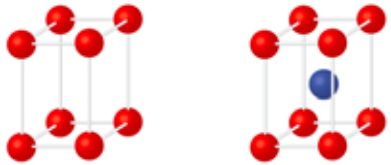
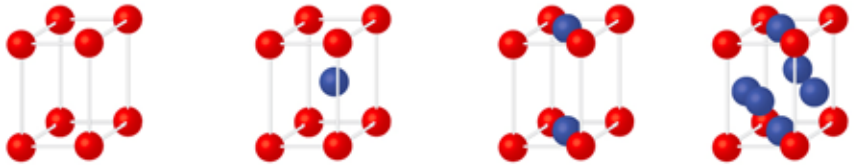
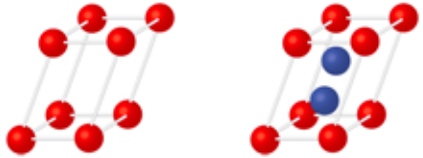
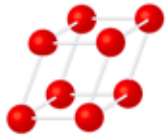
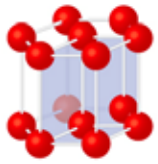
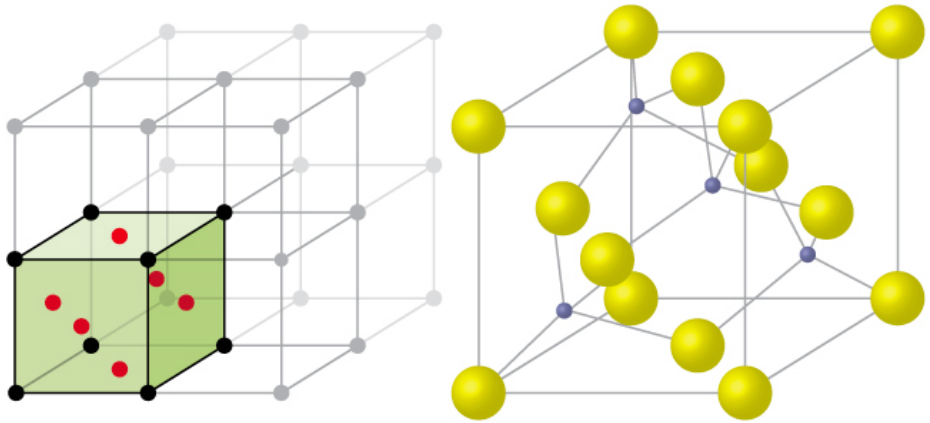
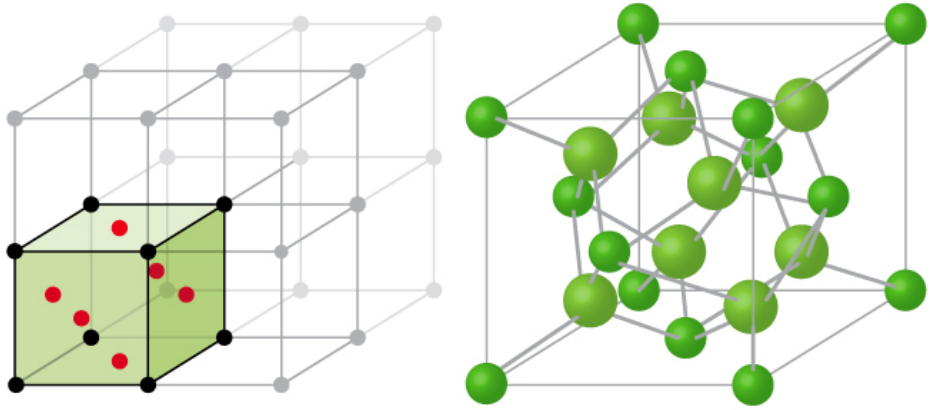
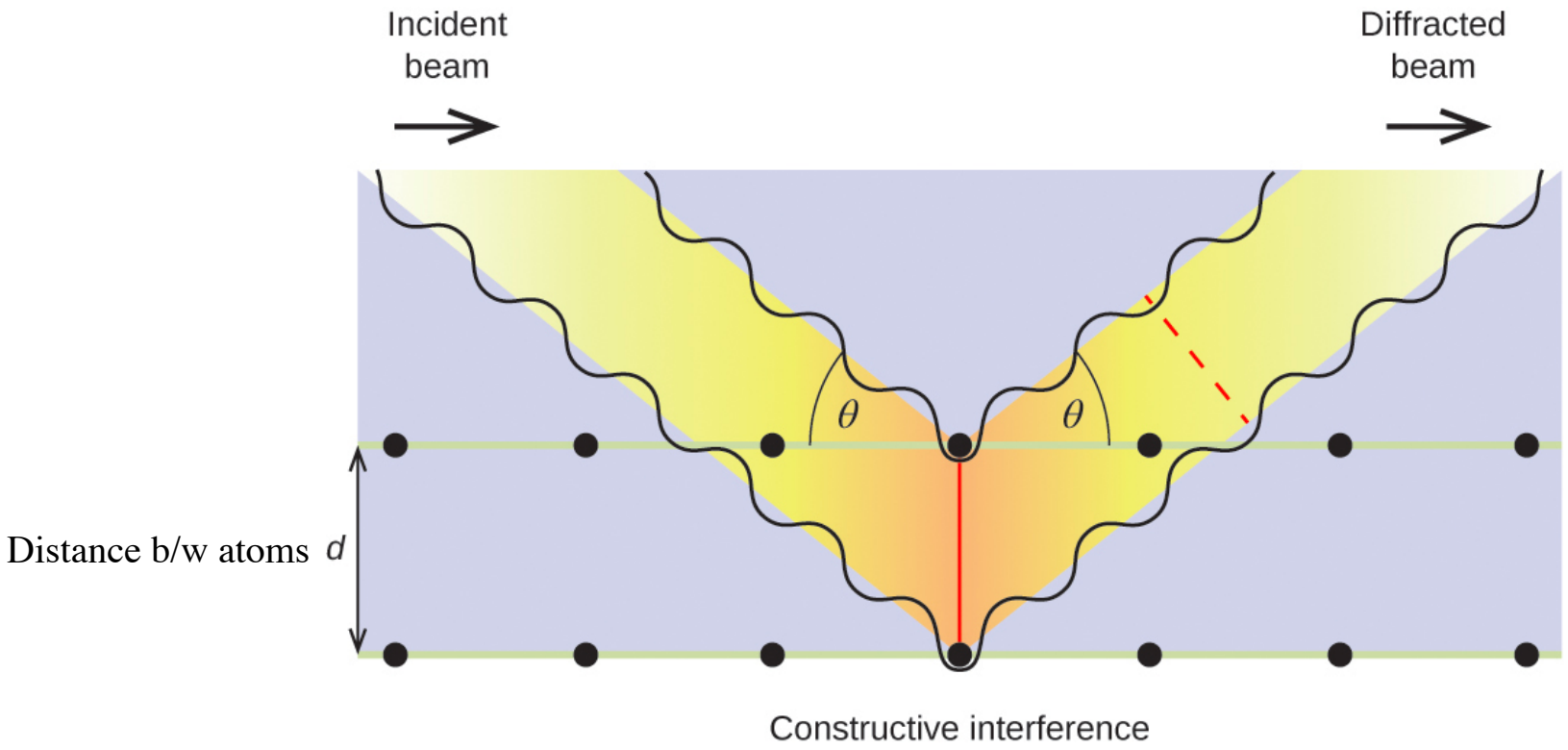
System/Axes/Angles	Unit Cells
Cubic $a = b = c$ $\alpha = \beta = \gamma = 90^\circ$	 Simple Face-centered Body-centered
Tetragonal $a = b \neq c$ $\alpha = \beta = \gamma = 90^\circ$	 Simple Body-centered
Orthorhombic $a \neq b \neq c$ $\alpha = \beta = \gamma = 90^\circ$	 Simple Body-centered Base-centered Face-centered
Monoclinic $a \neq b \neq c$ $\alpha = \gamma = 90^\circ; \beta \neq 90^\circ$	 Simple Base-centered
Triclinic $a \neq b \neq c$ $\alpha \neq \beta \neq \gamma \neq 90^\circ$	
Hexagonal $a = b \neq c$ $\alpha = \beta = 90^\circ; \gamma = 120^\circ$	
Rhombohedral $a = b = c$ $\alpha = \beta = \gamma \neq 90^\circ$	

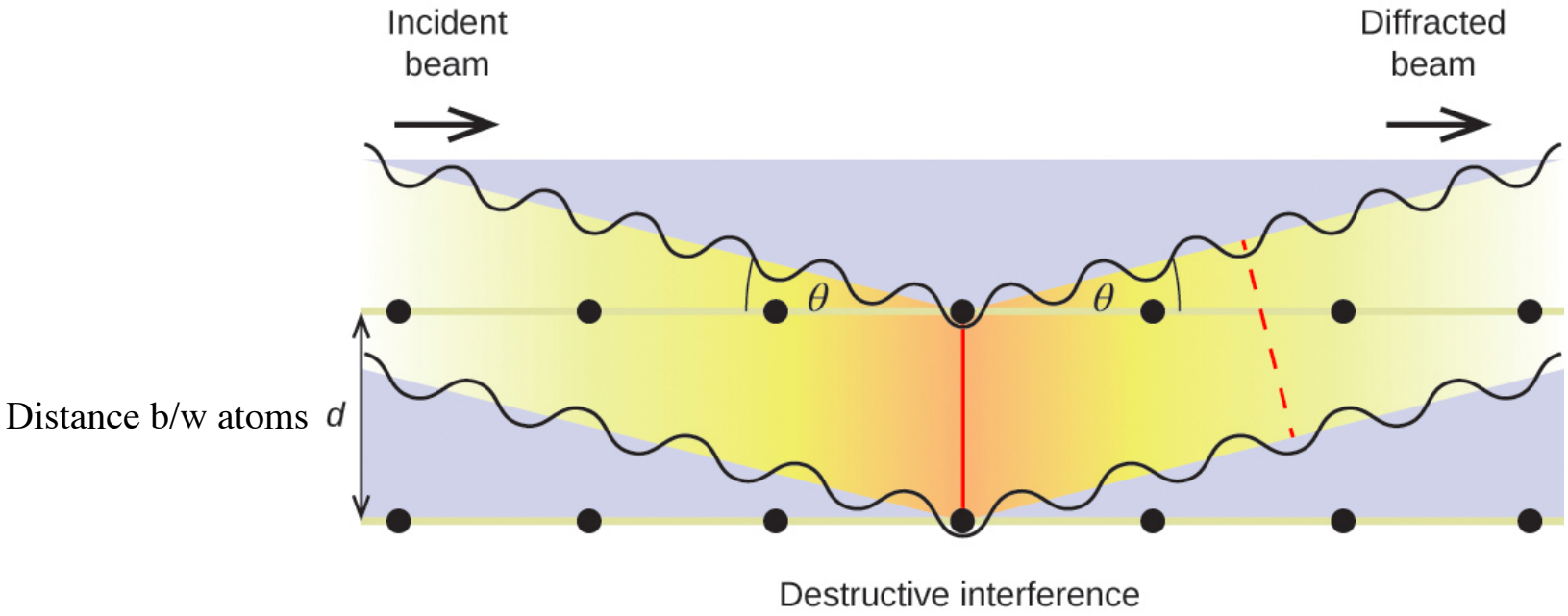


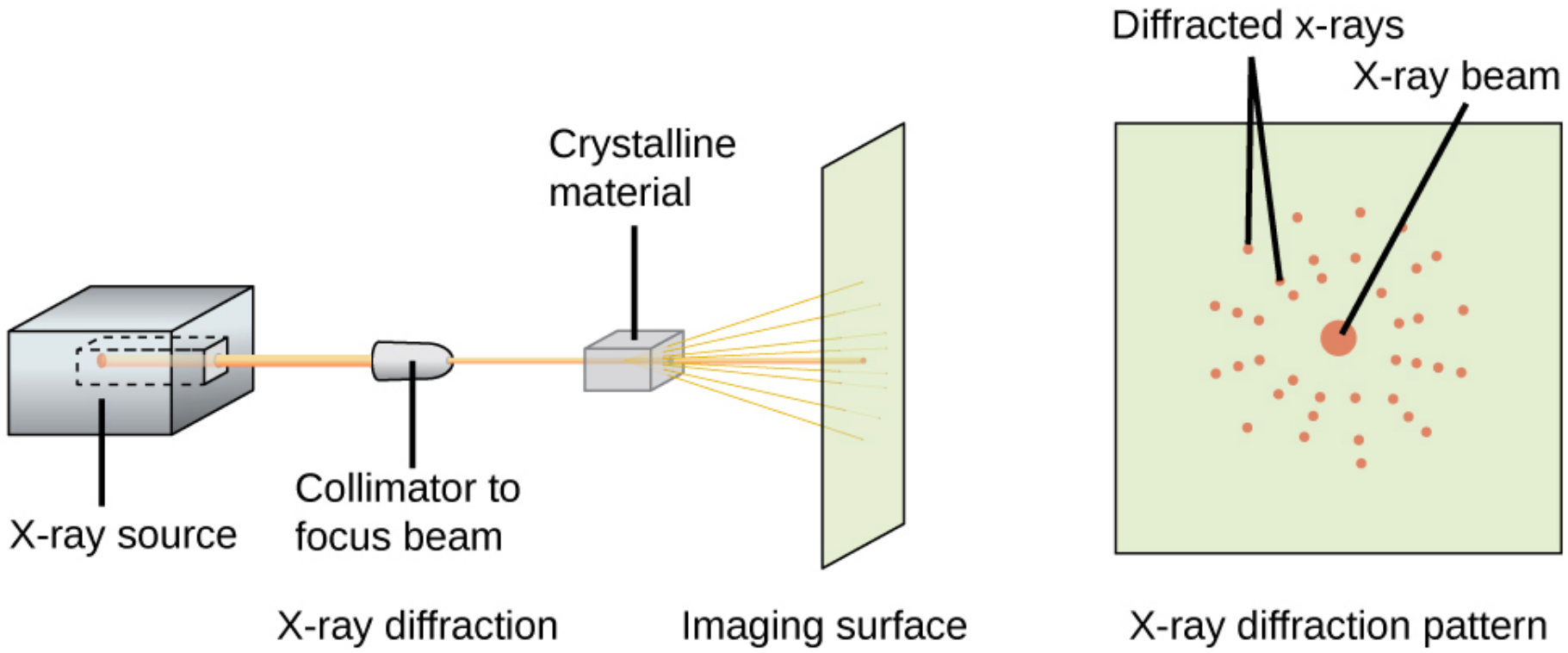
ZnS face-centered unit cell



CaF_2 face-centered unit cell


Incident beam
Diffracted beam
θ
θ
Distance b/w atoms d
Constructive interference


Incident beam
Diffracted beam
θ
θ
Distance b/w atoms d
Destructive interference


Crystalline material
X-ray source
Collimator to focus beam
X-ray diffraction
Imaging surface
(a)
Diffracted x-rays
X-ray beam
X-ray diffraction pattern
(b)

An 7-step program for protein structure determination by x-ray crystallography

1. Produce monodisperse protein either alone or as relevant complexes
2. Grow and characterize crystals
3. Collect X-ray diffraction data
4. Solve the phase problem either experimentally or computationally
5. Build and refine an atomic model using the electron density map
6. Validation: How do you know if a crystal structure is right**?**
7. Develop structure-based hypothesis

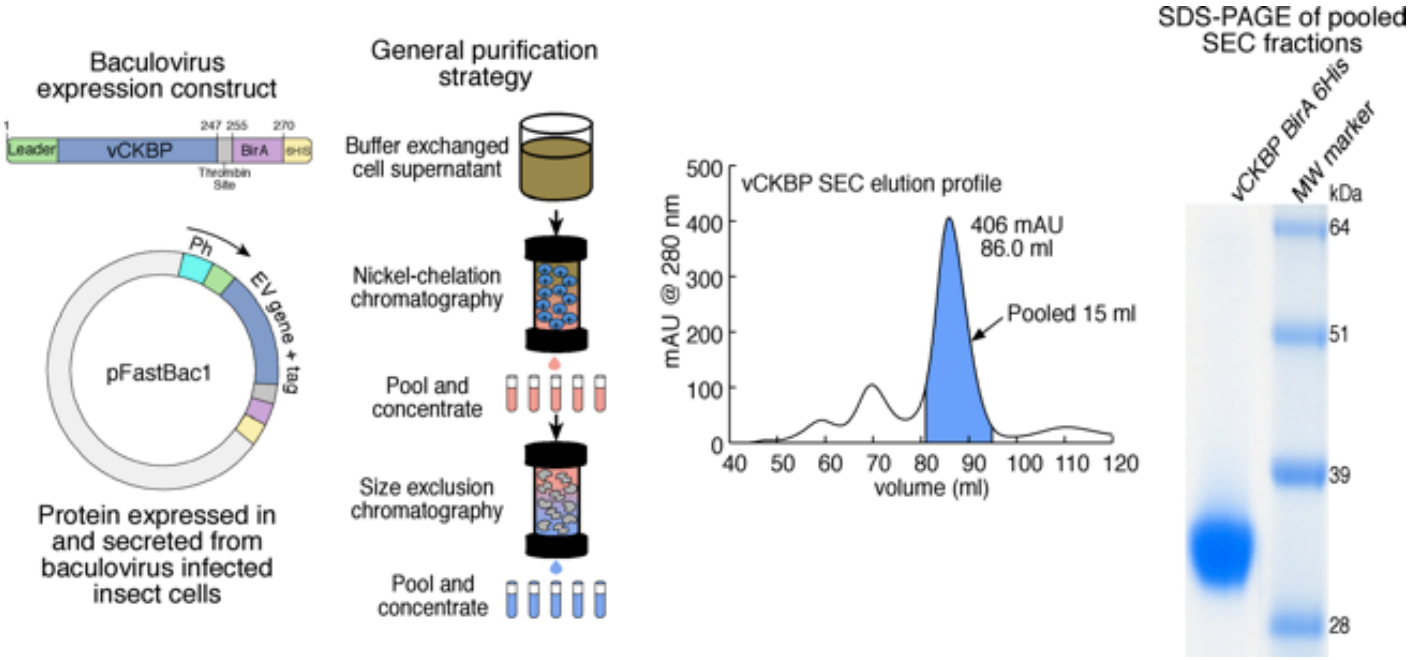
1. Produce monodisperse protein either alone or as relevant complexes

Methods to determine protein purity, heterogeneity, and monodispersity

- Gel electrophoresis (native, isoelectric focusing, and SDS-PAGE)
- Size exclusion chromatography
- Dynamic light scattering http://www.protein-solutions.com/
- Circular Dichroism Spectroscopy http://www-structure.llnl.gov/cd/cdtutorial.htm

Characterize your protein using a number of biophysical methods

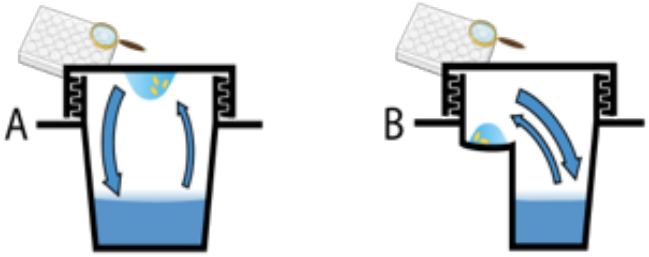
Establish the binding stoichiometry of interacting partners



2. Grow and characterize crystals

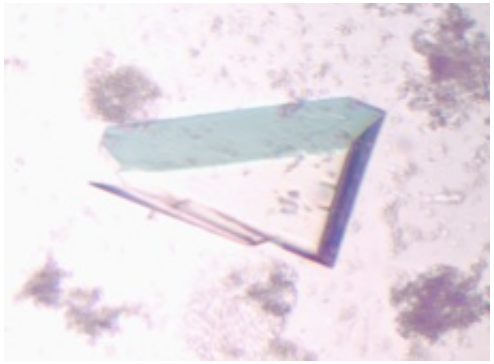
- Hanging Drop vapor diffusion
- Sitting drop, dialysis, or under oil
- Macro-seeding or micro-seeding
- Sparse matrix screening methods
- Random thinking processes, talisman, and luck

The optimum conditions for crystal nucleation are not necessarily the optimum for diffraction-quality crystal growth

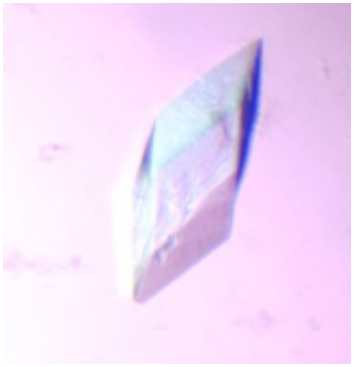


Hanging Drop Sitting drop

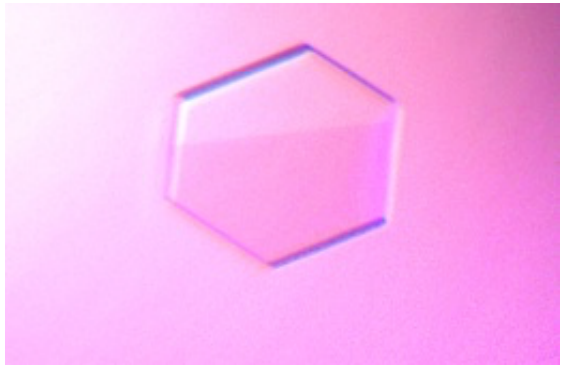
Commercial screening kits available from
http://www.hamptonresearch.com;
http://www.emeraldbiostructures.com



Space Group $P2_1$
4 M3 /ASU
diffraction >2.3Å
14.4% Peg6K
NaCacodylate pH 7.0
200mM $CaCl_2$



Space Group C2
2 M3 /ASU
diffraction >2.1Å
18% Peg4K
Malic Acid/Imidazole pH 5.1
100mM $CaCl_2$

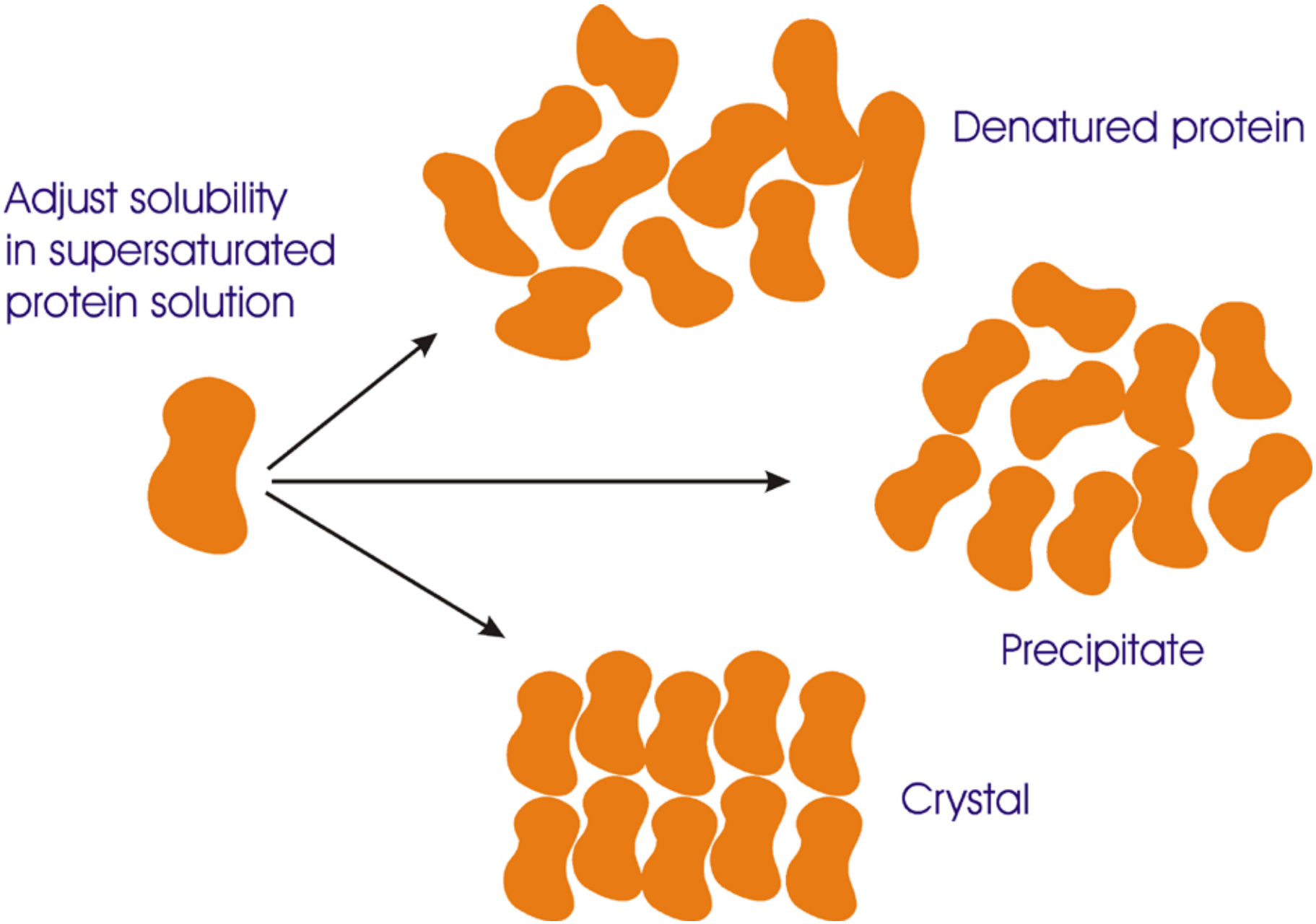


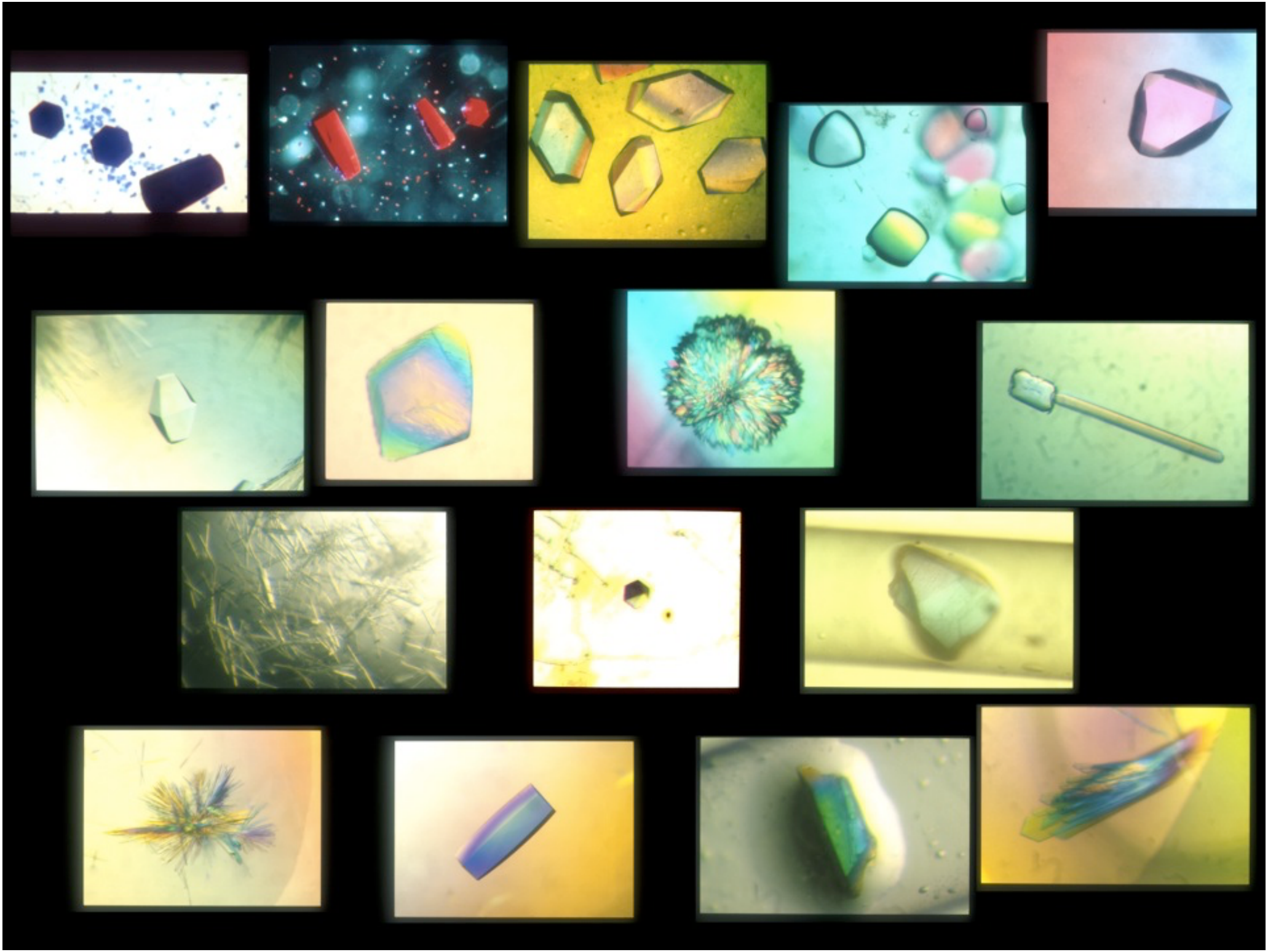
Space Group $P3_121$
3 M3 + 3 MCP-1/ASU
diffraction > 2.3Å
18% Peg4K
NaAcetate pH 4.1
100mM $MgCl_2$

No Crystals?

Decrease protein heterogeneity

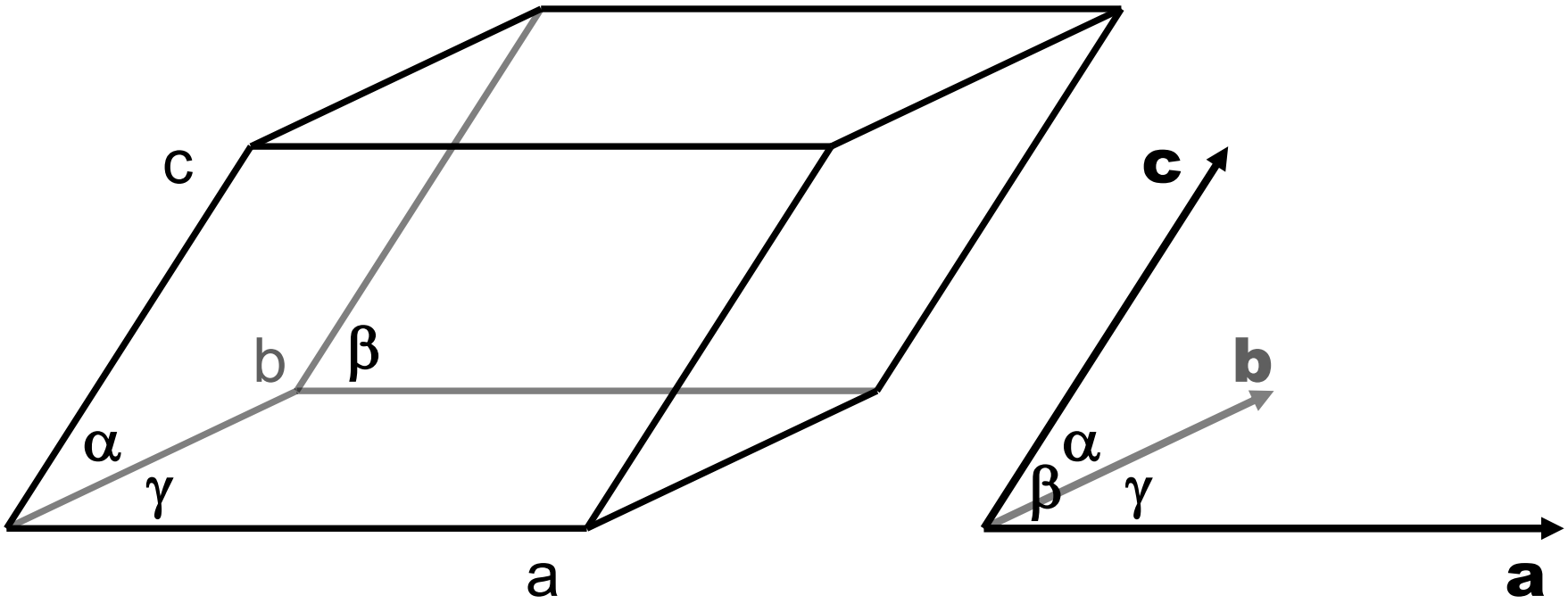
- Remove purification tags and other artifacts of protein production
- Remove carbohydrate and other residues
- Use MS to determine purity.
- **Think about the biochemistry of the system!** Does your protein have co-factors, accessory proteins, or interacting partners to prepare as complexes? Is their an inhibitor available? Are kinases or phosphatases available that will allow for the preparation of a homogeneous sample?
- Get a better talisman


Adjust solubility
in supersaturated
protein solution
Denatured protein
Precipitate
Crystal

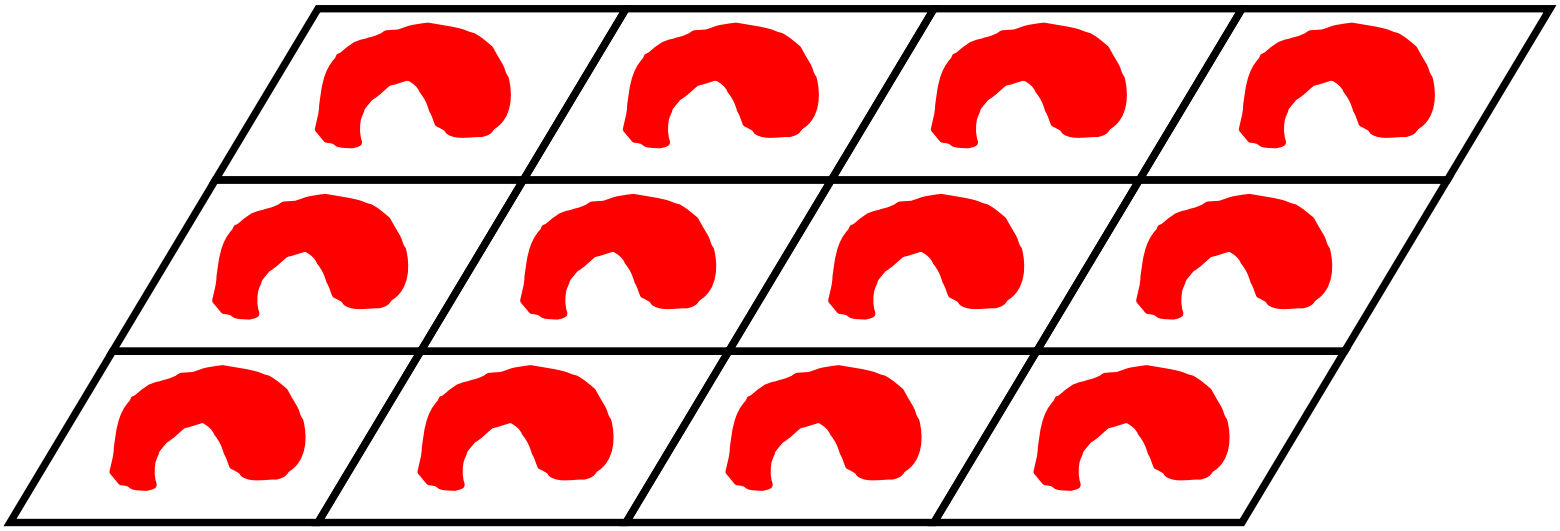


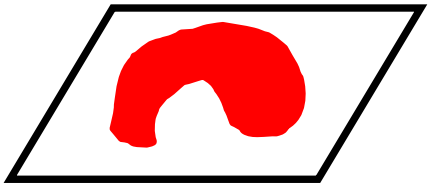
Building a crystal

The unit cell



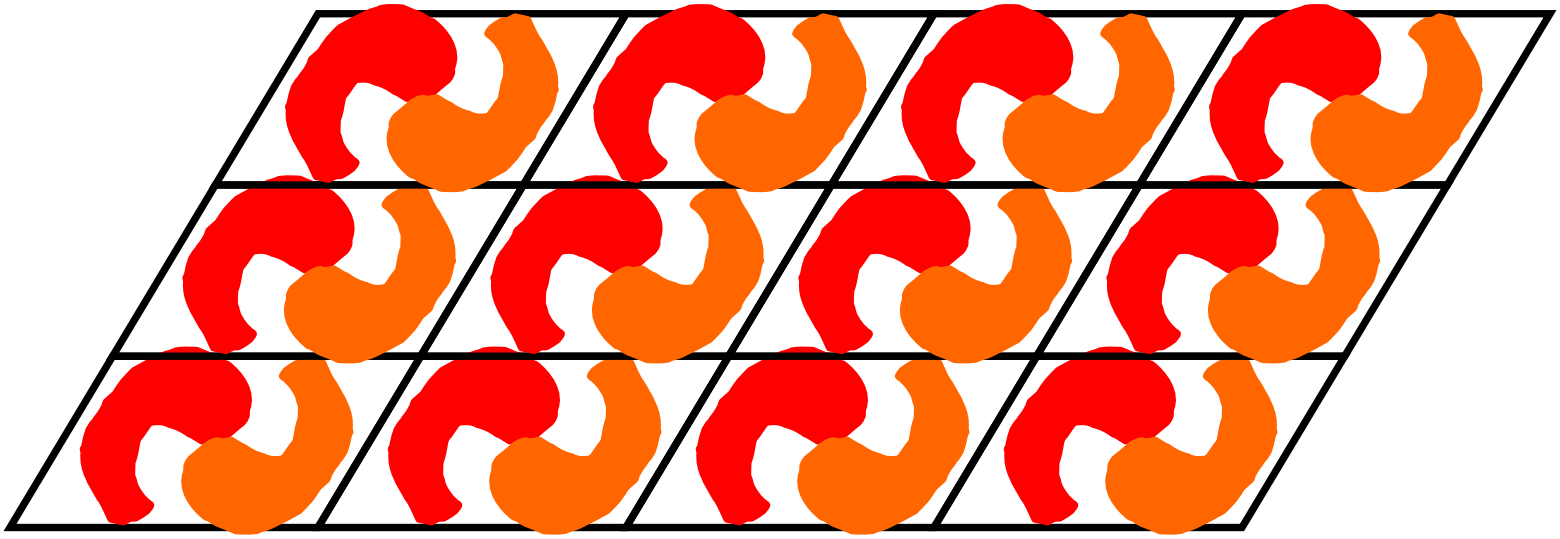
Crystal symmetries

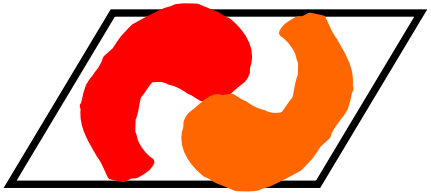




A **triclinic** lattice
(no symmetry)

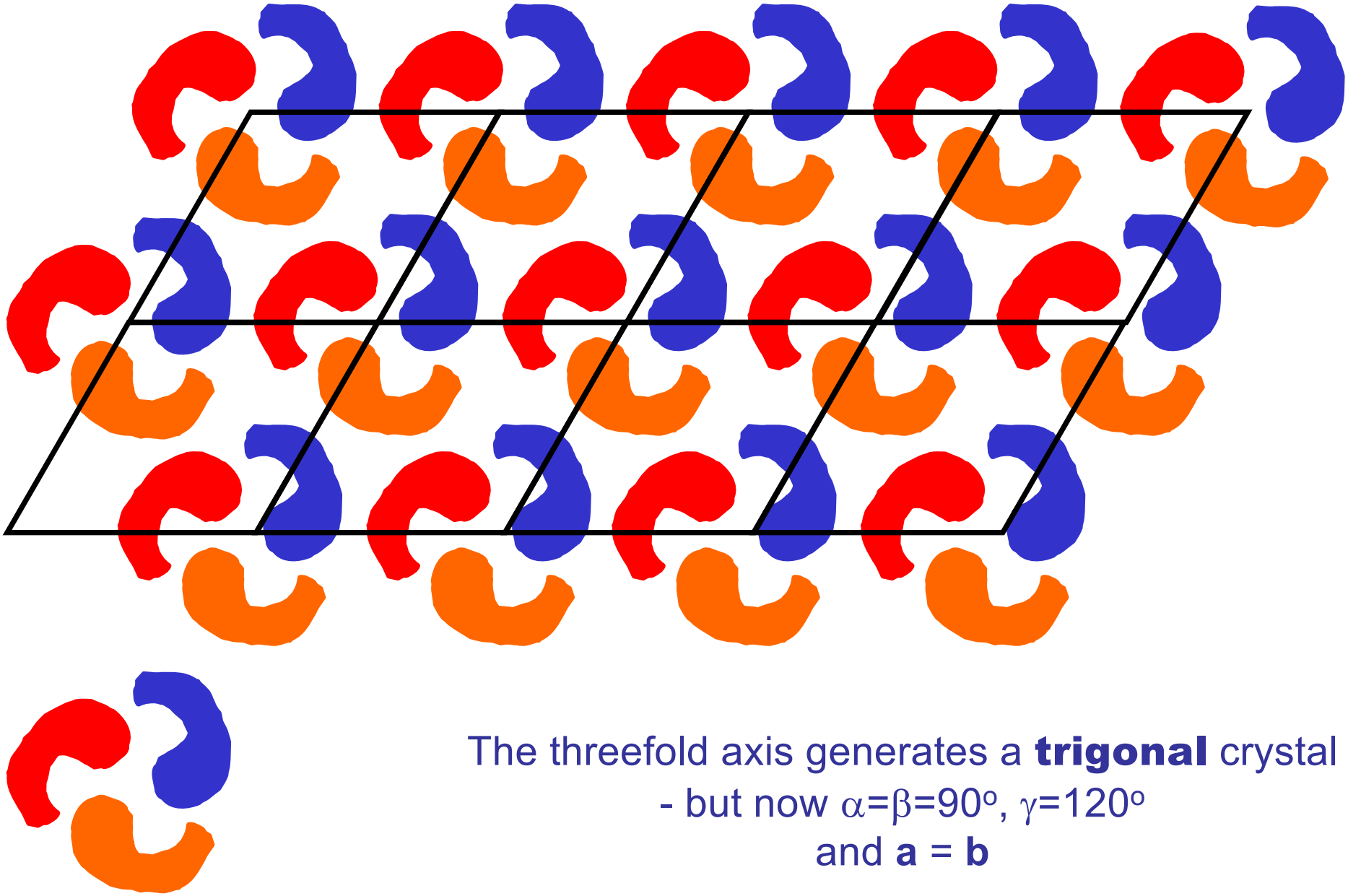
Crystal symmetries





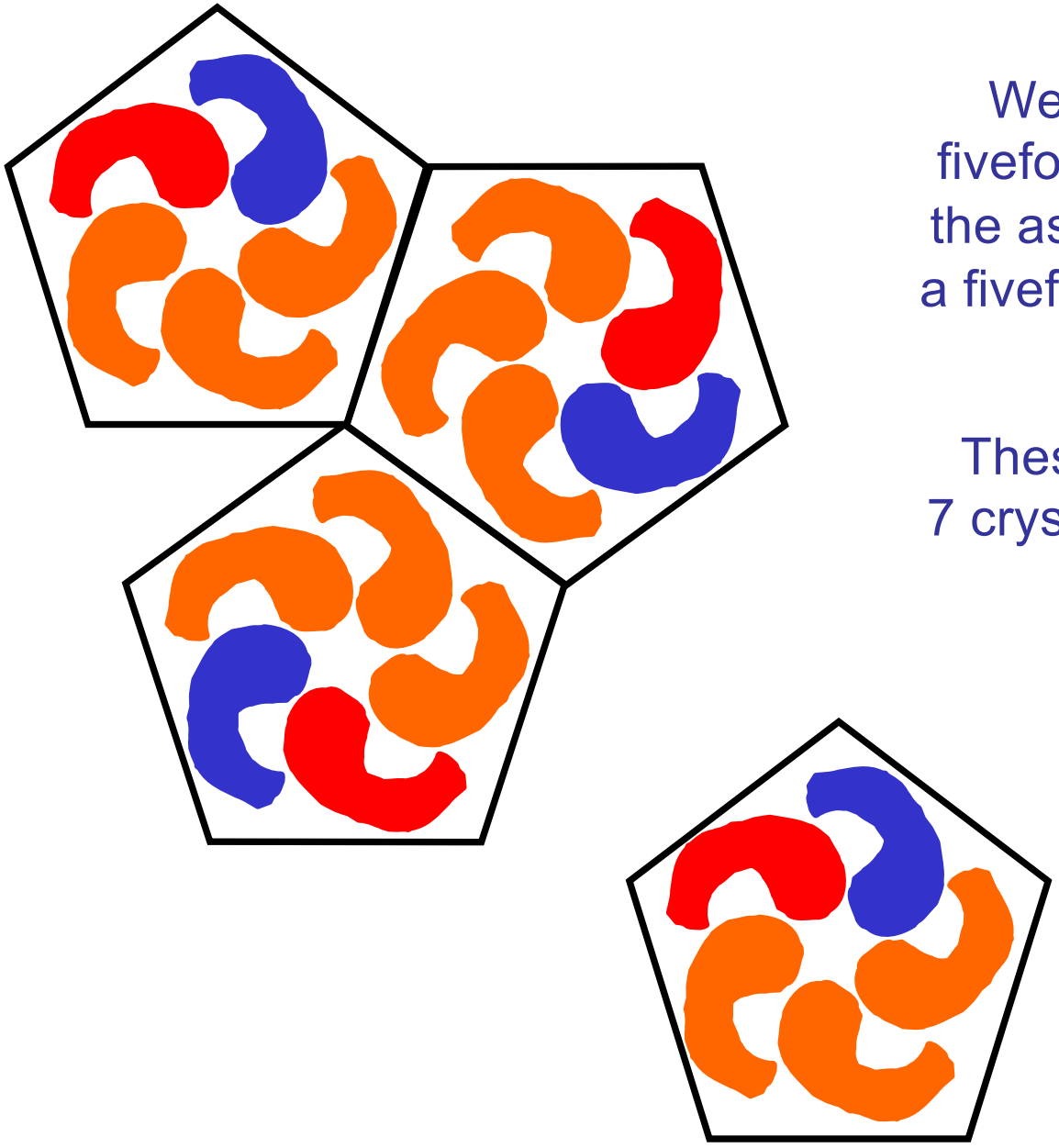
Introducing a twofold axis produces
a **monoclinic** lattice P2

Crystal symmetries



The threefold axis generates a **trigonal** crystal
- but now $\alpha=\beta=90^o$, $\gamma=120^o$
and **a** = **b**

Crystal symmetries

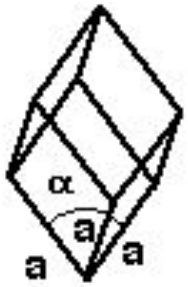


We cannot fill space with a fivefold arrangement – although the asymmetric unit can contain a fivefold axis (e.g. virus capsids)

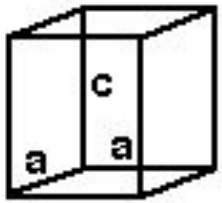
These restrictions give rise to 7 crystal classes in 3 dimensions

The seven crystal classes

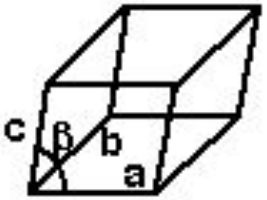
Rhombohedral $a = b = c$, $\alpha = \beta = \gamma \neq 90°$



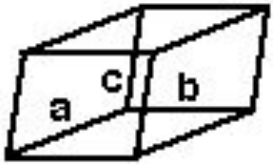
Hexagonal $a = b \neq c$, $\alpha = \beta = 90°$, $\gamma = 120°$



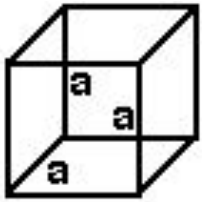
Monoclinic $a \neq b \neq c$, $\alpha = \gamma = 90° \neq \beta$



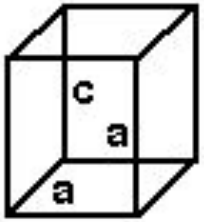
Triclinic $a \neq b \neq c$, $\alpha \neq \beta \neq \gamma \neq 90°$



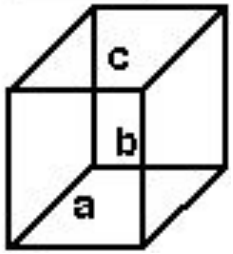
Cubic $a = b = c$, $\alpha = \beta = \gamma = 90°$

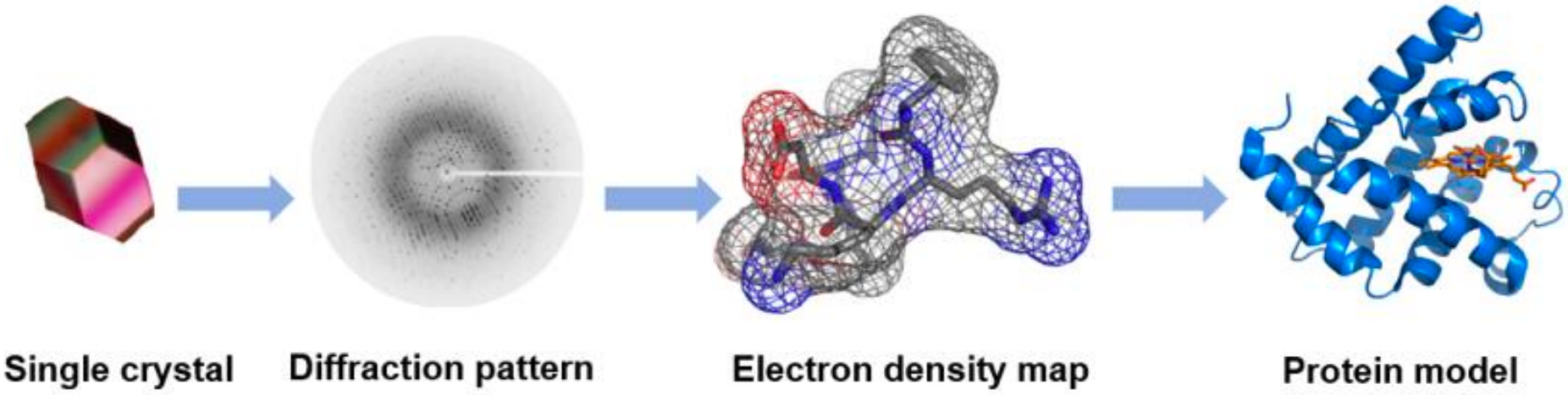


Tetragonal $a = b \neq c$, $\alpha = \beta = \gamma = 90°$



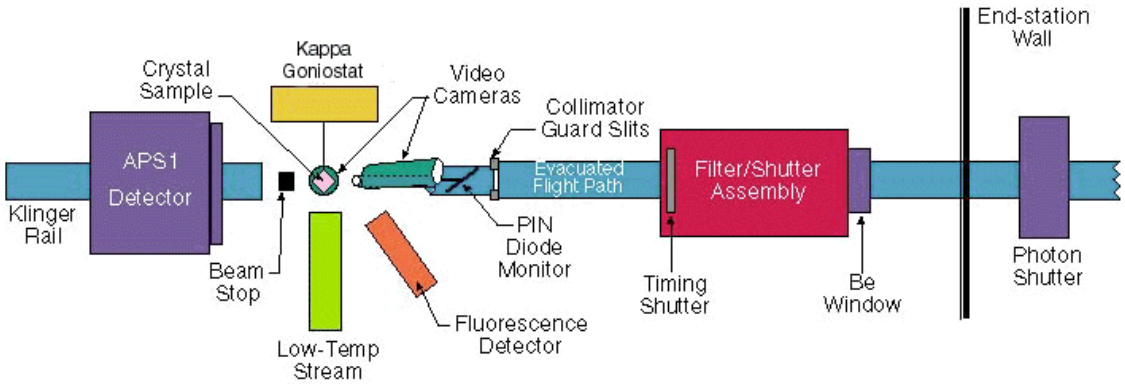
Orthorhombic $a \neq b \neq c$, $\alpha = \beta = \gamma = 90°$



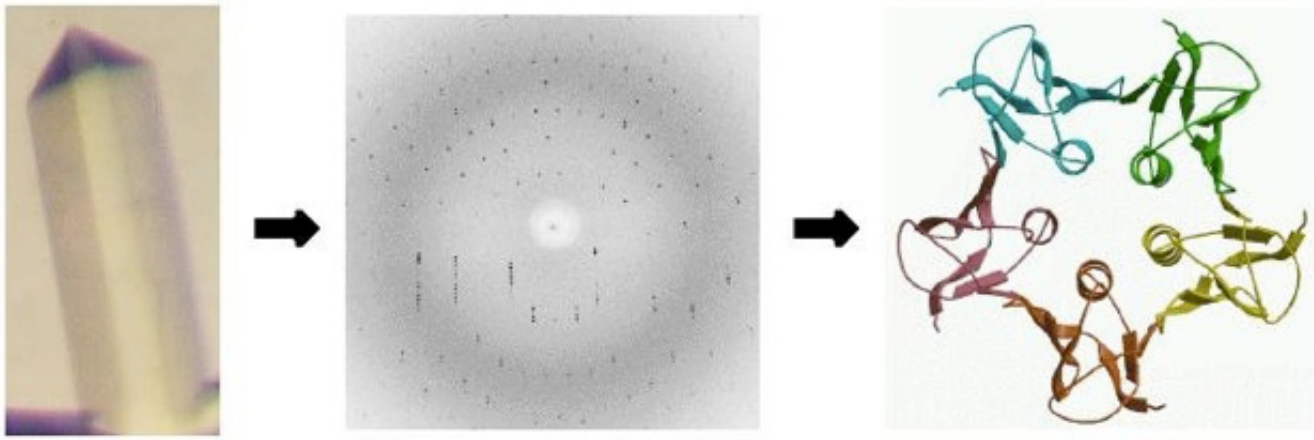

Single crystal
Diffraction pattern
Electron density map
Protein model

3. Collect X-ray diffraction data

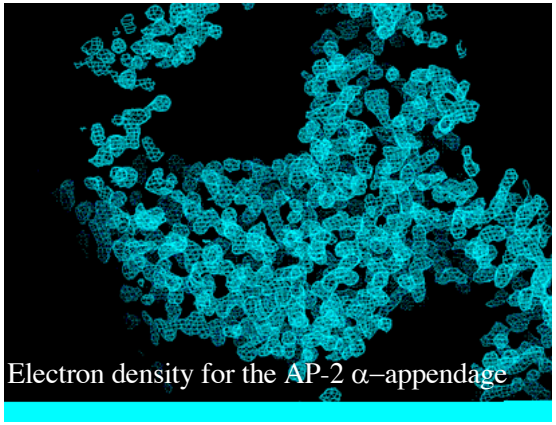
- Initiate experiments using home-source x-ray generator and detector
- Determine liquid nitrogen cryo-protection conditions to reduce crystal decay
- While home x-rays are sufficient for some questions, synchrotron radiation is preferred
- Anywhere from one to hundreds of crystals and diffraction experiments may be required



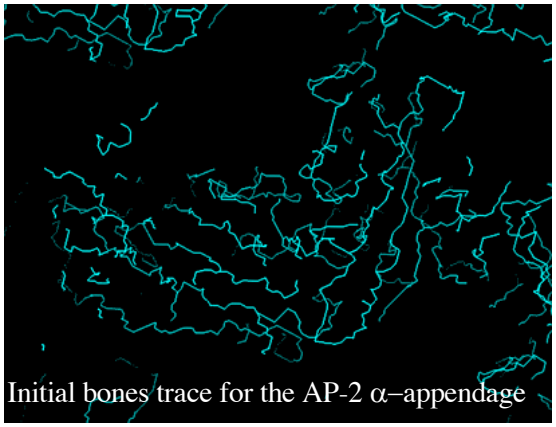
Argonne National Laboratory Structural Biology Center beamlineID19 at the Advanced Photon Source http://www.sbc.anl.gov



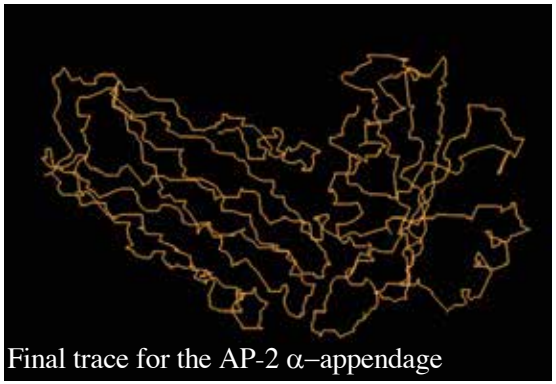
5. Build an atomic model using the electron density map



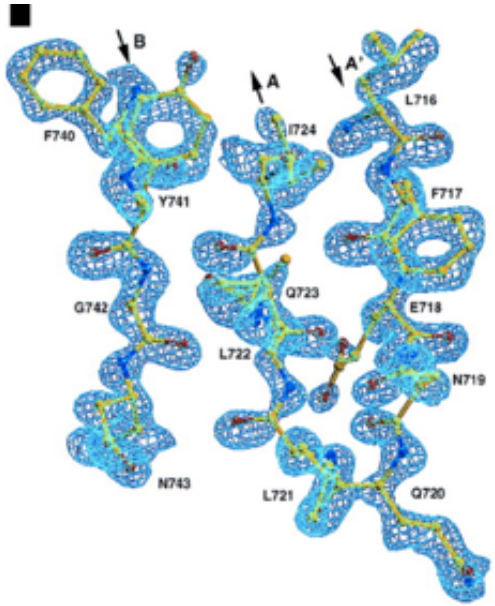
Electron density for the AP-2 α–appendage

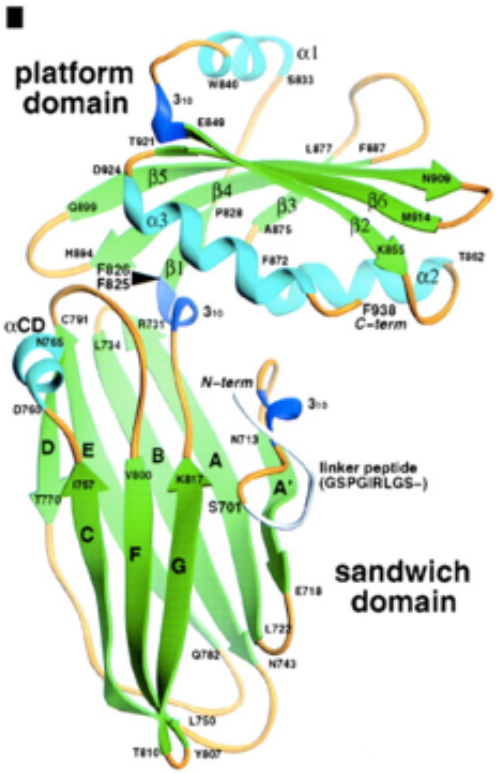


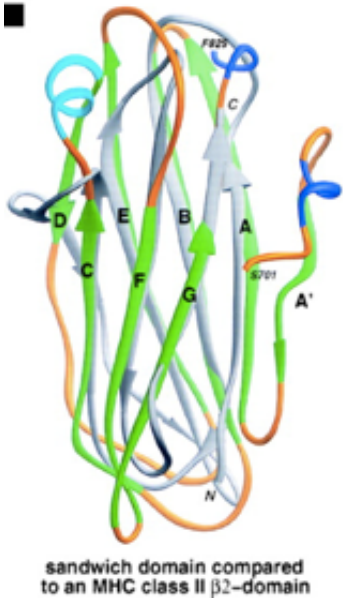
Initial bones trace for the AP-2 α–appendage



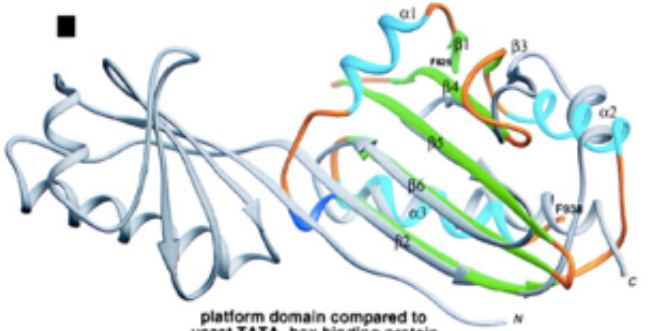
Final trace for the AP-2 α–appendage





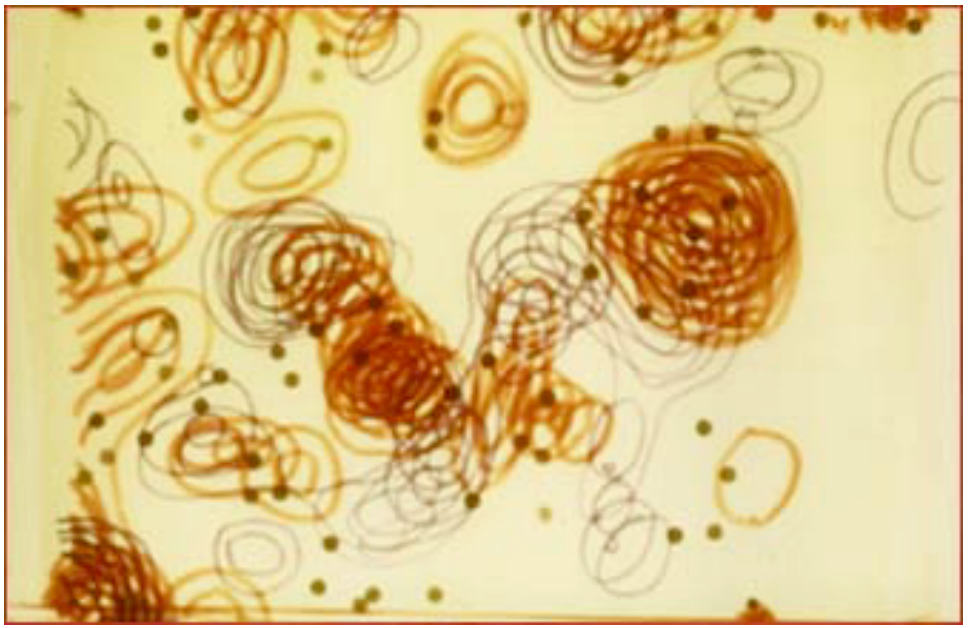


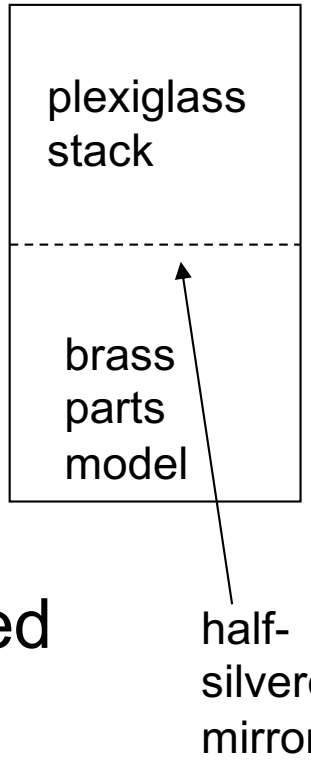
sandwich domain compared to an MHC class II β2–domain



platform domain compared to yeast TATA–box binding protein

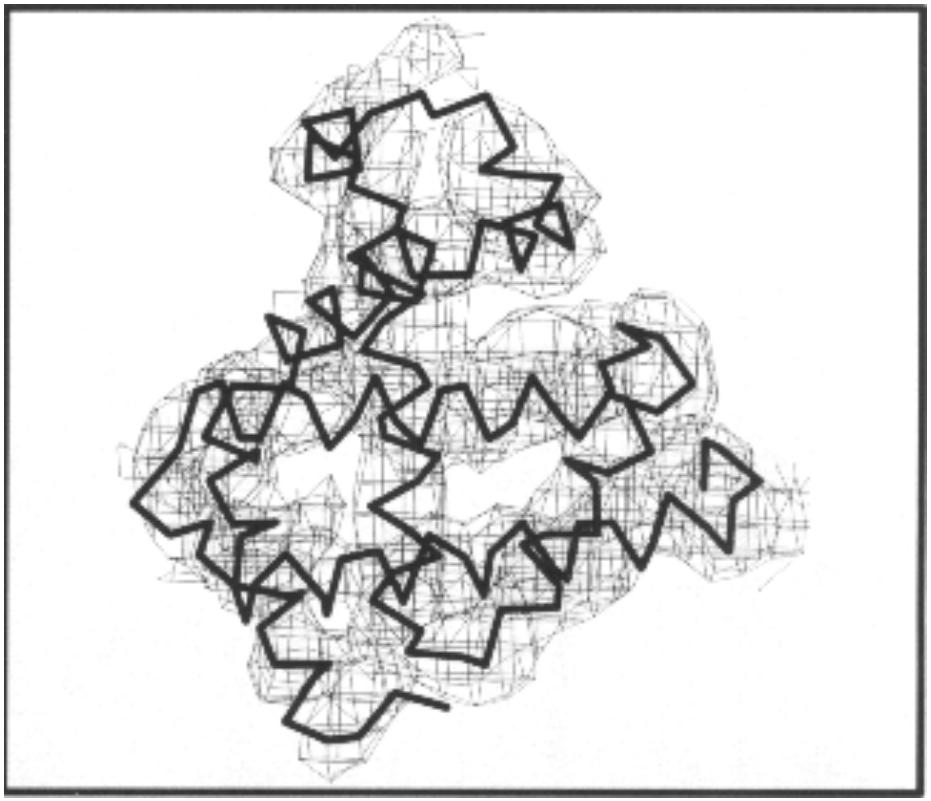
What does a good map look like?





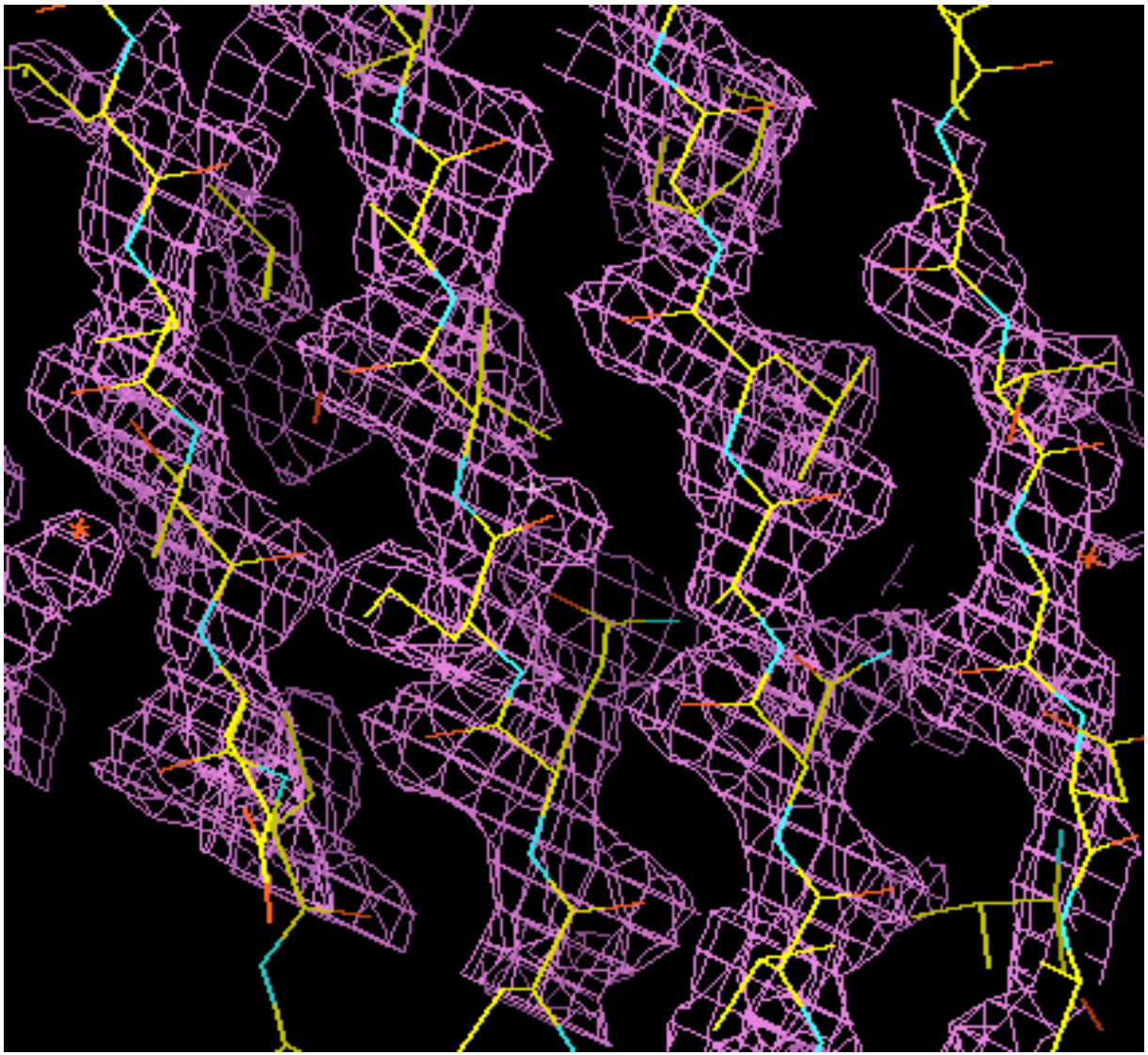
Before computers, maps were contoured on stacked pieces of plexiglass. A “Richards box” was used to build the model.

Low-resolution



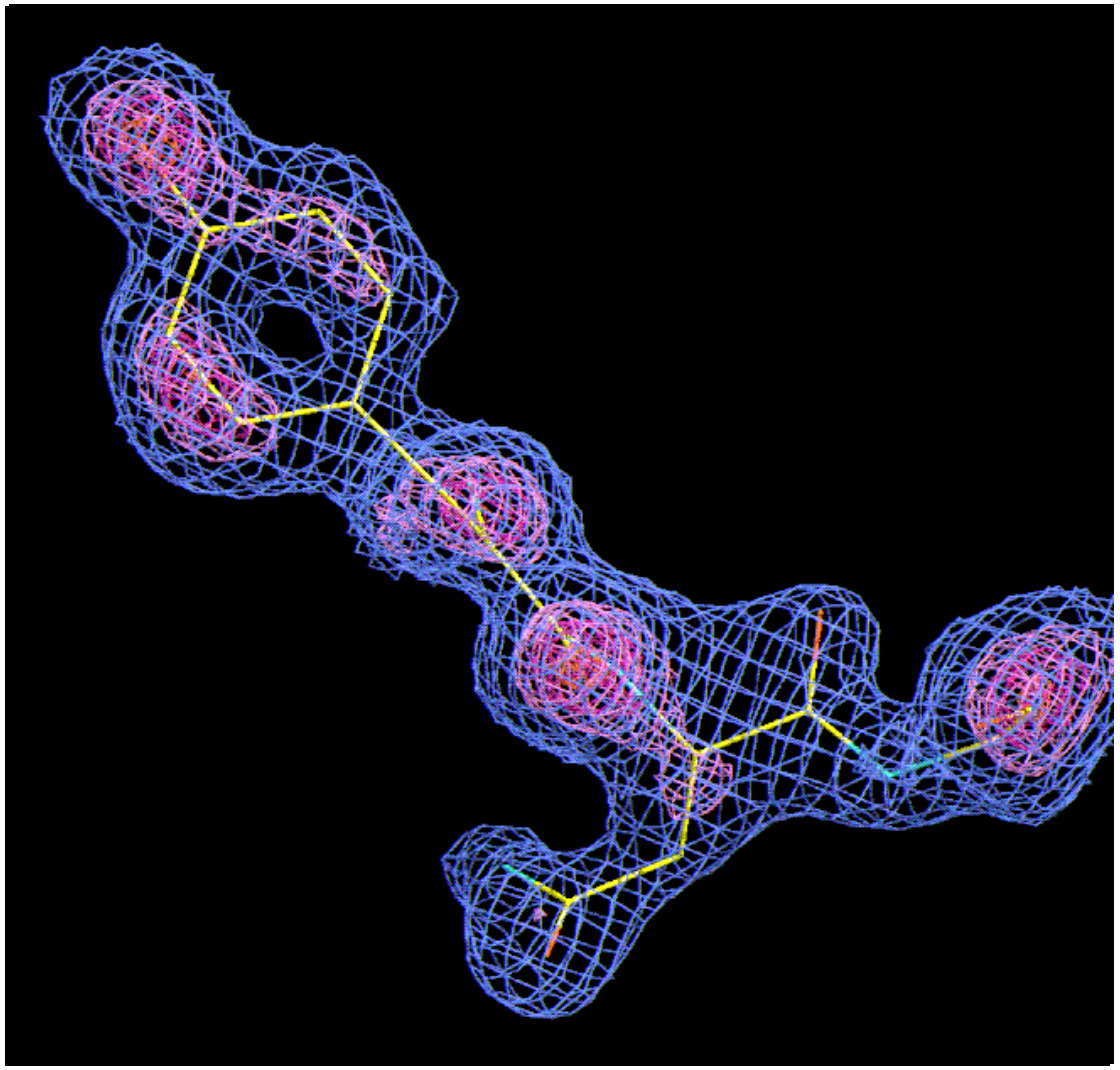
At 4-6Å resolution, alpha helices look like sausages.

Medium resolution



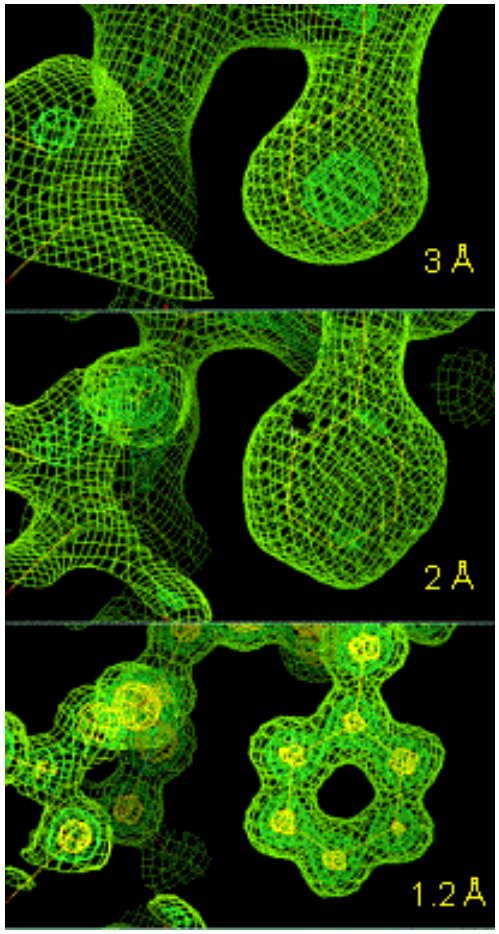
~3Å data is good enough to see the backbone with space in between.

Holes in rings are a good thing



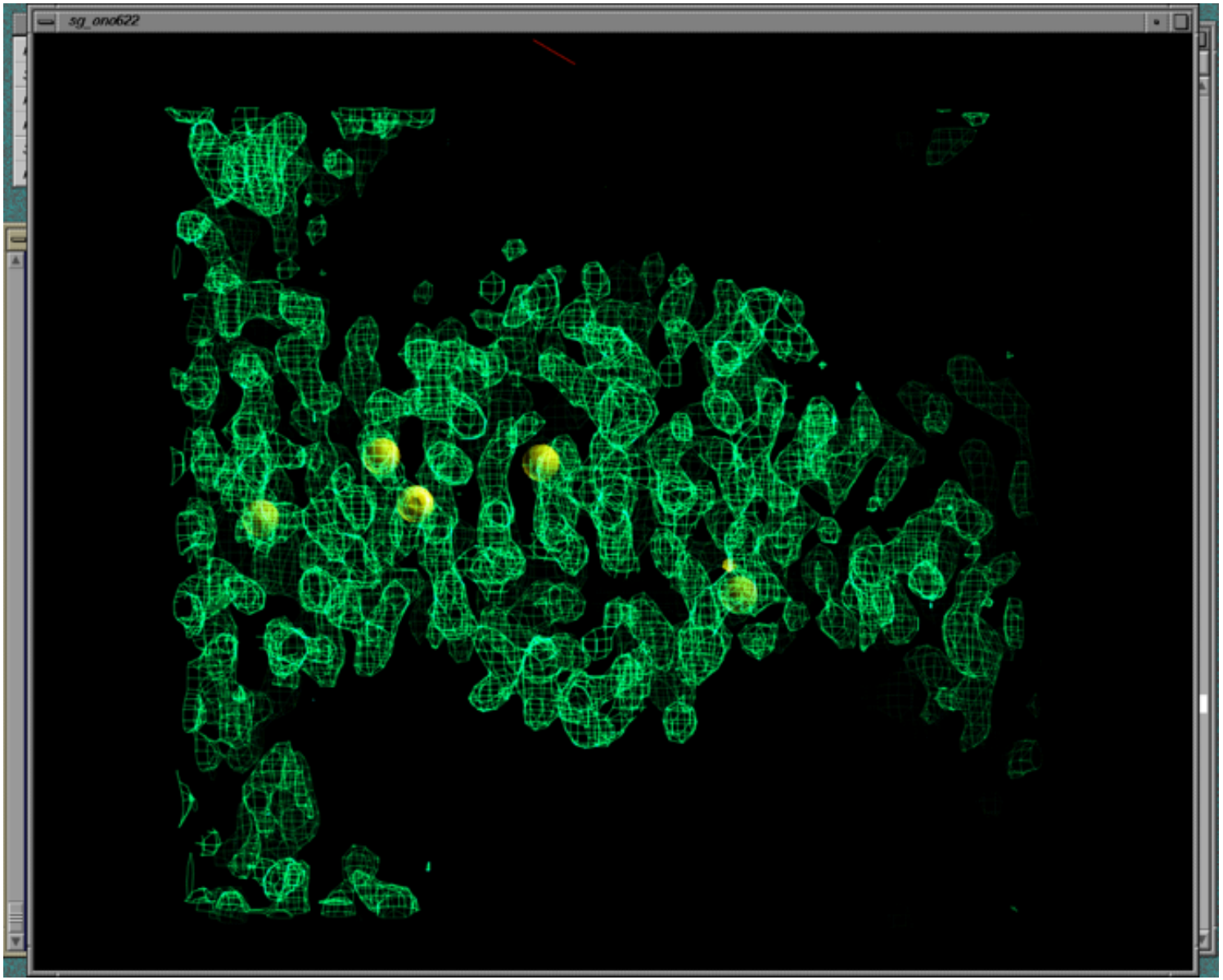
Seeing a hole in a tyrosine or phenylalanine ring is universally accepted as proof of good phases. You need at least 2Å data.

The resolution of the electron-density map and the amount of detail that can be seen

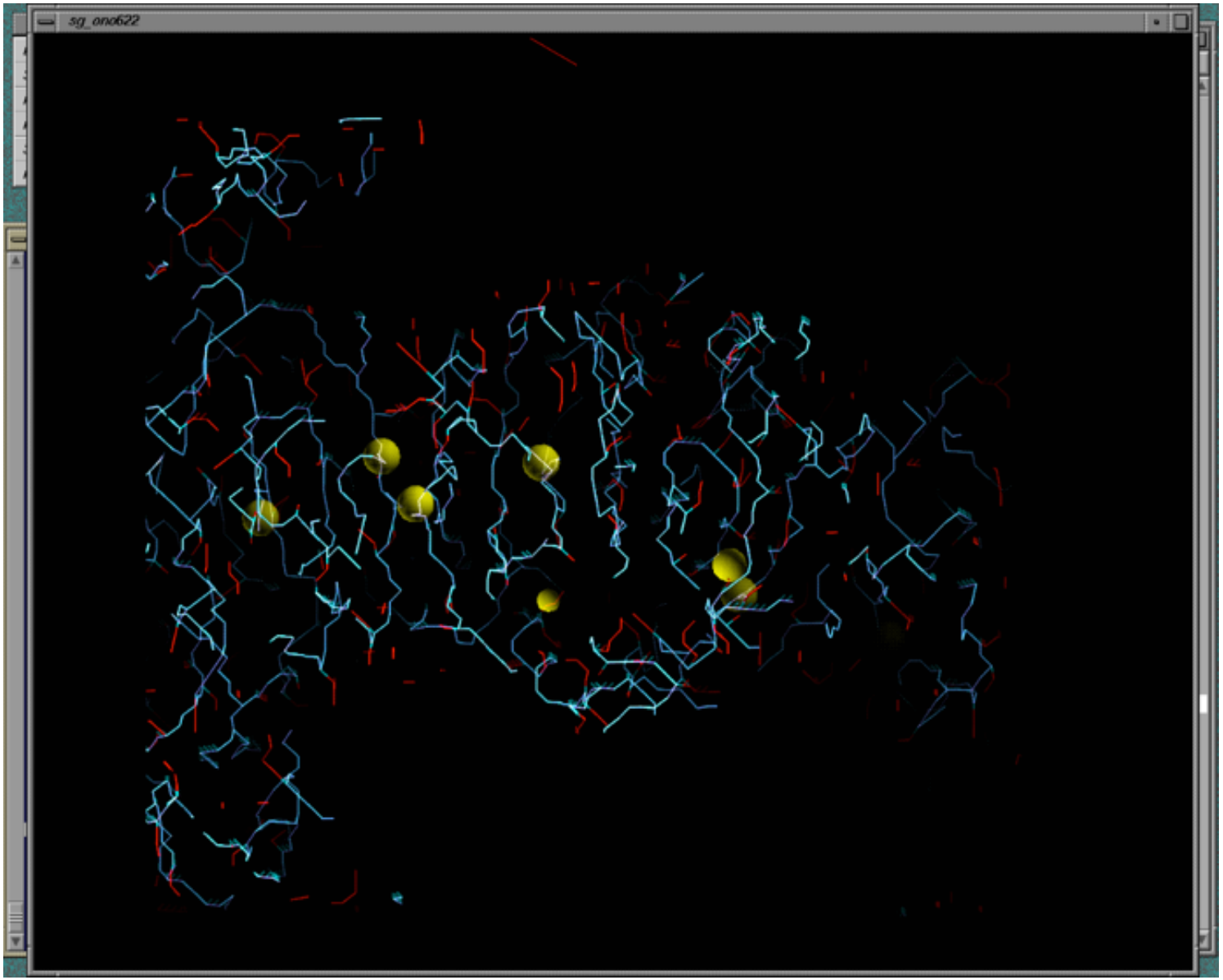


Resolution	Structural Features Observed
5.0 Å	Overall shape of the molecule
3.5 Å	Ca trace
3.0 Å	Side chains
2.8 Å	Carbonyl oxygens (bulges)
2.5 Å	Side chain well resolved, Peptide bond plane resolved
1.5 Å	Holes in Phe, Tyr rings
0.8 Å	Current limit for best protein crystals

The 2.8 Å density of SrfTE (Surfactin Thioesterase)



The 2.8 Å density of SrfTE could be skeletonised



6. Validation: How do you know if a crystal structure is right?

Geometric Distortions in bond lengths and angles

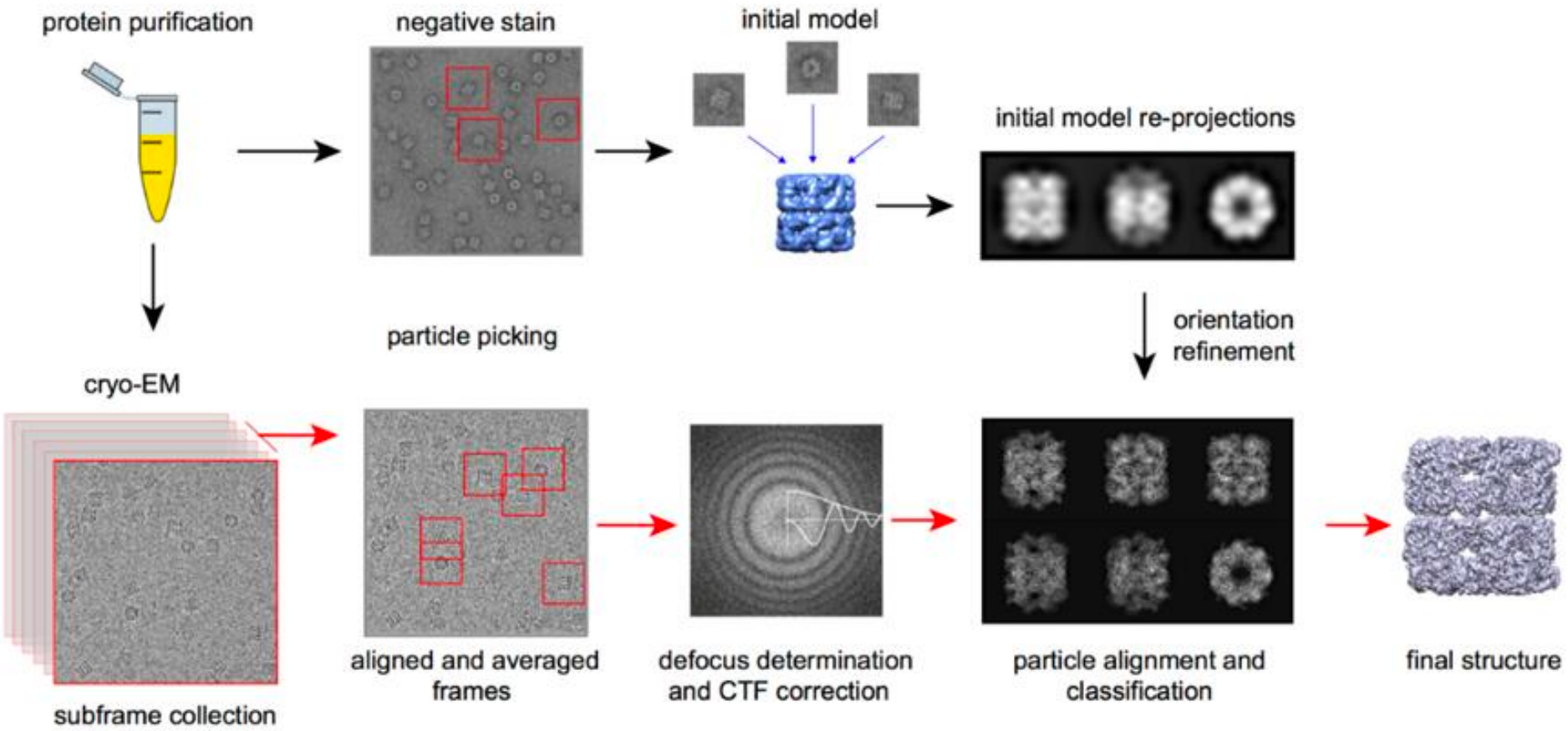
Favorable van der Waals packing interactions

Chemical environment of individual amino acids

Location of insertion and deletion positions in related sequences

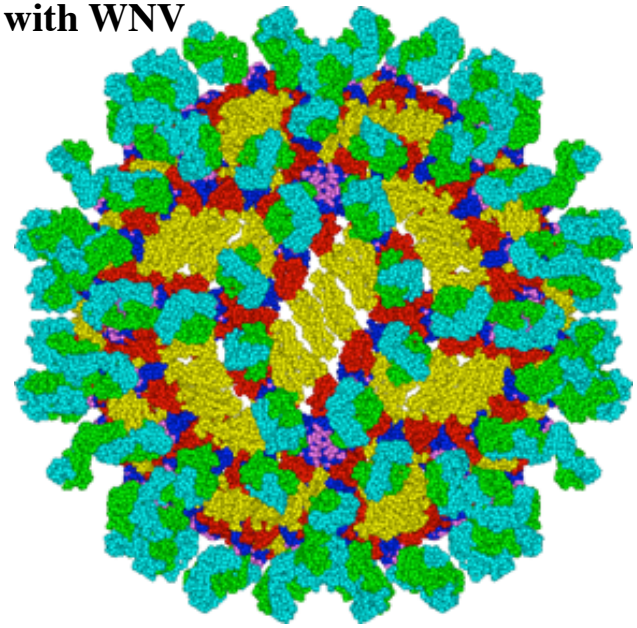
Confirm with other methods, Theory (as above), H-D exchange, ion mobility cross-sections, Cryo-EM

Cryo-EM

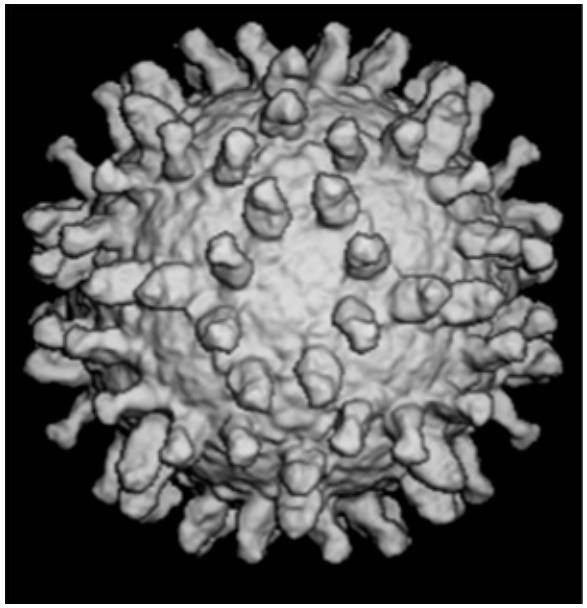

protein purification
negative stain
initial model
initial model re-projections
orientation
refinement
particle picking
cryo-EM
subframe collection
aligned and averaged
frames
defocus determination
and CTF correction
particle alignment and
classification
final structure

Combination of crystallographic and cryo-EM data - the E16 Fab/WNV complex

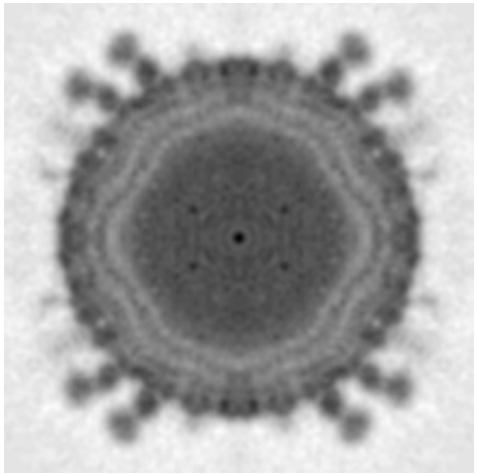
Model of E16 Fab complex with WNV



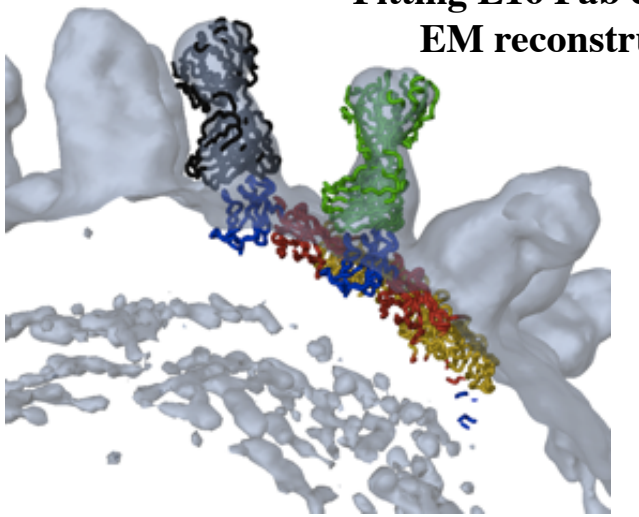
Cryo-EM reconstruction of E16 Fab complex with WNV



Cross-section of Cryo-EM reconstruction



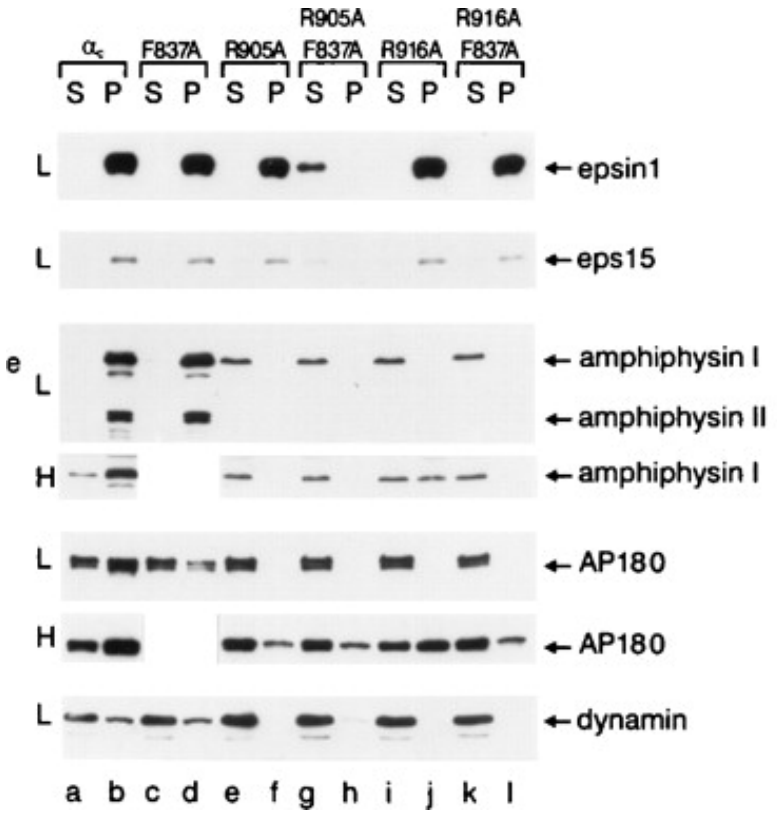
Fitting E16 Fab complex into Cryo-EM reconstruction of WNV

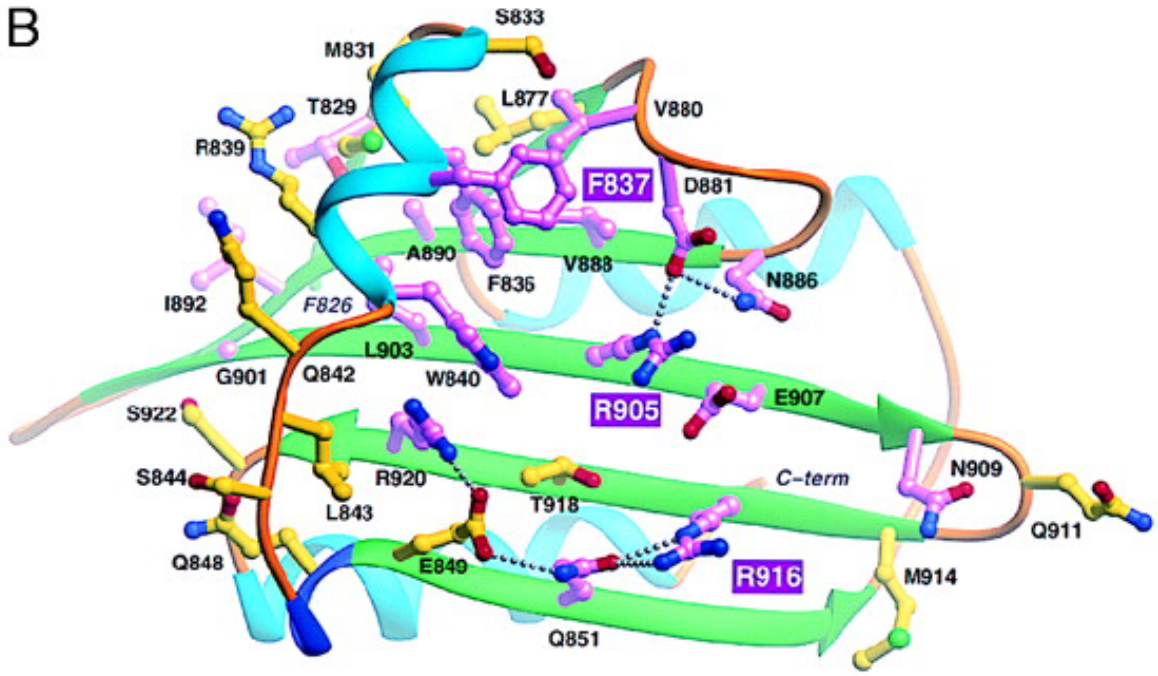


Cryo-EM work done in collaboration with Rossmann and Kuhn groups at Purdue University

7. Develop structure-based hypothesis

Structure-Based Mutagenesis of the α-appendage





Traub LM, Downs MA, Westrich JL, and **Fremont DH**: (1999) Crystal structure of the α-appendage of AP-2 reveals a recruitment platform for clathrin-coat assembly. *Proc. Natl. Acad. Sci. U.S.A.* **96**:8907-8912.

When things go wrong:

LETTERS

edited by Etta Kavanagh

Retraction

WE WISH TO RETRACT OUR RESEARCH ARTICLE "STRUCTURE OF MsbA from *E. coli*: A homolog of the multidrug resistance ATP binding cassette (ABC) transporters" and both of our Reports "Structure of the ABC transporter MsbA in complex with ADP•vanadate and lipopolysaccharide" and "X-ray structure of the EmrE multidrug transporter in complex with a substrate" (*1*–*3*).

The recently reported structure of Sav1866 (*4*) indicated that our MsbA structures (*1*, *2*, *5*) were incorrect in both the hand of the structure and the topology. Thus, our biological interpretations based on these inverted models for MsbA are invalid.

An in-house data reduction program introduced a change in sign for anomalous differences. This program, which was not part of a conventional data processing package, converted the anomalous pairs (I+ and I–) to (F– and F+), thereby introducing a sign change. As the diffraction data collected for each set of MsbA crystals and for the EmrE crystals were processed with the same program, the structures reported in (*1*–*3*, *5*, *6*) had the wrong hand.

The error in the topology of the original MsbA structure was a consequence of the low resolution of the data as well as breaks in the electron density for the connecting loop regions. Unfortunately, the use of the multicopy refinement procedure still allowed us to obtain reasonable refinement values for the wrong structures.

The Protein Data Bank (PDB) files 1JSQ, 1PF4, and 1Z2R for MsbA and 1S7B and 2F2M for EmrE have been moved to the archive of obsolete PDB entries. The MsbA and EmrE structures will be recalculated from the original data using the proper sign for the anomalous differences, and the new Cα coordinates and structure factors will be deposited.

We very sincerely regret the confusion that these papers have caused and, in particular, subsequent research efforts that were unproductive as a result of our original findings.

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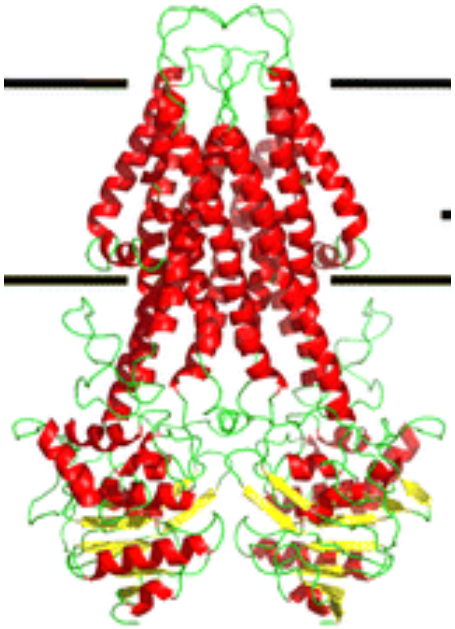
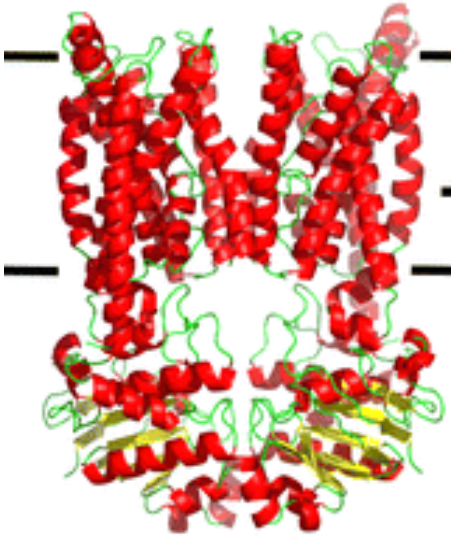
Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.

References

1. G. Chang, C. B. Roth, *Science* **293**, 1793 (2001).
2. C. L. Reyes, G. Chang, *Science* **308**, 1028 (2005).
3. O. Pornillos, Y.-J. Chen, A. P. Chen, G. Chang, *Science* **310**, 1950 (2005).
4. R. J. Dawson, K. P. Locher, *Nature* **443**, 180 (2006).
5. G. Chang, *J. Mol. Biol.* **330**, 419 (2003).
6. C. Ma, G. Chang, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2852 (2004).





Chang G, Roth CB. (2001) Structure of MsbA from E. coli: a homolog of the multidrug resistance ATP binding cassette (ABC) transporters. *Science* 293(5536):1793-800. PMID 11546864
Pornillos O, Chen YJ, Chen AP, Chang G. (2005) X-ray structure of the EmrE multidrug transporter in complex with a substrate. *Science* 310(5756):1950-3. PMID 16373573
Reyes CL, Chang G. (2005) Structure of the ABC transporter MsbA in complex with ADP.vanadate and lipopolysaccharide. *Science* 308(5724):1028-31. PMID 15890884
Chang G. (2003). Structure of MsbA from Vibrio cholera: a multidrug resistance ABC transporter homolog in a closed conformation. *J Mol Biol* 330(2):419-30. PMID 12823979
Ma C, Chang G. (2004). Structure of the multidrug resistance efflux transporter EmrE from Escherichia coli. *Proc Natl Acad Sci USA* 101(9):2852-7. PMID 14970332

When things go wrong:

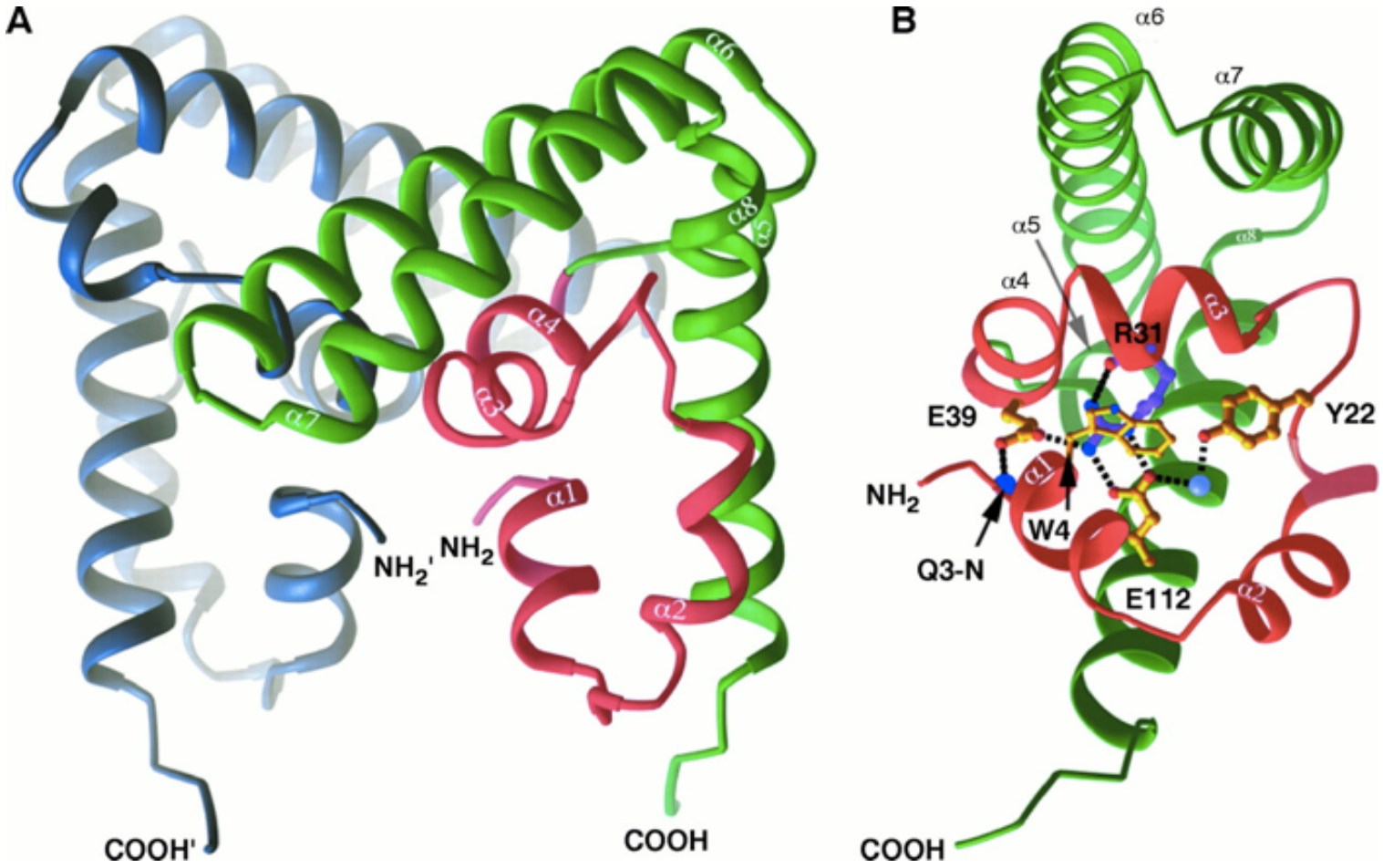


Figure 2. Tertiary structure of the N-domain of STAT-4. (**A**) Overall representation of two monomers (green and gray) in the crystallographic dimer, viewed approximately orthogonal to the molecular twofold axis, which is vertical. The ring-shaped NH2-terminal element is colored red in one monomer. (**B**) Orthogonal view of one of the N-domains shown in (A), depicting details of the architecture of the ring-shaped element. Side chains that participate in a charge-stabilized hydrogen-bond network are shown in a ball-and-stick representation. The side chain and backbone carbonyl of buried R31 are shown in magenta. For clarity, the indole ring of the invariant residue W4 that seals off this arrangement on the proximal side is drawn with thinner bonds. The blue sphere denotes a buried water molecule. Hydrogen bonds are indicated by dotted lines. Oxygen, nitrogen, and carbon atoms are red, blue, and yellow, respectively. Q3-N marks the position of the backbone amide group of residue Q3. The light-red segment of helix 2 highlights its 310 helical conformation. Fig. 2 and Fig. 3, B and C were created with the program RIBBONS, version 2.0 (28).

Structure of the Amino-Terminal Protein Interaction Domain of STAT-4
Uwe Vinkemeier, Ismail Moarefi, * James E. Darnell Jr., John Kuriyan
Science 13 February 1998:Vol. 279. no. 5353, pp. 1048 - 1052