Macromolecule Diffractive Imaging Using Imperfect Crystals

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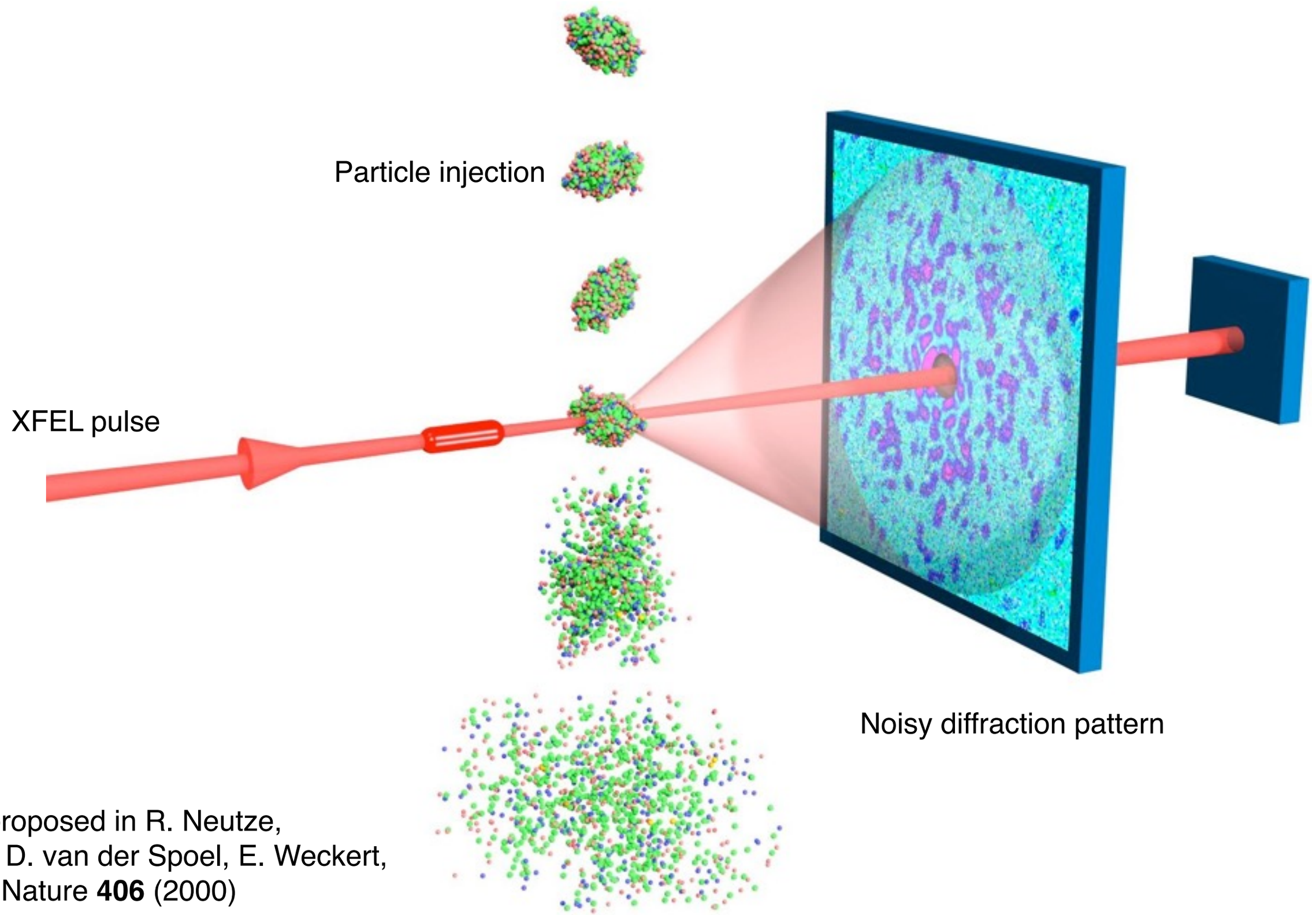
DESY and University of Hamburg

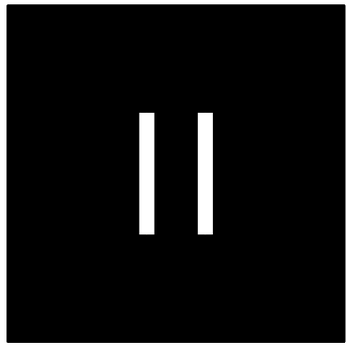
XFEL & DESY Users' Meeting, January 2016

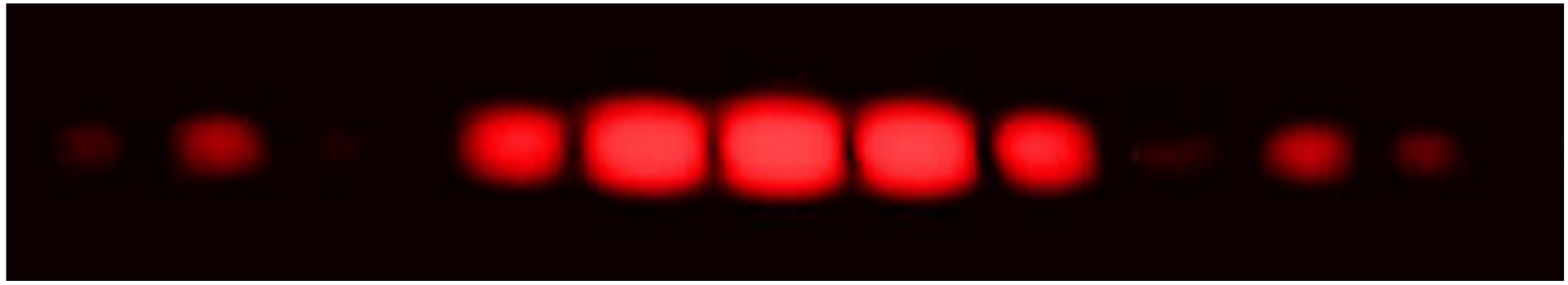
X-ray free-electron lasers may enable atomic-resolution imaging of biological macromolecules

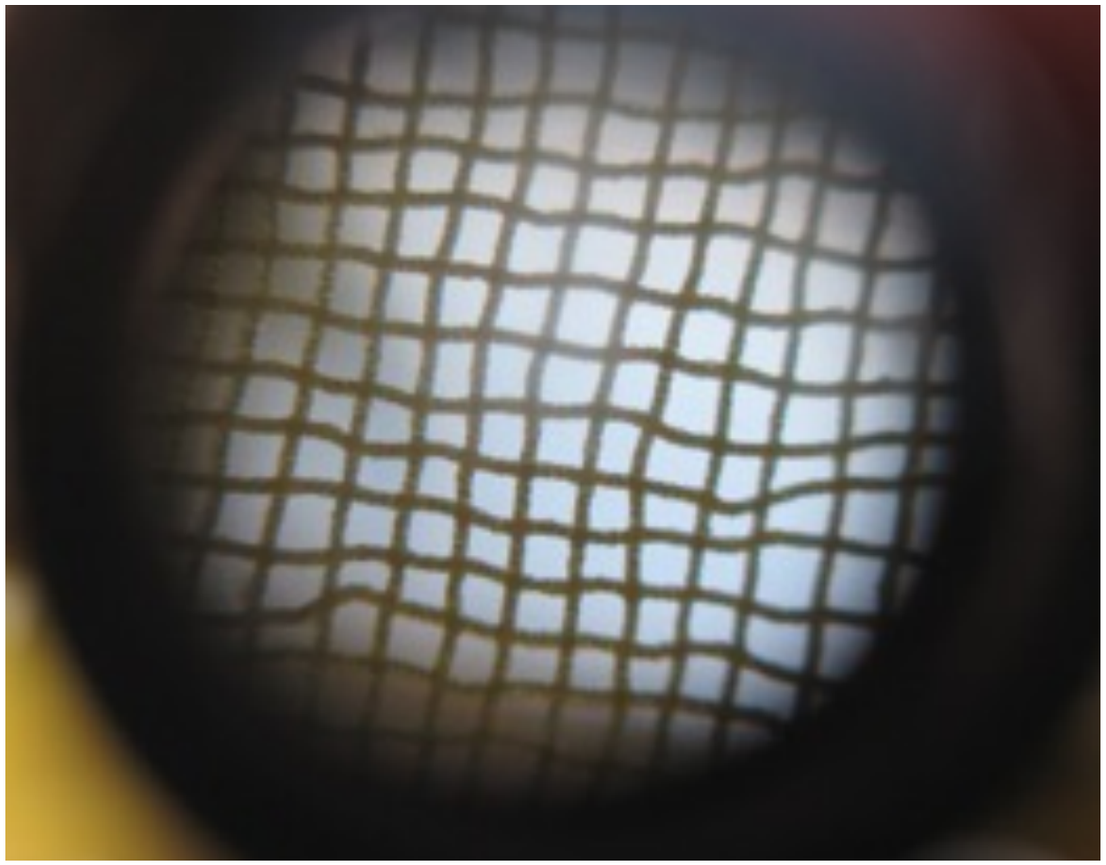


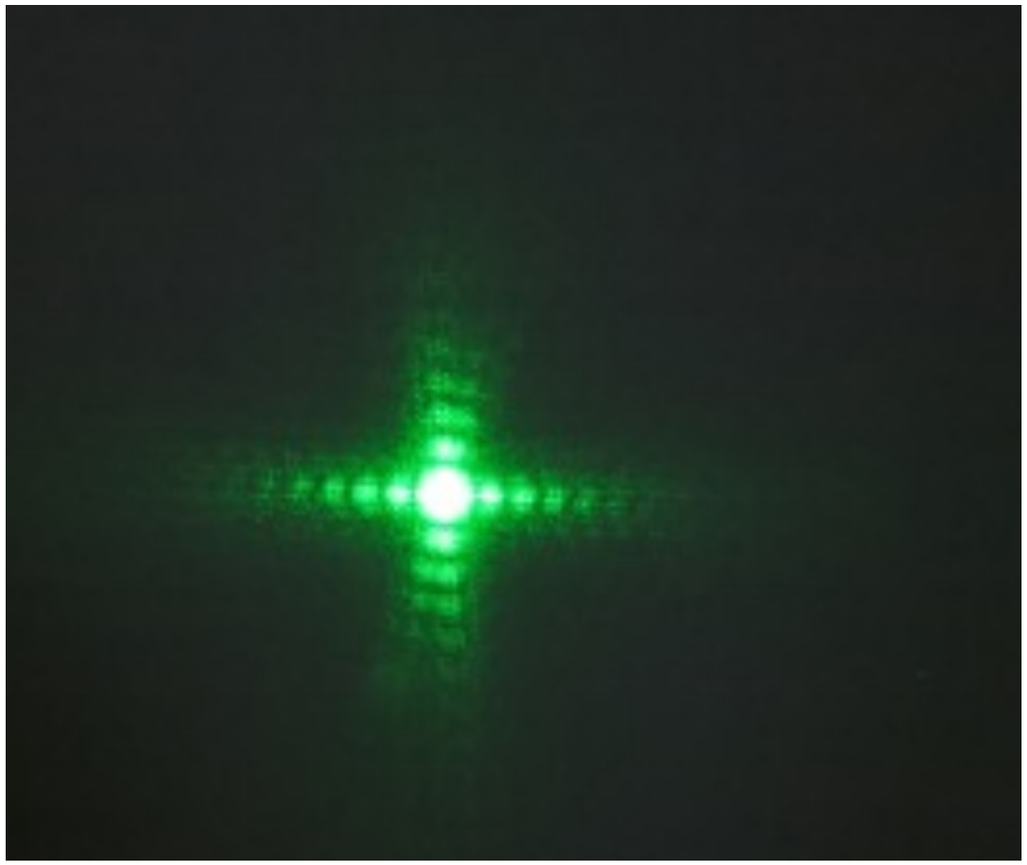
Scheme proposed in R. Neutze, R. Wouts, D. van der Spoel, E. Weckert, J. Hajdu, Nature **406** (2000)

Diffraction probes structure



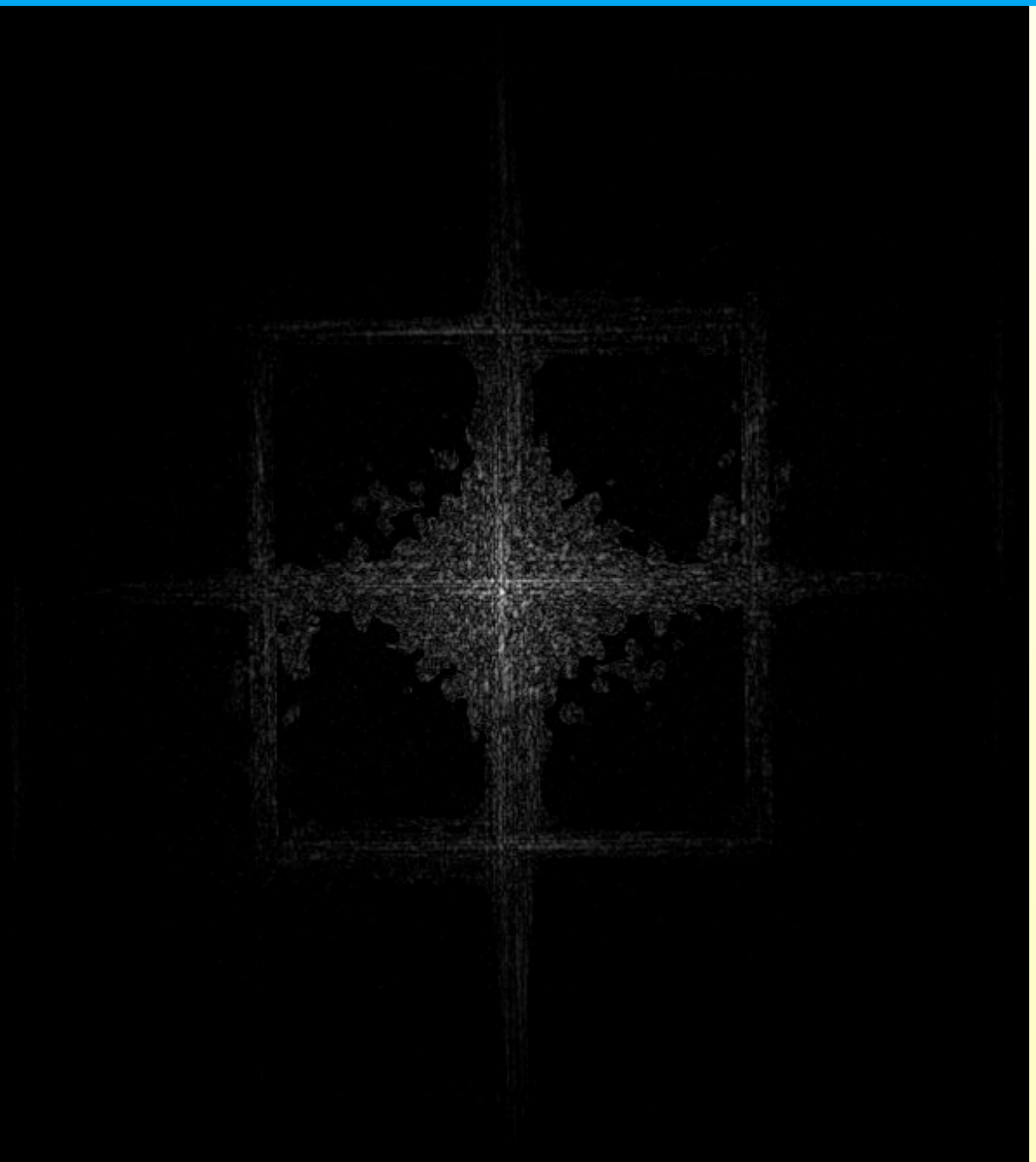


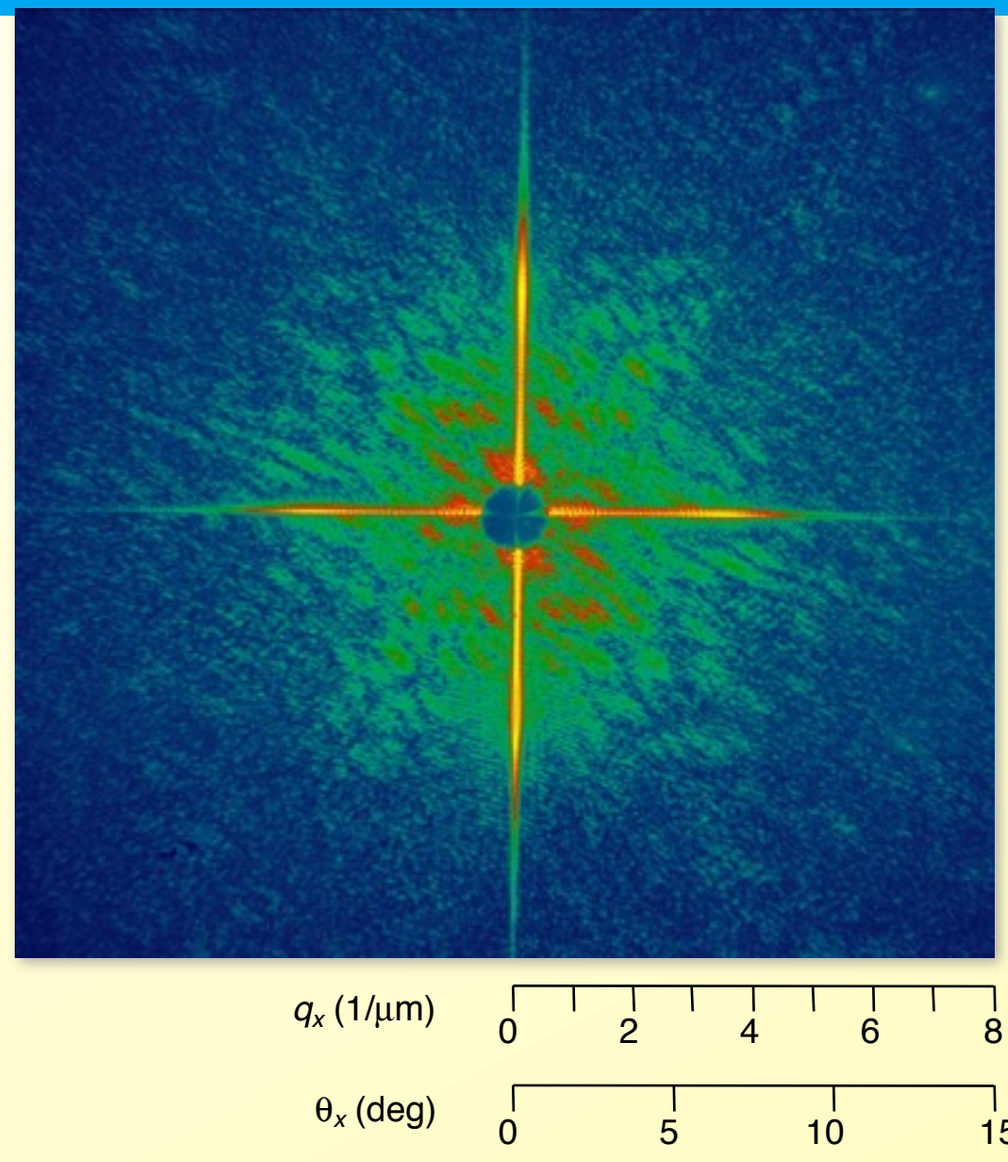




courtesy Katarina Chapman

We perform ab initio image reconstruction with our "Shrinkwrap" algorithm



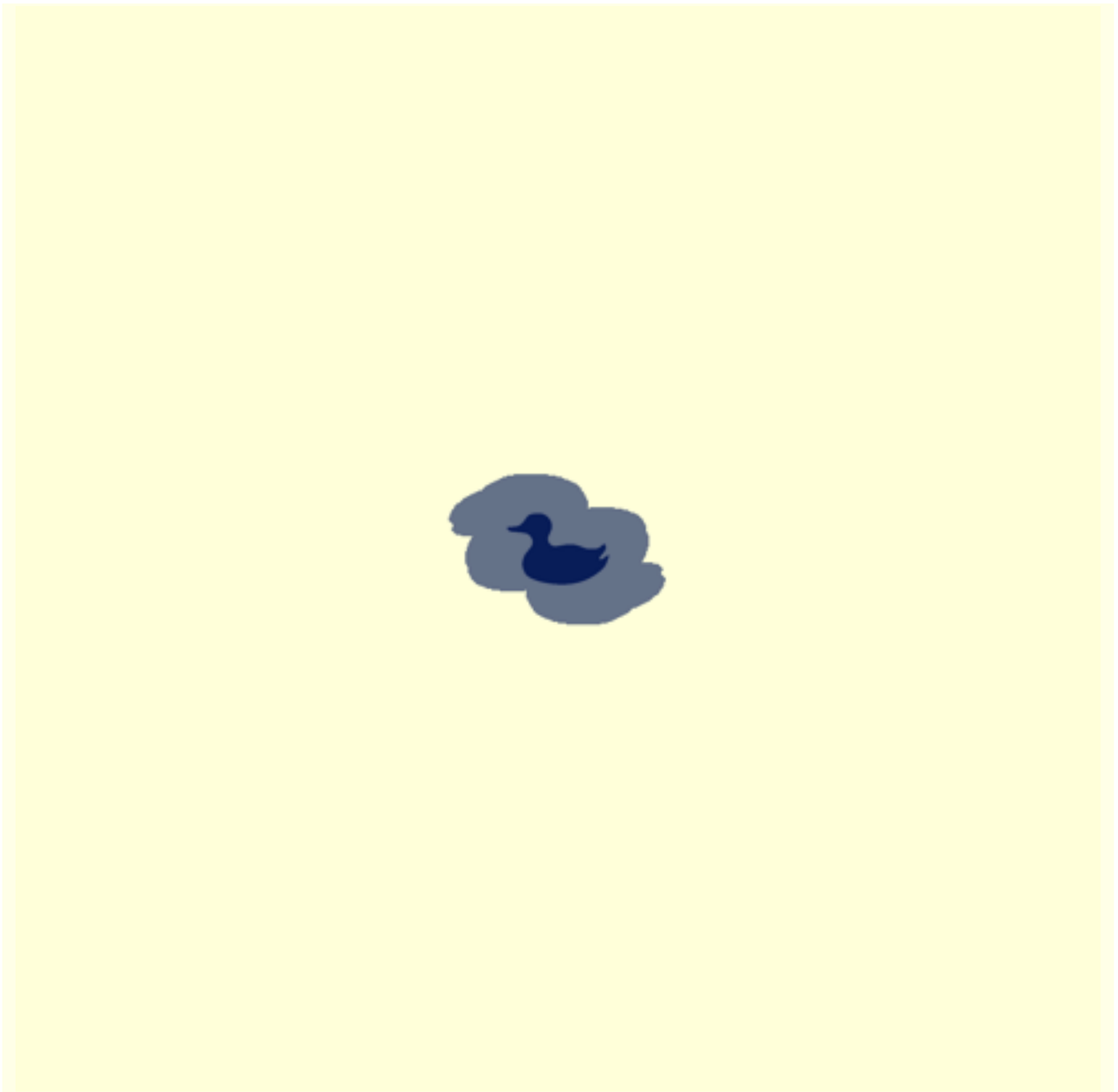


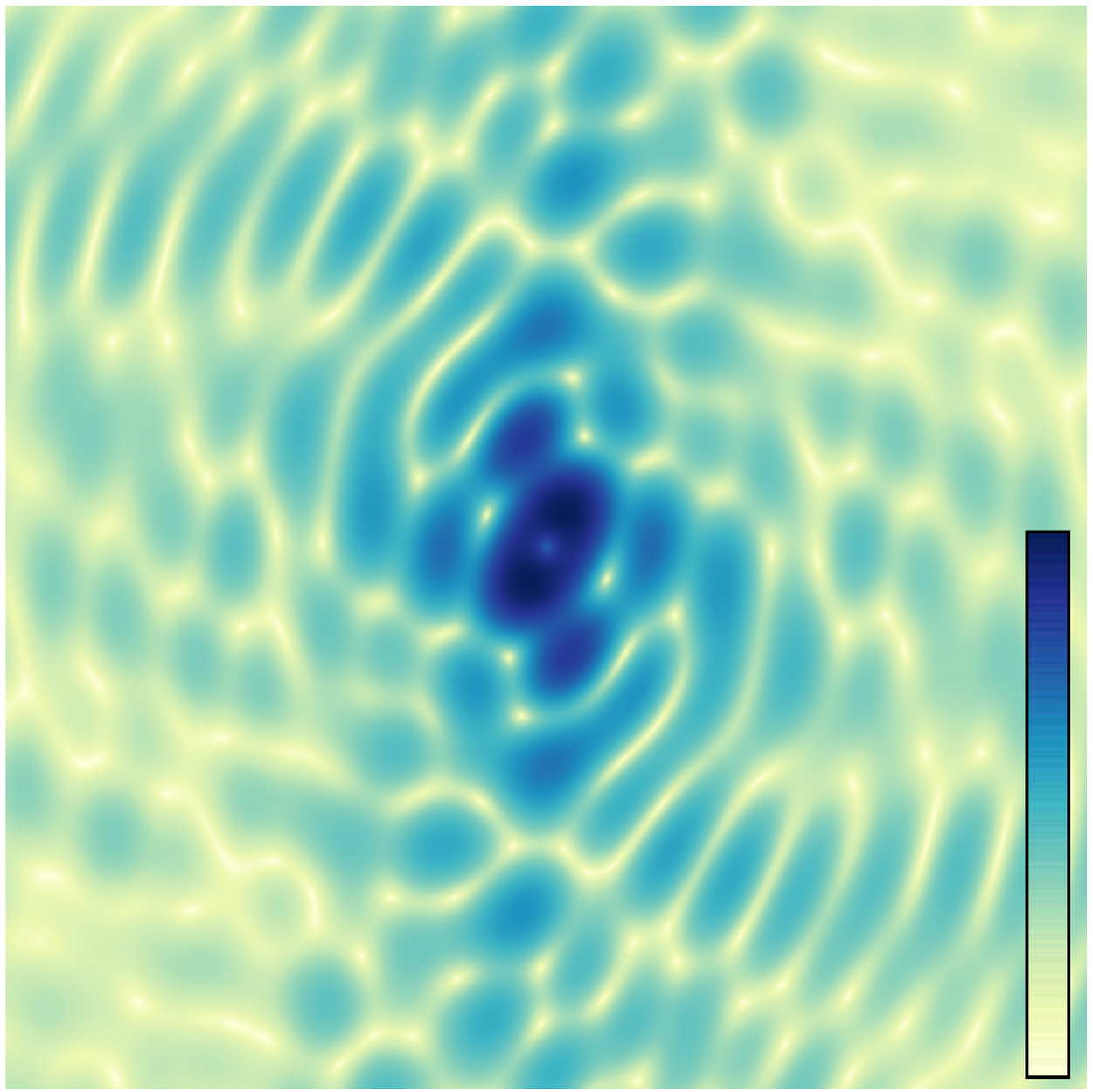
Marchesini et al. Phys Rev B **68** 140101 (2003)
Chapman et al, Nature Physics **2** 839 (2006)

Single particles

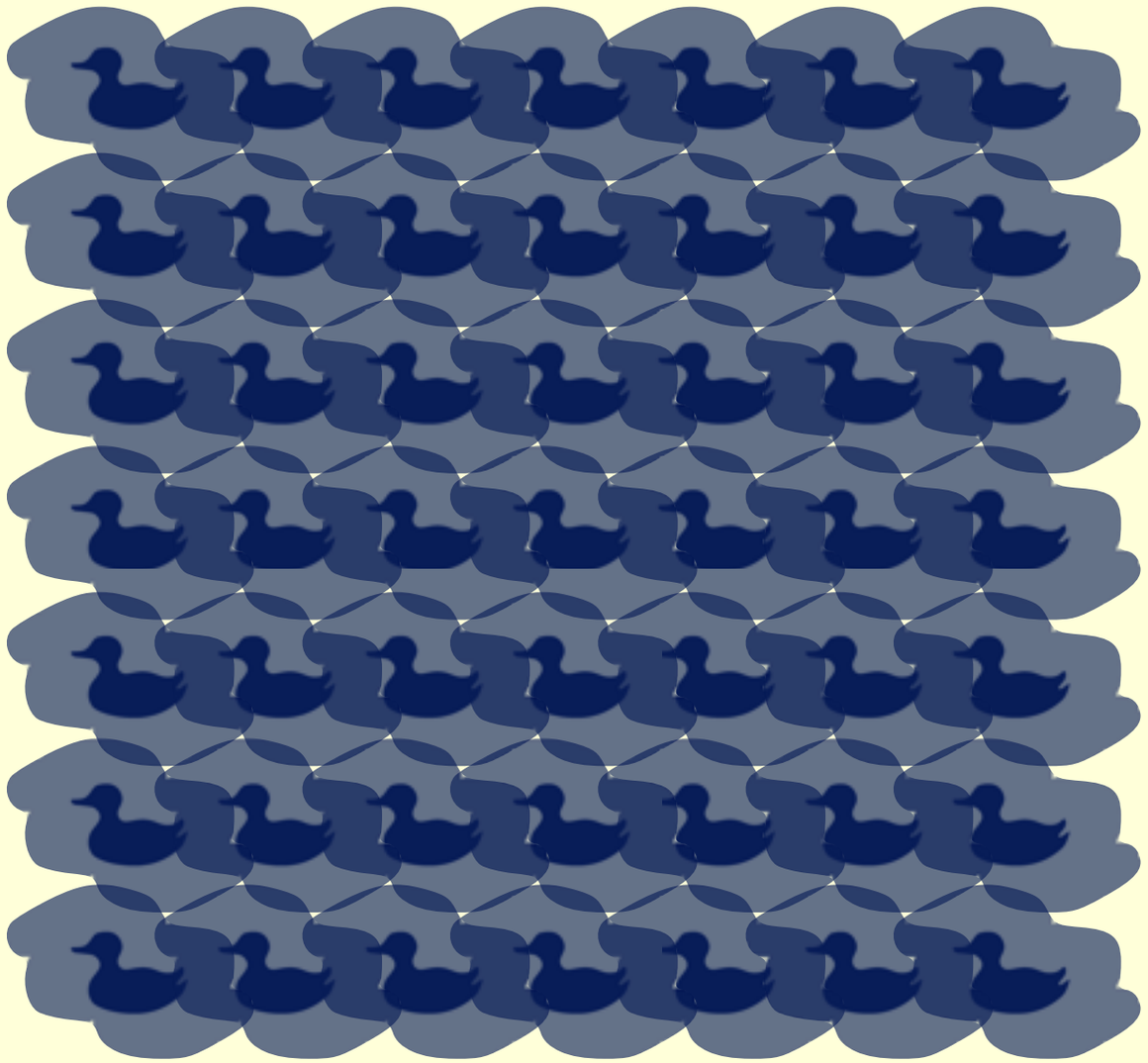
Crystal patterns: Bragg spots

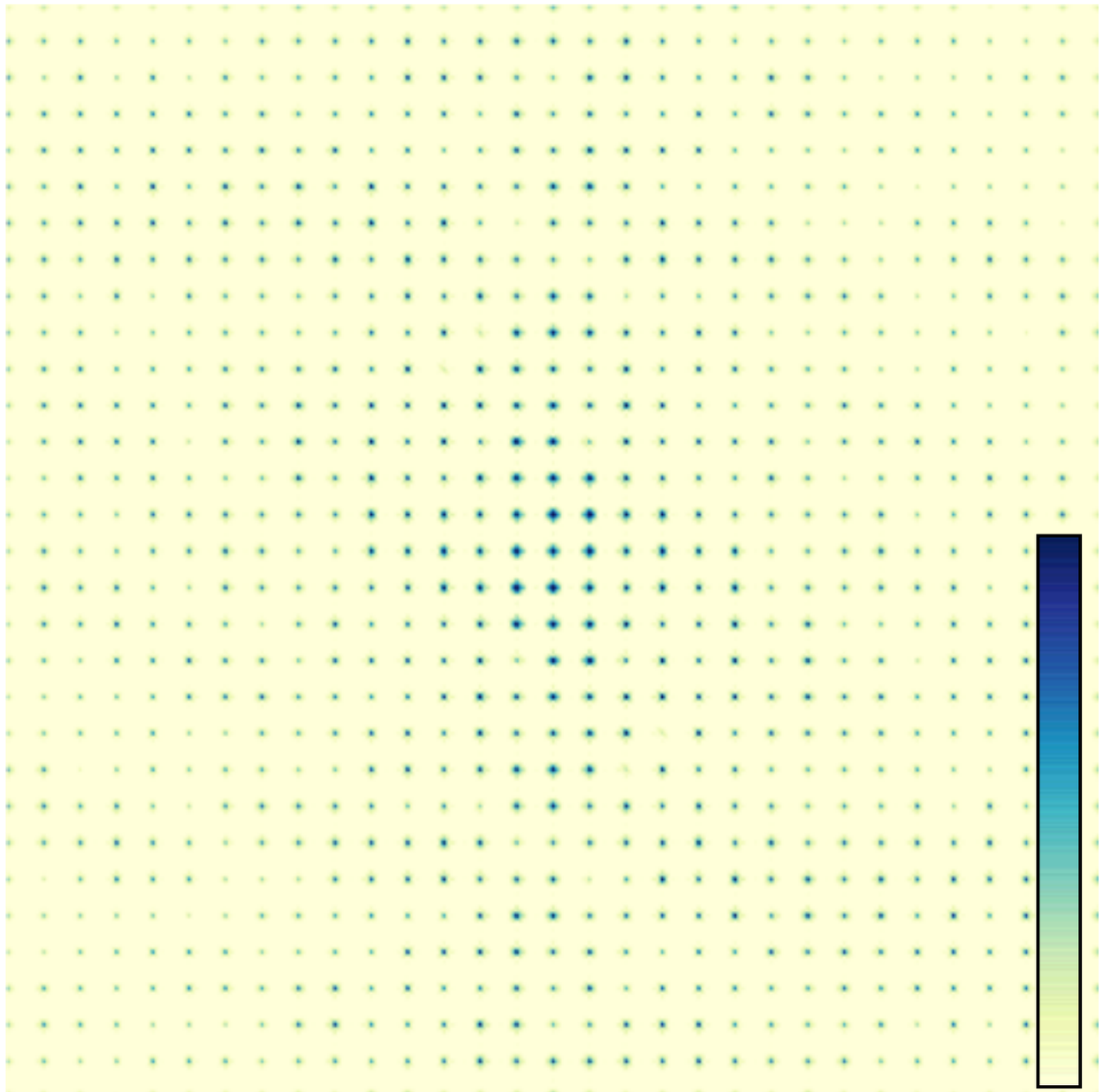
Continuous diffraction patterns



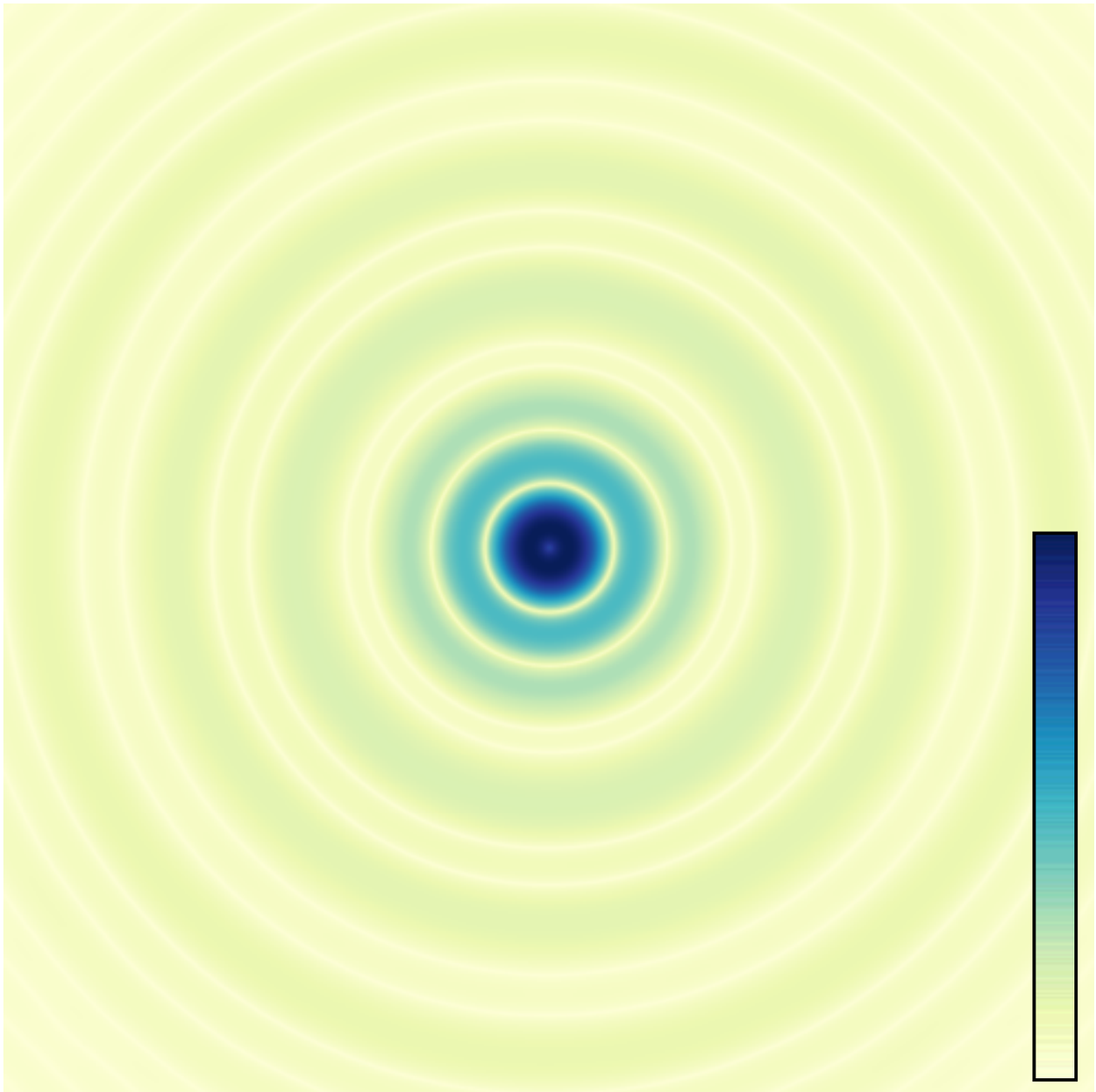


Crystals give Bragg spots

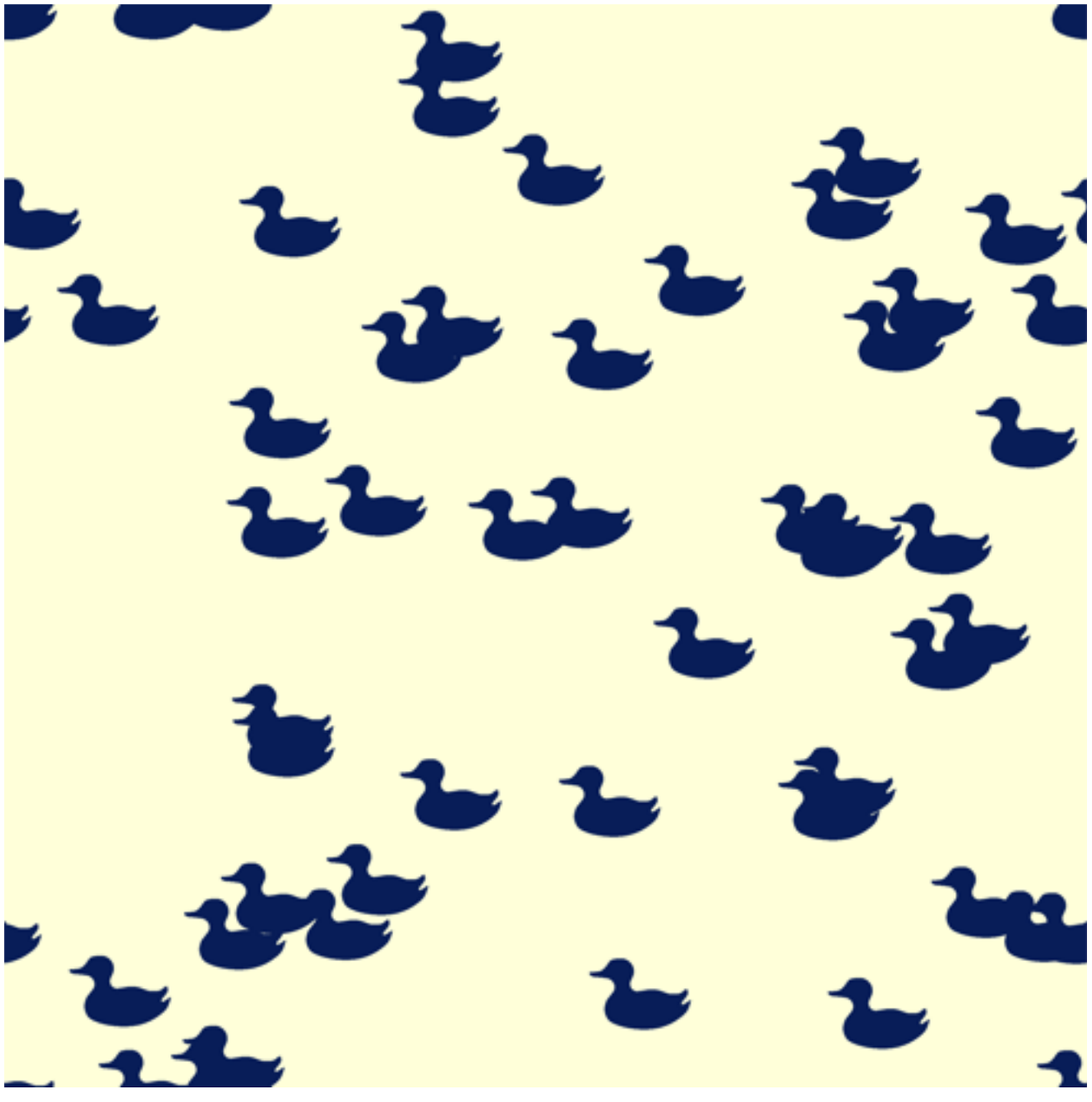


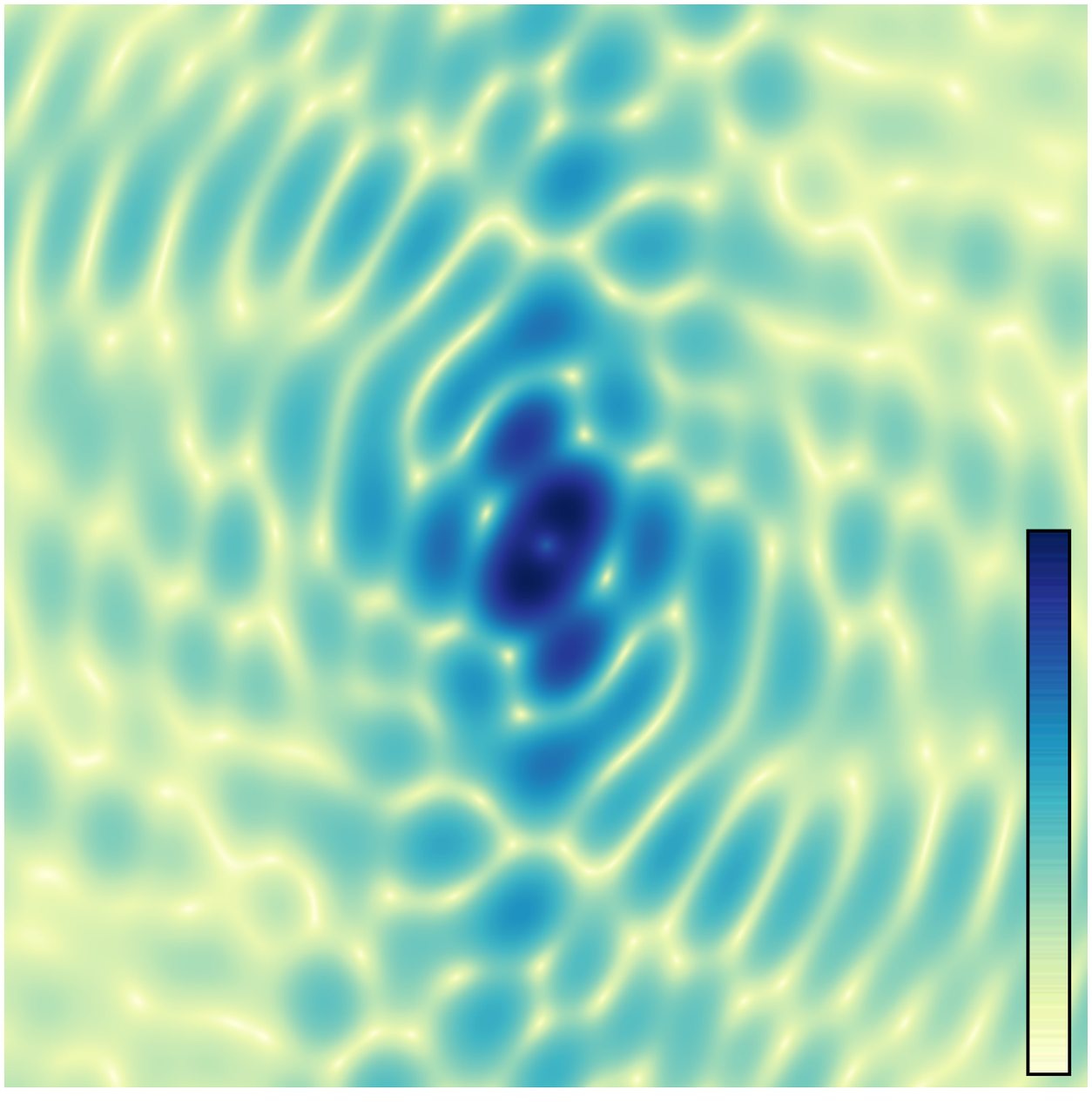


Solution scattering gives single-molecule diffraction, but orientationally averaged

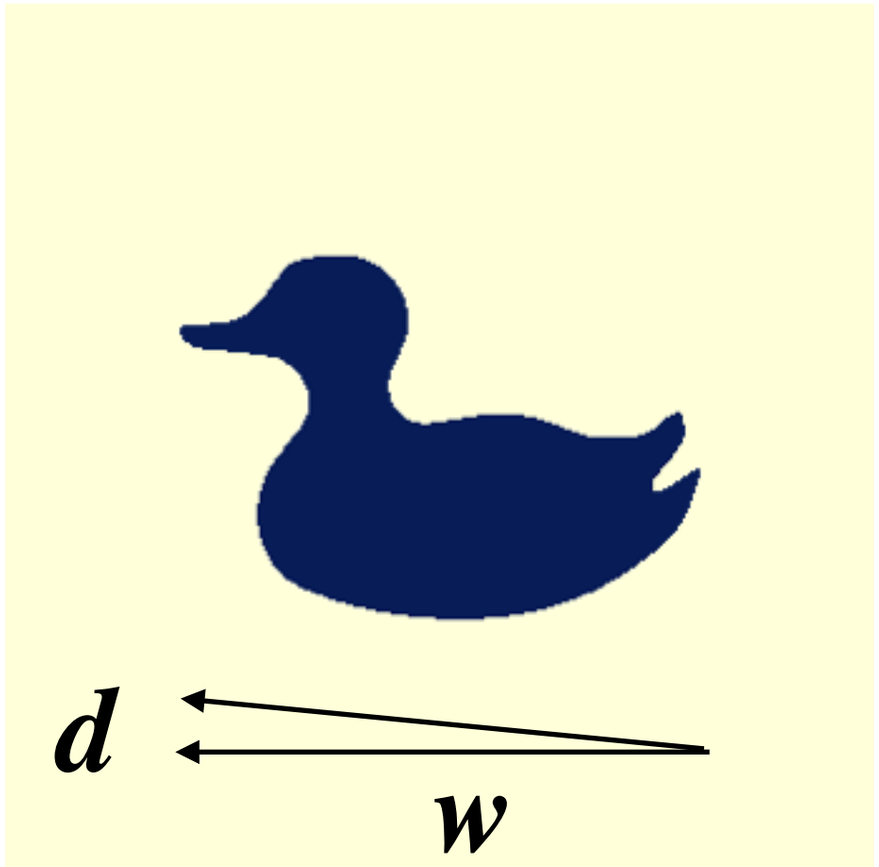


Aligned molecules yield a single-molecule pattern



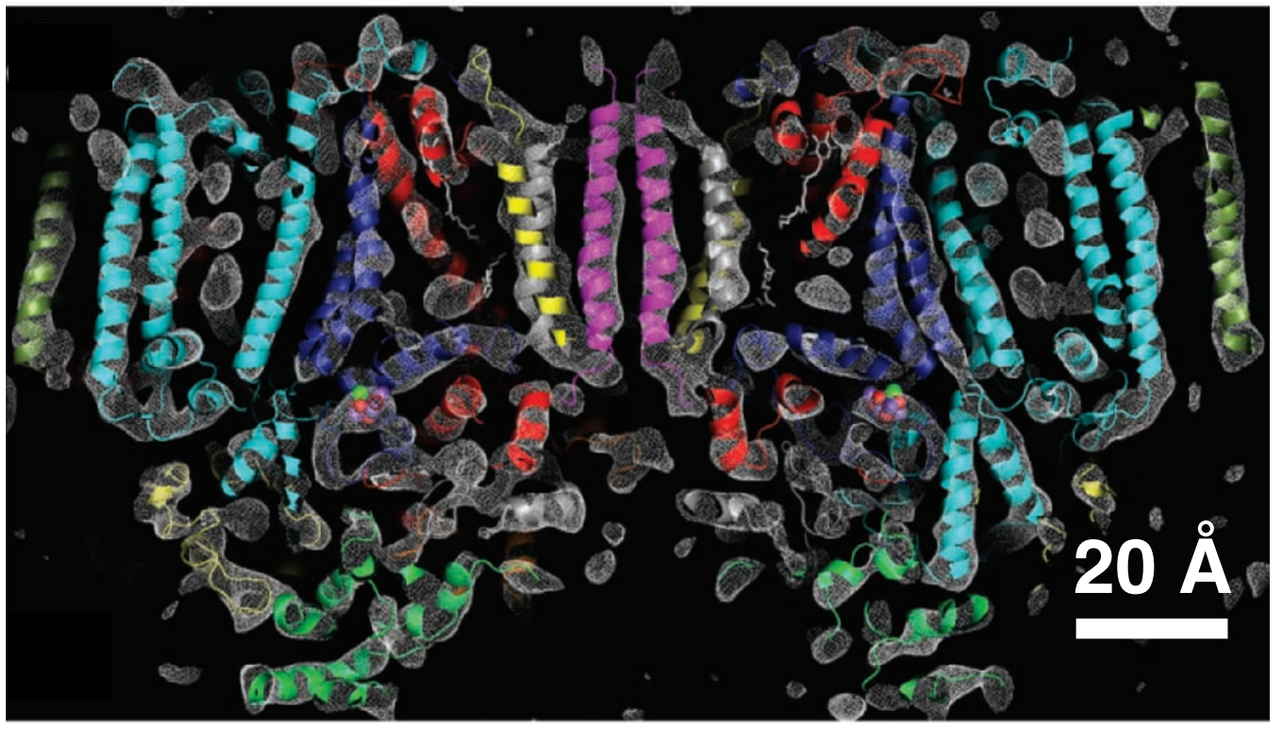


How well aligned do you need?



$$\Delta\phi = d/w$$

photosystem II

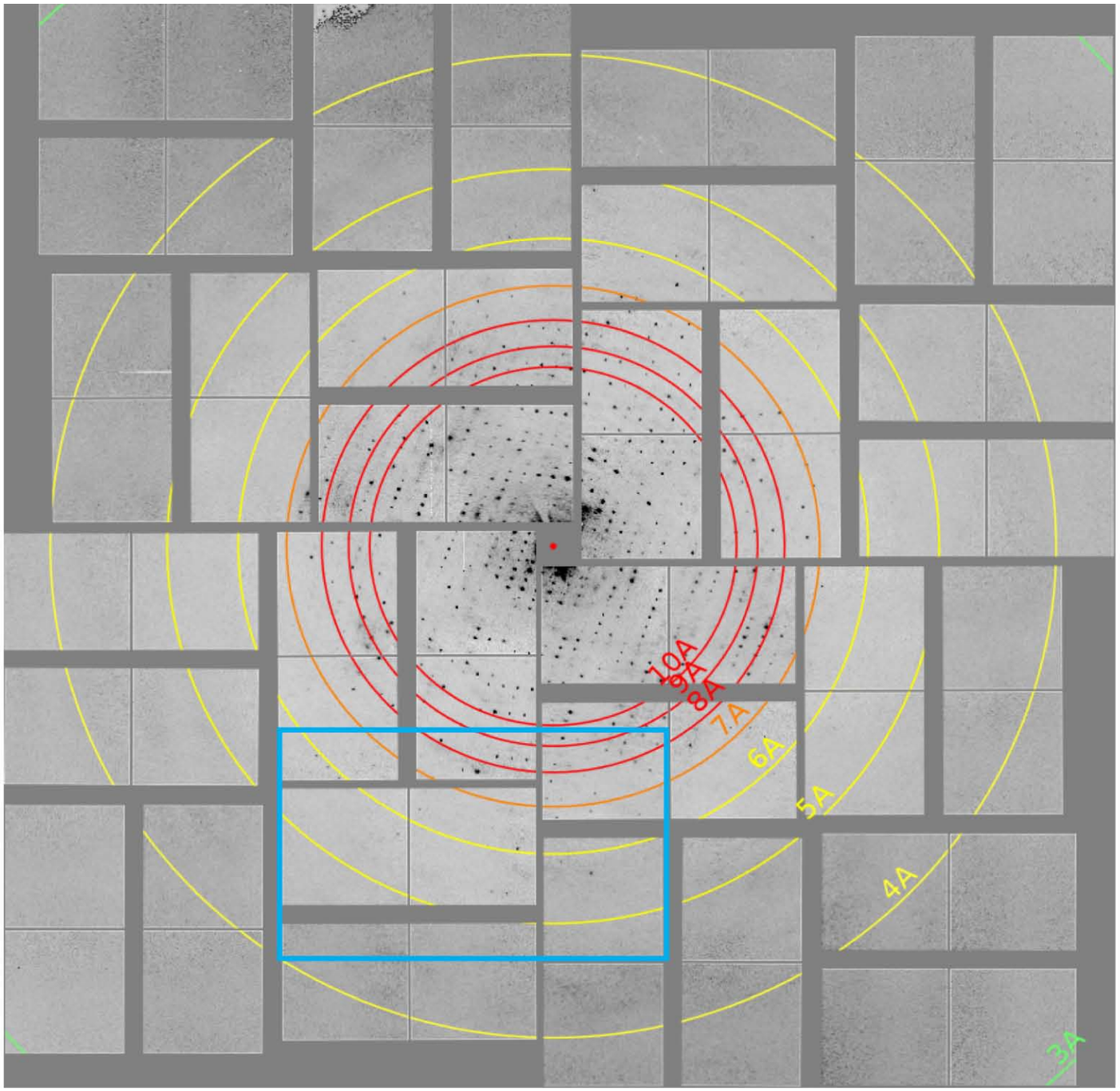


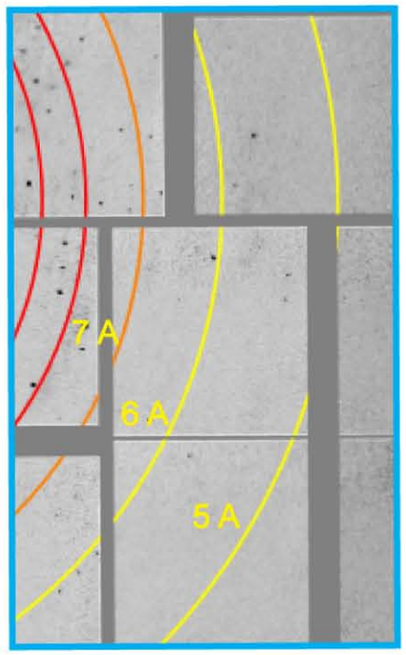
$$d = 3\text{Å}$$

$$w = 160\text{Å}$$

$$\Delta\phi = 1.1^\circ$$

Even bad crystals should attain the required level of alignment





$$d = 2\pi\sqrt{\langle D^2 \rangle}$$

$$d = 5\text{Å}$$

$$\sqrt{\langle D^2 \rangle} = 0.8\text{Å}$$

Extended Data Figure 2 | Background corrected diffraction pattern of a photosystem II microcrystal

Kuptiz et al, Nature **513**, 261 (2014)

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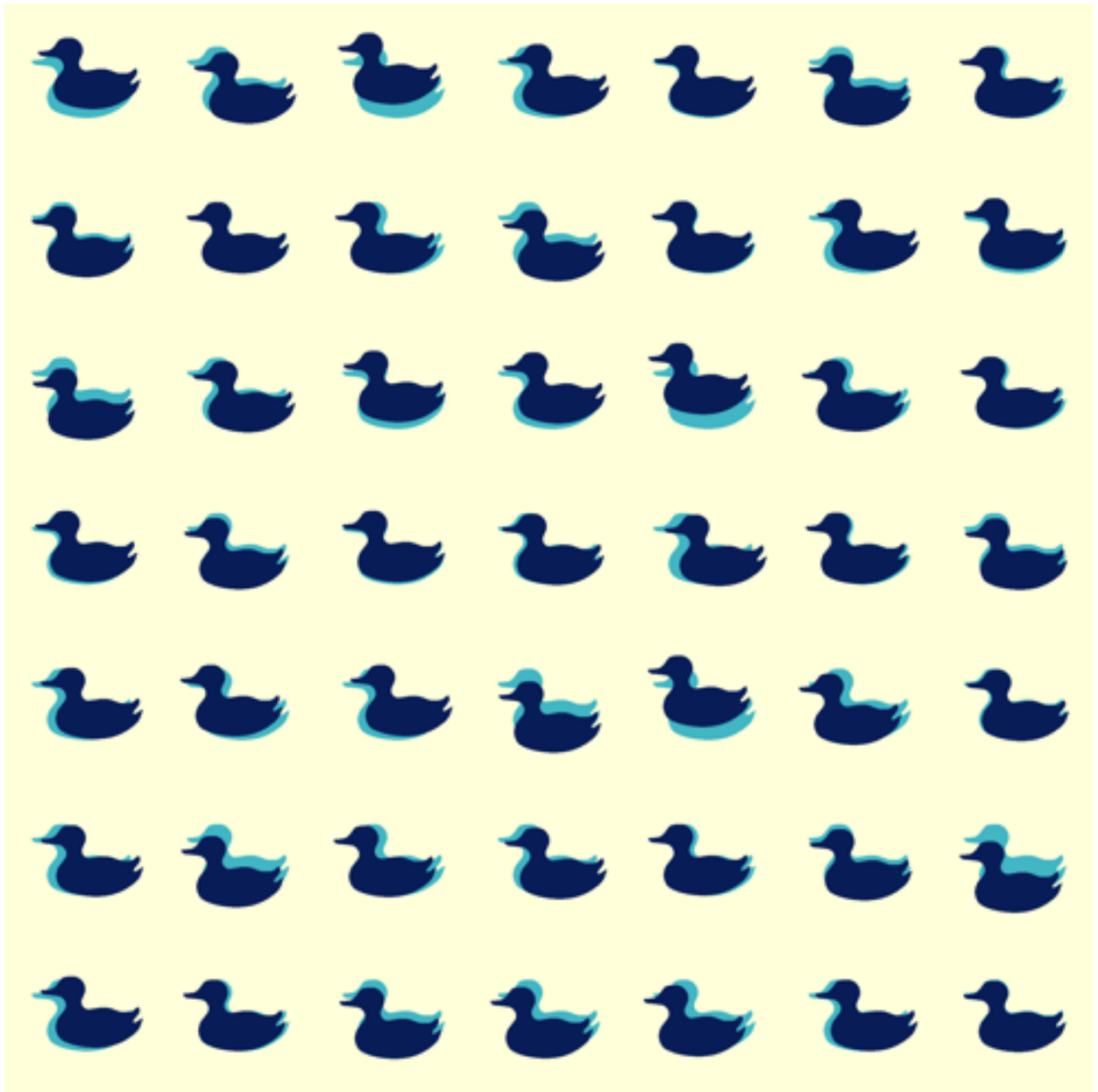
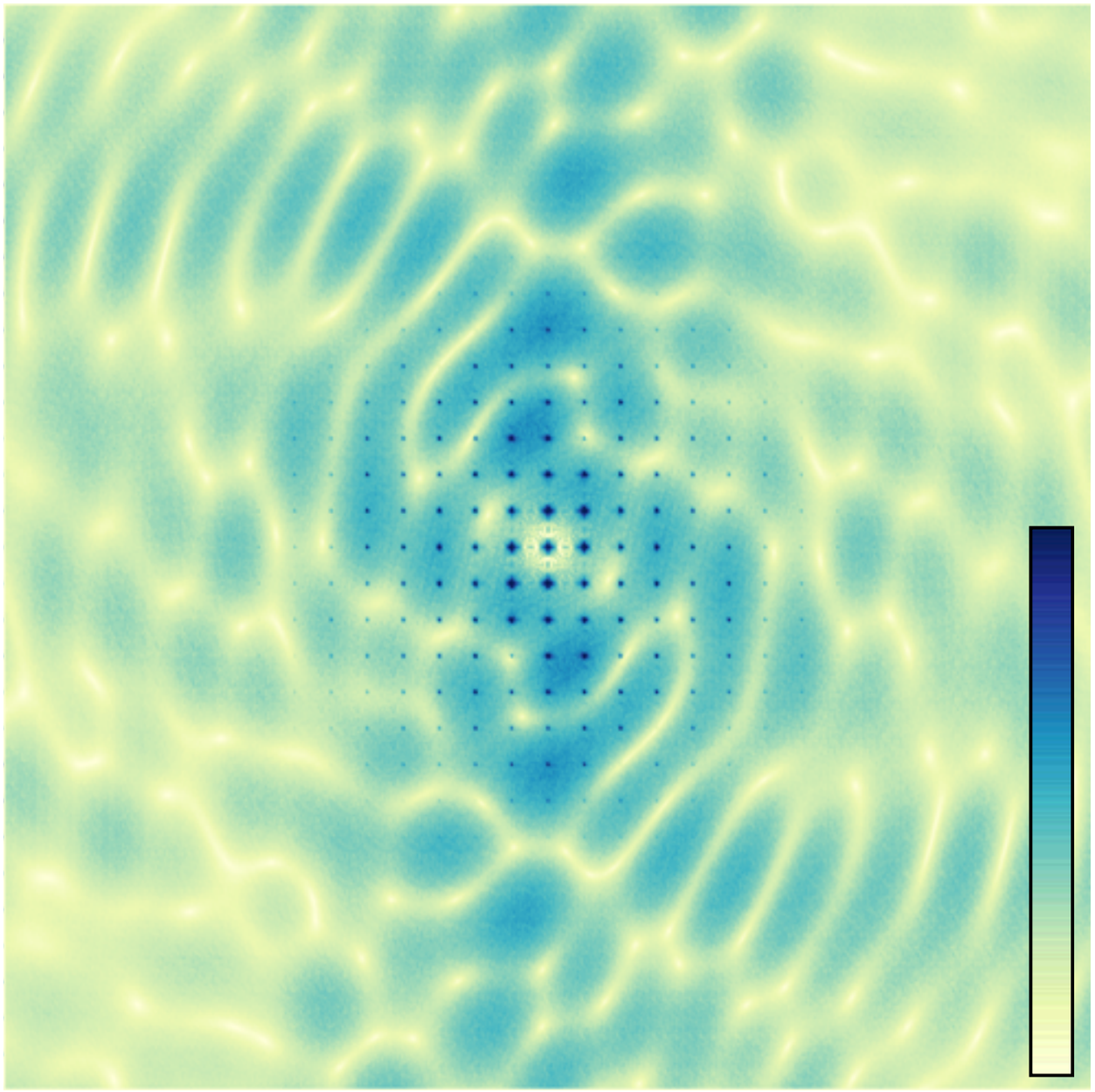




$$\langle I(\mathbf{q})\rangle = |f(\mathbf{q})|^2 \exp(-q^2\sigma^2) + \sum_i f_i^2 \left(1 - \exp(-q^2\sigma^2)\right)$$

$$\sigma^2 = \langle D^2 \rangle$$

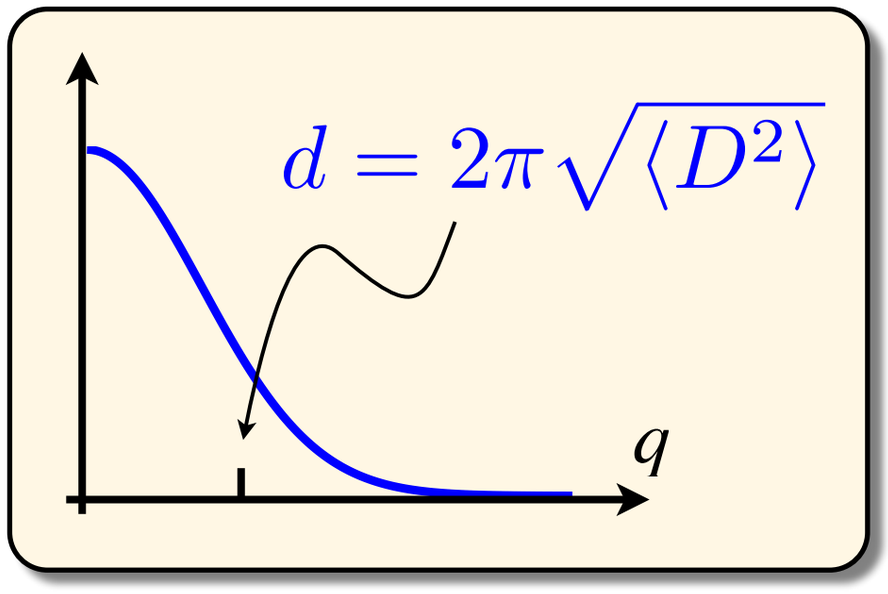
Crystal diffraction is sensitive to atomic displacements

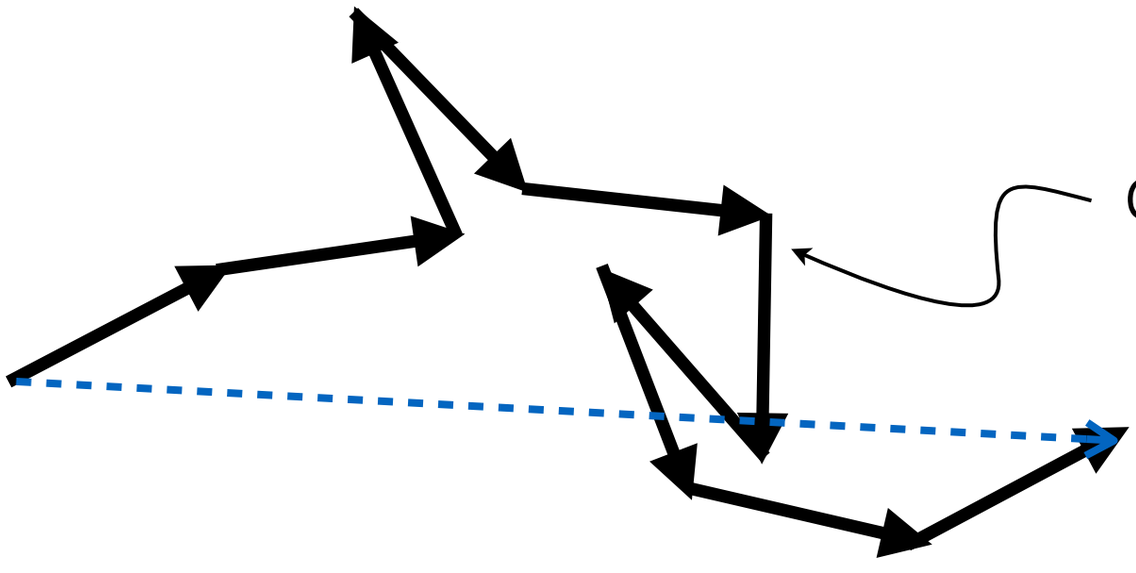
$$I(\mathbf{q}) = |f(\mathbf{q})|^2 = \left| \sum_i f_i \exp(i\mathbf{q}\cdot\mathbf{x}_i) \right|^2$$

$$|\mathbf{q}| = \frac{2\pi}{d}$$

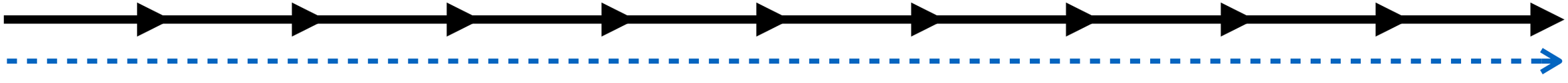
$$\mathbf{x}_i \to \mathbf{x}_i + \mathbf{D}_i \qquad \langle \mathbf{D}_i \rangle = 0$$

$$= |f(\mathbf{q})|^2 \exp(-q^2 \langle D^2 \rangle) + \sum_i f_i^2$$





displacement of atom by $d/4$



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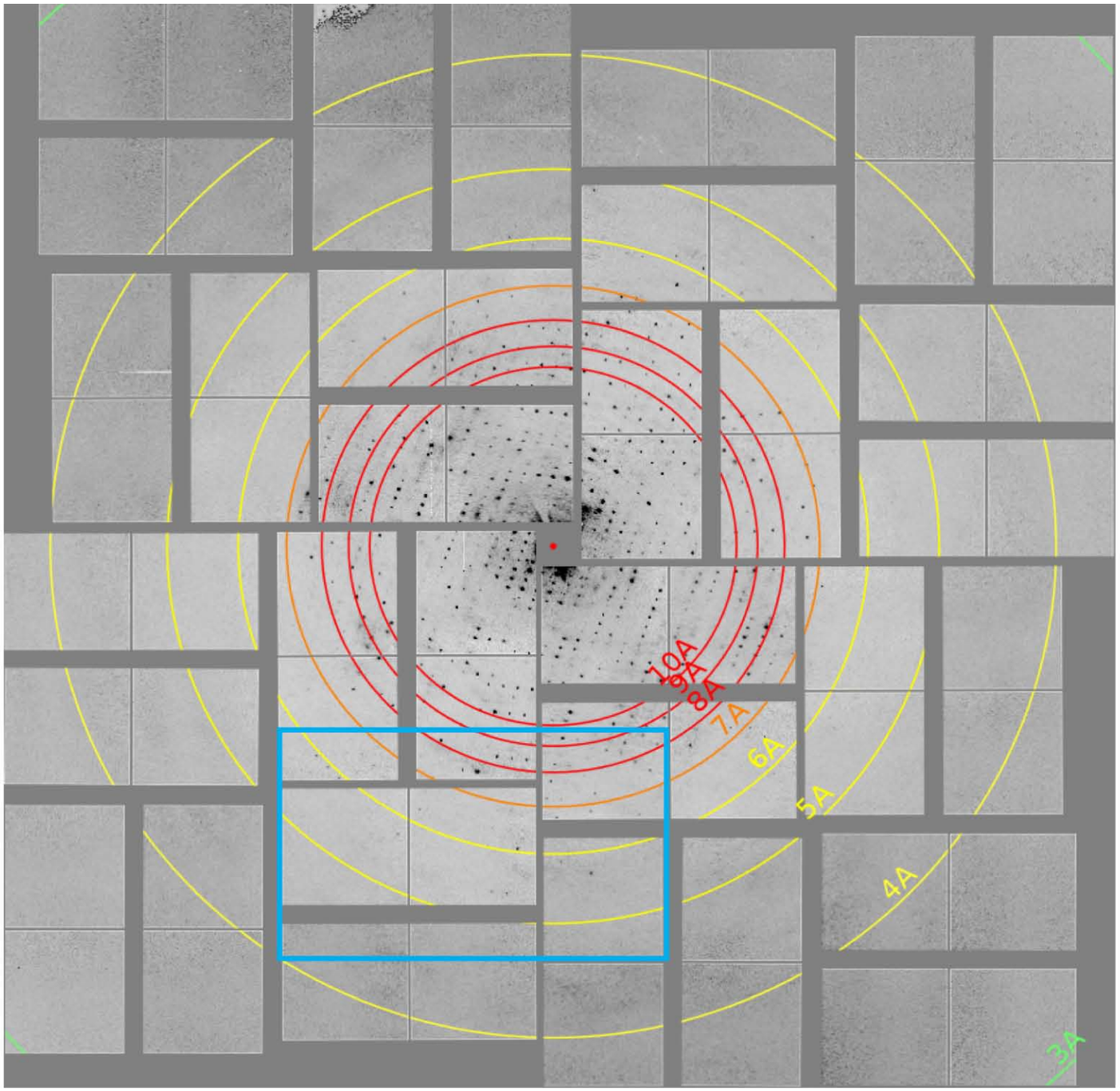

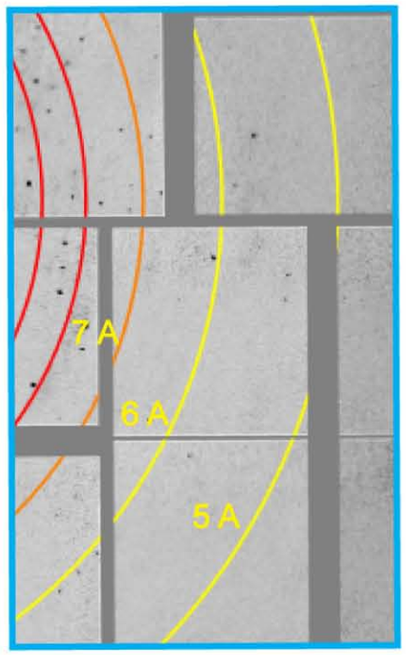

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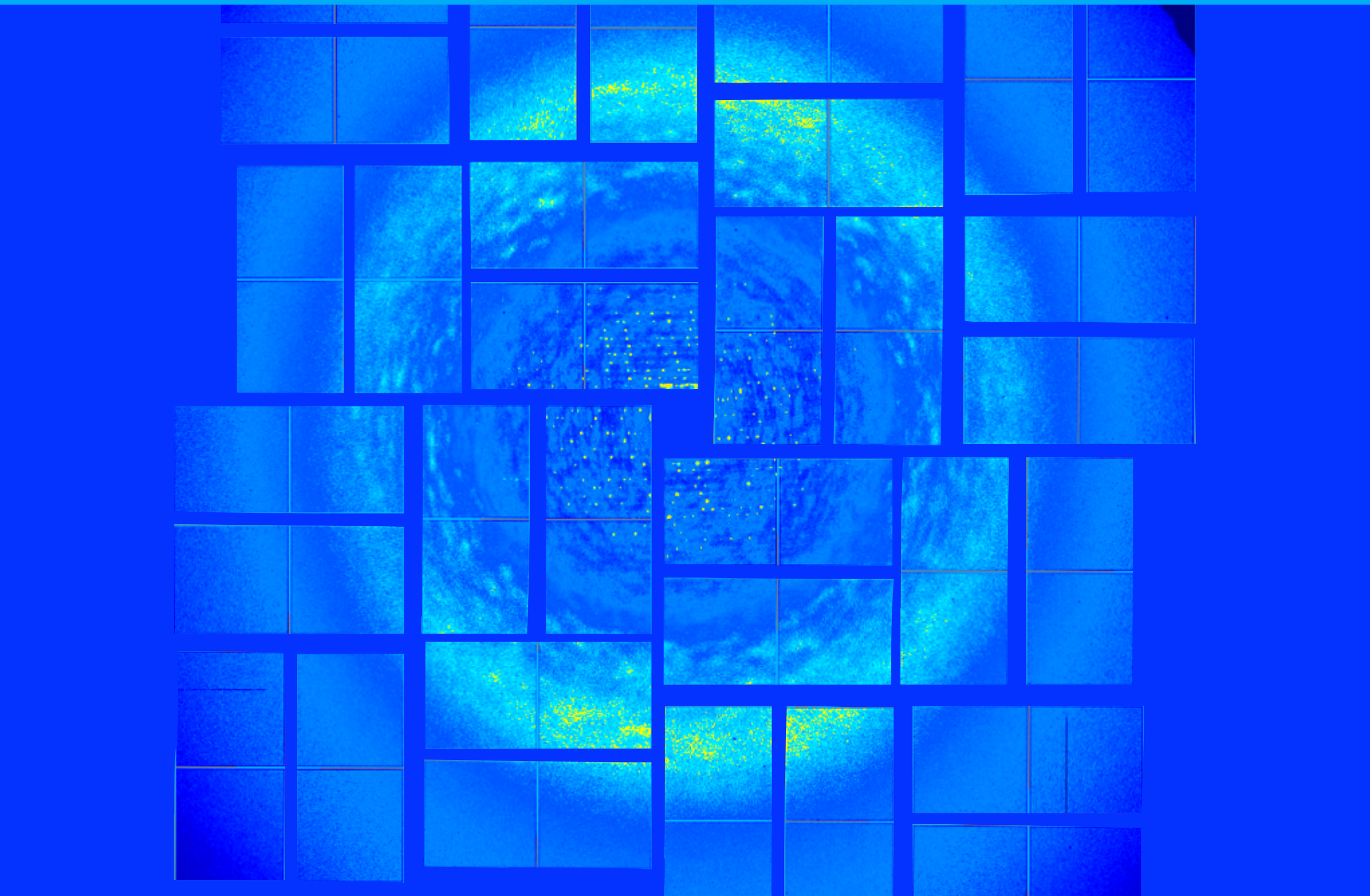
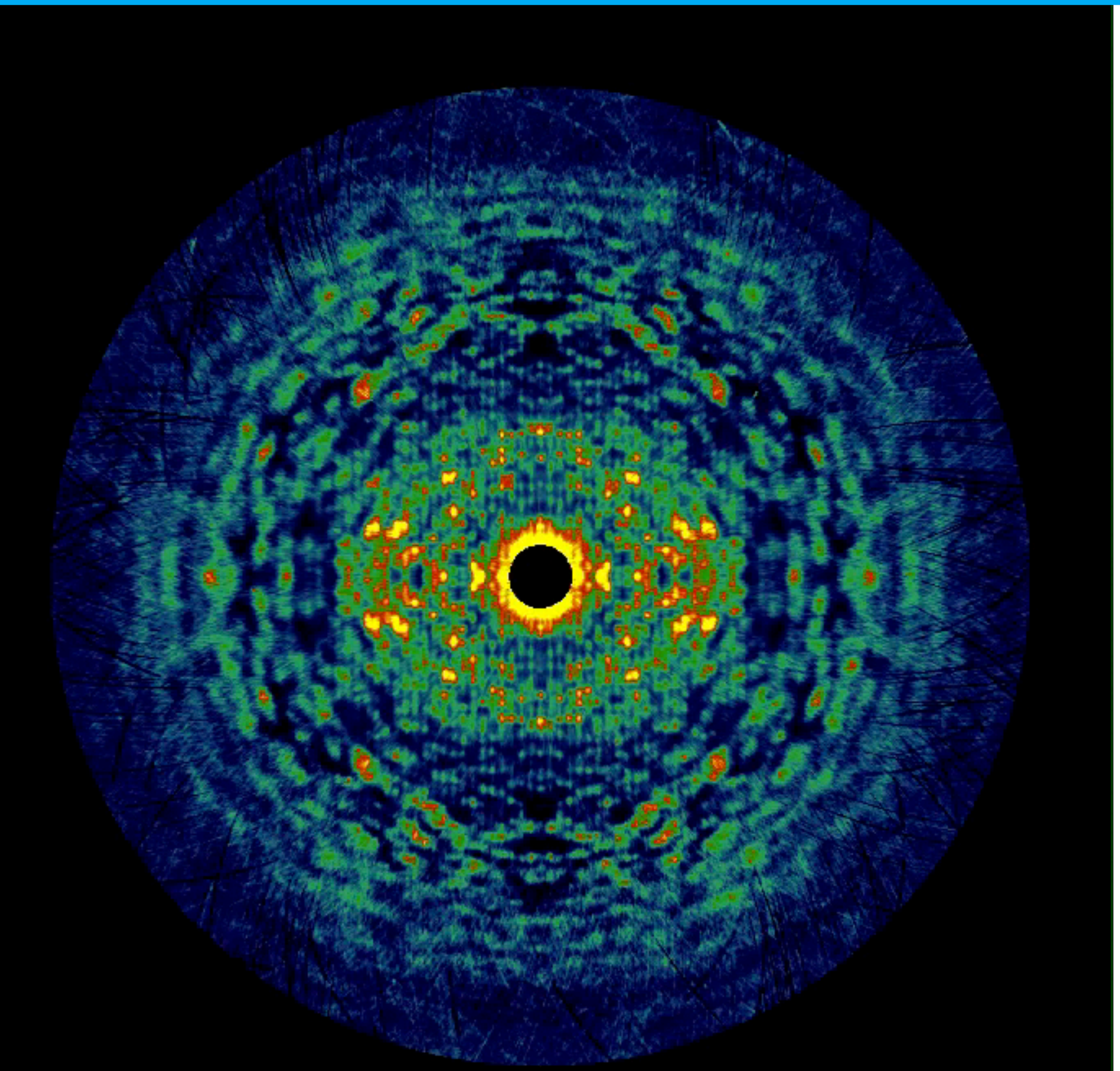
Extended Data Figure 2 | Background corrected diffraction pattern of a photosystem II microcrystal

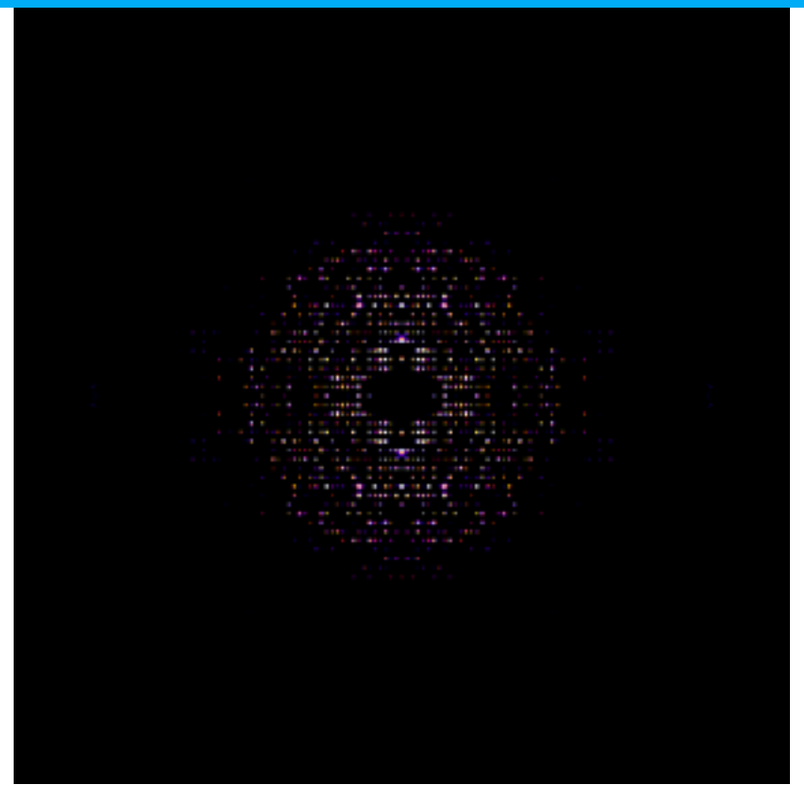
Kuptiz et al, Nature **513**, 261 (2014)

You can see a lot just by looking



By averaging thousands of patterns, a strong single molecule diffraction pattern emerges





Anton Barty
Oleksandr Yefanov

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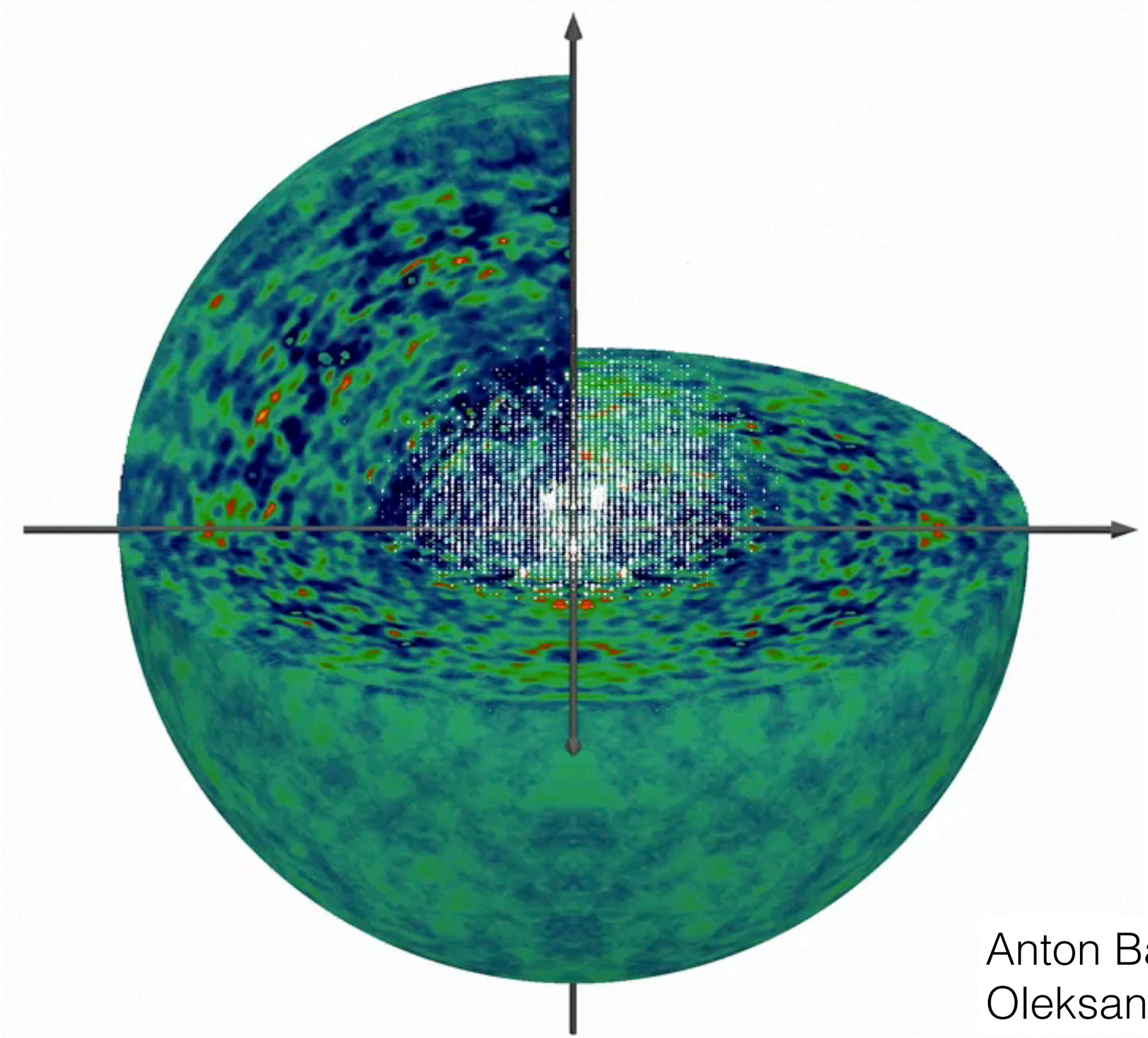
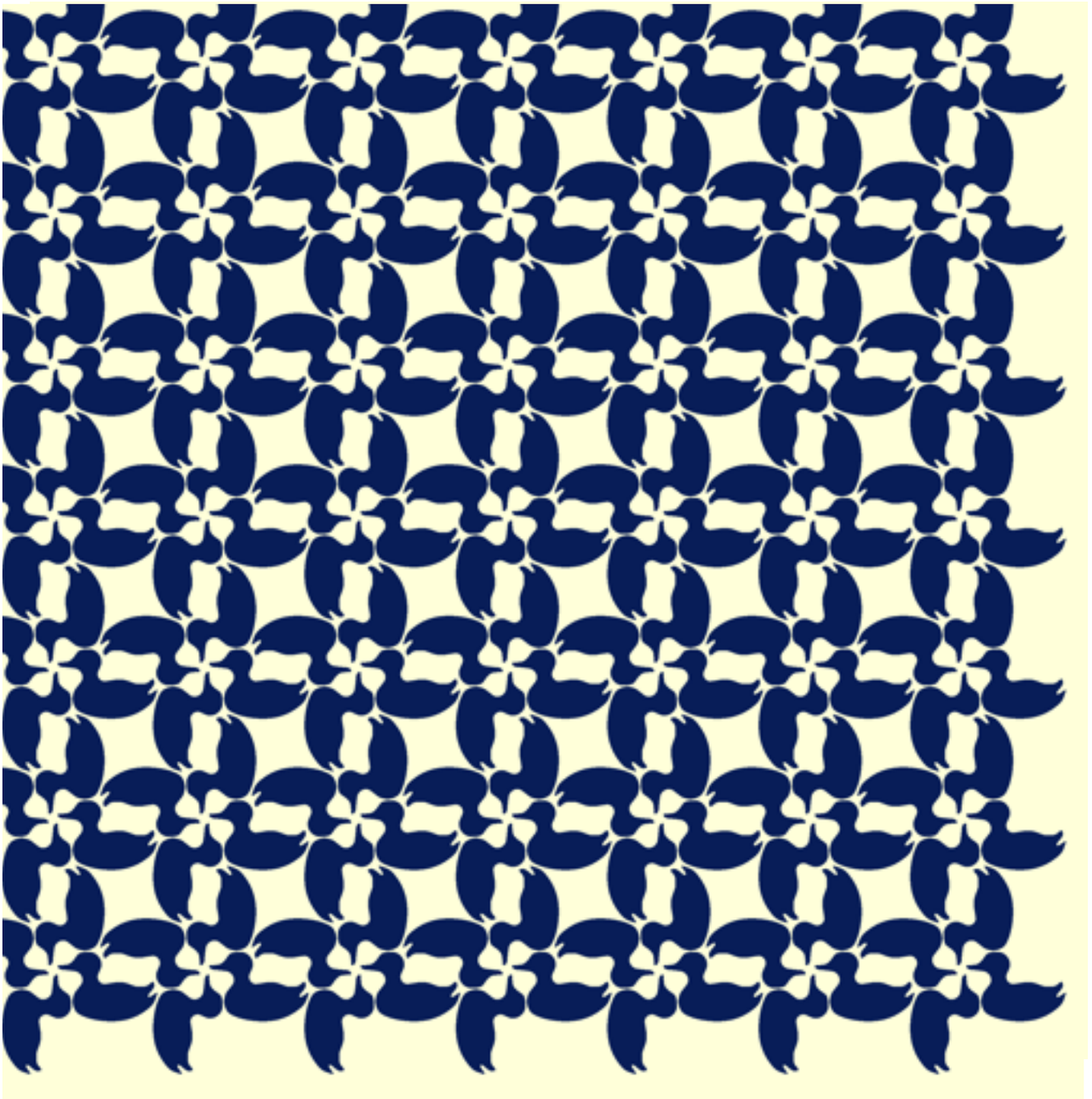
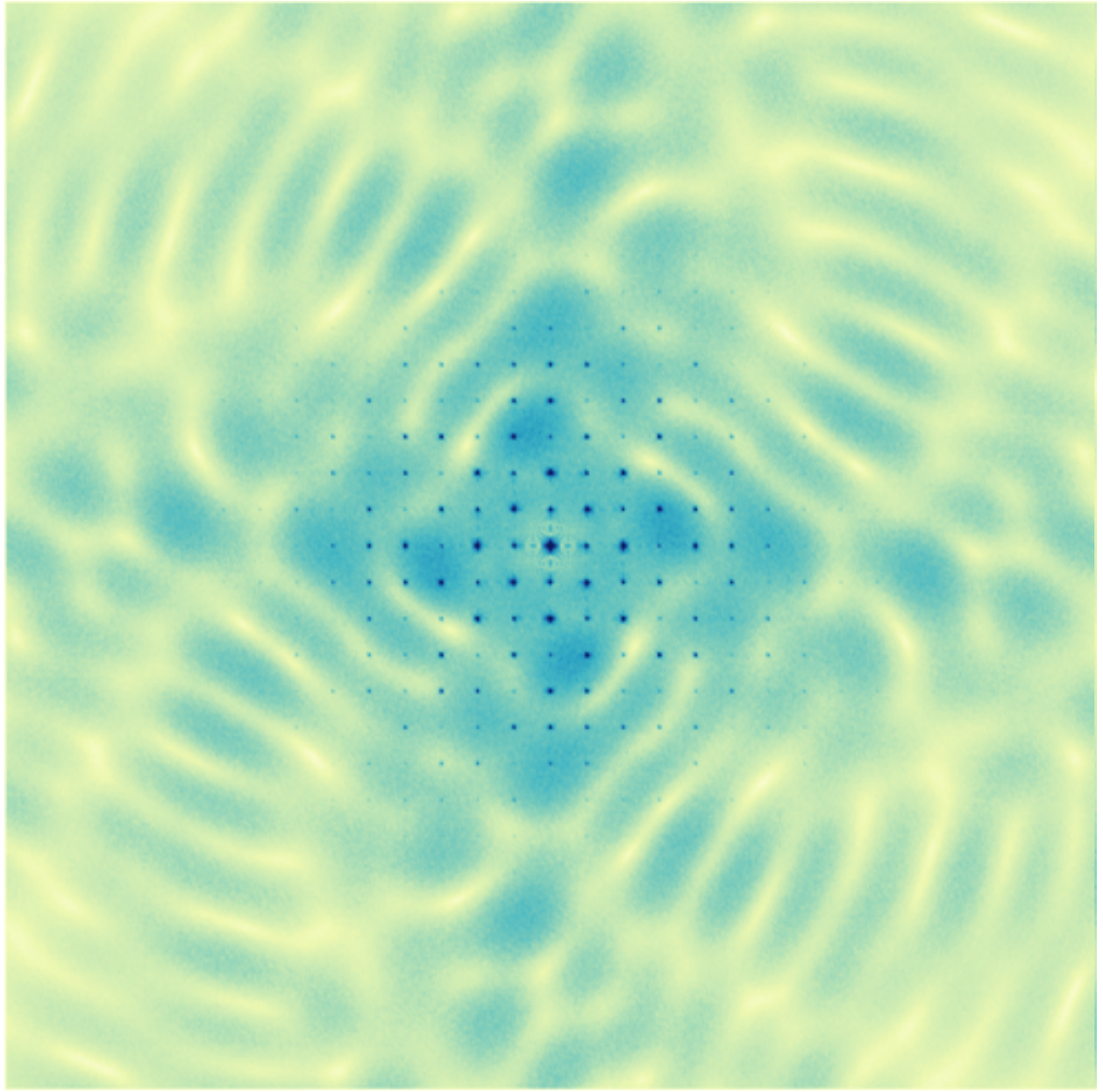


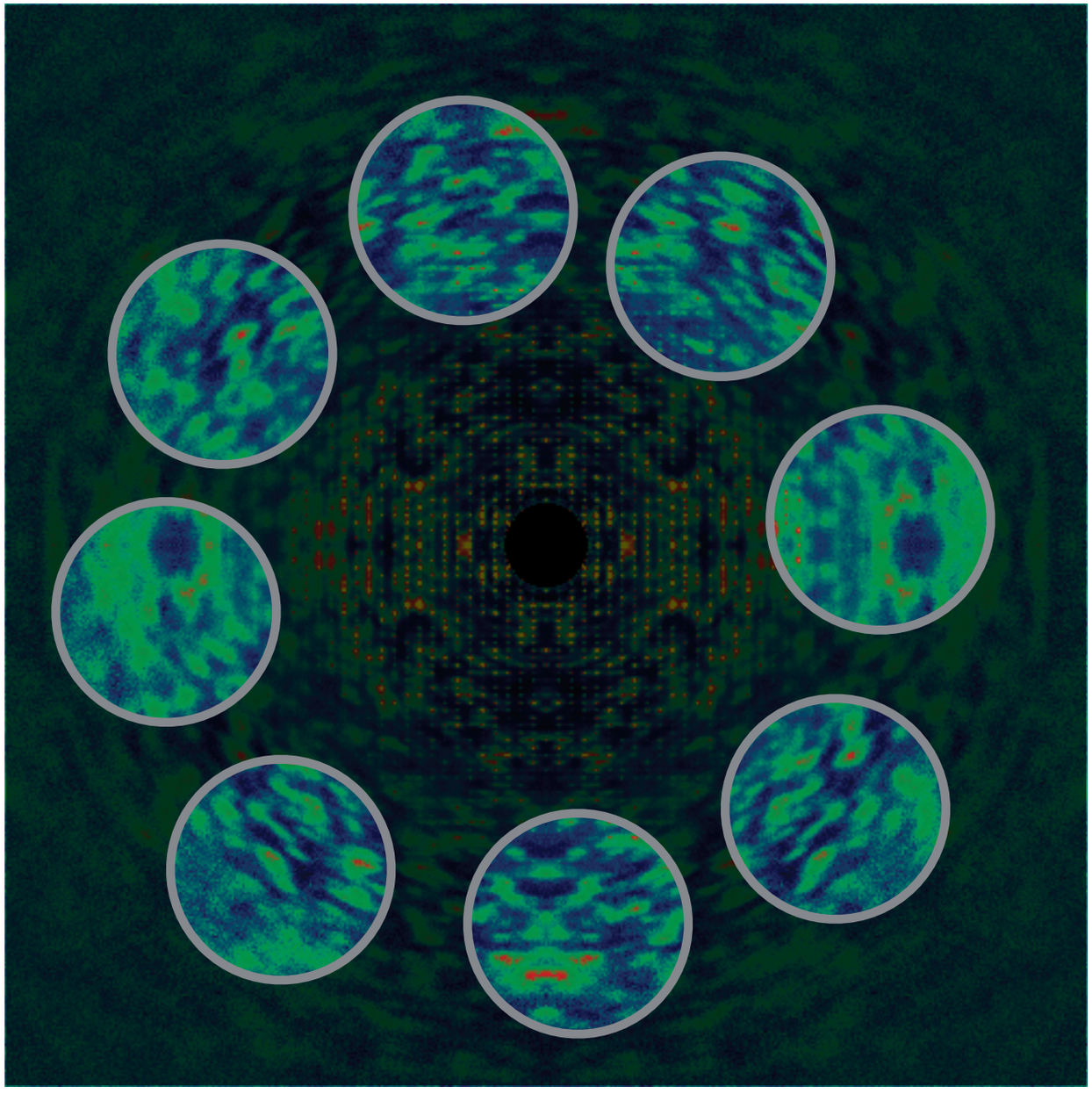
Anton Barty
Oleksandr Yefanov

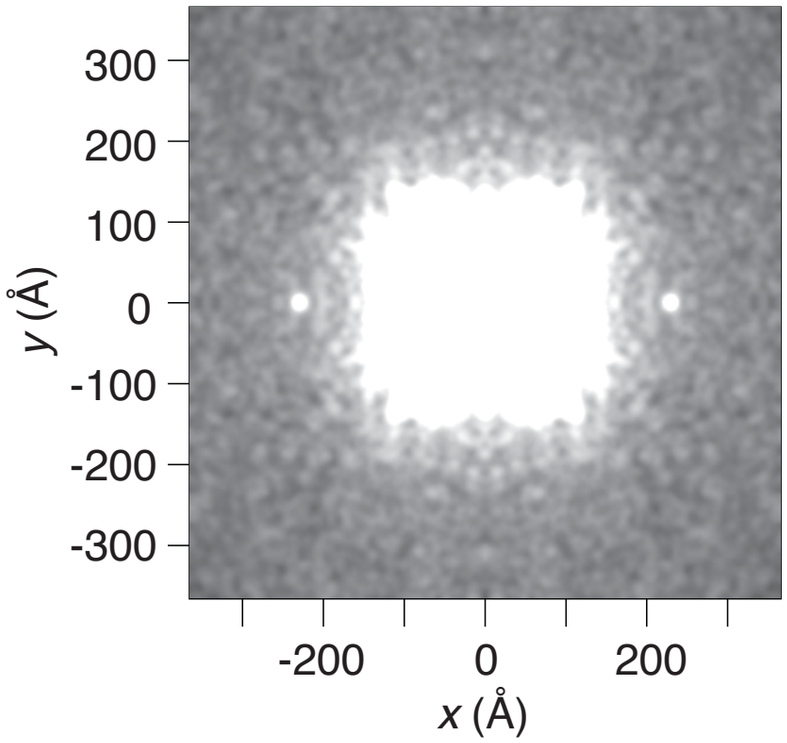
The orientational symmetry of the crystal is preserved, but not the translational symmetry





The rigid-body unit is consistent with the photosystem II dimer



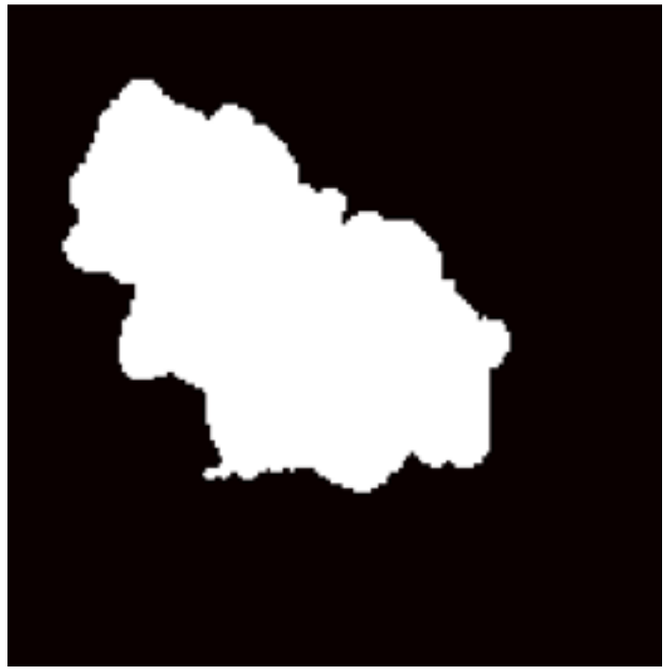


We performed iterative phasing of the “single molecule” diffraction

Phasing pipeline:

1. Obtain 4.5 Å refinement from Bragg peaks
2. Generate a support
3. Iterative phasing to 3.3 Å (using Elser’s difference map)
4. Improve the molecular model

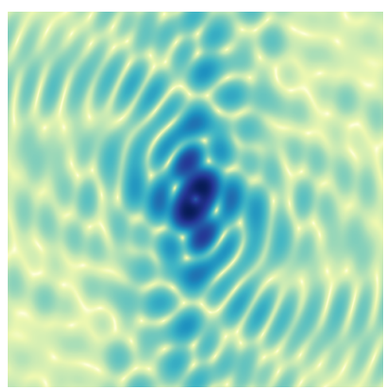
Real space constraint



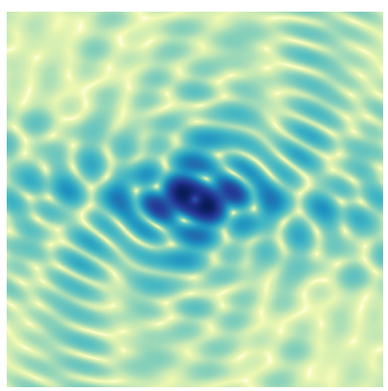
support

Reciprocal space constraint (n^{th} iterate)

$$\tilde{\psi}'_n(\mathbf{q}) = \sqrt{\frac{I(\mathbf{q})}{\sum_g \left|\tilde{\psi}_n(g\mathbf{q})\right|^2}}\,\tilde{\psi}_n(\mathbf{q})$$



$g = 1$



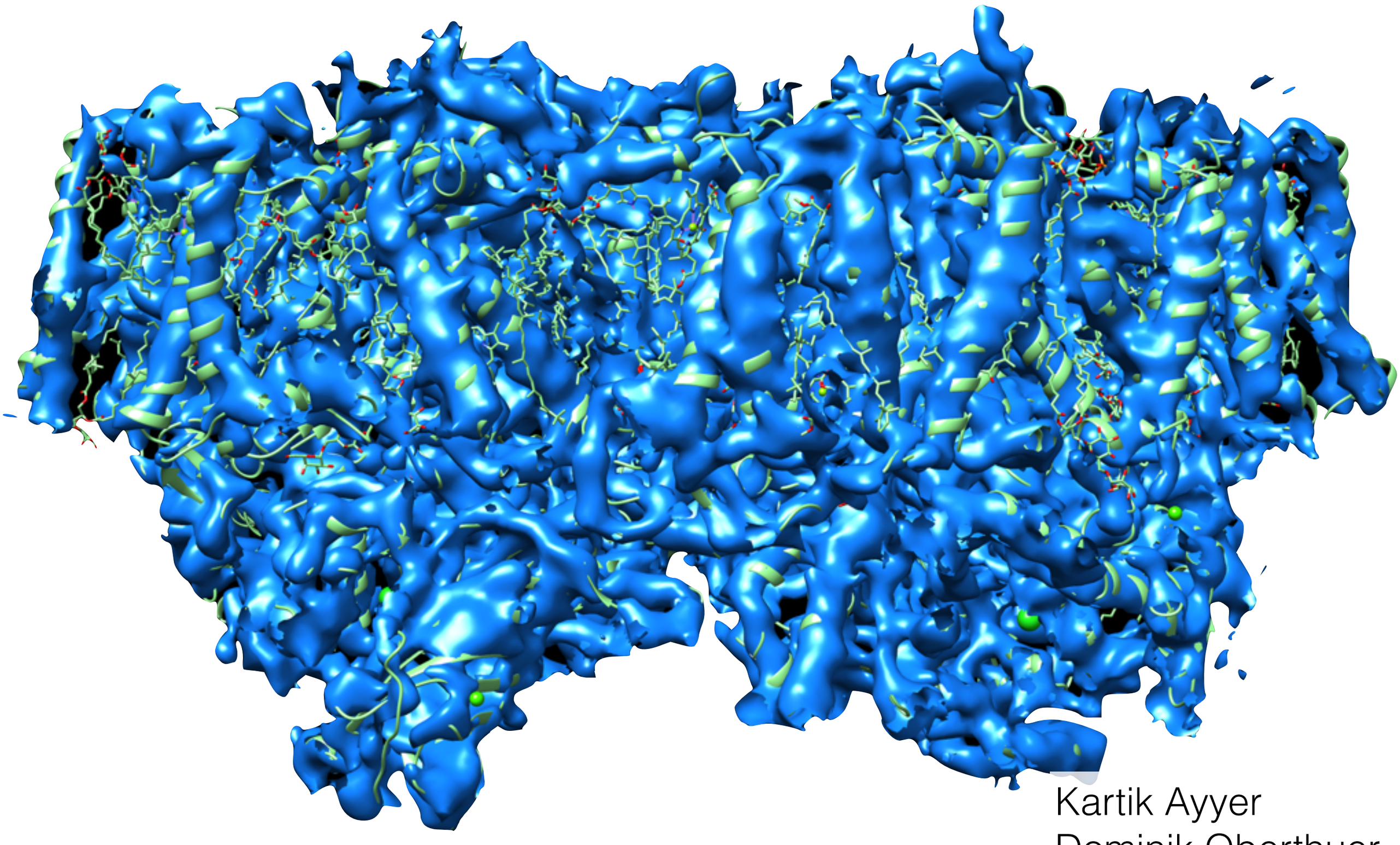
$g = 2$

…

Elser, JOSA A 20 (2003)
Elser & Millane, Acta Cryst A 64 (2008)

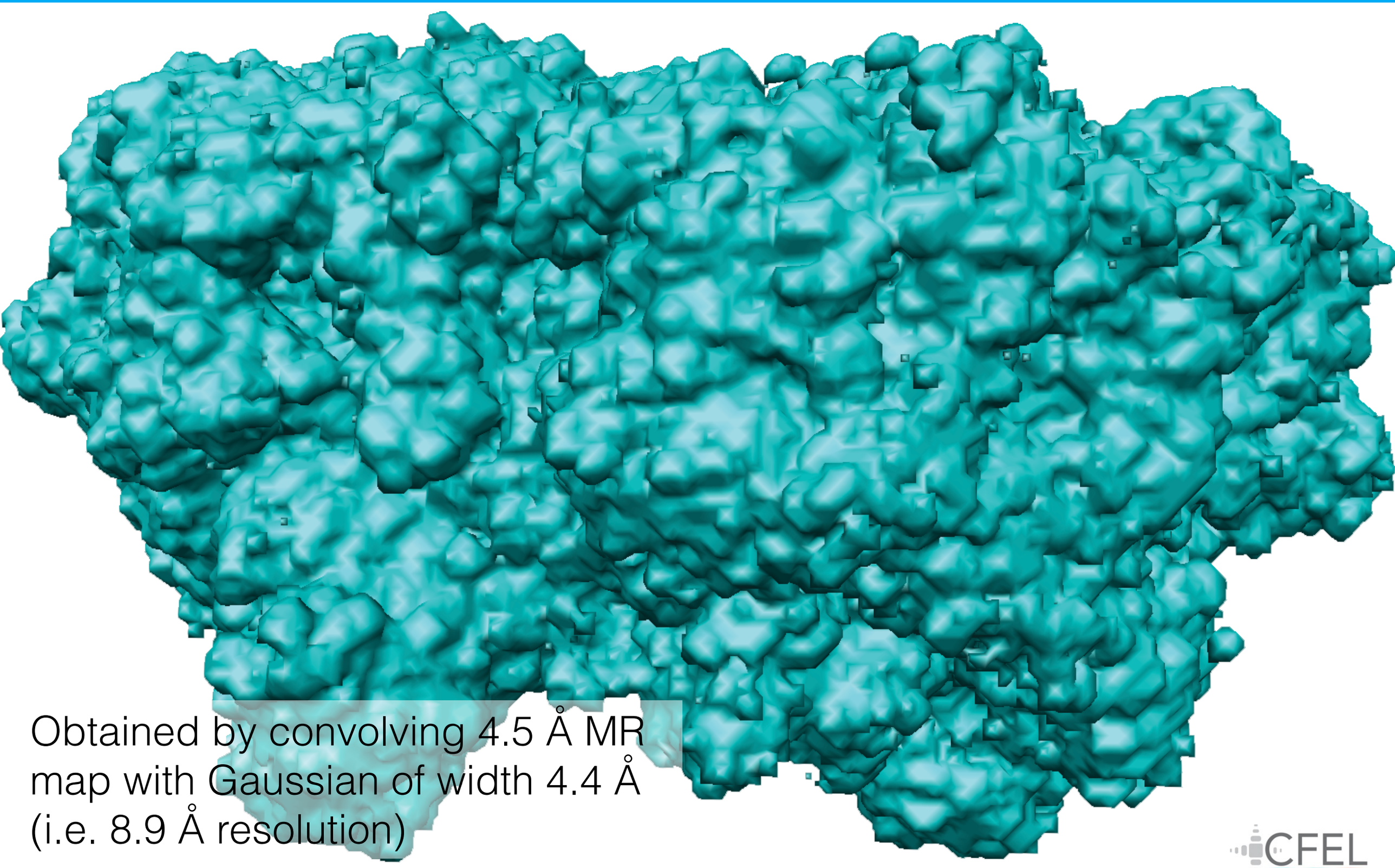
Kartik Ayyer

Electron density map from Bragg peaks alone (4.5 Å)



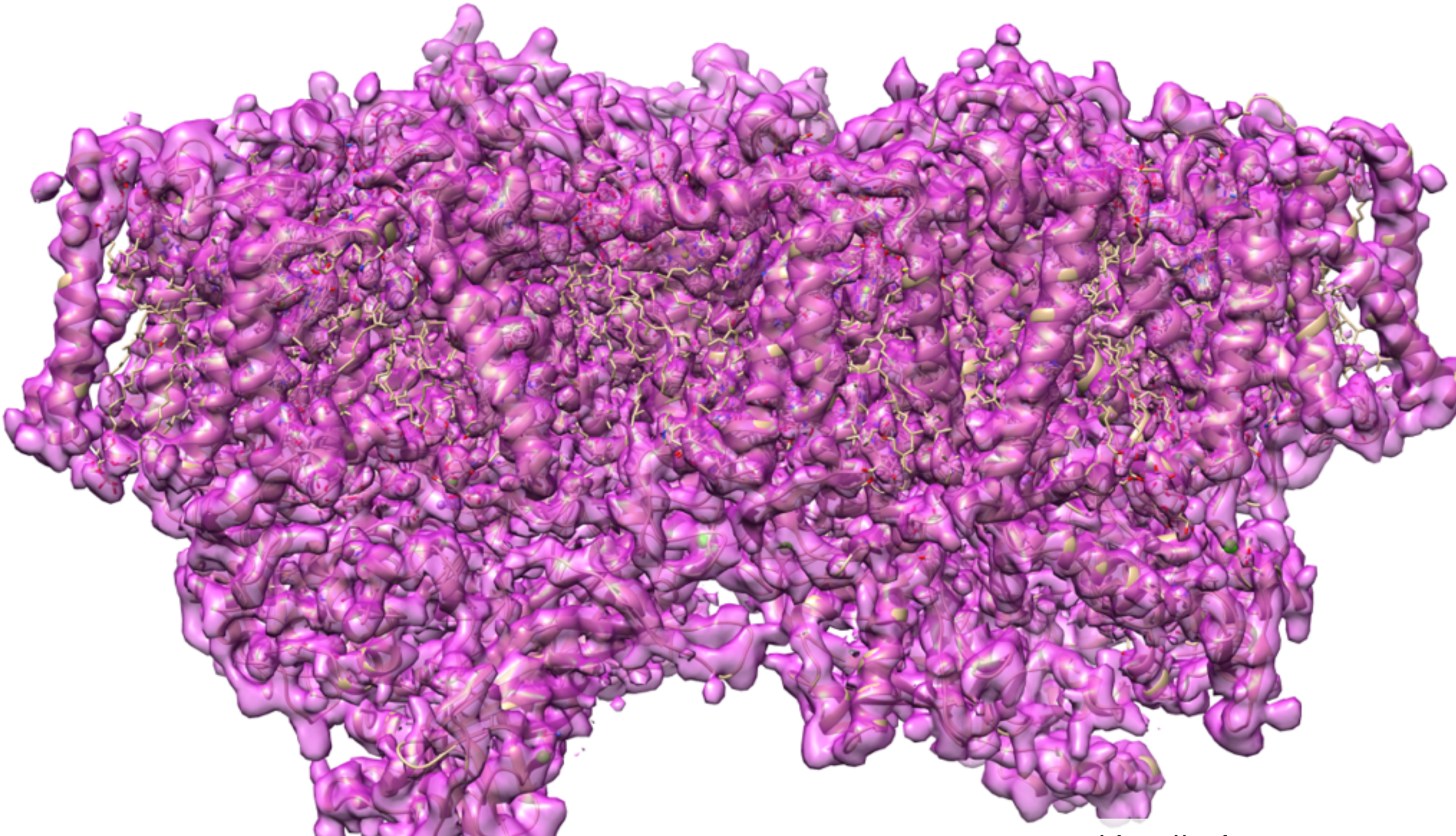
Kartik Ayyer
Dominik Oberthuer

The low-resolution support constrains the phases



Obtained by convolving 4.5 Å MR map with Gaussian of width 4.4 Å (i.e. 8.9 Å resolution)

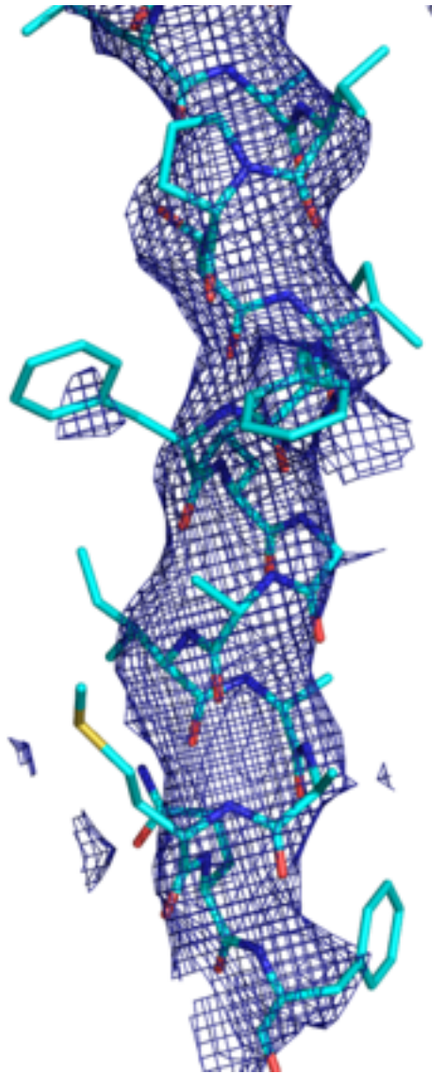
Electron density map including continuous diffraction



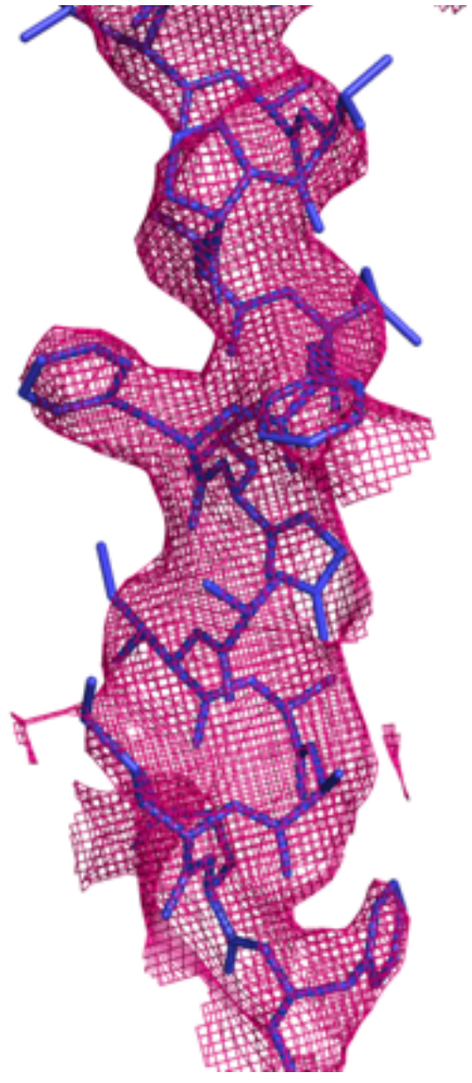
Kartik Ayyer
Dominik Oberthuer

The extended-resolution structure is superior

Bragg only
(4.5 Å)



Bragg and
continuous
(3.5 Å)



Higher diffraction sampling — model free phasing
— more reliable structure determination

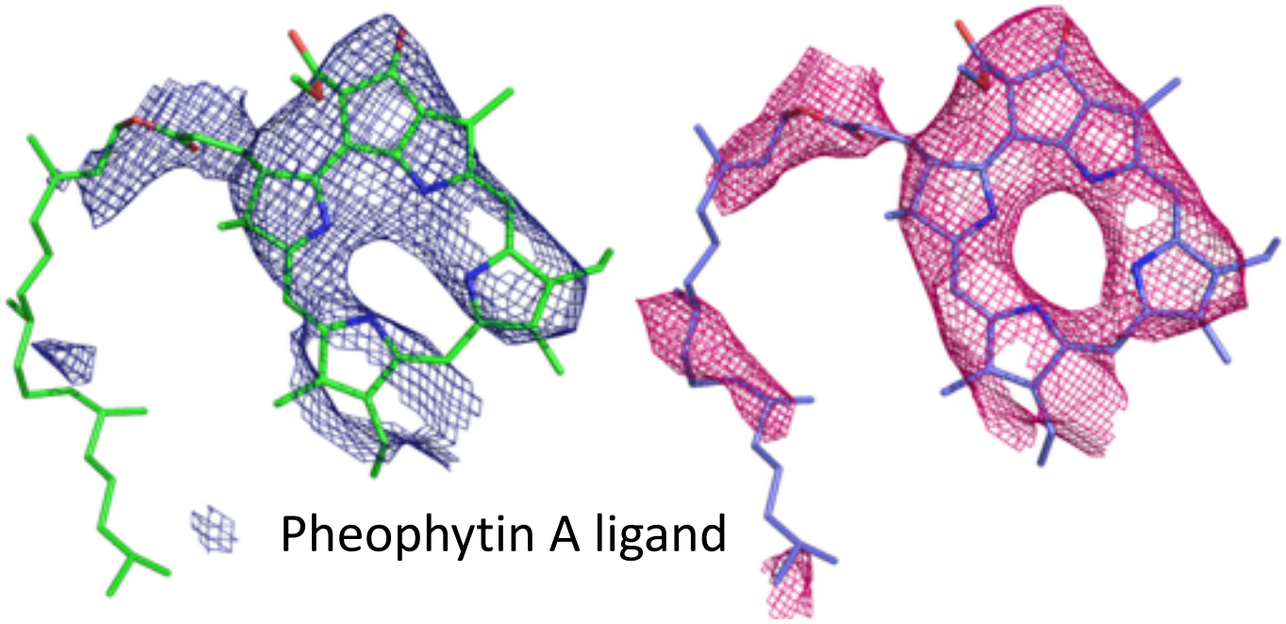
Resolution not limited by the crystal, just detector extent and shots

The best crystal is a “bad” crystal

Number of molecules per shot: 1 μm^3 x 4 / (9.2 x 10^6 $Å^3$) = 4 x10^5

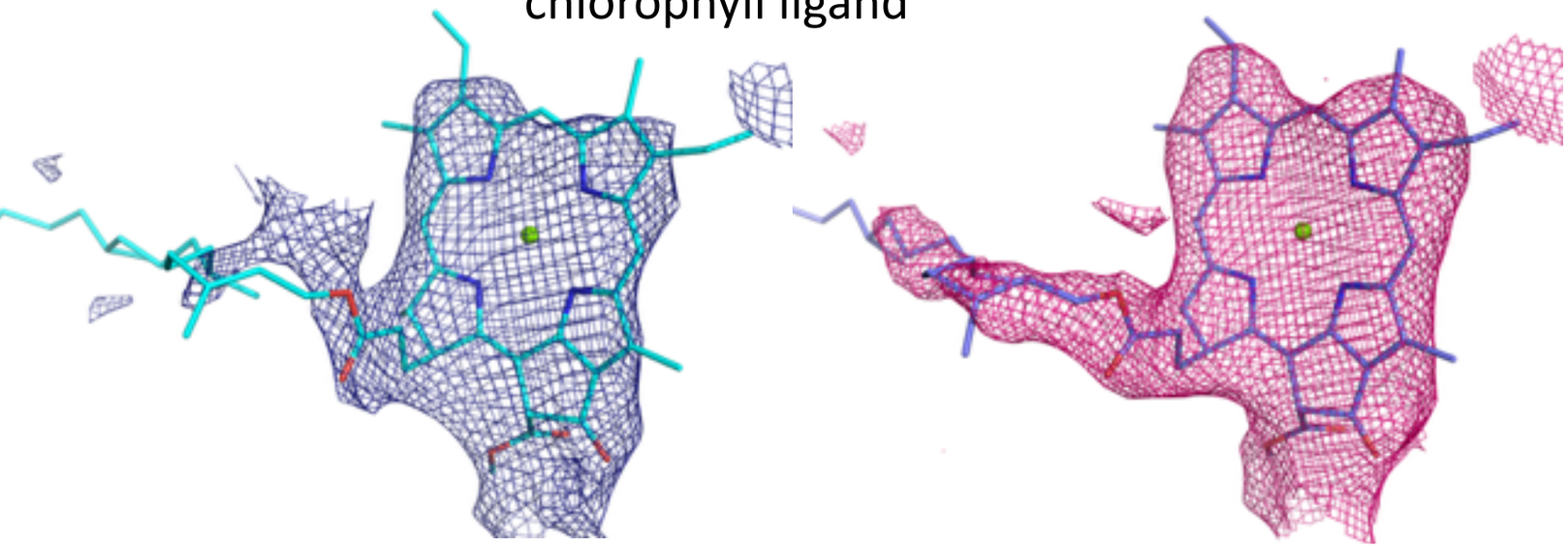
The extended-resolution structure is superior

Bragg only (4.5 Å)

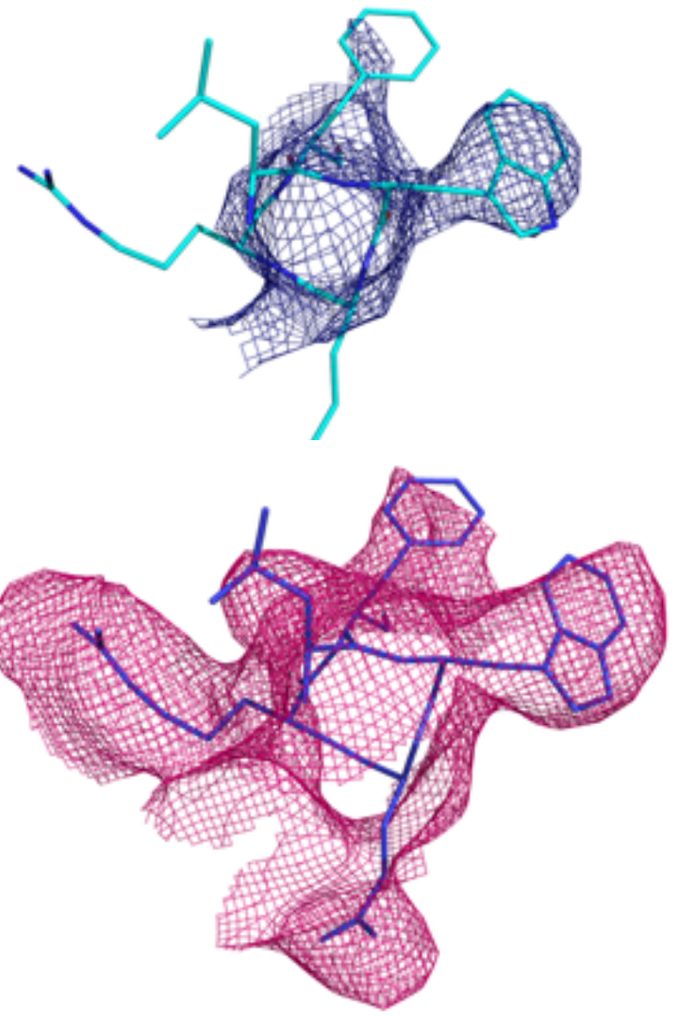


Pheophytin A ligand

chlorophyll ligand



Bragg and continuous (3.5 Å)



Aligned single particle diffraction overcomes the information deficit and crystallisation bottlenecks

Higher diffraction sampling

— More information than required to describe the object
— model free phasing
— more reliable structure determination
— first new phasing since MAD

Resolution not limited by crystal quality, just detector extent and number of shots

Acknowledgements

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Dominik Oberthür

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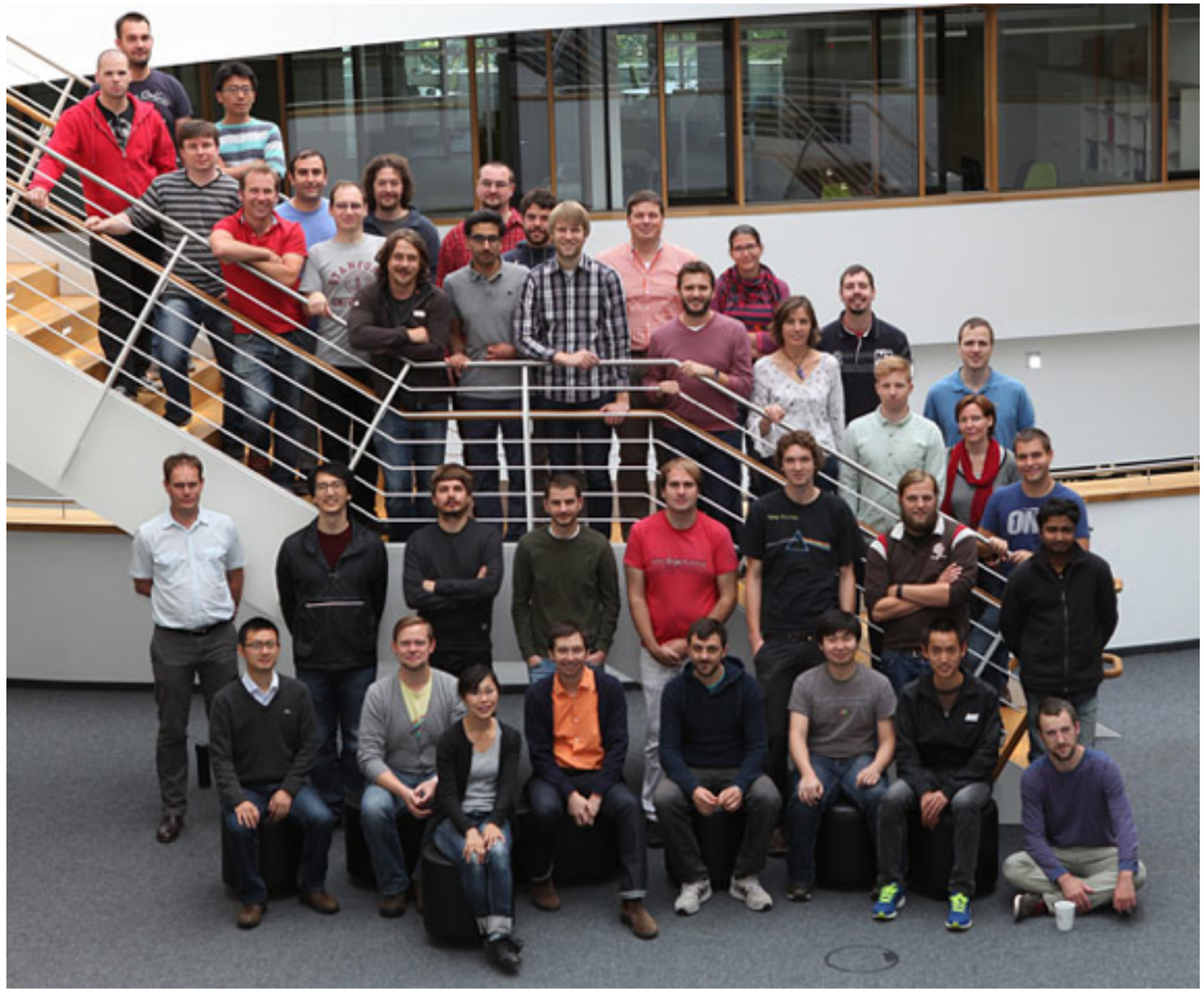
Valerio Mariani

Lorenzo Galli

The phasers



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DFG

CUI THE HAMBURG CENTER FOR ULTRAFAST IMAGING

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K. Ayyer et al. "Macromolecular Diffractive Imaging Using Imperfect Crystals," in press (2016)

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