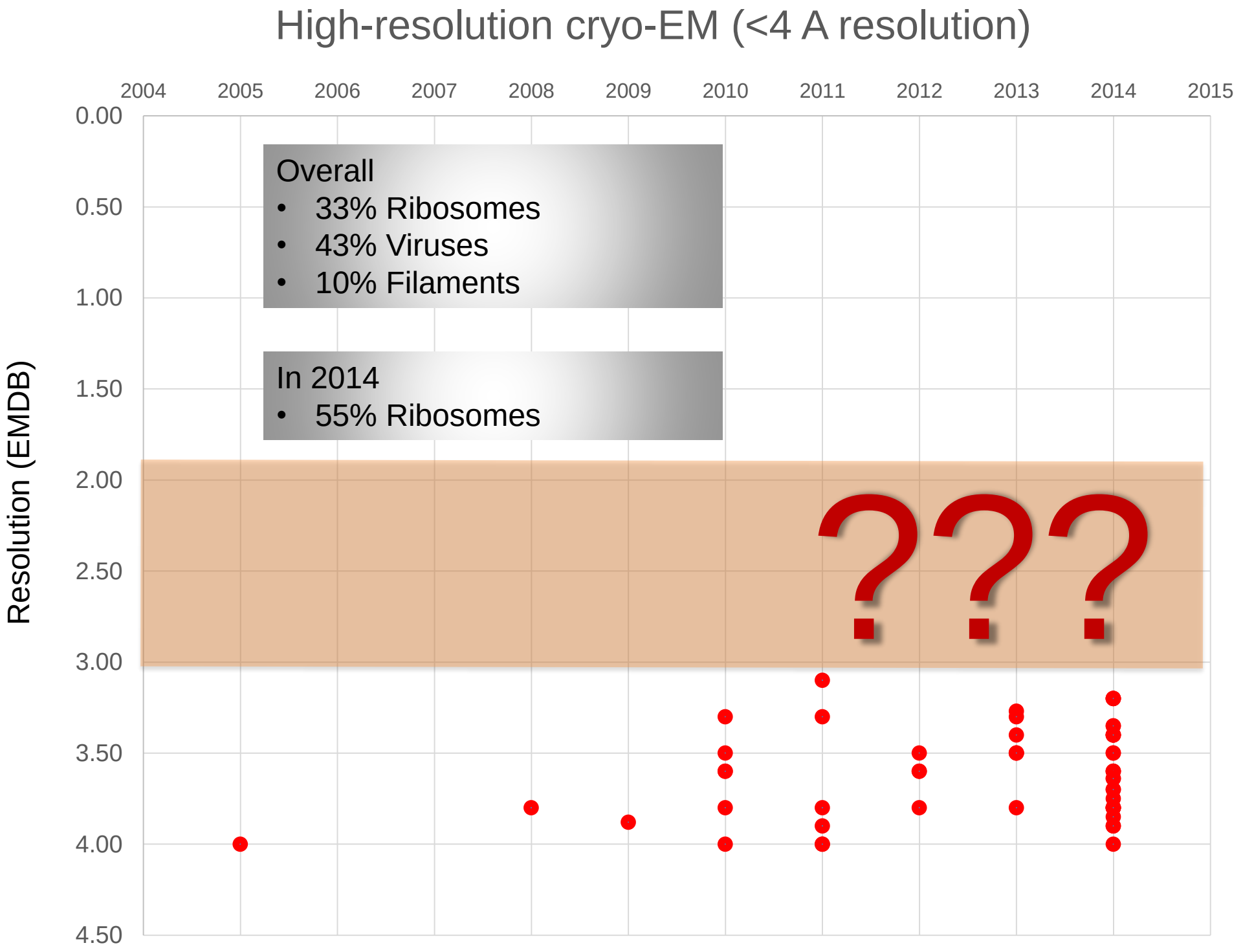
Higher Resolution Limitations ?

Even higher resolution?
Improving alignment and correcting optical aberrations may well be the future of reducing resolution limiting factors?

- Sample Quality
- Detectors
- Beam Damage
- EM Hardware/Alignments

Max-Planck-Institute for
Biophysical Chemistry


High-resolution cryo-EM (<4 A resolution)
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
Resolution (EMDB)
Overall
• 33% Ribosomes
• 43% Viruses
• 10% Filaments
In 2014
• 55% Ribosomes
???

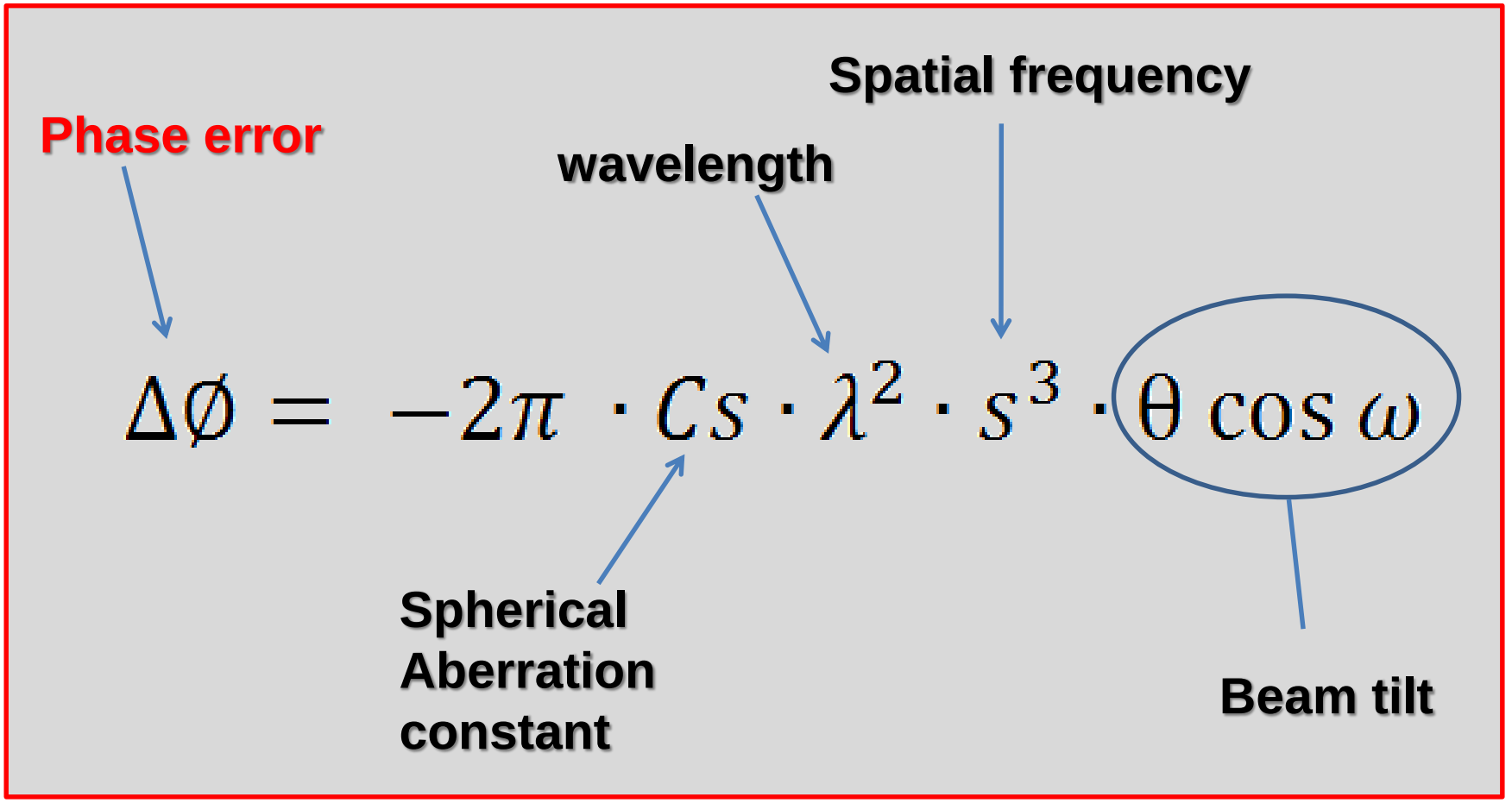
What are the major electron optical aberrations and distortions which may still be limiting ?

- Beam tilt induced Coma (Zemlin et al., Ultramicroscopy, 1978)
- Linear Distortion

Can both be optimized with a spherical aberration C_s corrector

For Coma see also: Glaeser, JSB, 2011 / Zhang and Zhou, JSB, 2011

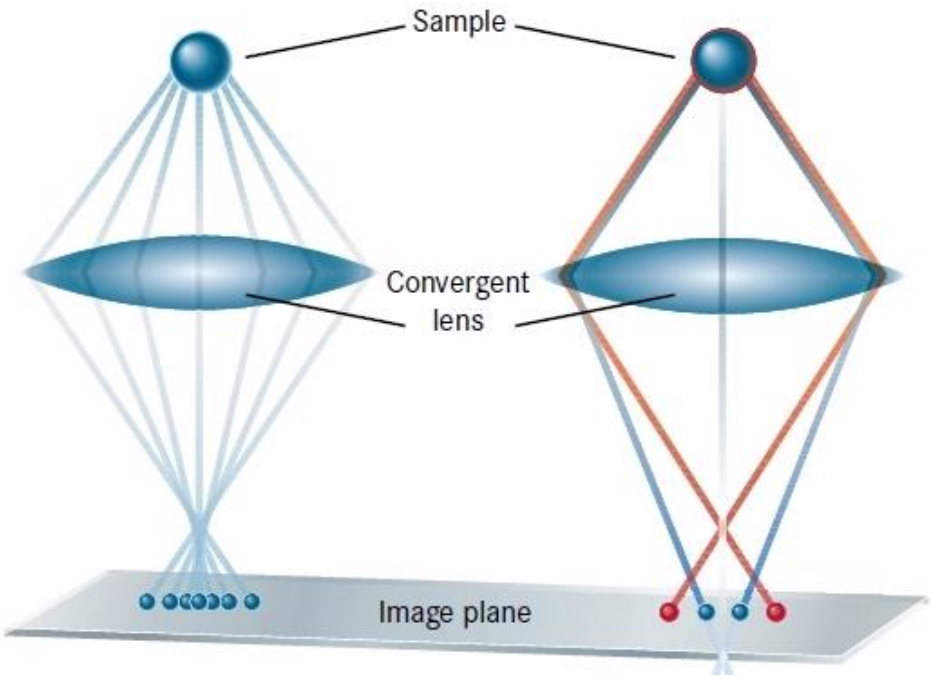
Coma is the most important optical aberration for high resolution imaging



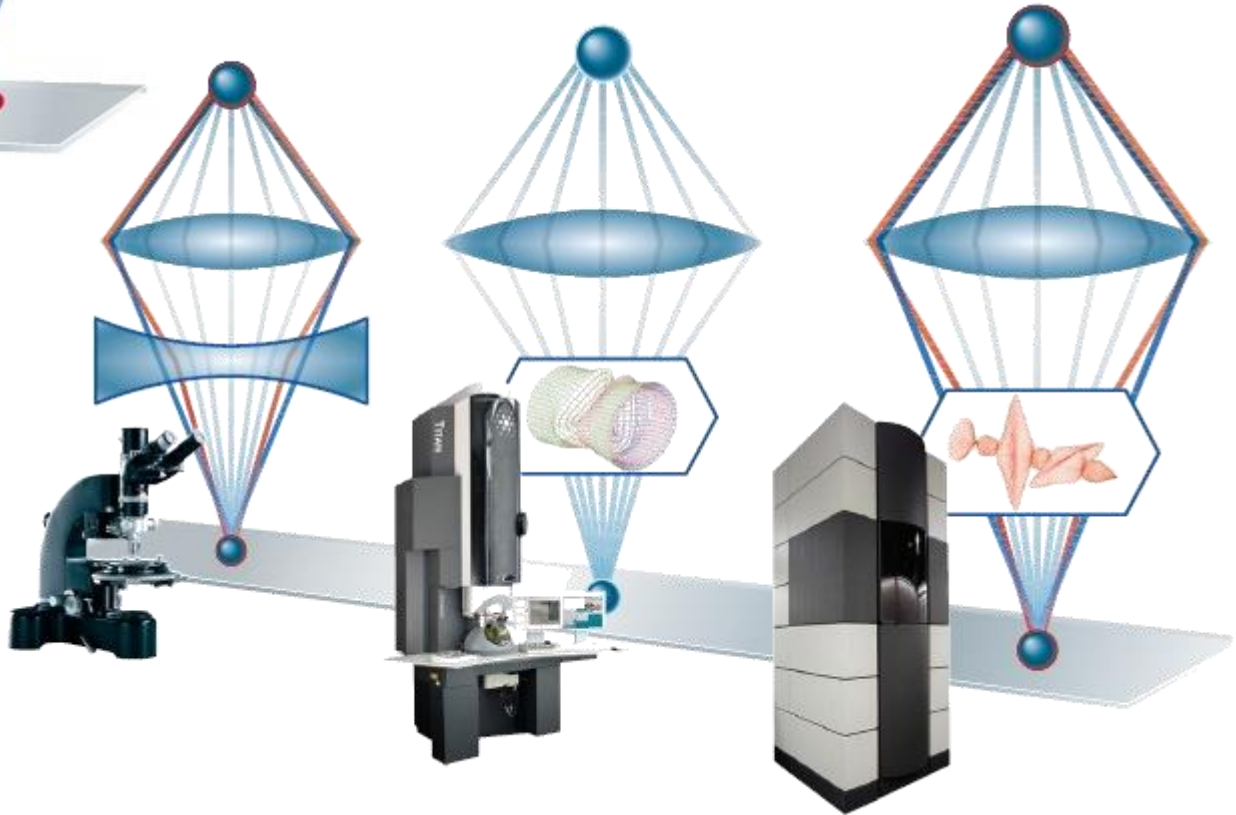
Beam tilt induces phase errors due to coma

(formula valid for non C_s corrected microscopes only)

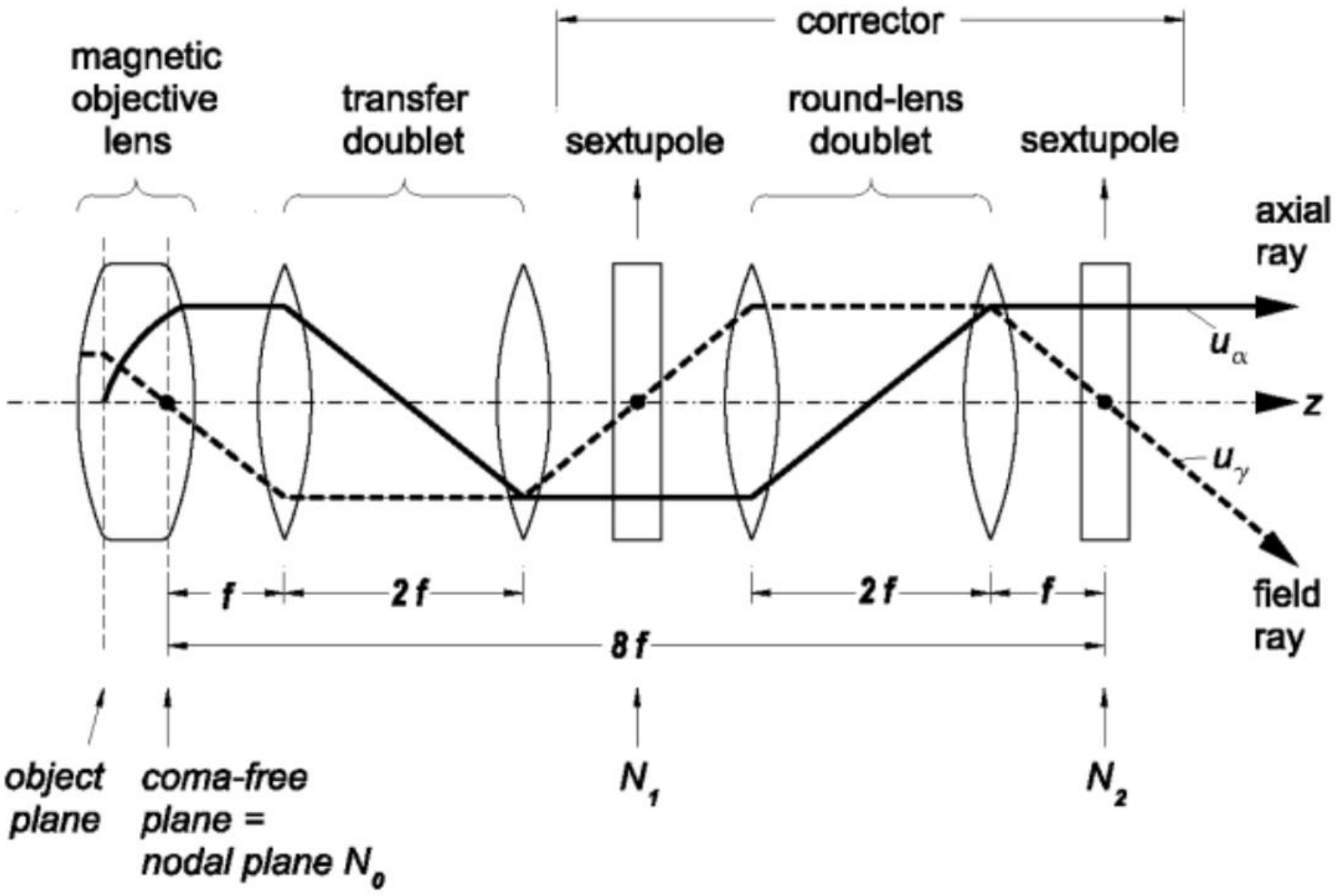
Spherical Aberration (C_s) Corrector



Scherzer Theorem:
Round lenses cannot be used to correct the spherical aberration caused by round lenses



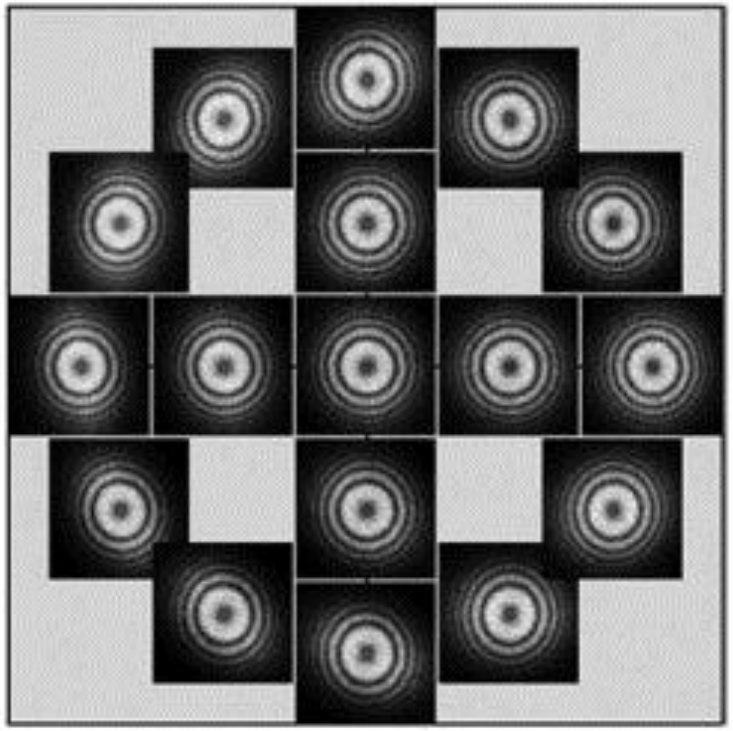
Symmetric design of a Hexapole C_s corrector



Designed by: Harald Rose Built by: Max Haider, CEOS Heidelberg

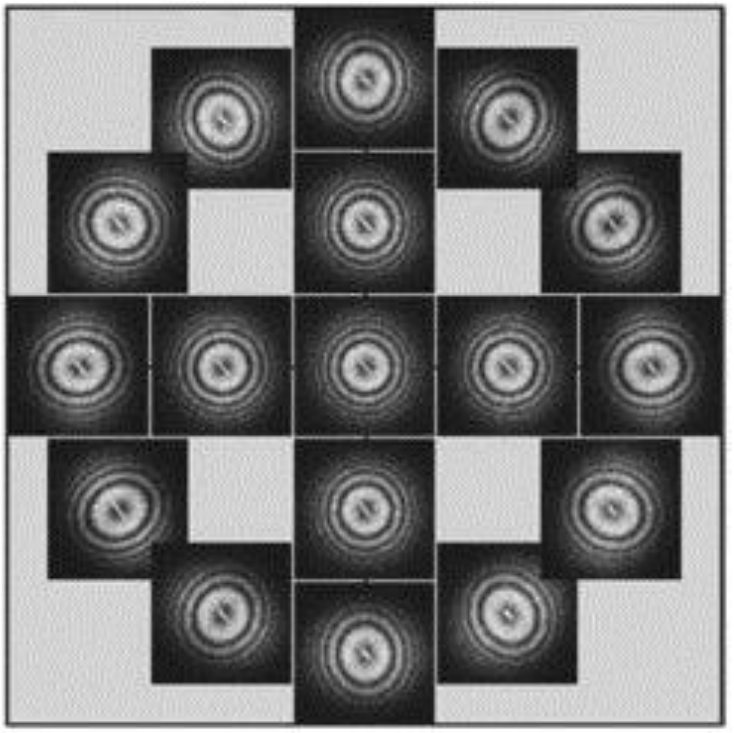
Zemlin Tableau

(a)



Fully corrected

(b)

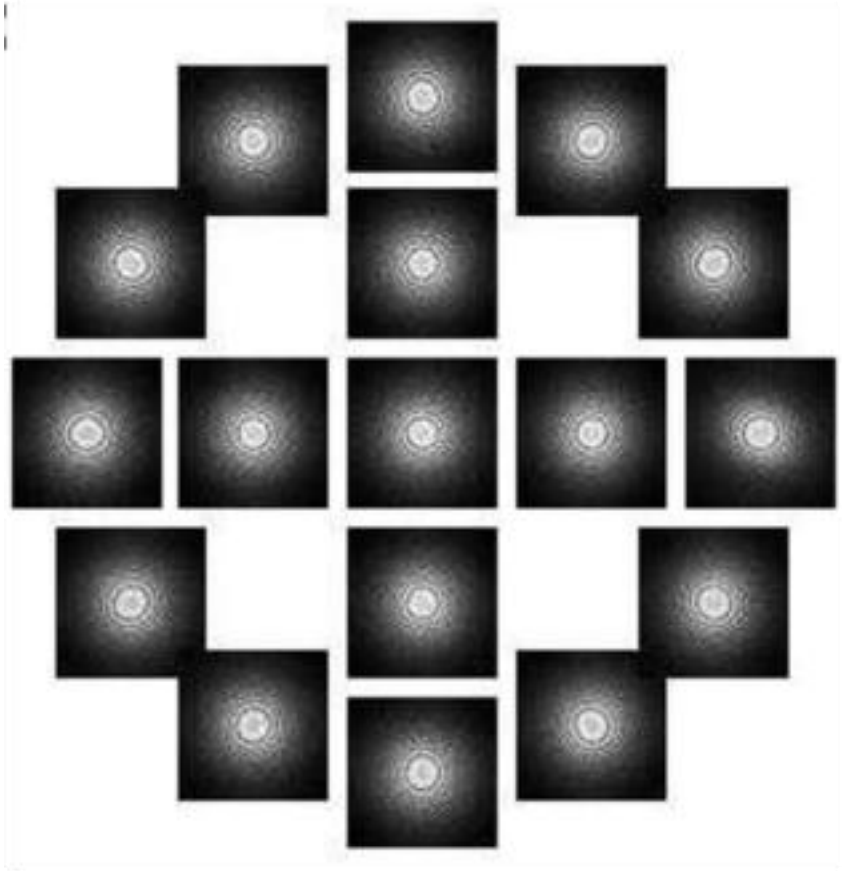


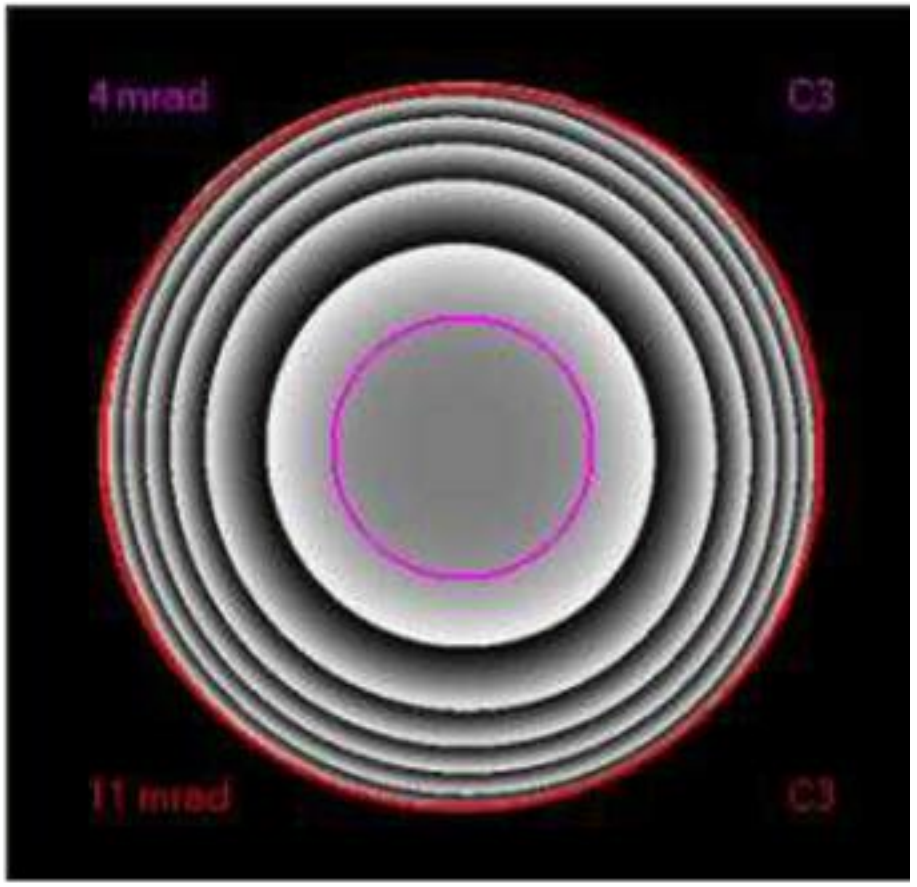
Imposed Cs of 0.1mm (18 mrad)

1. Measure beam-tilt dependent defocus and astigmatism
2. Determine phase errors
3. Correct up to 5th order aberrations

Zemlin et al., Ultramicroscopy 1978

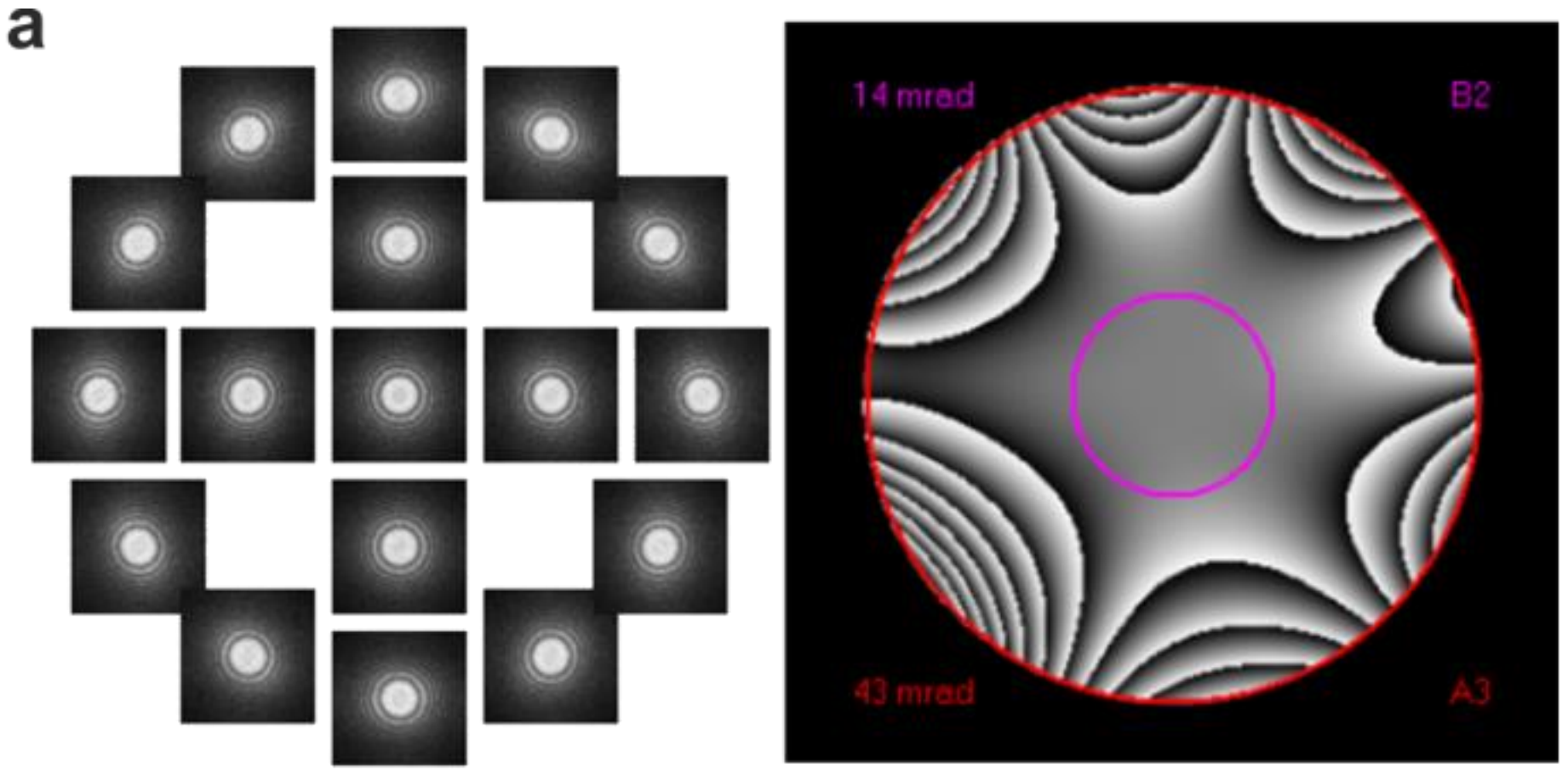
Cs Corrector Alignment





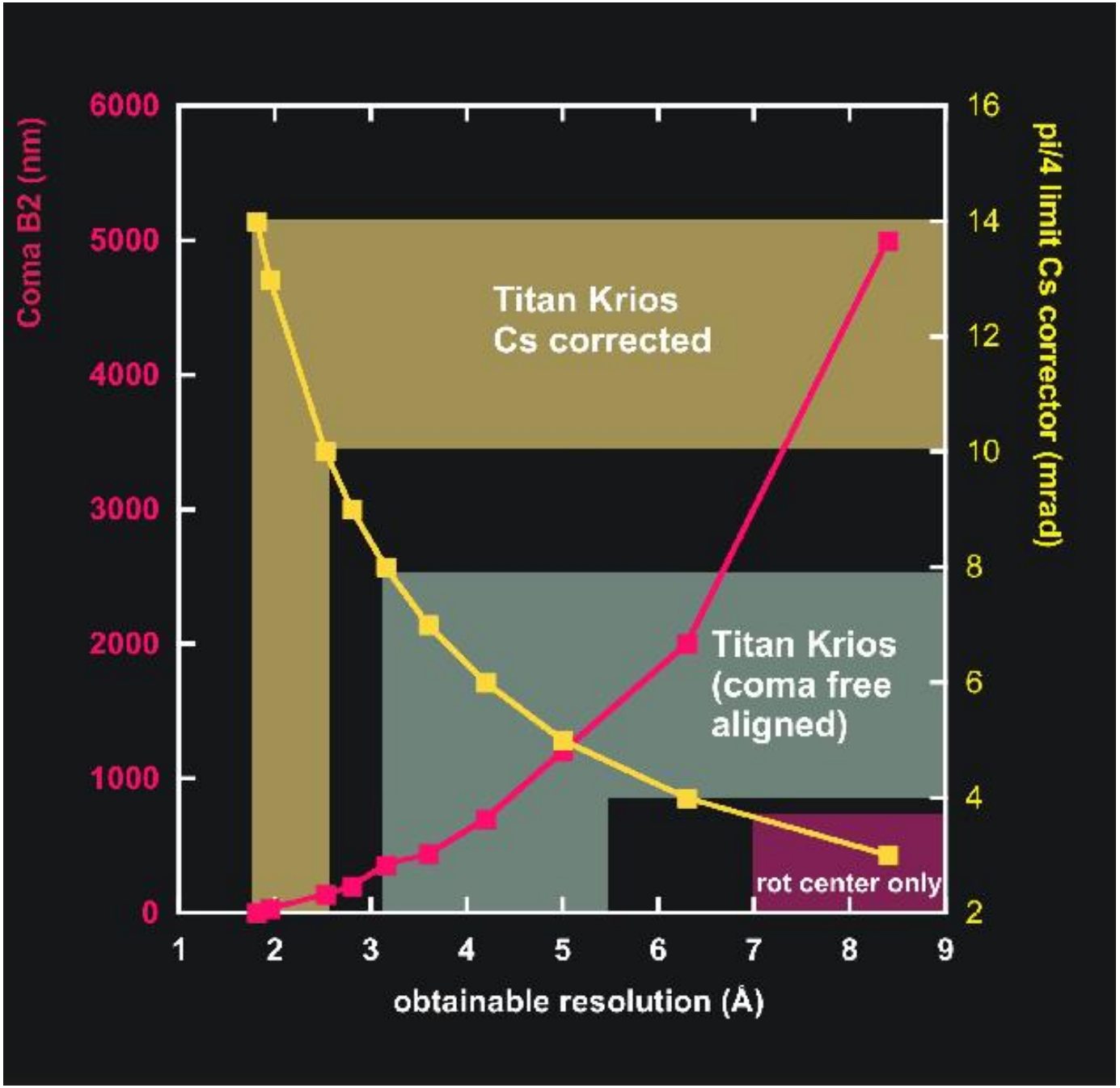
Hexapoles off: phase errors of 45 degrees at scattering angles of 4-7 mrad

Cs Corrector Alignment



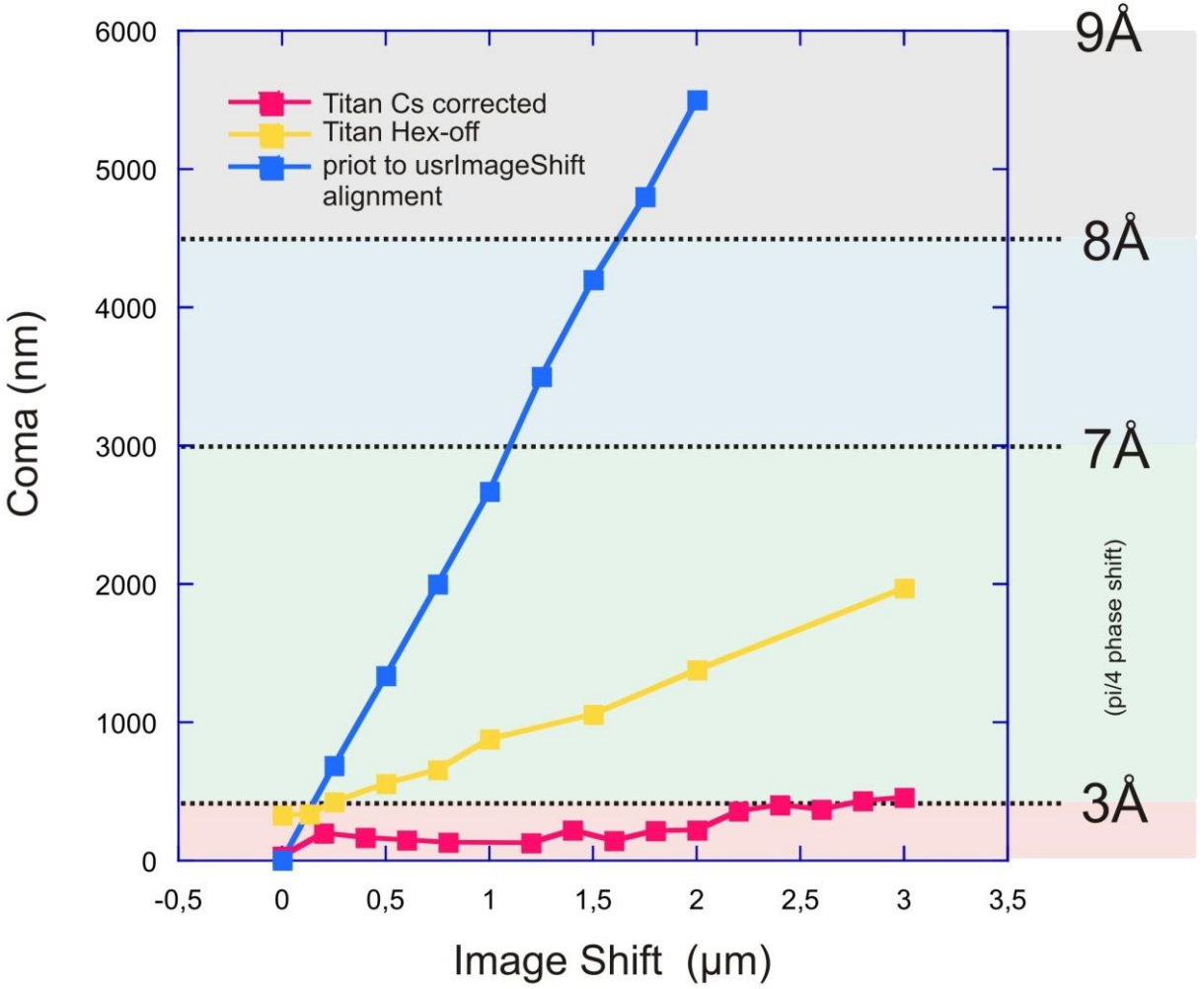
Hexapoles on: phase errors of 45 degrees at scattering angles of 12-15 mrad

Alignment accuracy in a C_s corrected Titan Krios

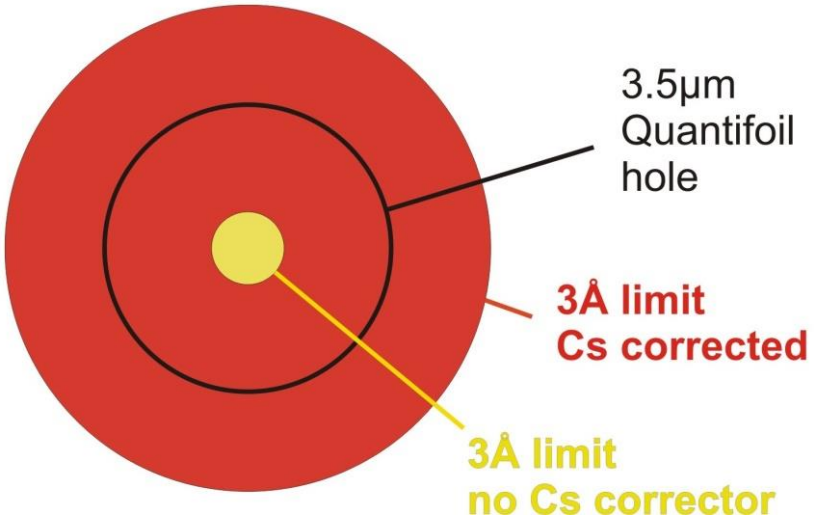


- pi/4 phase error is commonly used as resolution limiting criterion
- a phase error of pi/4 is not a sharp resolution limiting cutoff
- phase errors can be determined by the Zemlin tableau

Spotscan Imaging induced Coma



Implications for Spotscan on CCD:



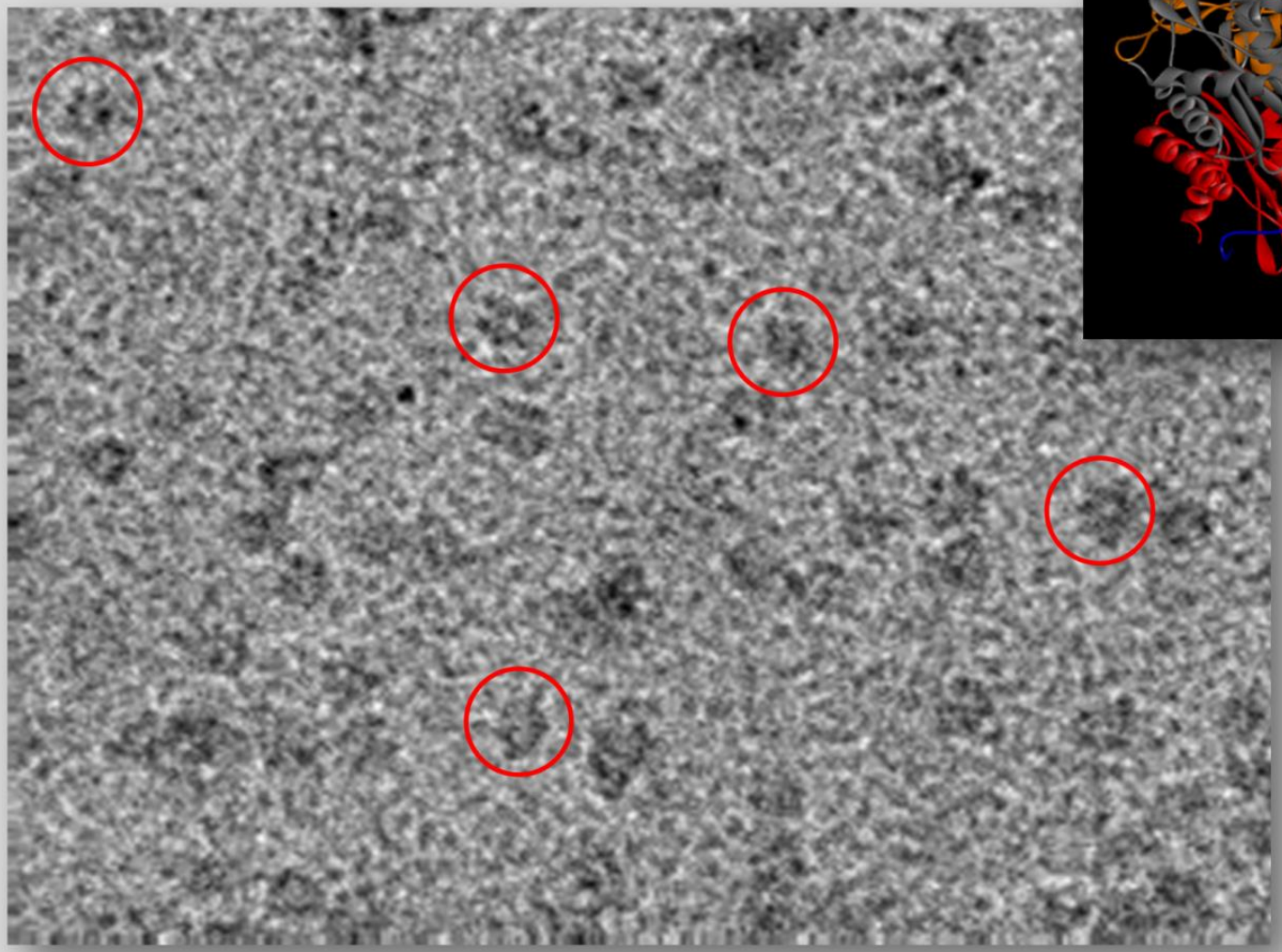
Coma dependent resolution (pi/4) limits

High Tension	With Cs Corrector on	With Cs Corrector off
300 kV	1.8 Å	3 Å
80 kV	3 Å	5 Å

$$\Delta\emptyset = -2\pi \cdot Cs \cdot \lambda^2 \cdot s^3 \cdot \theta \cos \omega$$

HbpS (heme binding protein)

Titan, Cs=0, 80kV, 1µm defocus, CRYO

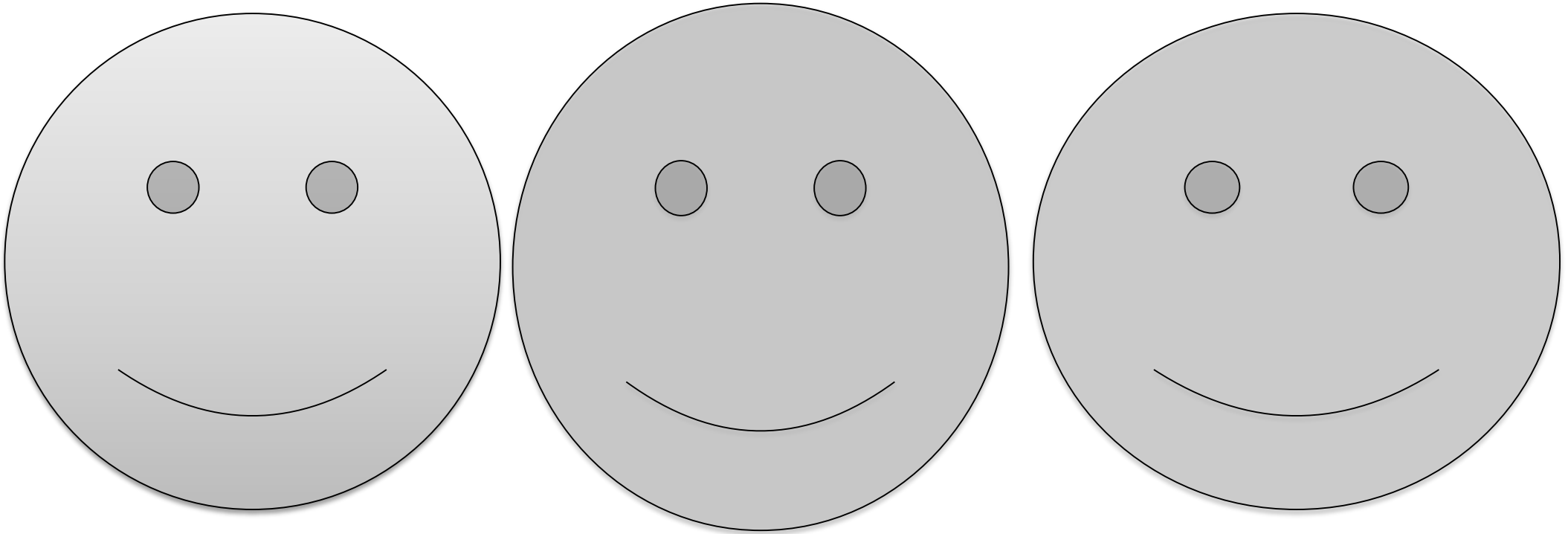


MW = 125 kDa

Linear Distortion

Linear Distortion

- Difference in magnification between x and y direction
- microscope specifications: 1-2% magnification accuracy
- some examples: CM200FEG (1.2%), Titan Krios (0.4%)



No distortion

X: 5% distortion

Y: 5% distortion

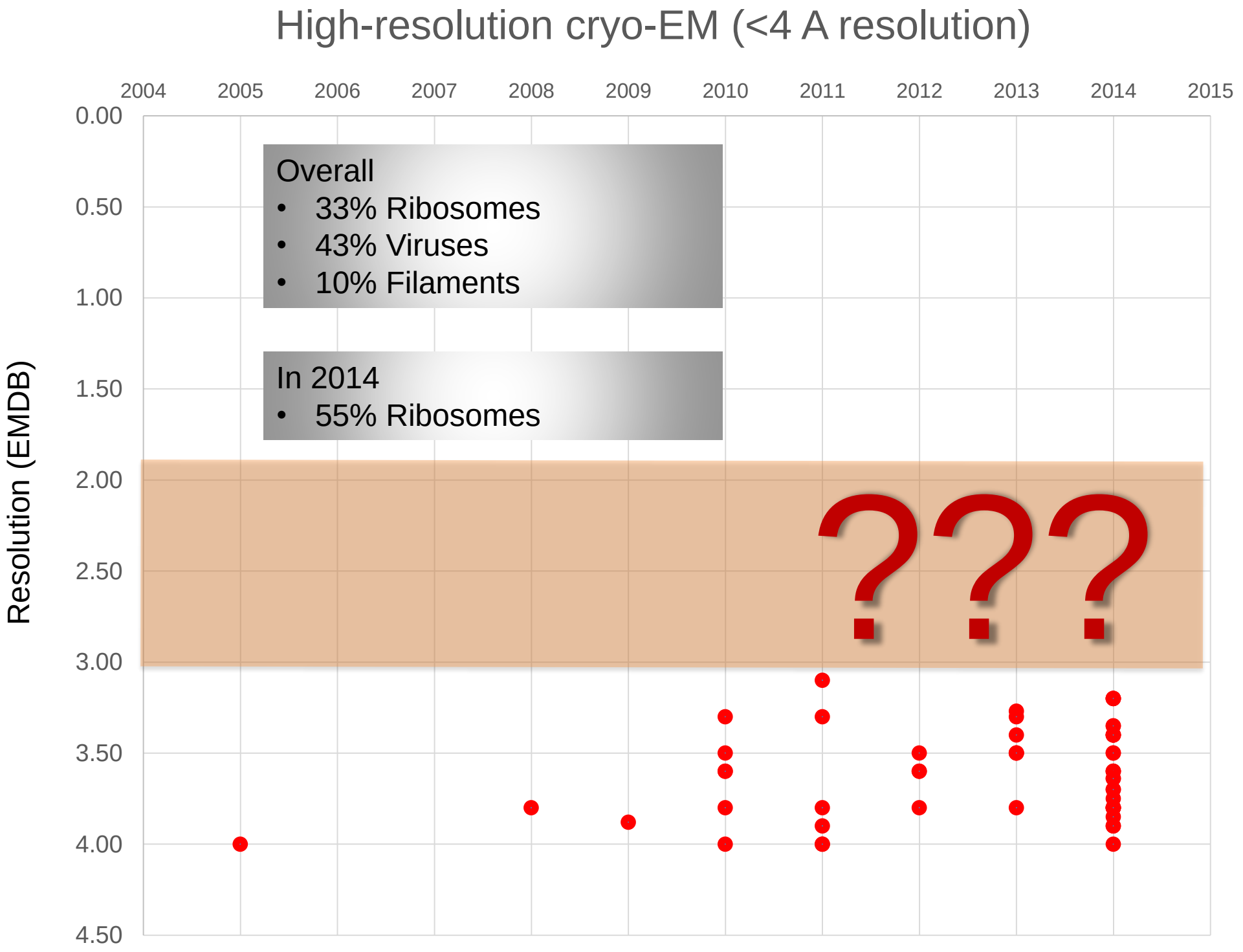
Can be reduced to <0.1% with a Cs corrector (TiltHexapole coils)

Virus with 1000A in diameter

Distortion (%)	Error in (A)	Expected min alignment error (A)	Max obtainable resolution
5	50	25A	25
3	30	15	15
2	20	10	10
1	10	5	5
0.5	5	2.5	2.5
0.1	1	0.5	0.5

Ribosome with 250A in diameter

Distortion (%)	Error in (A)	Expected min alignment error (A)	Max obtainable resolution
5	12	6	6
3	8	4	4
2	5	2.5	2.5
1	3	1.5	1.5
0.5	1.5	0.75	0.75
0.1	0.25	0.25	0.25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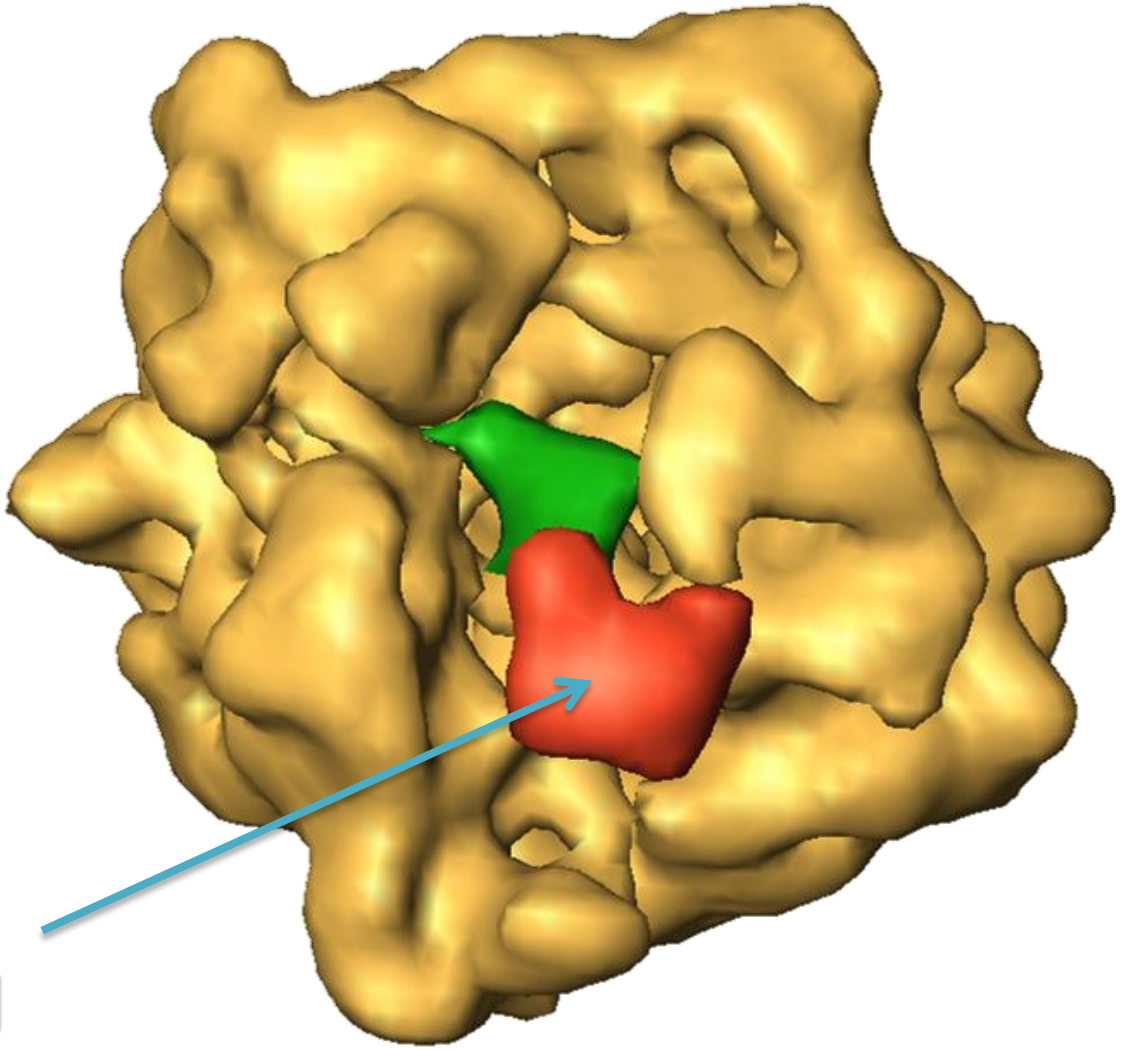
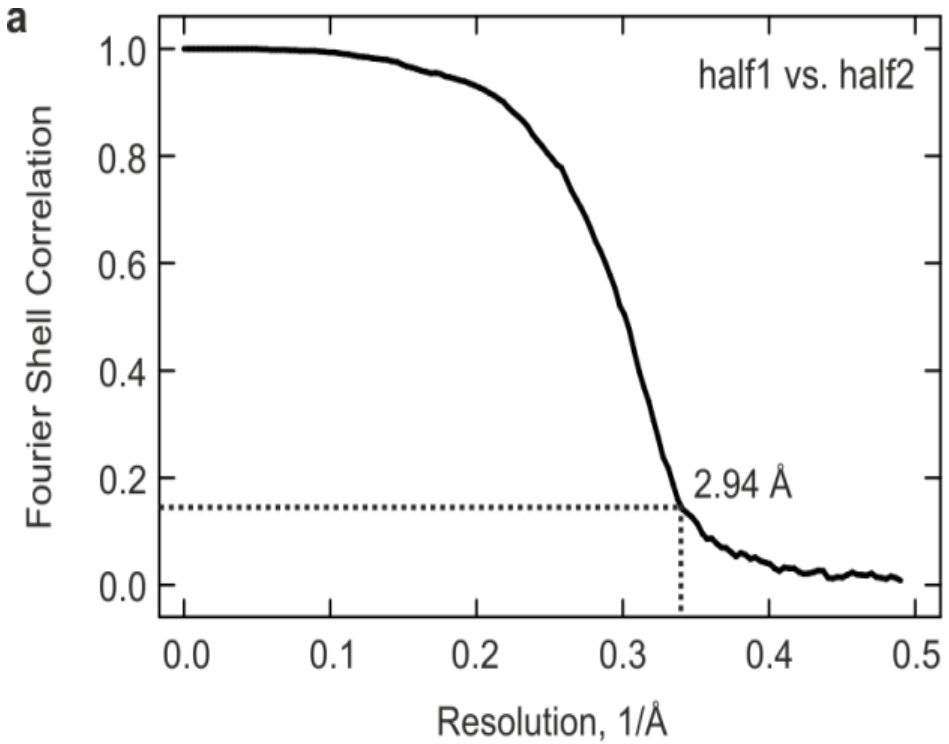
Ribosome – EF-Tu complex stalled by the Antibiotic kirromycin (18 Angstrom)

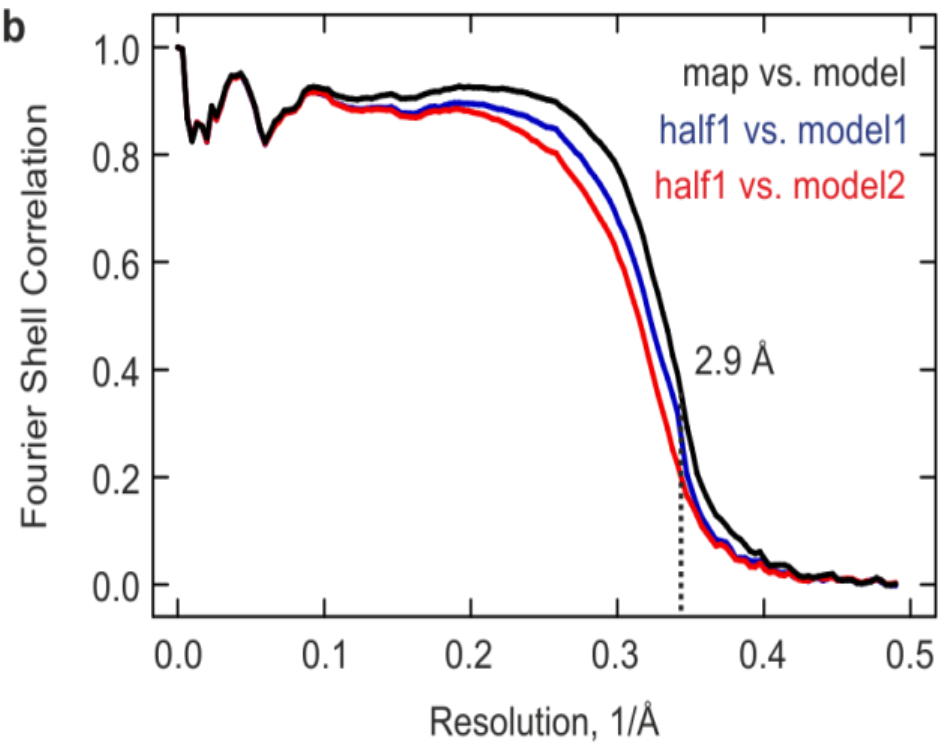


EF-Tu

Stark et al., 1997, Nature

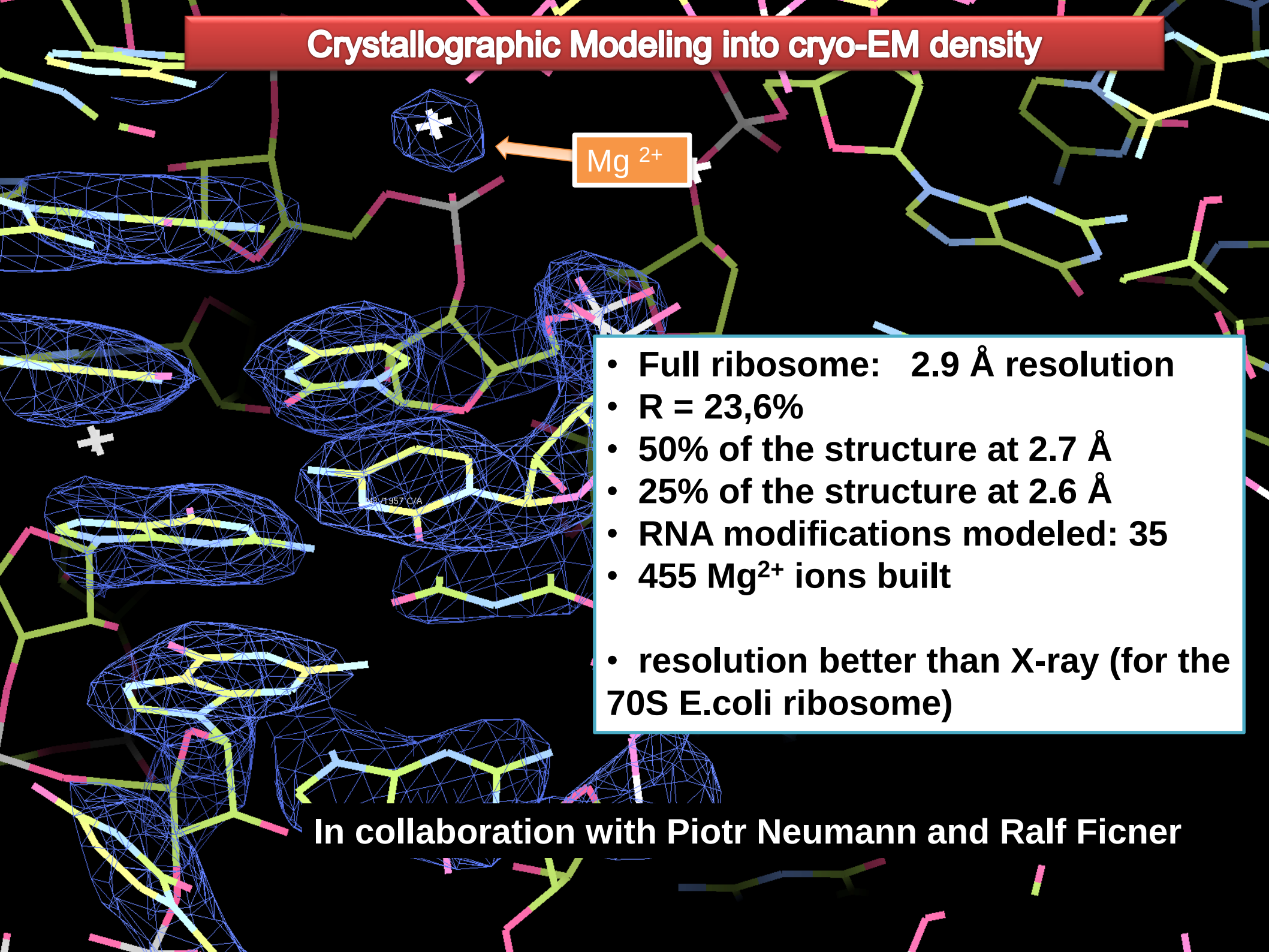
E coli 70S ribosome at <3A resolution





- **300 kV**
- **Falcon I**
- **No movie mode**
- **~40-45 e/A^2**

Crystallographic Modeling into cryo-EM density

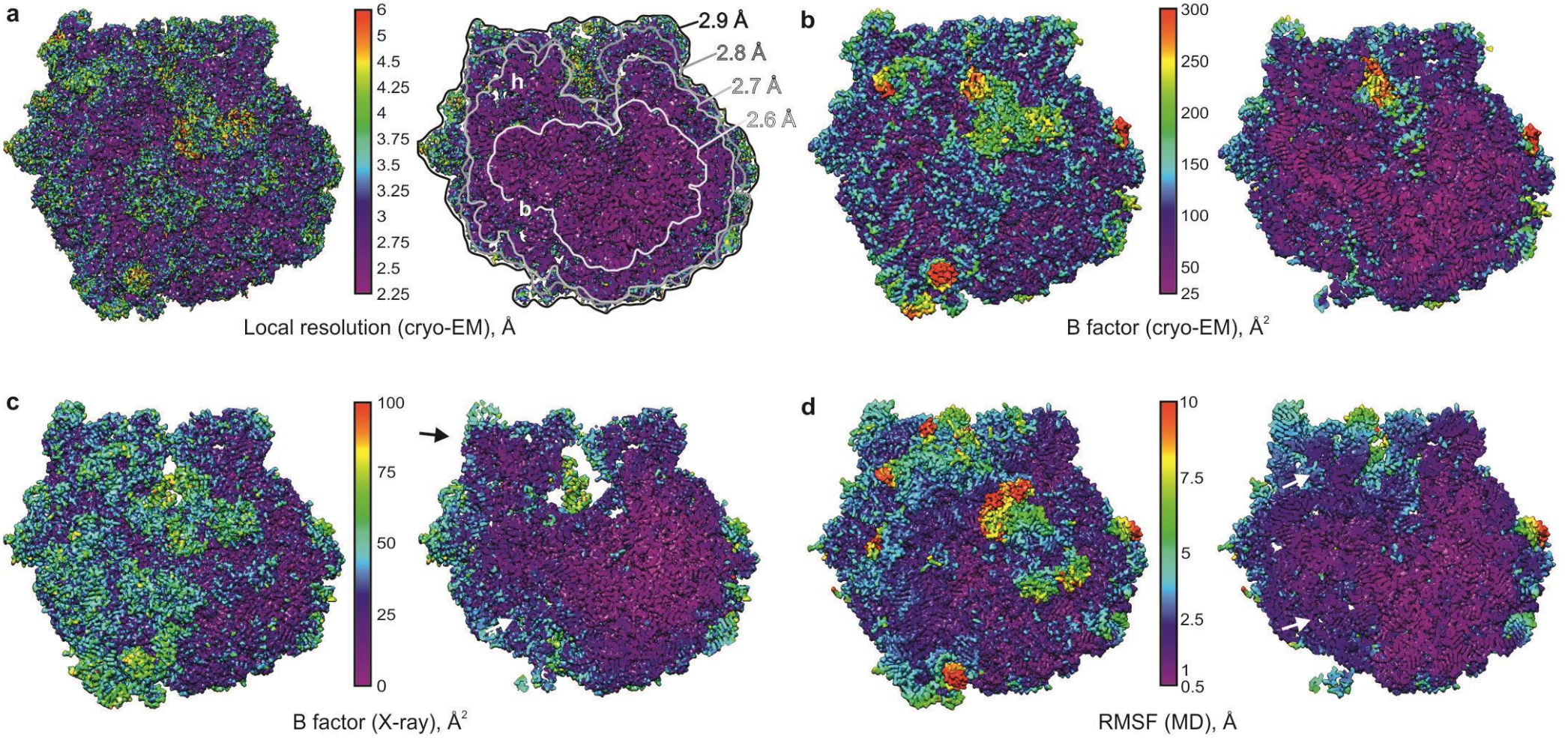


- **Full ribosome: 2.9 Å resolution**
- **R = 23,6%**
- **50% of the structure at 2.7 Å**
- **25% of the structure at 2.6 Å**
- **RNA modifications modeled: 35**
- **455 Mg^{2+} ions built**

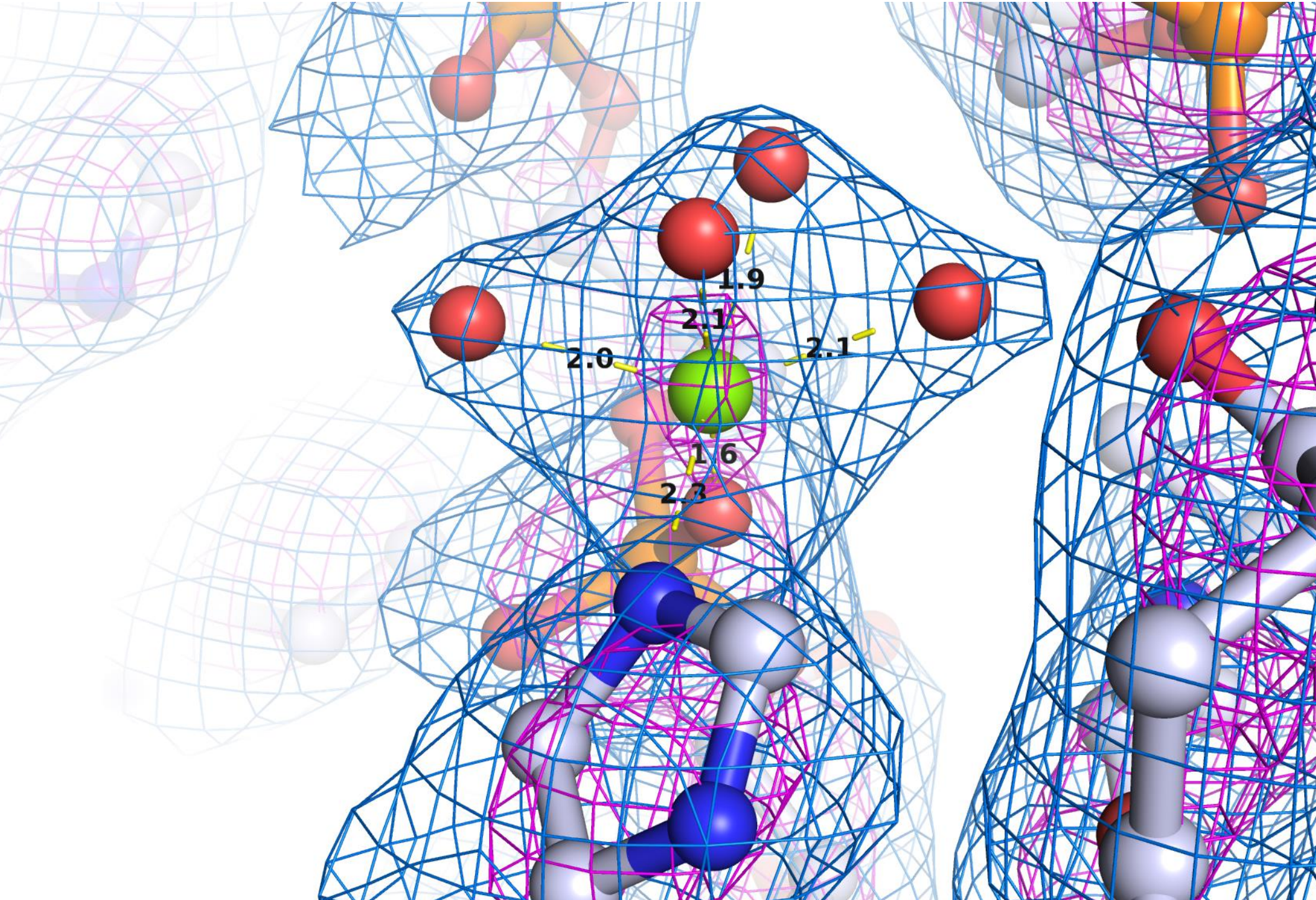
- **resolution better than X-ray (for the 70S E.coli ribosome)**

In collaboration with Piotr Neumann and Ralf Ficner

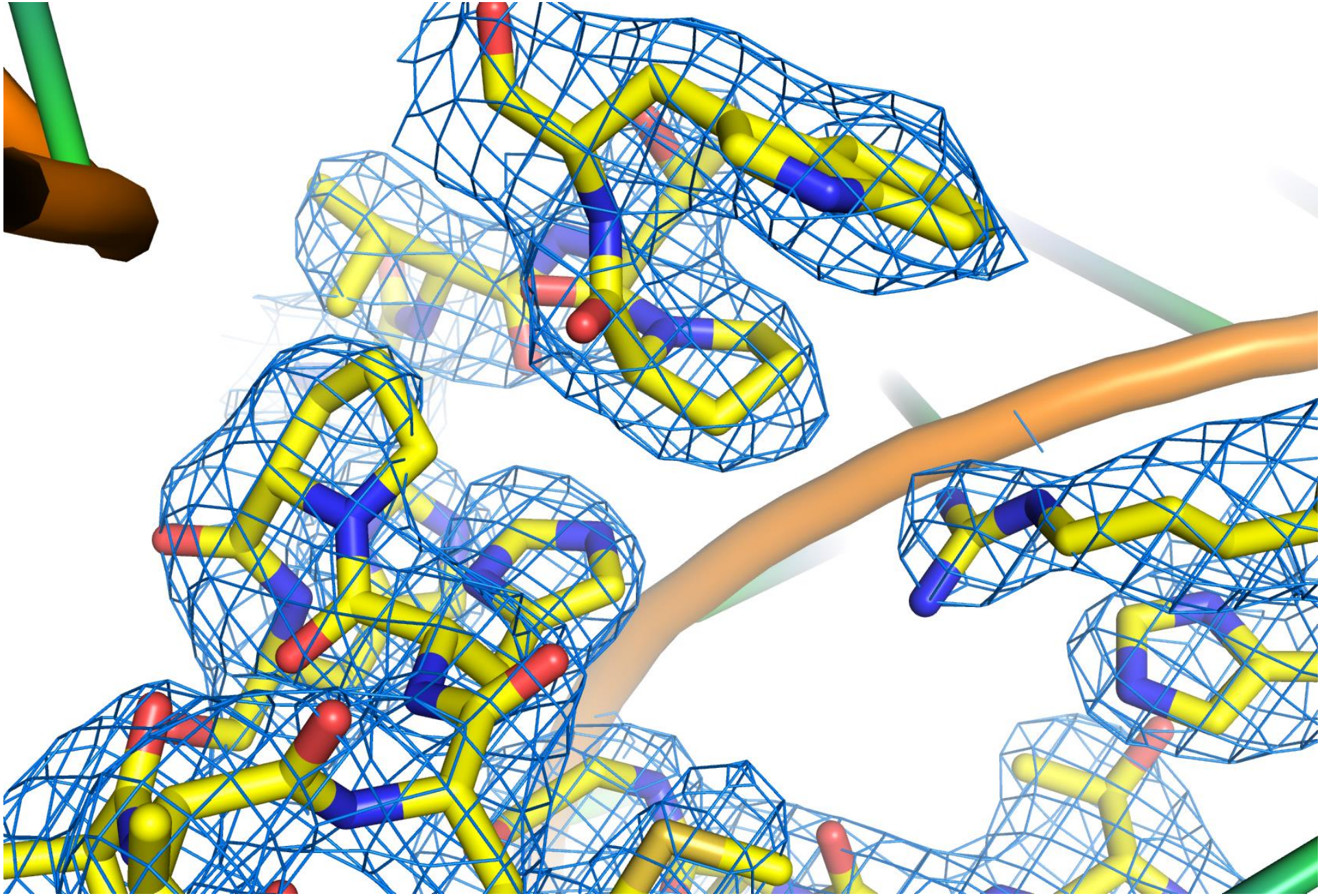
Local Structural Variations by Different Methods



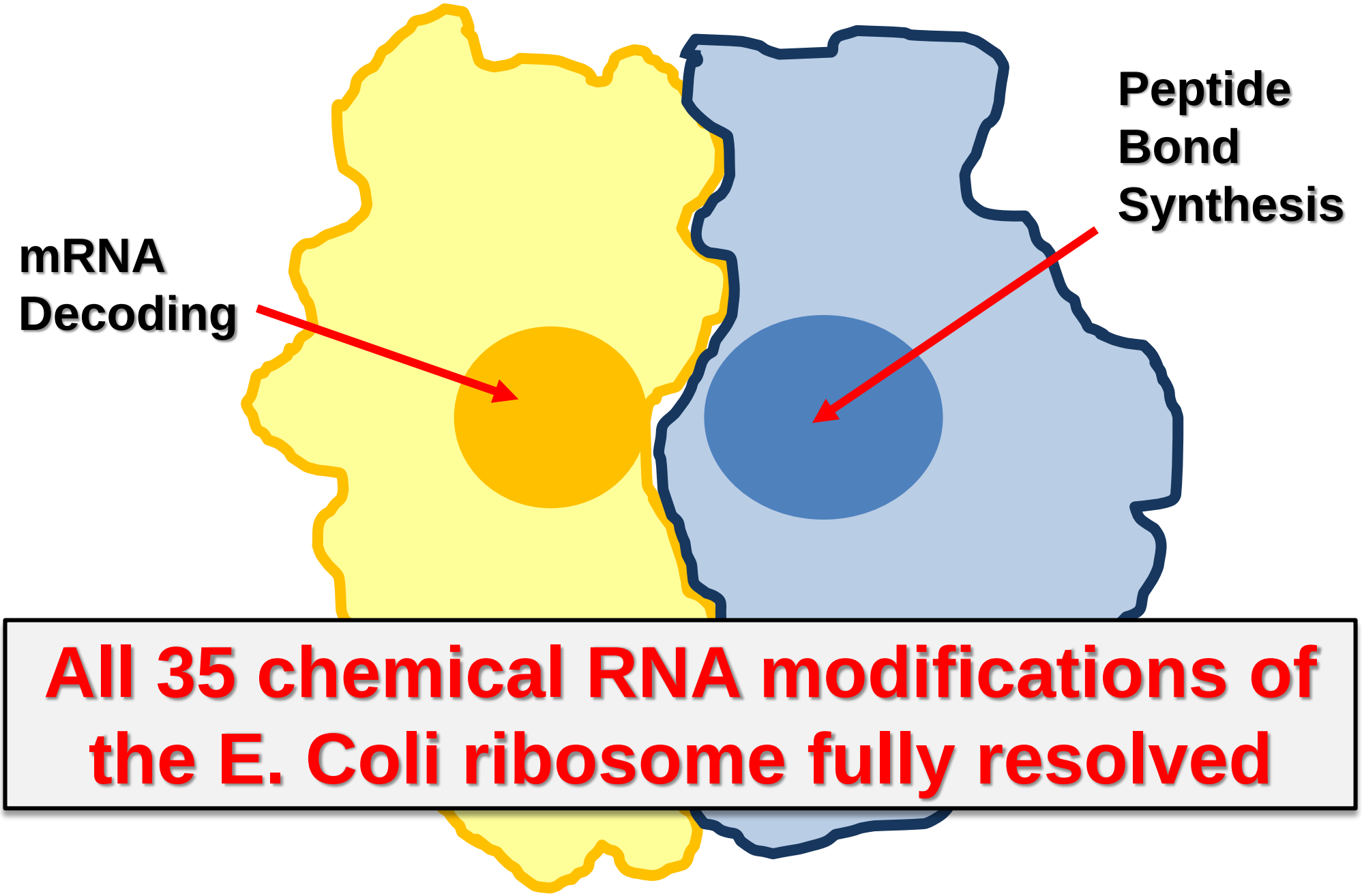
Mg coordinated by Water



Proteins at 2.6 Å Resolution

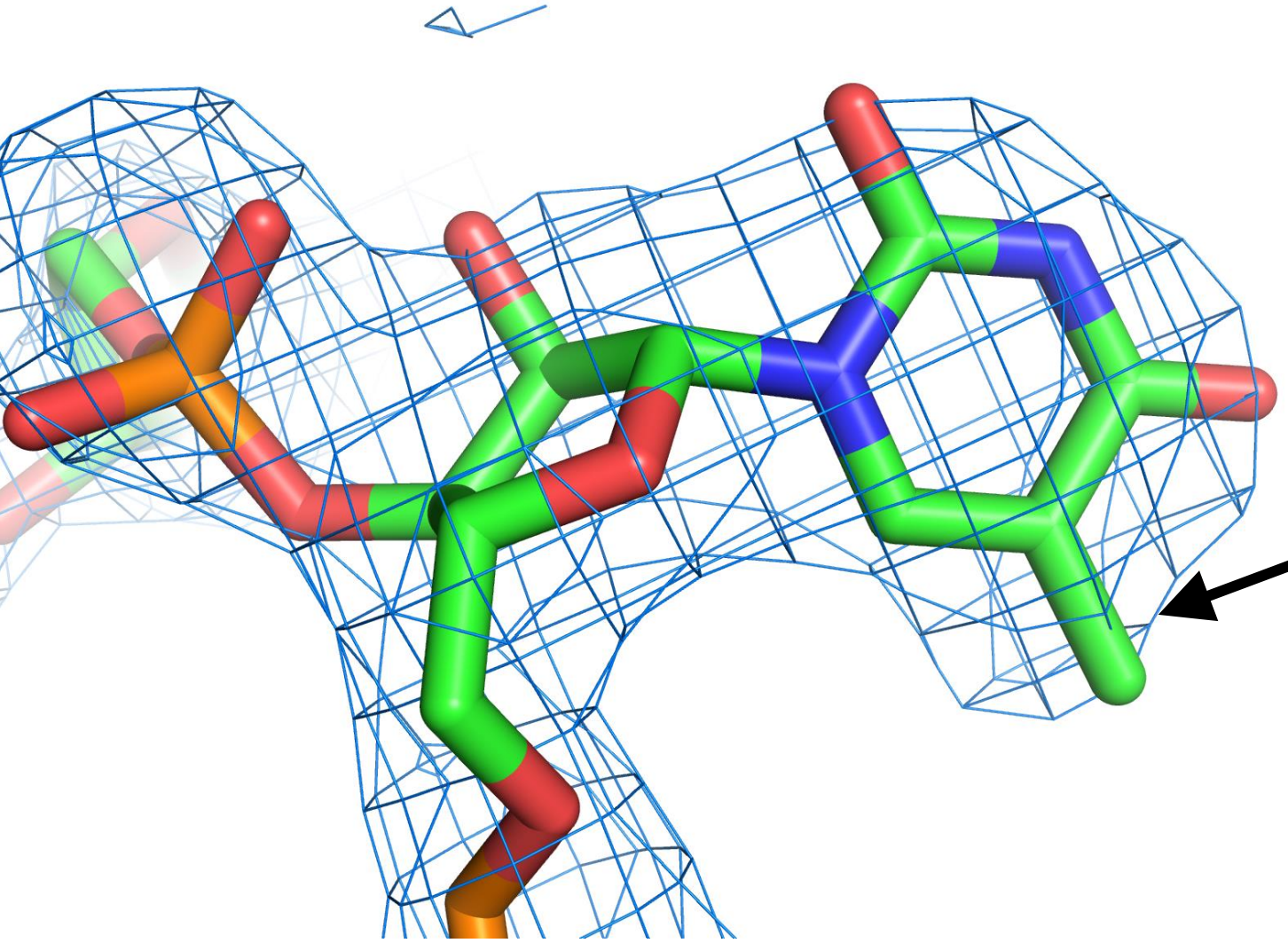


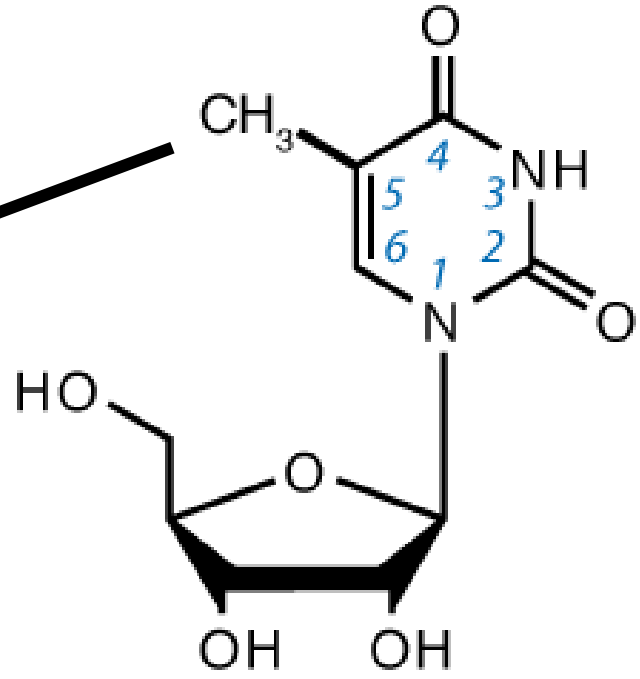
Clusters of RNA Modifications



All 35 chemical RNA modifications of the E. Coli ribosome fully resolved

Methylation of ribosomal RNA (U 1939)

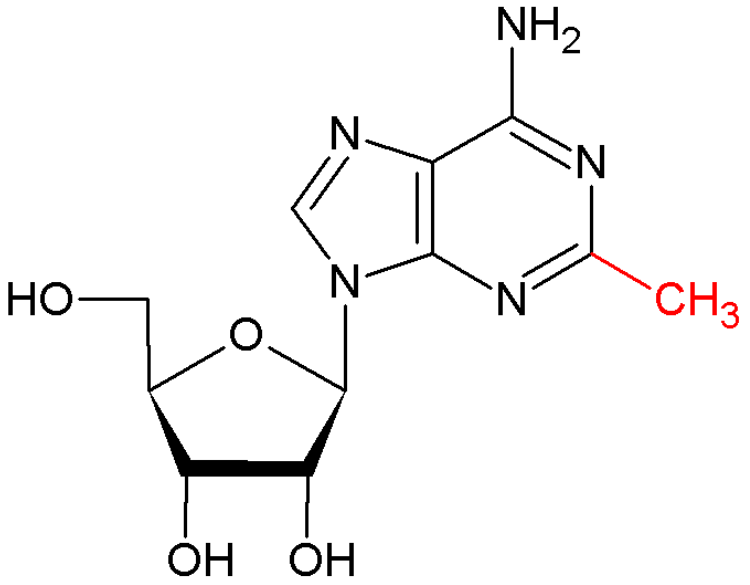




5-Methyl-Uridine

Local resolution: 2.6 Å

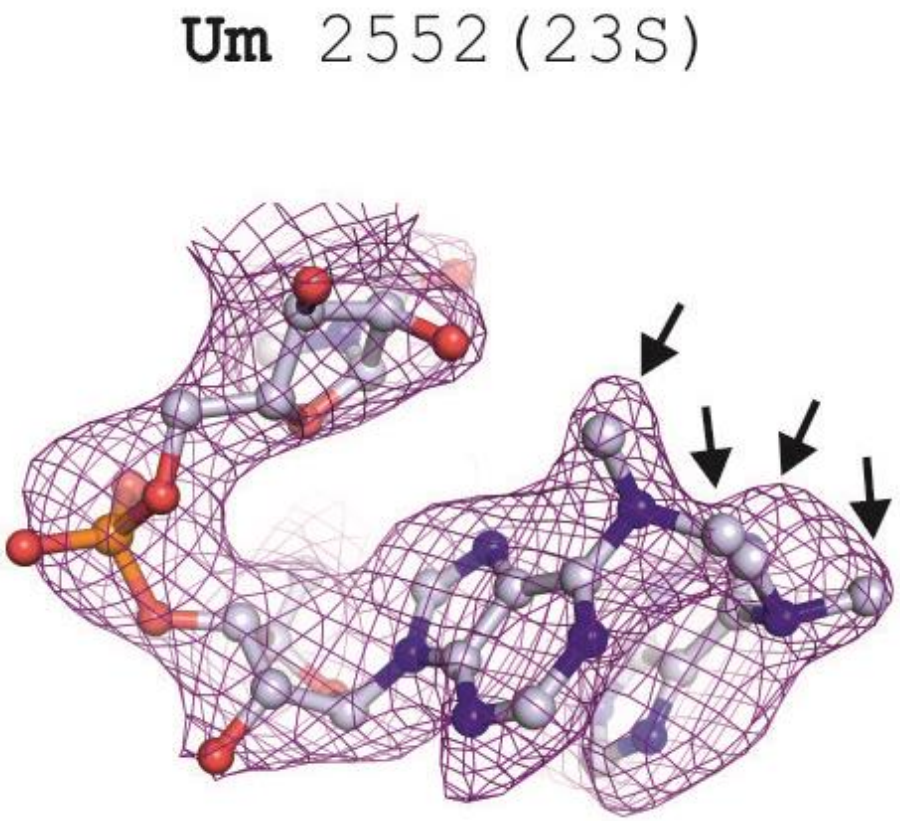
Methylation of ribosomal RNA (A 2503)

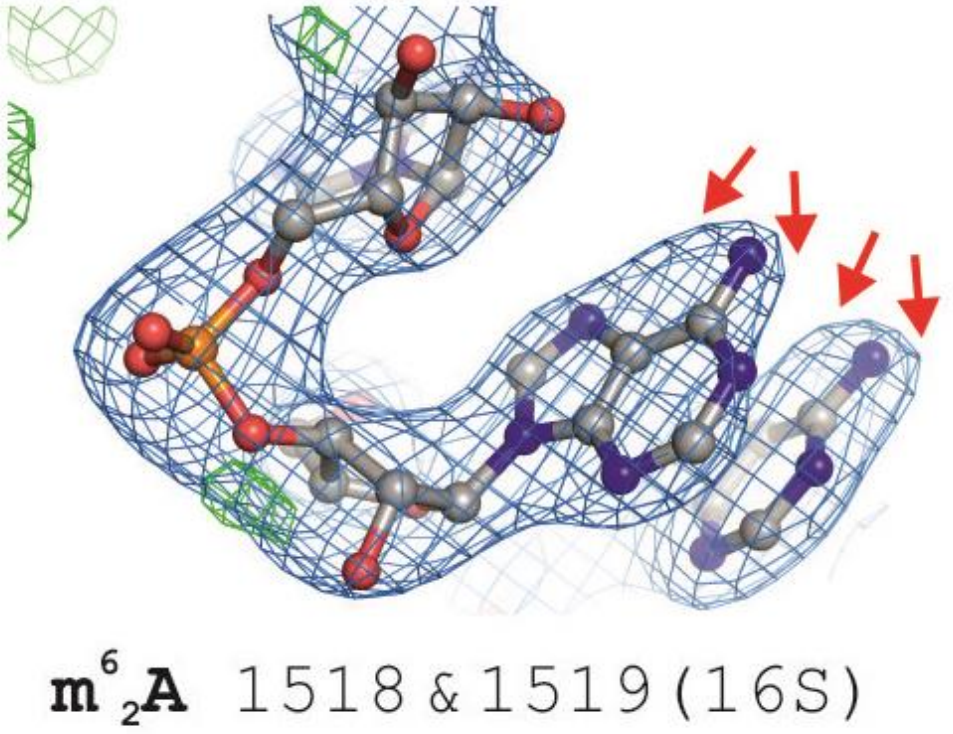


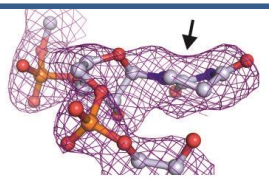
5-Methyl-Adenosine

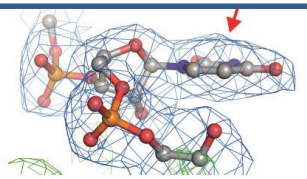
Local resolution: 2.6 Å

Comparison between X-ray and EM






D 2449(23S)


D 2449(23S)

X-ray 2mFo-DFc map at 1 σ

X-ray mFo-DFc map at 3 σ

density for modification

potential position of modification