Validation

EMVTF - conclusions in Sept 2010

- Development of positive 3DEM map validation methods remains an open research problem
- A number of conditions are necessary for map validity (but sufficient would be better)
- Some methods may detect whether a map is incorrect

EMVTF list of necessary conditions

- Absolute hand determination, requires either a tilt experiment or very high resolution (RCT, orthogonal tilt, SP-tomography, TPPP). If map has no discernible hand, how do you know it is not a mixture of the real structure and its enantiomorph?

- Data coverage (any missing views?)

- Agreement between raw images, class averages (if generated) and projections from 3D map (necessary but not sufficient). The author's "agreement" can easily be the referee's disagreement.

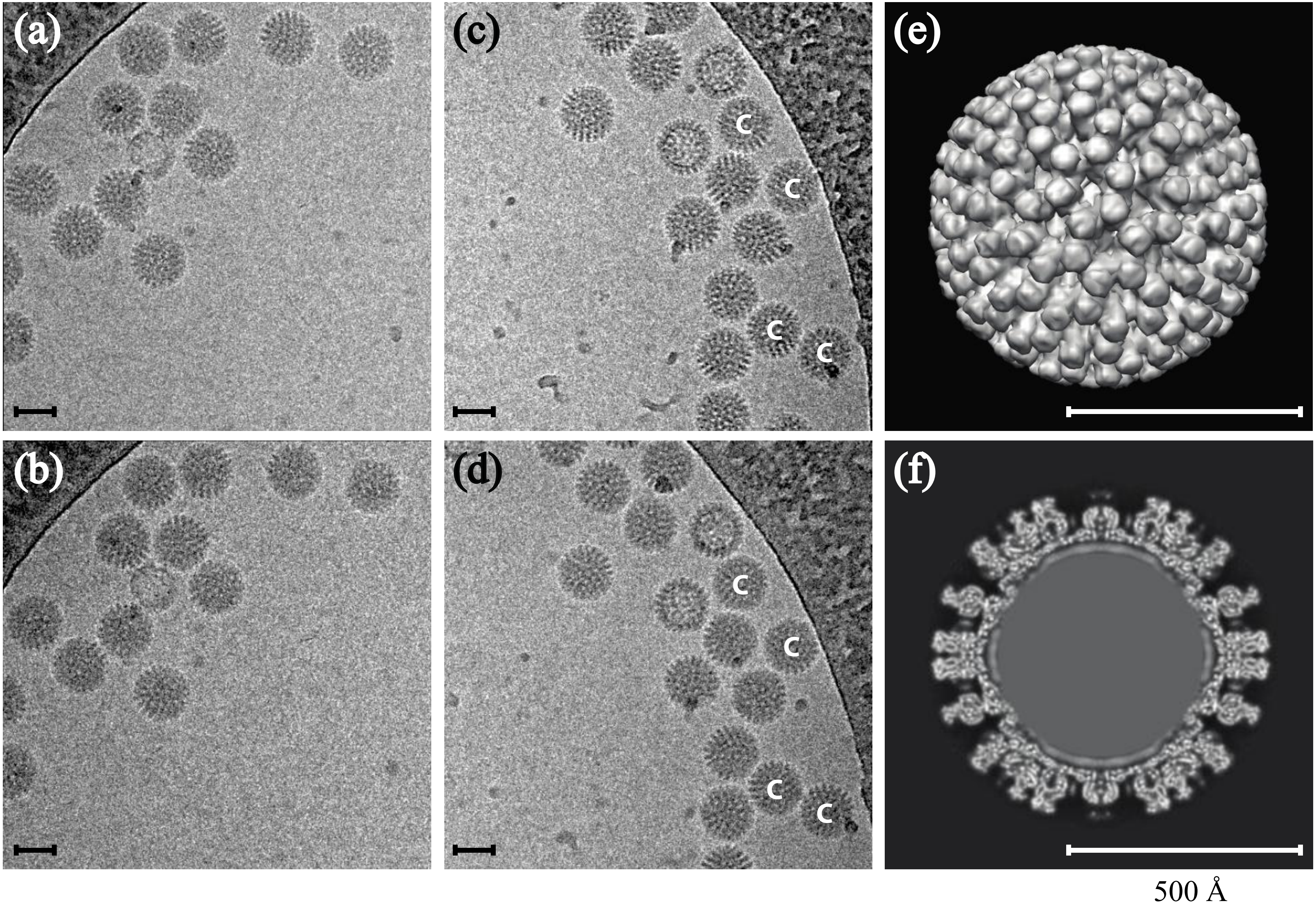
- Statistical assessment of map – map variance, local FSC in good and bad regions of map, preferably "gold standard FSC".

- Common sense

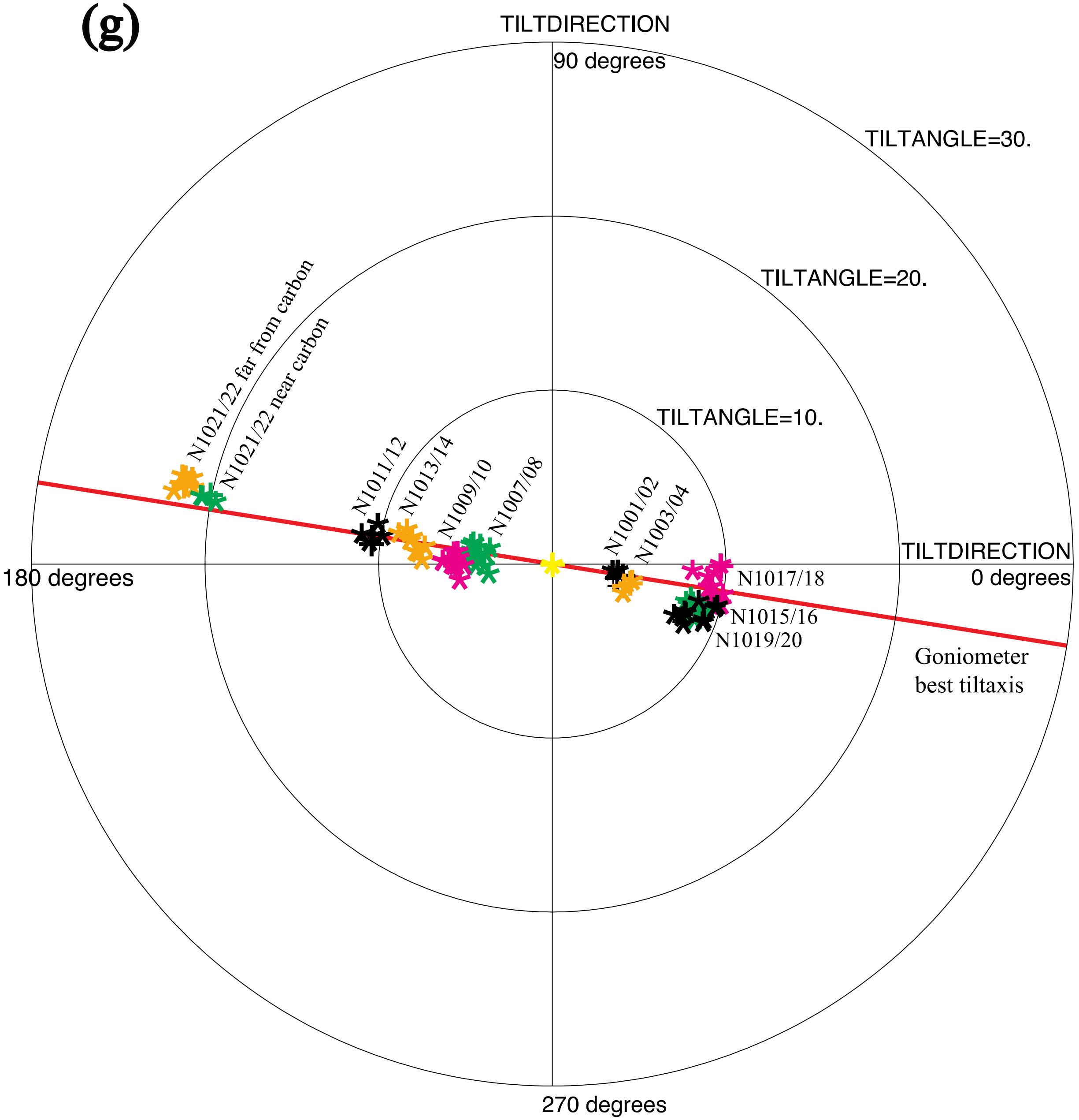
Two positive tests

- Tilt pair analysis to assess orientation determination (TPPP test)
- Calculate control map with high resolution random noise (HR-noise test)

Rotavirus (T=13, MW 50MDa) tilt pair images: James Chen, Brandeis

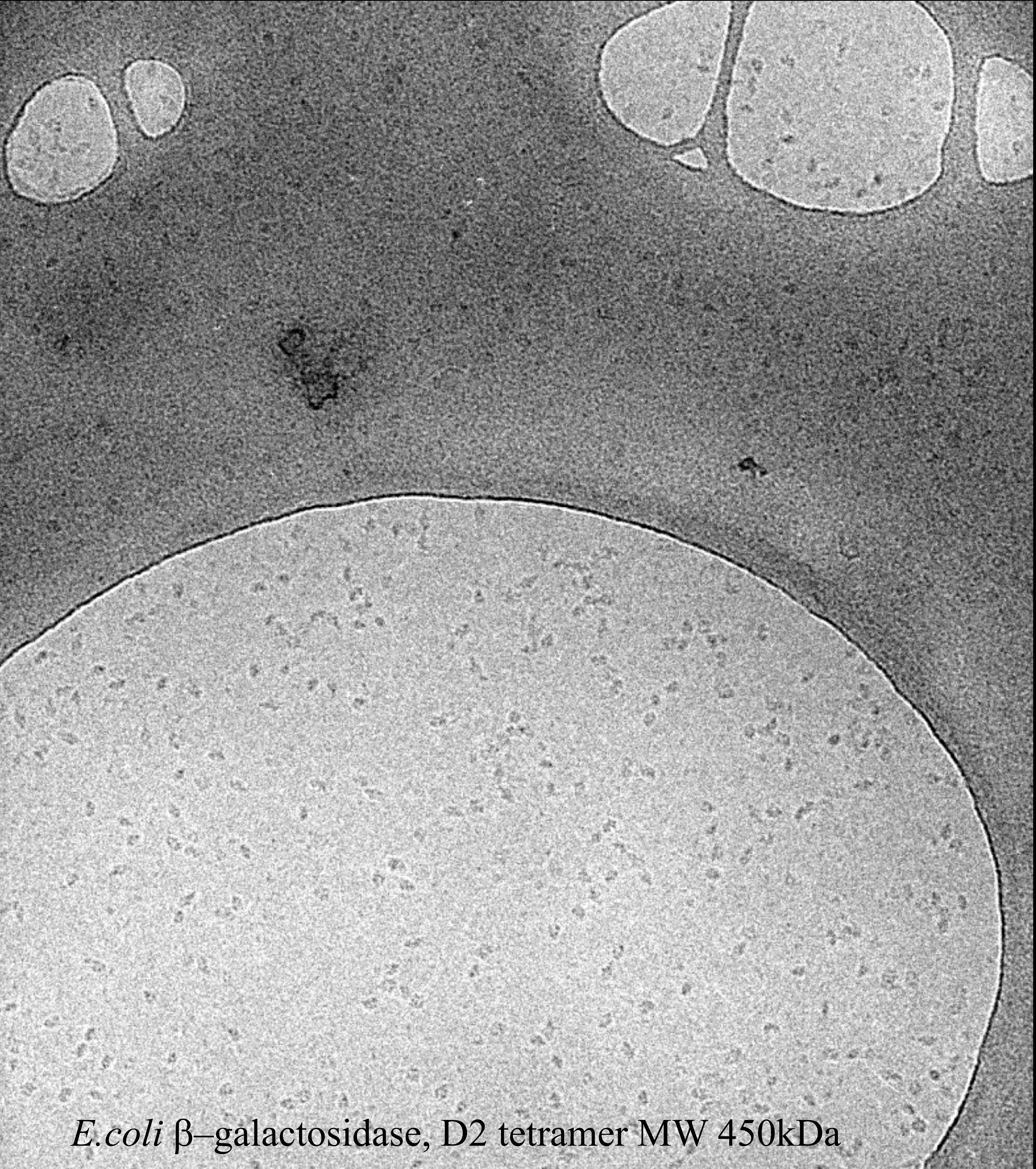


Rotavirus: 10 tilt pairs, Chen & Grigorieff

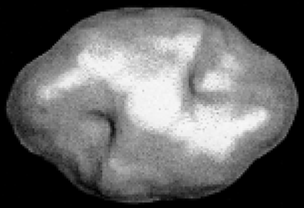


Film pair	<TANG> (sd)	Nom. TANG
N1001/2	+3.83 (±0.20)	+5.0
N1003/4	+4.50 (±0.21)	+5.0
N1007/8	-4.24 (±0.39)	-5.0
N1009/10	-5.67 (±0.33)	-5.0
N1011/12	-10.4 (±0.44)	-10.0
N1013/14	-8.07 (±0.63)	-10.0
N1015/16	+8.67 (±0.45)	+10.0
N1017/18	+9.34 (±0.53)	+10.0
N1019/20	+8.83 (±0.81)	+10.0
N1021/22	-21.14 (±0.95)	-20.0

Fig.1

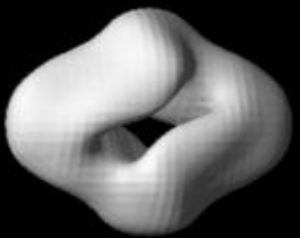


E.coli β–galactosidase, D2 tetramer MW 450kDa

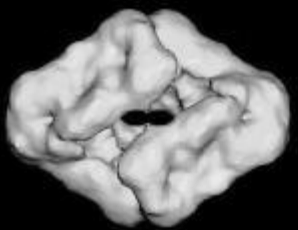


1997

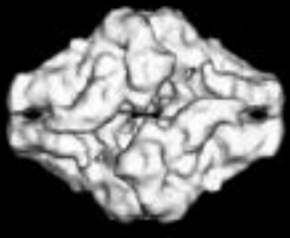
2002



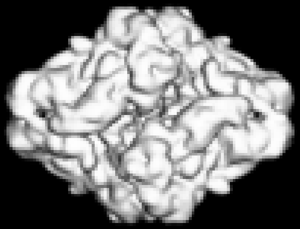
2005



2011



2012



2012

Shaoxia Chen: LMB Cambridge

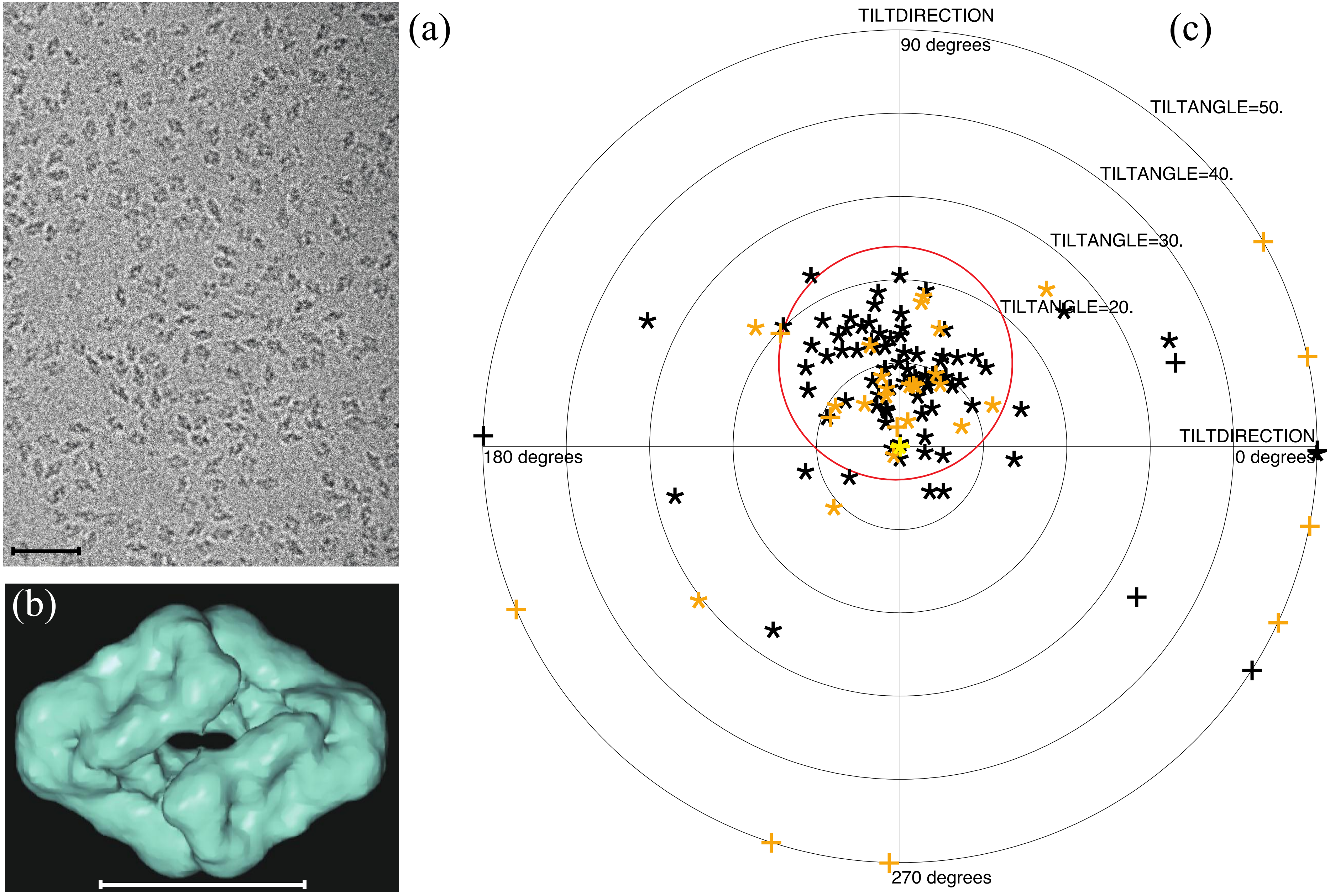
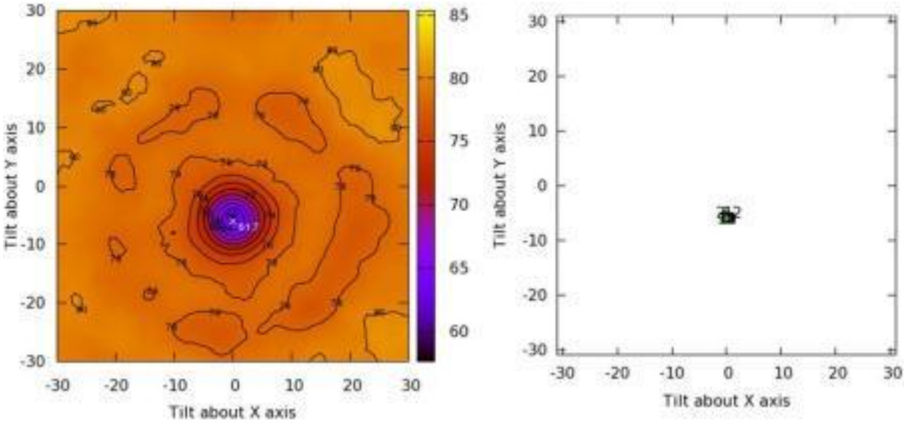


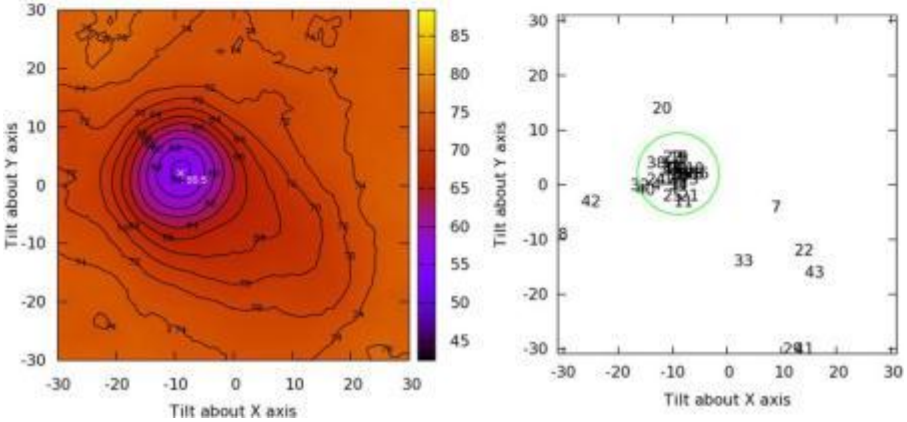
Fig.2

Experiment identifier: ROTAV 30 degrees

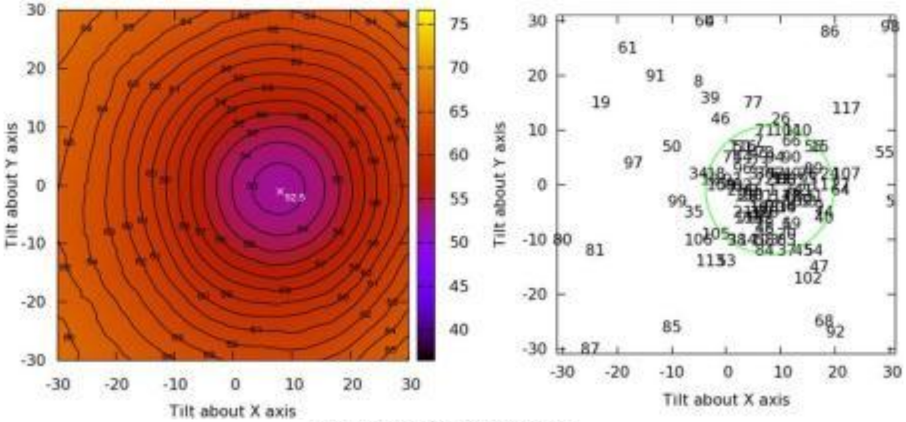


Sebastian Wasilewski & Peter Rosenthal, NIMR
Tilt pair web server at www.cryomicroscopy.org

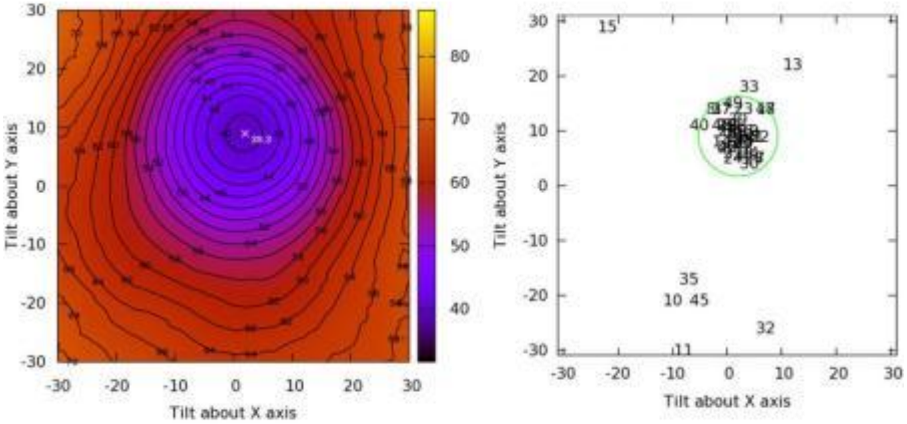
Experiment identifier: CAV 30 degrees



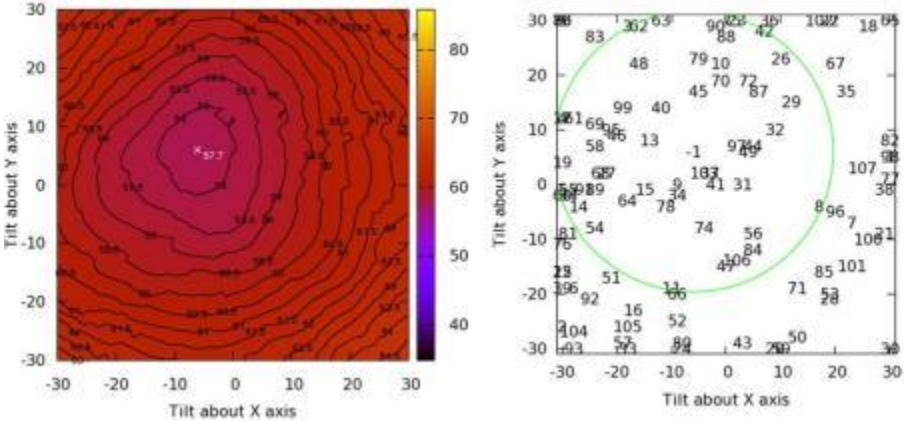
Experiment identifier: BGAL - 30 degrees



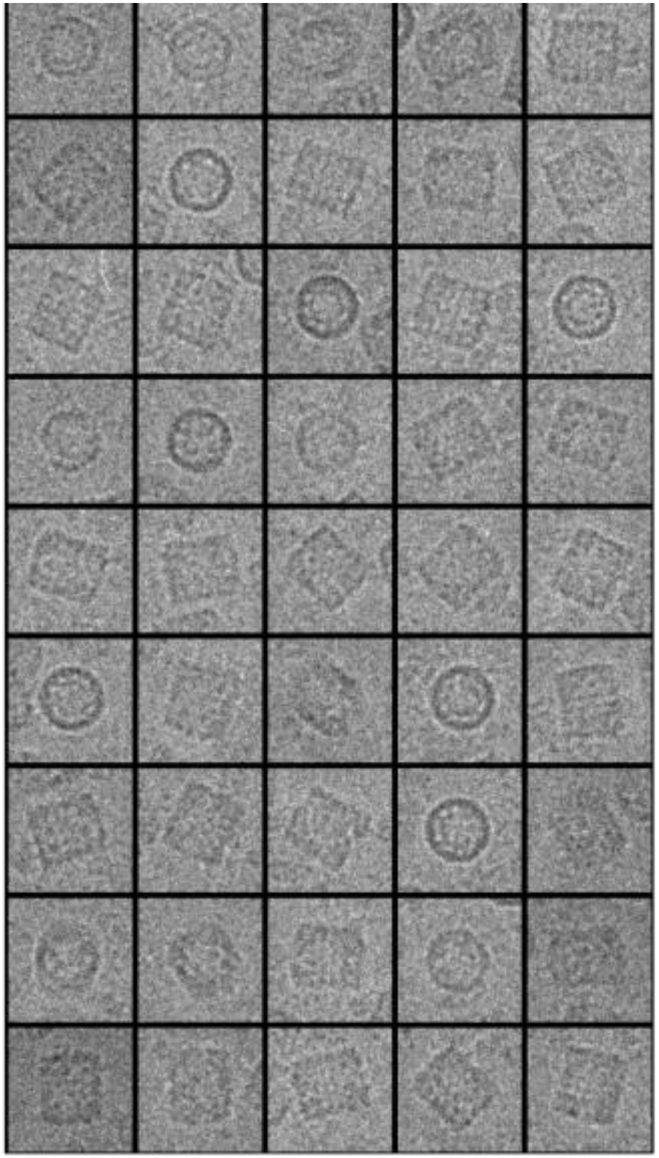
Experiment identifier: E2 - 30 degrees

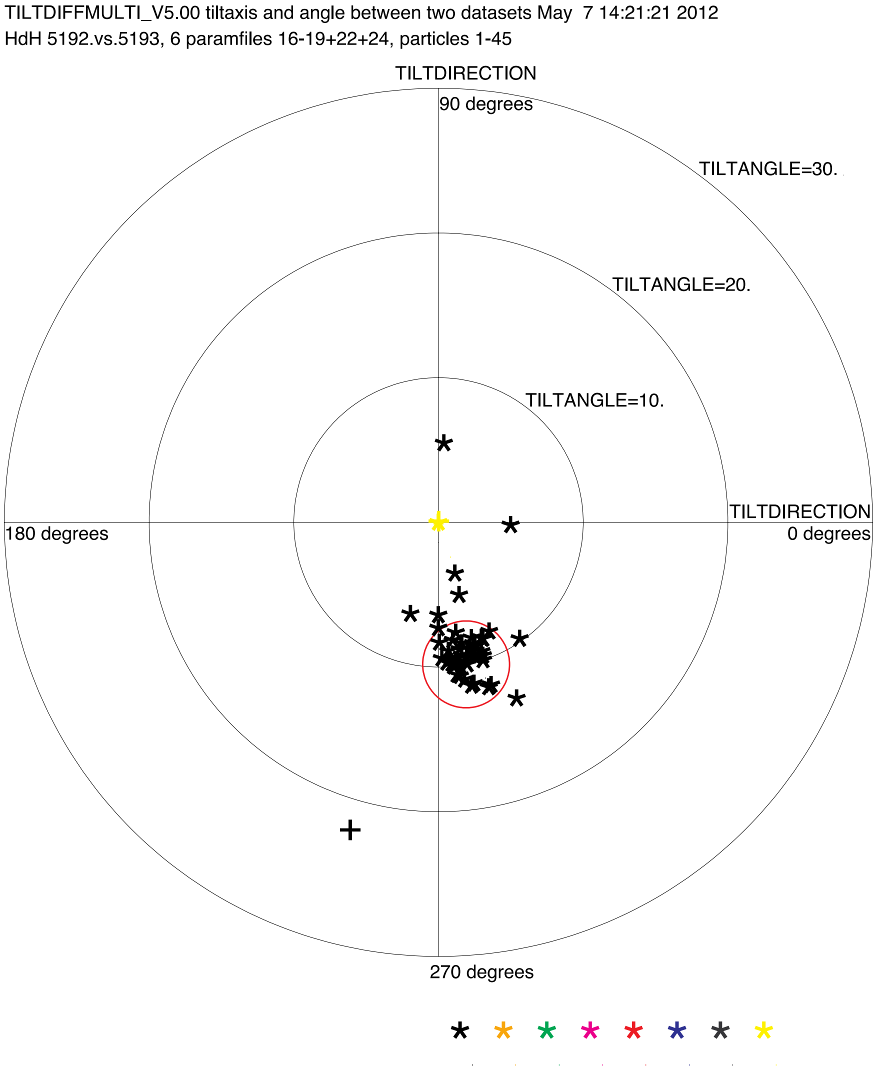


Experiment identifier: DPK - 30 degrees



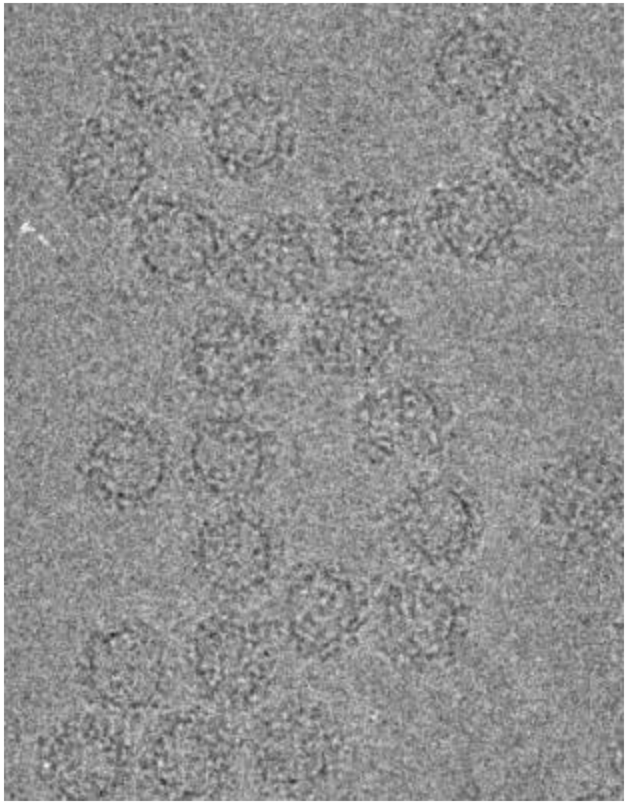
# *Haliotis diversicolor* hemocyanin – Qinfen Zhang, Zhongshan
M.W. = 8 MDa   200keV images on film, Shaoxia Chen



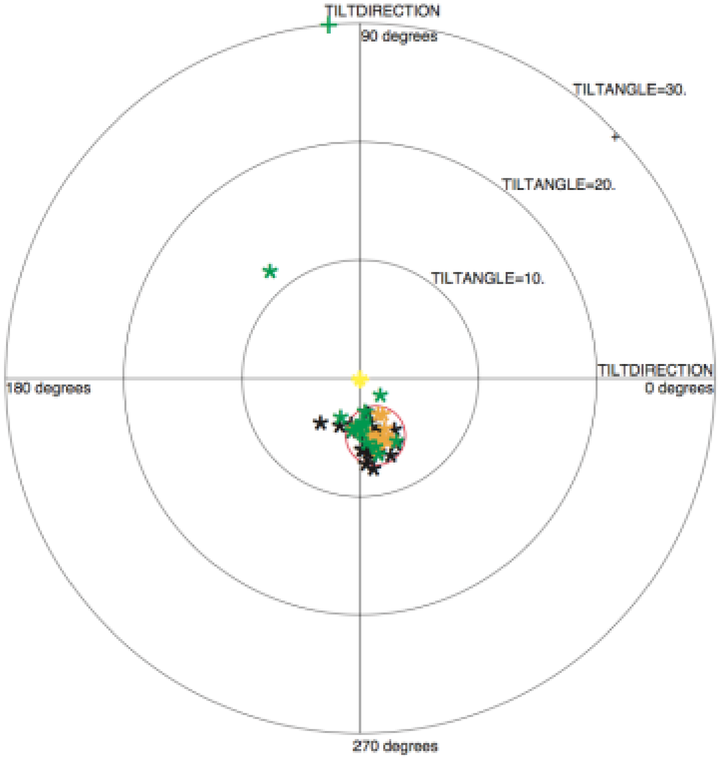


Norwalk virus (MW ~10MDa) – Prasad Venkatar, Shaoxia Chen

200keV images on film



TPPP for
NWVK_A04643 + A04644



3D models from cryoEM
and PDB X-ray

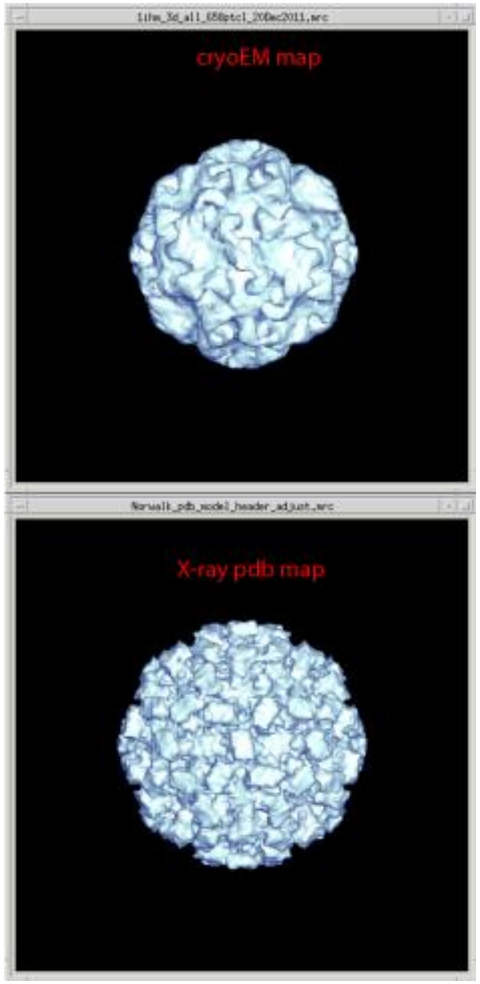
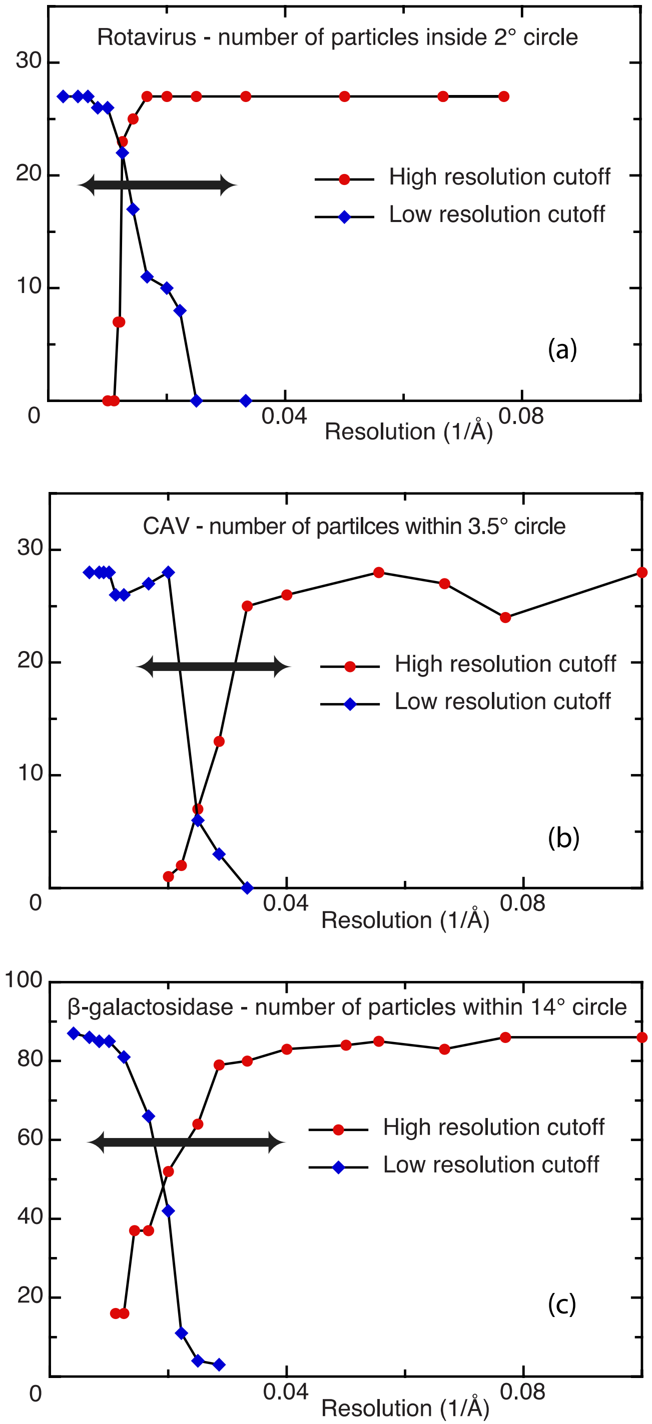


Table 2 – overview of TPPP statistics (extended May 2012)

Specimen	**Symmetry**	**Particle size**	**Molecular Weight**	**Number of tilt pairs**	**Number of particles**	**Successful alignment (%)**	**Mean/maximum angular error (degs)**	
Rotavirus DLP	I2	700 Å	50 MDa	10	95	100/100	0.25	1.0
Norwalk virus	I1	420 Å	10 MDa	1	51	98	1.5	2.5
HdH	D5	550 Å	8 MDa	3	45	78	1.5	3.0
CAV	I2	255 Å	2.7 MDa	1	45	62/82	2.5	3.5
FAS	D3	260x220 Å	2.6 MDa	2	44	59/95	4.0	6.0
70S ribosomes	C1	270x260 Å	2.6 MDa	12	220	45/75	4.0	5.0
PDH-E2CD	I1	280 Å	1.6 MDa	1	50	62/94	3.0	4.0
Thermus V-ATPase	C1	250x140 Å	0.6 MDa	1	50	54/80	10.0	16.0
Bovine F-ATPase	C1	250x140 Å	0.6 MDa	1	29	52/79	20.0	25.0
DNA-PKcs	C1	150x120 Å	0.47 MDa	14	108	44/81	15.0	17.0
β-galactosidase	D2	180x130x95 Å	0.45 MDa	2	119	74/91	10.0	14.0

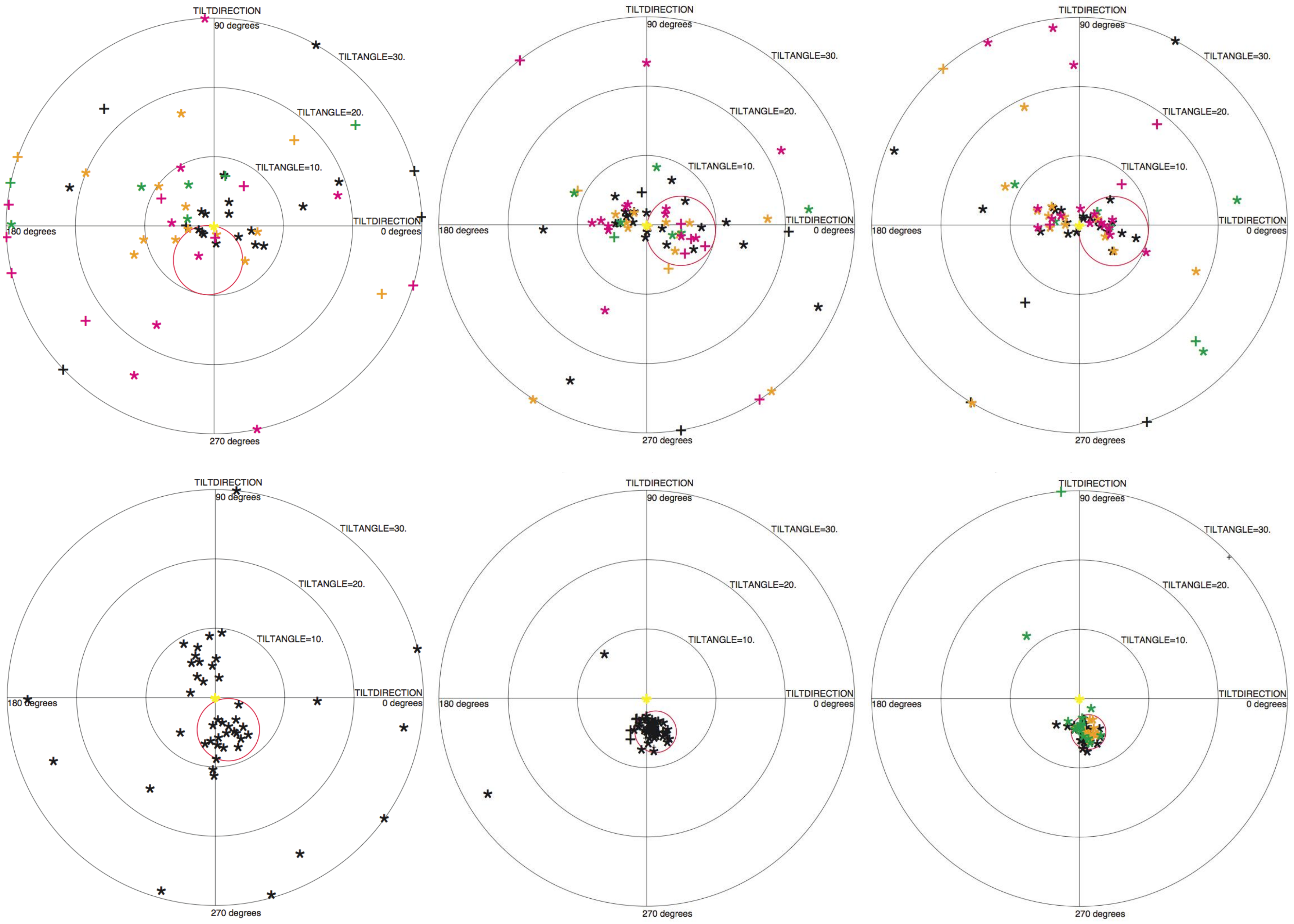


Number of particles in which the tilt pair relative orientations are clustered around the expected tiltaxis and tiltangle, plotted as a function of the lower and higher resolution cutoffs used in Frealign:

(a) Rotavirus within 2°
(b) CAV within 3.5°
(c) β-galactosidase within 14°

The double arrowhead shows the resolution range that contributes most to the orientation determination. When the low-resolution cutoff was varied, the high-resolution cutoff was set to its maximum value, and vice versa.

Improvements in Tilt-Pair Parameter Plots as the images and the 3D model are improved

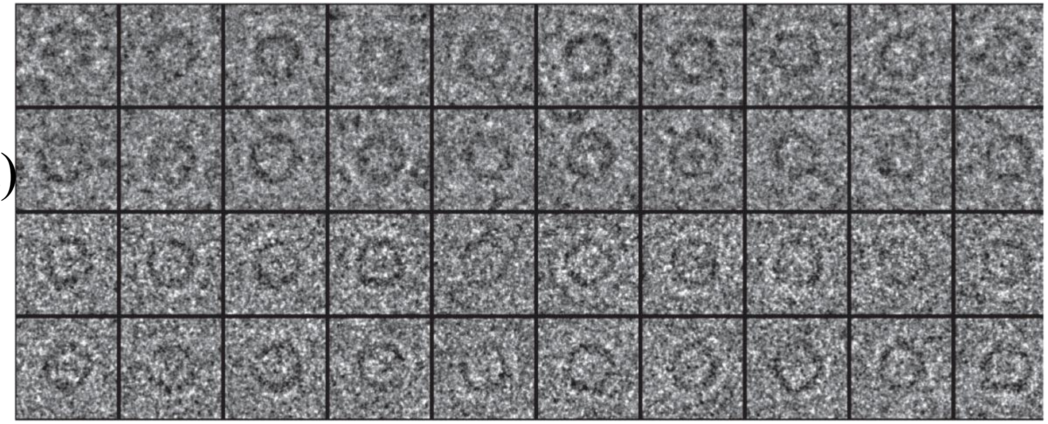


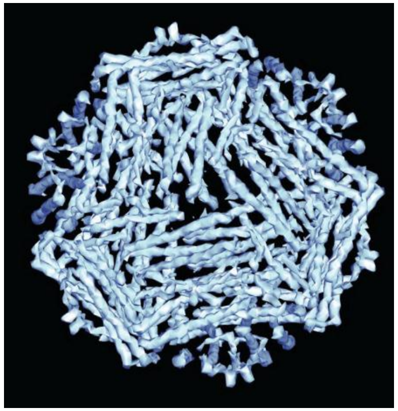
Norwalk virus (MW ~10MDa) – Prasad Venkatar, Shaoxia Chen

Apoferritin real tiltpair images compared with simulated images

real Falcon Images (Shaoxia)

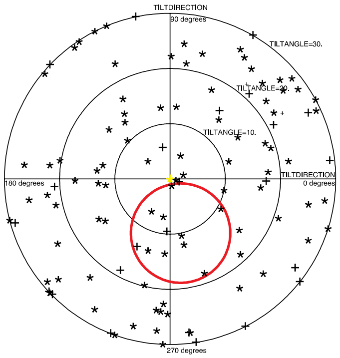
fake images (Greg)



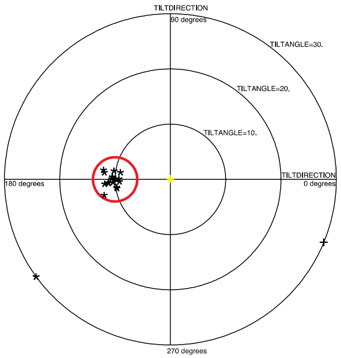


3D X-ray map

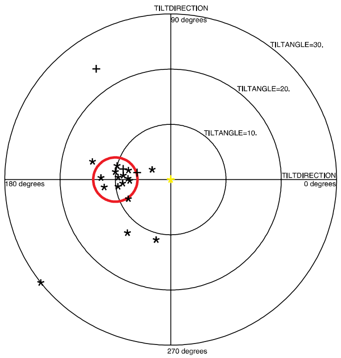
TPPPs



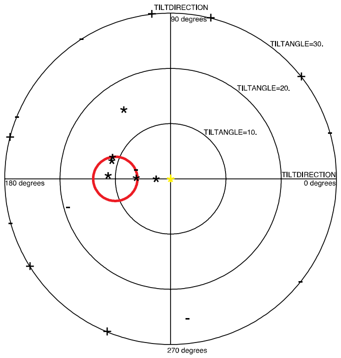
(a) experimental



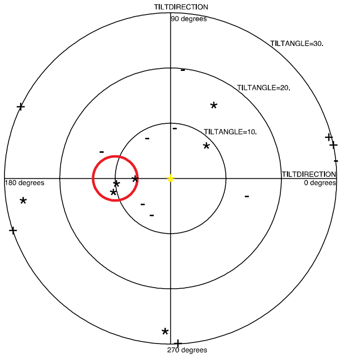
(b) B = 100



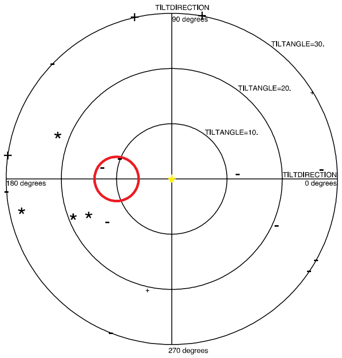
(c) B = 200



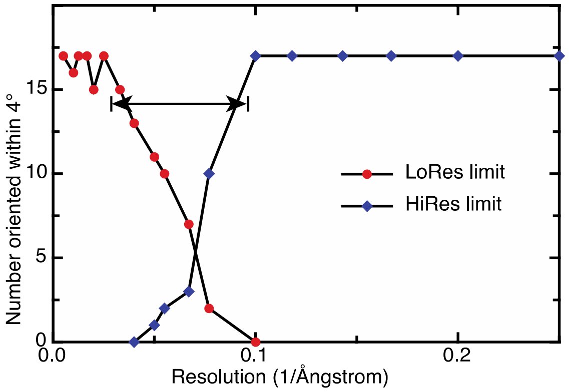
(d) B = 300



(e) B = 400



(f) B = 600



(g) resolution dependence of orientation success

Reliable tests for resolution determination?

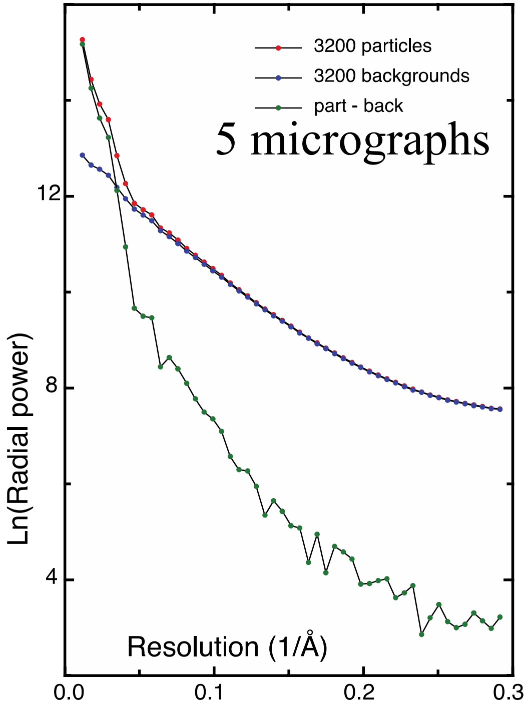
Existing options

(i) Divide data into two halves and keep completely separate (gold standard)
(ii) Refine particle orientations using low-pass cut-off/filtered data (e.g. 15 Å)

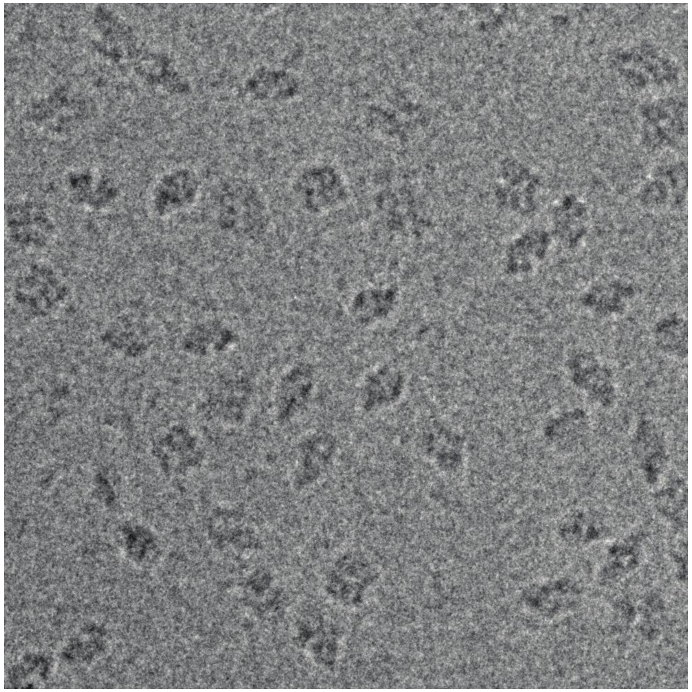
(iii) Proposal **<u>that avoids dividing data in half or omitting high resolution</u>**

(a) Perform Single Particle EM analysis by any chosen procedure.
(b) Substitute random phases beyond a selected resolution (HR-noise)
(c) Repeat entire analysis as in (a)
(d) Any overfitted noise will show up as non-zero FSC : genuine information will show up as the area between the two curves

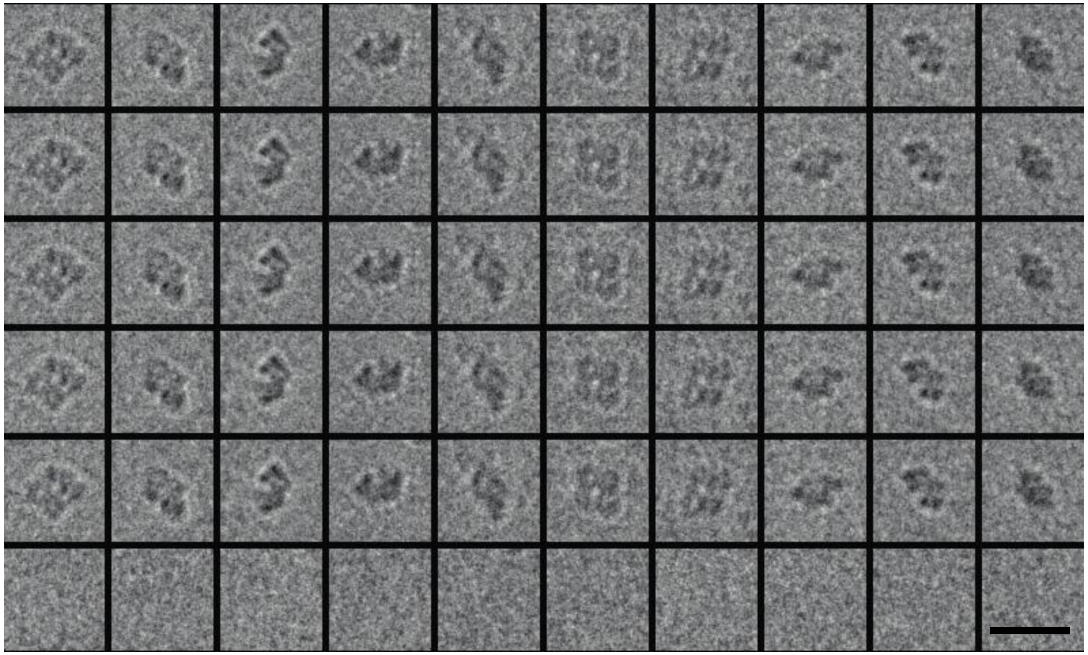
(a)



(b)



β-galactosidase
part of Falcon
micrograph
01.49.47



(c) original particles

(d) background A,φ > 1/10Å^{-1}

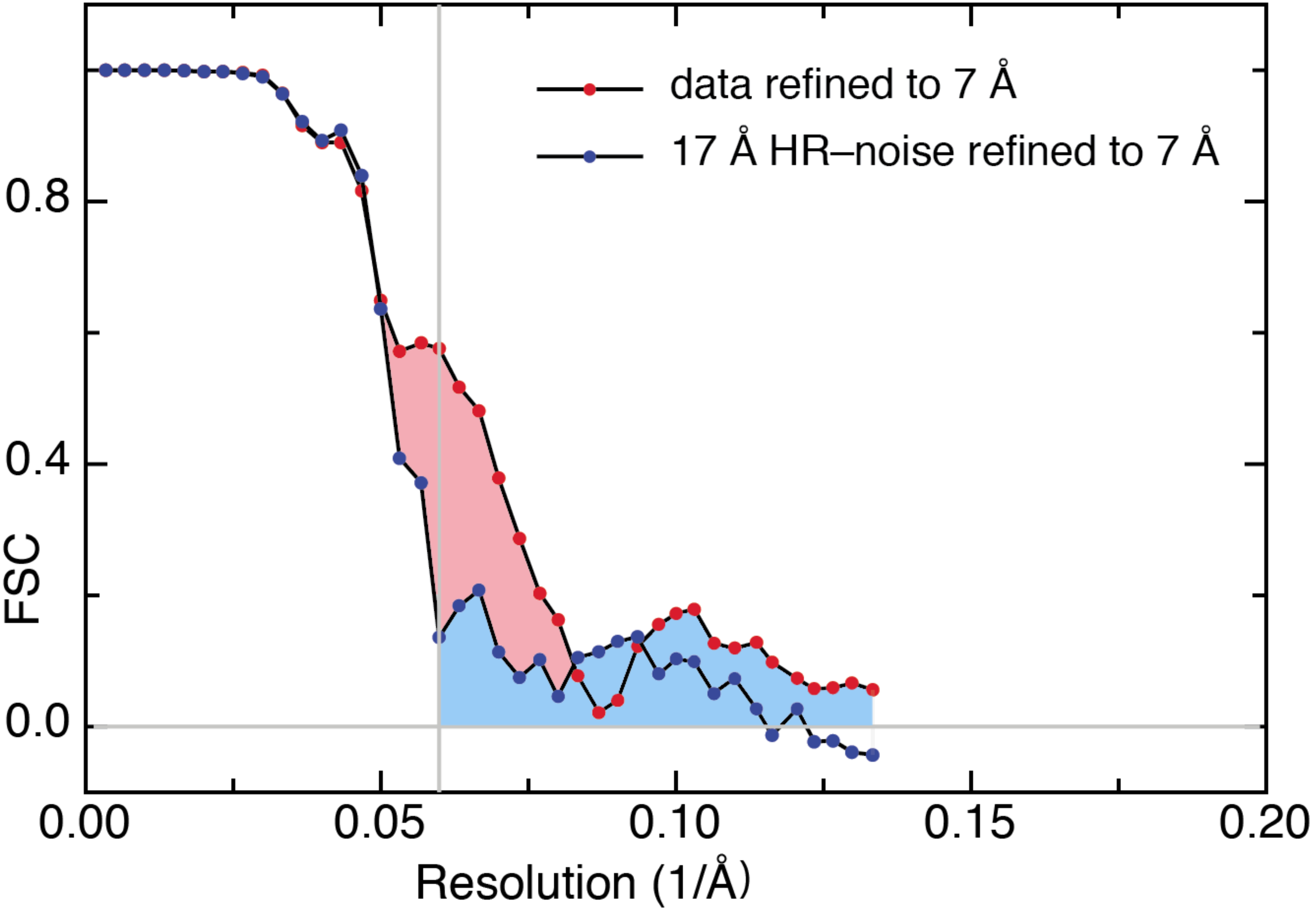
(e) random φ > 1/10Å^{-1}

(f) background A,φ > 1/17Å^{-1}

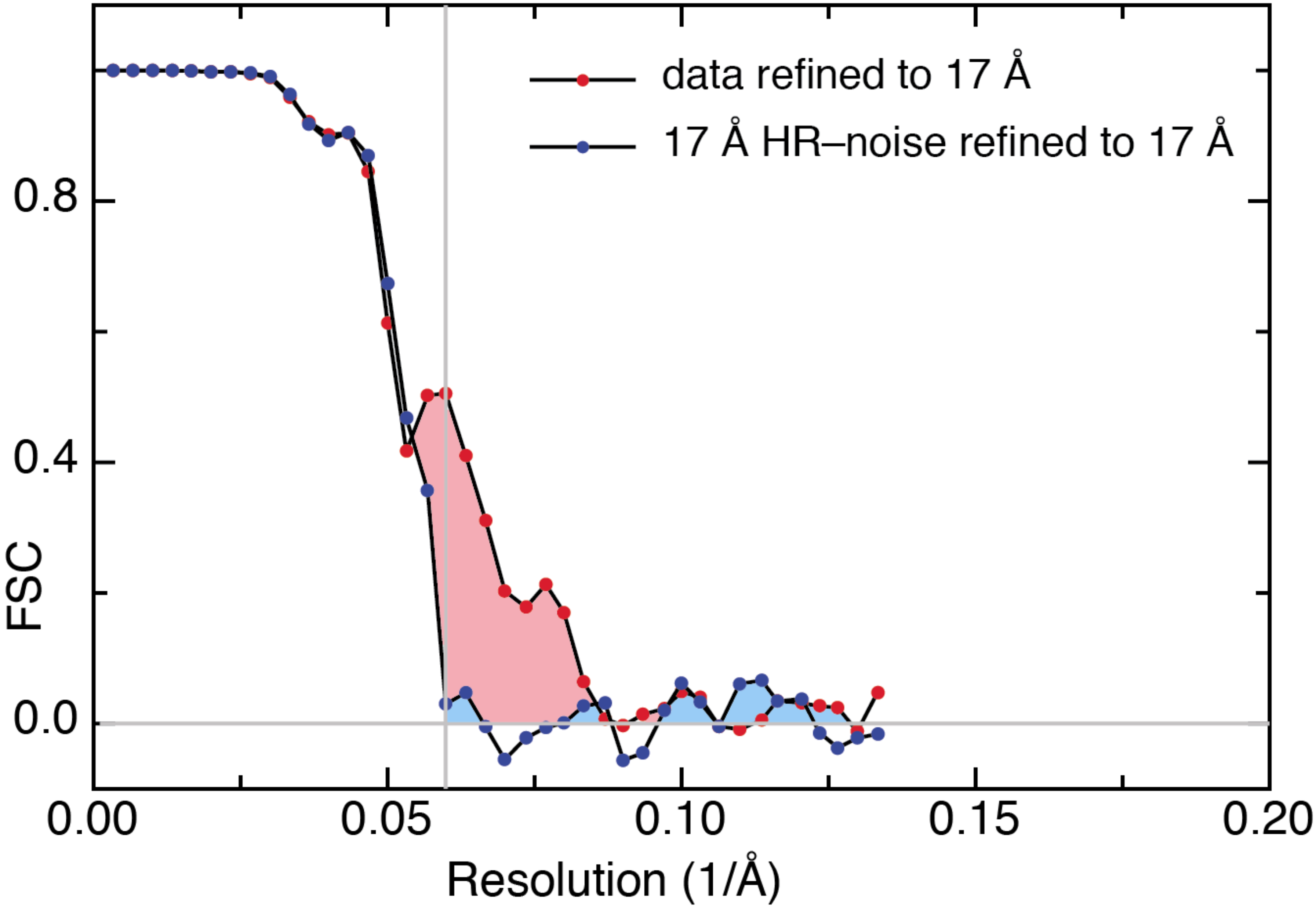
(g) random φ > 1/17Å^{-1}

(h) background A,φ

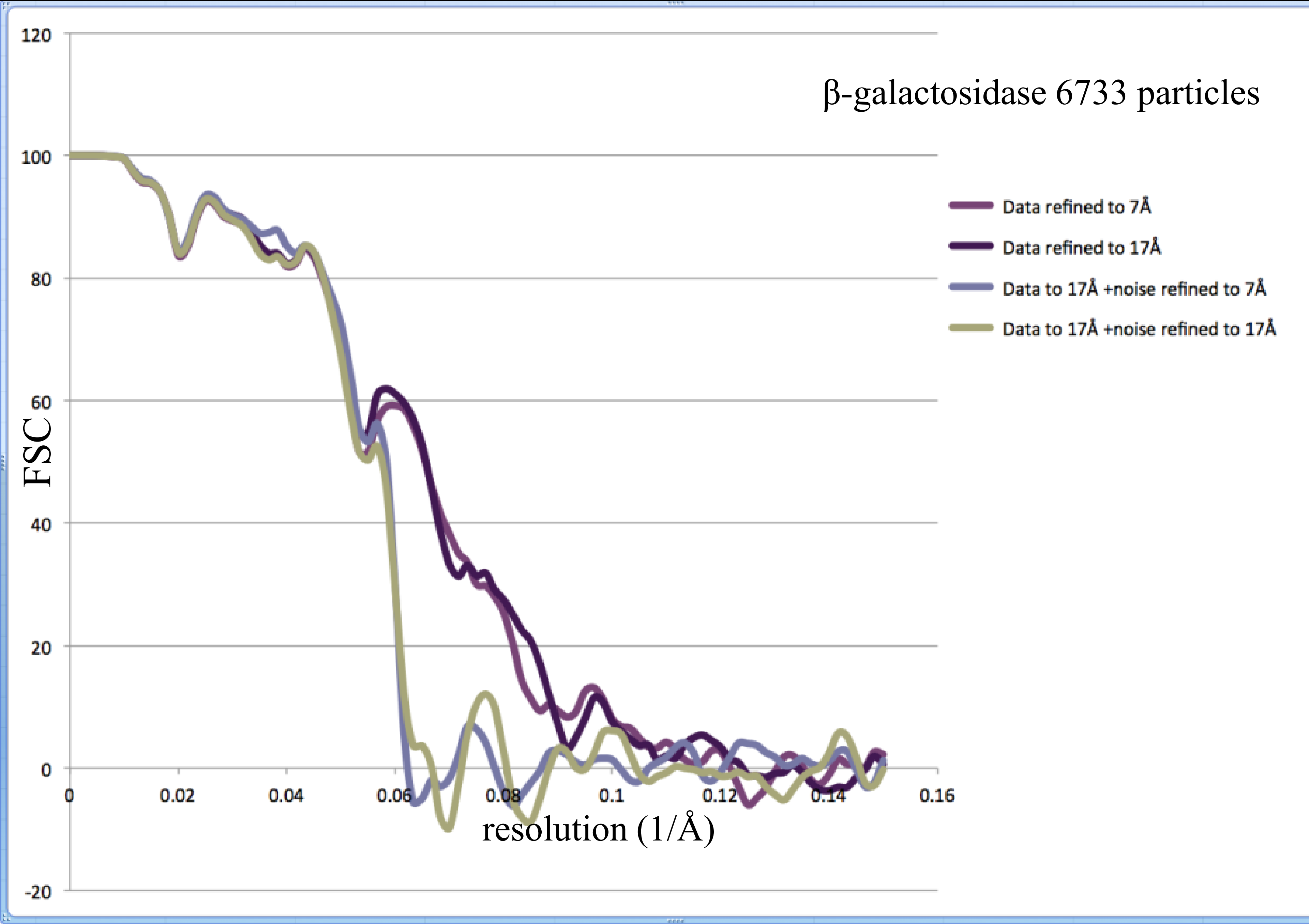
6733 film particles, orientations refined to 7 Å



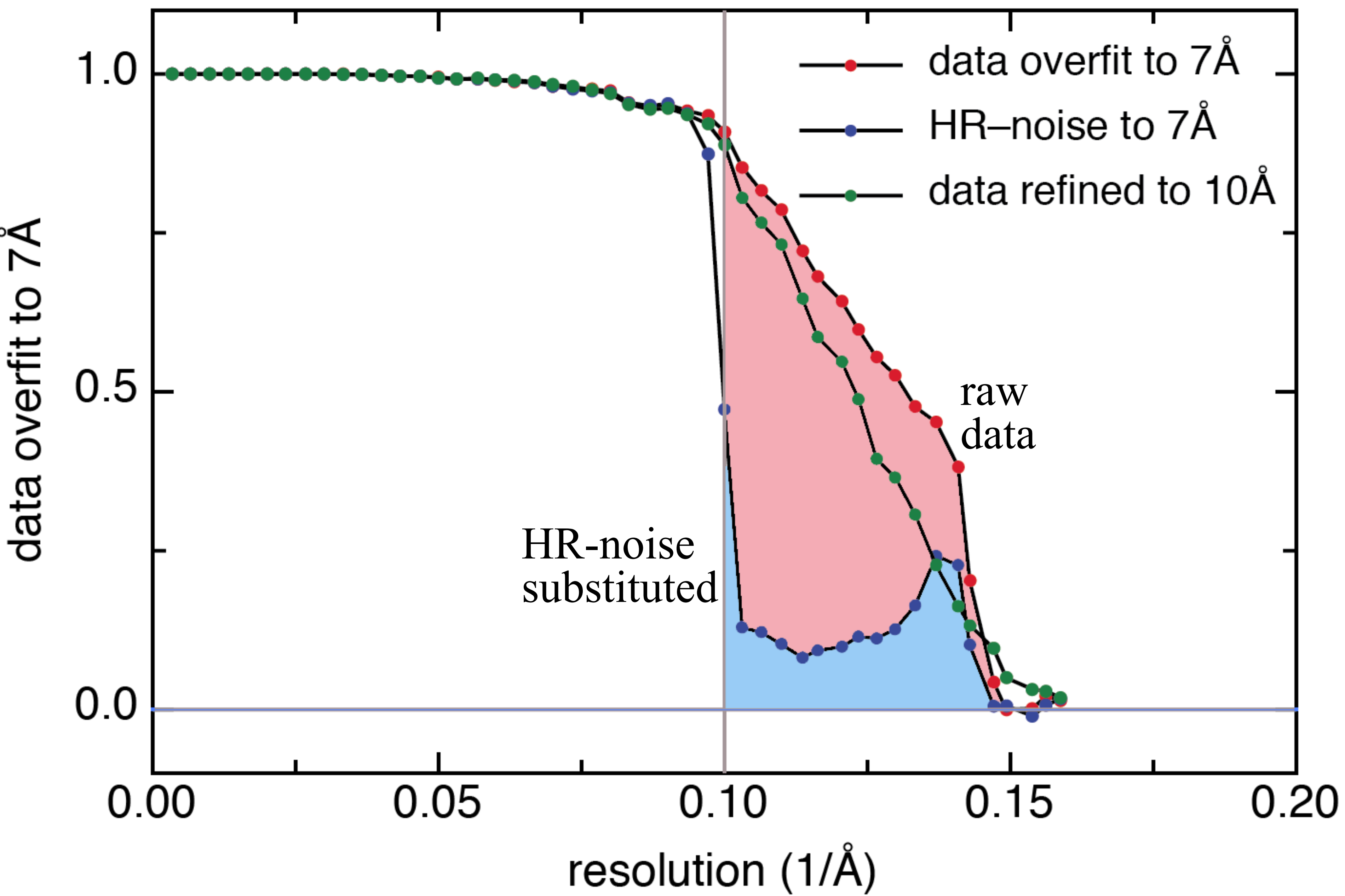
6733 film particles, orientations refined to 17 Å



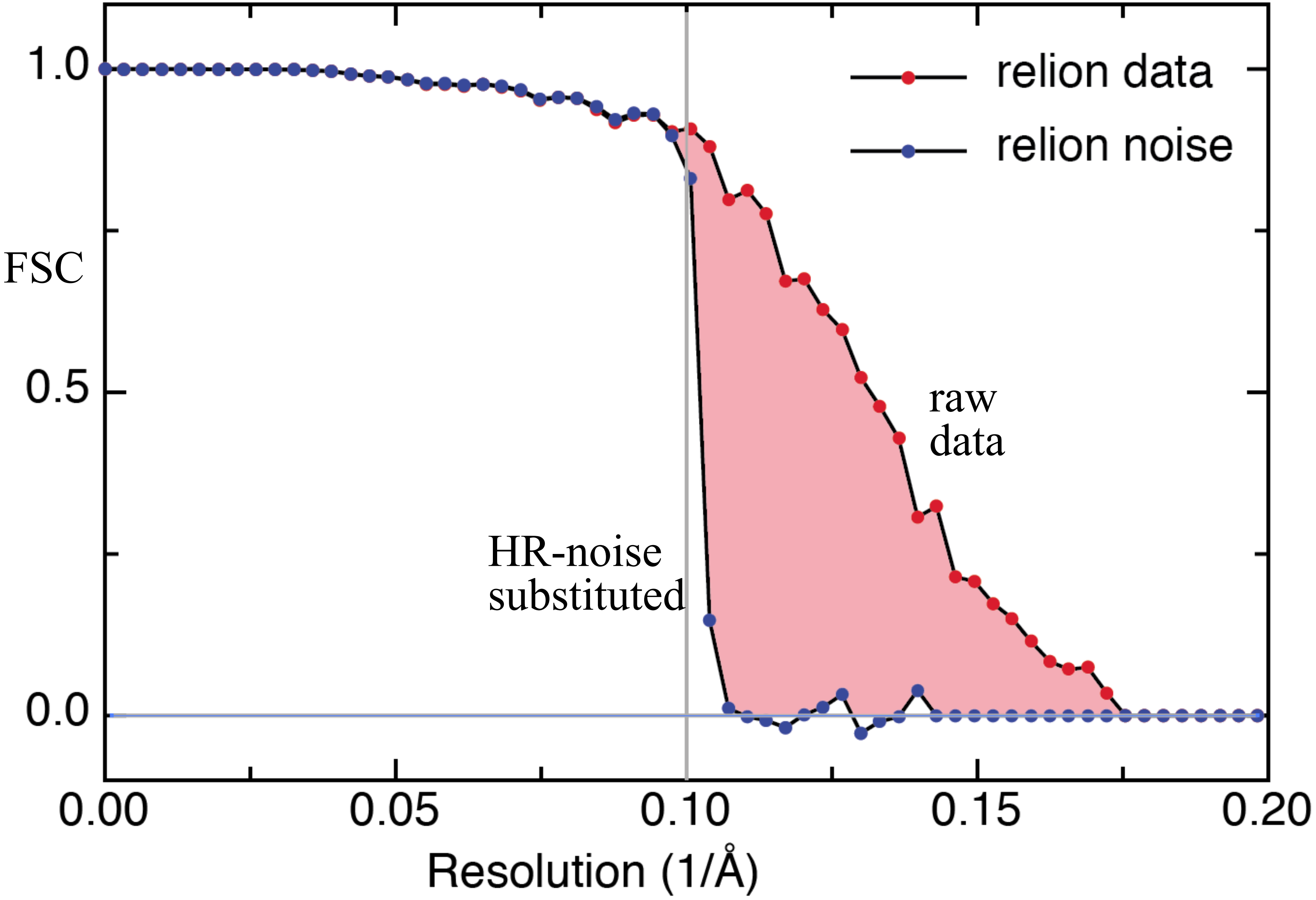
FSC versus map from the X-ray model coordinates



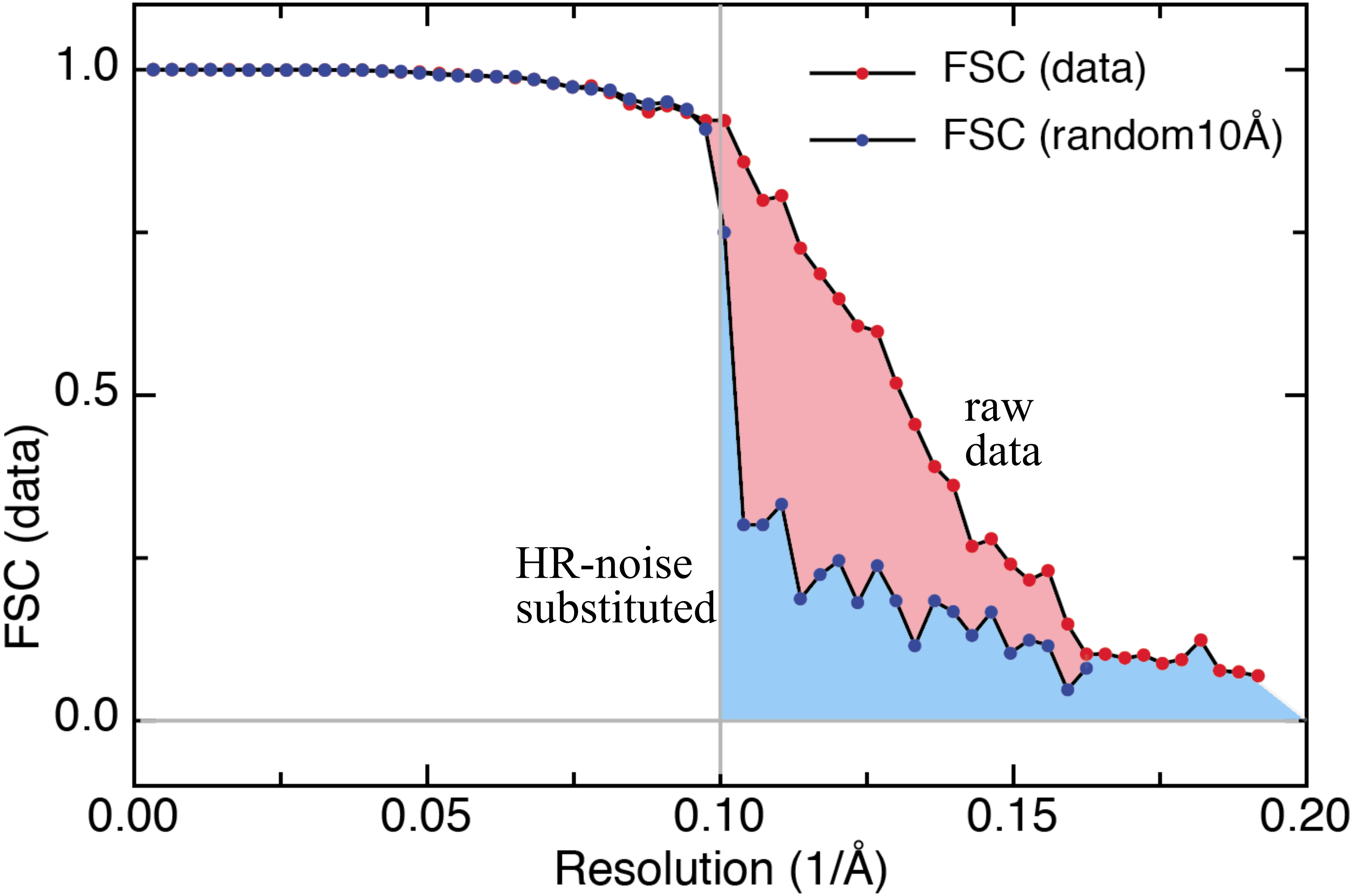
43758 Falcon particles, orientations refined to 7 Å



43758 Falcon particles, orientations refined with Relion weights using “gold-standard” FSC – no overfitting visible.



43758 Falcon particles, orientations refined with xmipp
using FSC low pass filtering



Conclusions from two validation tests

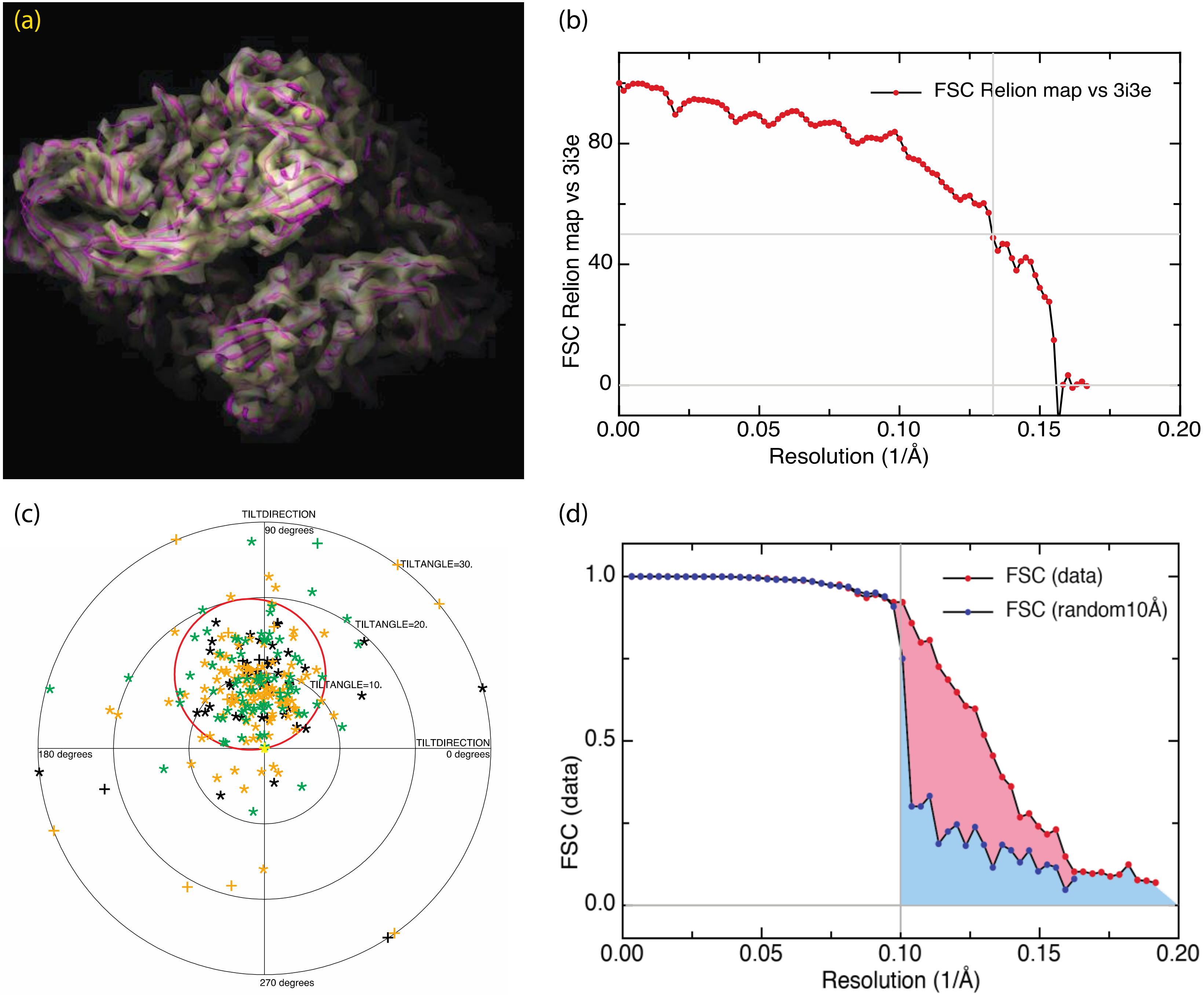
Test 1 : TPPP

- Tilt pair parameter plots can prove the orientation of the individual particles that go into the 3D map are correct. Provided the data that is used to calculate the map is of similar quality, the resulting structure must be right (at a certain resolution).

Test 2 : HR-noise

- The difference between a FSC plot using the full data and that using data with randomised high resolution phases represents real information. Everything else is overfitted noise.

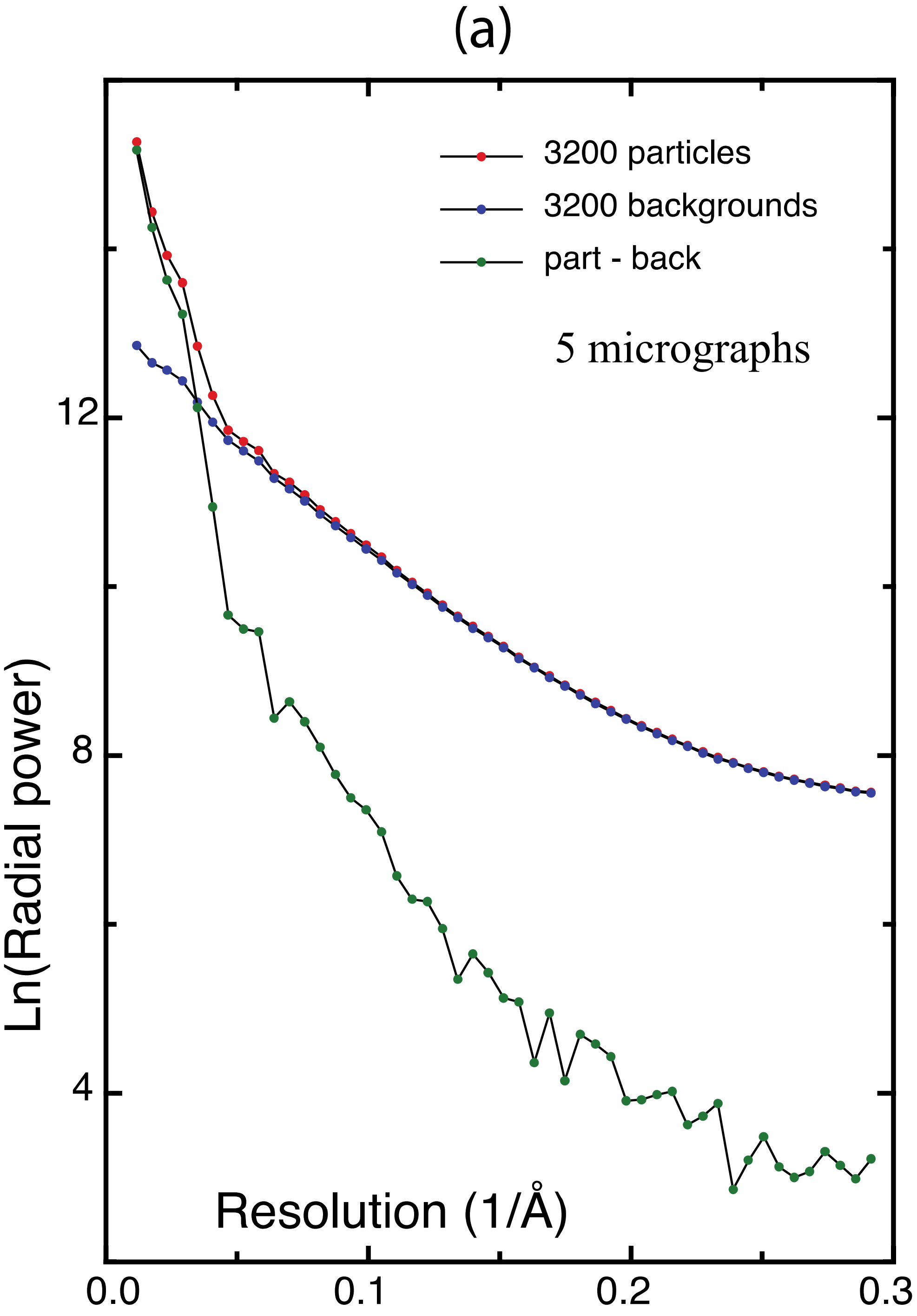
4 validations – map:model superposition, map:model_FSC, TPPP, HR-noise

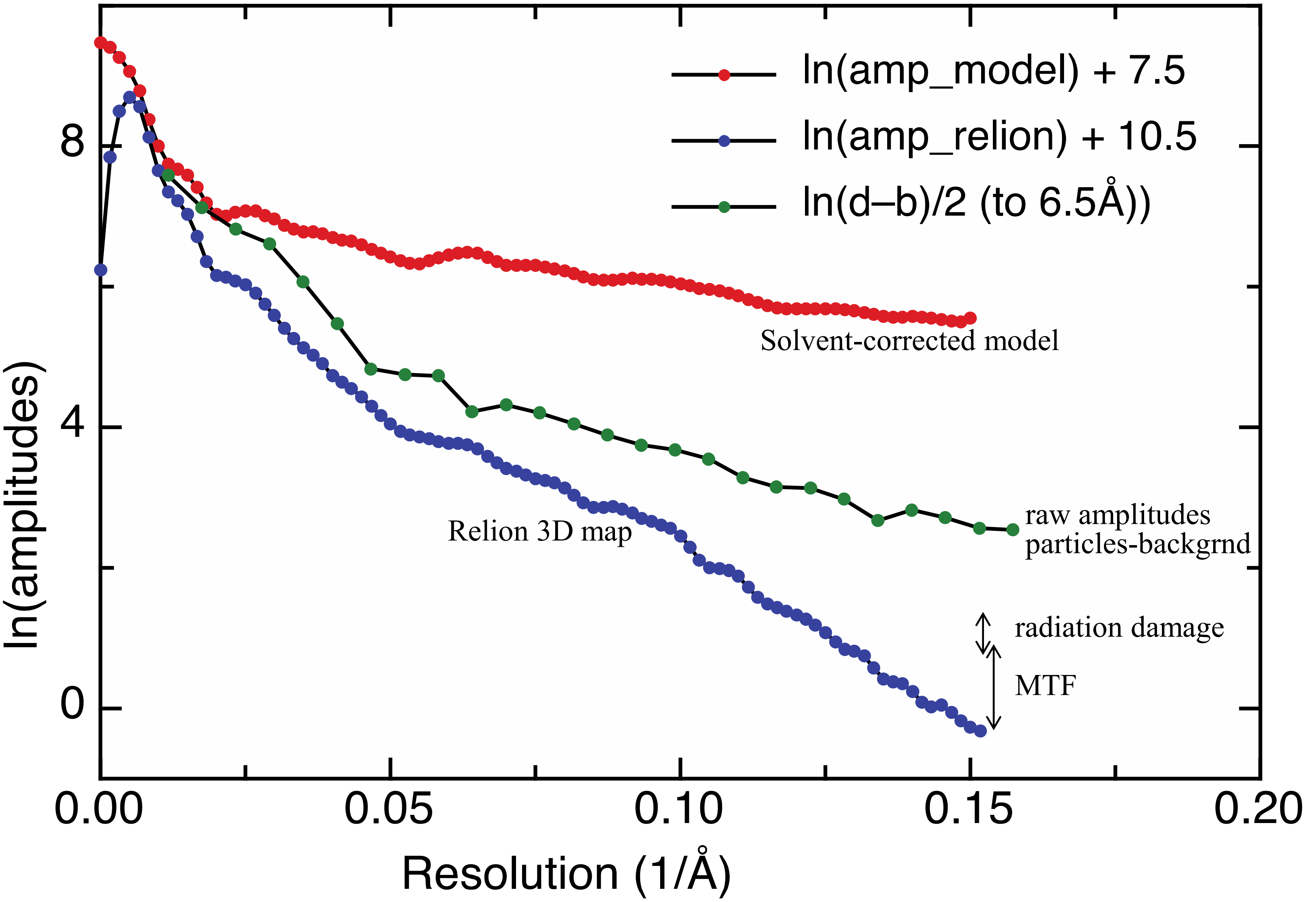


Quantitative SPEM

- are images as good as they should be?
- is 3D map as good as it should be?
- what is missing and why?

- detector DQE
- beam-induced movement
- specimen charging


(a)
3200 particles
3200 backgrounds
part - back
5 micrographs
Ln(Radial power)
12
8
4
Resolution (1/Å)
0.0
0.1
0.2
0.3



Radial amplitudes in X-ray B30 model, EM images and 3D EM map

Thanks to

- Shaoxia Chen
- Greg McMullan
- Sjors Scheres
- Wasi Faruqi

- Prasad Venkatar (Norwalk virus)
- Qinfen Zhang (hemocyanin)

Some useful references

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