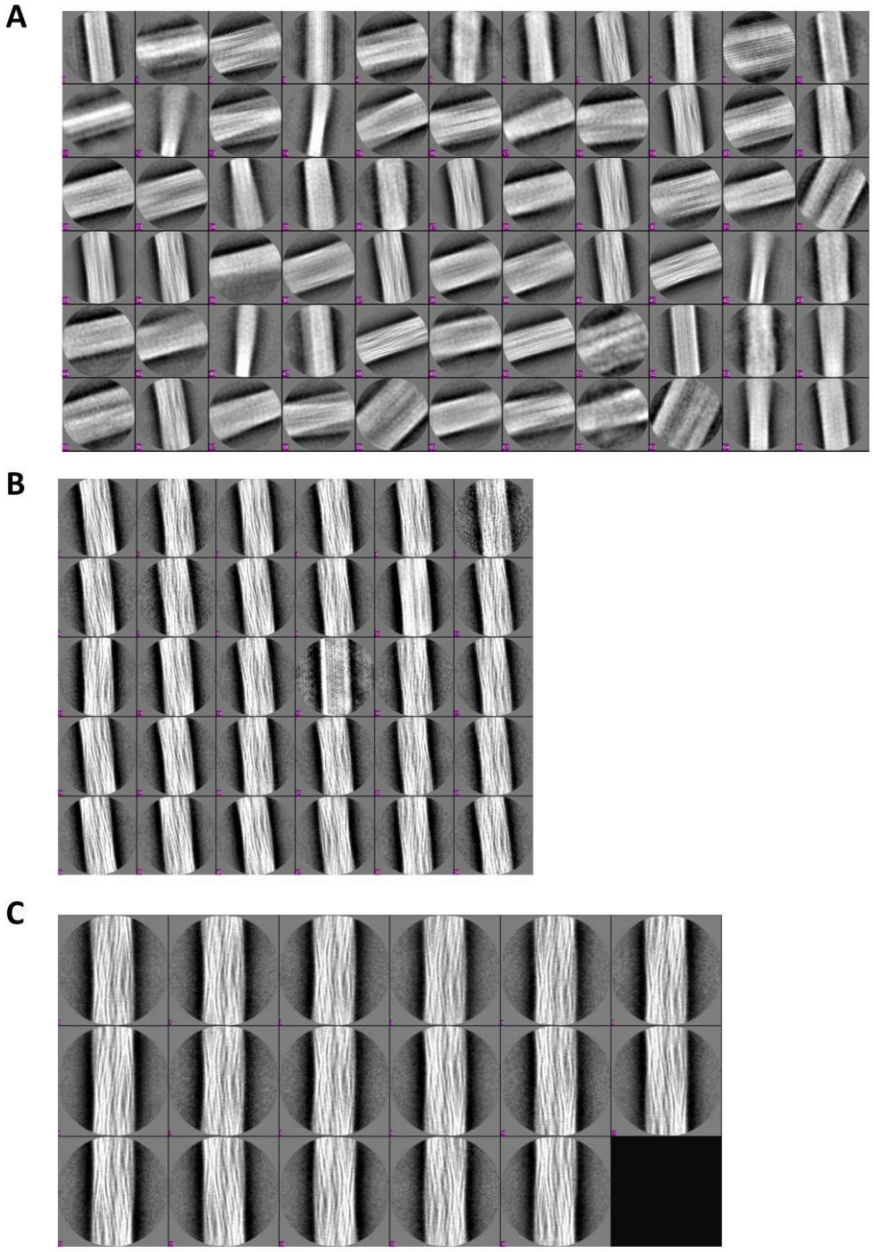
C



Supplementary Figure 3

Eight unique fibril morphologies are observed in the 247DLIIKGISVHI257 cryo-EM sample

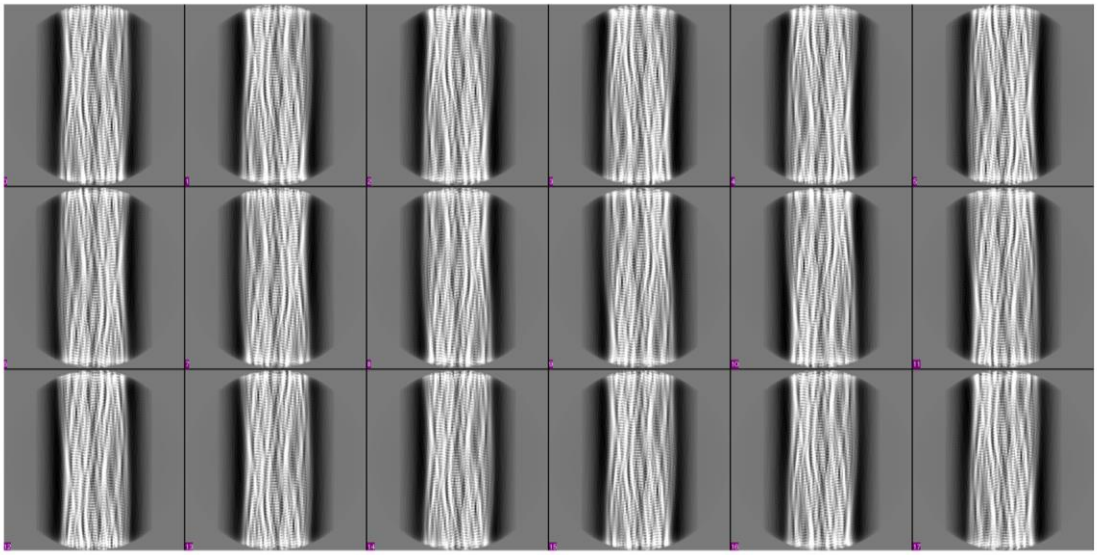
(A) Negative stain EM image of the 247DLIIKGISVHI257 cryo-EM sample. 247DLIIKGISVHI257 fibrils were grown by shaking for one week at 1mM in water. Sample was stained with 2% uranyl acetate and visualized on the FEI T12 transmission electron microscope. Fibril samples illustrate multiple morphologies, including twisted helical assemblies. (B) Illustration of the eight different types of fibrils observed by cryo-EM. The eight fibrils demonstrate four different conformations of amyloid fibrils: helices, twists, cylinders, and sheets. (C) The relative abundance of the eight unique fibrils are displayed in a table. The 3-start helix represents the most abundant species, comprising approximately 60% of the sample.



Supplementary Figure 4

Classification of images for the 247DLIIKGISVHI257 cryo-EM sample

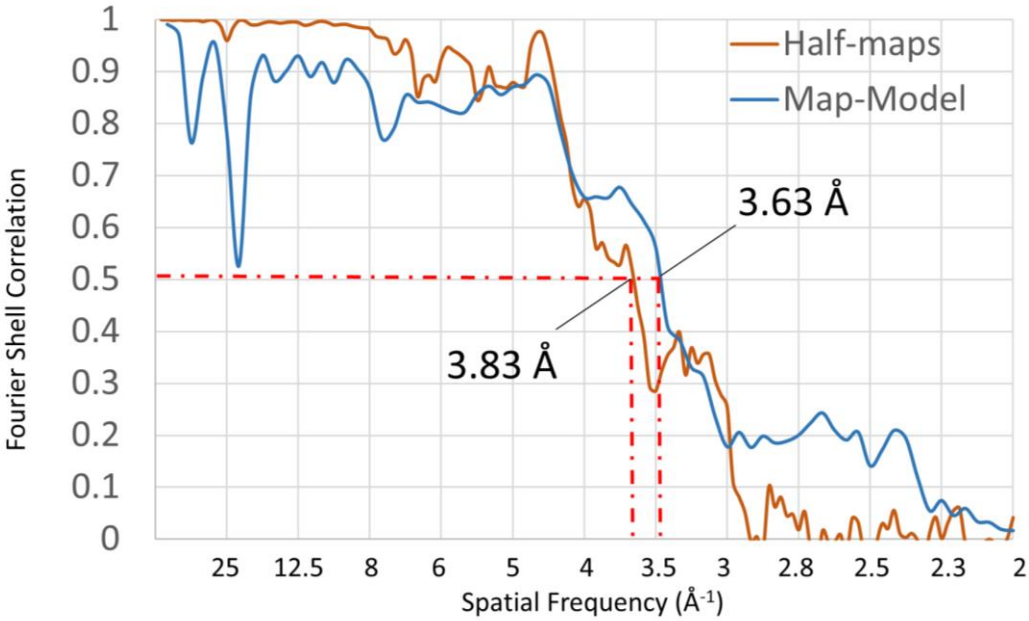
(A) Round 1 classification illustrates the polymorphic assemblies observed in the fibril sample. (B and C) Round 2 and 3 class averages focus on fibrils exhibiting the 3-start helix morphology. Each panel illustrates a unique face of the fibril as it twists along the EM grid. 5-7Å features of the structure become apparent during these rounds of class averaging.



Supplementary Figure 5

2D projection of the final 3D model of the 247DLIIKGISVHI257 cryo-EM fibril structure

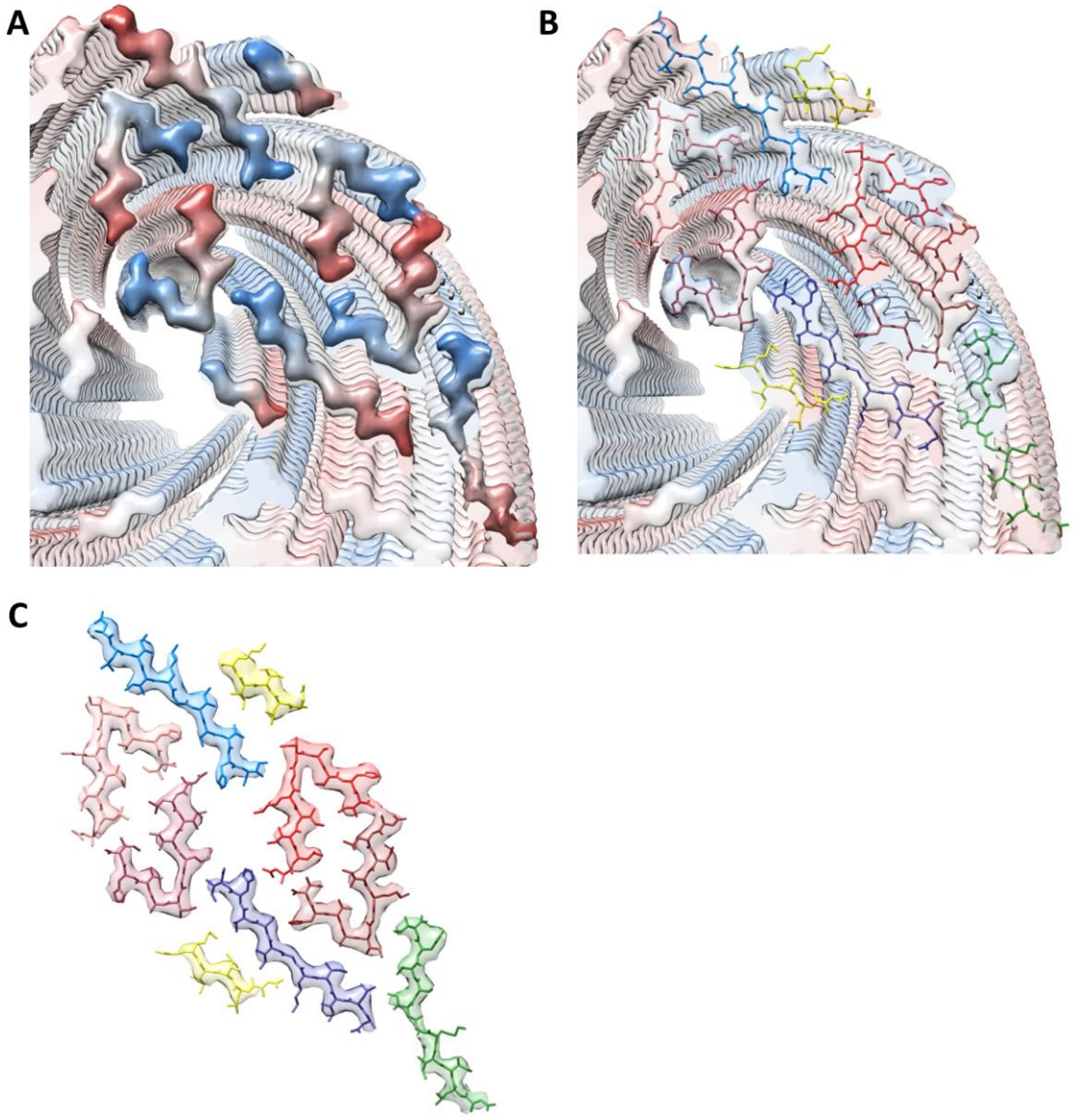
The panels illustrate a side view of the fibril, perpendicular to the fibril axis. Each panel represents an 8° azimuthal rotation of the fibril. The hydrogen bonding network is visualized by the stacking of β-strands.



Supplementary Figure 6

Fourier shell correlation (FSC) curve for the single-particle cryo-EM structure of RRMcore

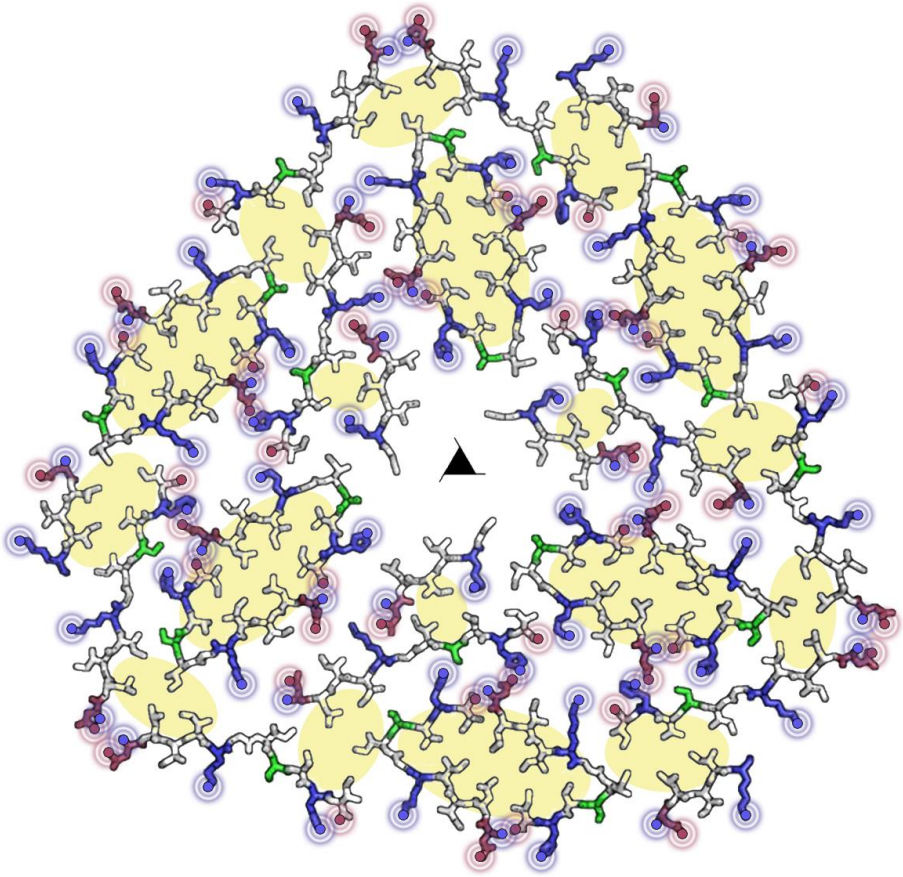
FSC cutoff value of 0.5 results in spatial frequency of 3.83Å for the half-maps and 3.63Å for the map-model.



Supplementary Figure 7

The asymmetric unit of the three-start helix is composed of nine strands

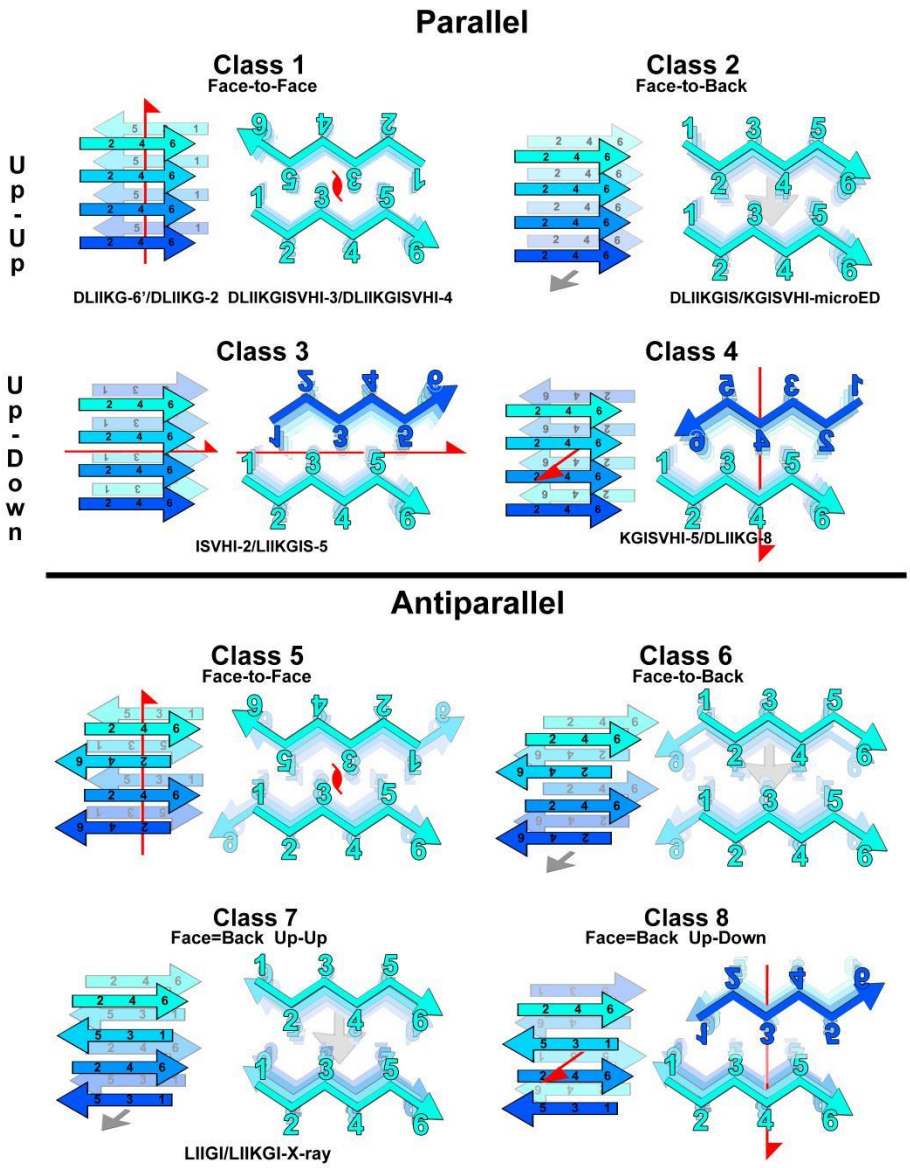
(A) The electron density of the asymmetric unit is displayed. Here, the N-terminus is displayed in red and the C-terminus is displayed in blue. (B,C) The peptide strands are displayed within the electron density. The β-sheets are classified by their relative conformation; kinked = red, straight = blue, curve = green and partial = yellow. Each strand is represented by a unique color within its designated family.



Supplementary Figure 8

The protofilaments of the three-start helix fill space, exclude water, bury hydrophobic surfaces, and exhibit electrostatic interactions

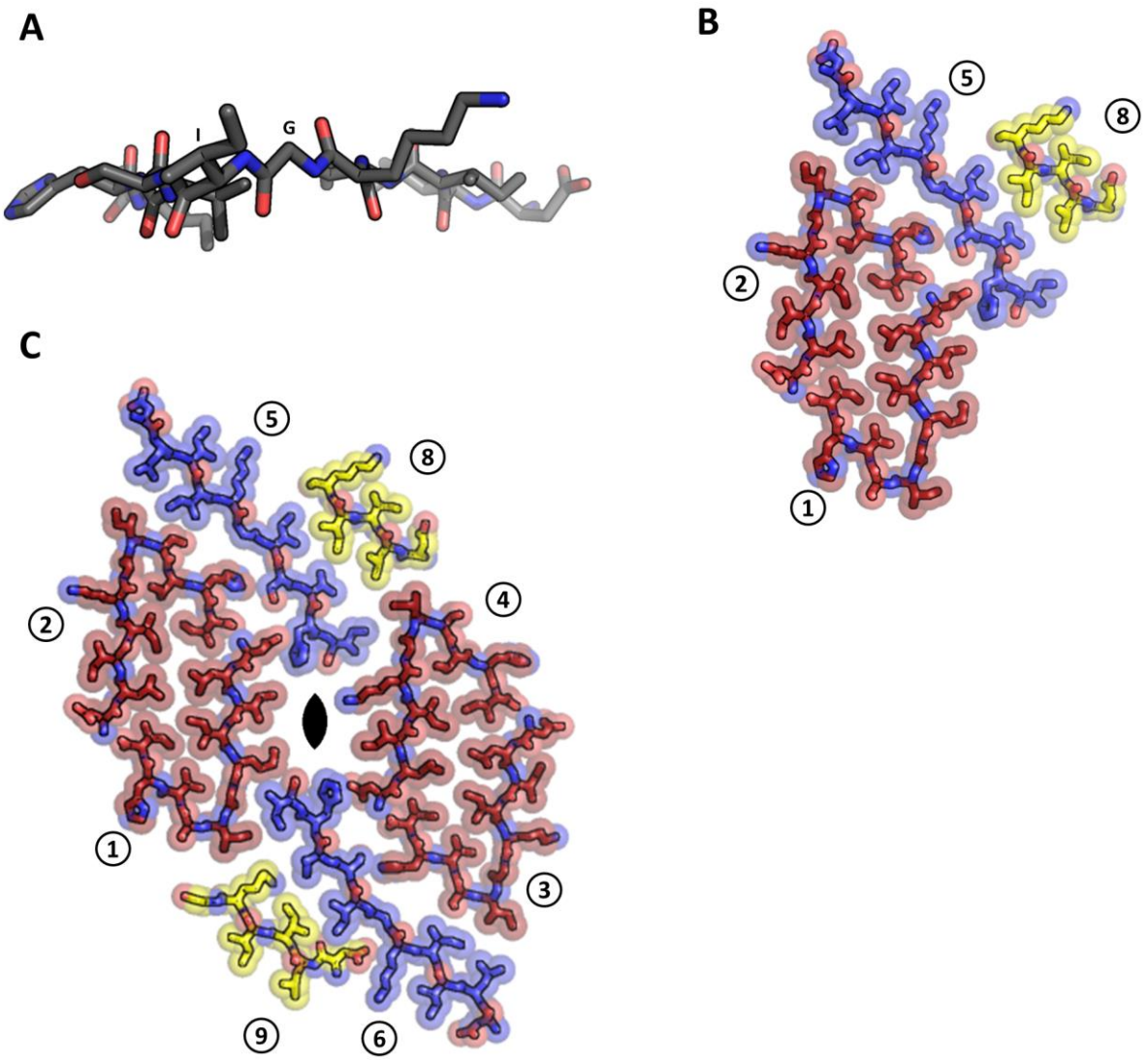
Here, glycines and apolar residues are white, polar residues are green, positively charged residues are blue, and negatively charged residues are red. The pH of this sample is ~4 and therefore histidine residues are charged. Red and blue circles indicate the presence of electrostatic interactions between the N and C termini as well as between the aspartic acid and histidine side chains. Yellow ellipses illustrate the presence of hydrophobic cores. The three fold screw axis is indicated by the triangle.



Supplementary Figure 9

Eight different symmetry classes of amyloid steric zippers

Examples of their occurrence among RRMcore segments of TDP-43 are cataloged below their respective classes.



Supplementary Figure 10

The cryo-EM structure demonstrates key features including a ^{247}DLIIKGISVHI^{257} kinked backbone that flips orientation and a pseudo-two-fold symmetry is observed in the asymmetric unit

(A) Below, the kinking of the 247DLIIKGISVHI257 backbone allows for the carbonyl of the glycine and isoleucine to both face down. The glycine and isoleucine residues have been marked by one letter abbreviations. (B-C) Strands 1, 2, 5 and 8 form a complex which exhibits two fold symmetry with strands 3, 4, 6 and 9. The oval shape indicates two fold symmetry.

Table S1: Charge and hydropathicity values for the RRM2 segments. Total number of charges and net charges are displayed for fibrils prepared in PBS at pH 7.5. GRAVY value represents the grand average of hydropathy values as calculated with EXPASY Protparam.

Segment	Sequence	# of Charges	Net Charge	GRAVY
1	DLIIKG	4	0	0.83
2	DLIIKGISV	4	0	1.43
3	DLIIKGISVH	5	1	0.97
4	DLIIKGISVHI	5	1	1.29
5	LIIKGI	3	1	2.17
6	LIIKGISVH	4	2	1.47
7	LIIKGISVHI	4	2	1.77
8	KGISVHISN	4	2	0.07
9	GISVHI	3	1	1.47
10	ISVHISN	3	1	0.70
11	SVHISN	3	1	0.07

Table S2: Area buried and shape complementarity values for the 5 interfaces formed in the cryo-EM, 3-start helix structure. Area buried is abbreviated as Ab and shape complementarity is abbreviated as Sc.

Interface	Segment	Ab (Å^2)	Average Ab (Å^2)	Sc
ISVHI/LIIKGIS	2	175	166	0.36
	5	157		
DLIIKG/KGISVH	9	164	168	0.47
	6	172		
KGISVHI/DLIIKG	5	127	131	0.59
	8	135		
DLIIKG/DLIIKG	6’	87	85	0.24
	2	82		
D11I (kink)	4	226	223	0.52
	3	220		

Table S3: RMSD values the ^{247}DLIIKGISVHI^{257} backbone illustrates that all strands are unique. The RMSD values in Å^2 illustrate that none of the backbones overlay perfectly. Strands 1-4 represent the kinked morphologies while strands 5-8 represent the linear morphologies.

Strand	1	2	3	4	5	6	7	10
1	0.00							
2	0.67	0.00						
3	0.64	0.50	0.00					
4	0.72	0.41	0.47	0.00				
5	6.38	6.20	6.08	6.16	0.00			
6	6.54	6.34	6.23	6.29	0.59	0.00		
7	5.60	5.36	5.25	5.29	2.00	2.00	0.00	
10	6.96	6.76	6.65	6.71	1.13	1.06	2.34	0.00