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THE UNIVERSITY *of* TEXAS

HEALTH SCIENCE CENTER AT HOUSTON

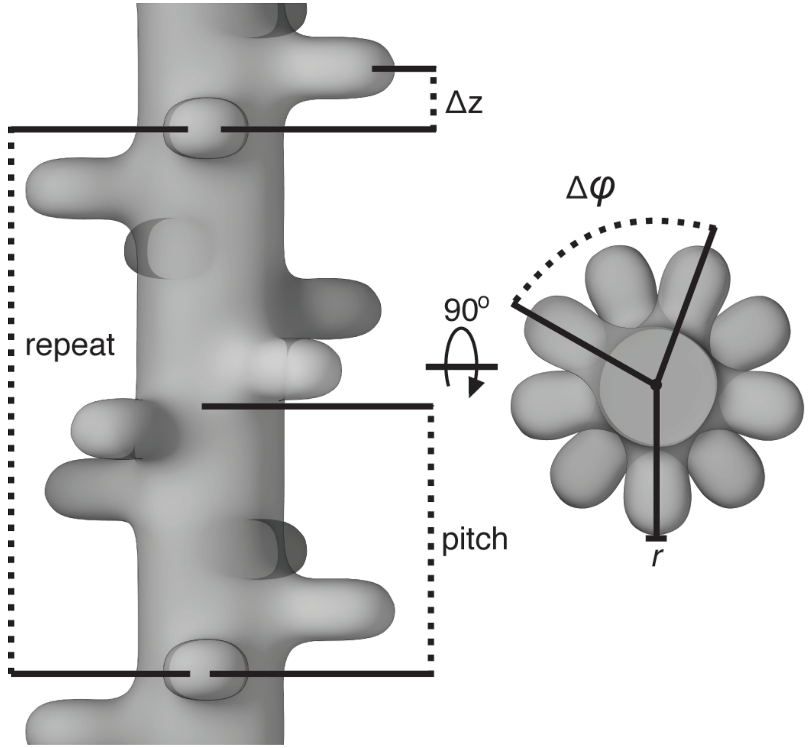
MEDICAL SCHOOL

3D reconstruction of helical filaments

helical symmetry:
azimuthal rotation per subunit $\Delta\varphi$
axial subunit translation (rise) Δz

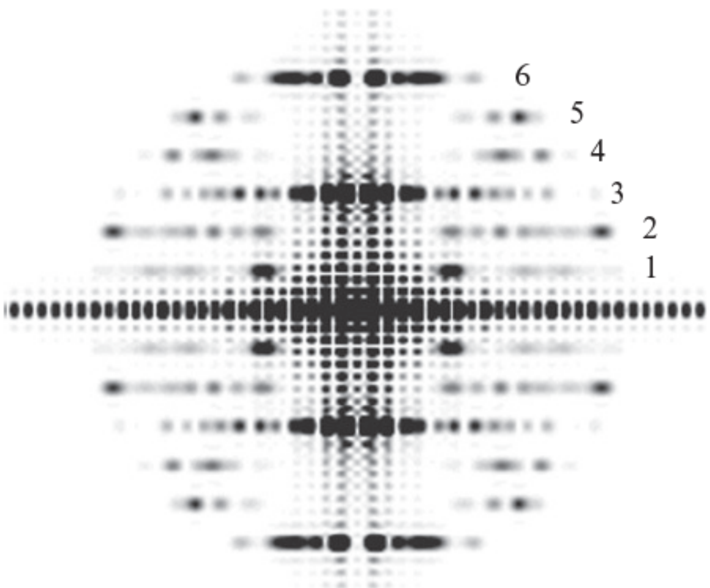
$$f(r,\varphi,z) = f(r,\varphi + n\Delta\varphi, z + n\Delta z), \quad n = \pm 1, \pm 2, \ldots$$

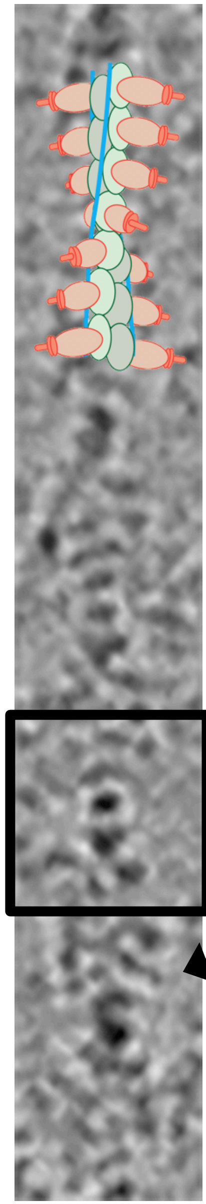
$$r = \sqrt{x^2 + y^2}$$



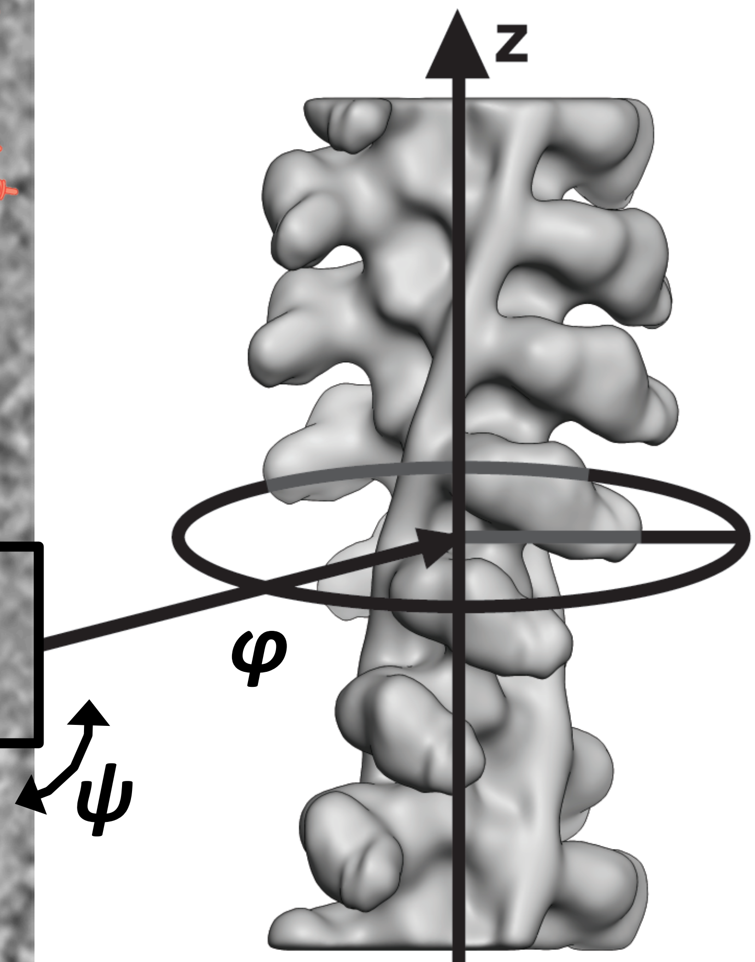
Crystal or Single Particle with Symmetry?

Fourier-Bessel Formalism





Single Particle Reconstruction

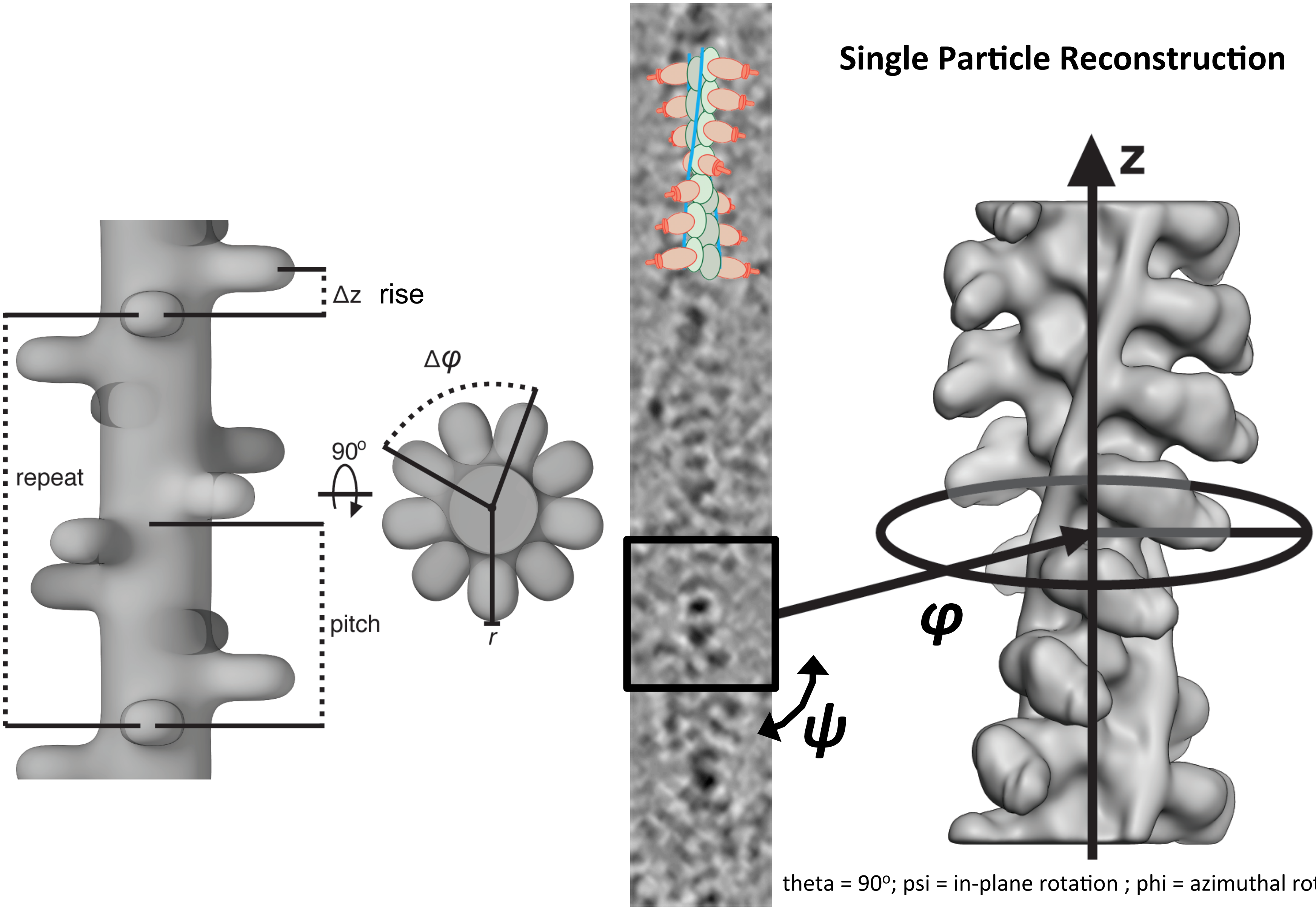


theta = 90°; psi = in-plane rotation ; phi = azimuthal rotation

Diaz *et al*: *Meth. Enz.* **2010** 482:131; Egelman: *Meth. Enz.* **2010** 482:167

Egelman: Ultramicroscopy **2000** 85:225.

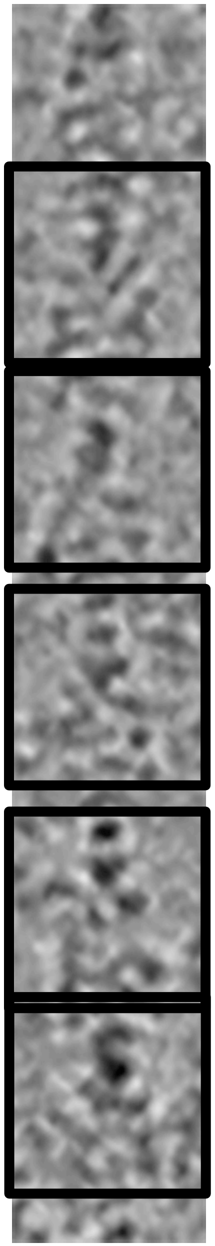
Crystal or Single Particle with Symmetry?

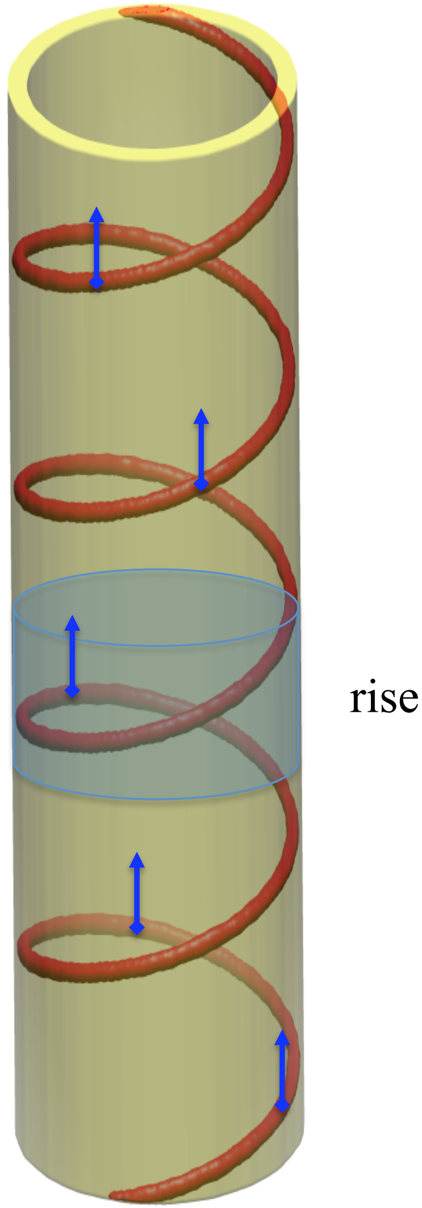


theta = 90°; psi = in-plane rotation ; phi = azimuthal rotation

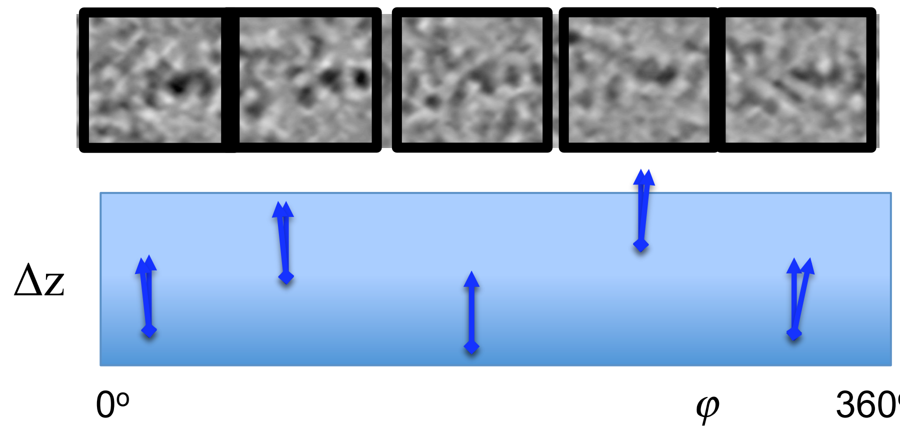
Behrmann, Tao, Stokes, Egelman, Raunser, Penczek: JSB **2012** 177:302.

Single Particle Approach to Helical Filaments



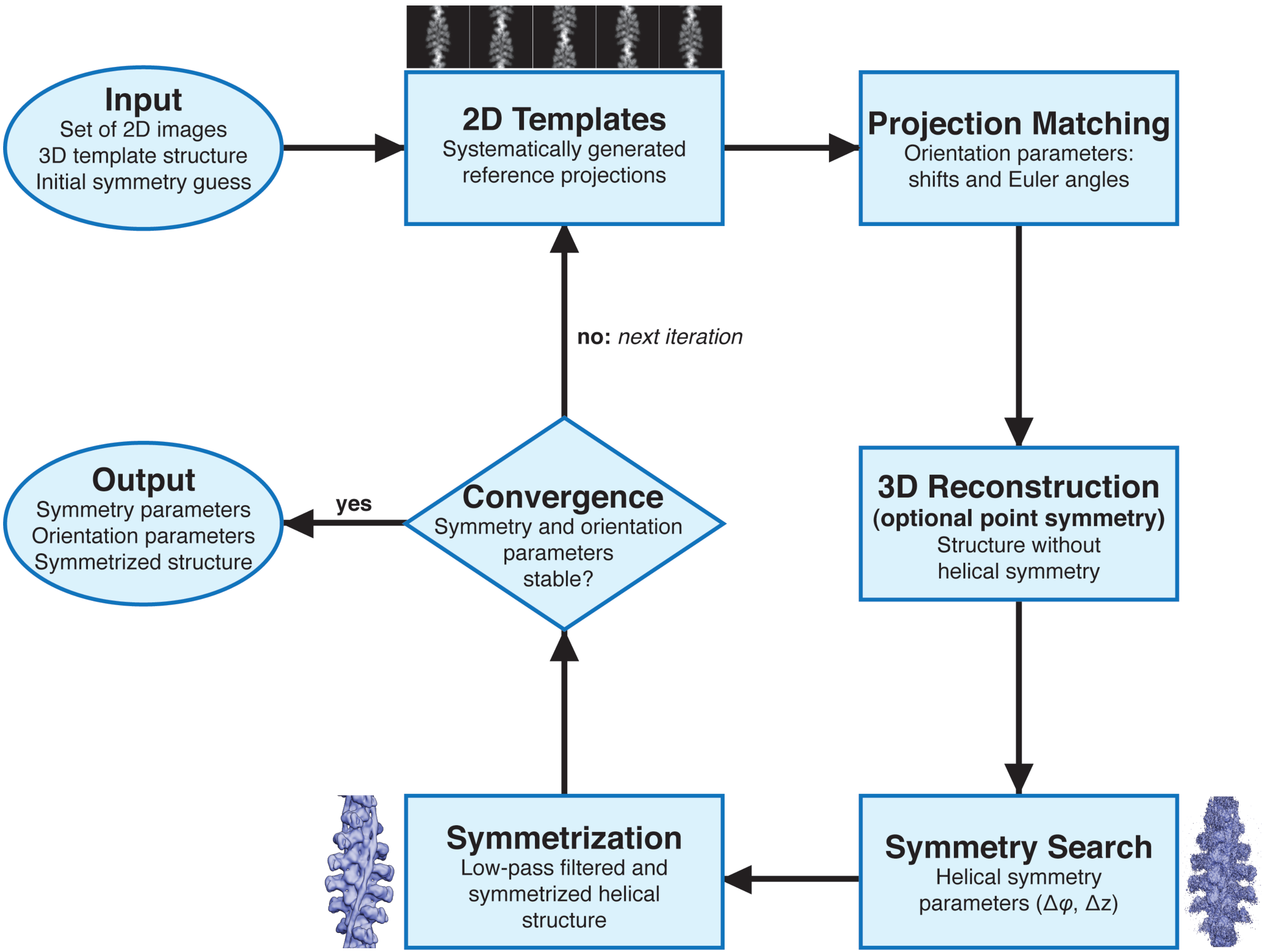


$$f(r,\varphi,z) = f(r,\varphi + n\Delta\varphi, z + n\Delta z), \quad n = \pm 1, \pm 2, \ldots$$



If filaments were perfectly flat within the ice layer, all EM projection images would constitute orthoaxial projections of the filament and the problem would be to find three orientation parameters for each segment: angles *phi* and *psi* (*theta*=90) and translation along the main axis z

IHRSR



Behrmann, Tao, Stokes, Egelman, Raunser, Penczek: JSB **2012** 177:302.

IHRSR implementation in SPARX

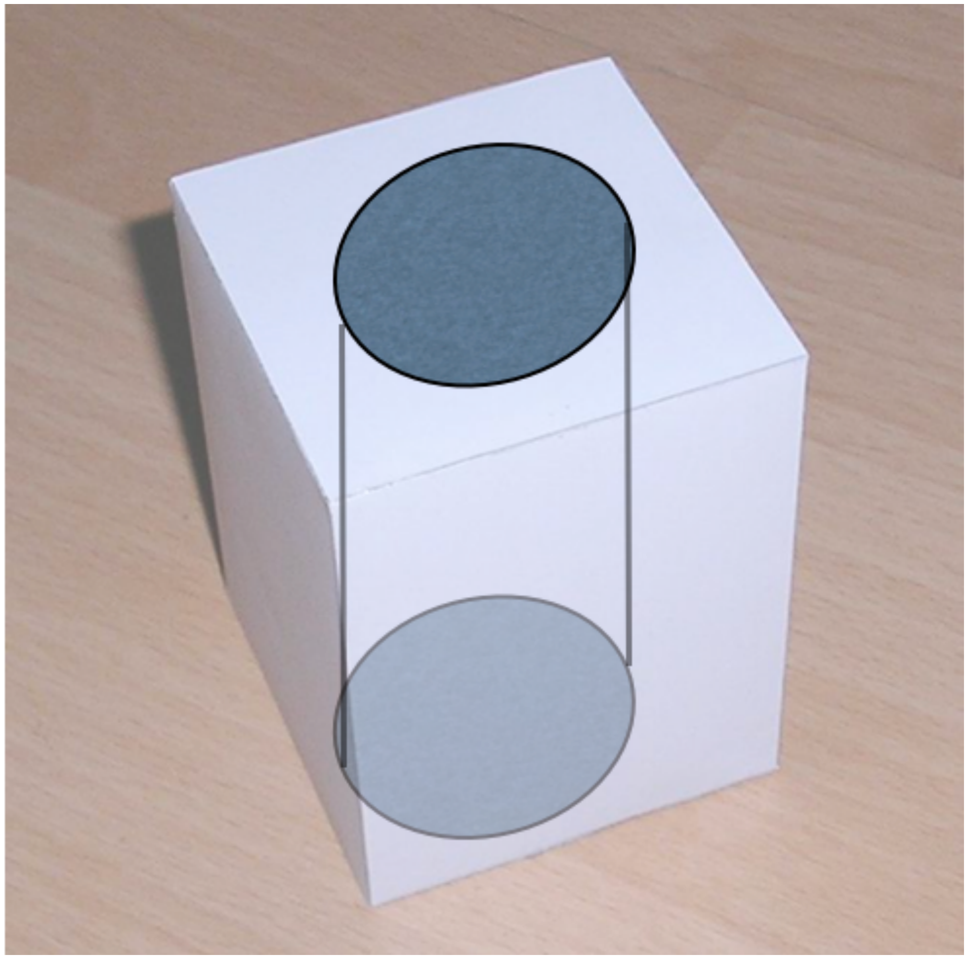
1. new implementation offers more flexibility
2. orientation searches are done in a sensible way
3. point-group symmetries of helical filaments

New features:

1. parallelization using python-level MPI makes it possible to execute the refinement rapidly on large clusters
2. restricted (constrained) search for in-plane rotation (psi) makes the procedure more robust (segments are pre-aligned along z-axis)
3. search for translation restricted to axial rise
4. search for helical symmetry implemented under MPI (it tends to be time consuming)
5. search for translation adapts itself to the current axial rise
6. out-of-plane tilt (theta not equal 90) implemented!
7. 3D reconstruction and reprojections done within rectangular prism

Behrmann E, Tao G, Stokes DL, Egelman EH, Raunser S, Penczek PA. Real-space processing of helical filaments in SPARX. J Struct Biol 2012, 177:302-13.

Rectangular prism geometry saves computer memory and time of calculations.



Behrmann E, Tao G, Stokes DL, Egelman EH, Raunser S, Penczek PA. Real-space processing of helical filaments in SPARX. J Struct Biol 2012, 177:302-13.

Actin-Tropomyosin-Myosin Complex

- JEOL 200 kV EM, 8k CCD

  Selected decorated filaments (B):

  Number of filaments: 7,696

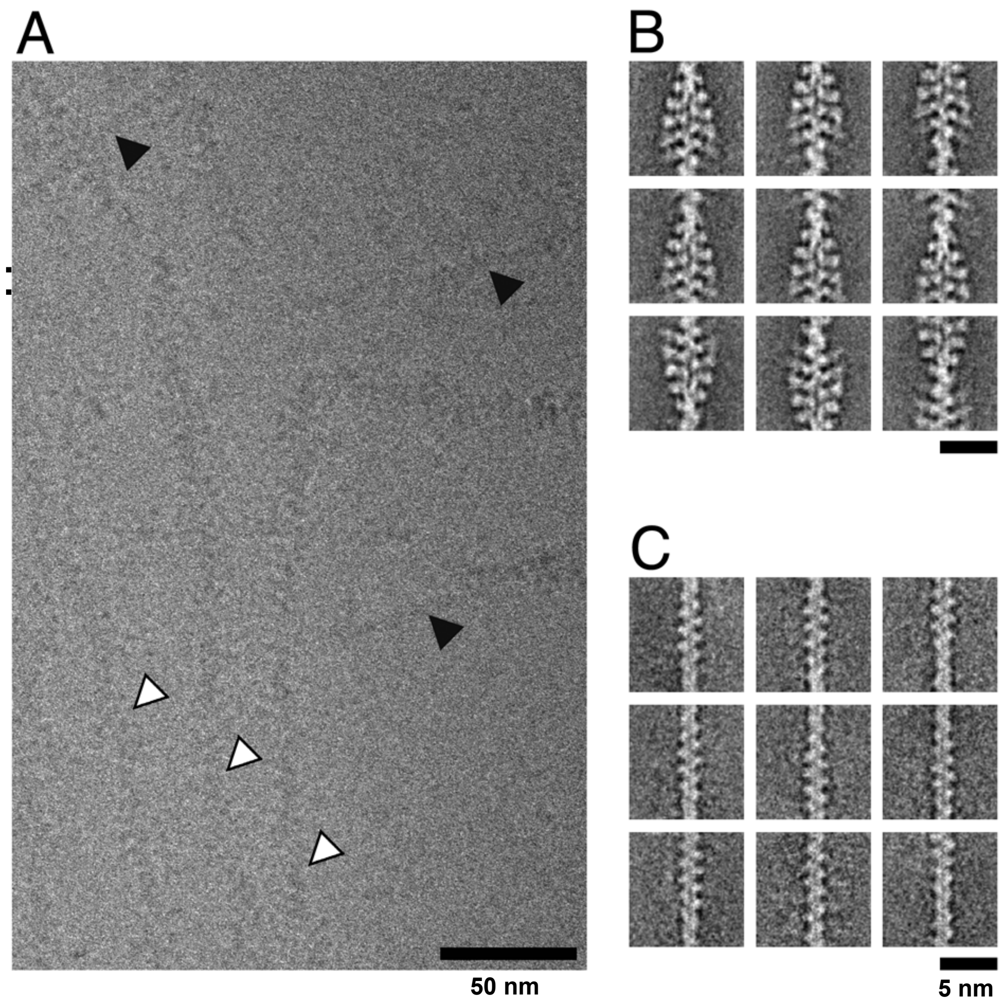
  Number of segments: 35,319

  Pixel size 1.84 Å

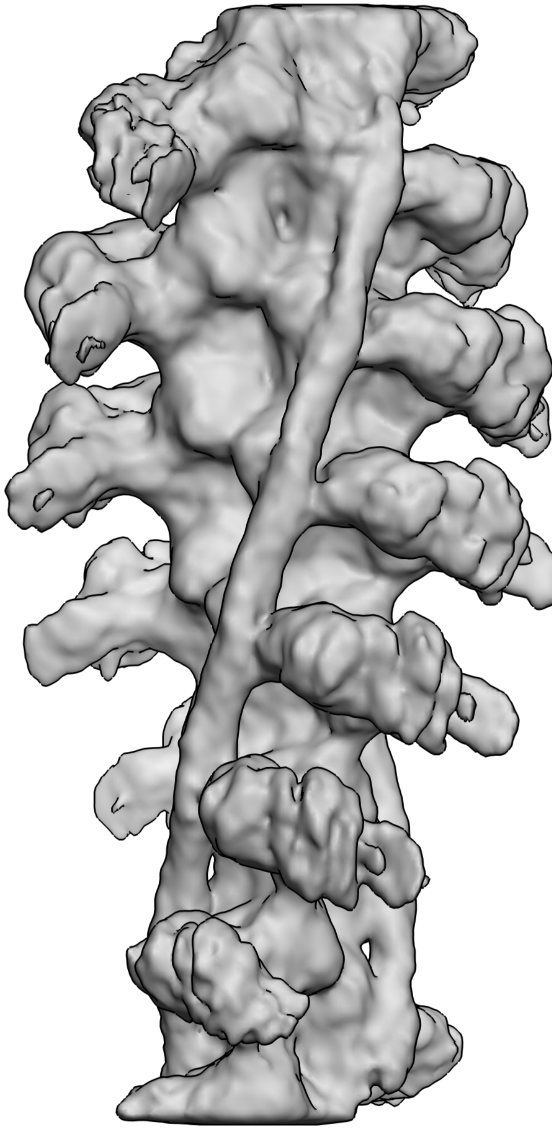
- Helical symmetry parameters:

  rise Δz= 27.6 Å

  azimuthal rotation 166.5°

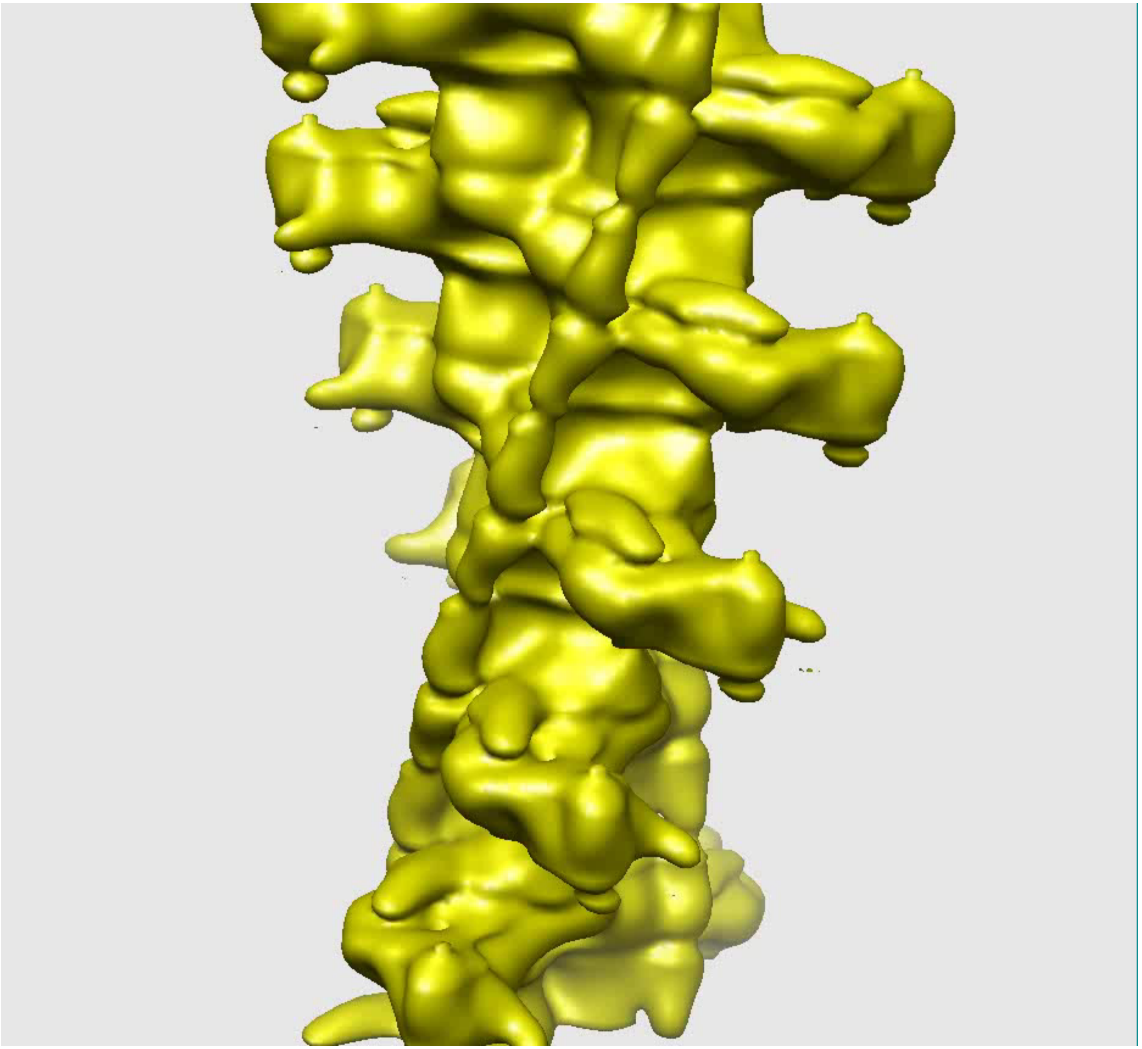


Using the Asymmetric Unit for helical PCA



Behrmann, Tao, Stokes, Egelman, Raunser, Penczek: JSB **2012** 177:302.

Conformational modes of the Actin-Tropomyosin-Myosin complex



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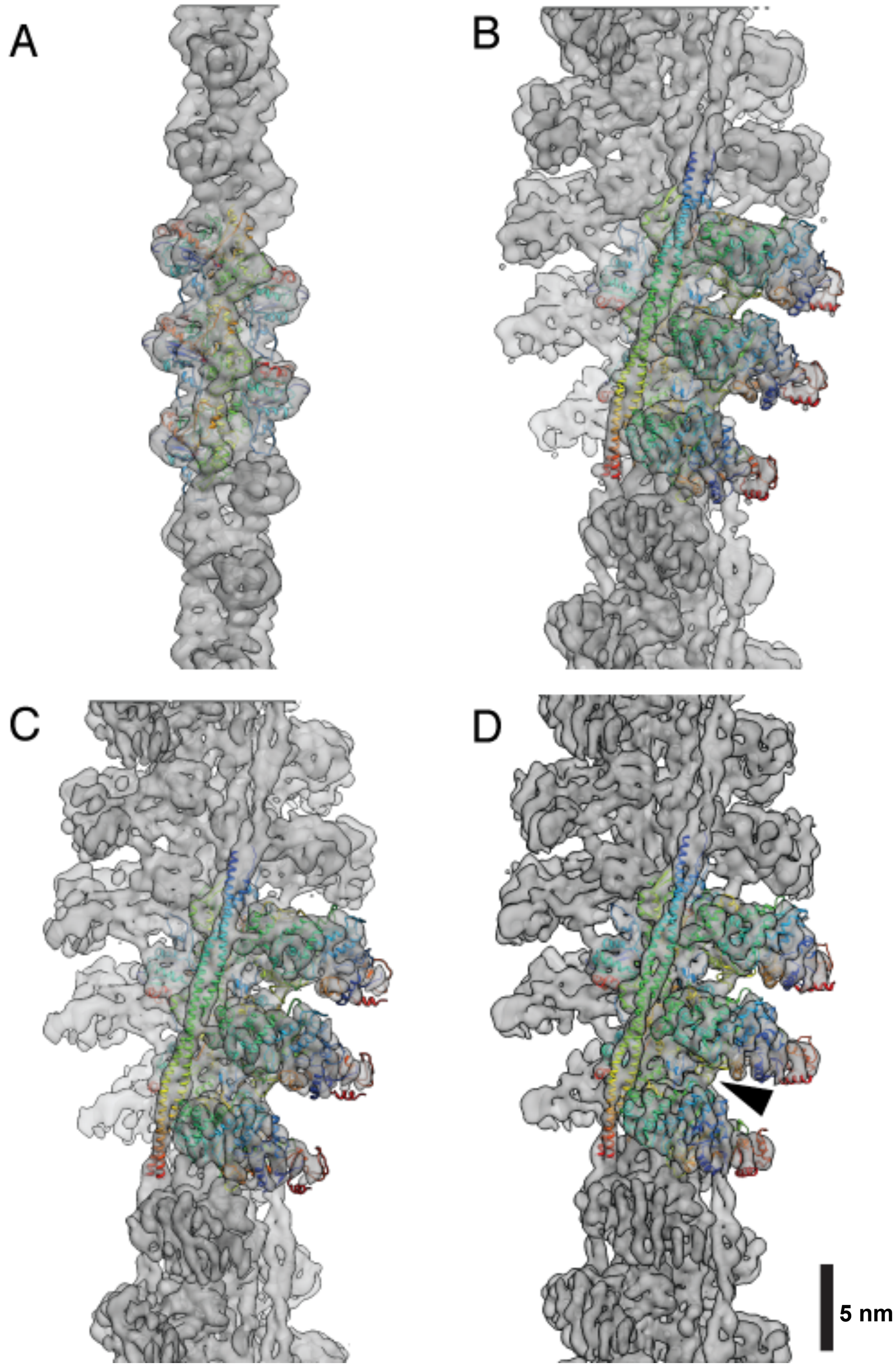
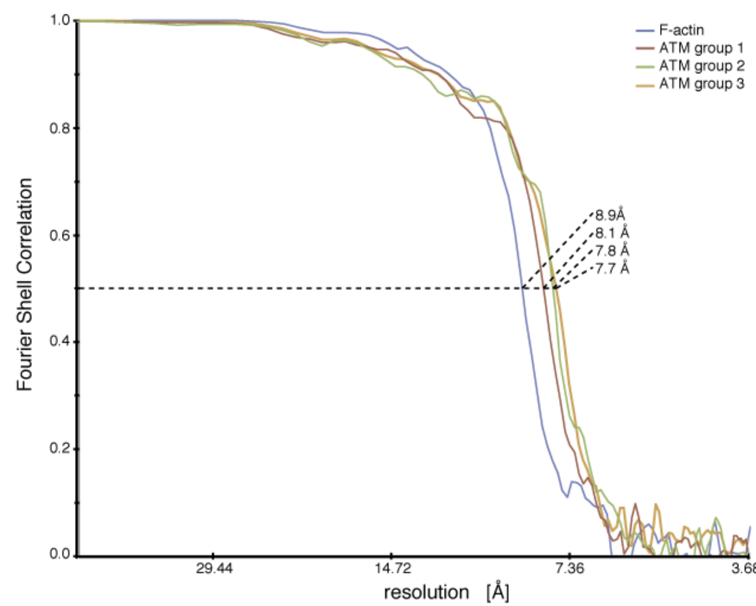


Structures of:

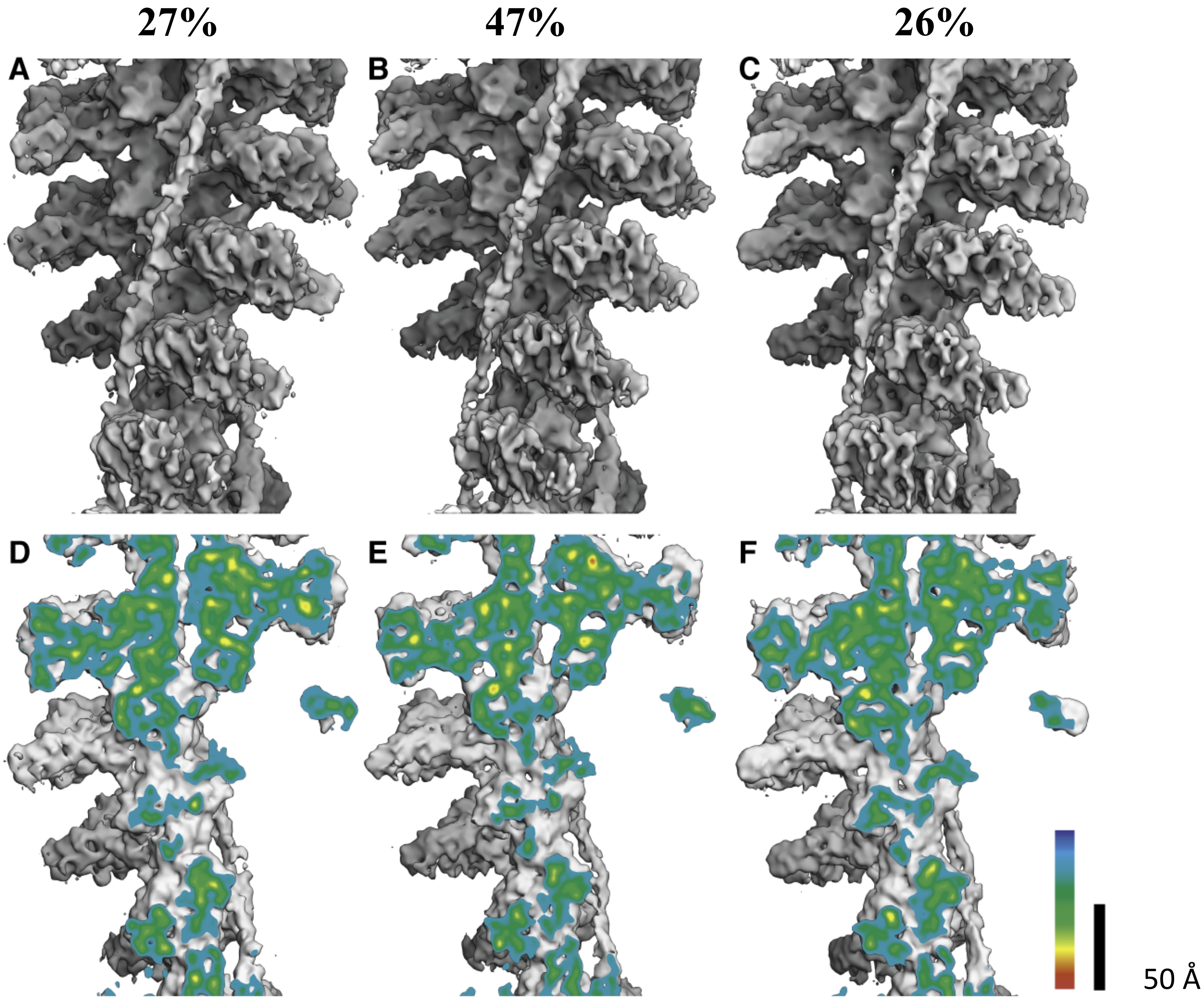
(A) undecorated F-actin filament

(B-D) three groups of decorated Actin-Tropomyosin-Myosin complex conformers (B-D)



Three states of the Actin-Tropomyosin-Myosin complex determined by hPCA (resolution 8Å)

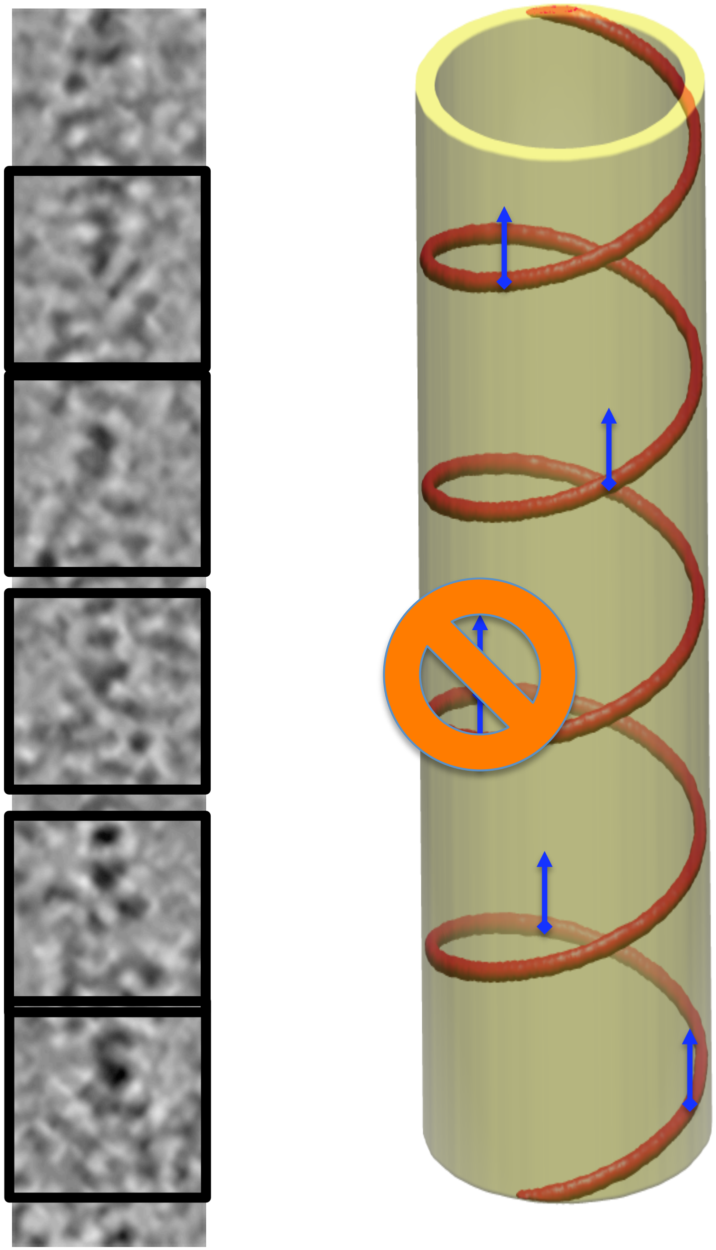
20,686 segments



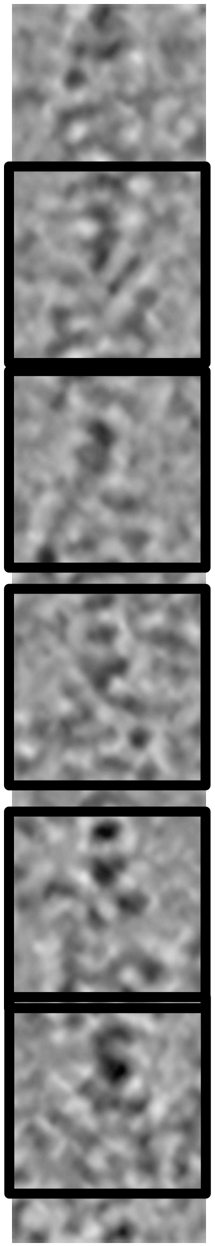
Behrmann E, Muller M, Penczek PA, Mannherz HG, Manstein DJ, Raunser S. Structure of the rigor Actin-Tropomyosin-Myosin complex. Cell 2012, 150:327-38.

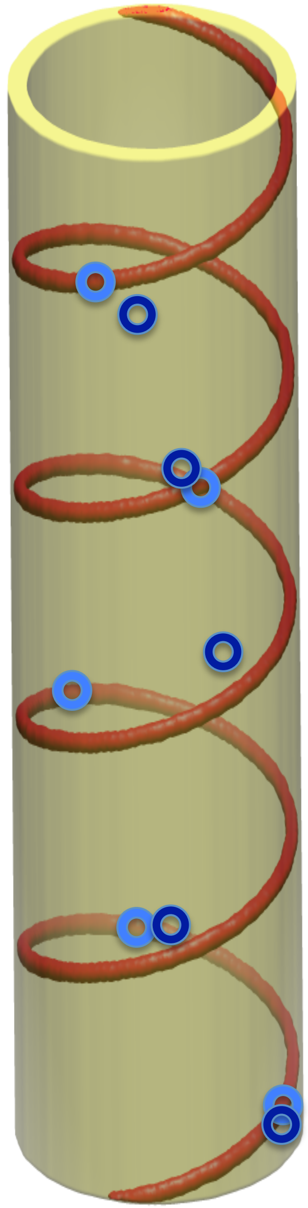
IHRSR segments are not independent

Fundamental Geometrical Consistency up/down



Geometrical Consistency
helical





- Helical consistency would require a physical model of filament flexibility

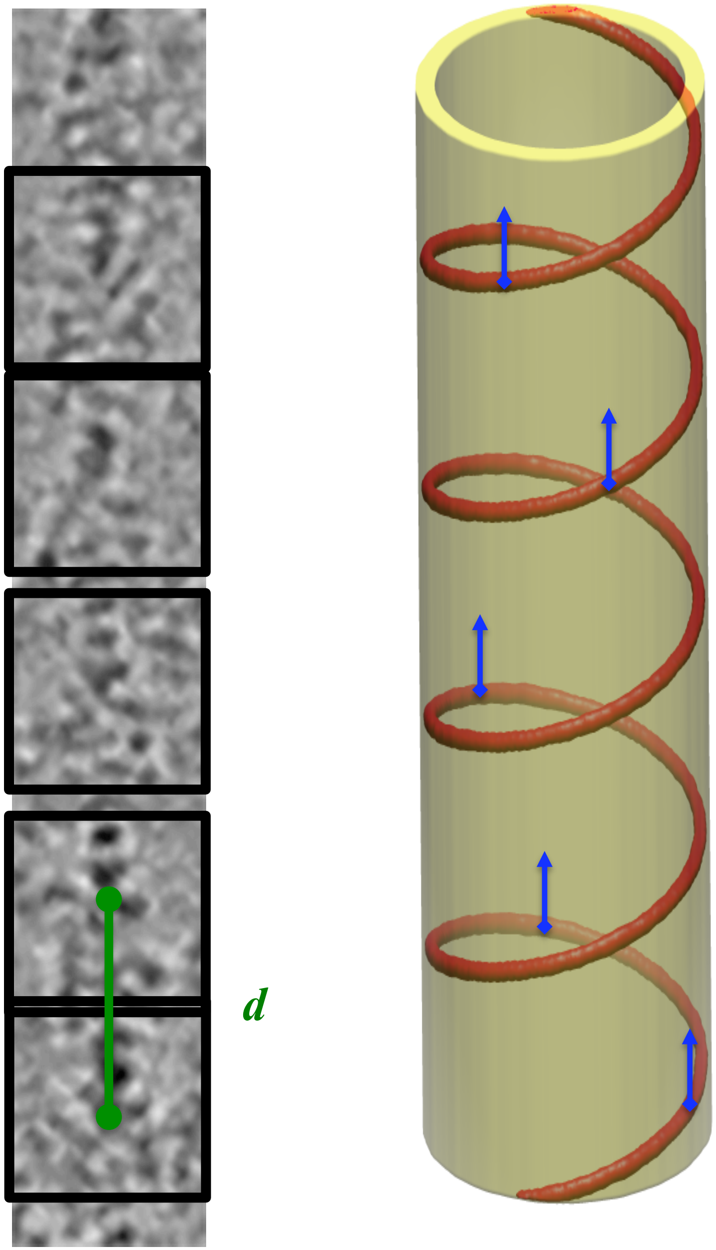
The Design of Geometrically Consistent IHRSR (gcIHRSR)

1. Prediction of orientation parameters based on assumed helical symmetry

2. Cooperative initial structure determination (only per-filament changes are allowed)

3. High-accuracy structure refinement (only restricted changes per segment are allowed)

1. Prediction of orientation parameters based on assumed helical symmetry

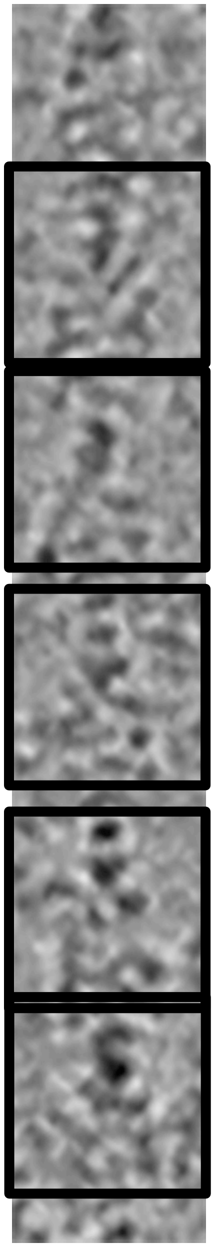


$$f\left(r,\varphi,z\right)=f\left(r,\varphi+n\Delta\varphi,z+n\Delta z\right),\quad n=\pm1,\pm2,\ldots$$

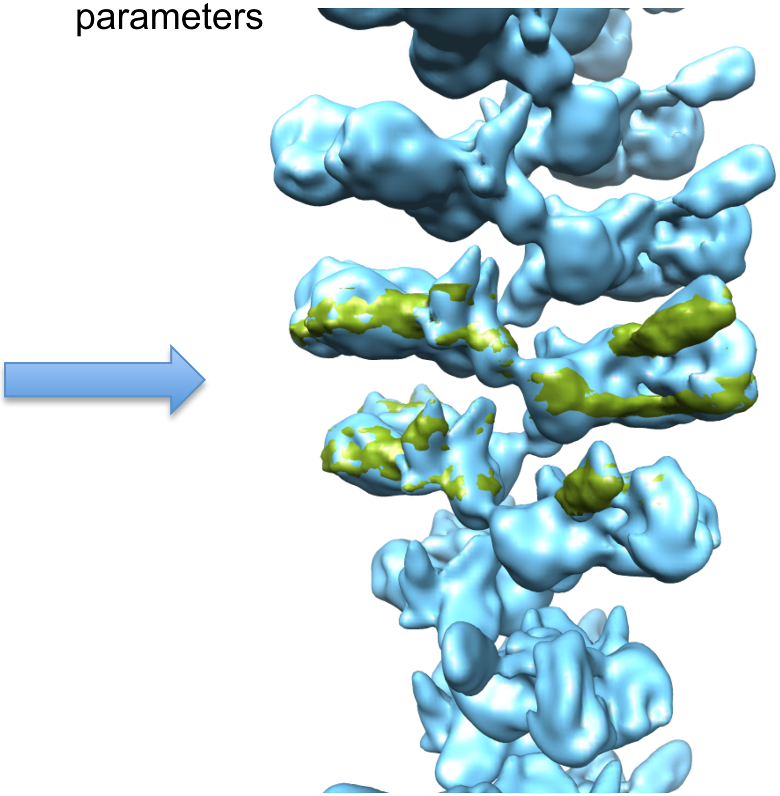
For each segment we have to assign φ and z:

1. first segment: φ=0 and z=0
2. second segment:
   given d, $\varphi = (d/\Delta z)\,\Delta\varphi$ and z=mod(d, Δz)
3. and so on ….

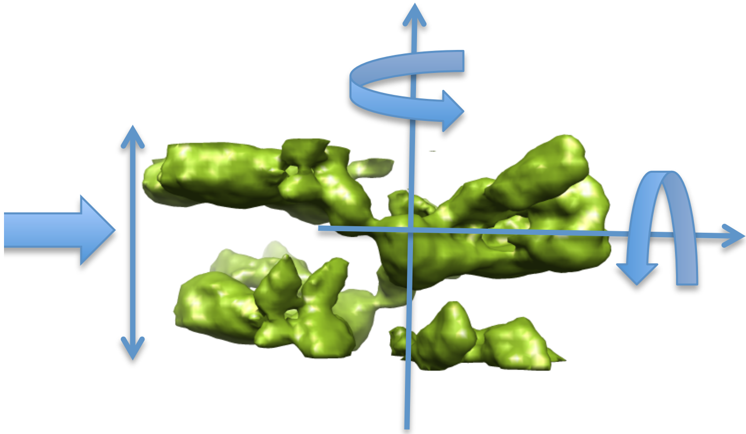
2. Cooperative initial structure determination, aka *Disk Alignment*



For each filament compute corresponding helical structure using predicted parameters



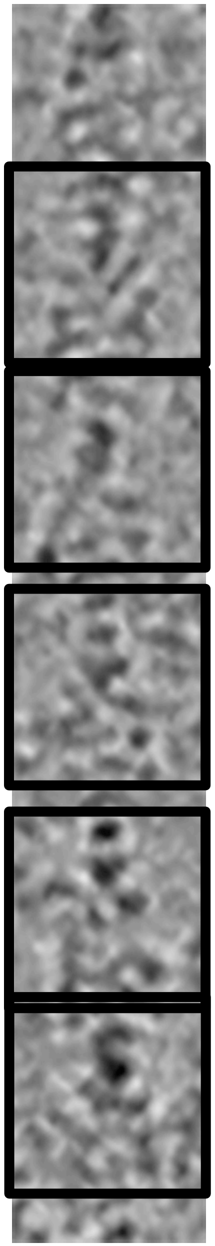
Extract a disk one rise high

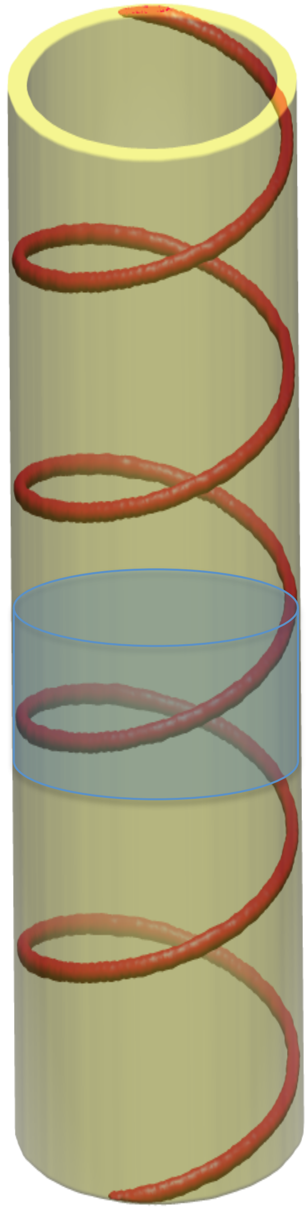


Reference-free alignment of all filament disks determines rotation and translation of each filament/disk.

The resulting parameters are transferred to segments.

3. High-accuracy structure refinement (only restricted changes per segment allowed)





$$f(r,\varphi,z) = f(r,\varphi + n\Delta\varphi, z + n\Delta z), \quad n = \pm 1, \pm 2, \ldots$$

Restriction of searches:

translation t_y no more than one rise: $|t_y| < \Delta z/2$

azimuthal angle $\varphi \cong (t_y / \Delta z)\, \Delta\varphi$

OUT OF PLANE TILT!

Actin-Tropomyosin-Myosin Complex

- JEOL 200 kV EM, 8k CCD

  Selected decorated filaments (B):

  Number of filaments: 7,696

  Number of segments: 35,319

  Pixel size 1.84 Å

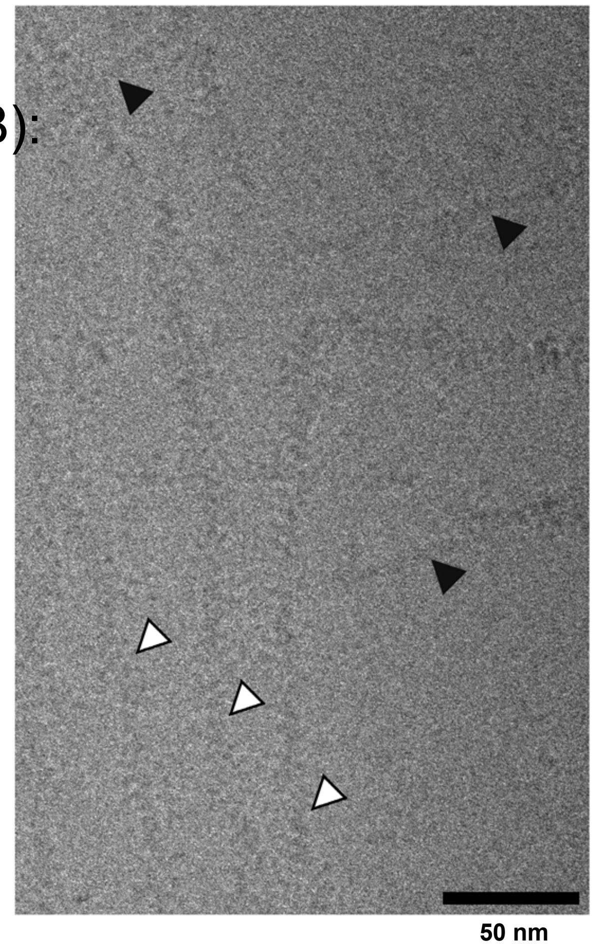
- Helical symmetry parameters:

  rise Δz= 27.6 Å

  (15 pixels)

  azimuthal rotation 166.5^{o}

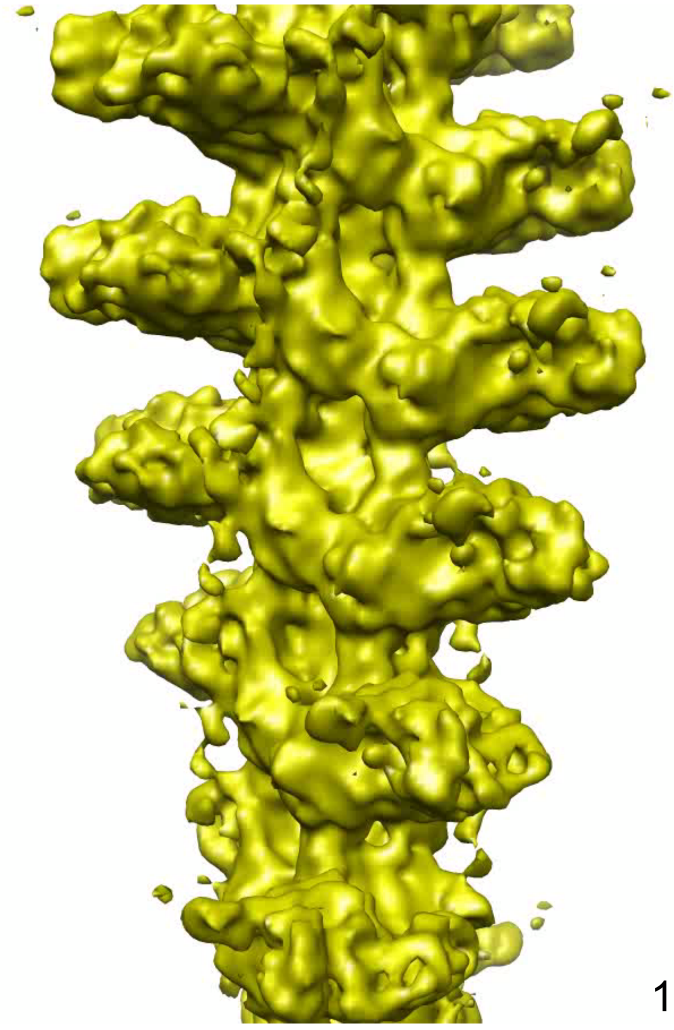
A



segments per filament	segments	cumulative
	51%	
7	2541	18055
8	2520	15514
9	1962	12994
10	2130	11032
11	1419	8902
12	984	7483
13	1066	6499
14	1050	5433
15	660	4383
16	592	3723
17	612	3131
18	288	2519
19	247	2231
20	240	1984
21	336	1744
22	242	1408
23	161	1166
24	120	1005
25	175	885
26	156	710
27	81	554
28	84	473
29	116	389
30	60	273
31	62	213
32	0	151
33	0	151
34	34	151
35	35	117
36	0	82
37	0	82
38	38	82
39	0	44
40	0	44
41	0	44
42	0	44
43	0	44
44	44	44

ATM complex
IHRSR versus GCihrsr

Disk alignment
number of filaments 1,705

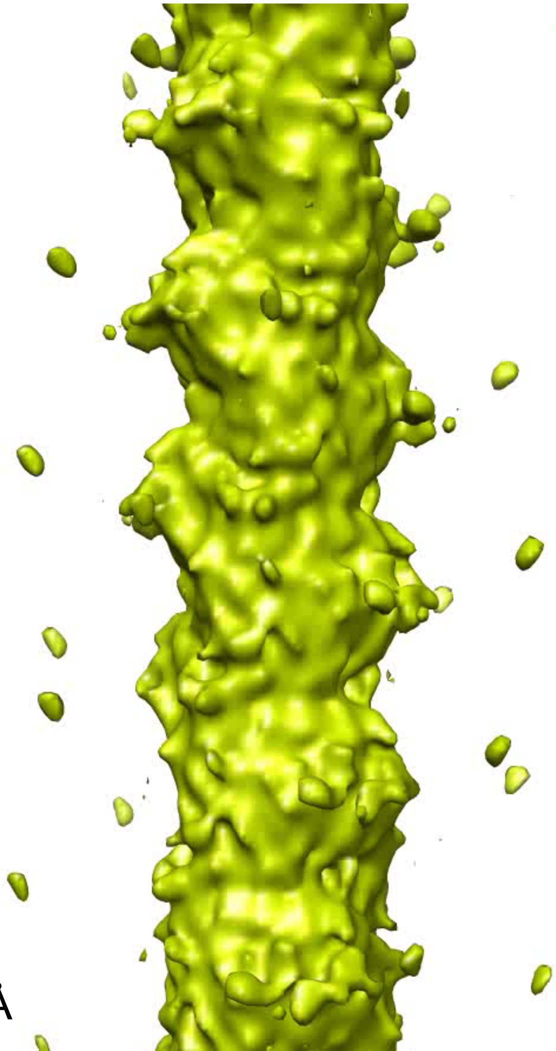


18.4Å

100% consistent

ATM complex
IHRSR versus GCihrsr

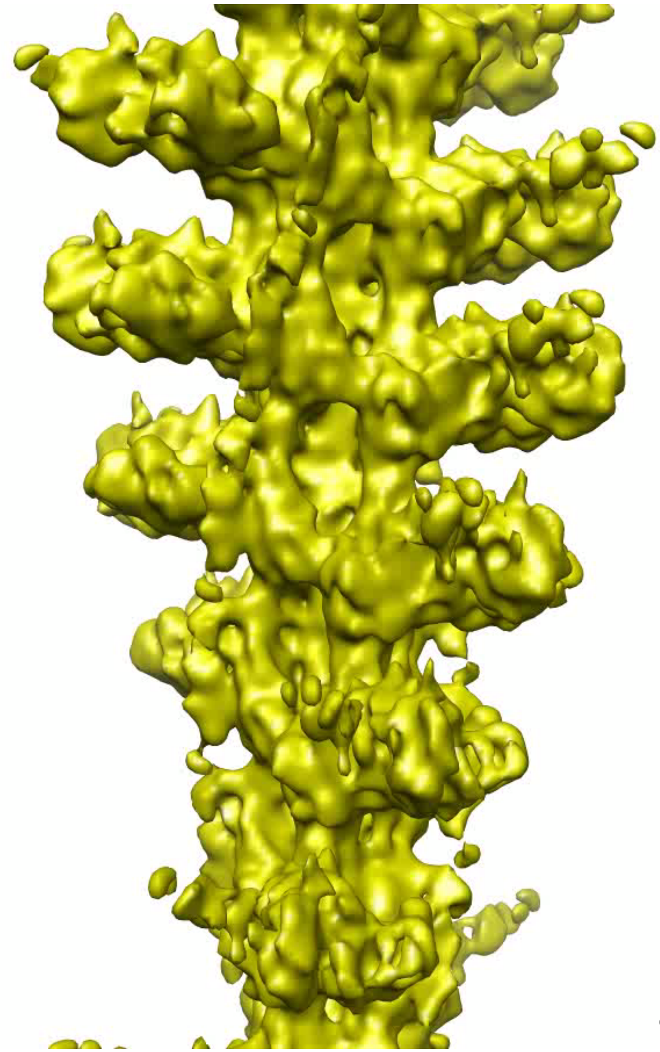
Exhaustive search



8.8Å

85% psi-consistent

Constrained search



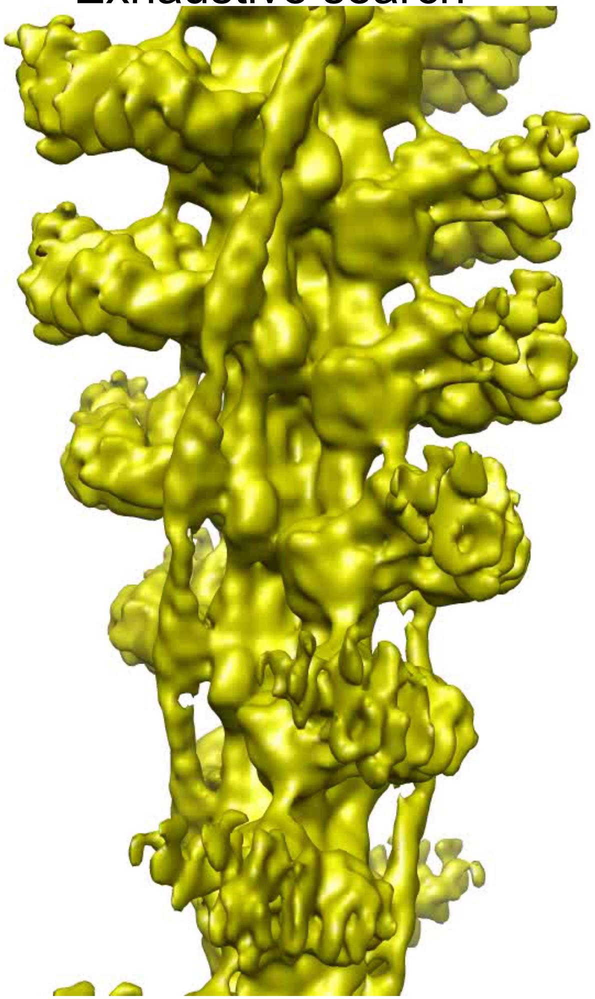
8.3Å

100% psi-consistent

ATM complex
IHRSR versus GCihrsr

out-of-plane tilt: 85<theta<95

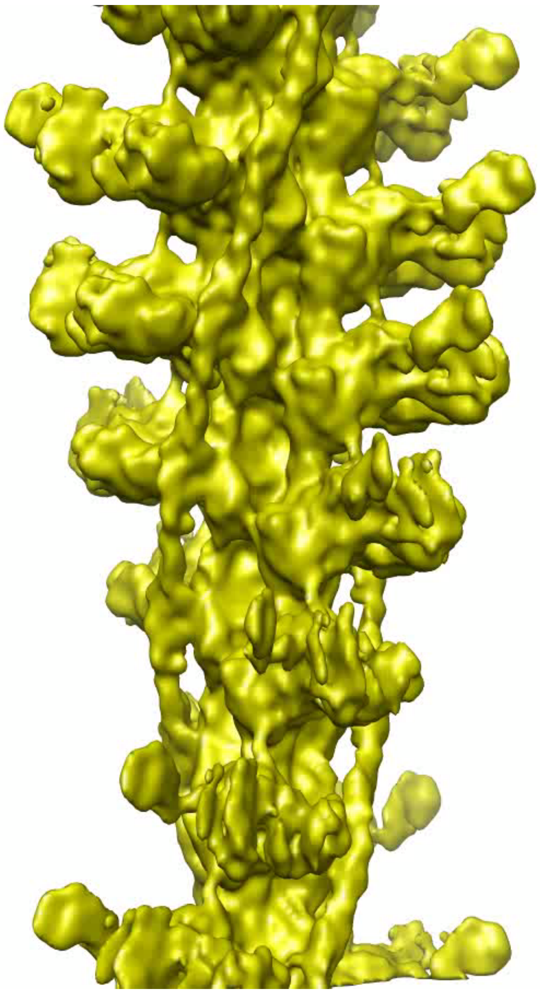
Exhaustive search



8.4Å

85% psi-consistent

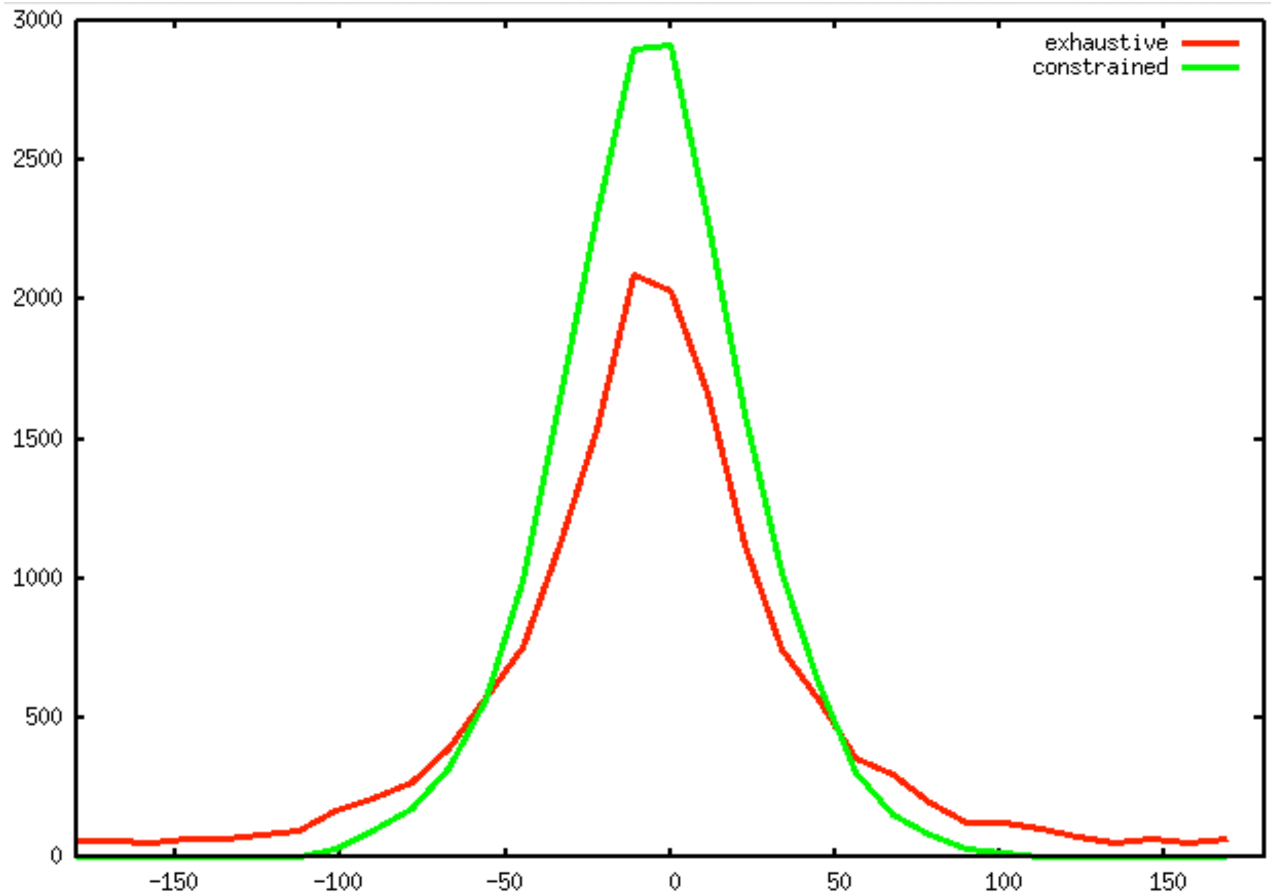
Constrained search



8.3Å

100% psi-consistent

ATM complex
IHRSR versus GCihrsr



$\Delta\varphi = 166.5^o$ $\Delta z = 15$

azimuthal angle error
difference between actual and predicted

ATM complex
IHRSR versus GCihrsr

$$\Delta\varphi = 166.5^{o} \qquad \Delta z = 15$$

Predicted

φ	θ	ψ	t_x	t_y
0	90	90	0	0
333	90	90	0	-9.3681e-05
279	90	90	0	6.7365e-05
117	90	90	0	5.1956e-05
90	90	90	0	-4.1725e-05
63	90	90	0	1.4733e-05
36	90	90	0	-7.8948e-05
9	90	90	0	-2.249e-05

Disk/t_x alignment

φ	θ	ψ	t_x	t_y
329.9	90	270.0	-18.0	6.0
356.9	90	270.0	-15.0	6.0
50.9	90	270.0	-18.0	6.0
212.9	90	270.0	-15.0	6.0
239.9	90	270.0	-15.0	6.0
266.9	90	270.0	-15.0	6.0
293.9	90	270.0	-15.0	6.0
320.9	90	270.0	-15.0	6.0

Exhaustive

φ	θ	ψ	t_x	t_y
47.0	90	87.2	-4.0	0.0
91.0	90	276.7	3.0	-2.8
122.0	90	277.4	8.0	-3.8
217.0	90	271.8	-5.7	-2.0
308.0	90	277.4	2.0	1.9
291.0	90	88.9	5.4	4.8
305.0	90	90 .0	1.0	0.0
1.0	90	85.7	-13.0	1.9

Local

φ	θ	ψ	t_x	t_y
326.0	90	277.4	-13.0	3.6
359.0	90	263.3	-12.0	5.4
94.0	90	263.3	-19.0	2.4
223.0	90	273.2	-23.0	6.4
250.0	90	271.1	-24.0	7.5
273.0	90	265.4	-20.0	9.6
305.0	90	272.8	-23.0	9.2
311.0	90	267.9	-21.0	9.2

Conclusions/Future work

✓ Geometrically Consistent IHRSR is the *correct* approach to helical filament structure determination:

- improved details
- reduced fake resolution

? Cooperative local searches.

? Modeling of flexibility ?

Acknowledgments

Stefan Raunser
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University of Virginia

David Stokes
NYU Medical Center

TEMIMPS

NIH