



ÉCOLE POLYTECHNIQUE  
FÉDÉRALE DE LAUSANNE

# Biomolecular Structure and Mechanics

Master SV - Spring 2018

Lecture 3 - March 6<sup>th</sup>

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# Outline of lecture 3

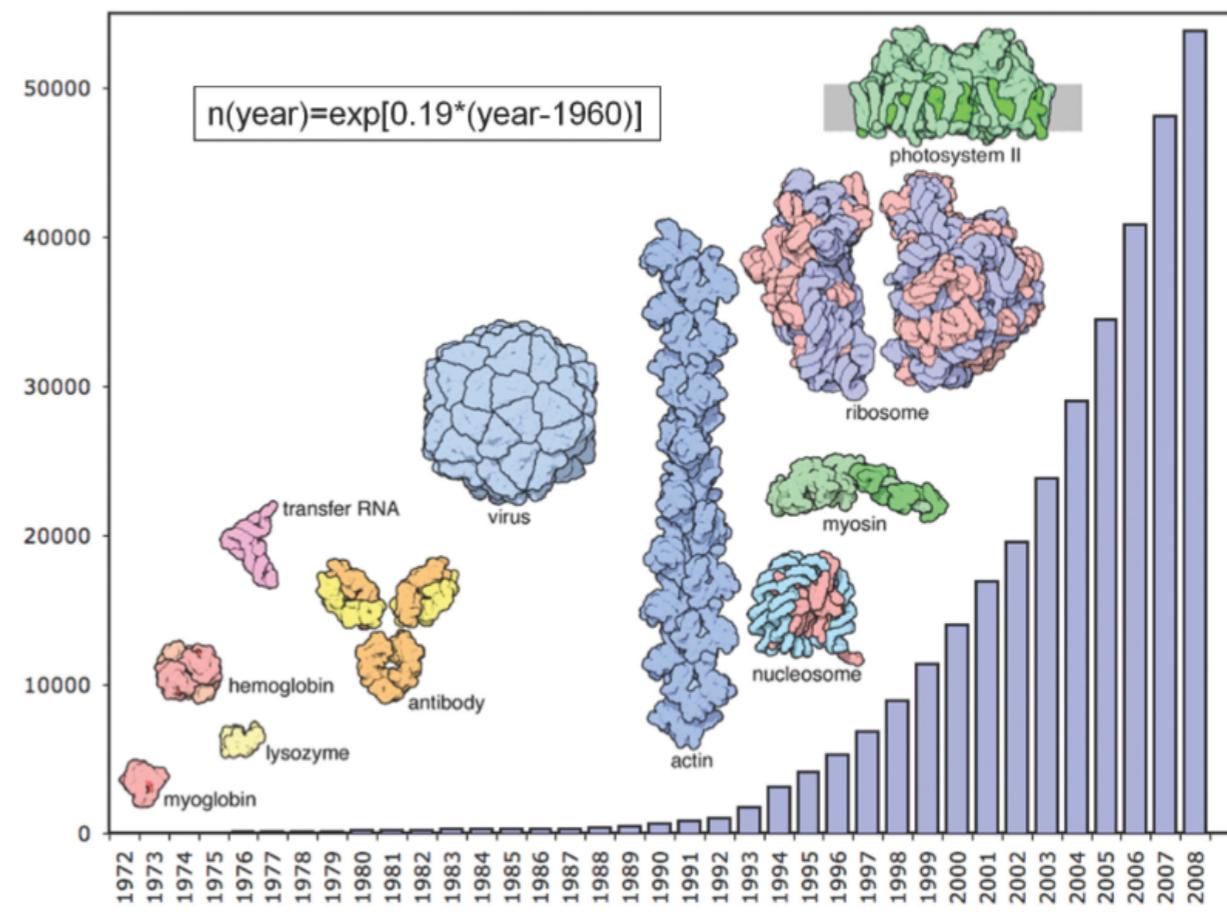
- X-ray crystallography and SAXS
  - cryo electron microscopy (cryo-EM)
  - wrap-up on experimental techniques for SB
  - integrative modeling
- Lab session 3: completing Coot experience

# Experimental structure determination

goal is set to atomic resolution ( $\sim 1\text{-}2 \text{ \AA}$ ,  $0.1\text{-}0.2 \text{ nm}$ )

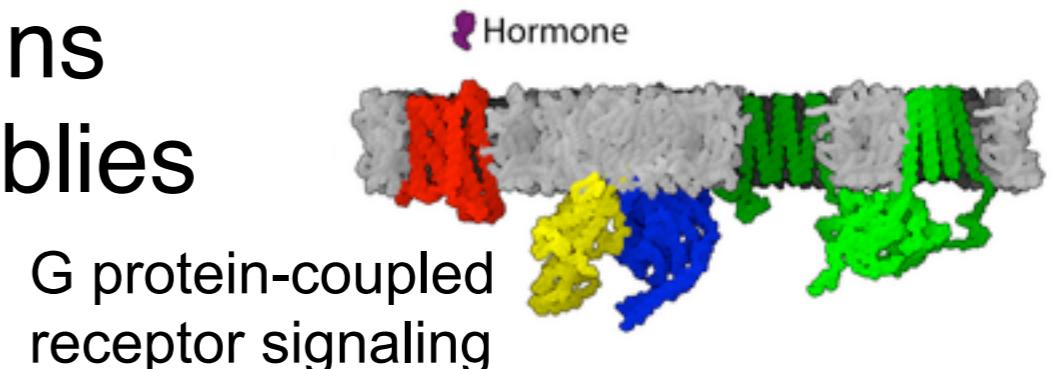
- **x-ray crystallography** (high-res  $\sim 1\text{-}2 \text{ \AA}$ )
- **nuclear magnetic resonance** (high-res  $\sim 1\text{-}2 \text{ \AA}$ )
- **cryo-electron microscopy** (medium-res  $3\text{-}20 \text{ \AA}$ )

to learn how to interpret  
and use structures solved  
by each given technique for  
understanding **biological  
function**



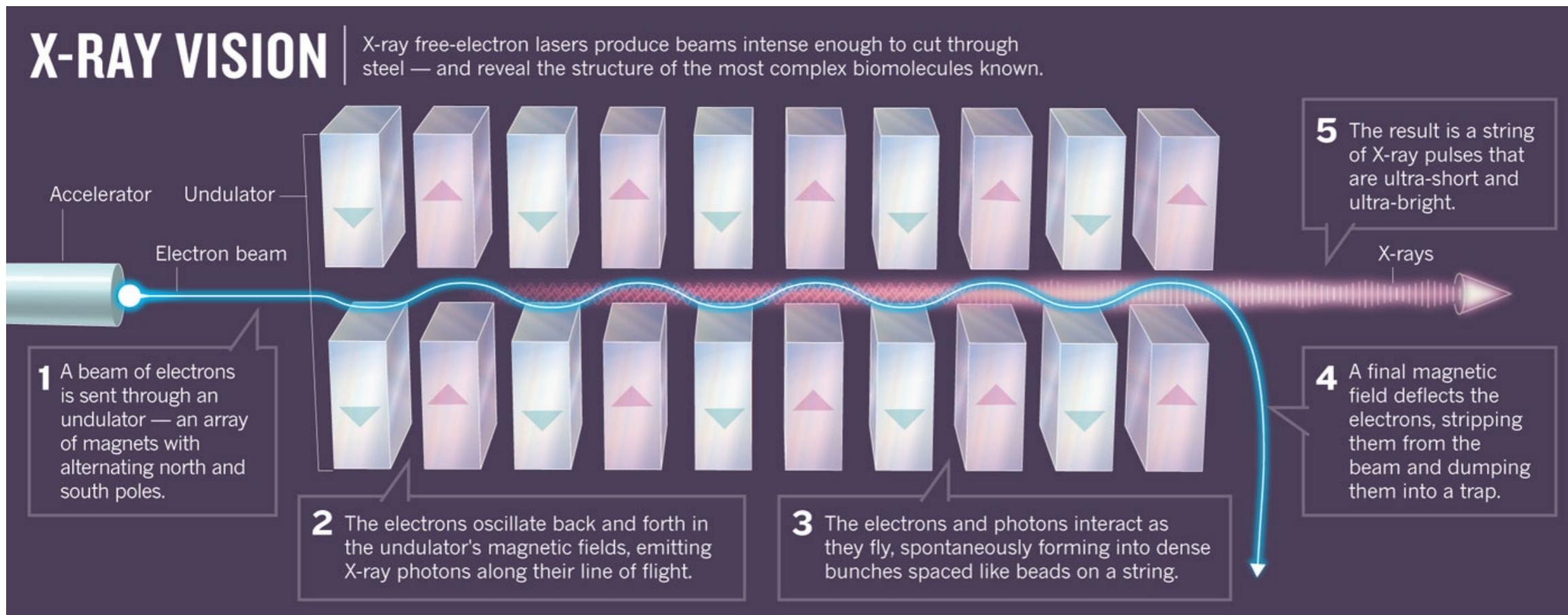
# Caveat for X-ray structures

- at usual resolution no information about **hydrogens** and protonation of titratable groups (Asp, Glu, His, etc.)
- cryogenic **temperature** (~100 K) can dump dynamics (B-factors) and produce perturbation on biological unit
- crystal **packing** can perturb biological assembly 
- difficult to solve **membrane** proteins and large macromolecular assemblies (co-crystallization problems)
- it's a **static** picture (no information about kinetics), and usually of **inactive** form (cannot trap metastable states)



# New frontiers in X-ray crystallography

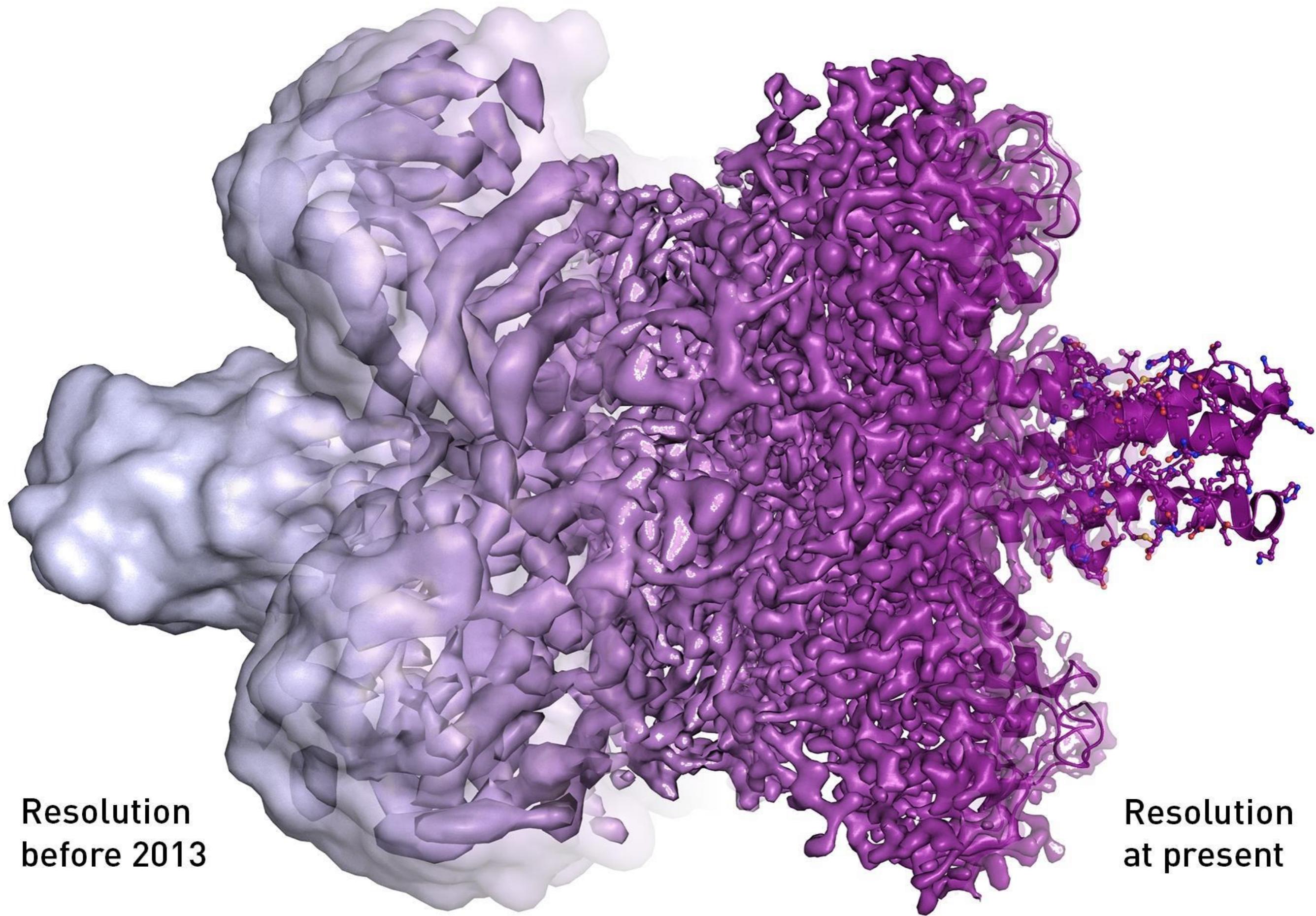
## • X-ray free electron laser (XFEL)



use of nanocrystals, ~10,000 pulses per second, big data problem, promising for membrane proteins, look at molecular dynamics in real time

**100 years of crystallography:** in 1914, Max von Laue won the Nobel Prize in Physics for discovering how crystals can diffract X-rays

>> <http://www.nature.com/news/specials/crystallography-1.14540>



# The Nobel Prize in Chemistry 2017

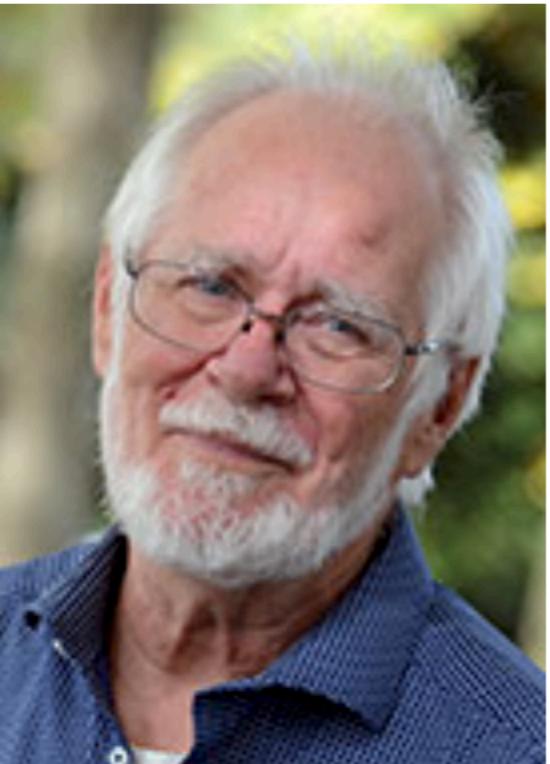


Photo: Félix Imhof ©  
UNIL [CC BY-SA 4.0]

**Jacques Dubochet**

Prize share: 1/3



Photo: B. Winkowski ©  
Columbia University  
Medical Center

**Joachim Frank**

Prize share: 1/3



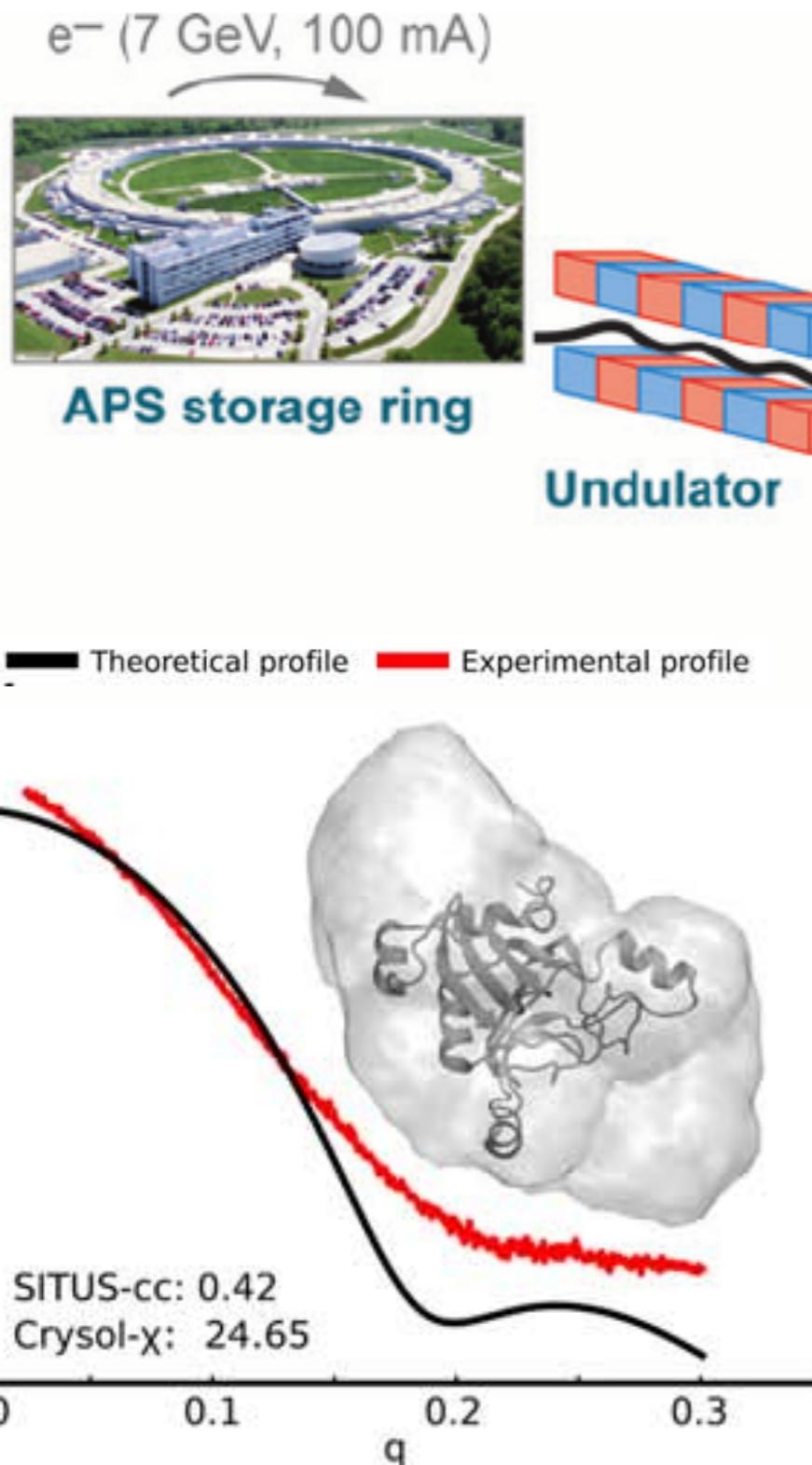
Photo: MRC Laboratory of  
Molecular Biology

**Richard Henderson**

Prize share: 1/3

The Nobel Prize in Chemistry 2017 was awarded to Jacques Dubochet, Joachim Frank and Richard Henderson *"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"*.

# Blob-ology remains for SAXS



## Small-Angle X-ray Scattering

Monochromator and X-ray optics

Sample cell

Scattering pattern

Vacuum path (0.5 - 4 m)

CCD detector

Circular averaging and data processing

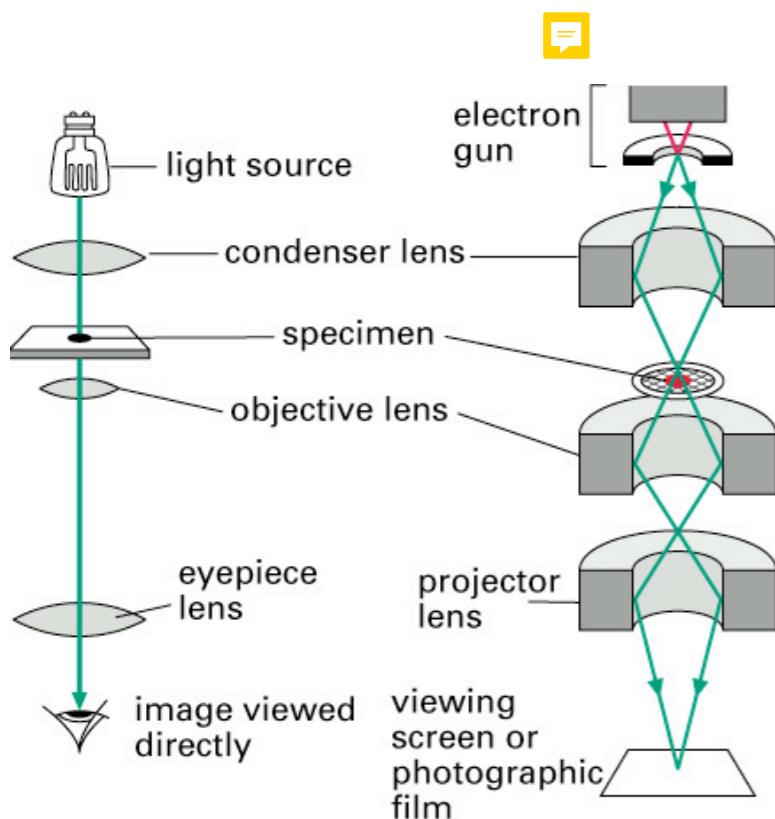
X rays (3-27 keV)



θ

# Electron microscopy

- in EM a cathode emits electrons which are scattered by the specimen (need for vacuum)
- wavelength of electrons decreases as their velocity increases (de Broglie eq:  $\lambda=h/p$ )
- with an accelerating voltage of 100 keV, wavelength is 0.004nm : resolution is  $\sim 0.002$  nm
- spherical aberrations of electron lens more difficult to correct than light microscope
- this gives practical resolving power is 0.1 nm
- negative staining (with uranyl acetate, uranyl formate etc.) no native environment
- image reconstruction algorithms which in turn decrease resolution



**cryo-EM 3D image reconstruction of West Nile Virus in complex with the Fab of the neutralizing anti-DIII mAb E16**

**3D image  
reconstruction**

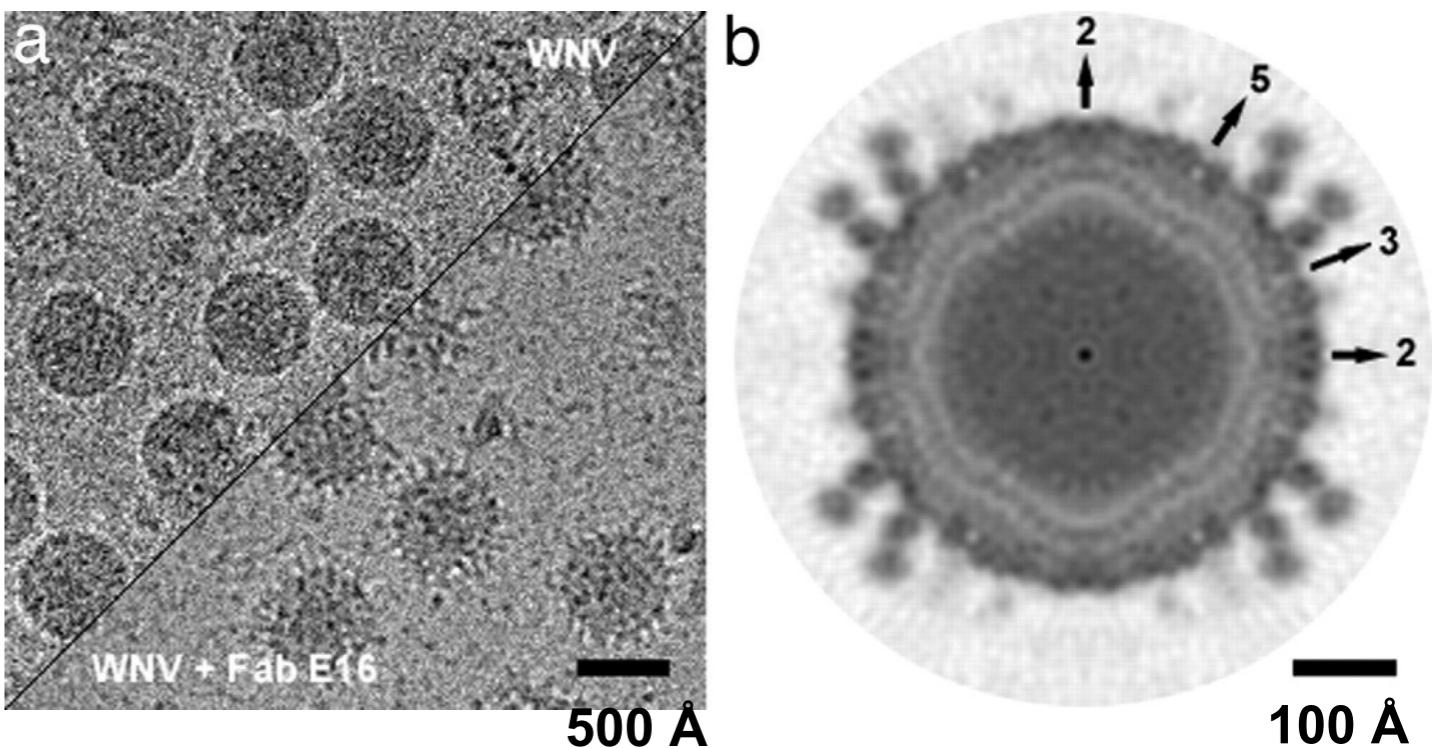
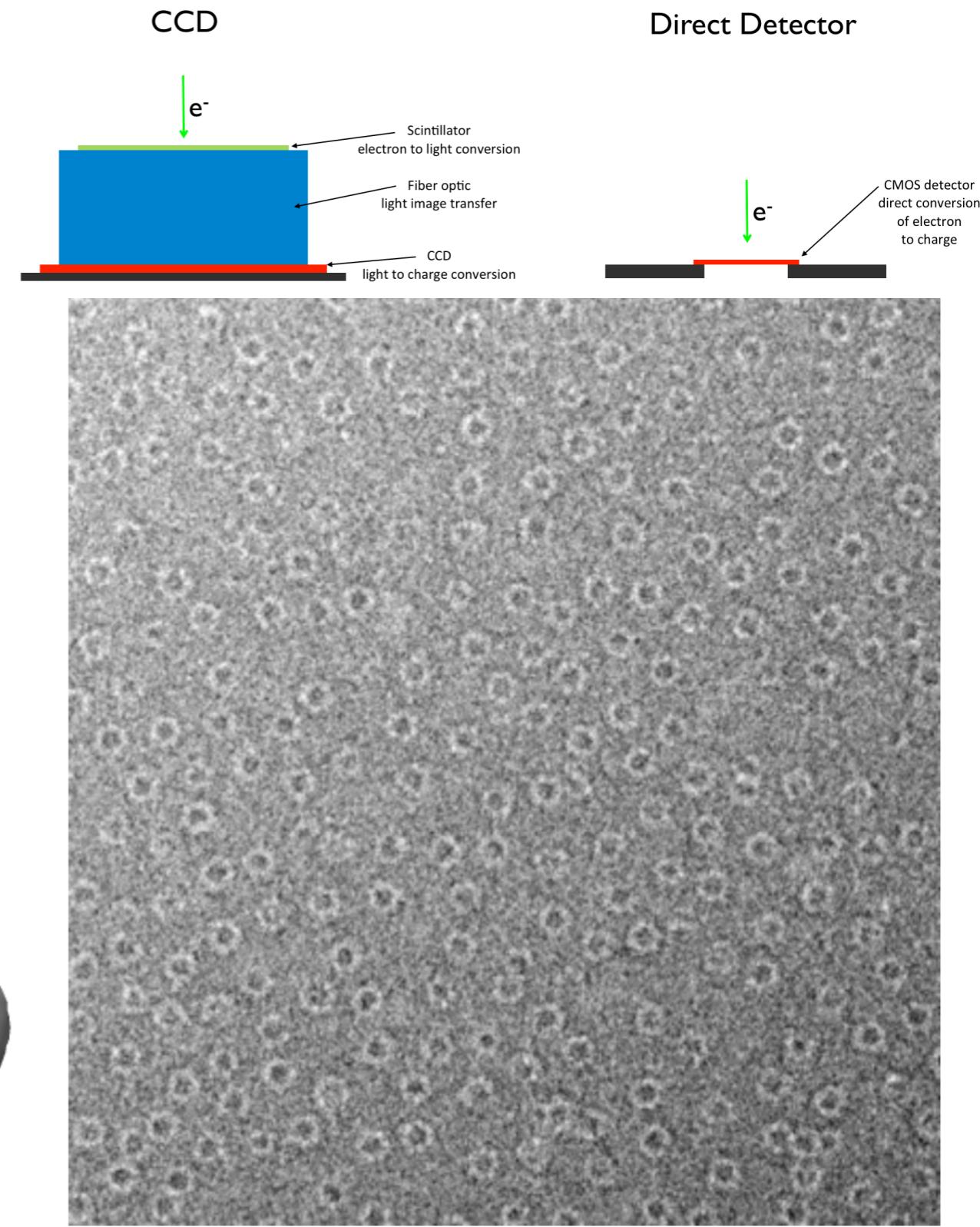
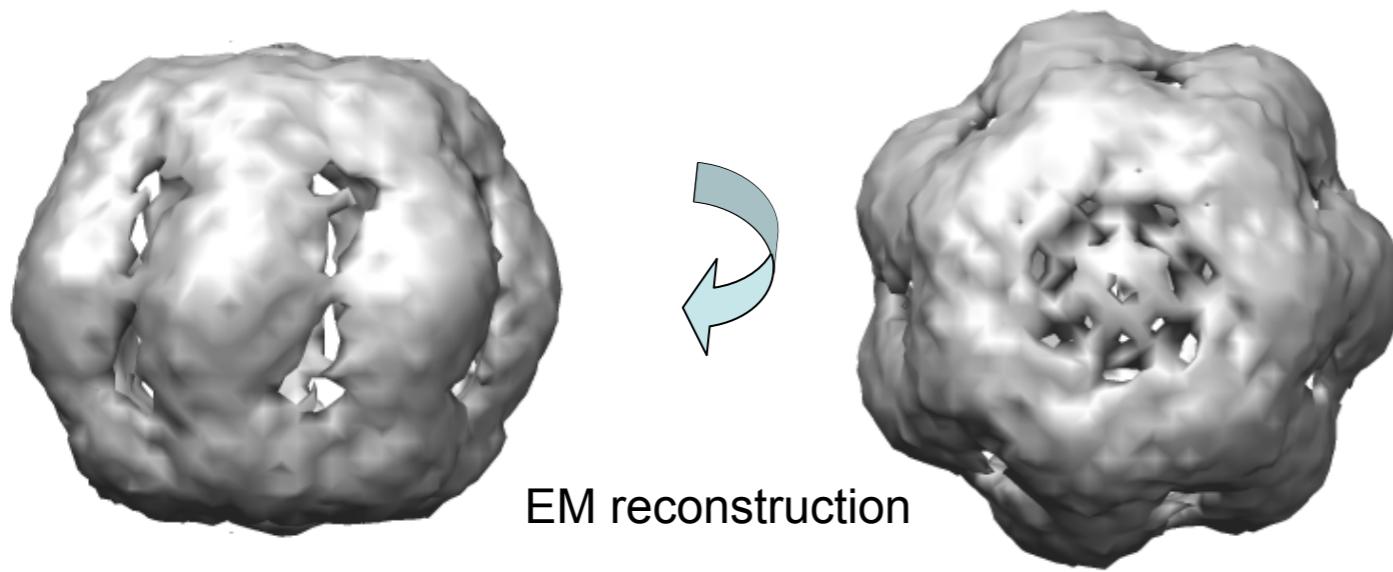


Figure 9-22. Molecular Biology of the Cell, 4th Edition.

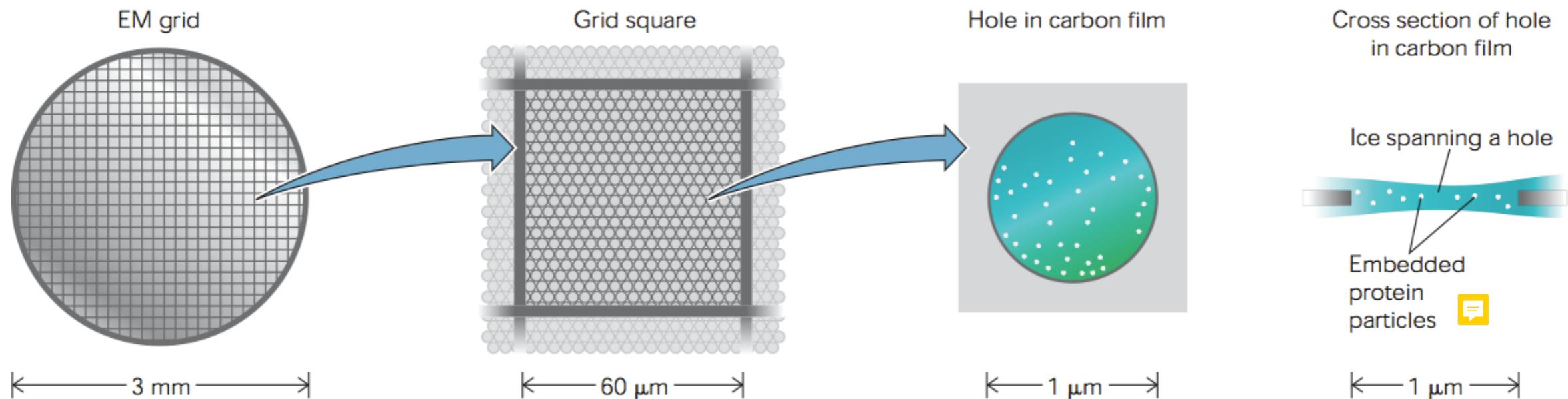
# Cryo-electron microscopy

- able to look at large molecular assemblies (min. 70 kDa)
- resolution is getting better: 30Å to below 3Å
- cryo-EM preserves aqueous environment (no staining)
- can provide a 3D volumetric reconstruction
- suitable for the study of membrane proteins and dynamics

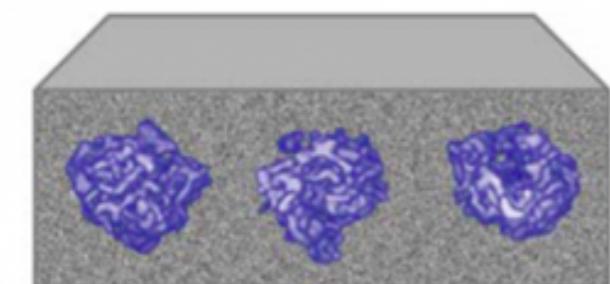


Cryo-EM image of a secreted protein of *M. tuberculosis*

# Cryo-electron microscopy

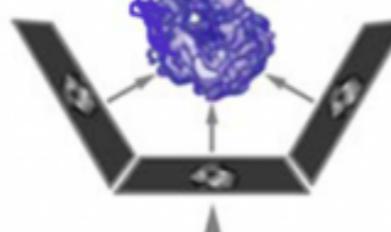


Electron source

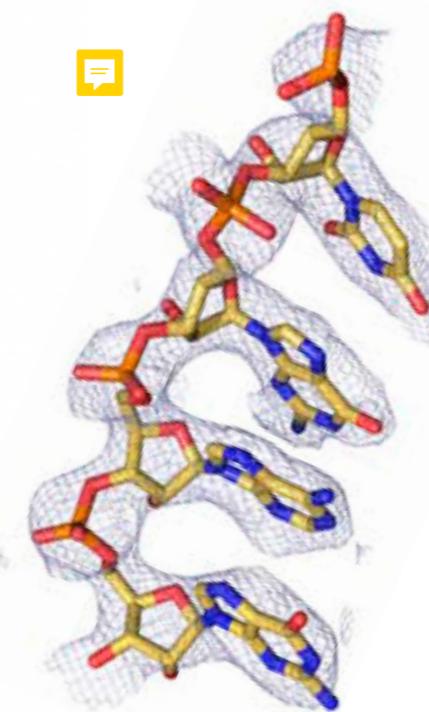


- Classify 2D images according to orientation
- Align and average images within an orientation class

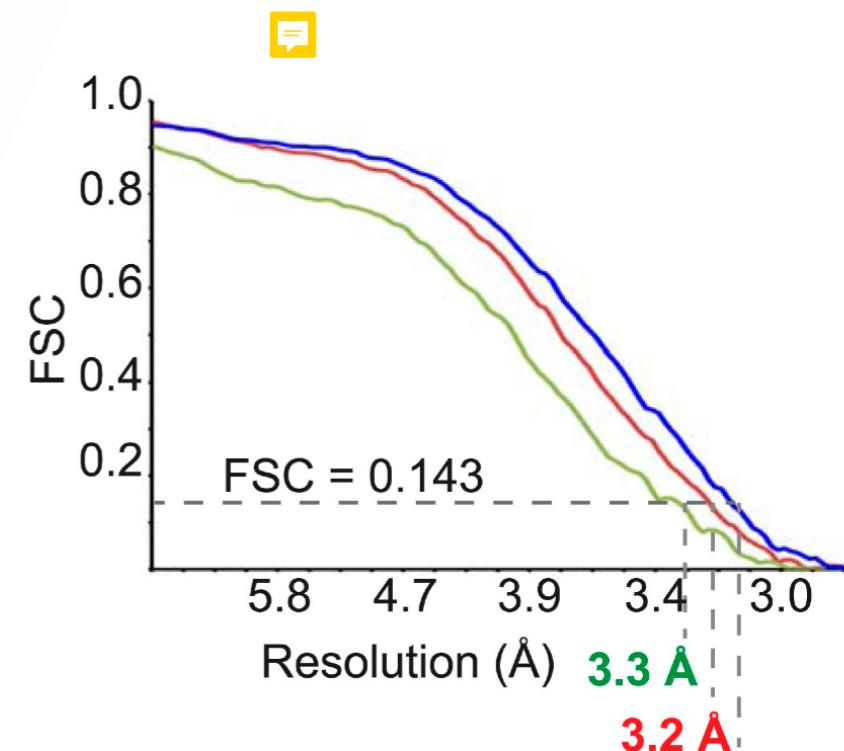
Reconstruct the 3D density map of the protein

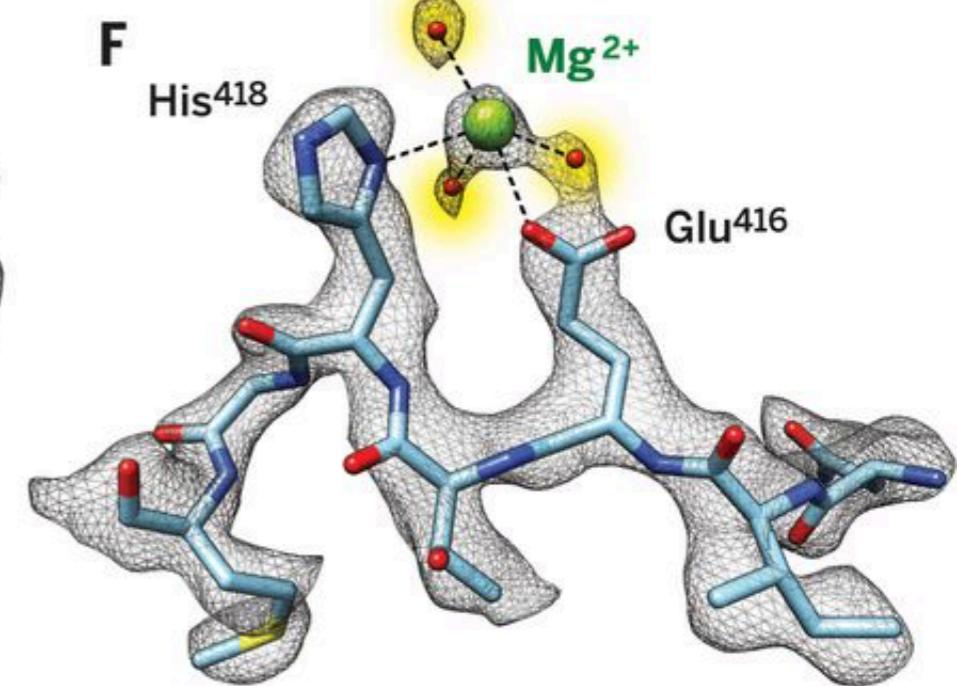
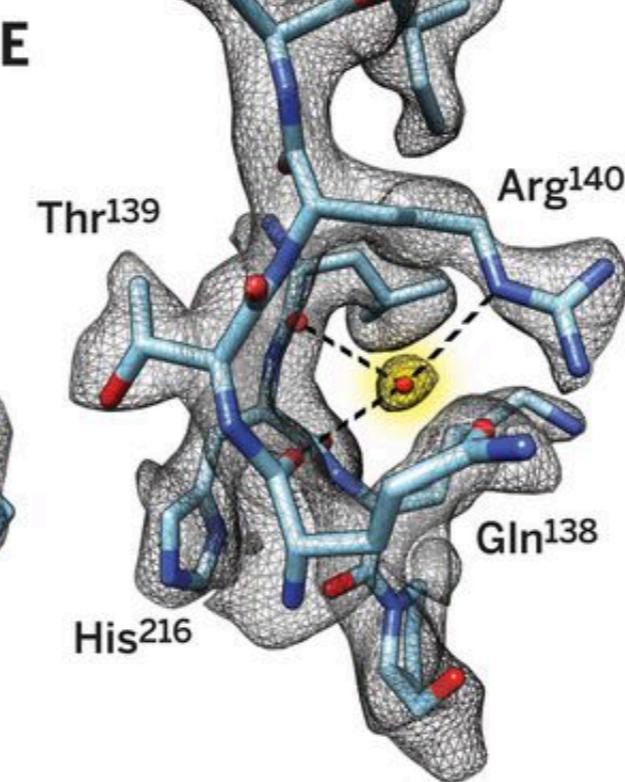
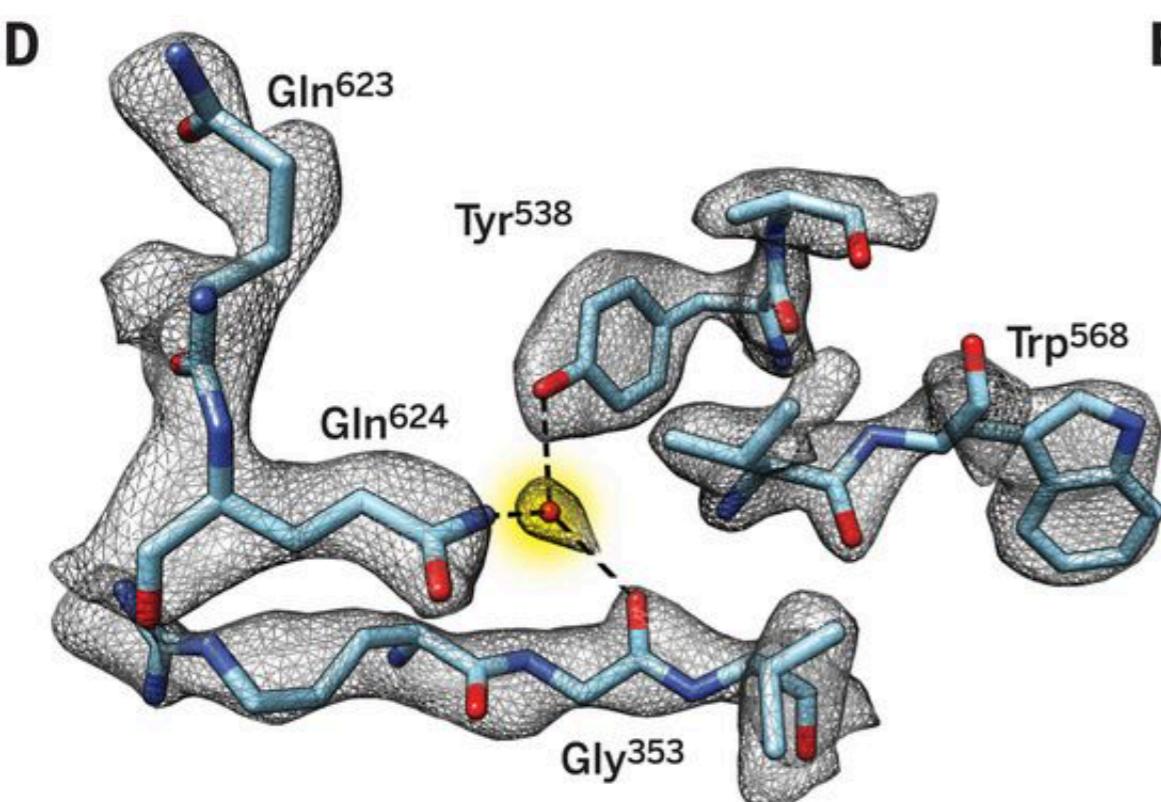
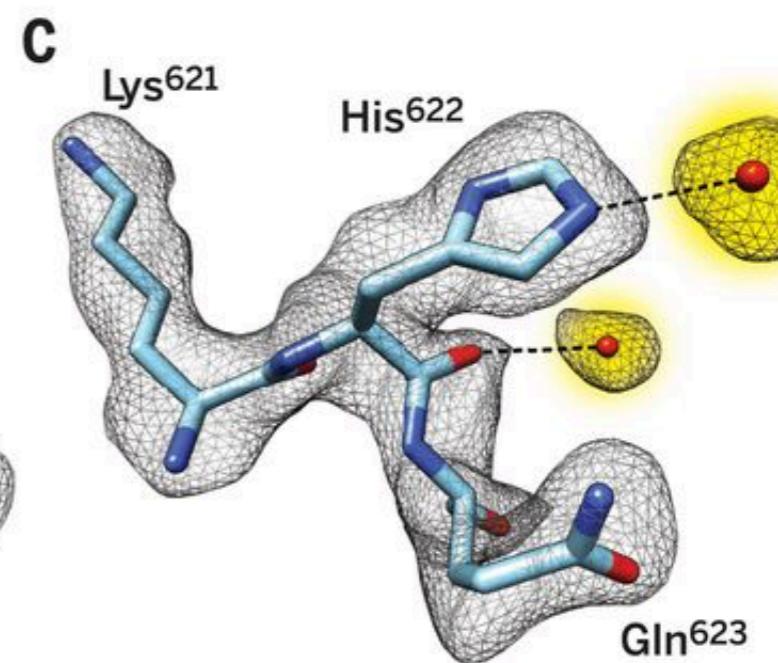
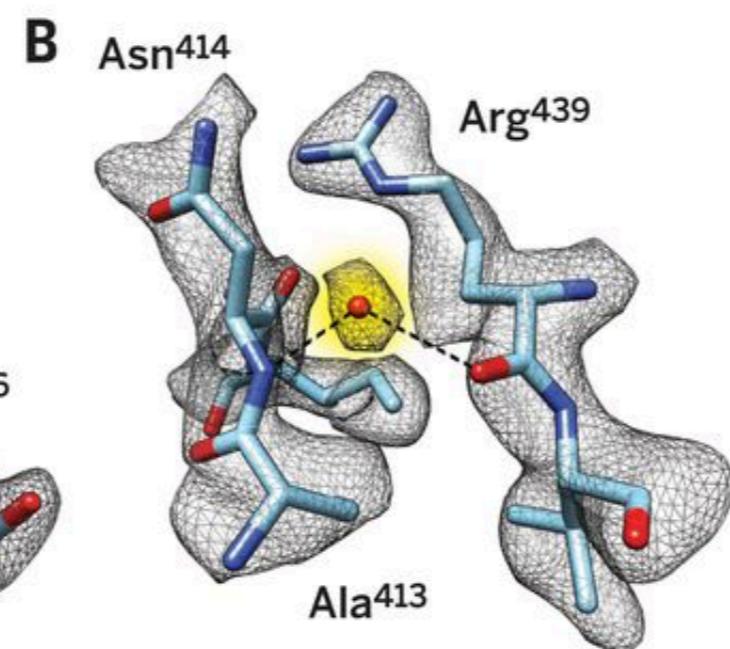
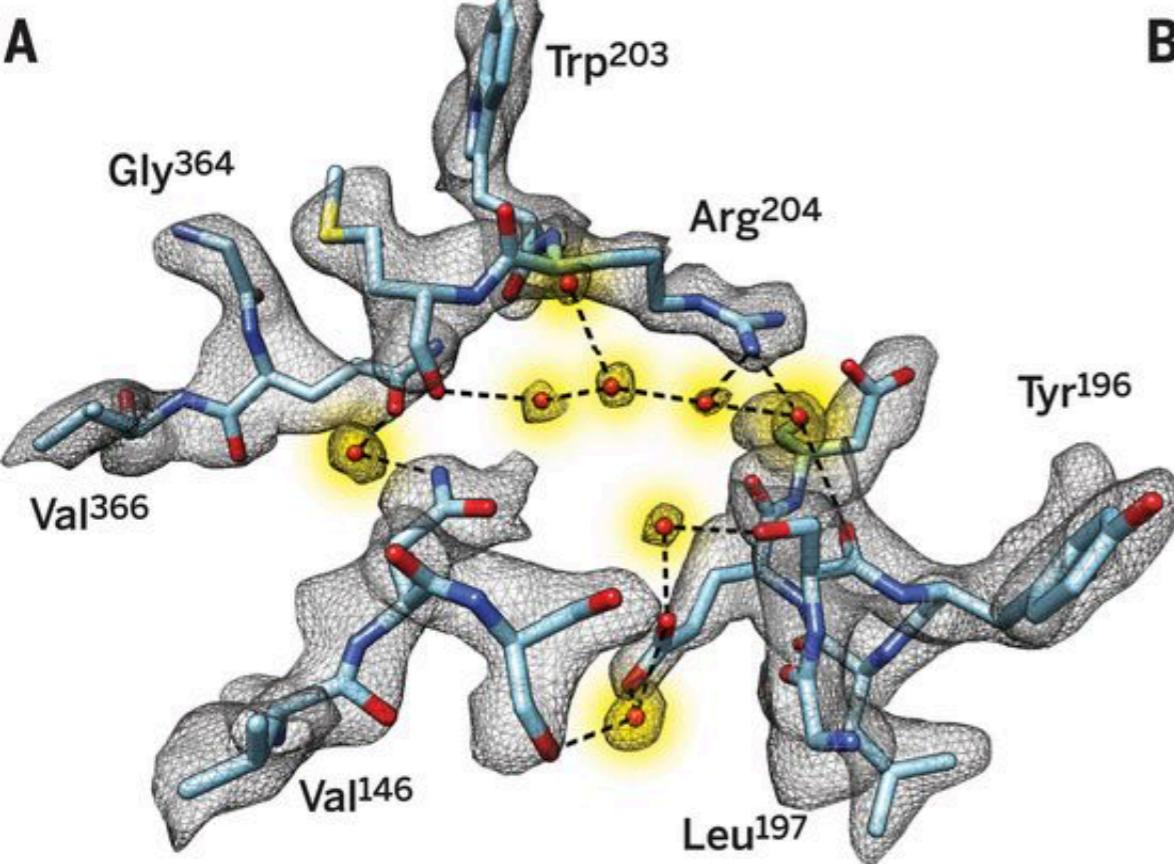


Correct the Contrast Transfer Function

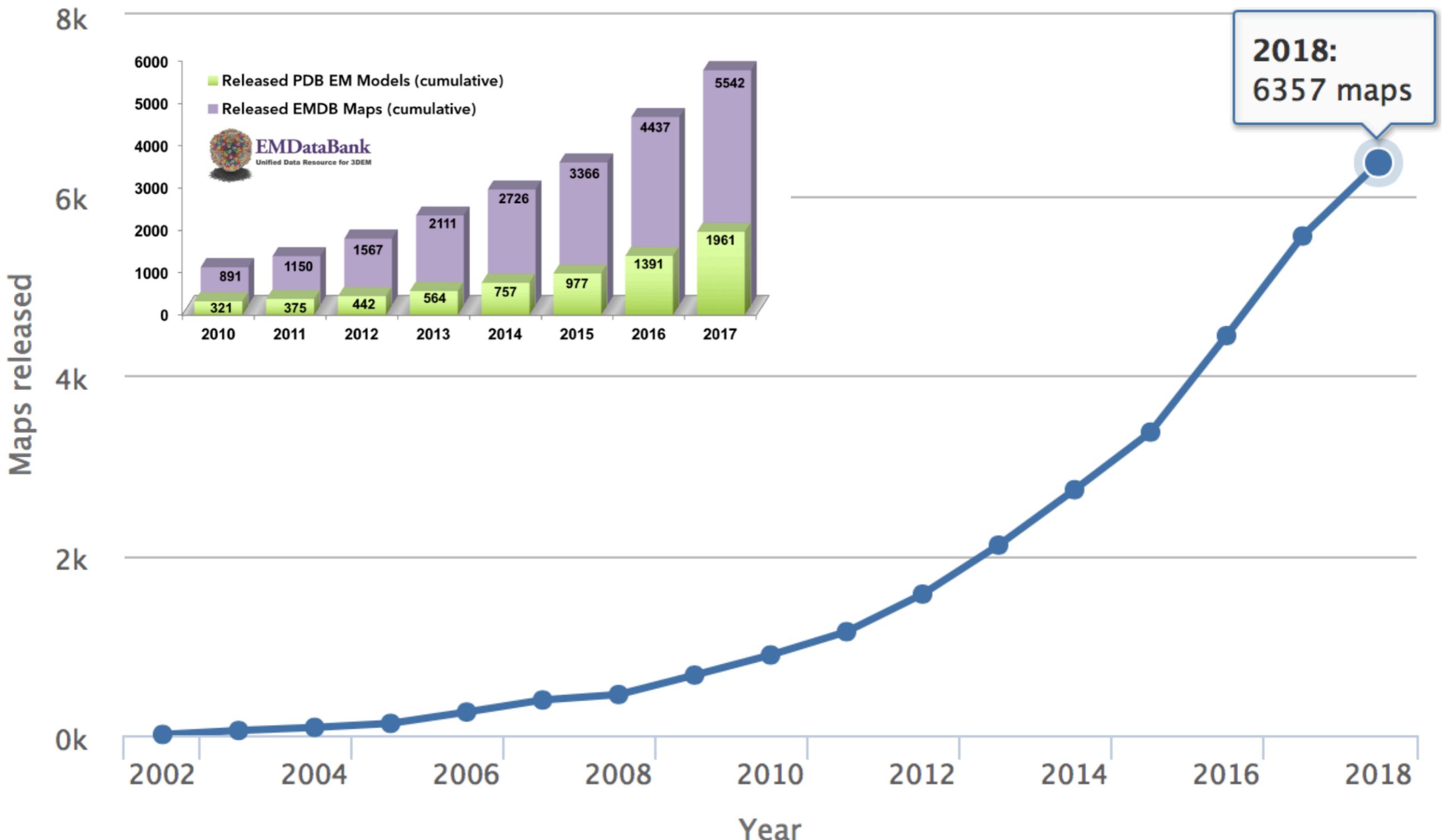


$$FSC(r) = \frac{\sum_{r_i \in r} F_1(r_i) \cdot F_2(r_i)^*}{\sqrt{2 \sum_{r_i \in r} |F_1(r_i)|^2 \cdot \sum_{r_i \in r} |F_2(r_i)|^2}}$$

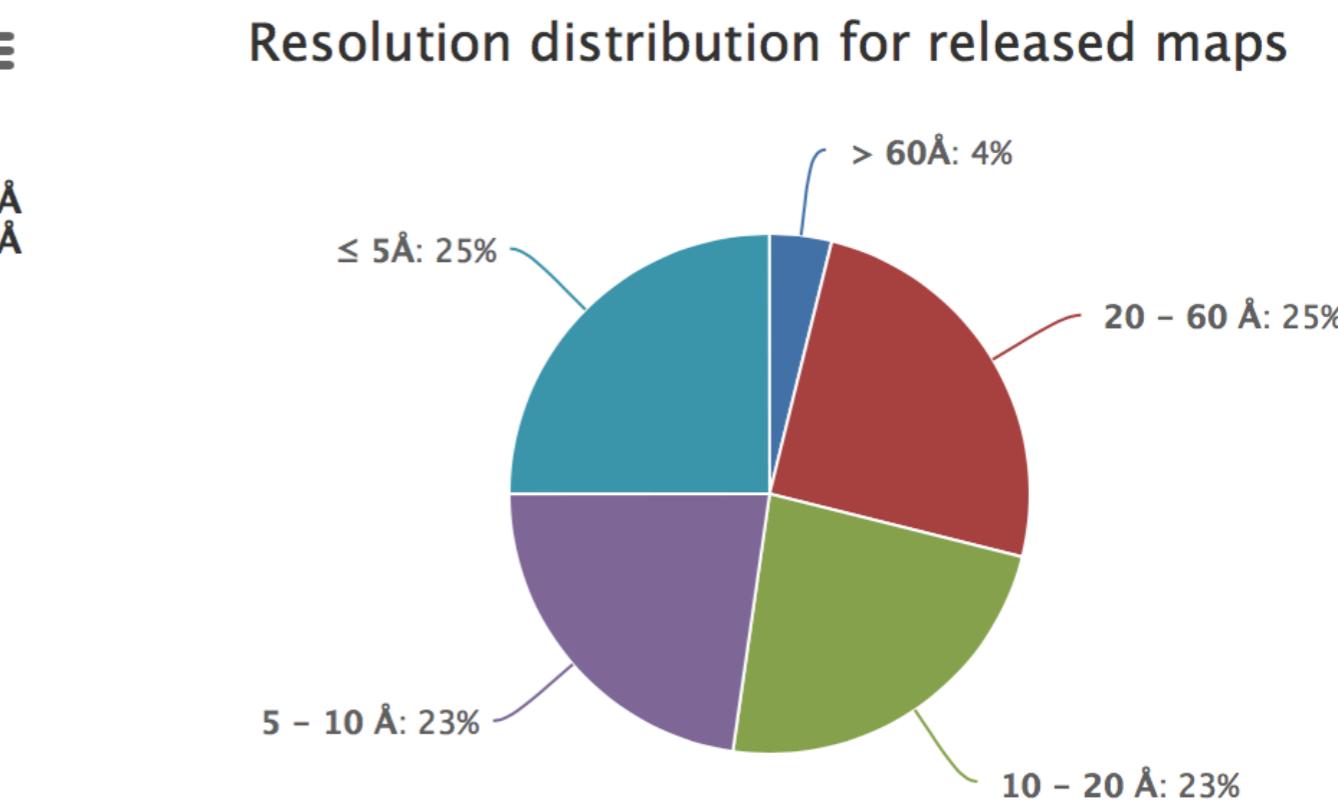
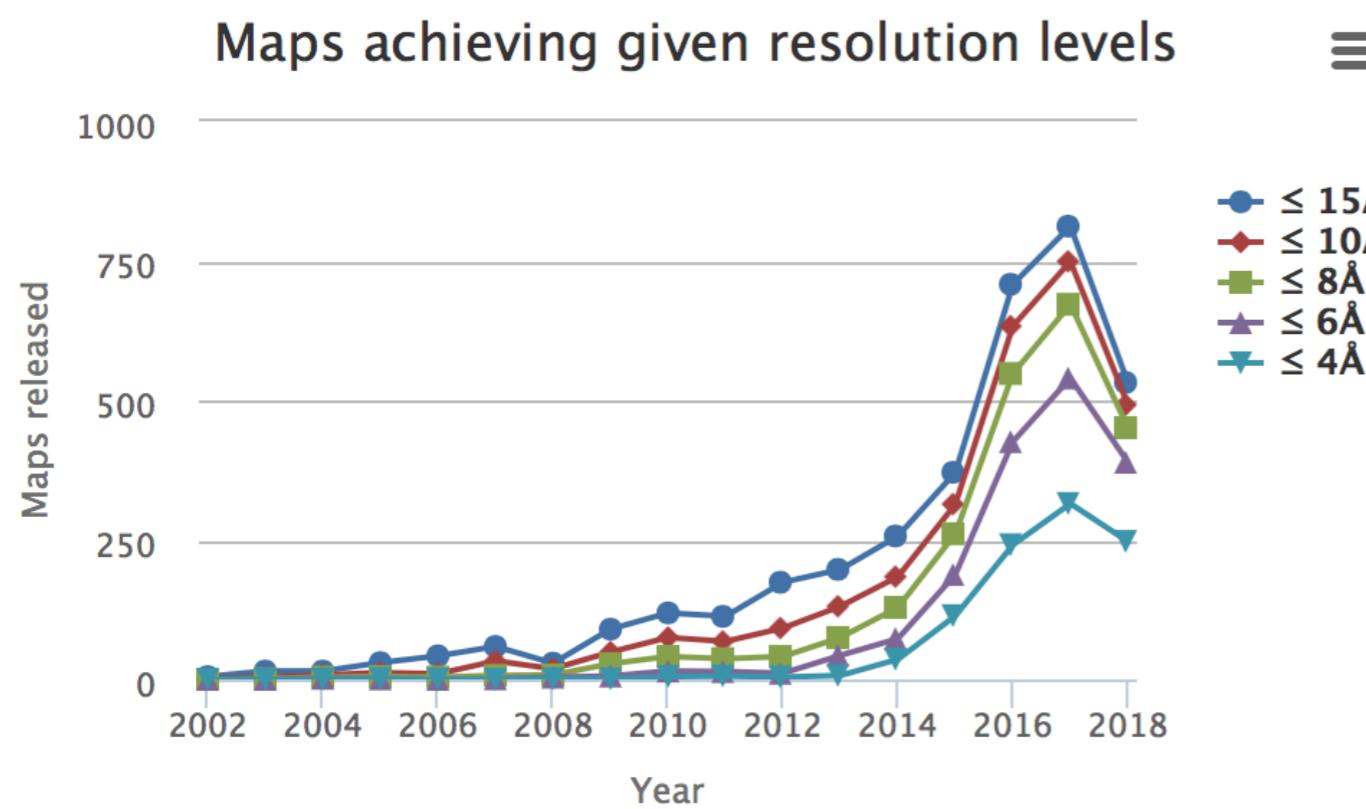
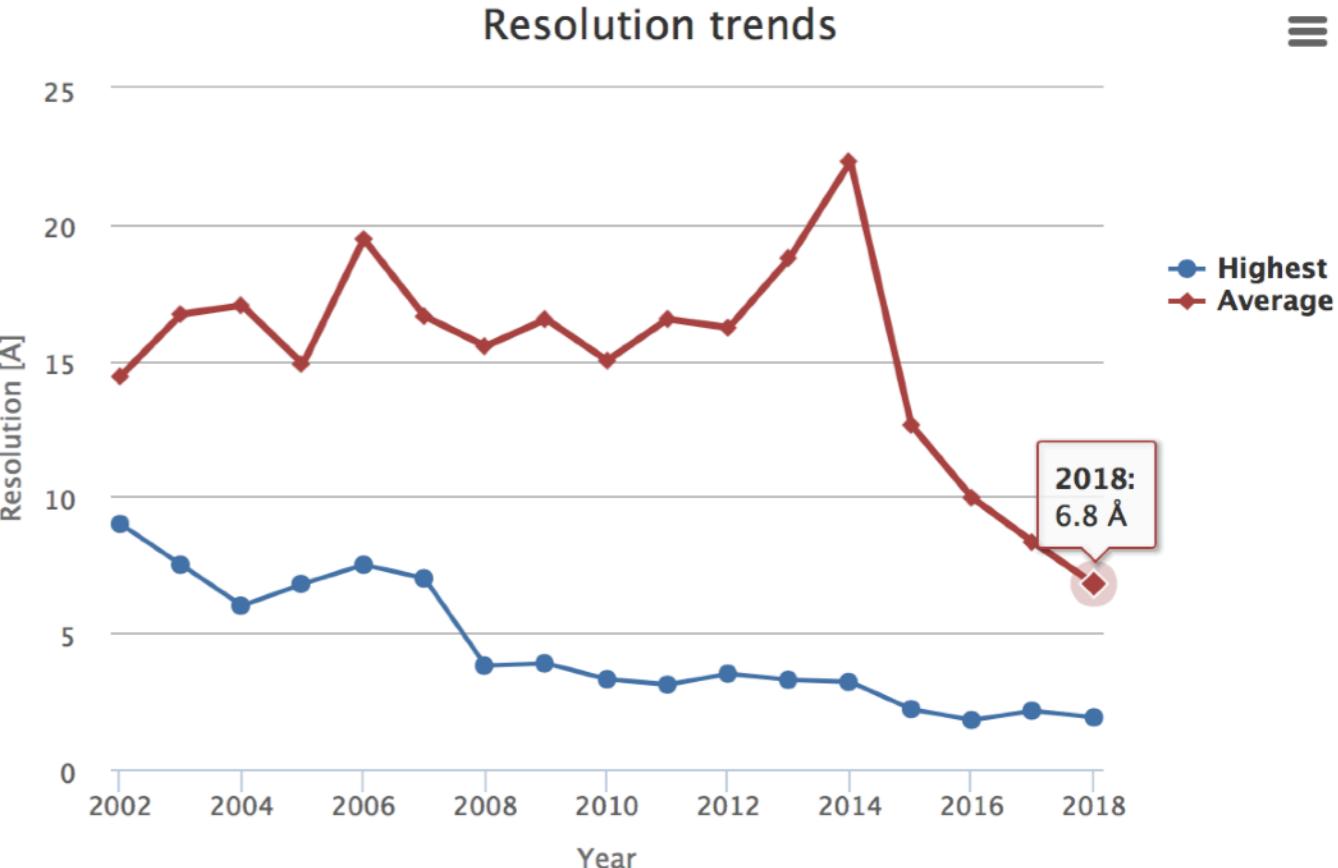
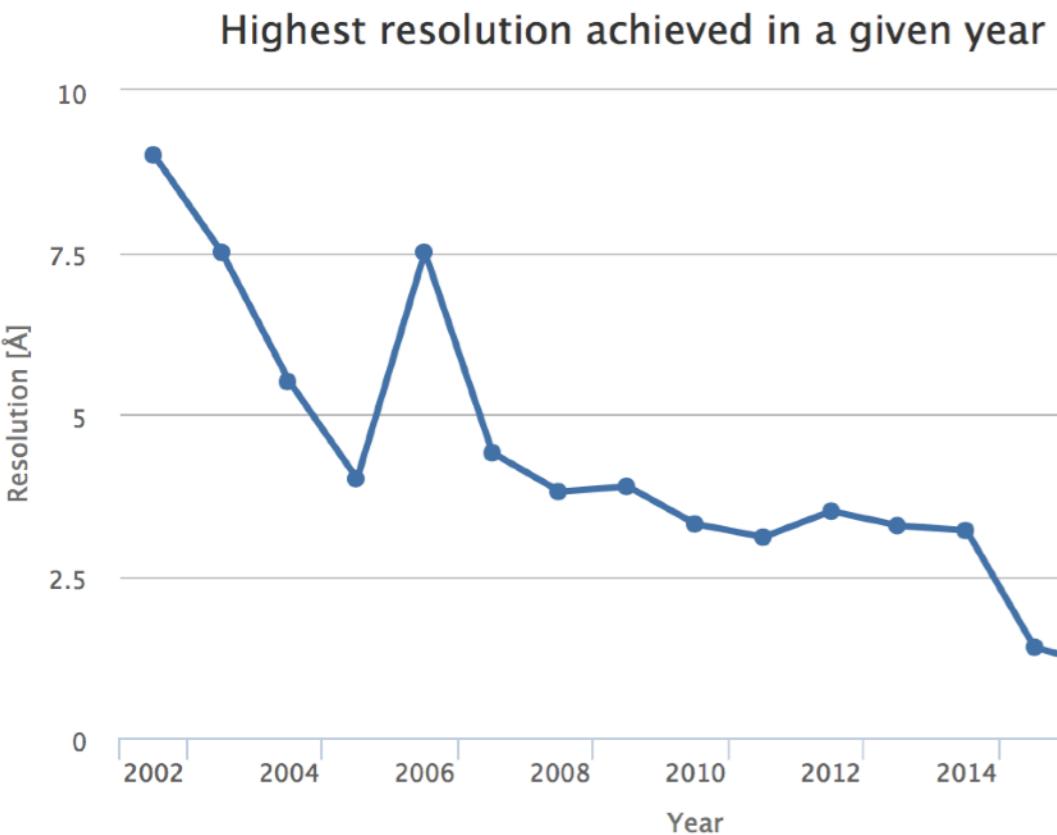




# Cumulative number of maps released



# • FEI Titan Krios



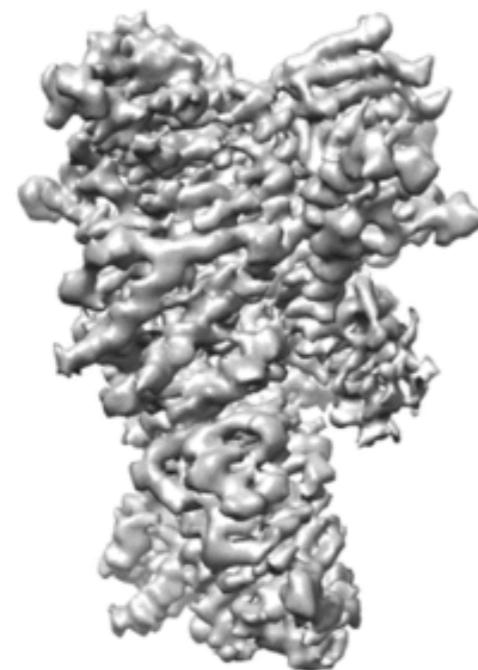


## Unified Data Resource for 3-Dimensional Electron Microscopy

EMDataBank is a unified global portal for deposition and retrieval of 3DEM density maps, atomic models, and associated metadata, as well as a resource for news, events, software tools, data standards, validation methods for the 3DEM community.

For up-to-date information about map and model challenges, visit [challenges.emdatabank.org](http://challenges.emdatabank.org).

### Recently released entries



>All recent entries

#### EMD-3337

[PDBe](#) | [RCSB](#)

**Feb. 17, 2016** RELEASED ON June 29, 2016 singleParticle 3.9Å **NEW**

*Atomic cryoEM structure of Hsp90/Cdc37/Cdk4 complex*

Verba KA, Wang RYR, Arakawa A, Liu Y, Shirouzu M, Yokoyama S, Agard DA



### News

[All news](#)

#### New NMR and 3DEM Validation Reports for Archived PDB Structures

The wwPDB Partners and the EMDataBank are pleased to announce that validation reports for all Nuclear Magnetic Resonance (NMR) and 3D Cryo Electron Microscopy (3DEM) structures already represented in the global PDB archive have been publicly released.

[Read more...](#)

#### EMDep, AutoDep, ADIT Deposition Systems to Be Retired, Effective September 30th 2016

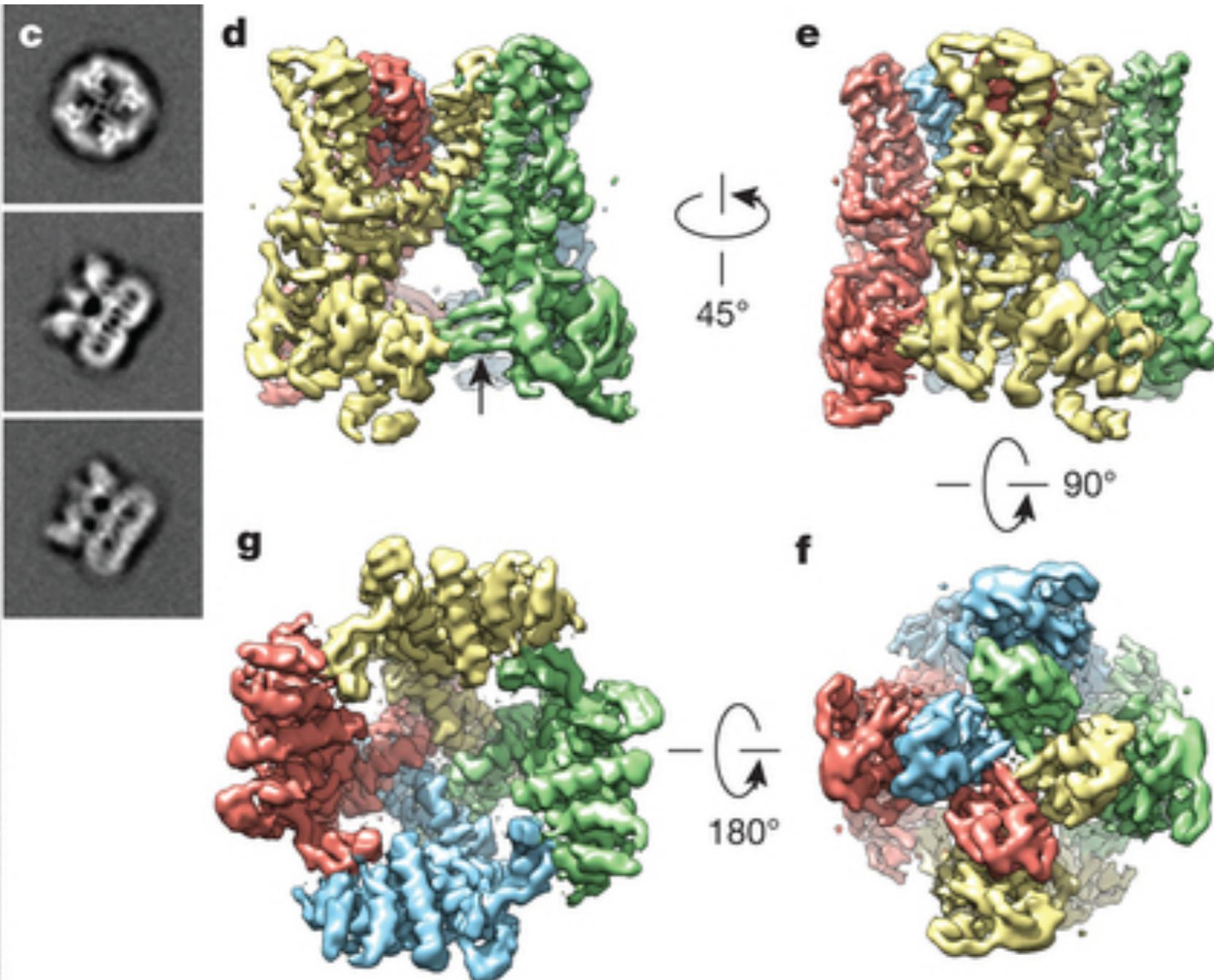
Starting May 31st 2016, the legacy deposition systems [EMDep](#), [AutoDep](#), and [EM-ADIT](#) will no longer accept new EMDB and PDB submissions. Depositors with in-progress sessions on the legacy systems will be able to access and complete their deposition sessions until September 30th 2016.

[Read more...](#)

[RSS](#)

# New frontiers in cryo electron microscopy

## Transient receptor potential (TRP) channels



3D reconstruction of TRPV1 determined by single-particle cryo-EM.

- newly developed direct electron detector
- new image-processing algorithms to correct motion-induced image blurring and improve signal and contrast of single-particle cryo-EM images
- possible to reach routinely  $\sim 3 \text{ \AA}$  resolution in the near future

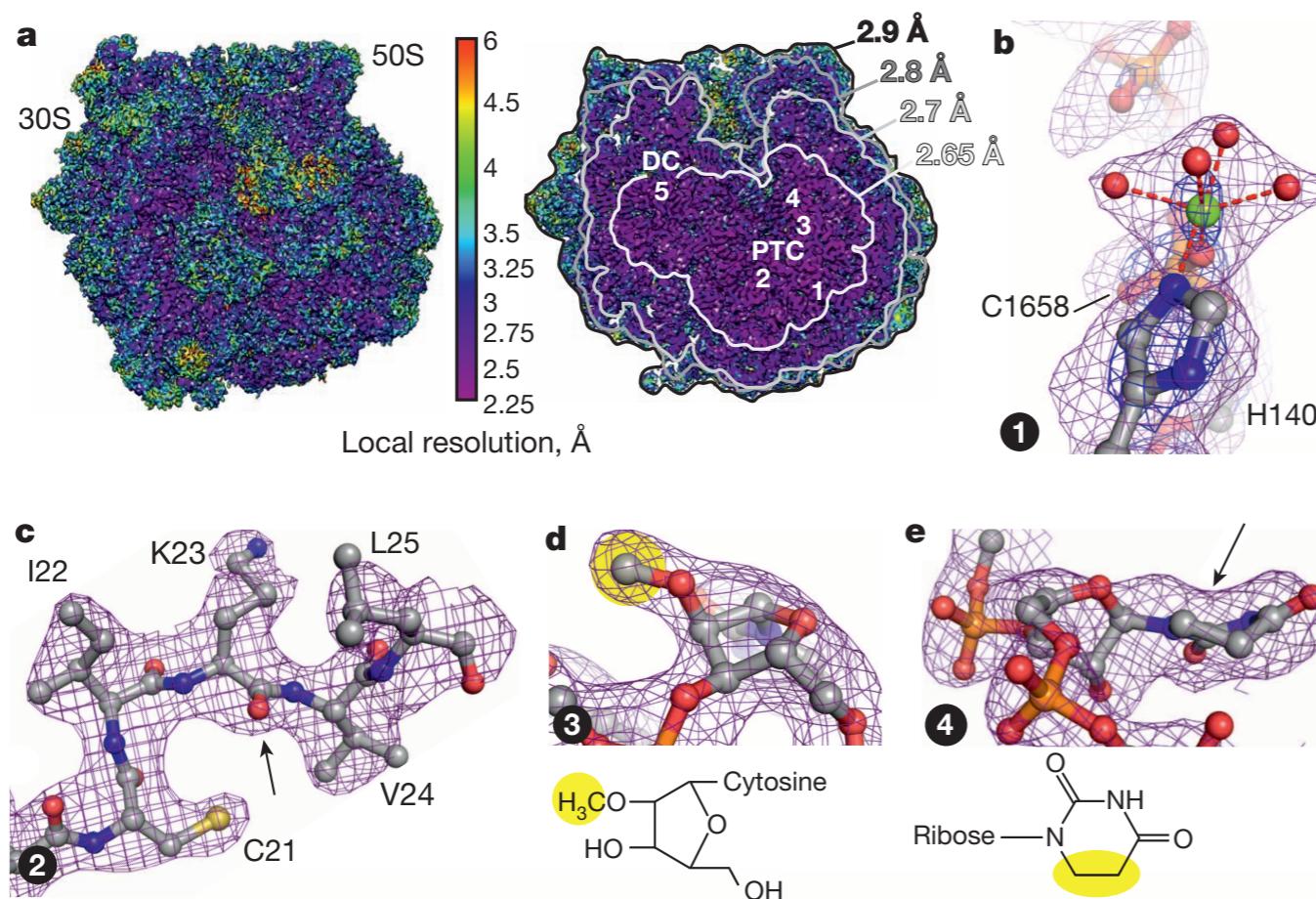
# Structure of the *E. coli* ribosome–EF–Tu complex at <3 Å resolution by C<sub>s</sub>-corrected cryo–EM

Niels Fischer<sup>1\*</sup>, Piotr Neumann<sup>2\*</sup>, Andrey L. Konevega<sup>3,4,5</sup>, Lars V. Bock<sup>6</sup>, Ralf Ficner<sup>2</sup>, Marina V. Rodnina<sup>5</sup> & Holger Stark<sup>1,7</sup>

Single particle electron cryomicroscopy (cryo–EM) has recently made significant progress in high-resolution structure determination of macromolecular complexes due to improvements in electron microscopic instrumentation and computational image analysis. However, cryo–EM structures can be highly non-uniform in local resolution<sup>1,2</sup> and all structures available to date have been limited to resolutions above 3 Å<sup>3,4</sup>. Here we present the cryo–EM structure of the 70S ribosome from *Escherichia coli* in complex with elongation factor Tu, aminoacyl-tRNA and the antibiotic kirromycin at 2.65–2.9 Å resolution using spherical aberration (C<sub>s</sub>)-corrected cryo–EM. Overall, the cryo–EM reconstruction at 2.9 Å resolution is comparable to the best-resolved X-ray structure of the *E. coli* 70S ribosome<sup>5</sup> (2.8 Å), but provides more detailed information (2.65 Å) at the functionally

important ribosomal core. The cryo–EM map elucidates for the first time the structure of all 35 rRNA modifications in the bacterial ribosome, explaining their roles in fine-tuning ribosome structure and function and modulating the action of antibiotics. We also obtained atomic models for flexible parts of the ribosome such as ribosomal proteins L9 and L31. The refined cryo–EM-based model presents the currently most complete high-resolution structure of the *E. coli* ribosome, which demonstrates the power of cryo–EM in structure determination of large and dynamic macromolecular complexes.

Determining the structure of large, dynamic biological macromolecules at a uniformly high resolution provides a challenge both for X-ray crystallography and cryo–EM. Here we have used aberration-corrected cryo–EM in combination with extensive computational sorting to solve

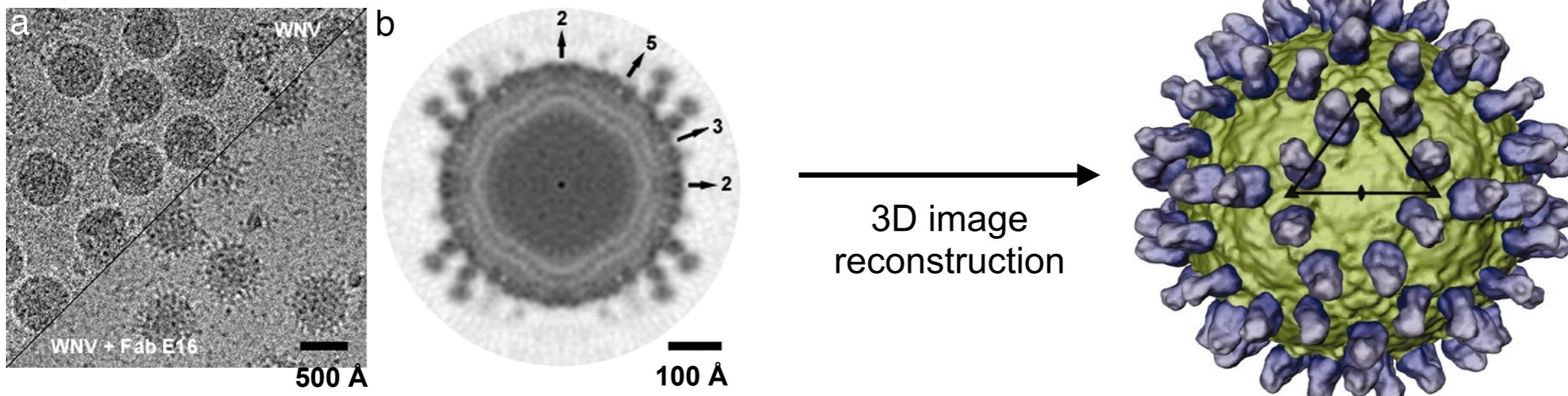
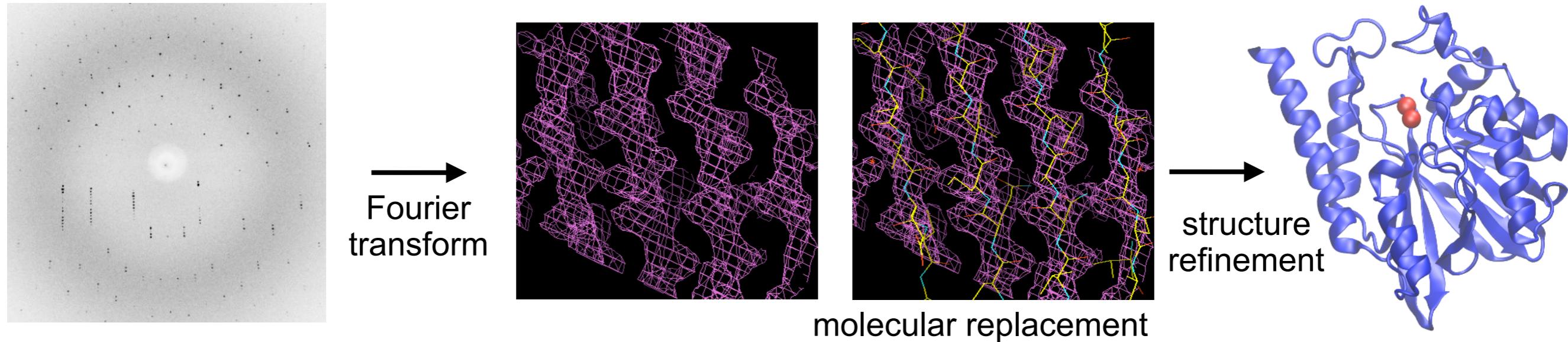


**Figure 1 | High-resolution features of the cryo-EM map.** **a**, 3D cryo-EM map of the kirromycin-stalled 70S–EF–Tu–Phe–tRNA<sup>Phe</sup> complex from *E. coli* coloured according to local resolution. Left, surface view; right, cut-away view. PTC, peptidyl-transferase centre; DC, decoding centre. Numbers (1–5) mark the densities shown in **b–f** rendered at 3σ (purple mesh). **b**, Mg<sup>2+</sup> ion (green) in octahedral coordination by four water molecules, C1658 of 23S rRNA and His 140 of the protein L3 (dark blue mesh, density at 6σ). **c**, Structure of the protein L14 revealing details such as the thiol group of Cys 21, the branched side chain of Ile 22, the zigzag pattern of Lys 23 side chain, and the carbonyl of the backbone (arrow). **d**, The 2'-O-methyl group (yellow) of Cm2498 in 23S rRNA. **e**, Nucleobase ring of D2449 in 23S rRNA with a characteristic distortion (arrow) of the planar geometry. **f**, The two methyl groups in m<sup>4</sup>Cm1402 of 16S rRNA (yellow) resolved in the cryo-EM map (left), which are not seen (red arrows) in the X-ray map (right) of the 70S ribosome<sup>9</sup> at 2.4 Å (PDB ID: 4RB5; blue mesh,

# Cryo-EM and dynamics

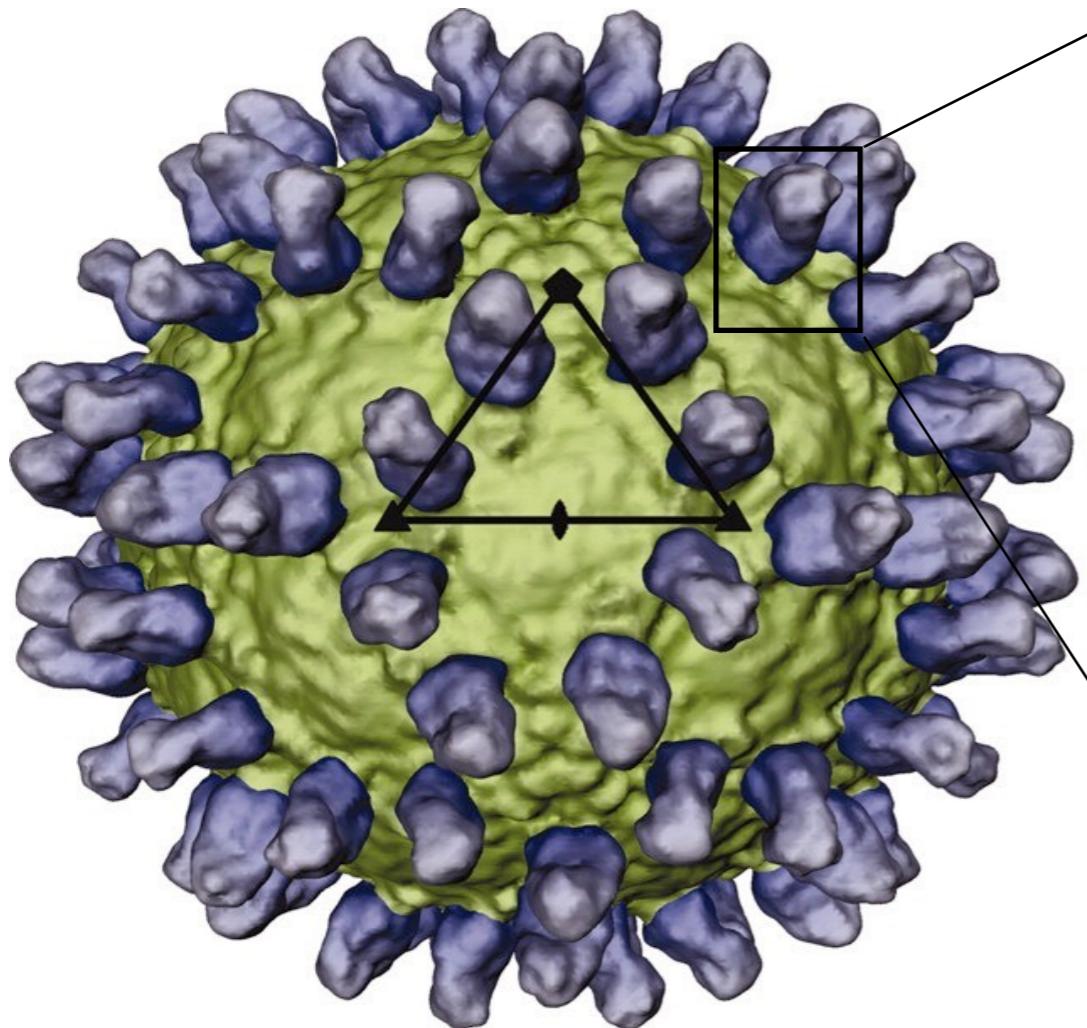
“I would like to express some **heretic ideas** spawned by the new findings. In hindsight, after seeing a growing body of evidence from single-particle cryo-EM of molecules in solution, I suggest that the idea of “**a**” **molecular structure** has been largely created by X-ray crystallographic practice, but one needs to see that such a **structure is just one selected from numerous states** by the energy minimization implicit in the formation of a crystal. However, when cryo-EM came along, and with it clear evidence of heterogeneity in the sample, the idea was again to look—this time by maximum likelihood methods—for a small number of distinct structures, still perpetrating the myth of the existence of fixed structures, albeit now with a few, rather than one. **It is time now to recognize that molecules may as a rule exhibit a large continuous variation in conformational space and that gathering information about this continuum should be the true goal of functionally oriented structure research.”**

# Structural biology methods are strongly based on theory and computation!

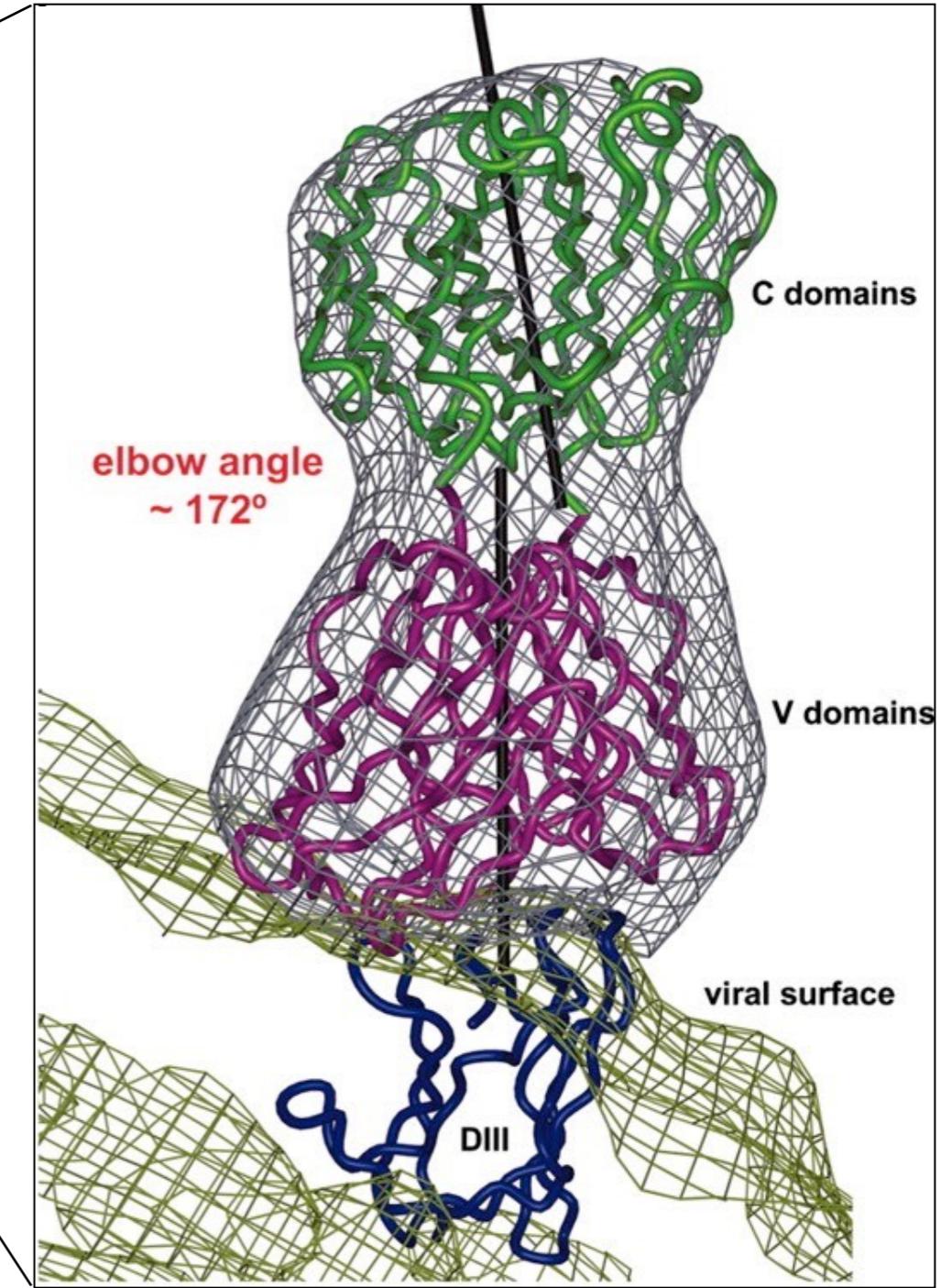


# Combine cryo-EM and X-ray structures

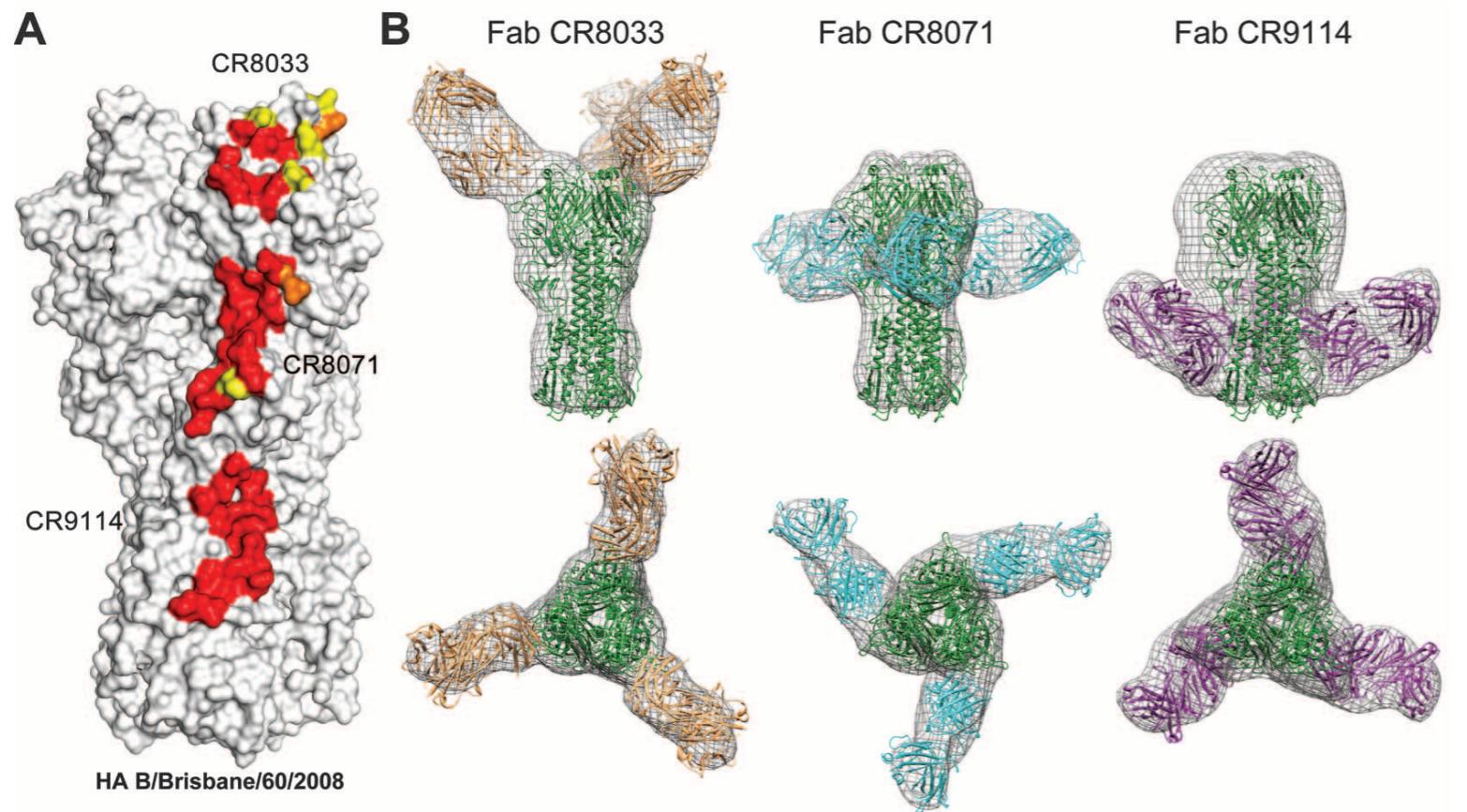
surface rendering of the 3D image  
reconstruction of WNV (green) in complex  
with Fab E16 (blue) at 15-Å resolution



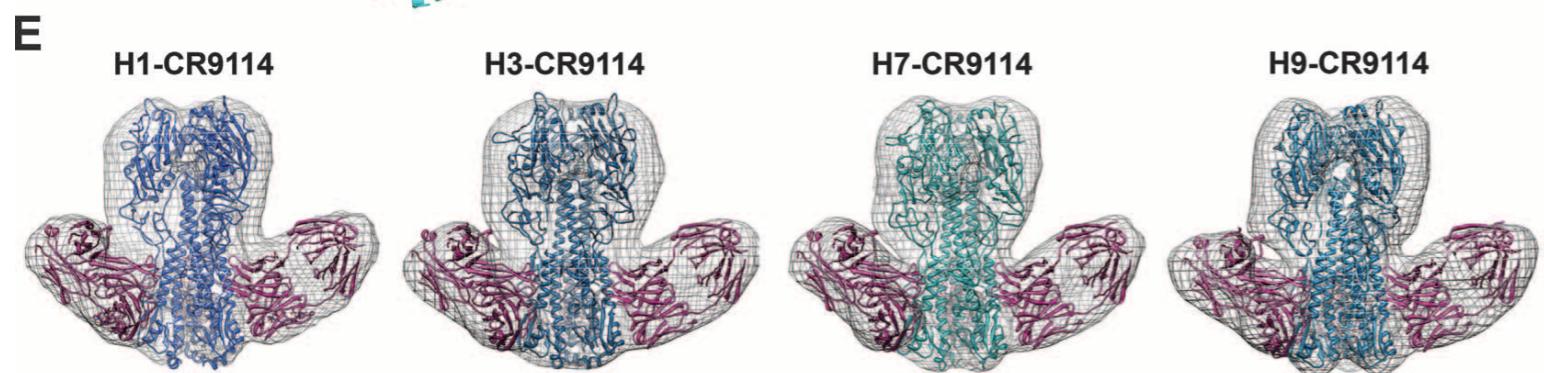
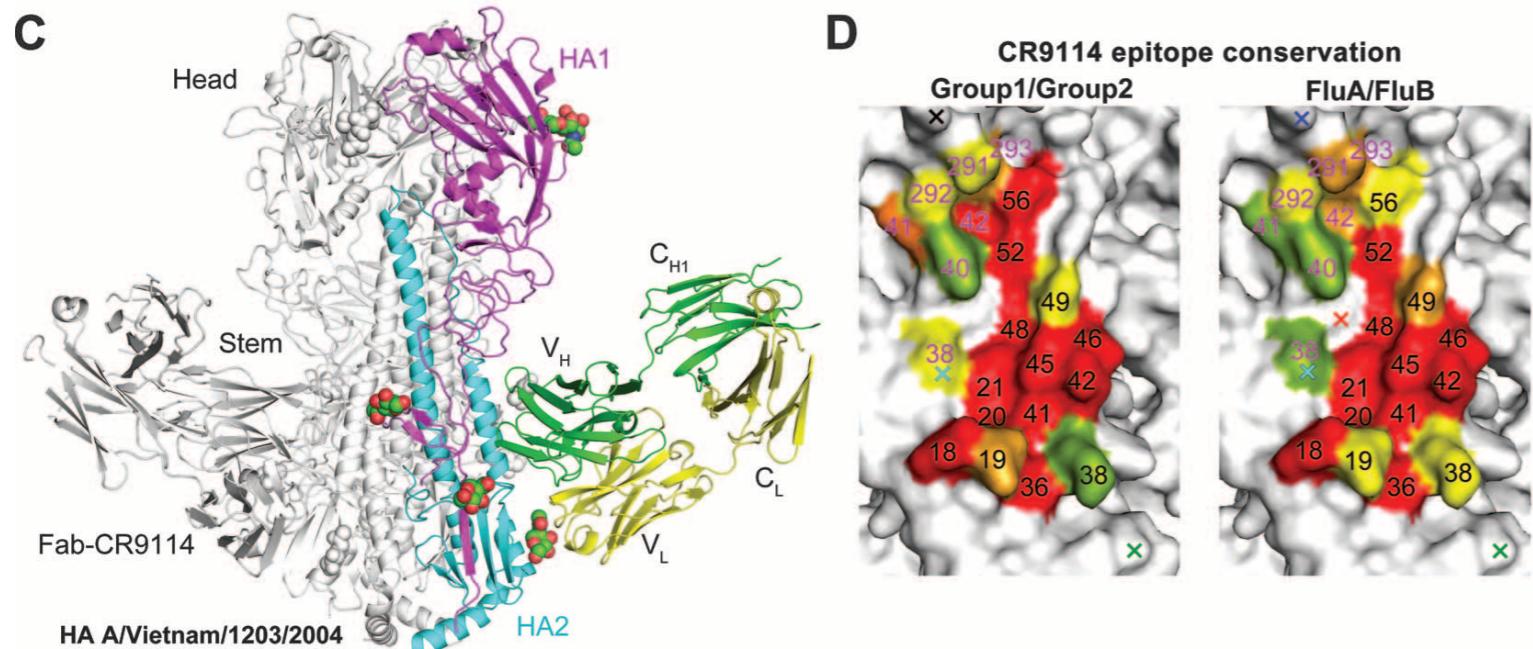
integrative modeling



Kaufmann B et al. PNAS 2006;103:12400-12404



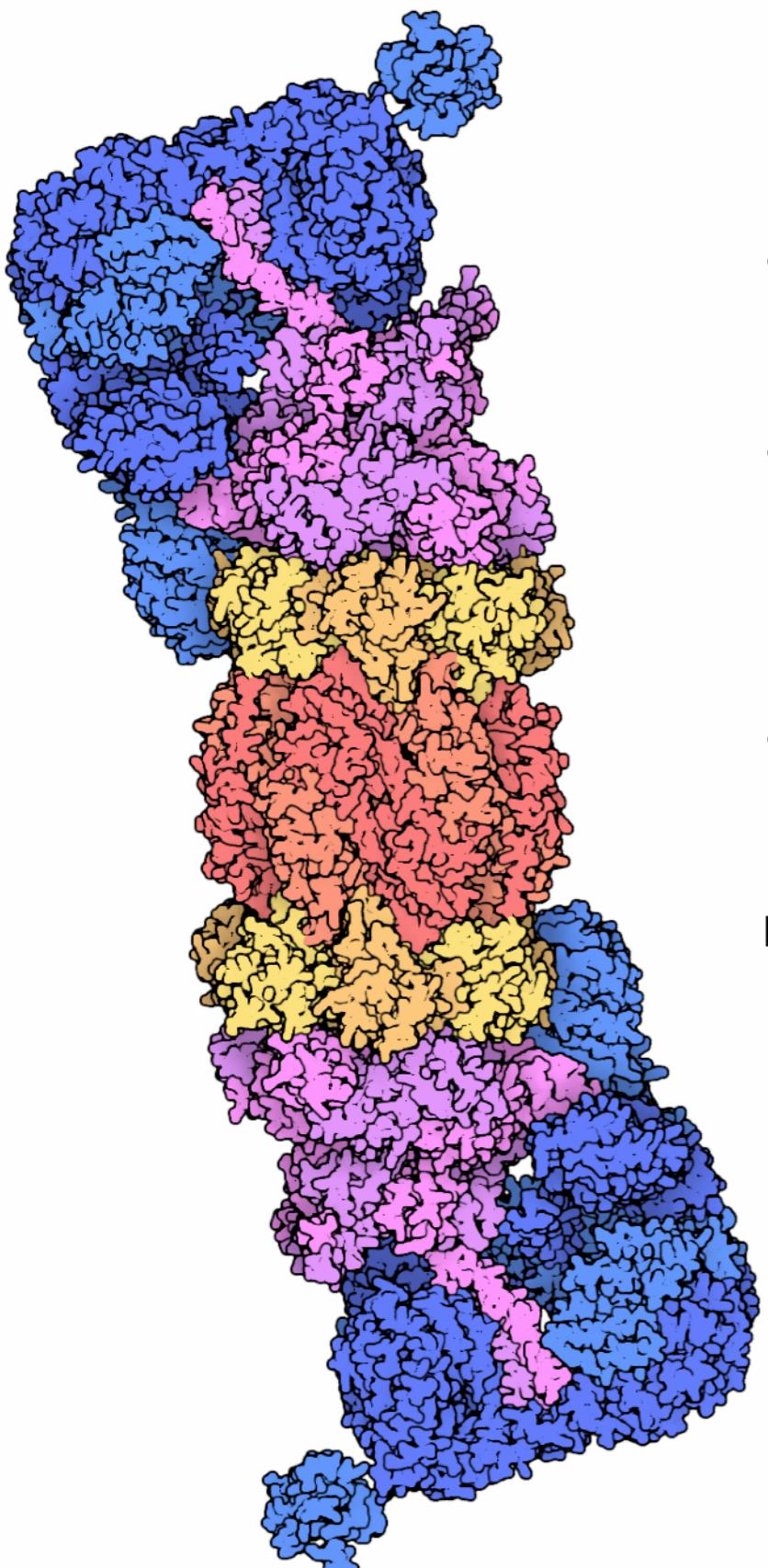
speed-up the development of broadly neutralizing antibodies as universal flu vaccine for all influenza A and B viruses



Highly Conserved Protective Epitopes on Influenza B Viruses  
Dreyfus et al. *Science* 337, 1343 (2012);

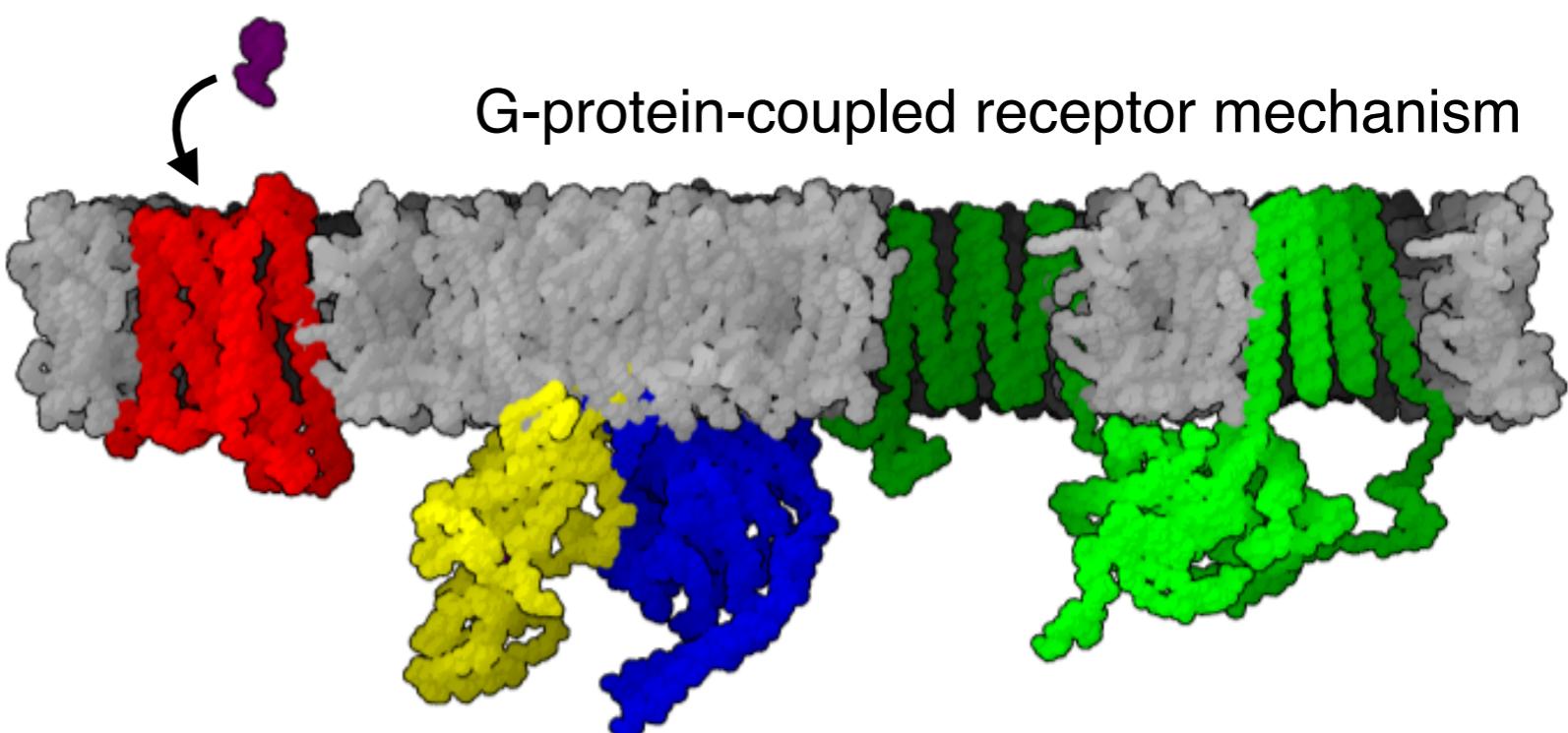
# Large molecular assemblies

- multimeric assembly defines function
  - it is hard to get atomistic resolution with traditional techniques (e.g., X-ray, cryo-EM)
  - assemblies are highly *dynamic*
- integration *via* molecular modeling

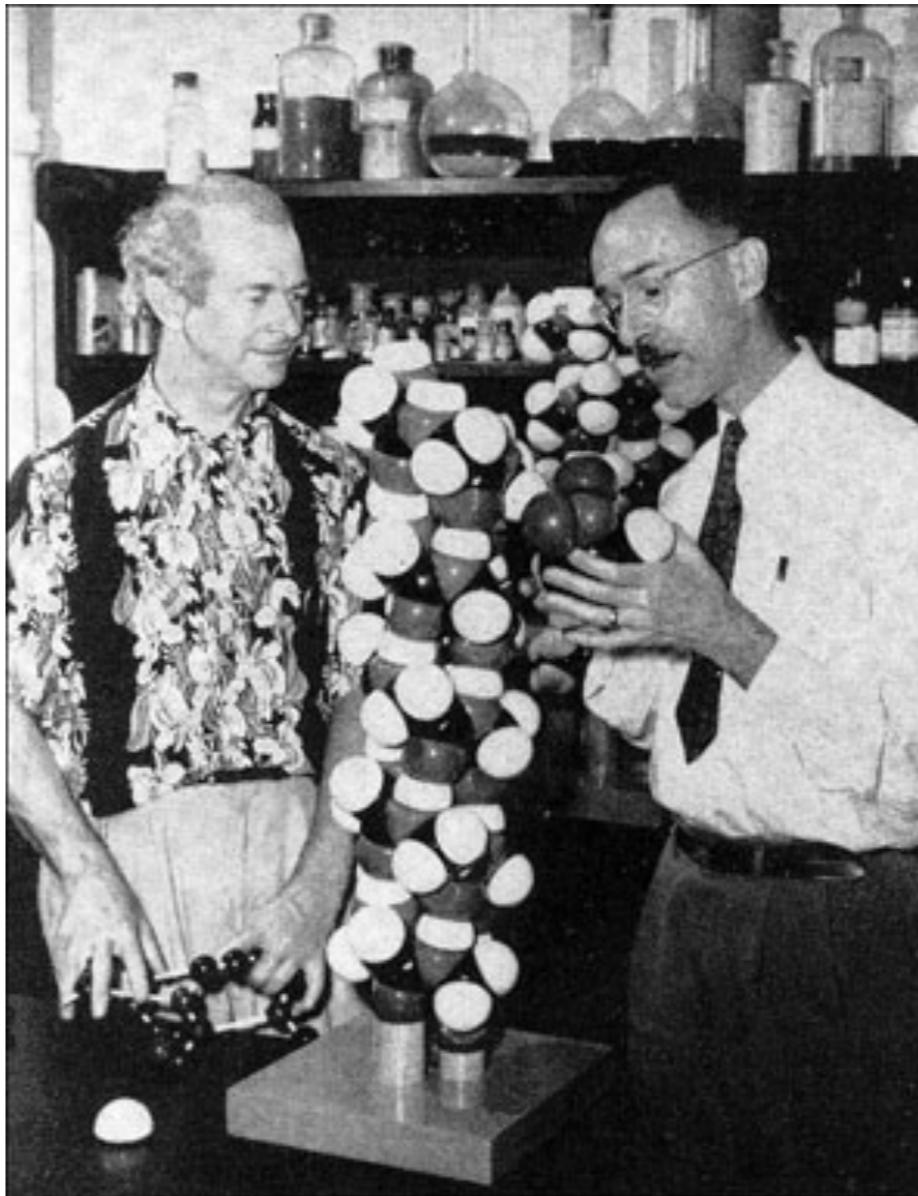
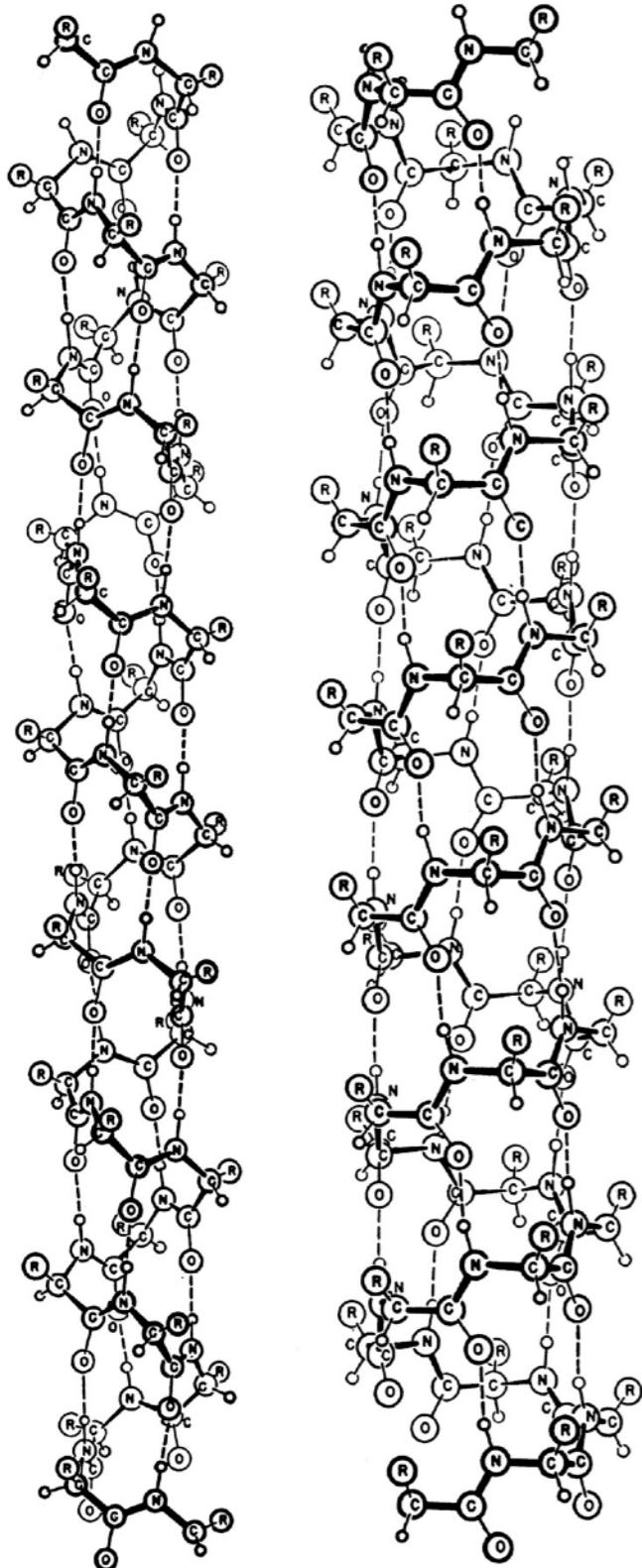


yeast proteasome 26S

October 2013 Molecule of the Month  
doi: 10.2210/rcsb\_pdb/mom\_2013\_10



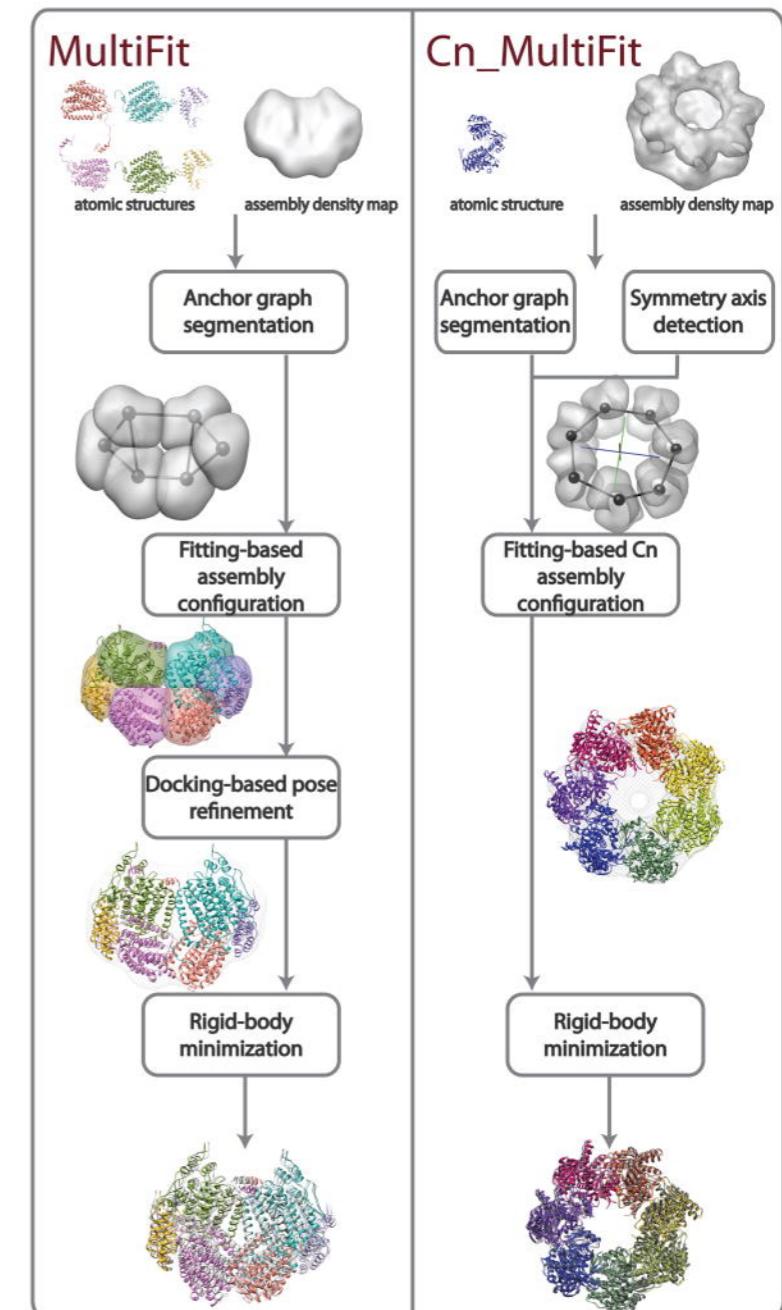
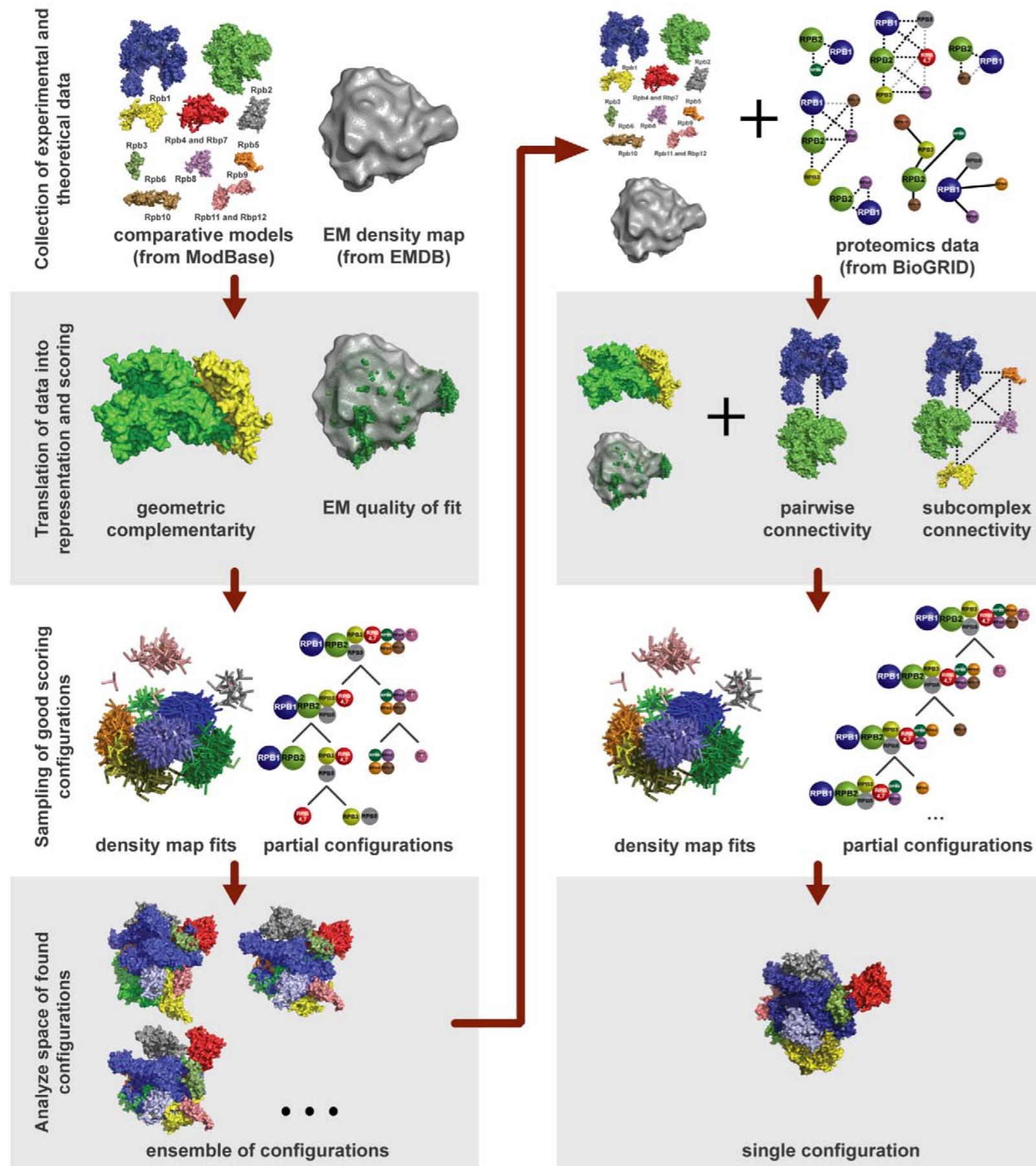
# (the dawn of) Integrative modeling



Pauling and Corey protein structure papers (1951):  
<http://www.pnas.org/site/misc/classics1.shtml>  
X-ray myoglobin 1959 (Kendrew and Perutz)

# Integrative modeling

IMP: integrative modeling platform  
<http://salilab.org/imp/>



<https://modbase.compbio.ucsf.edu/multifit/>

Russel et al. (2012) Putting the Pieces Together: Integrative Modeling Platform Software for Structure Determination of Macromolecular Assemblies.

PLoS Biol 10(1): e1001244. doi:10.1371/journal.pbio.1001244

Home

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Deposit

## Welcome to PDB-Dev

PDB-Dev is a prototype deposition and archiving system for structural models obtained through integrative/hybrid (I/H) methods. Structural characterization of many complex macromolecular assemblies are increasingly carried out using [I/H methods](#), where a combination of complementary experimental and computational techniques are used to determine the structure. The structural models obtained through I/H methods are collected, archived and disseminated to the public through PDB-Dev.

Deposit new structures [here](#).

More information of how to deposit structures can be found in the [FAQ](#) section.

## Visualization of structures downloaded from PDB-Dev

The multi-scale I/H structures downloaded from PDB-Dev can be visualized using the [ChimeraX](#) software.

Since ChimeraX is under active development, it is recommended to use the [Daily Build](#) of ChimeraX for visualization.

Please refer to the [Daily Build Notes](#) for information regarding operating systems currently supported by [ChimeraX](#).

The I/H structures can be downloaded as text files (use right click + save as) and opened on ChimeraX using the "format ihm" command line option along with the complete path to the downloaded file:

```
open path/to/myfile.cif format ihm
```

Alternately, the following command can be used to directly visualize an entry from PDB-Dev, eg: PDBDEV\_00000010.

```
open 10 from pdbdev
```

## Browse PDB-Dev

The PDB-Dev prototype system currently consists of integrative/hybrid models that follow the specifications defined in the I/H methods [dictionary](#), which is a modular extension of the [PDBx/mmCIF](#) dictionary. These include atomistic models as well as multi-scale models consisting of different coarse-grained representations. Browse all released structures below.

### Structure of the Nup84 sub-complex of the Nuclear Pore Complex

The Nup84 structure from budding yeast has been determined by IMP using spatial restraints derived from two-dimensional Electron Microscopy (2DEM) and chemical crosslinking followed by mass spectrometry (CX-MS) experiments.

Publication: Shi et al., Mol Cell Proteomics. 2014 Nov;13(11):2927-43. doi: [10.1074/mcp.M114.041673](https://doi.org/10.1074/mcp.M114.041673)

Related resource: [10.5281/zenodo.820724](https://zenodo.360724)

Accession code: [PDBDEV\\_00000001](#)

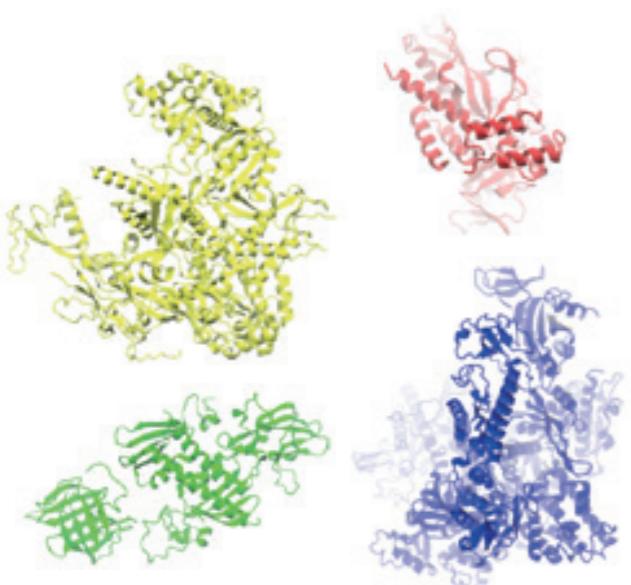
[Download](#) the structure.



# Integrative Modeling @ LBM

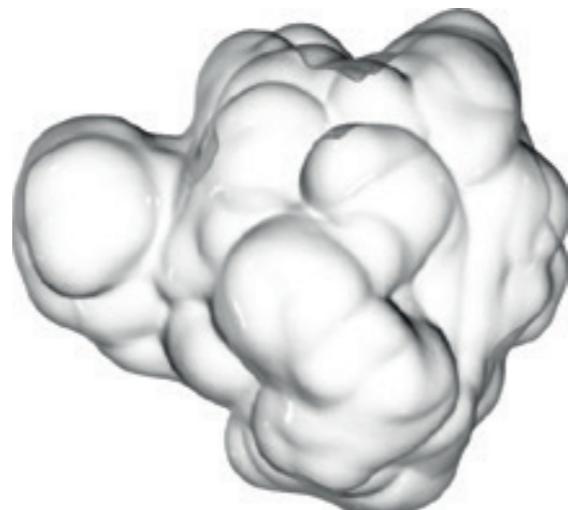
## Individual subunits

- X-ray crystallography
- NMR
- Cryo-EM
- Homology models



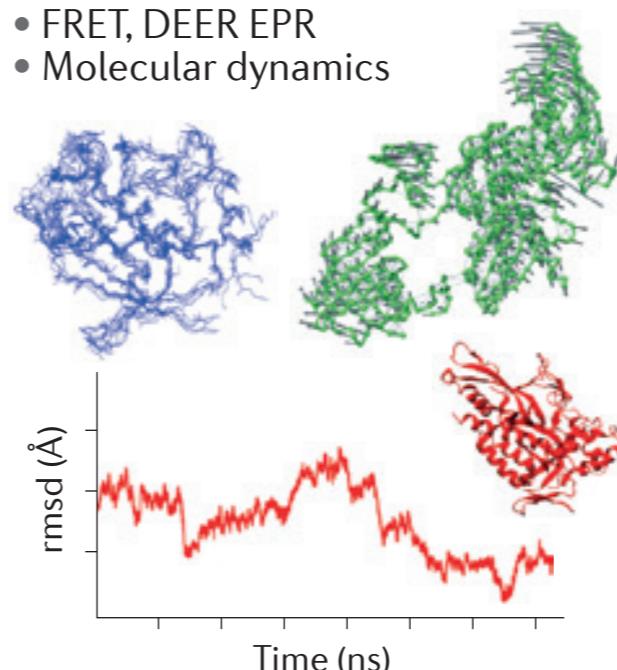
## Volumetric maps

- Cryo-EM
- Electron tomography
- SAXS, SANS
- AFM



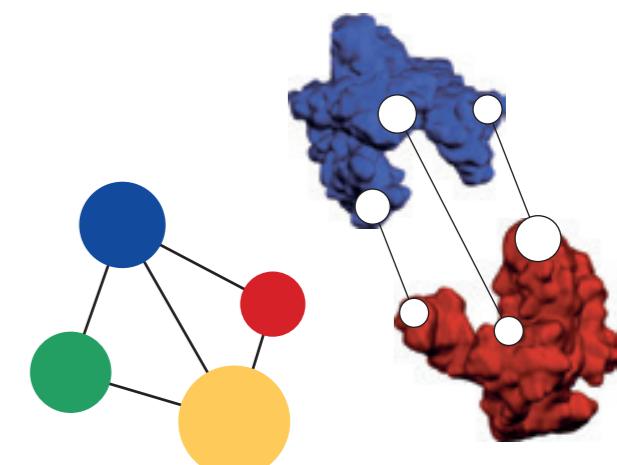
## Structural flexibility

- Side-chain and backbone sampling
- Elastic network models
- NMR ensembles
- FRET, DEER EPR
- Molecular dynamics

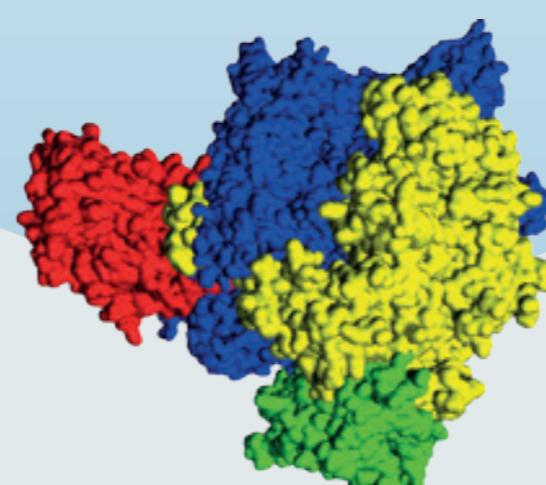


## Spatial connectivity

- Mutagenesis
- Evolutionary couplings
- Chemical crosslinking
- Proteomics
- H/D exchange
- ChIP-seq and ChIP-exo
- 3C, 4C, 5C and Hi-C



3C, chromatin conformation capture;  
4C, circularized 3C; 5C, carbon-copy  
3C; AFM, atomic force microscopy; ChIP-exo,  
ChIP-seq with an exonuclease sample preparation  
step; ChIP-seq, chromatin immunoprecipitation followed  
by sequencing; DEER EPR, double electron-electron  
resonance electron paramagnetic resonance; FRET,  
fluorescence resonance energy transfer; H/D exchange,  
hydrogen-deuterium exchange; NMR, nuclear magnetic  
resonance; Hi-C, genome-wide 3C; rmsd, root-mean-square  
deviation; SANS, small-angle neutron scattering; SAXS,  
small-angle X-ray scattering.



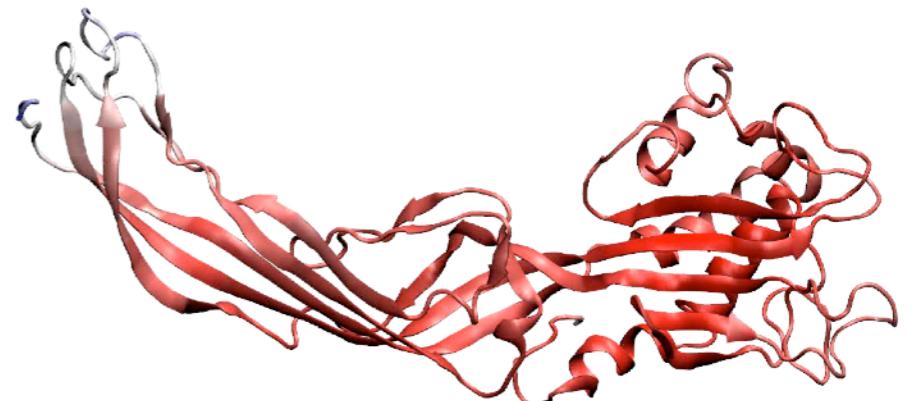
Near-atomic-resolution structure  
of supramolecular assemblies

# Integrative dynamic modeling

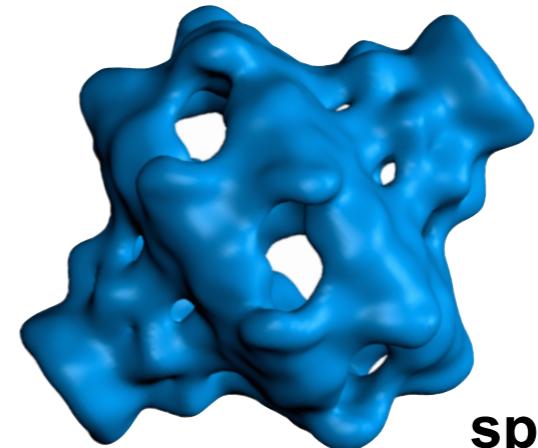
Degiacomi et al., *Structure* 2013



► input: monomeric structure

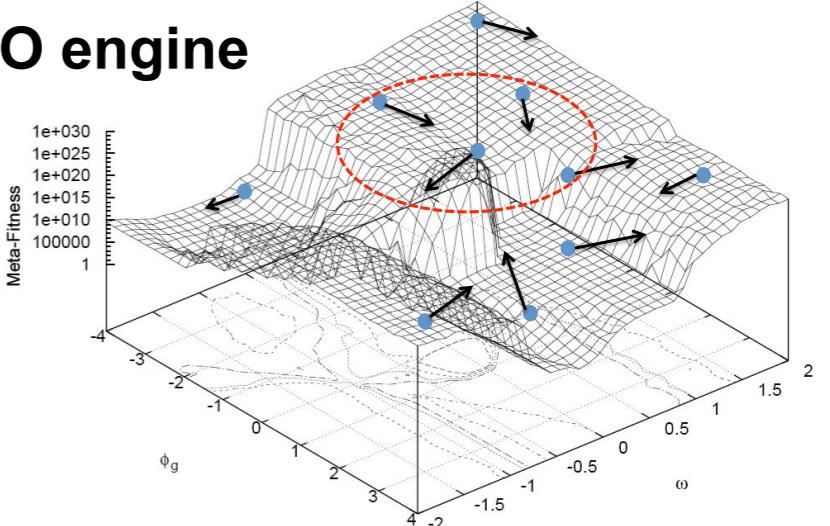


molecular dynamics, *time*  $\sim 10^{2-3}$  ns



low-resolution  
experimental  
spatial restraints

PSO engine



# Assembly by swarm intelligence

```
for every timestep t do:  
    for every particle p do:  
        inertia ← w*v(p,t-1)  
        personal ← cp*rand(0,1)*(x(p,t-1) - xbest(p))  
        global ← cn*rand(0,1)*(x(p,t-1) - x'best)  
        v(c,t) ← inertia + personal + global  
        if |v(p,t)| ≥ size(space) then  
            v(p,t) ← norm(v(p,t))*size(space)  
            x(p,t) ← x(p,t-1) + v(p,t)  
        else if |v(p,t)| ≤ vmin and f(x(p,t)) ≥ fmin then  
            v(p,t) ← rand(0,1)*vmin  
            x(p,t) ← x(p,t-1) + v(t)  
        else if |v(p,t)| ≤ vmin and f(x(p,t)) ≤ fmin then  
            v(p,t) ← rand(0,1)*vmin  
            x(p,t) ← rand(0,1)*space  
  
        else  
            x(p,t) ← x(p,t-1) + v(p,t)  
        end if  
        if f(x(p,t)) ≤ fbest(p) then  
            fbest(p) ← f(x(p,t))  
            xbest(p) ← x(p,t)  
        end if  
    end for  
end for
```

## Particle swarm optimization (PSO):

Kennedy & Eberhart (1995)

- converges efficiently to global minima

- particles are associated with a score (**fitness function**)

$$f(x) = c E_{phys} + (1-c) E^*_{data}$$

based on experimental derived restraints (cross-linking, cryo-EM, SAXS, ss-NMR, mutagenesis, etc.) and physical-based energy function (e.g. hard-wall, CG and atomistic potentials)

- a particle is affected by its **inertia** (w), its **personal best solution** (cp), and by the **global best solution** (cn)

- novel implementation: *KaR (kick and reseed)* has shown to be robust against complex benchmarks (e.g. Rastrigin function)



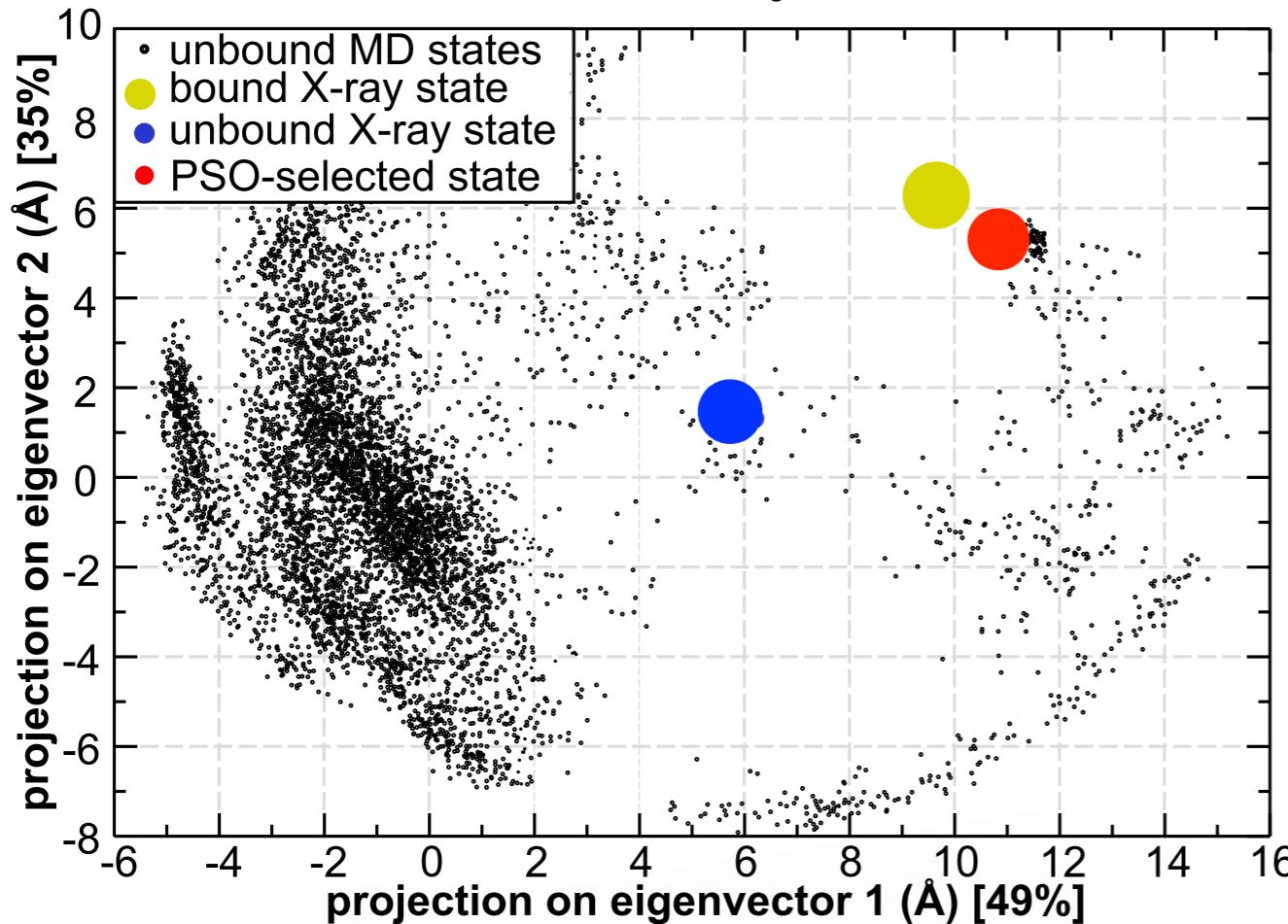
**power**

a parallel optimization workbench  
to enhance resolution in biological systems

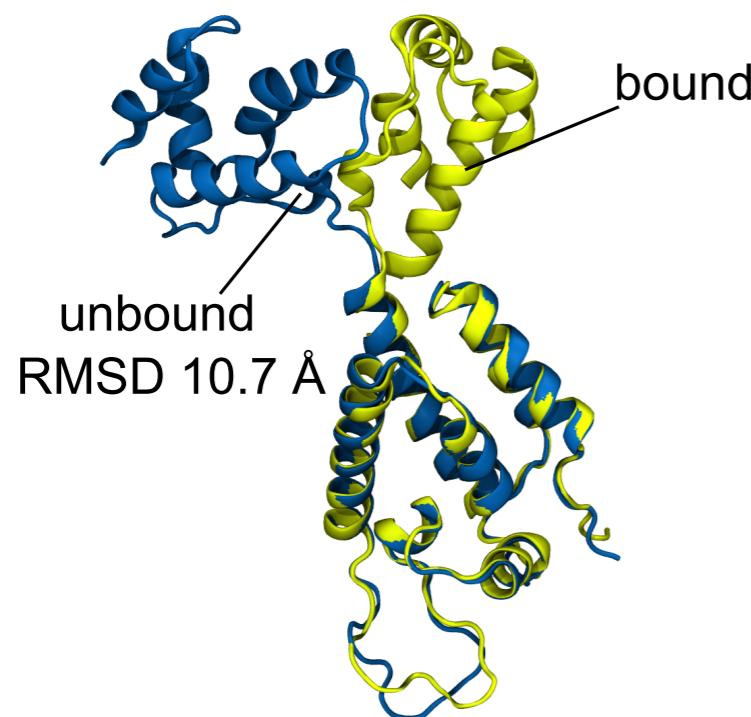
@ <http://lmb.epfl.ch/resources>  
Degiacomi *et al.*, **Structure** 2013

# *In silico* conformational capture

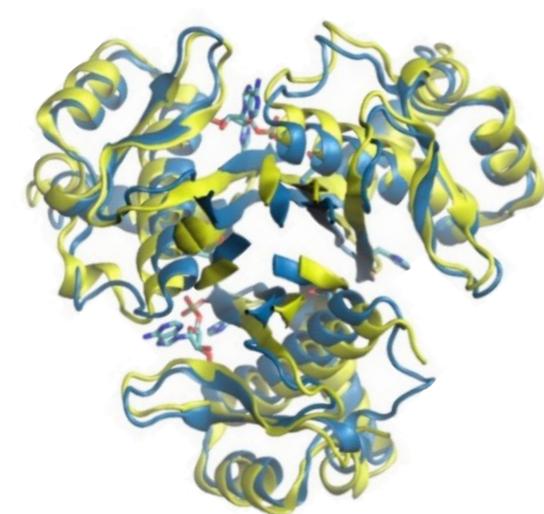
Hexameric capsomer ( $C_6$ ) ( $\sim 10 \text{ \AA RMSD}$ )



- large conformational changes upon assembly (up to  $\sim 10 \text{ \AA}$ ) can be taken into account
- within a **conformational selection** paradigm our approach is able to find the best bound state

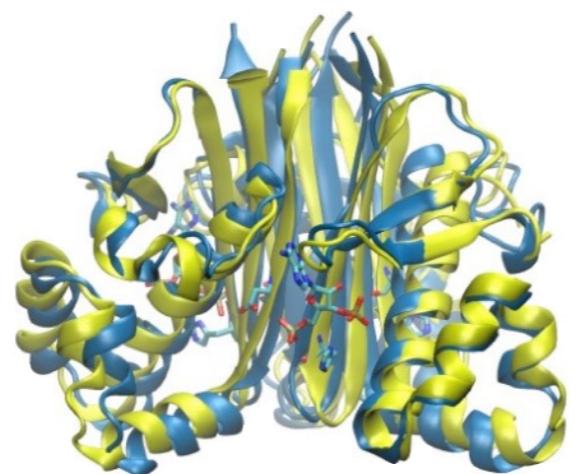


# Prediction of symmetrical assembly

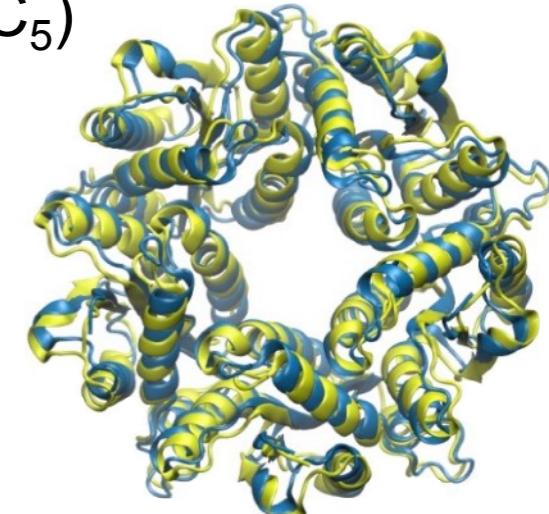
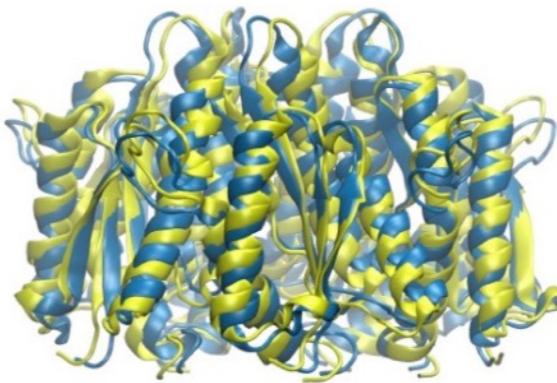


Acyl Carrier (C<sub>3</sub>)

RMSD 1.9 Å  
(restrained location of two residues at the active site)

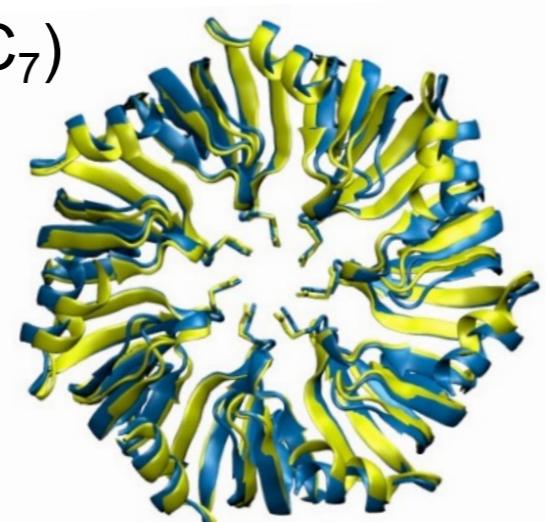
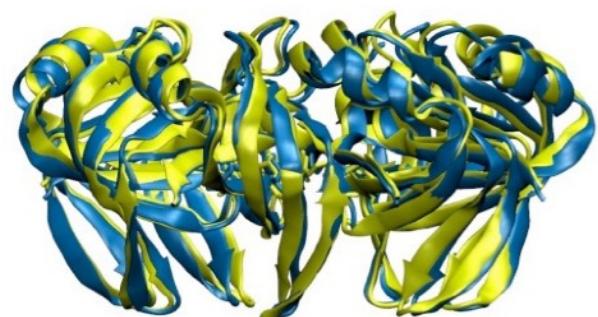


Lumazine Synthase (C<sub>5</sub>)



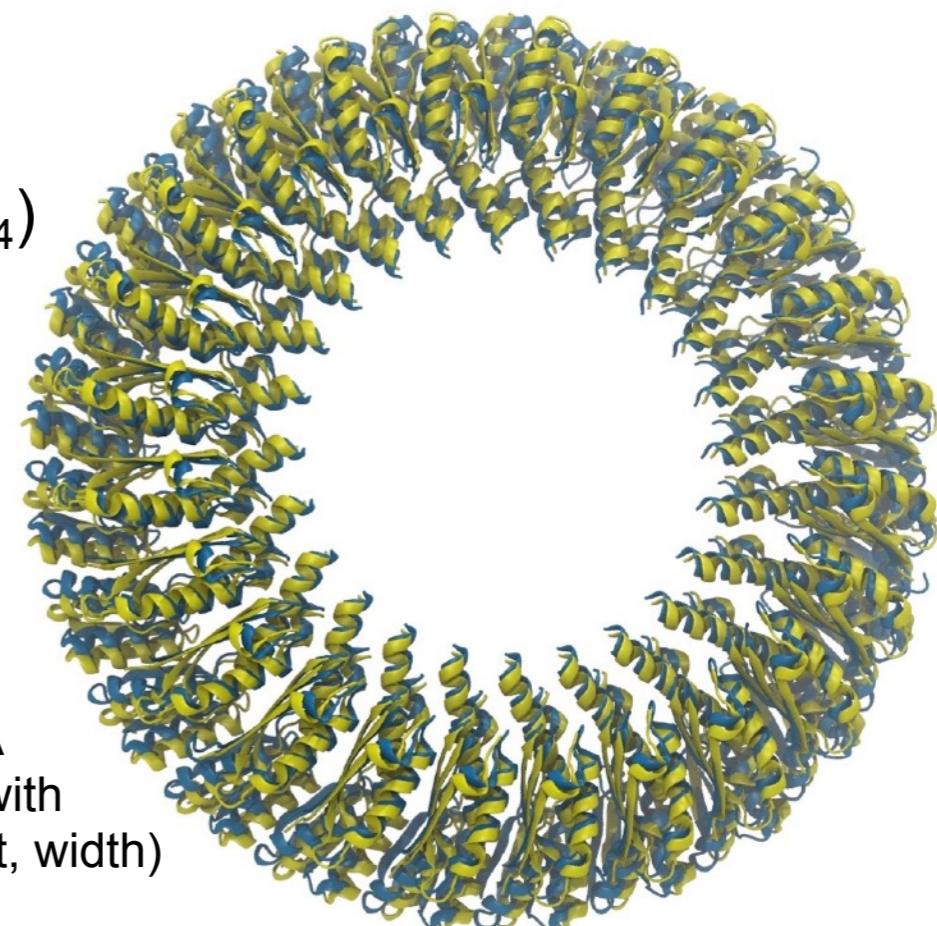
RMSD 1.7 Å  
(restrained with global height, width)

Archaeal SM protein (C<sub>7</sub>)



RMSD 0.9 Å (with X-ray reference)  
(restrained with position of a residue)

Yersinia  
YscJ (C<sub>24</sub>)



RMSD 2.4 Å  
(restrained with  
global height, width)

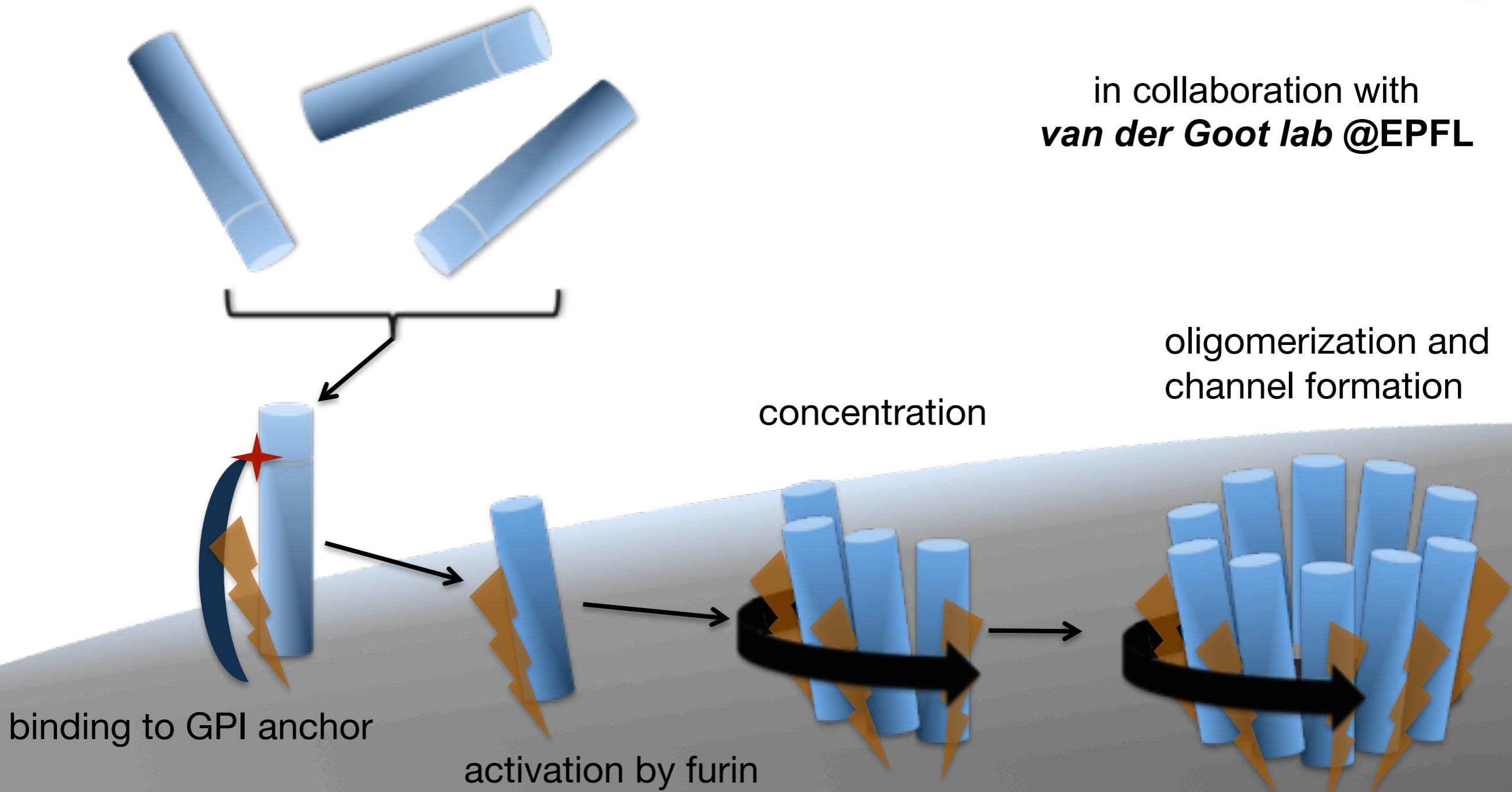
# Pore-forming toxin Aerolysin



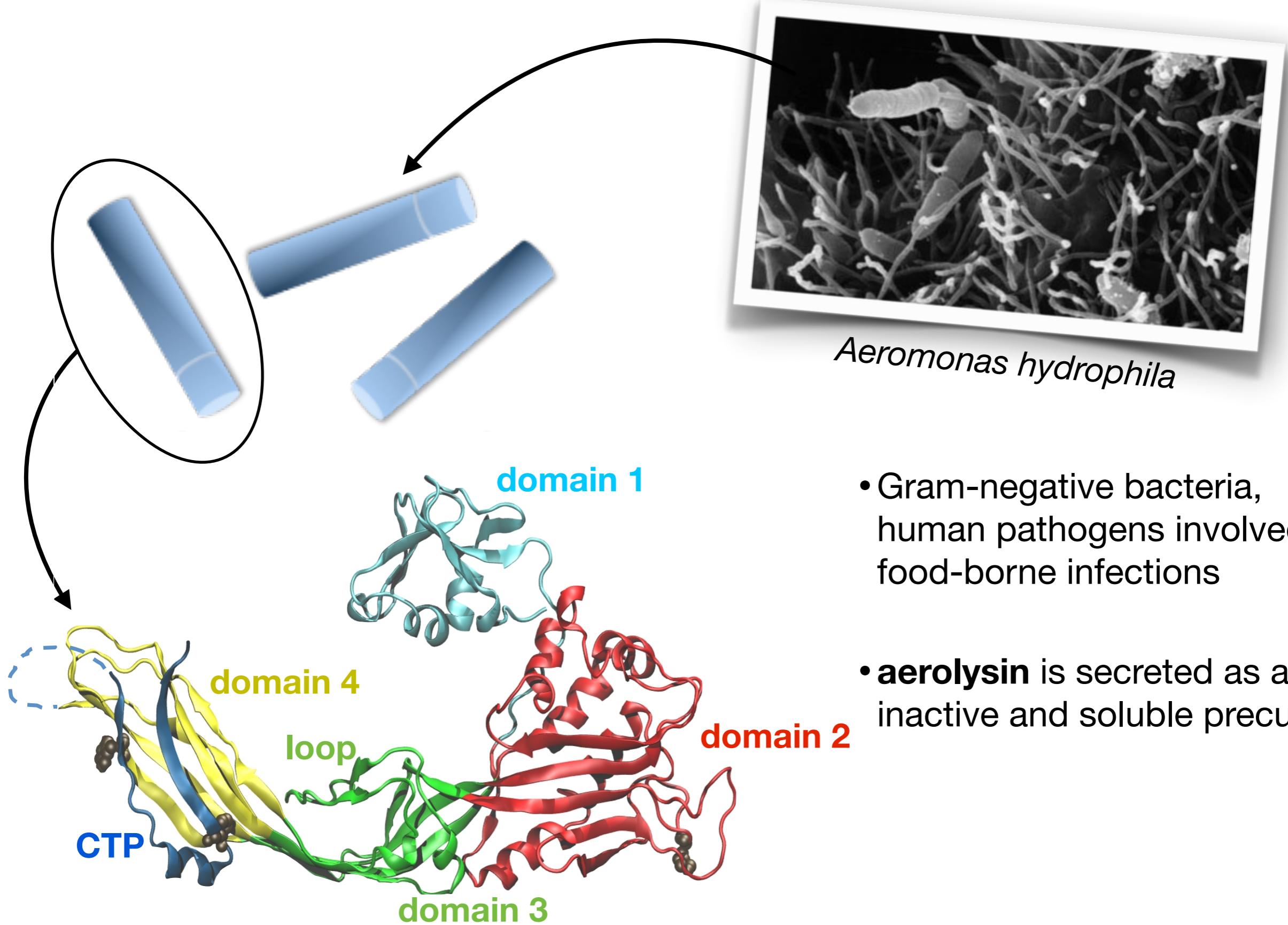
*Aeromonas hydrophila*

secretion  
monomerization

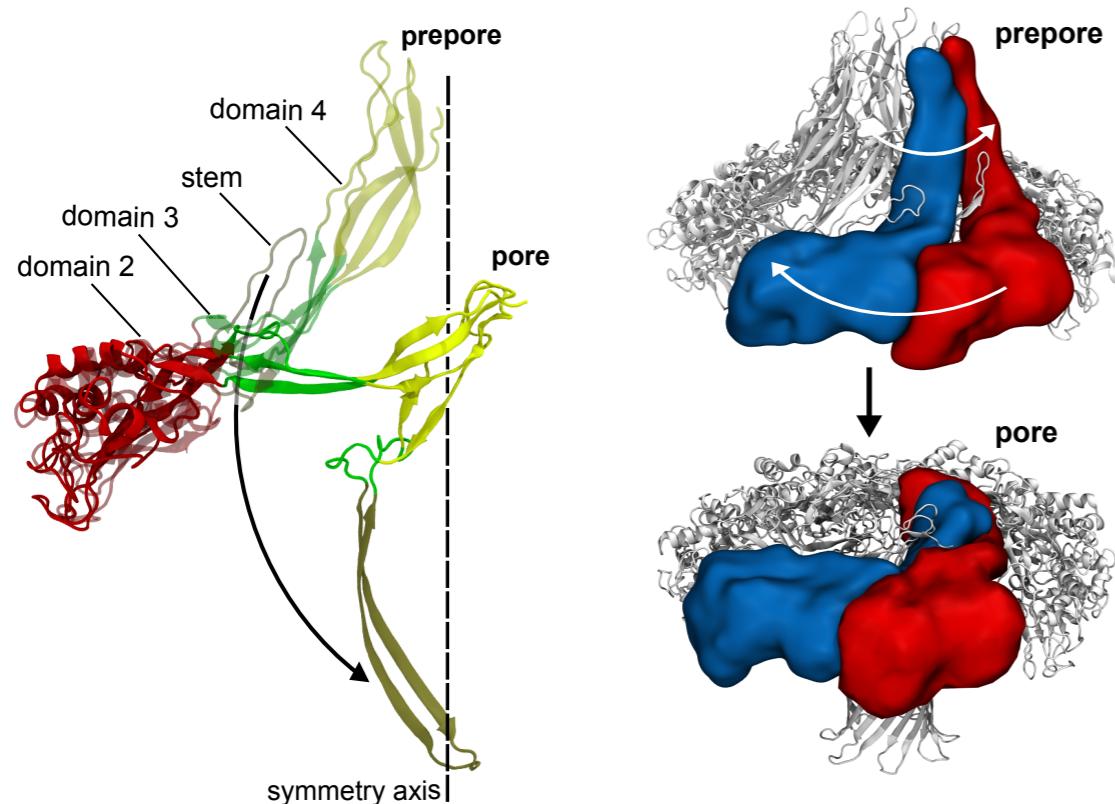
in collaboration with  
**van der Goot lab @EPFL**



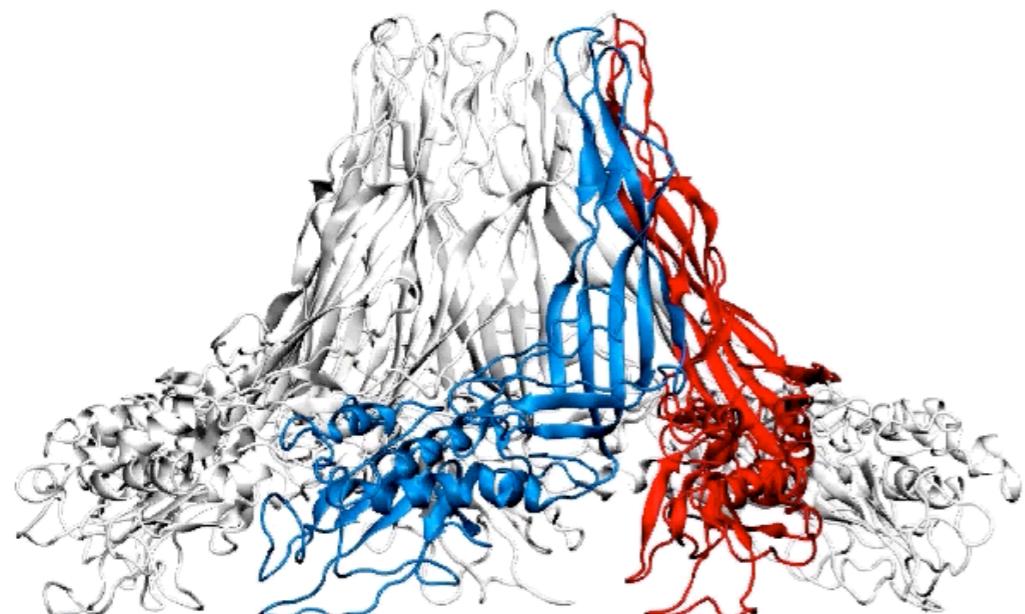
# PFT aerolysin from *Aeromonas* spp.



# A swirling mechanism of pore formation



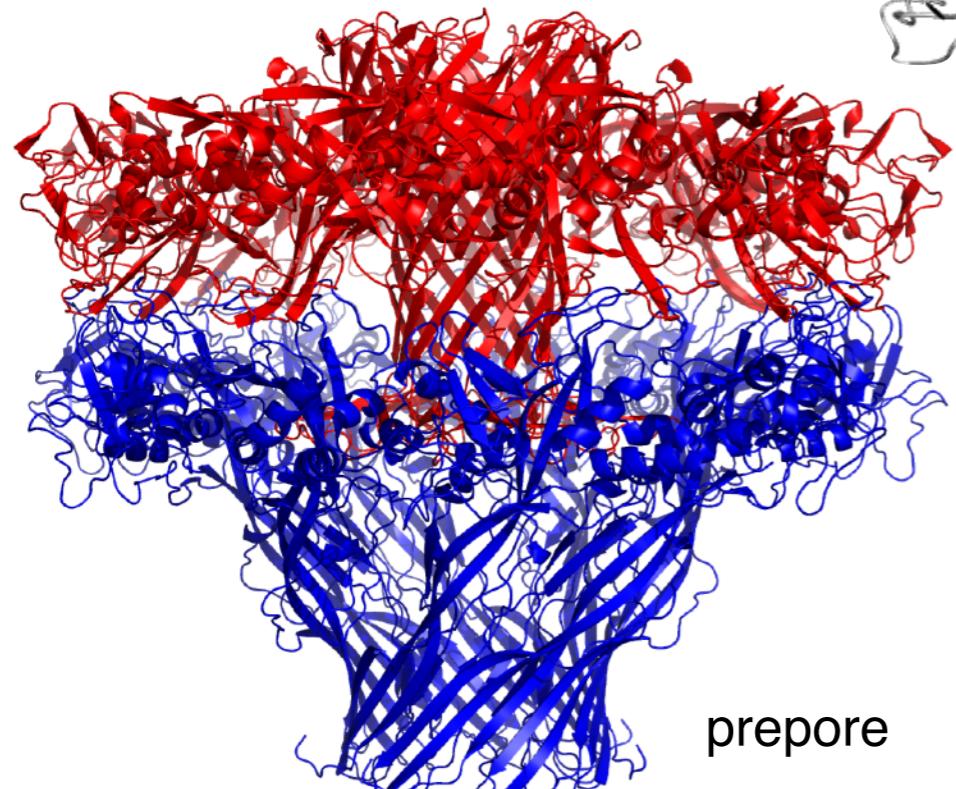
- flattening of domain 4 triggered by extraction of the stem loop
- global **swirling mechanism** for pore assembly and transmembrane barrel formation
- transition between prepore and pore is topologically consistent and does not involve structural clashes



Degiacomi et al. *Nature Chemical Biology* 2013

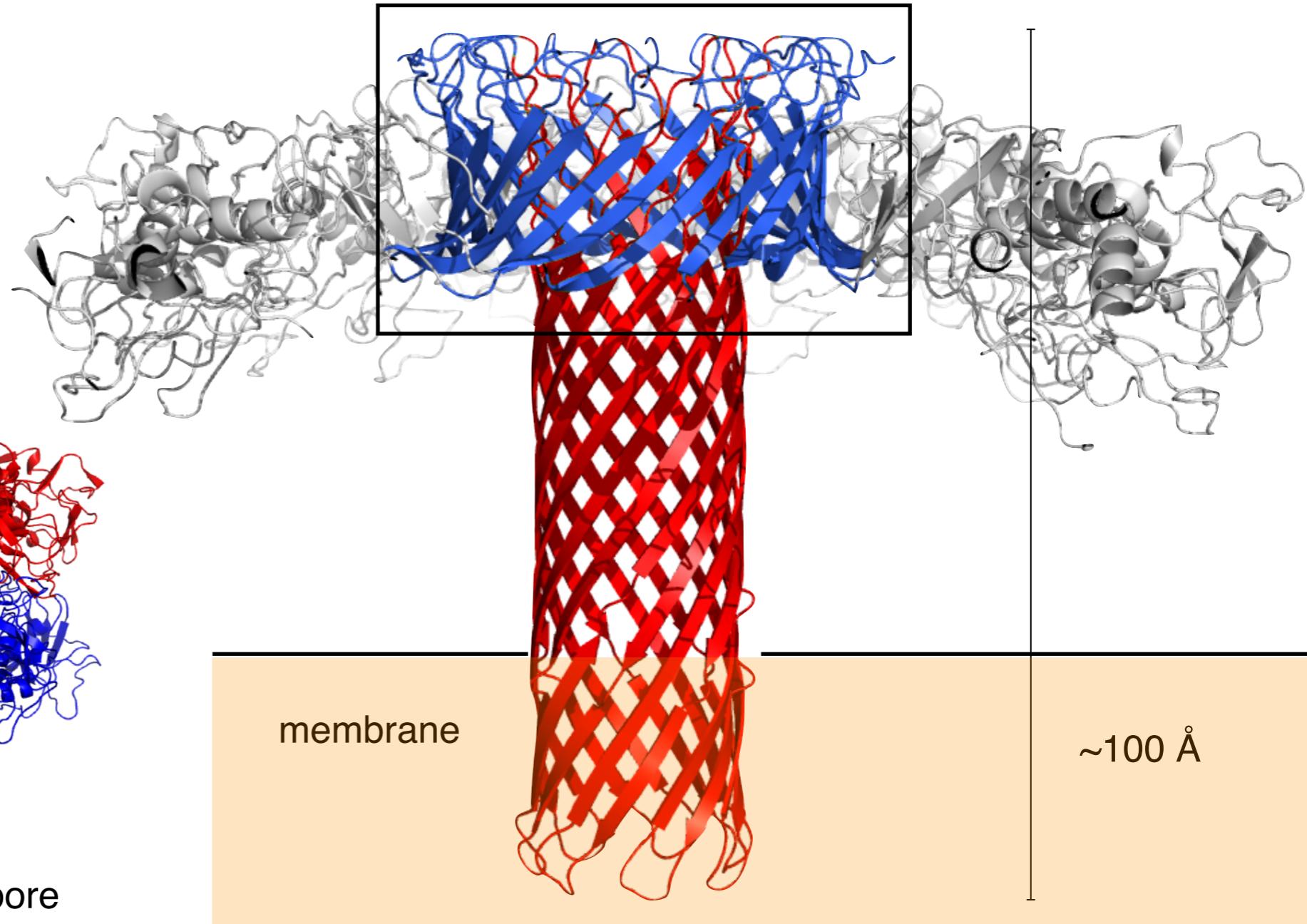
# Down to atomic resolution

quasi-pore



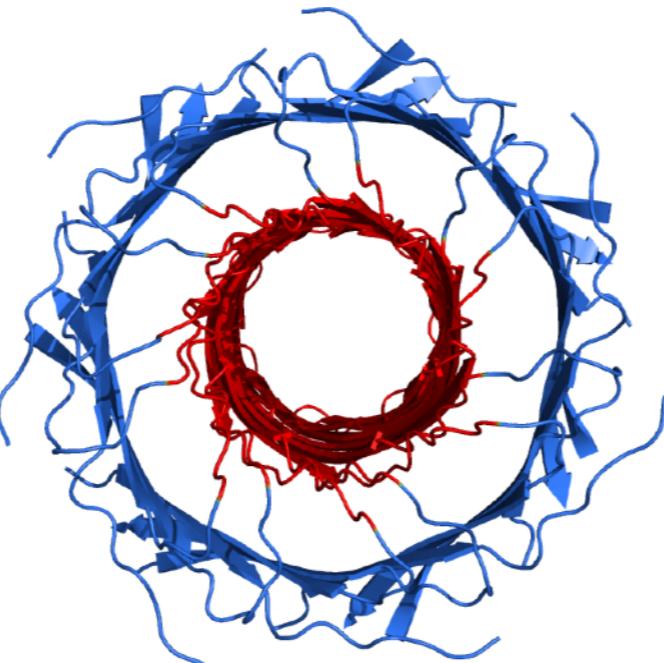
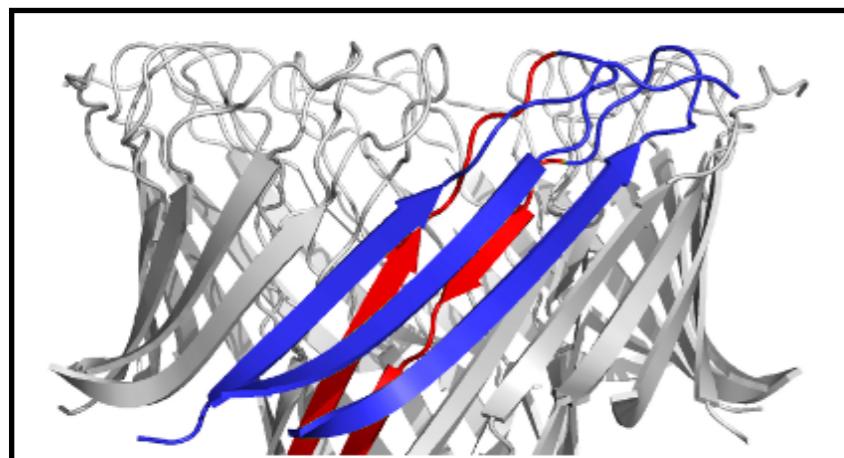
[Ioan Iacovache]

**new cryo-EM map at 3.5 Å  
(Zuber's lab, UniBern)**



[Nuria Cirauqui]

**an atomistic model of the mature pore**



a novel double  $\beta$ -barrel fold

inverted  $\beta$ -barrel topology

