Electron Crystallography Workshop Basel, August 2008

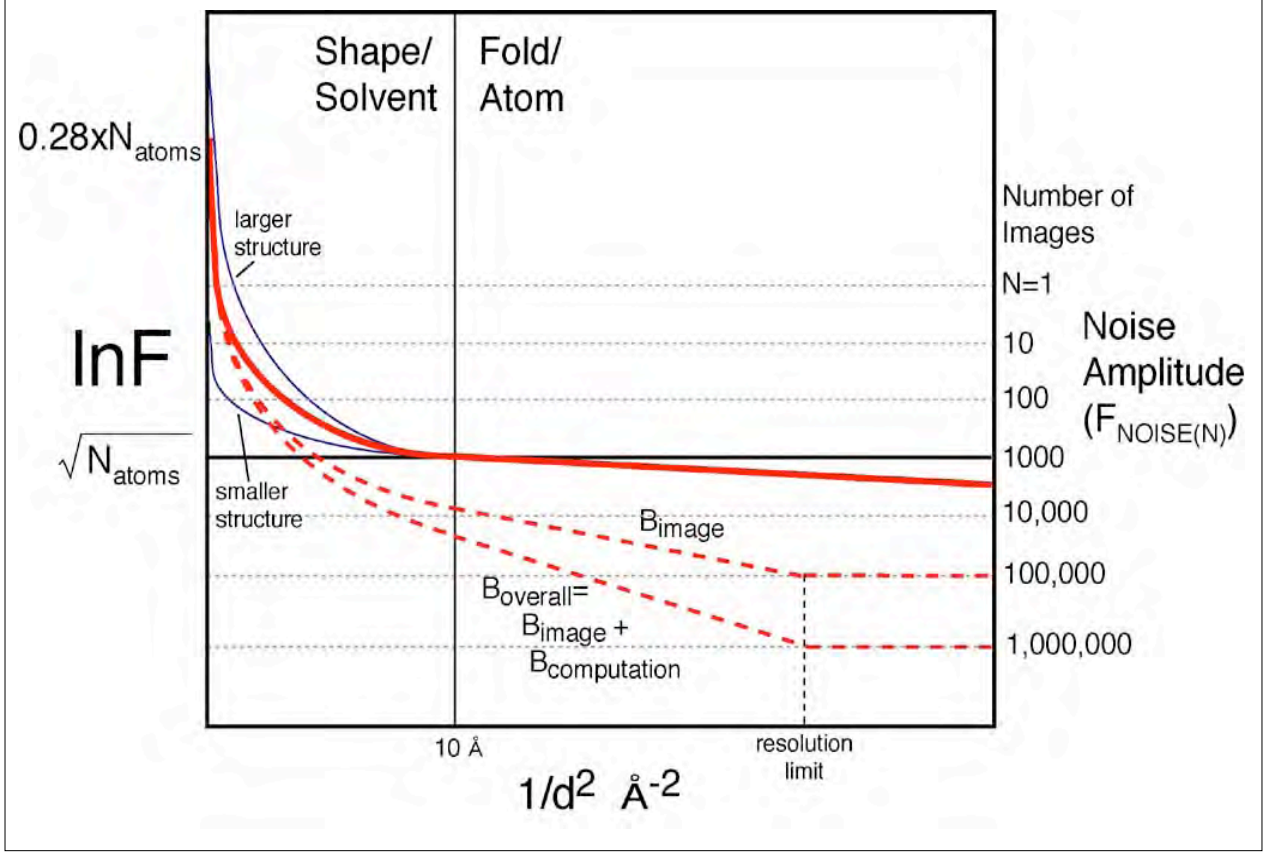
Richard Henderson

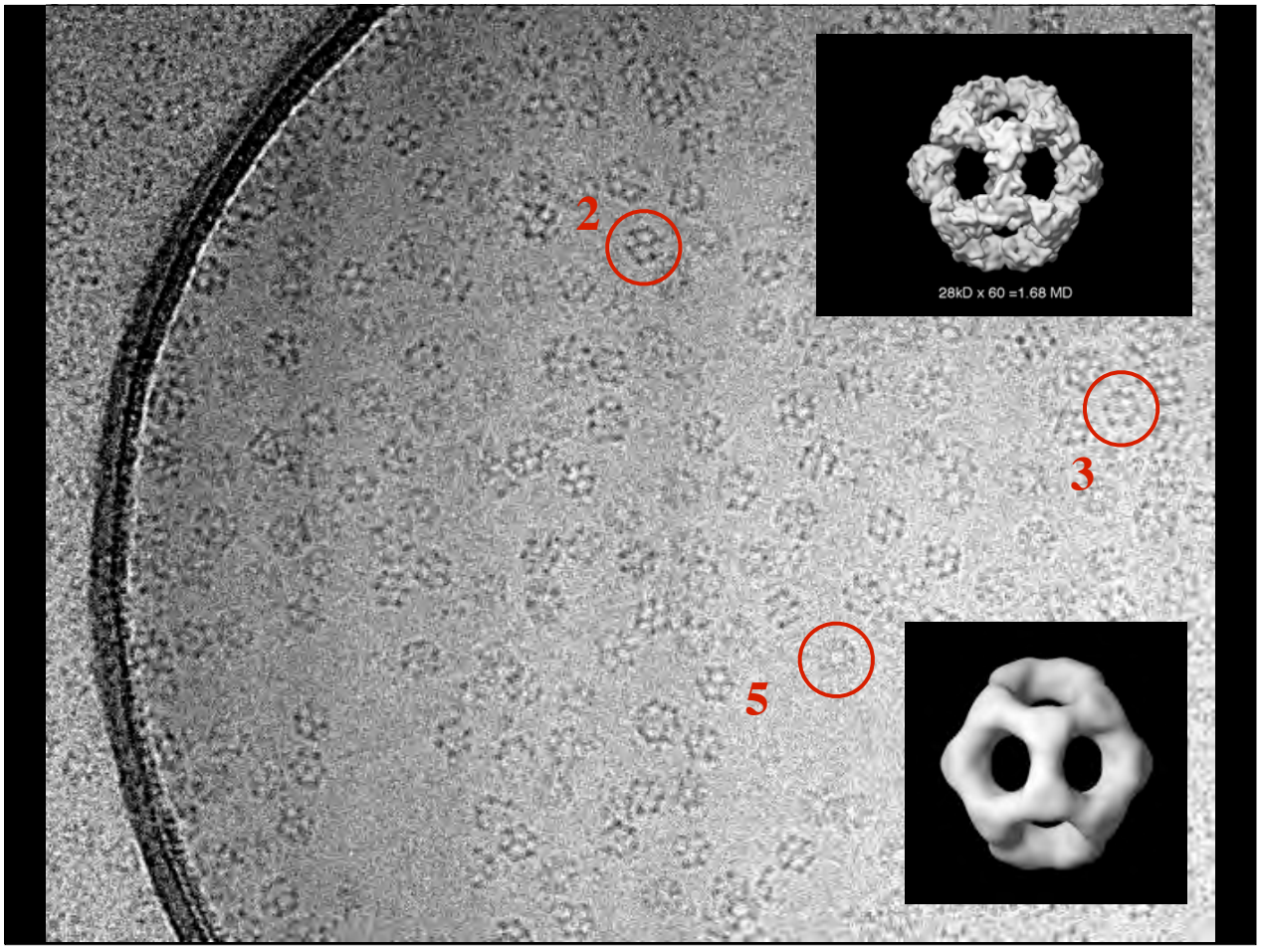
Tilt pair validation, B-factors, charging and movement

Rosenthal & Henderson, (2003) - three main points

- More realistic (less conservative) resolution criterion (FSC = 0.14) derived in Appendix with Tony Crowther
- Sharpening map and f.o.m. weighting EM-Bfactor (Fernandez et al, JSB 2008)
- Tilt pair validation of orientation angle determination not yet very popular
- Also, tomography resolution limit of 20 Å

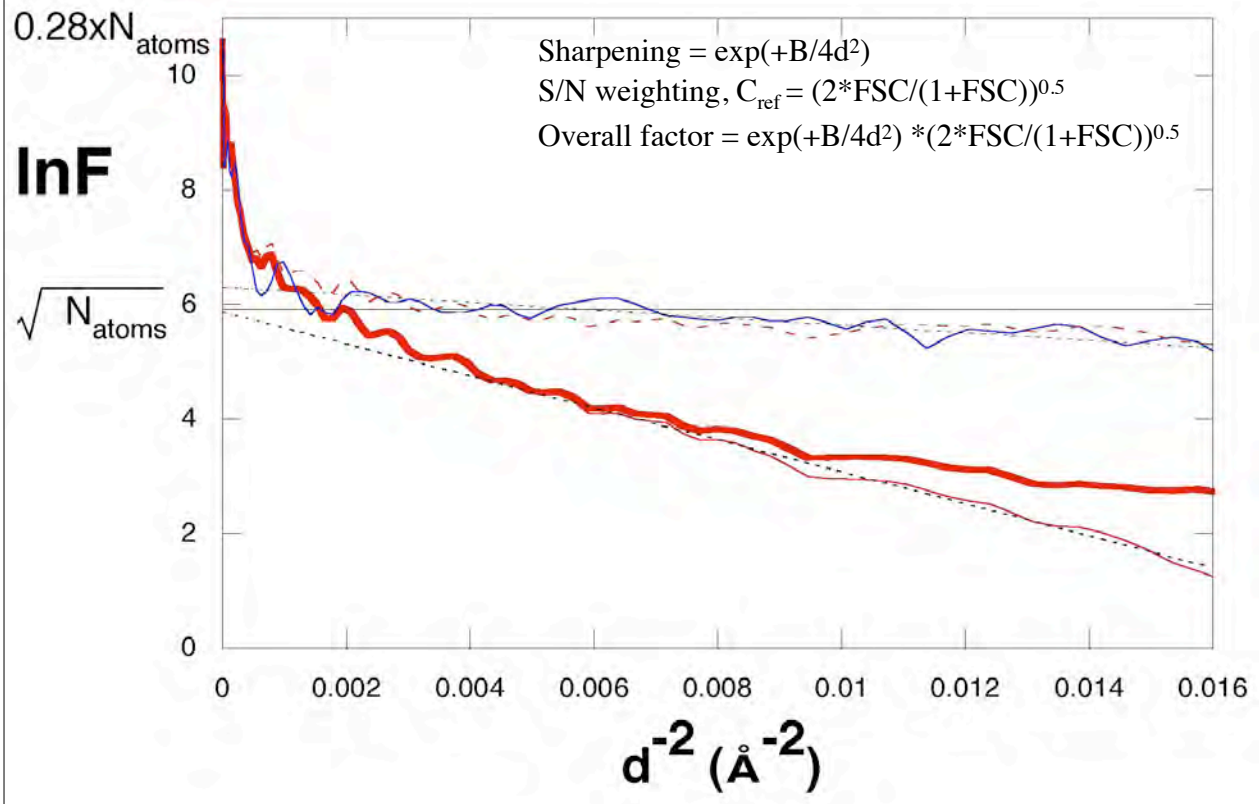
Theory – single particles in ice



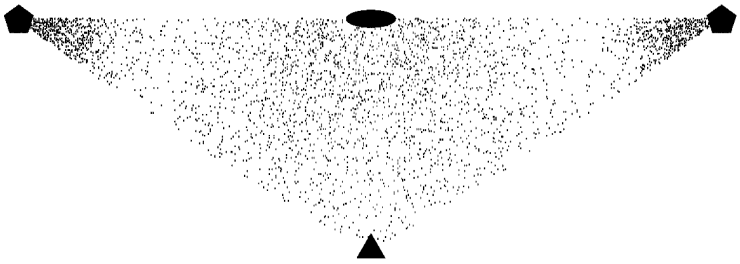


Experimental data

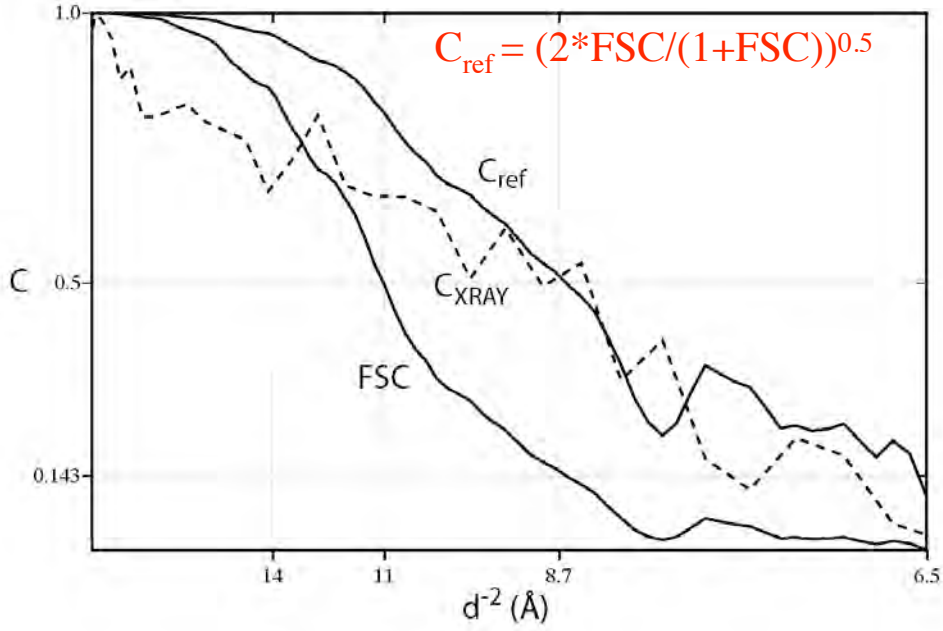
Rosenthal (2003) JMB **333**, 225-36
Fernandez (2008) JSB **164**, 170-5



Particle distribution

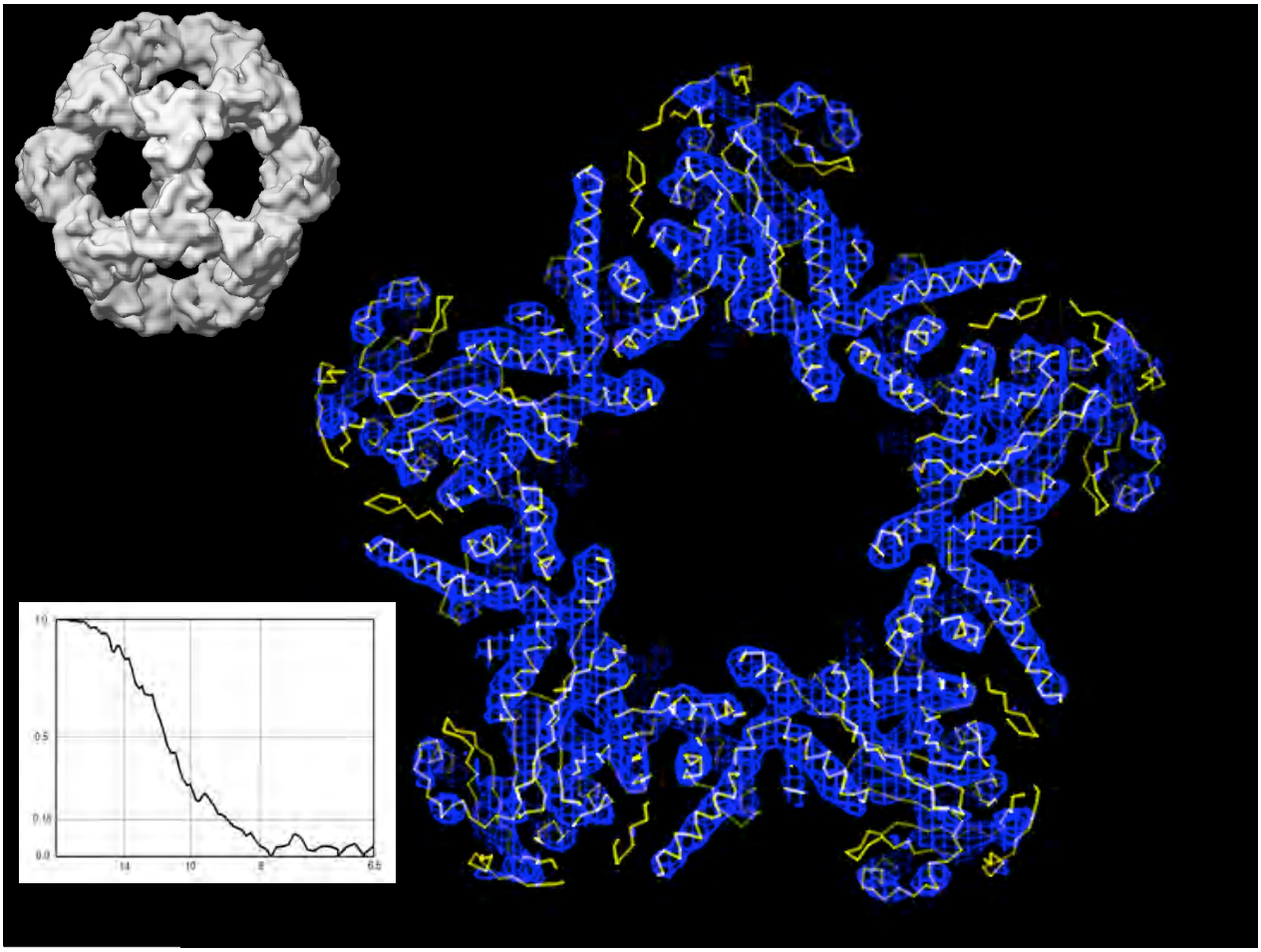


Fourier shell correlations

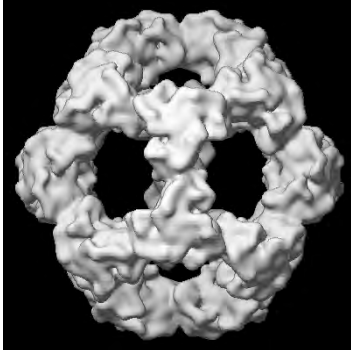


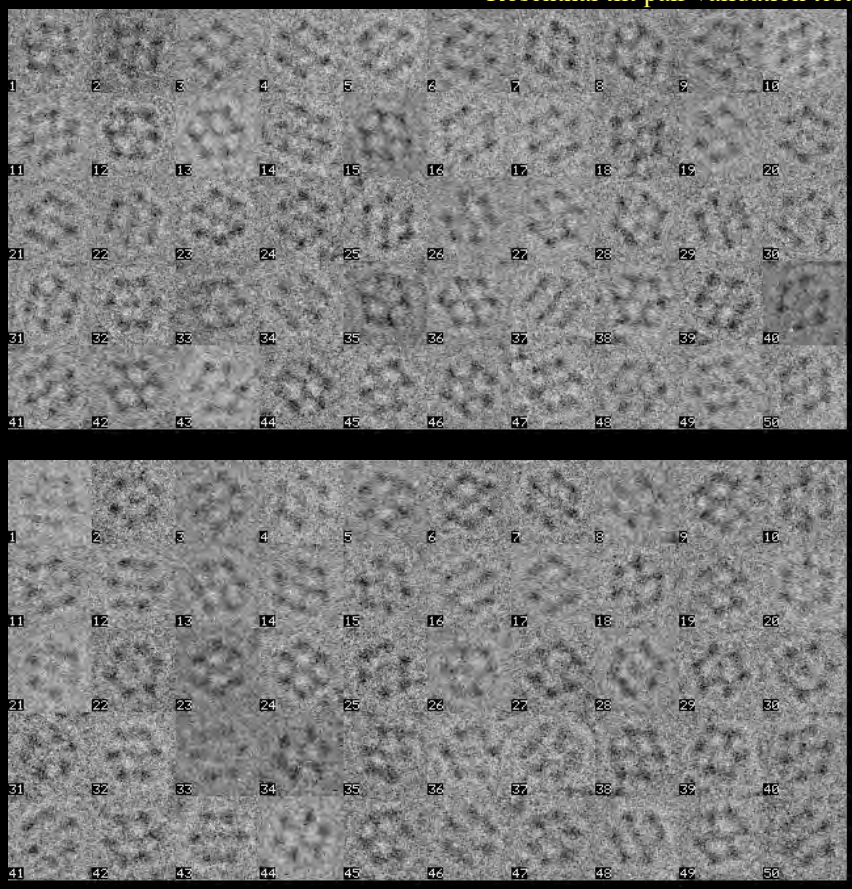
Application of Rosenthal & Henderson tilt pair validation approach
(9/112 citations up to August 2010)

- Pyruvate dehydrogenase : R & H (2003) JMB **333**, 721-42
- *Neurospora* P-type ATPase : Rhee et al (2002) EMBO J. **21**, 3582-89
- Bovine ATPase : Rubinstein et al (2003) EMBO J. **22**, 6182-92
- Chicken anaemia virus : Crowther et al (2003) J.Virol. **77**, 13036-41
- HepB surface antigen : Gilbert et al (2005) PNAS **102**, 14783-88
- Hsp104, yeast AAA+ ATPase : Wendler et al (2007) Cell **31**, 1366-77
- Yeast ATPase : Lau et al (2008) JMB **382**, 1256-64
- V-type ATPase, *T.thermophilus* : Lau & Rubinstein (2010) PNAS **107**, 1367-72
- DNA-dependent PKase : Williams et al (2008) Structure **16**, 468-77



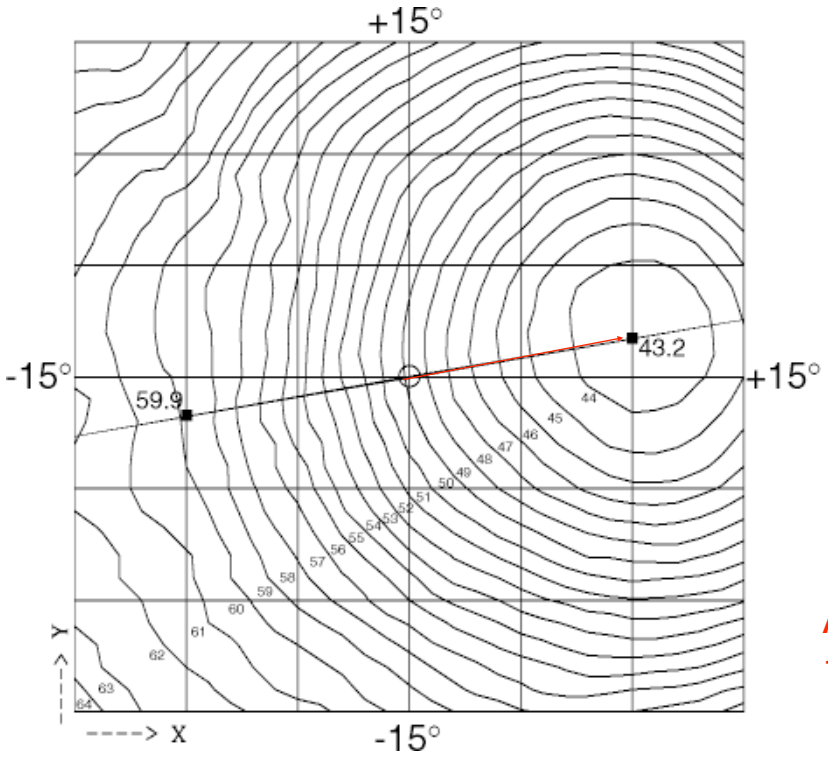
UNTILTED
$(\psi,\theta,\varphi)_u$





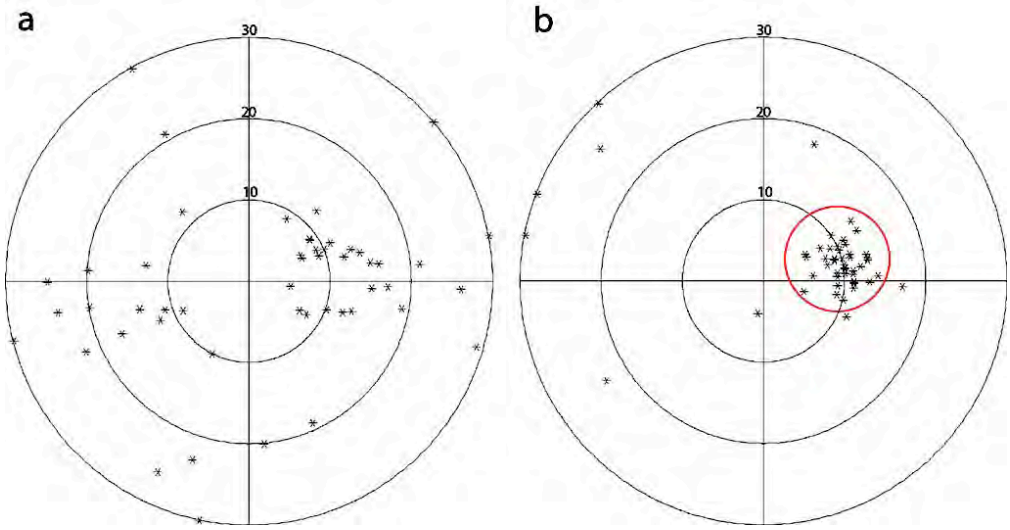
TILTED
10 degrees
$(\psi,\theta,\varphi)_t$

Rosenthal tilt pair validation test



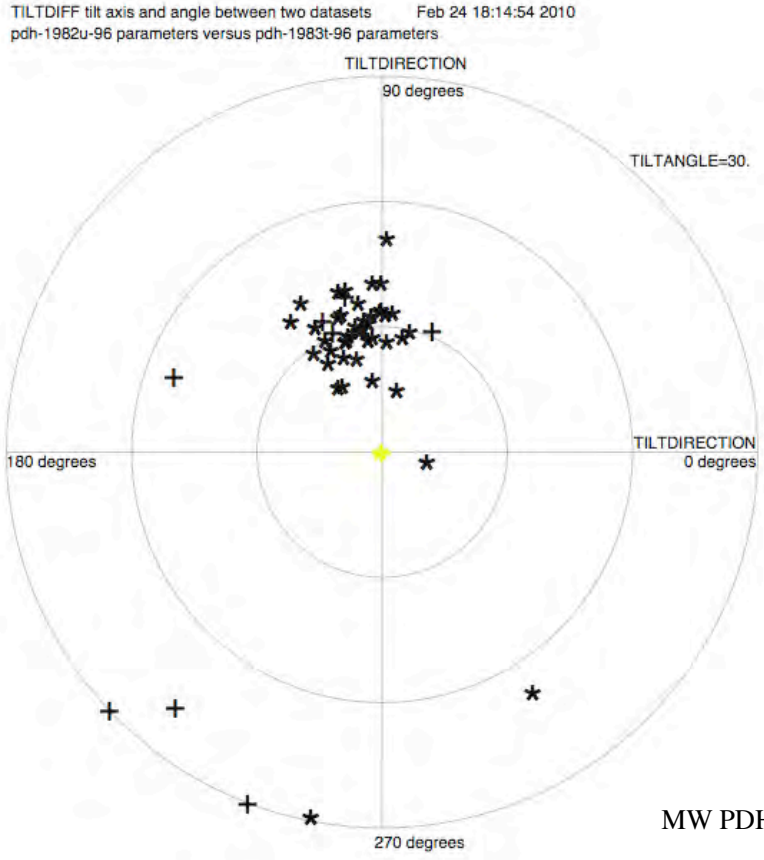
ANGLE
10 deg

Mean phase residual for 50 particle image pairs – ANGPLOT + FREALIGN



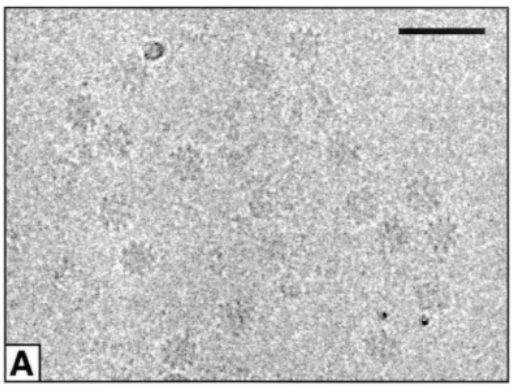
Individual particle image pairs – TILTDIFF output

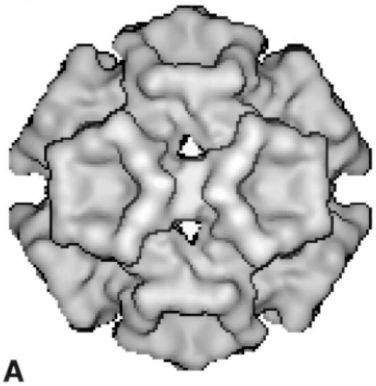
Pyruvate dehydrogenase, E2 catalytic domain, Rosenthal & Henderson JMB, 2003

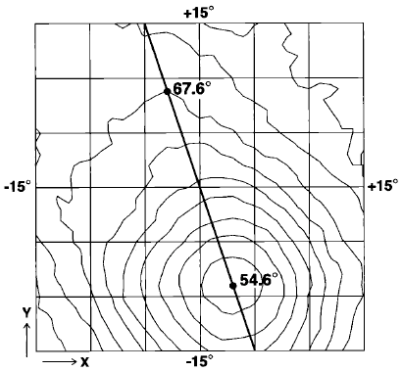


MW PDH_E2CD = 1.6 MDa

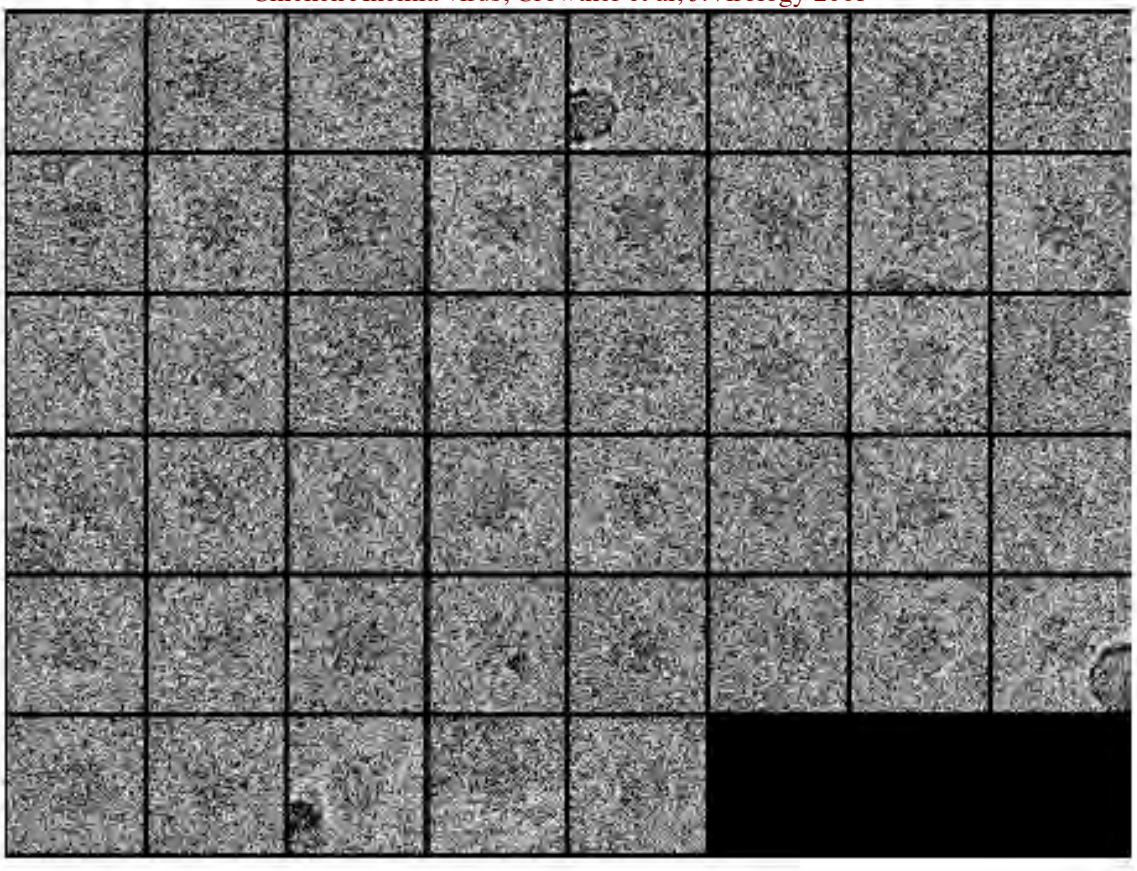
Chicken Anemia virus, Crowther et al, J.Virology 2003



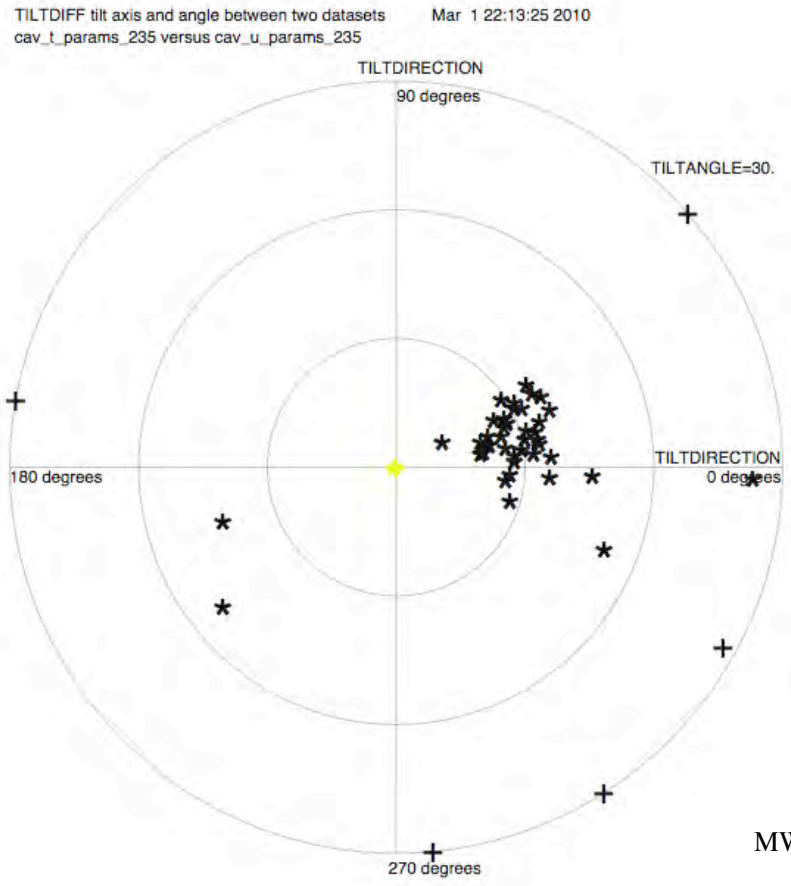




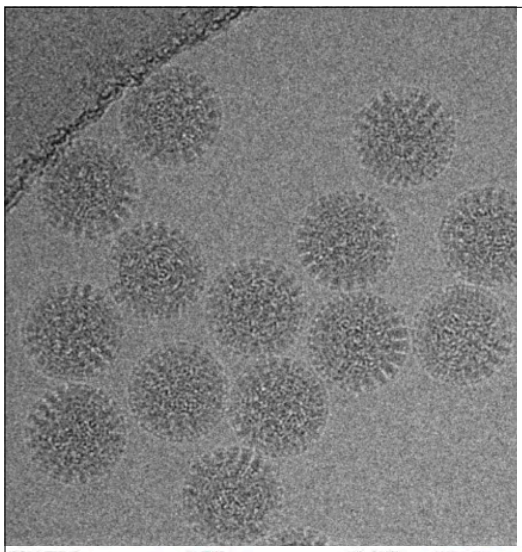
Chicken Anemia virus, Crowther et al, J.Virology 2003



Chicken Anemia virus, Crowther et al, J.Virology 2003



MW CAV = 3.3 MDa



Human Rotavirus DLP

Zhang et al & Grigorieff (2008) PNAS **105**, 1867-72.

3.8 Å, B-factor 450Å^2

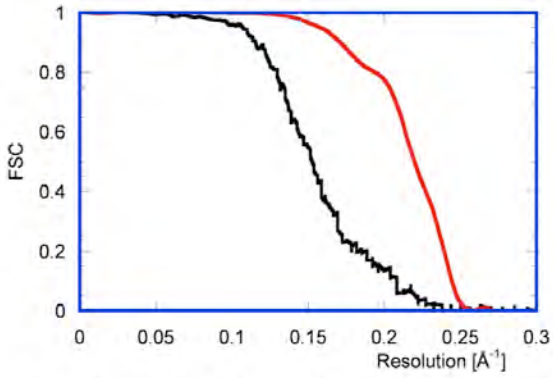
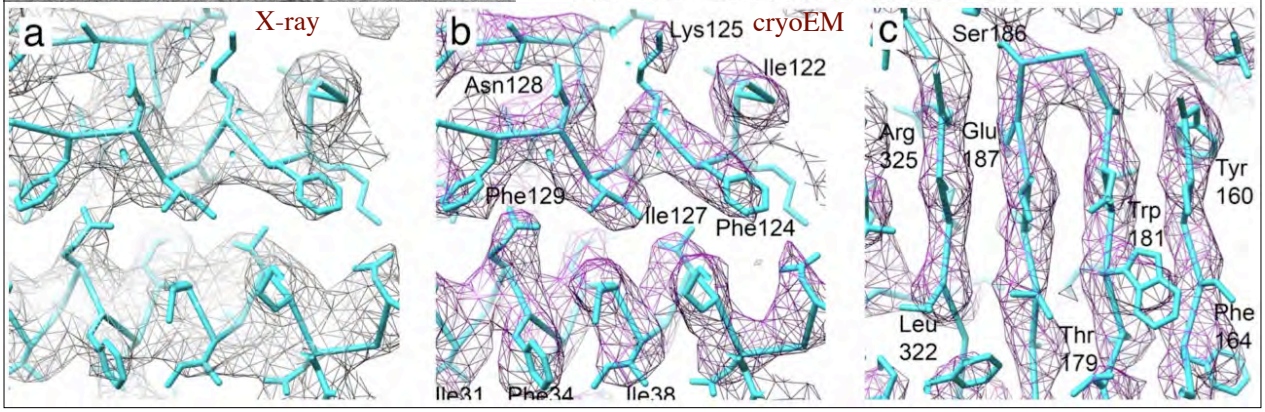
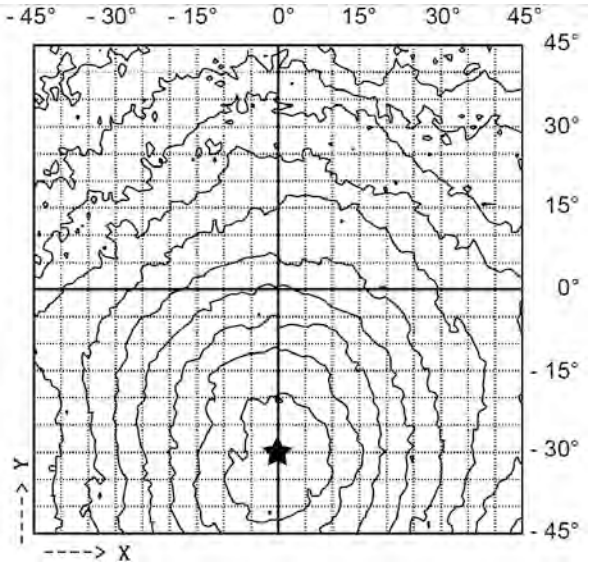


Fig. 4. FSC curves before (black) and after (red) 13-fold nonicosahedral averaging. The black curve suggests a resolution of 5.1 Å (0.143 threshold value), and the red curve indicates a resolution of 4.1 Å.



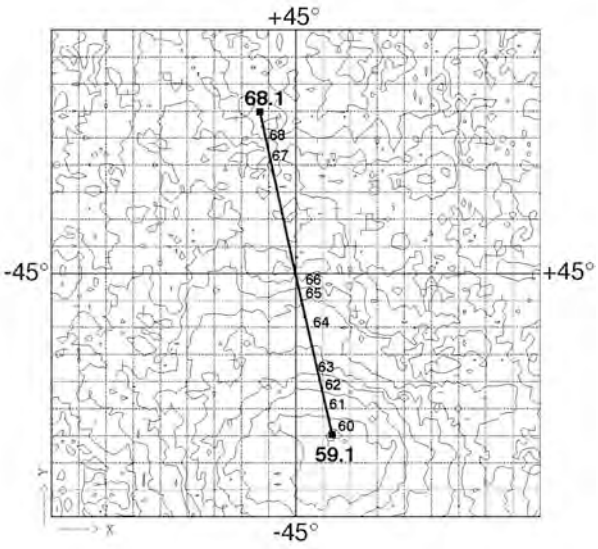
ATP-synthase, John Rubinstein, 2003 - 2010

Phase residual difference = 14.9°
Lau et al, PNAS 2010



MW Thermus V_1V_o = 600kDa

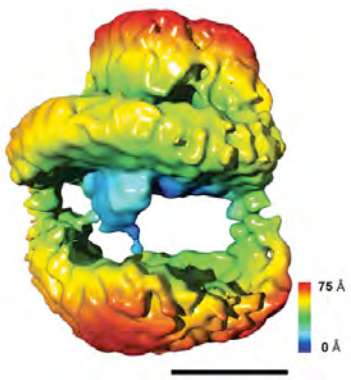
Phase residual difference = 9.0°
Rubinstein et al, EMBO J. 2003



MW bovine F_1F_o = 600kDa

Williams et al & Stewart
Structure (2008) 16, 468-477.

DNA-dependent protein kinase
~500kDa, 300,000 particles
7 Å resolution



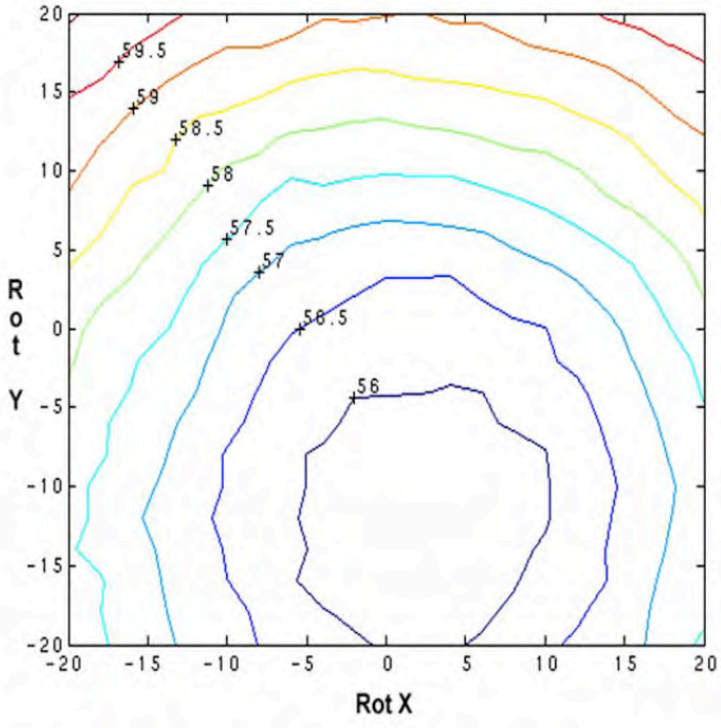


Figure S2. Determination of the Absolute Hand of DNA-PKcs

Peter Rosenthal and Sebastian Wasilewski

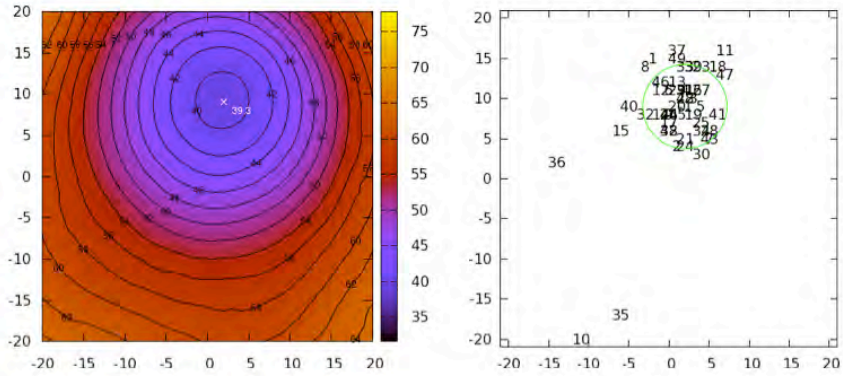
Tilt analysis report

Thu, 22 Jul 2010 02:02:18 +0100

Save as archive

3D model:	/home/swasile/Hand/combine_22av_halfp.map2k.mrc
Untilted stack:	/home/swasile/Hand/e2f301982.partpadred.mrc
Tilted stack:	/home/swasile/Hand/e2f301983.partpadred.mrc
Parameters file:	/home/swasile/Hand/e2_1982u_96.par

Experiment identifier: Sample demo job



Magnification	4.98 A/px
Defocus	58626 ; 59084
Astigmatism	55.7
Voltage	300 kV
Resolution Interval	100.0 - 30.0 A
Tilt Interval	20
Particle radius	20 px
Optimized box size :	128
Effective binning:	1

Average for all particles submited:

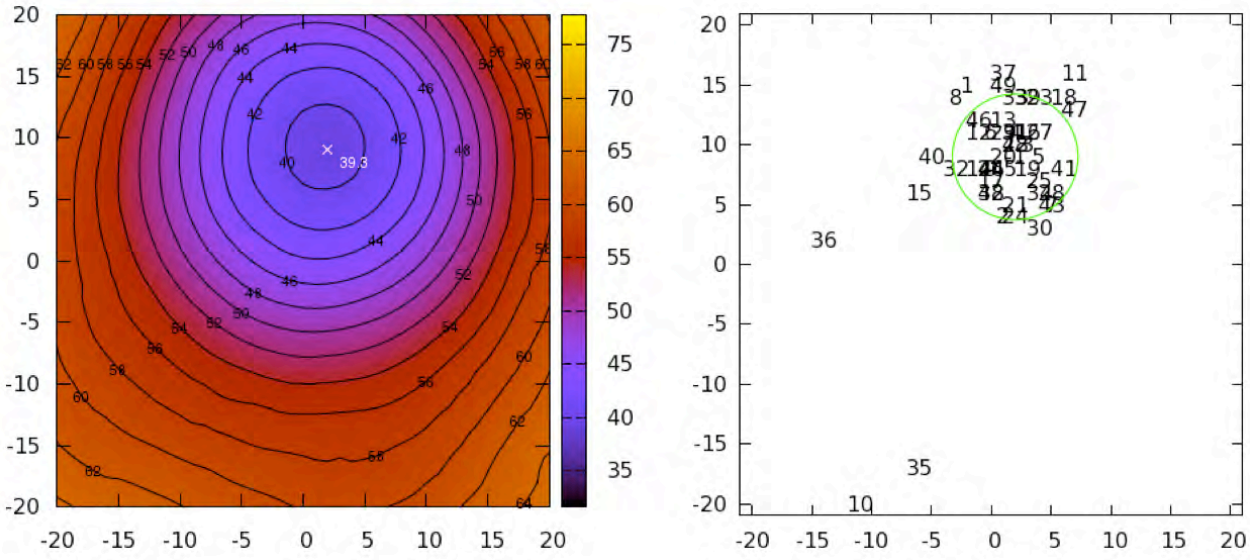
Minimal Phase Residue: **39.26 °**
Minimum at position: **2.0°, 9.0°**
Hand Phase Difference: **14.13 °**
Average distance from the mean minima: **5.25 °**

Particles with the hand difference below the average:
2 7 9 11 12 14 15 17 19 20 21 24 26 30 32 35 36 38 41 45

Particles with minima distant from the determined tilt transfromation:
1 8 10 11 15 18 23 30 35 36 37 40 47 49

Particles contributing to the determined minimum:
0 3 4 5 6 13 16 22 25 27 28 29 31 33 34 39 42 43 44 46 48

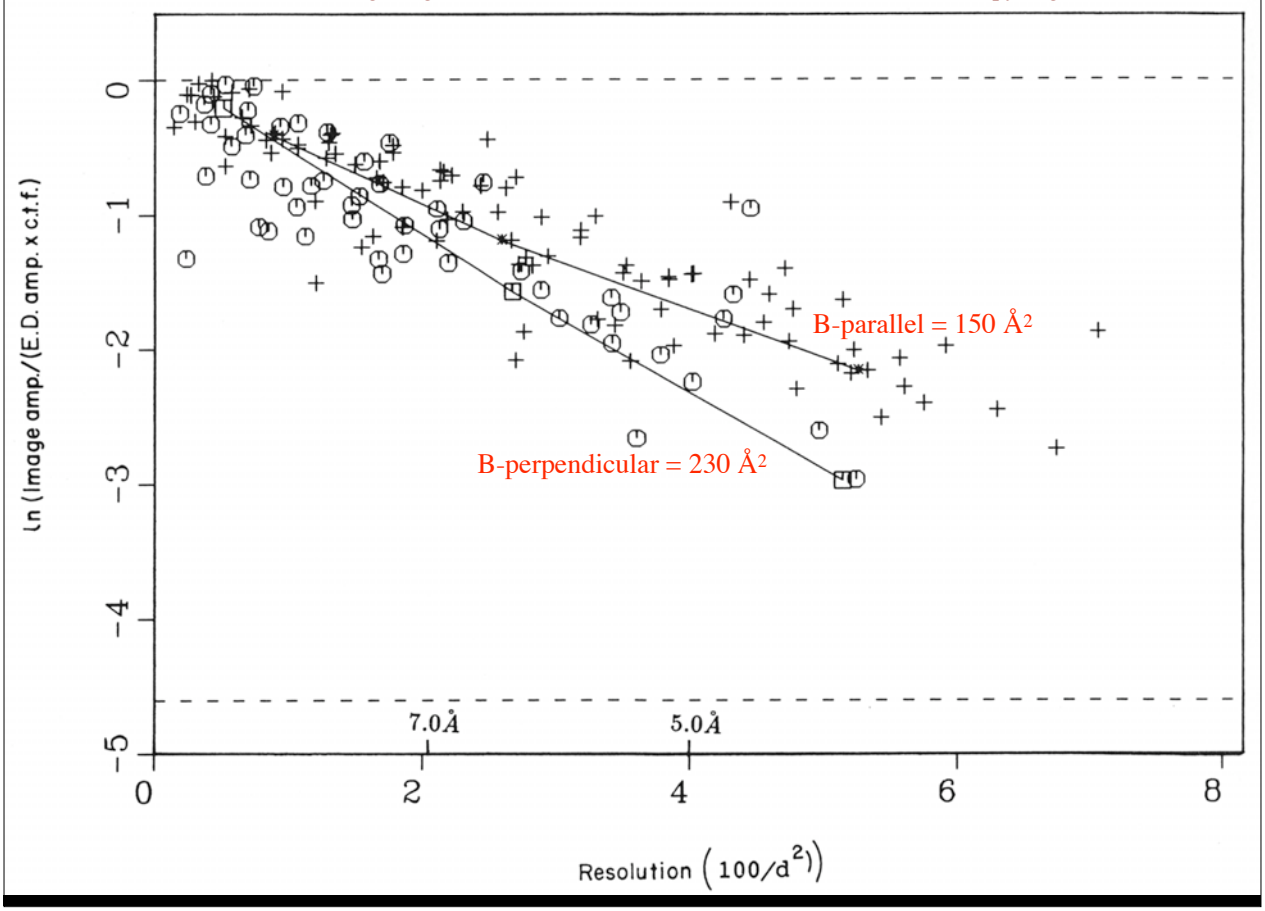
Peter Rosenthal and Sebastian Wasilewski (swasile@nimr.mrc.ac.uk)
http://www.cryomicroscopy.org/software/tilt-analysis-manual/
"Demo results page"



Conclusion - value of tilt pairs

- Works really well for big particles (20MDa); because the orientation determination is so accurate, it provides another piece of information about the magnitude of beam-induced specimen motion for particles in ice
- Works quite well for medium sized particles, but orientation determination has larger error bars (+/- 2-3°)
- For particles less than 1MDa, the success rate for orientation determination becomes less. More work is needed

Ken Downing image of tilted 2D xtal, from Henderson, 1992, Ultramicroscopy, Fig 6



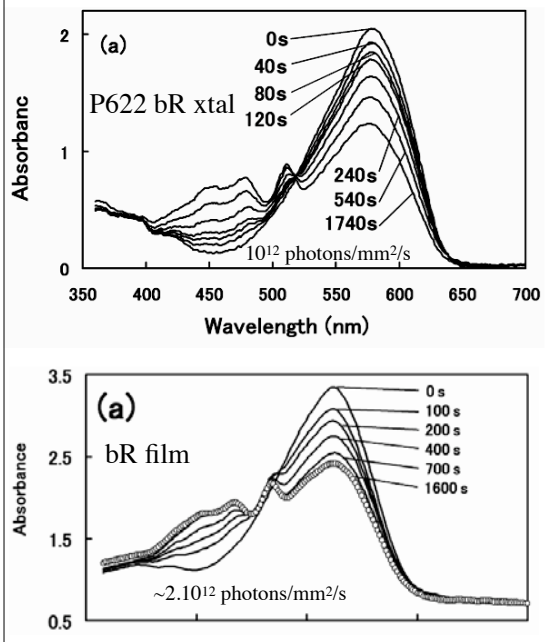
Radiation damage in structural biology

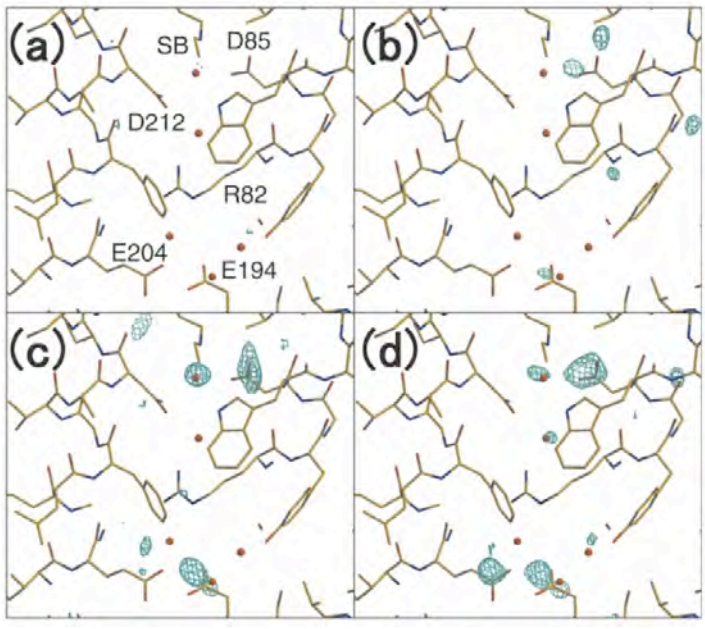
- Three-dimensional crystals (X-ray) contain ~10^{10} molecules
- Two-dimensional crystals (EM) contain ~10^{4} molecules
- Single particles contain 1 or a small number of copies

- Radiation damage unfortunately makes it impossible
  to determine the structure, except at > 2-4 nm resolution,
  without some averaging
- Current challenge is to understand how much averaging is necessary
  in theory and to try to get close to this in practice

Matsui .. & Kouyama (2002) JMB 324, 469-81

Damage induced by X-irradiation of bacteriorhodopsin





Doses = 4, 8, 12, 16*10^{15} photons/mm²

bR in crystals or membranes show similar sensitivity to irradiation
10^{16} photons/mm² => 5 el/Å² = normal cryo-EM exposure - carboxyl groups fall off
4*10^{15} photons/mm² => 2 el/Å² = dose/frame in above X-ray sequence
2*10^{14} photons/mm² => 0.1 el/Å² = safe dose where no damage of any kind is detectable

Unwin & Henderson (1975) JMB

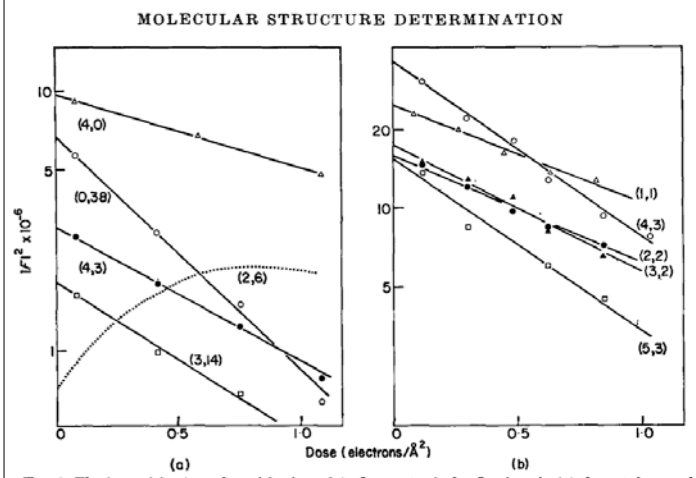
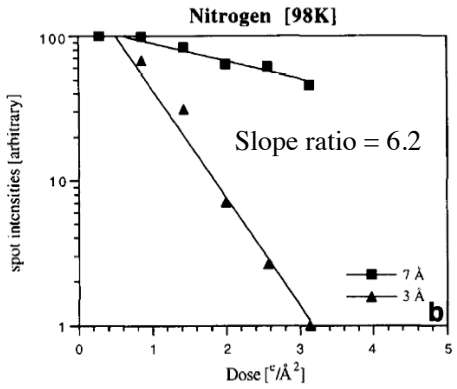
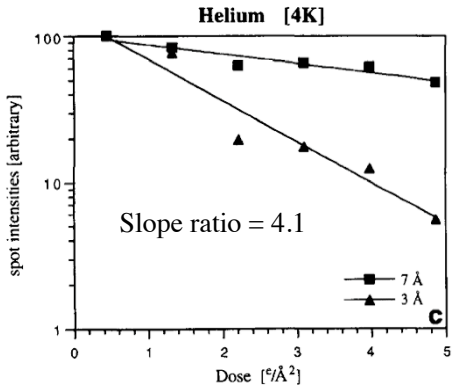


FIG. 1. The intensities (on a logarithmic scale) of some typical reflections in (a) the catalase and (b) the purple membrane electron diffraction pattern, plotted as a function of electron dose.

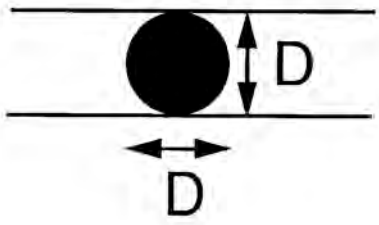
Stark, Zemlin & Boettcher (1996) Ultramicroscopy

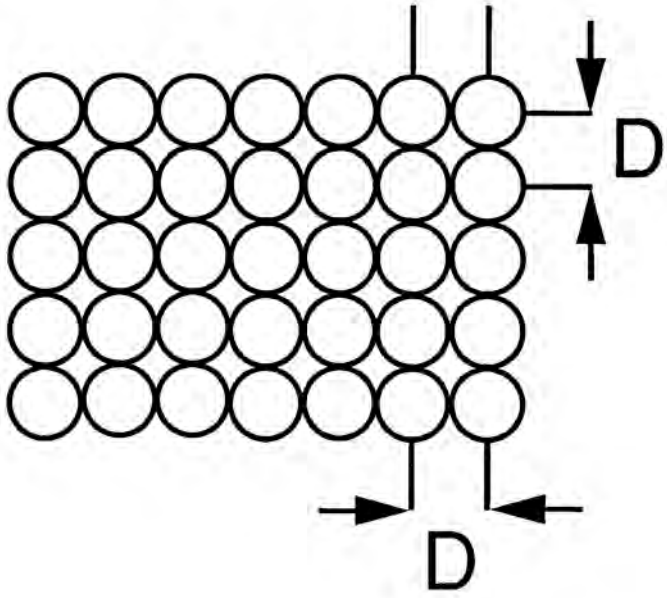




Conclusions

- 3Å data is more radiation sensitive than 7Å data by a factor of 4.1x to 6.2x.
- This translates into a B-factor due to radiation damage of B = 90Å² at 98K, or B = 70Å² at 4K





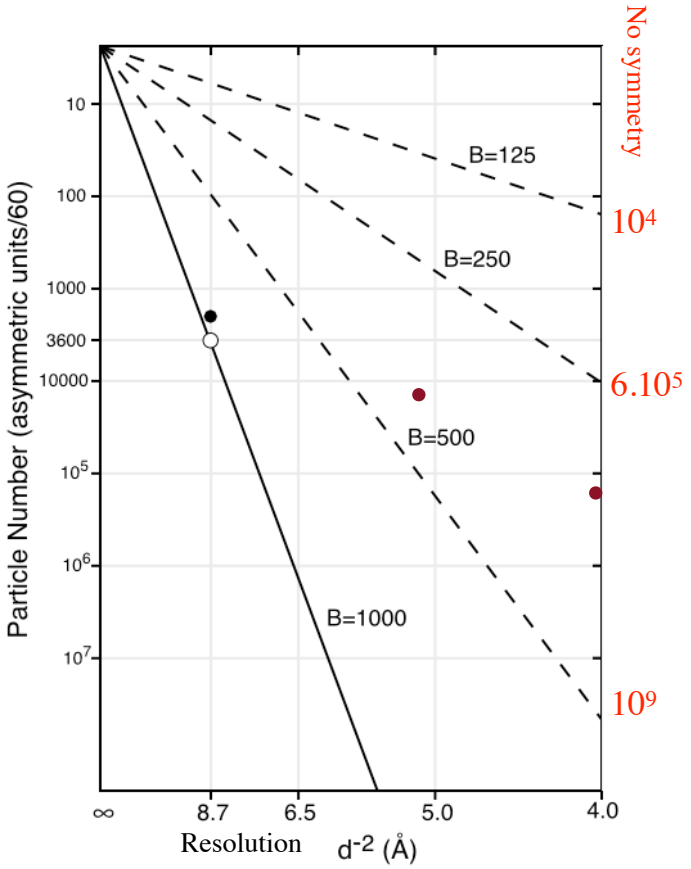
Henderson (1995) QRB **28**, 171-93.

TABLE 2

Type of molecule	Approx. M.W. (Daltons)	D (Å)	N_c, number of carbon atom equivalents	N_s, number of unique diffraction spots to resolution of d = 3Å in projection	f, fraction of electrons elastically scattered out to 3Å resolution	$\langle I_{OBS} \rangle$ / Io	$\langle F_{OBS} \rangle$ / Fo	Phase contrast = total image fractional contrast = signal	Fractional noise level in pixel of dimension $\left(\frac{d}{2}\right)^2$ = 1.5Å x 1.5Å	Can single molecule be detected? How many times > noise	Multiple of sigma expected within unit cell at random	Multiple of sigma expected within entire volume of 5 parameter space at random	Can single molecule alignment be carried out in practice?	Minimum number of images needed for structure with average Fourier component to be >3 σ in projection	Total number of images in 3D x $\left[\frac{\sigma_3}{\sigma_2}\right]$ De Rosier & Klug (1967)
large virus	300M	900	25,000,000	141,371	0.0520	0.184×10^{-6}	0.429×10^{-3}	0.322	0.30	644	5.2	8.5	yes	13	12600
small virus	11M	300	936,000	15,707	0.0173	0.552×10^{-6}	0.743×10^{-3}	0.186	0.30	124	4.8	7.7	yes	40	12600
ribosome	3.3M	200	277,000	6,981	0.0115	0.827×10^{-6}	0.910×10^{-3}	0.152	0.30	68	4.7	7.5	yes	60	12600
	1.4M	150	117,000	3,926	0.0087	1.103×10^{-6}	1.050×10^{-3}	0.132	0.30	44	4.6	7.3	yes	80	12600
multimeric enzyme	420K	100	35,000	1,745	0.0058	1.654×10^{-6}	1.286×10^{-3}	0.107	0.30	24	4.4	7.1	possibly	120	12600
	180K	75	14,600	981	0.0043	2.206×10^{-6}	1.485×10^{-3}	0.093	0.30	16	4.2	6.8	possibly	160	12600
	52K	50	4,330	436	0.0029	3.309×10^{-6}	1.819×10^{-3}	0.076	0.30	8.4	4.1	6.7	possibly	240	12600
small protein	18K	35	1,500	213	0.0020	4.727×10^{-6}	2.174×10^{-3}	0.064	0.30	4.9	3.9	6.3	no	345	12600
very small protein	7K	25	540	109	0.00144	6.618×10^{-6}	2.572×10^{-3}	0.054	0.30	3.0	3.5	5.9	no	480	12600
equation	(1)	-	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(13)	(16)	(17)	(10)	(11)
relation to D	$0.418 \times D^3$	D	$0.0346 \times D^3$	$0.01745 \times D^2$	$5.7 \times 10^{-5} \times D$	$1.654 \times 10^{-4} \times D^{-1}$	$0.0128 \times D^{-\frac{1}{2}}$	$0.0107 \times D^{\frac{1}{2}}$	-	$0.02388 \times D^{\frac{1}{2}}$				$12087 \times D^{-1}$	-
dependence on resolution d	-	-	-	$\alpha\ 1/d^2$	-	-	-	$\alpha\ 1/d$	$\alpha\ 1/d$	$\alpha\ 1/d$				-	$38,000/d$

Parameters in electron microscopy of single protein molecules or molecular assemblies. To simplify the presentation, it is assumed that the molecules are arranged in a closely-packed 2-dimensional crystal with a square unit cell as shown in Fig. 3. The formulae used to derive Table 2 are given in the Appendix.

Number of particles needed to reach given resolution as a function of B-factor



Conclusion

Contributions of different factors to contrast loss

- Radiation damage degrades structure factors $\Delta B = 80$
- Detectors (e.g. film) poor high resolution MTF (and DQE) $\Delta B = 60$
- Charging and mechanical movement $\Delta B = 60$ to 500
- Intrinsic molecular flexibility $\Delta B = 30$ to 500

Technical challenge is to reduce contribution of everything except radiation damage to near zero