Structural Basis for the Assembly of Amyloid Fibrils by the Master Cell-Signaling Regulator Human Receptor-Interacting Protein Kinase 1

Paula Polonio[1], Jorge Pedro López-Alonso[2,3], Hanxing Jiang[2], Fátima Escobedo[1], Gustavo Titaux[1], Iban Ubarretxena-Belandia[2,4*], Miguel Mompeán[1*]

*Correspondence: mmompean@iqf.csic.es and ivan.ubarrechena@ehu.eus

1. Instituto de Química Física Blas-Cabrera (IQF-CSIC), Madrid, Spain
2. Instituto Biofisika (UPV/EHU, CSIC), Leioa, Spain
3. Basque Resource for Electron Microscopy, Leioa, Spain
4. Ikerbasque Foundation for Science, Bilbao, Spain

Figure S1 | Workflow of cryo-EM data processing and helical reconstruction. A total of 2,814,672 filament segments were automatically picked from 8,650 micrographs and subjected to iterative 2D classification to remove noise and poor-quality segments. An initial 3D model was generated using ab initio reconstruction without imposing symmetry constraints. Helical parameters (rise and twist) were determined through a symmetry search, followed by refinement using non-uniform reconstruction. To improve the map, several rounds of local and global CTF refinement, as well as reference-based motion correction, were performed. The local resolution of the final map, estimated using the 0.5 FSC criterion, is shown.

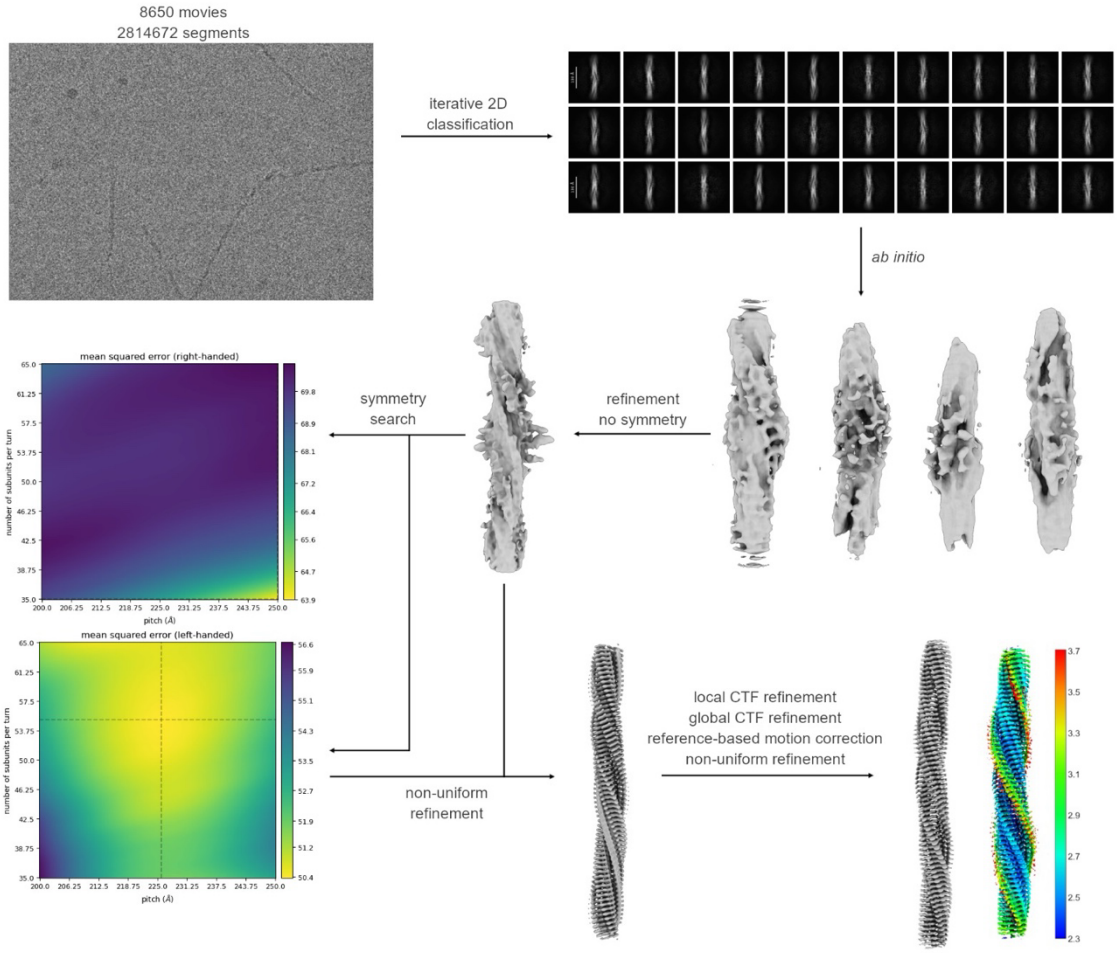

Table S1 | SSNMR acquisition parameters.

14.1 T (600 MHz 1H frequency), 3.2 mm HCN CPMAS CryoProbe										
Experiment	NS	MAS (kHz)	d1 (s)	Acq time (ms)	Sweep width (kHz)	Dec power (kHz)	Mixing (ms)	CP time (ms)	Field Strength (kHz)	Sample T (K)
2D NCA	8	14	2	t2: 15; t1: 11	ω2: 52.6; ω1: 2.8	100	-	HN: 1.00; NC: 1.50	H: 54; N: 40 (transfer 1); N: 4; CA: 18 (transfer 2)	315
2D NCACX	8	14	2	t3: 15.0; t1: 7.9	ω3: 52.6; ω2: 3.5; ω1: 2.8	100	50	HN: 1.00; NC: 1.50	H: 54; N: 40 (transfer 1); N: 4; CA: 18 (transfer 2)	315
3D NCACX	8	14	2	t3: 15.0; t2: 8.0; t1: 7.9	ω3: 52.6; ω2: 3.5; ω1: 2.8	100	50	HN: 1.00; NC: 1.50	H: 54; N: 40 (transfer 1); N: 4; CA: 18 (transfer 2)	315
3D NCOCX	8	14	2	t3: 15.0; t2: 8.0; t1: 7.9	ω3: 52.6; ω2: 3.5; ω1: 2.8	100	50	HN: 1.00; NC: 1.50	H: 54; N: 40 (transfer 1); N: 8.4; CA: 5.6 (transfer 2)	315
3D CANCOC	64	14	2	t3: 17.4; t2: 7.9; t1: 6.0	ω3: 58.8; ω2: 2.8; ω1: 4.7	100	50	HC: 1.20; CN: 1.25; NC: 7.00	H: 69; CA: 55 (transfer 1); CA: 3; N: 11 (transfer 2); N: 8.4; CO: 5.6 (transfer 3)	315
2D CORD	4	14	2	t2: 20; t1: 17	ω2: 44.2; ω1: 35	100	5, 20, 100, 500	HC: 1.75	H: 69 ; C: 55	315

Table S2 | Restraints identified in the spectra.

Unambiguous restraints

Residue 1	Residue 1 type	Atom 1 type	Residue 2	Residue 2 type	Atom 2 type	
532	THR	C	541	ILE	CD	*
536	SER	CA	538	GLY	CA	*
536	SER	CB	538	GLY	CA	*
538	GLY	CA	550	GLY	CA	*
538	GLY	CA	547	MET	CG	*
538	GLY	CA	547	MET	CE	*
538	GLY	C	547	MET	CE	*
540	GLN	CG	545	ASN	CB	*
540	GLN	CG	547	MET	CE	*
540	GLN	CD	547	MET	CE	*
542	GLY	CA	545	ASN	CA	*
543	ALA	CB	545	ASN	CB	

Ambiguous restraints

Residue 1	Residue 1 type	Atom 1 type	Residue 2	Residue 2 type	Atom 2 type	
533	ILE	CA	536	SER	CB	
533	ILE	CB	536	SER	CB	
533	ILE	CG1	536	SER	CB	**
533	ILE	CG2	536	SER	CB	**
533	ILE	CD	536	SER	CB	**
533	ILE	CD	536	SER	CA	**
535	ASN	CA	537	THR	CB	
536	SER	CA	539	ILE	CG1	**
536	SER	CB	539	ILE	CA	
536	SER	CB	539	ILE	CB	
536	SER	CB	539	ILE	CG1	**
536	SER	CB	539	ILE	CG2	**
537	THR	CA	535	ASN	CA	
537	THR	CB	535	ASN	CA	
537	THR	CA	539	ILE	CG1	
537	THR	CB	539	ILE	CG1	
537	THR	CA	539	ILE	CD	
537	THR	CB	539	ILE	CD	
537	THR	CG	539	ILE	CD	
537	THR	CB	550	GLY	CA	
537	THR	CG	550	GLY	CA	**
537	THR	CB	549	ILE	CB	
537	THR	CB	549	ILE	CG2	
537	THR	CG	549	ILE	CG2	

537	THR	CG	550	ILE	CD	
538	GLY	CA	540	GLN	CA	
538	GLY	CA	540	GLN	CB	
538	GLY	CA	540	GLN	CG	
538	GLY	CA	547	MET	CA	
538	GLY	CA	547	MET	CB	
538	GLY	CA	549	ILE	CG2	
540	GLN	CA	542	GLY	CA	
540	GLN	CA	547	MET	CE	**
540	GLN	CB	547	MET	CE	**
542	GLY	CA	544	TYR	CA	
542	GLY	CA	545	ASN	CB	**
545	ASN	CA	547	MET	CE	
546	TYR	CB	548	GLU	CB	
547	MET	CA	549	ILE	CD	

In light green: restraints between nuclei that are 2 residues apart in the sequence
In dark green: restraints between residues that are 3 or more residues apart in the sequence
* Distance restraints used in first structure calculation
** Low ambiguity restraints between nuclei that are 3 or more residues apart
and that can be resolved based on the preliminary model

Table S3 | SSNMR structure calculation statistics.

Statistic description	Value
Total distance restraints	121
Intra-residue (|i-j|=0)	4
Sequential (|i-j|=1)	44
Medium range (|i-j|>1 and |i-j|<5)	48
Long range (|i-j>_5)	25
Total dihedral-angle restraints	35
Number of restraints per residue	7.1
Number of long range restraints per residue	1.1

Table S4 | Cryo-EM data collection, refinement and validation statistics

	Human RIPK1 (EMDB-52356) (PDB 9HR6)
Data collection and processing	
Magnification	165000
Voltage (kV)	300
Electron exposure (e–/Å^2)	49.3
Defocus range (μm)	0.8-1.6
Pixel size (Å)	0.5054
Axial symmetry	C1
Twist (degree)	-7.319
Rise (Å)	4.667
Initial segment images (no.)	2814672
Final segment images (no.)	460173
Map resolution (Å)	2.57
FSC threshold	0.143
Map resolution range (Å)	2.32-2.86
Refinement	
Initial model used	*In silico* (ModelAngelo)
Model resolution (Å)	2.57
FSC threshold	0.143
Model resolution range (Å)	2.32-2.86
Map sharpening *B* factor (Å^2)	-57.4
Model composition	
Non-hydrogen atoms	930
Protein residues	120
Ligands	0
B factor (Å^2)	65.70
R.M.S. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.789
Validation	
MolProbity score	0.82
Clashscore	0
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	95.45
Allowed (%)	4.55
Disallowed (%)	0.00