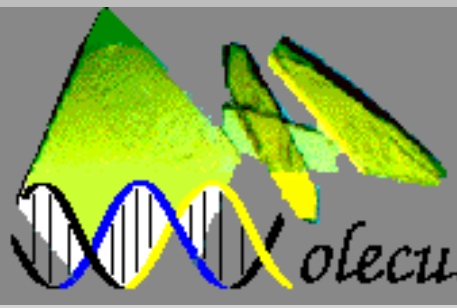


Pixels and Proteins: Detectors for Protein Crystallography

Edwin M. Westbrook, John Morse, Sherwood Parker, Chris Kenney

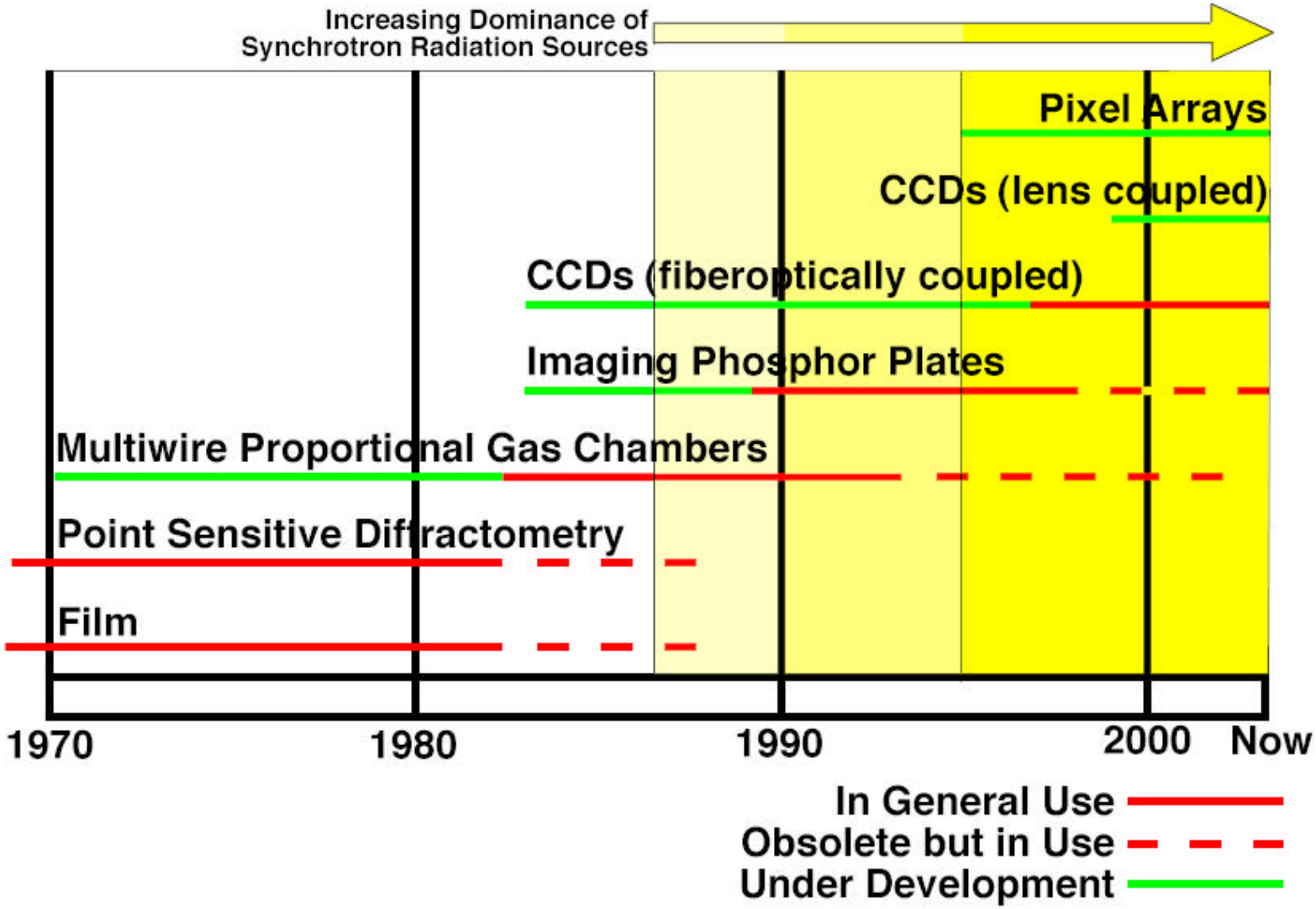
Molecular Biology Consortium Inc.

Collaborations and Acknowledgements

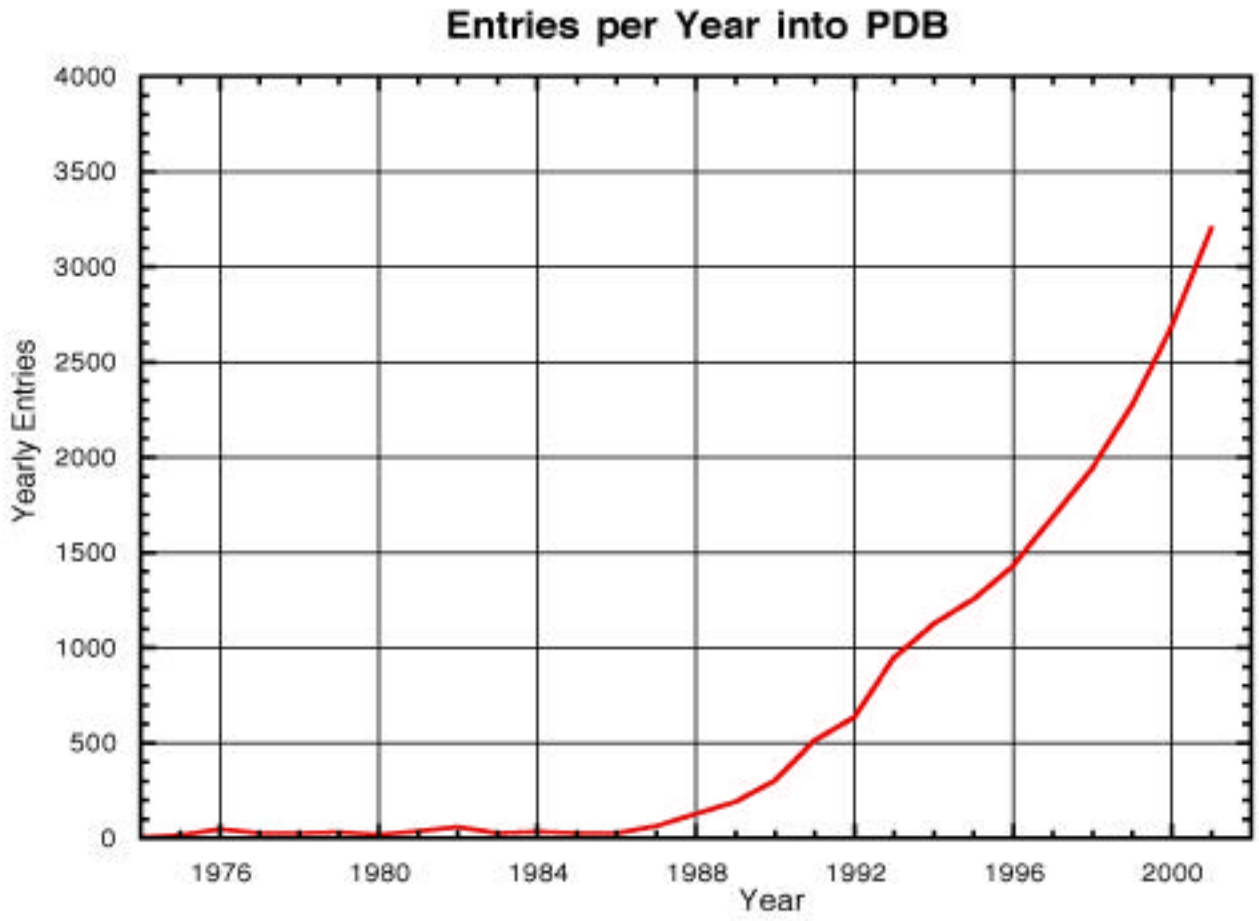
- At Berkeley: Emanuelle Mandelli, Gerrit Meddeler,
  - Many others

- Supported by:
  - NIH grant R01 RR16334
  - NIH grant R01 RR16230
  - The Molecular Biology Consortium Inc.

Historical Perspective of Protein Crystallographic Detectors



Entries into the Protein Data Bank are increasing exponentially



There are now more than 20,000 entries in the PDB.

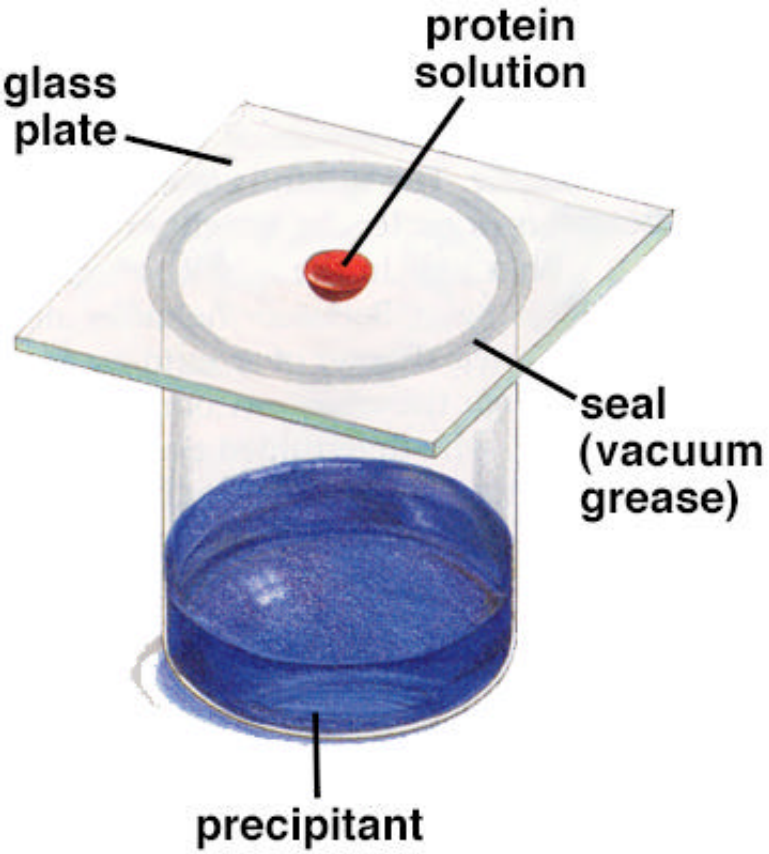
Quality Parameters For Protein Crystallographic X-ray Detectors

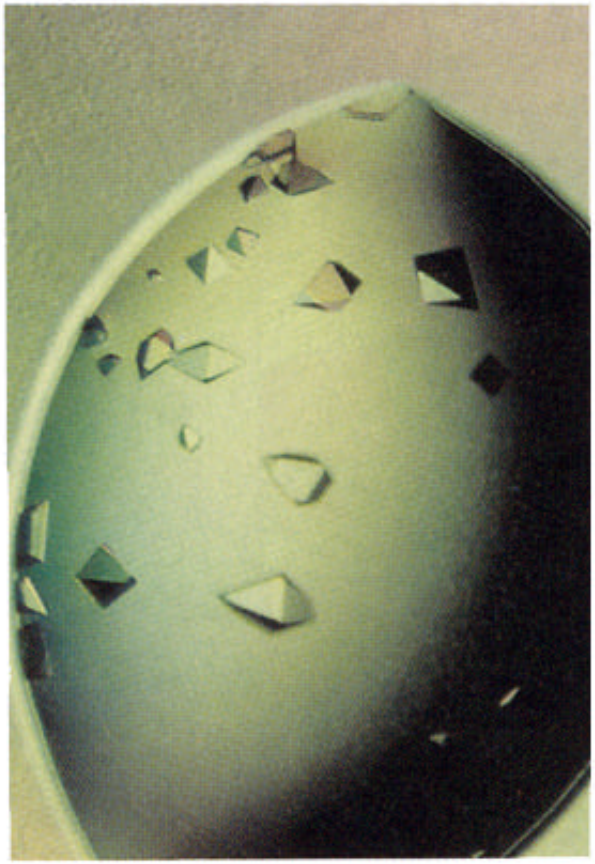
(A) Dynamic Range
Noise
Efficiency
Sensitivity

(B) Speed: driven by crystal sample decay

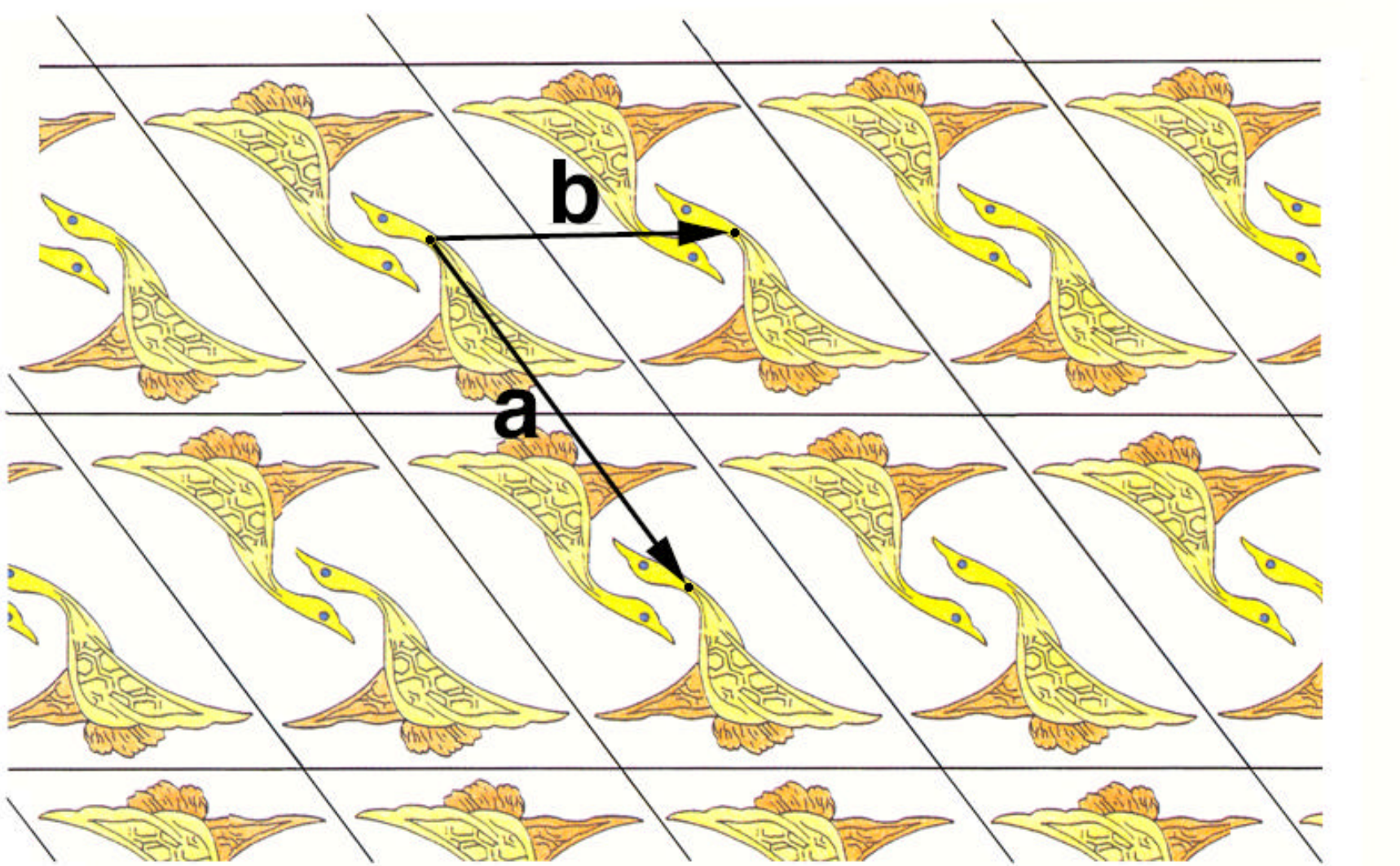
(C) Resolving Power =
(# Bragg orders across face)
Physical Size

Protein Crystals are (almost) always grown from vapor diffusion droplets.

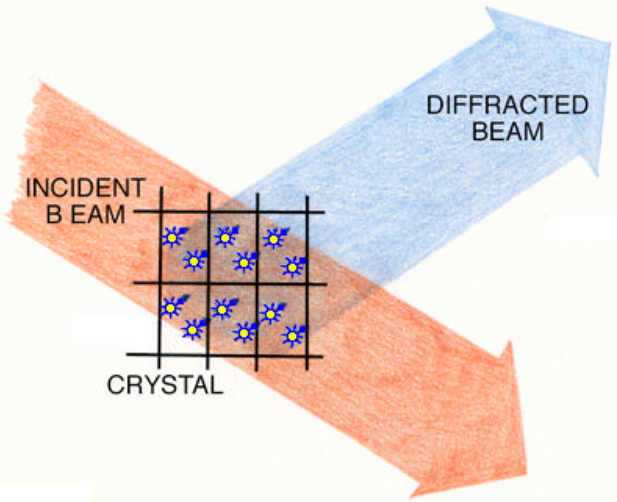


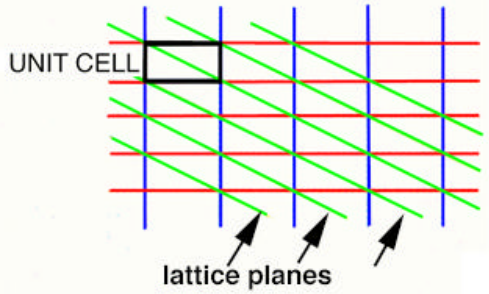


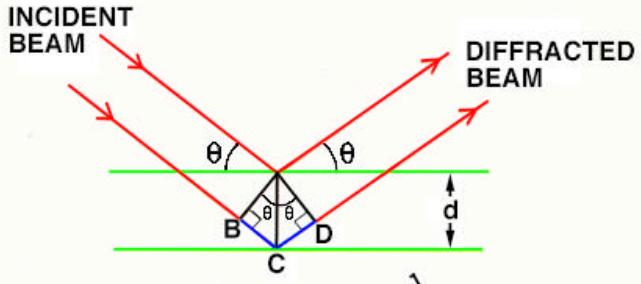
Crystals are symmetric w.r.t. translation operations:
(x',y',z') = (x,y,z) + (na,mb,lc)
a,b,c vectors, n,m,l integers



Crystal Diffraction Occurs only at discrete momentum transfer directions: the Bragg Reflections.



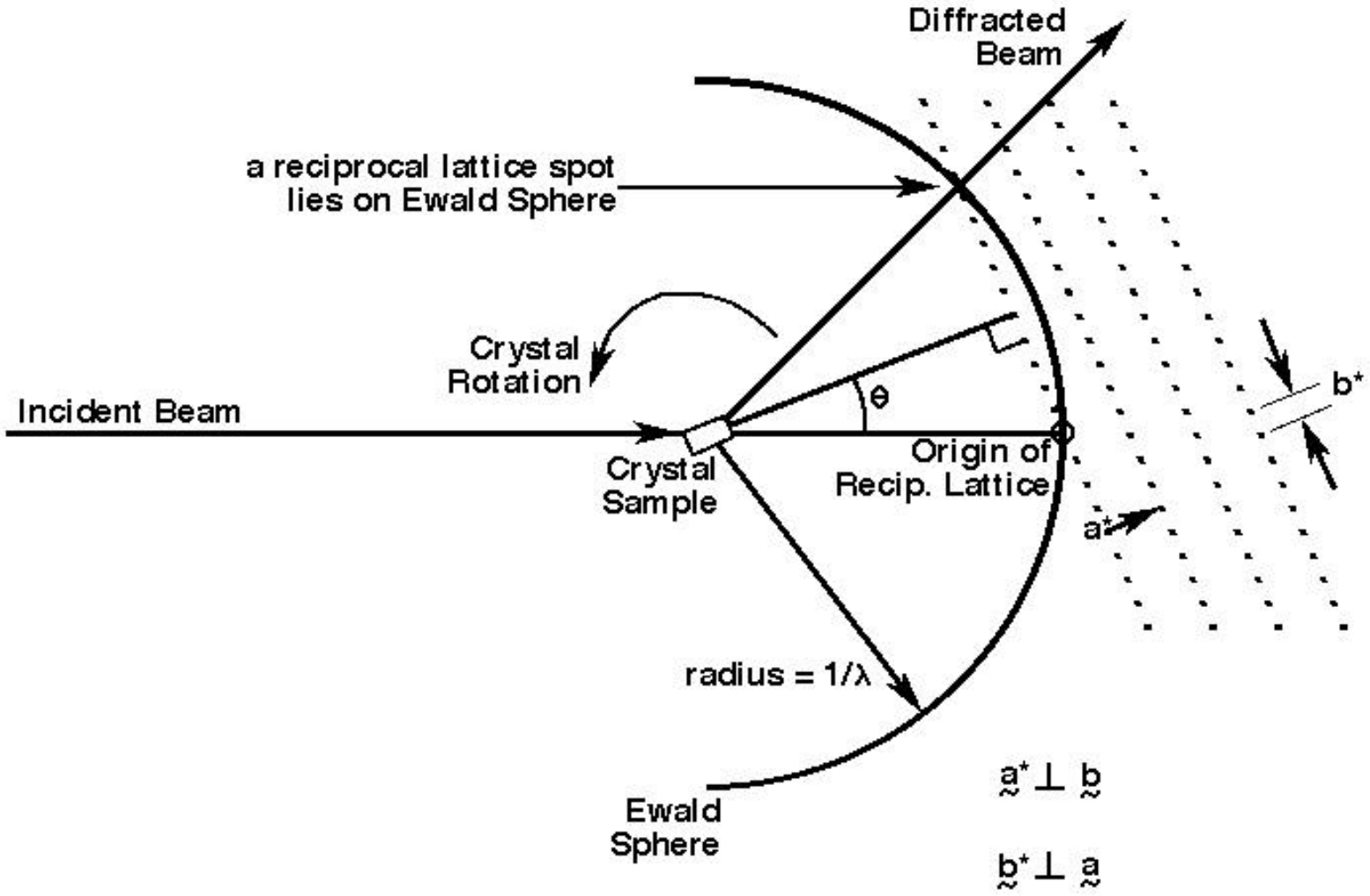




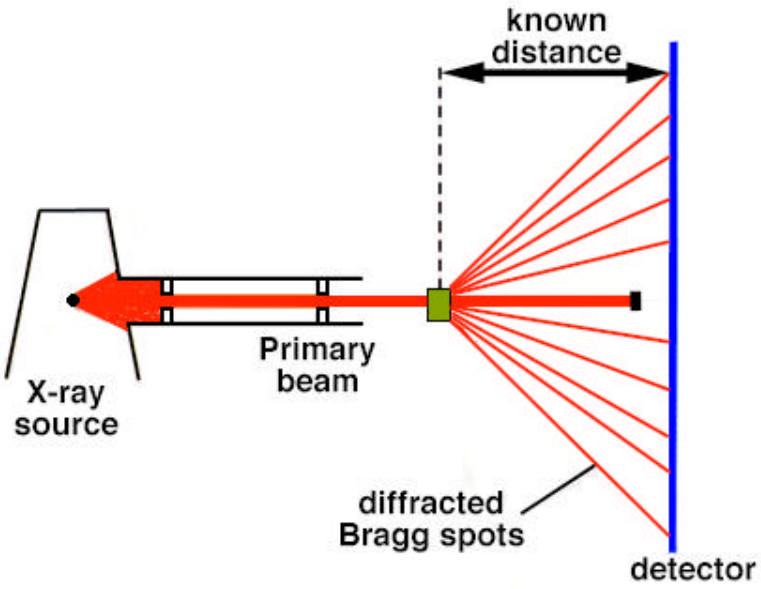
$BC = CD = d \cdot \sin(\Theta) = \frac{\lambda}{2}$ Wavelength = λ

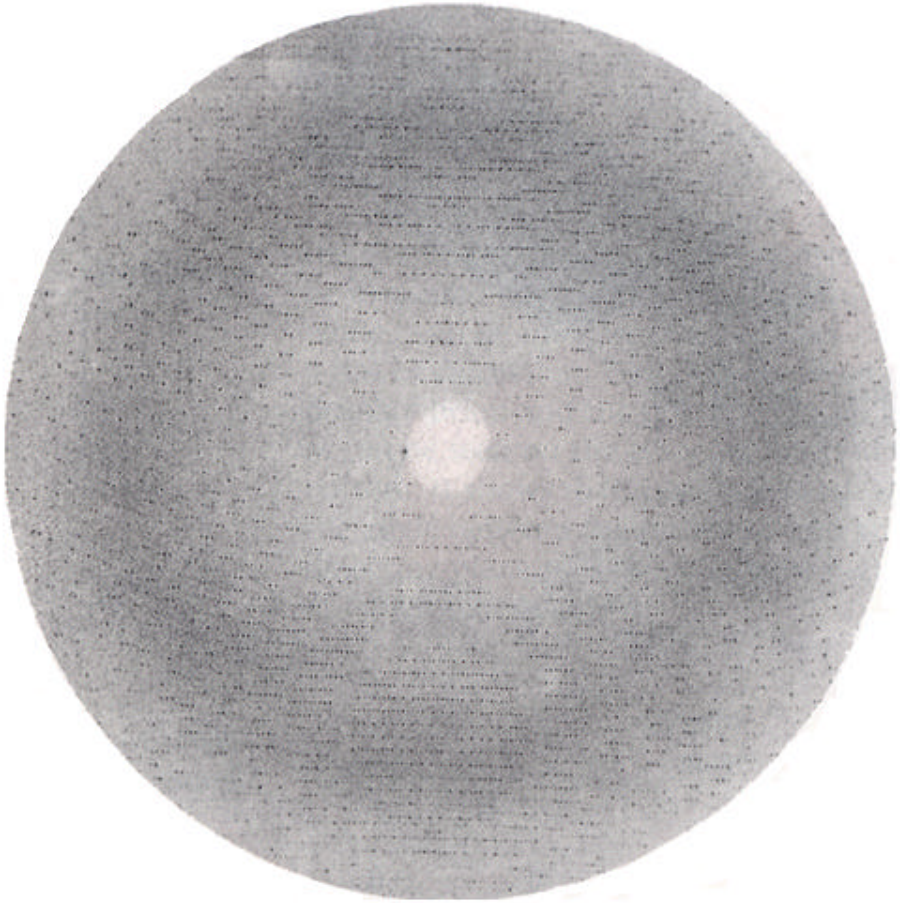
Bragg's Law: $\sin(\Theta) = \frac{\lambda}{2d}$

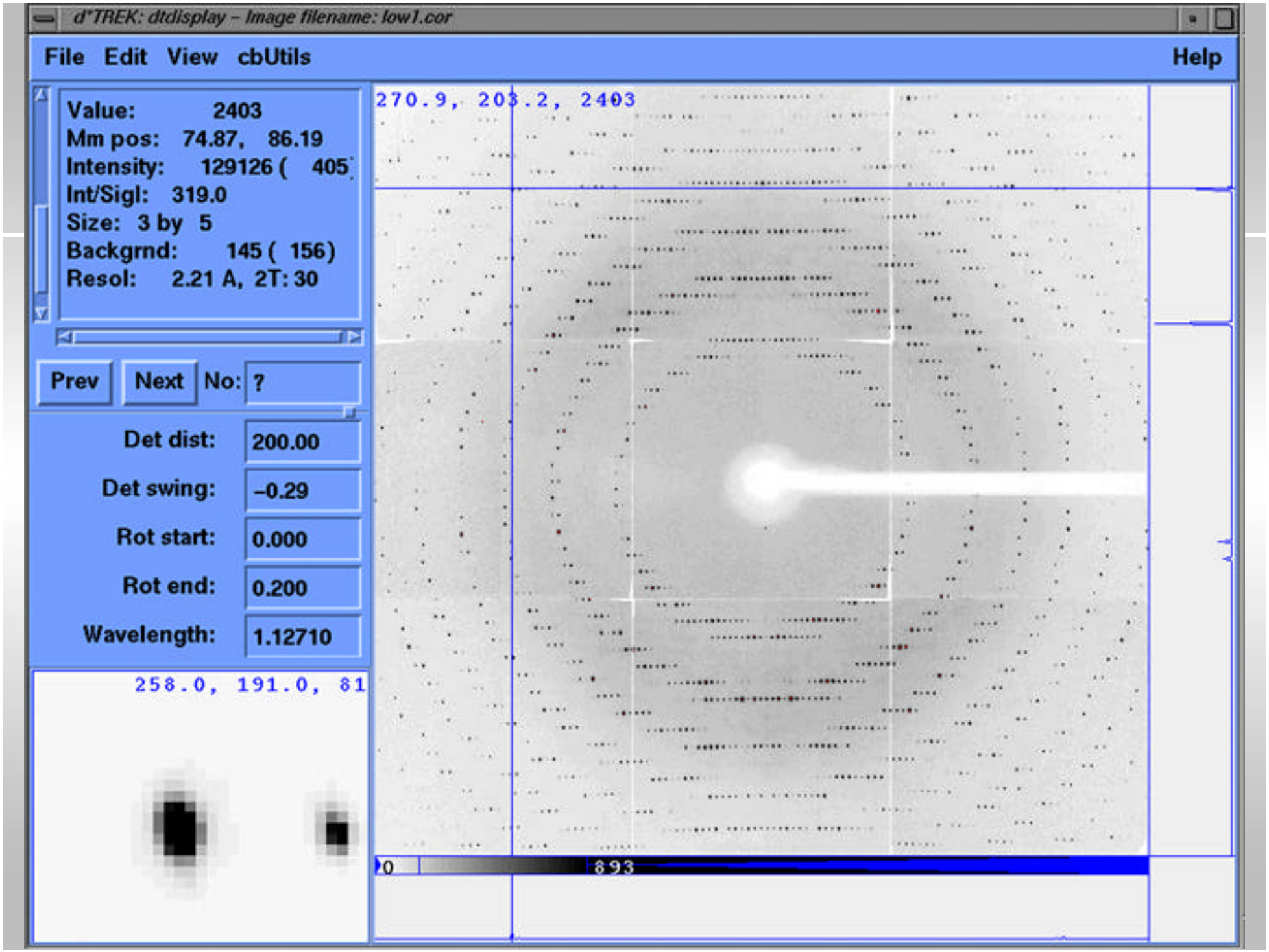
Each Bragg spot diffracts only at its Bragg angle. The crystal must be rotated to record all Bragg spot intensities.



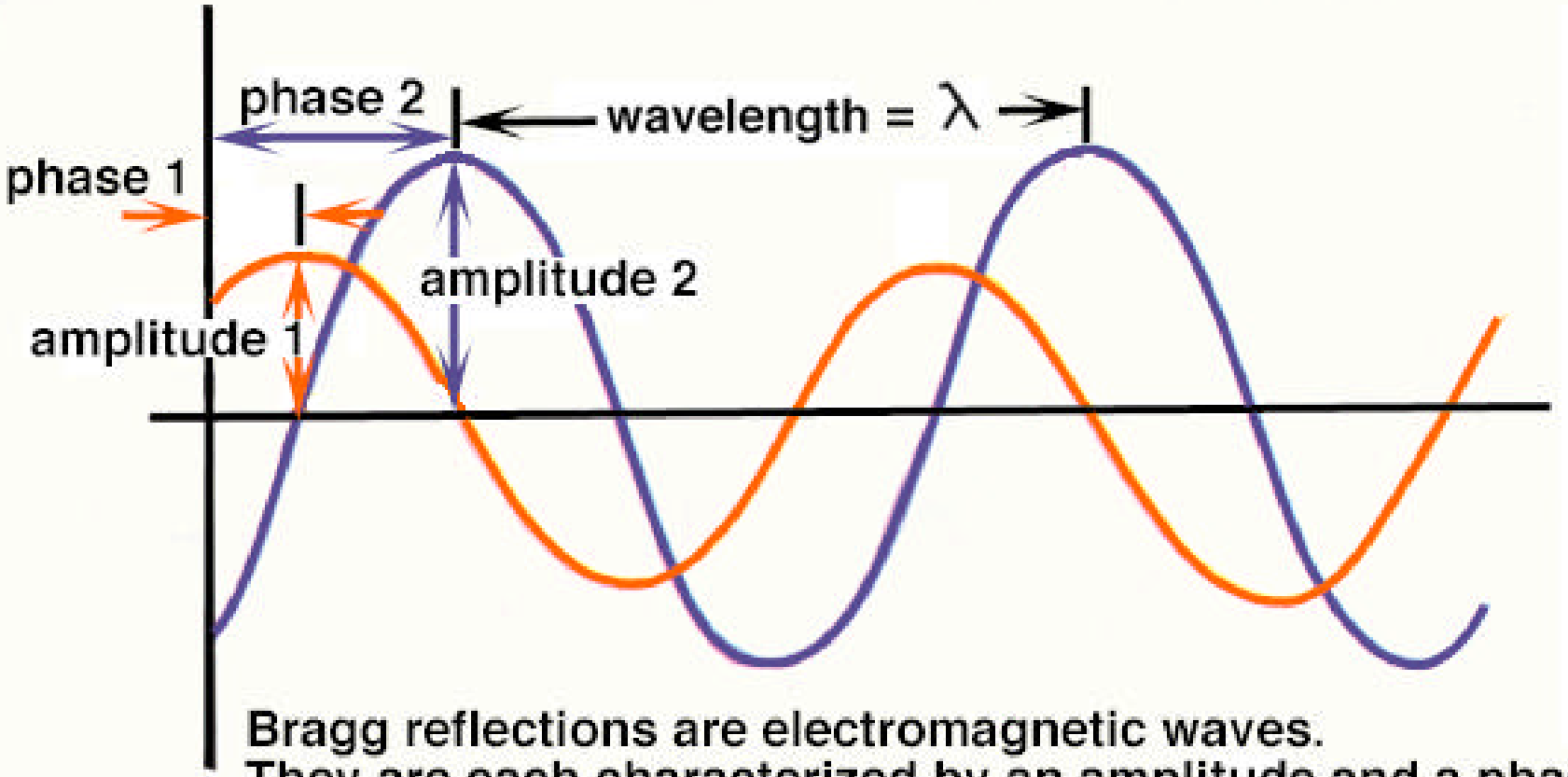
The Diffraction Pattern of Discrete Bragg Spots is Captured by the Detector






d*TREK: dtdisplay - Image filename: low1.cor
File Edit View cbUtils Help
Value: 2403
Mm pos: 74.87, 86.19
Intensity: 129126 (405
Int/Sigl: 319.0
Size: 3 by 5
Backgrnd: 145 (156)
Resol: 2.21 A, 2T: 30
Prev Next No: ?
Det dist: 200.00
Det swing: -0.29
Rot start: 0.000
Rot end: 0.200
Wavelength: 1.12710
258.0, 191.0, 81
270.9, 203.2, 2403
0
893

You can measure amplitude, but phases must be derived indirectly.

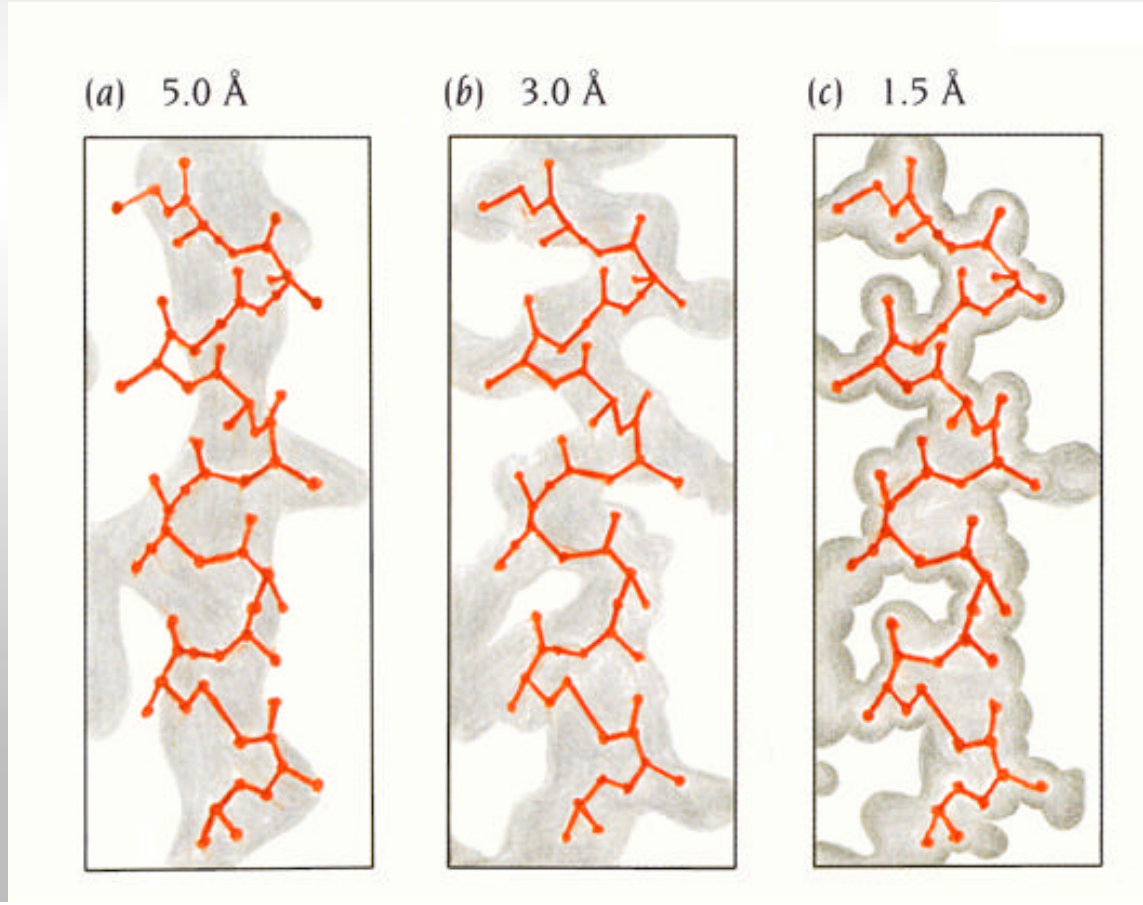


Bragg reflections are electromagnetic waves.
They are each characterized by an amplitude and a phase.

$$F(h,k,l) = A(h,k,l)\, e^{i\theta(h,k,l)}$$

Once phases are known, the electron density map can be calculated by a Fourier transform.

$$\rho(x,y,z) = \sum_h \sum_k \sum_l |F(h,k,l)| e^{i\phi(h,k,l)} e^{-2\pi i(hx+ky+lz)}$$

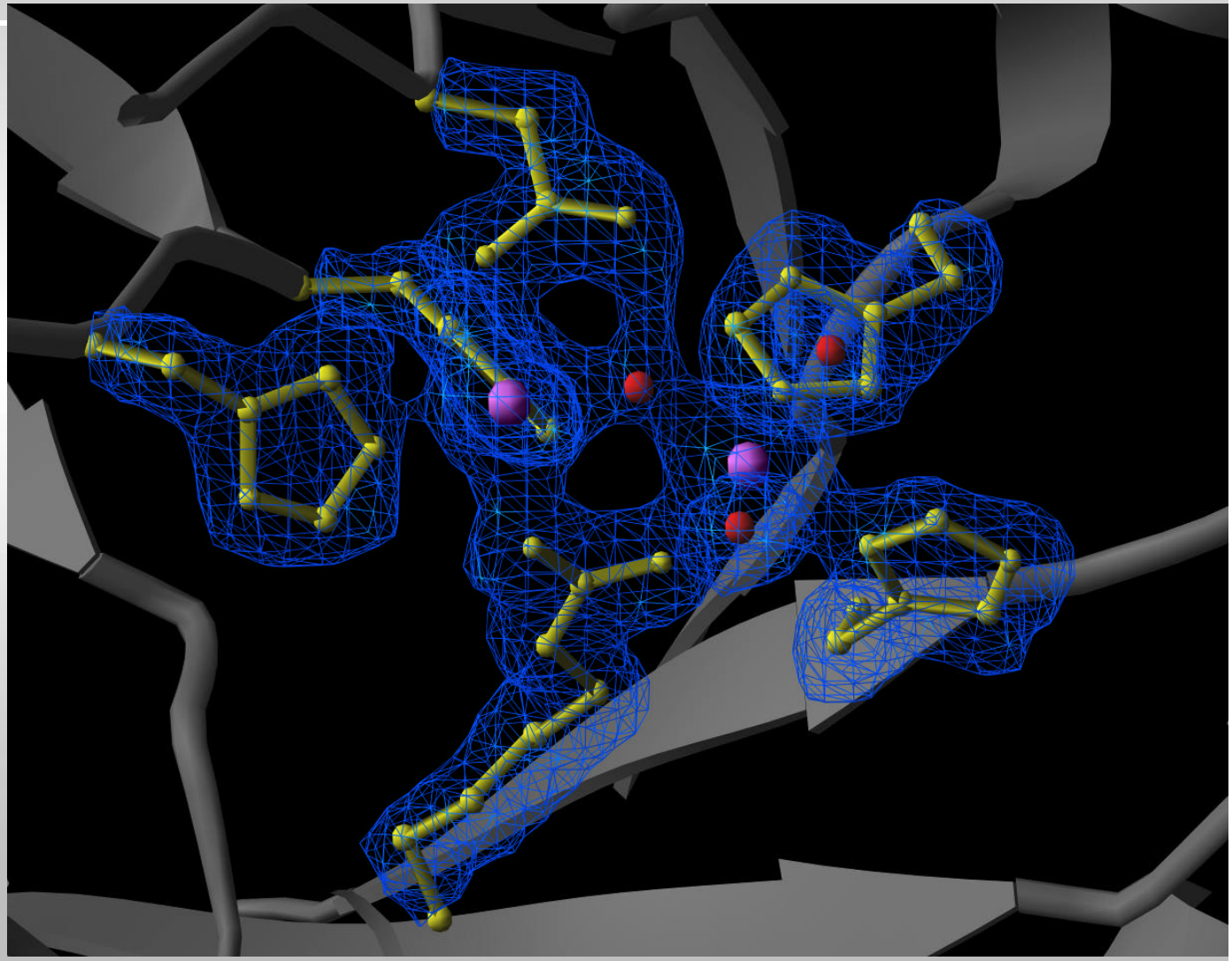


The molecular structure must be inferred from the map by someone competent in physical biochemistry.

The precision of the map interpretation depends on the resolution of the data to which you collected your data.

Example: Structure of an Enzyme Active Site

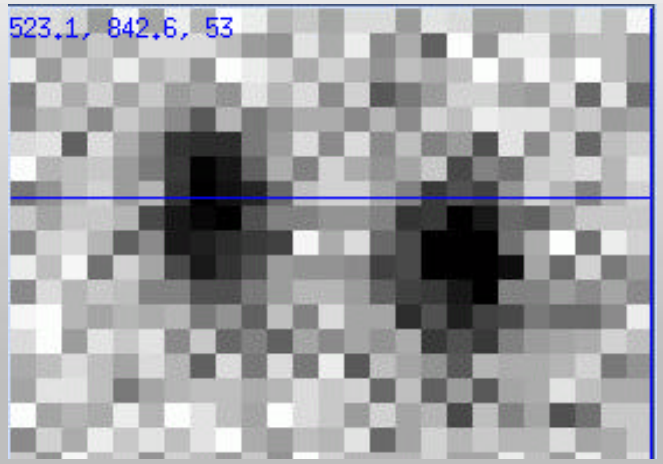
- ***Pseudomonas diminuta* phosphotriesterase**: This enzyme catalyzes the hydrolysis of organophosphorus pesticides and nerve agents. Its crystal structure is being studied by Hazel Holden's research group at the University of Wisconsin, Madison (see PDB file 1DPM).
- **Purple atoms: zinc**
- **Red: bound water**
- **Yellow: side chains**
- **1.8 Å resolution map,**
- **21% R-factor**

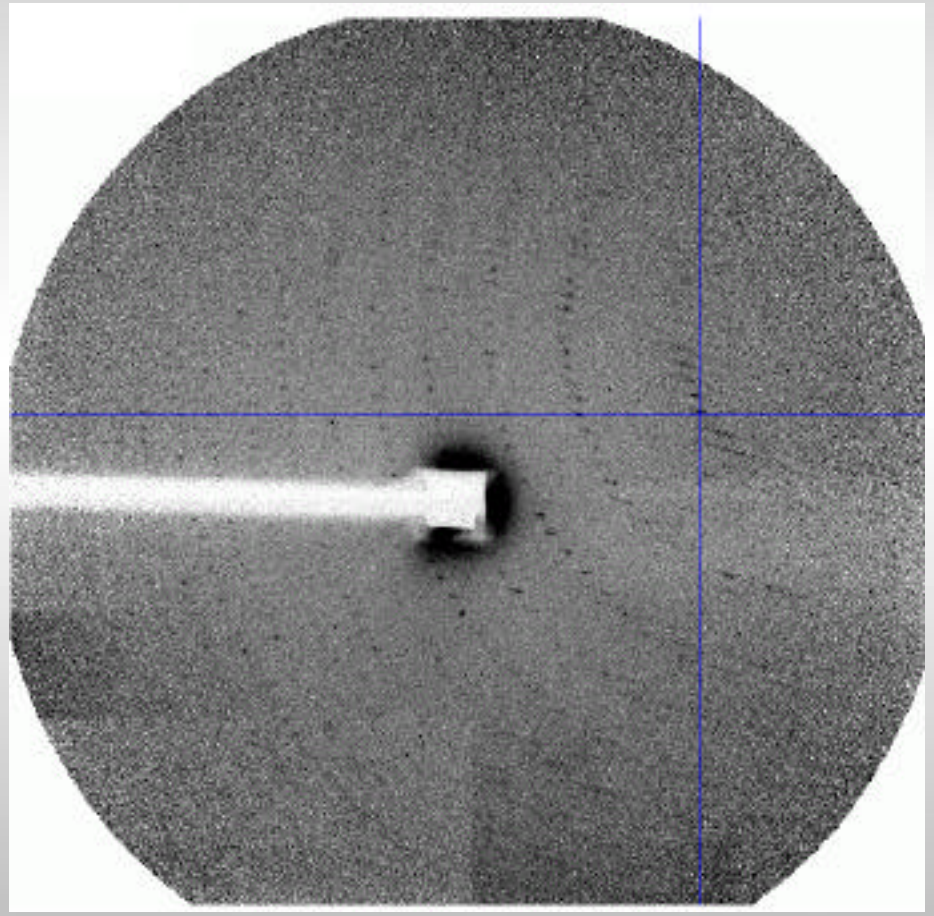


Virus Crystal Diffraction: 600 Å Unit Cell

- Data taken at SBCCAT bending magnet line, 19BM, at APS.
- Image of virus diffraction pattern, detector distance = 550mm, Wavelength λ = 1.5 Å.

  Data to edge of detector, 5.8 Å. Below - pixel blowup showing separation of 600 Å axis spots.

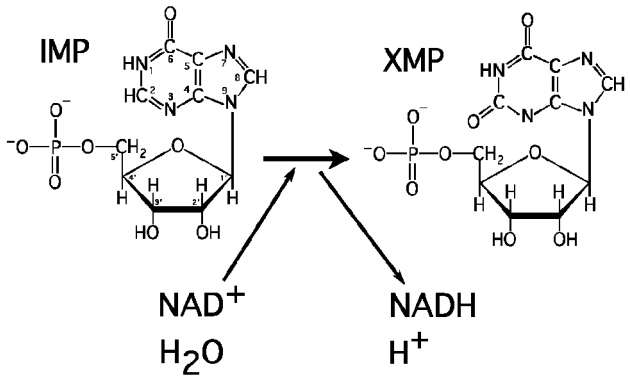


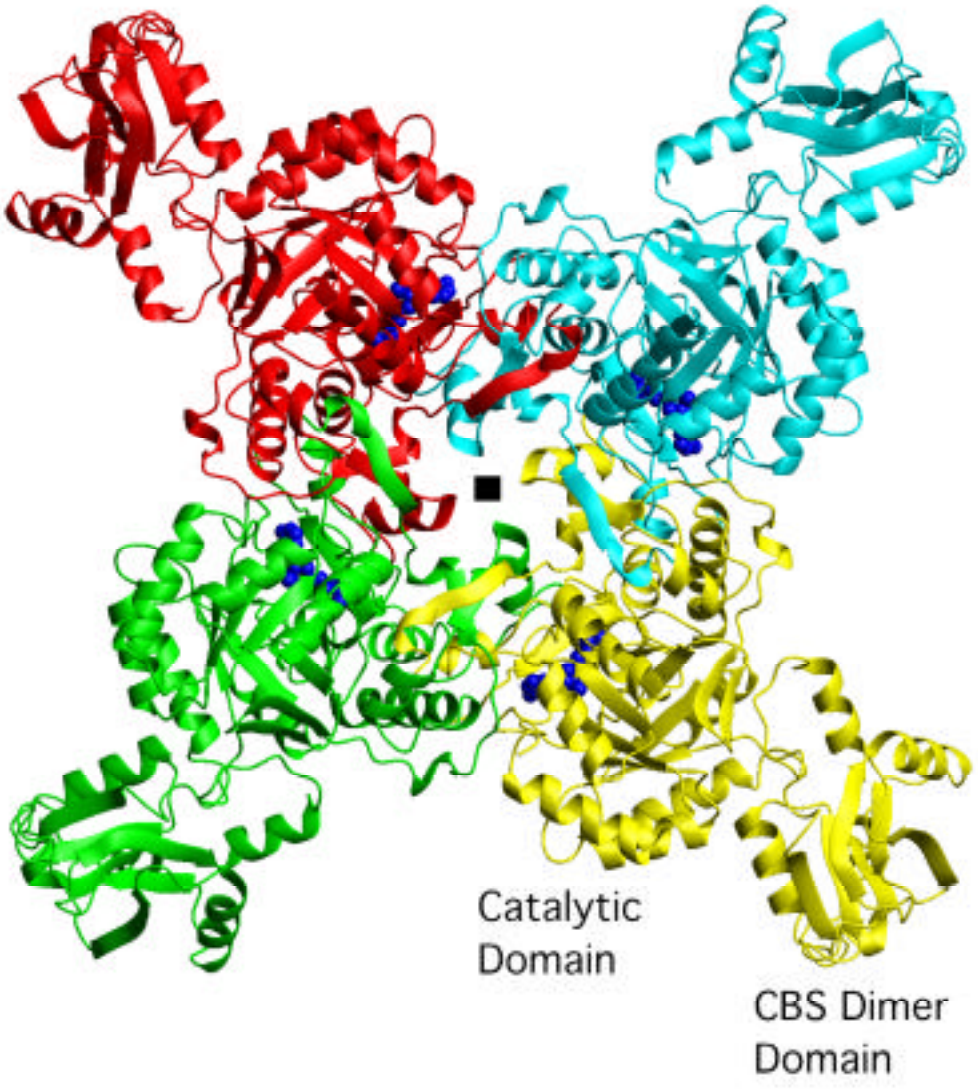


- Thanks to Michael Chapman, Florida State University

IMPDH Story: Compare Bacterial (Staphylococcus) Enzyme with Human: drug design

Structure and Function
of
Inosine Monophosphate
Dehydrogenase



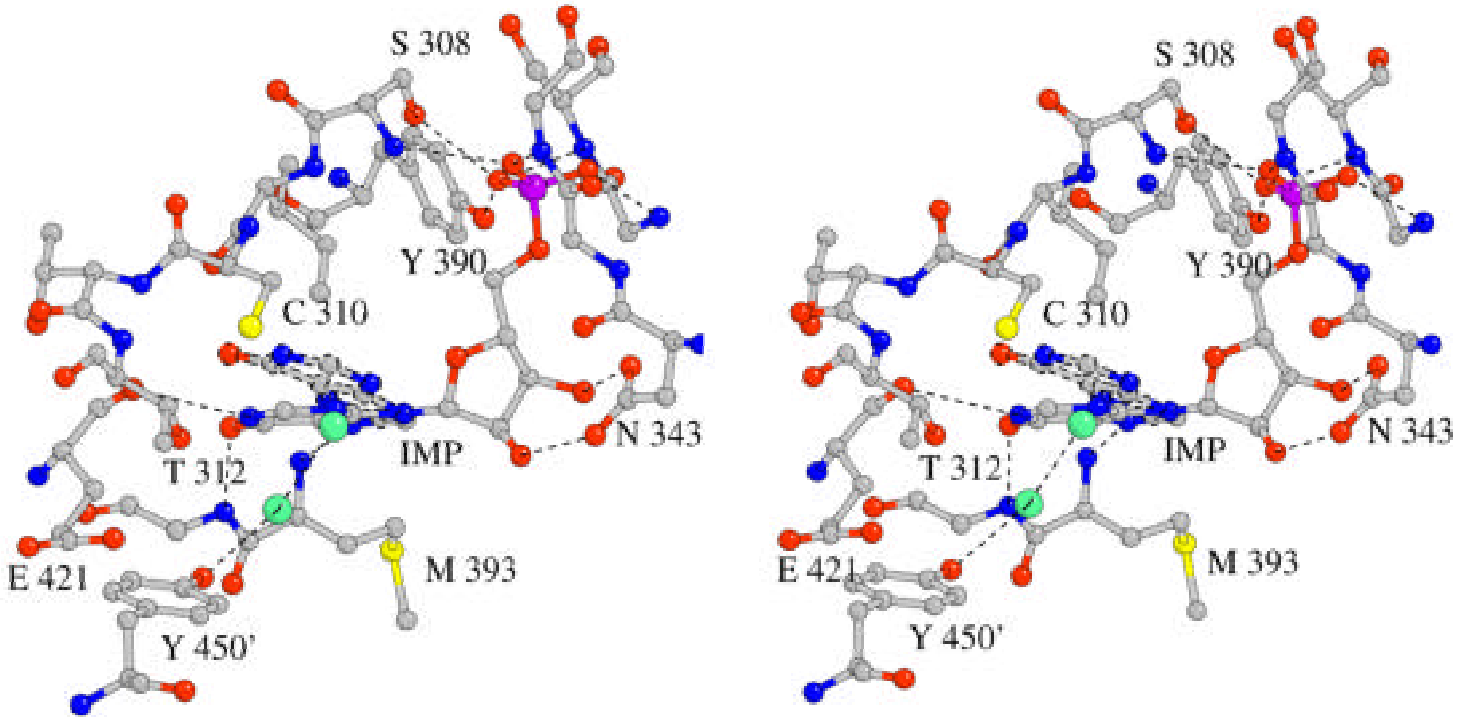


S. pyogenes IMPDH: Crystal Parameters and Data Summaries (data from fiber-optic/CCD detector)

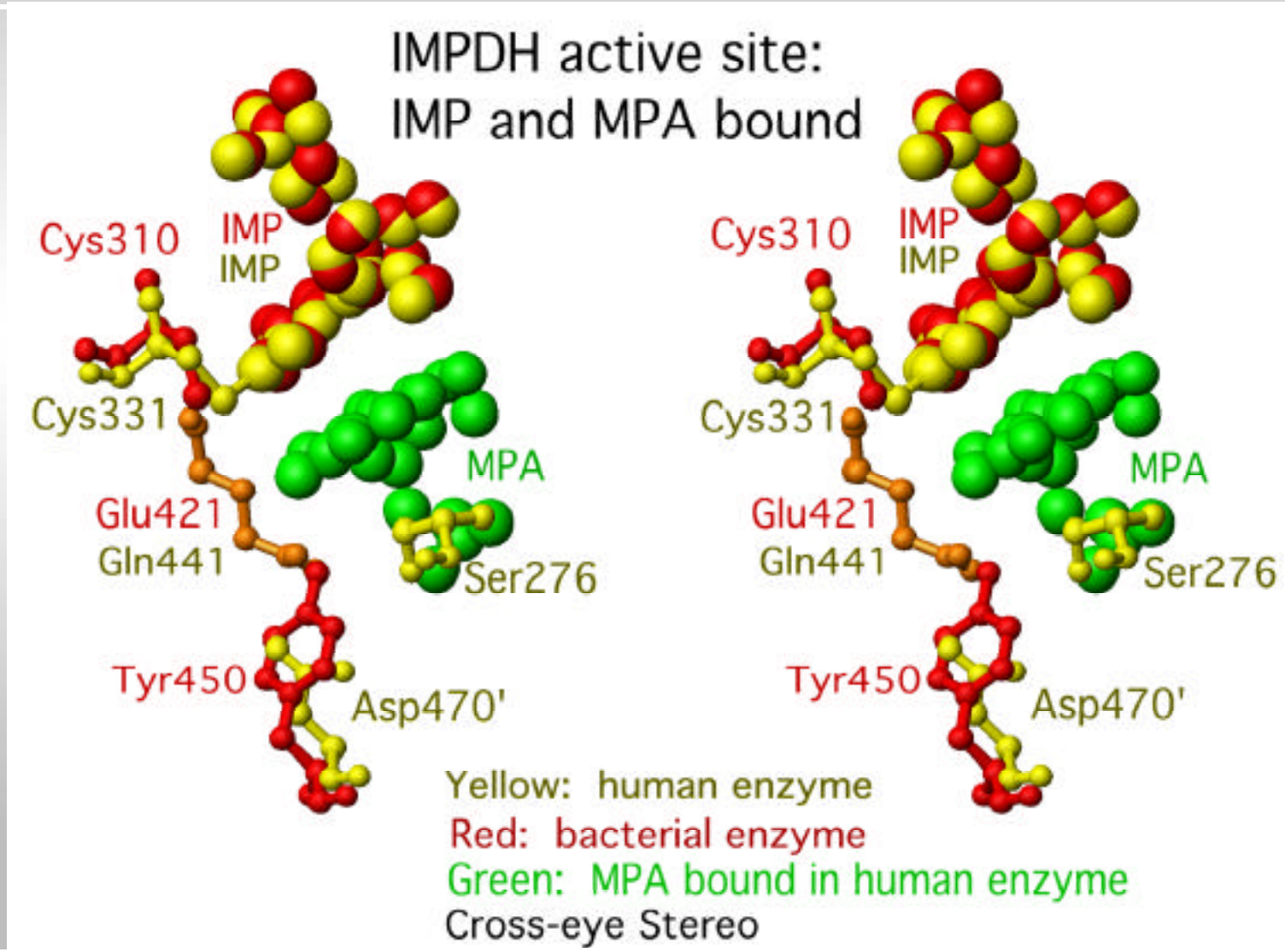
- **Unit cell:** **Space group: I422**
- **a = b = 151.5 Å, c = 101.7 Å**
- **High Resolution data set (one crystal):**
  - **Resolution: 10 Å - 1.9 Å**
  - **Wavelength: 1.0332 Å**
  - **Exposure times: 8 seconds/degree of rotation**
  - **# Bragg spots observed: 263,355**
  - **# Unique reflections: 44,921**
  - **Redundancy: 5.9-fold**
  - **Completeness: 96.5%**
  - **R_{merge} 6.8%**

100 data images
20 minutes to record all data

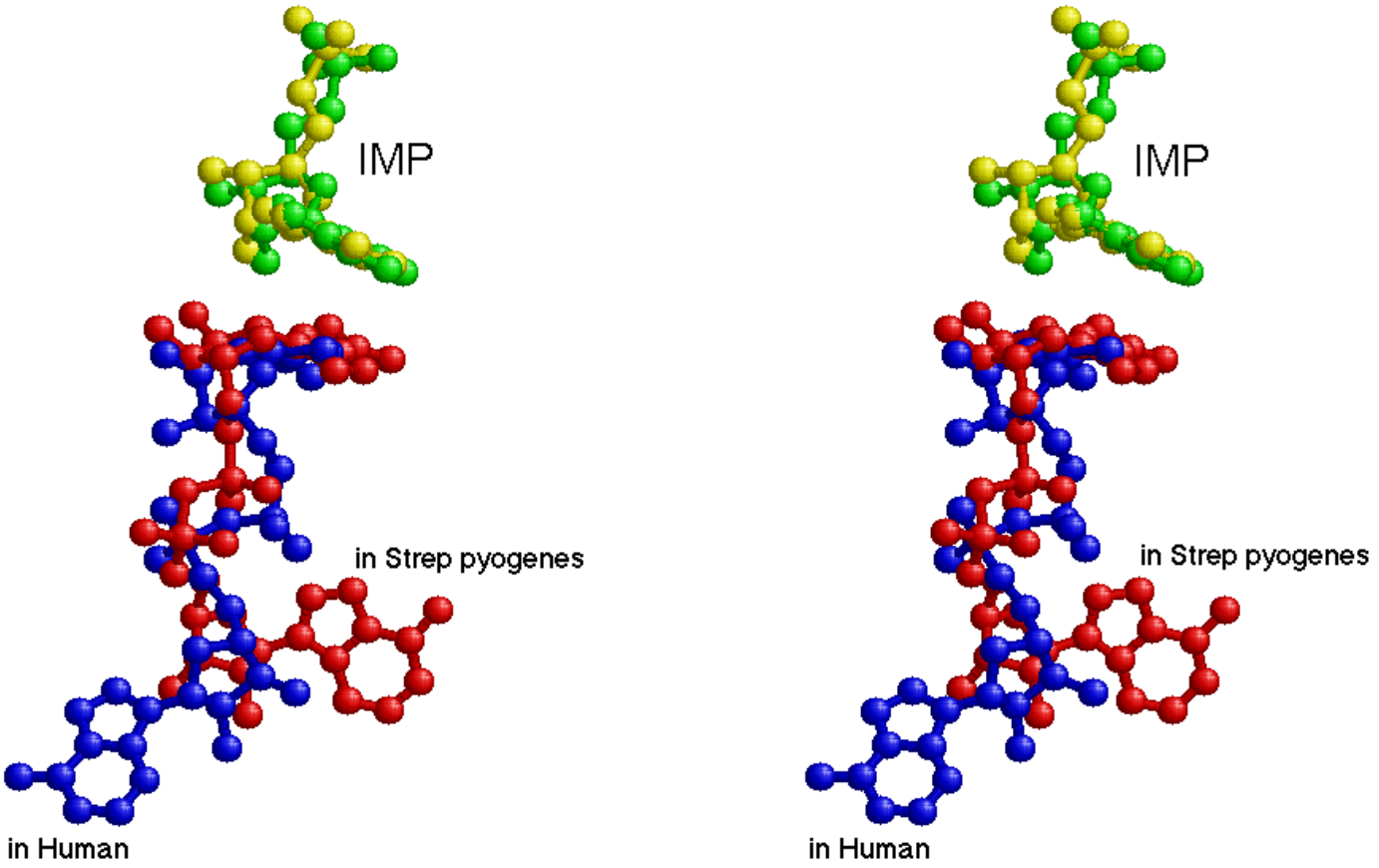
Molecular Model of Bacterial IMPDH Enzyme Active Site



Correspondence between Bacterial and Human IMPDH Active Sites: IMP/MPA binding

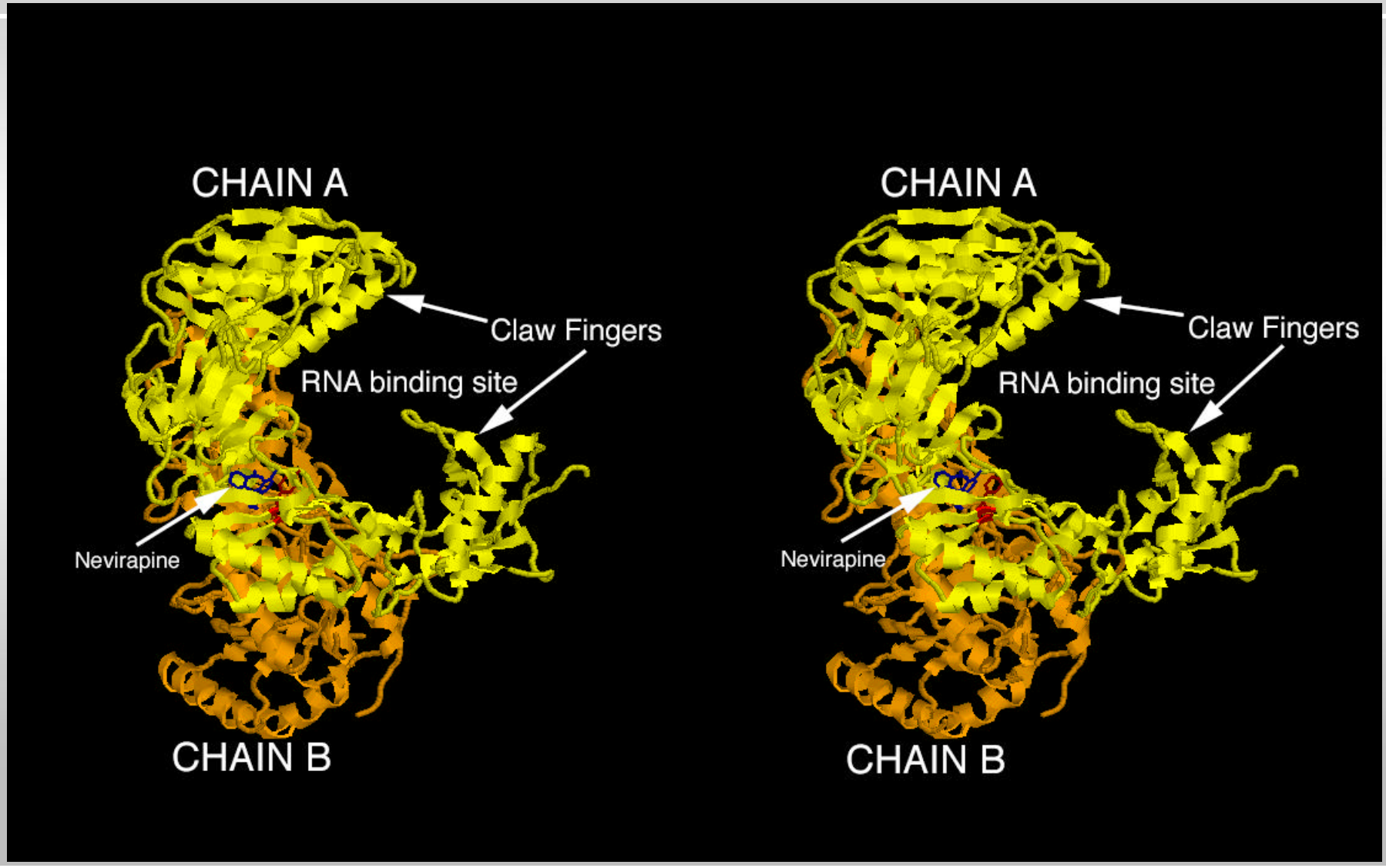


A Striking Difference Between Human and Bacterial Binding of NAD Analog TAD

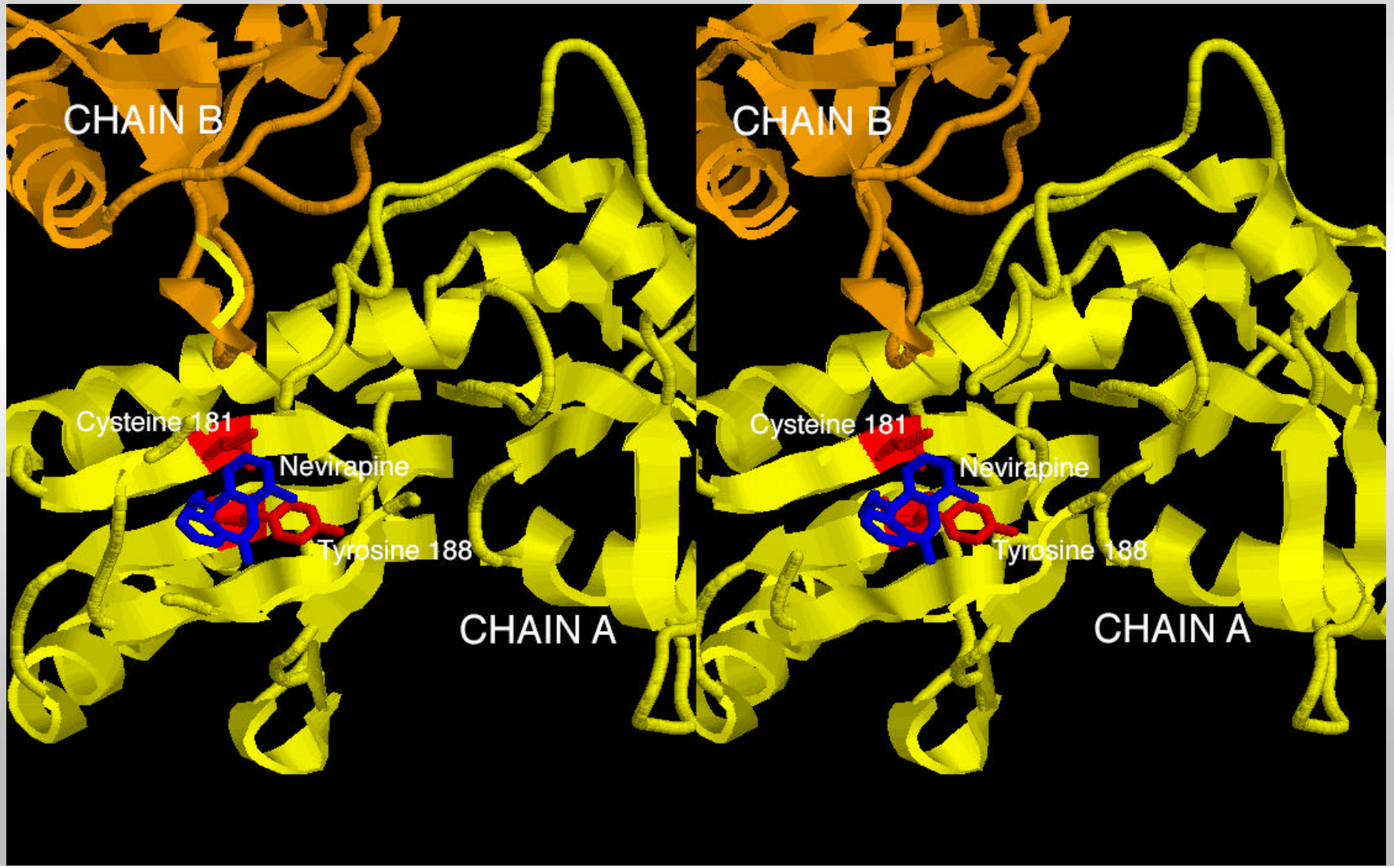


Alternate sites of Adenine in 2 crystal structures

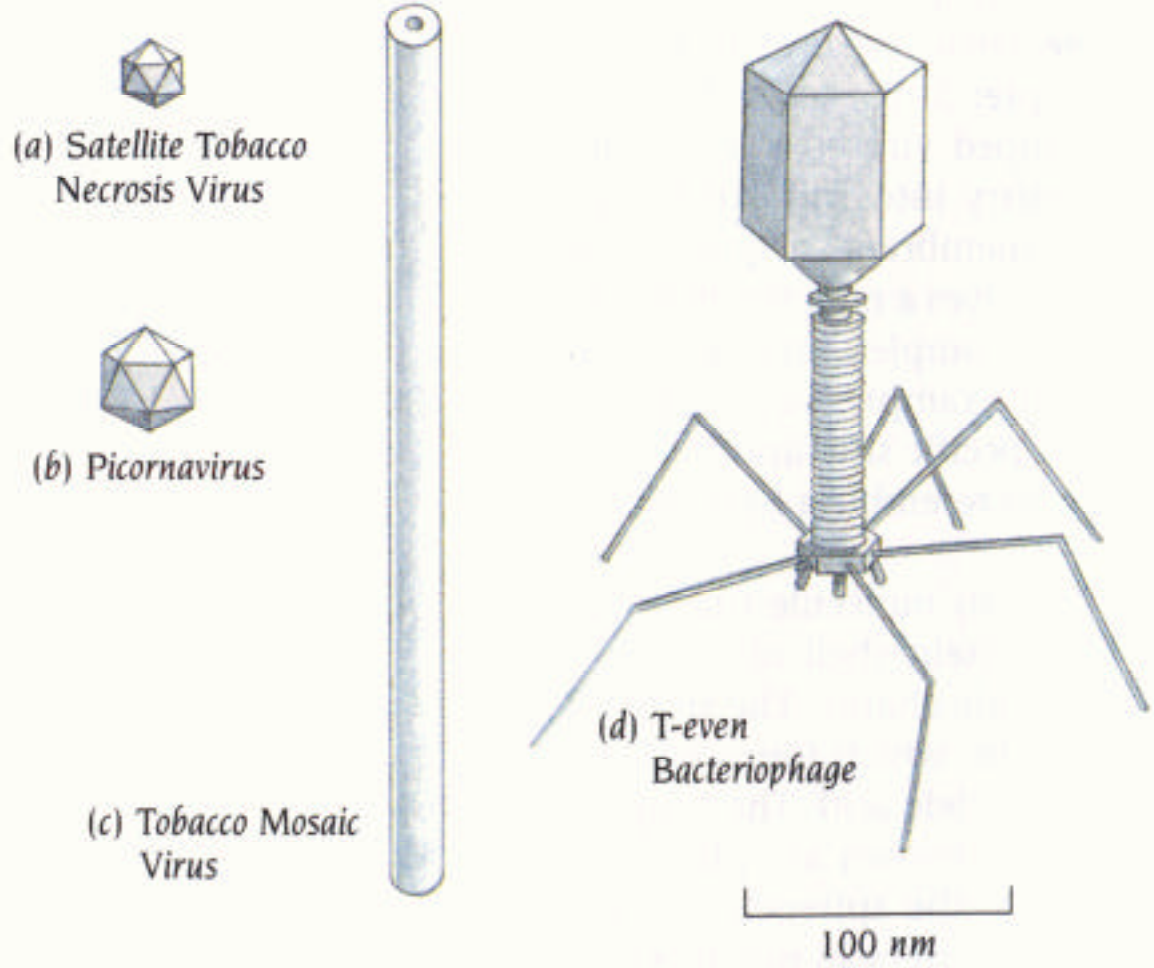
HIV Reverse Transcriptase, with inhibitor Nevirapine (J. Ren et al. 2001, J.Mol.Bio. 312)

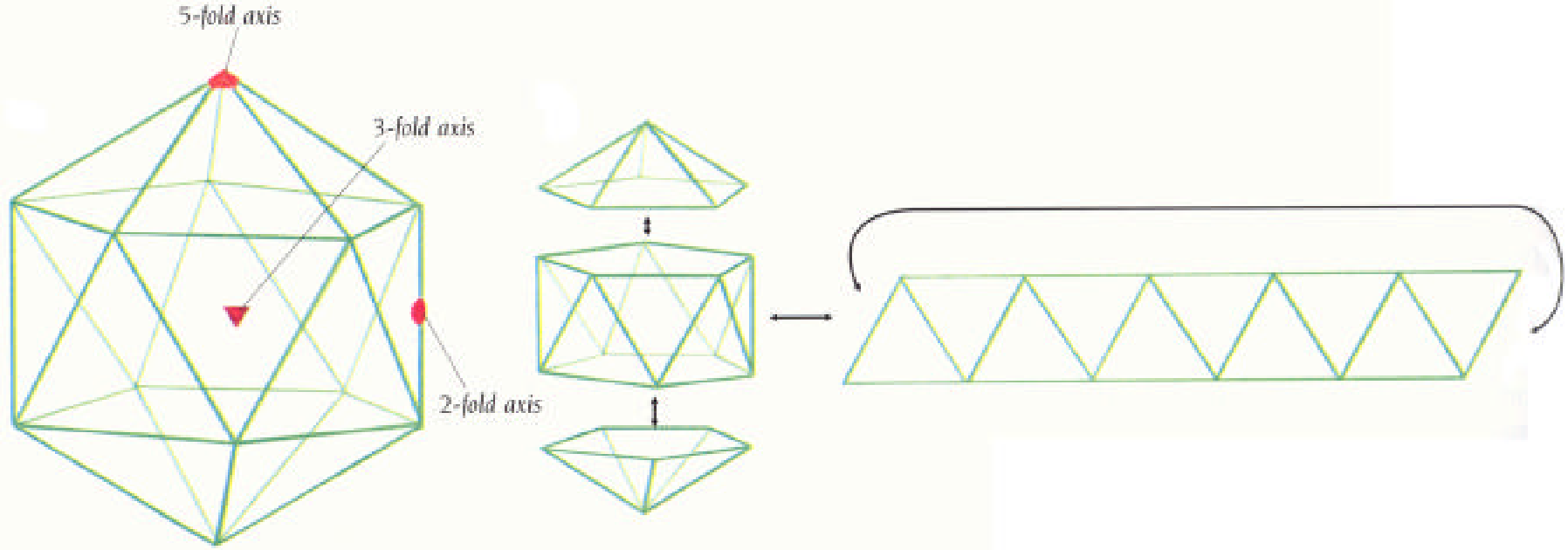


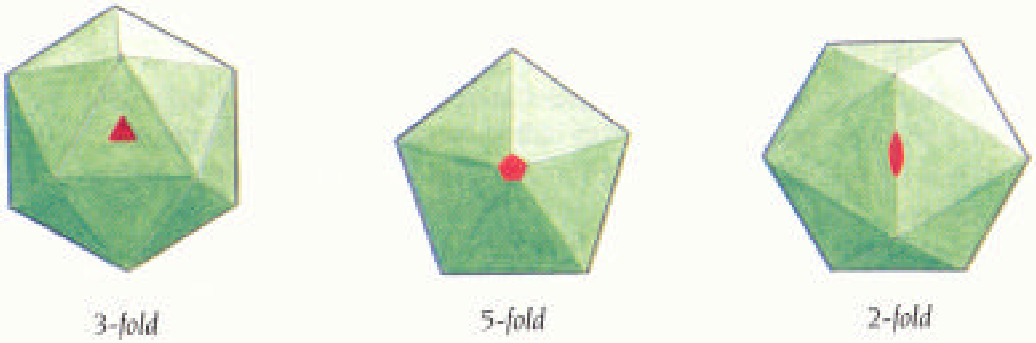
Nevirapine alters the RNA binding site. RT resistance results from mutations of Cys181 or Tyr188



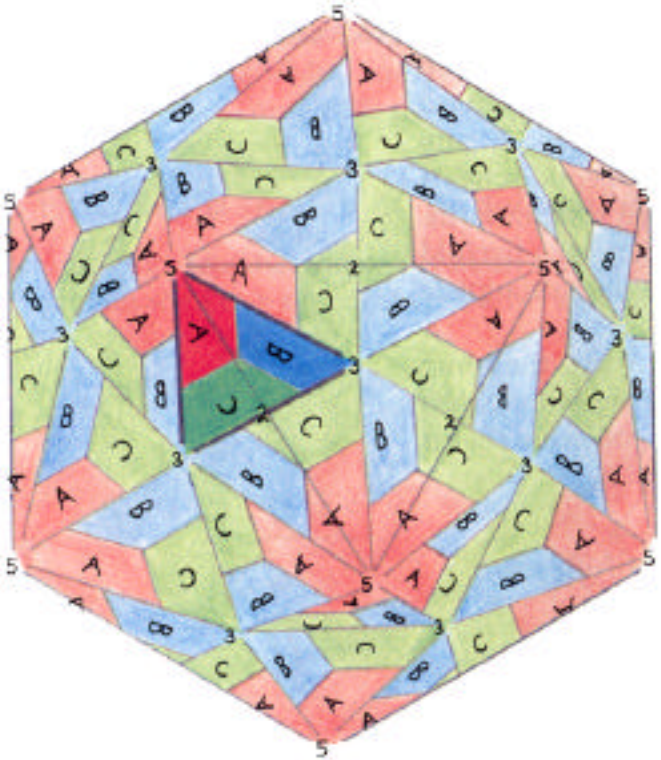
Viruses


(a) Satellite Tobacco Necrosis Virus
(b) Picornavirus
(c) Tobacco Mosaic Virus
(d) T-even Bacteriophage
100 nm

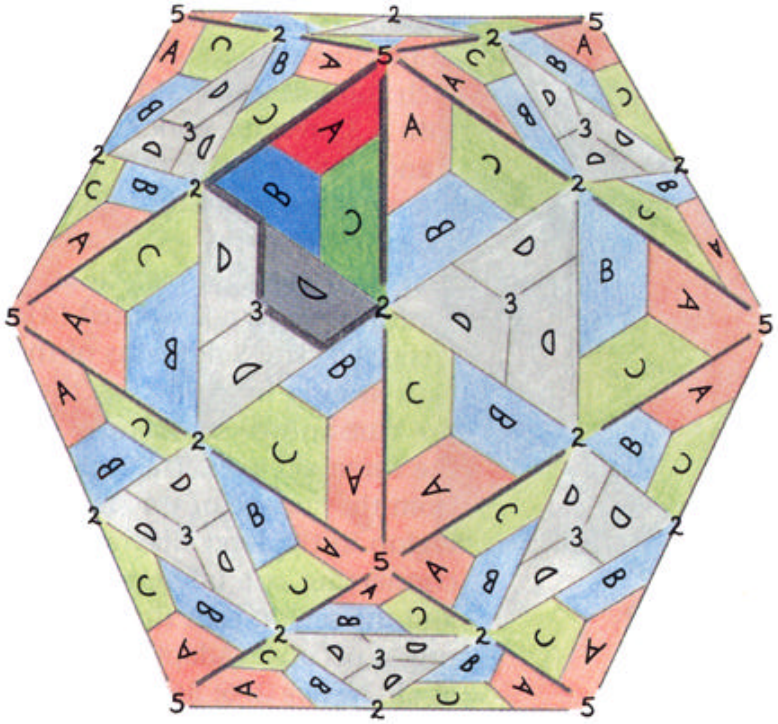

5-fold axis
3-fold axis
2-fold axis


3-fold
5-fold
2-fold

Viruses with T-numbers >1 have identical coat proteins with *quasi-equivant* shapes

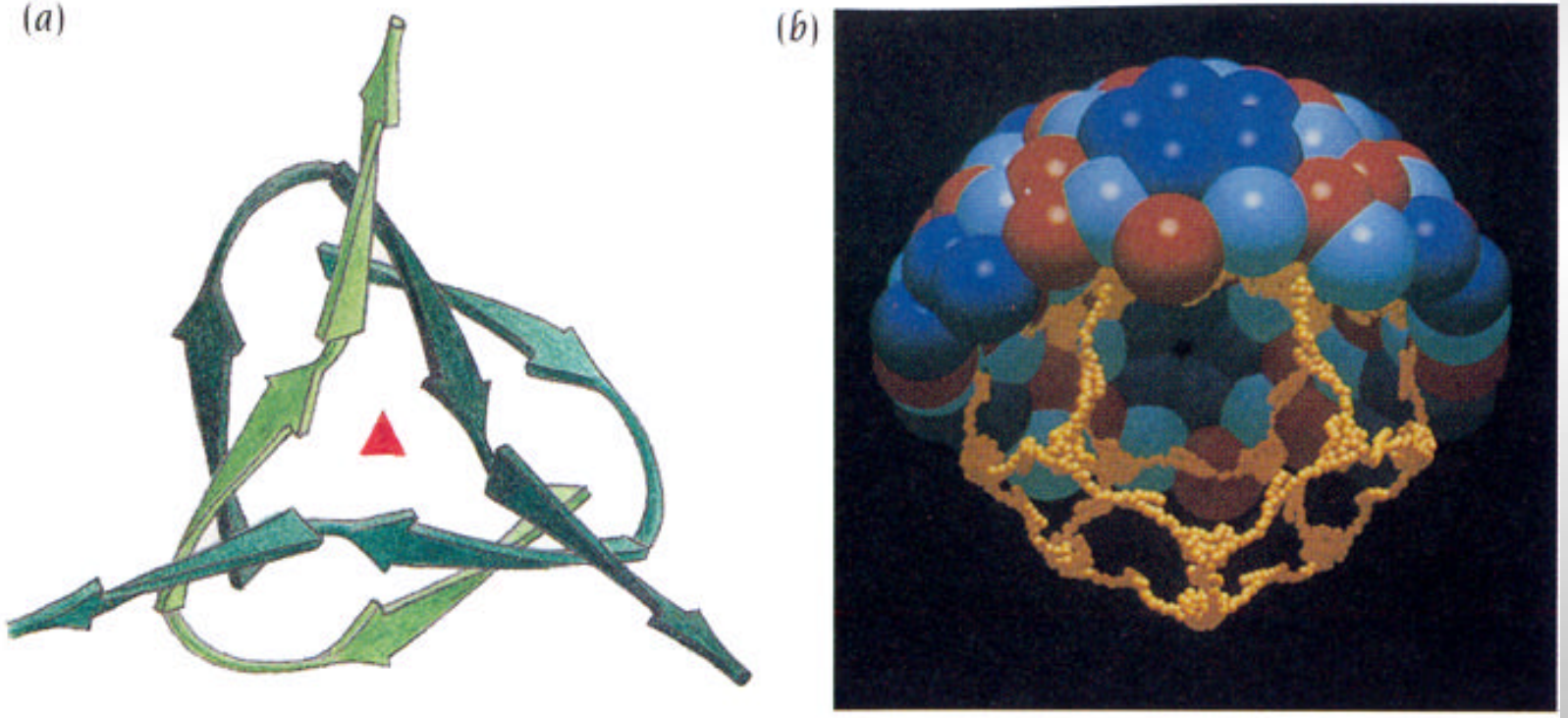


T3 symmetry

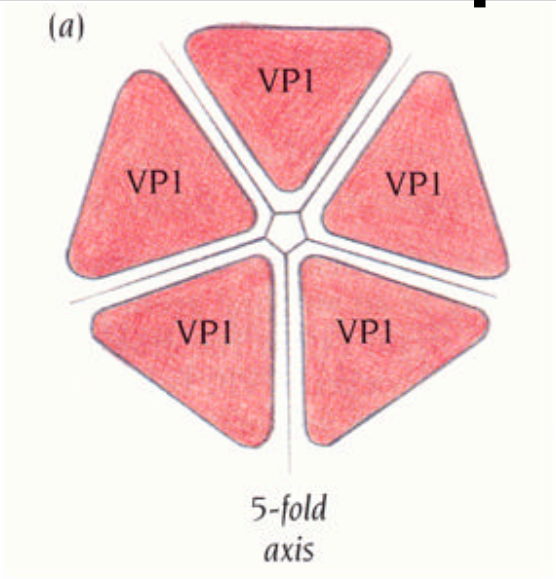


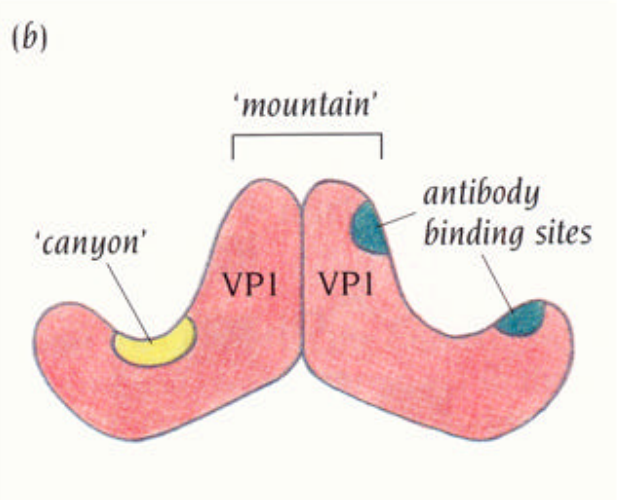
T4 symmetry

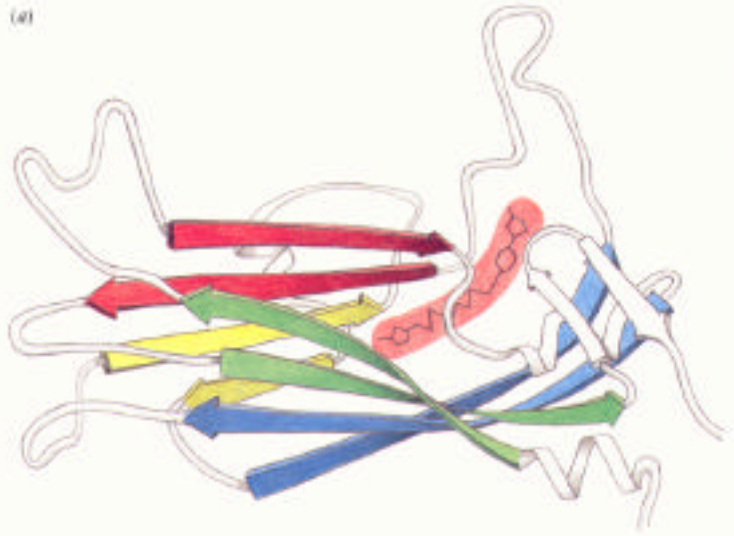
In the Tomato Bushy Stunt Virus, the C-termini tie knots (actually each is a tight 3-strand β-sheet) at each 3-fold vertex.

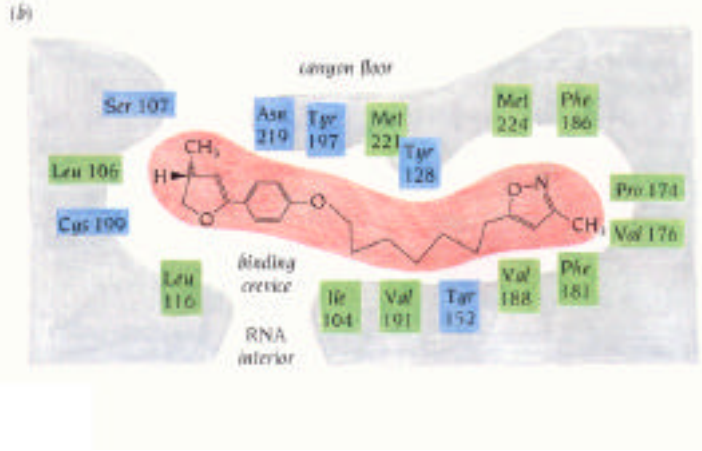


In Human Cold Virus, a “Canyon” surrounds the 5-fold. Antibodies cannot bind there, but ICAM-1 (the Rhinovirus receptor adhesin) does.









Multiwire Detectors

- Counting detector: Poisson statistics, no dark image
- VERY efficient at low-to-medium count rates: excellent choice for rotating-anode x-ray source, or low-signal synchrotron applications
- Can be made large, but not VERY large
- Histogram image; electronic dead time for each count, but no “readout” lag
- Not expensive

- Problem #1: pixels are big (0.2-1.0 mm), limiting spatial resolution
- Problem #2: limited speed/count rate

Imaging Phosphor Plates

- Integrating detector, but no dark image
- Can be made VERY large (at expense of speed)
- Modest spatial resolution (0.15 mm), but large size permits substantial resolving power
- More expensive than multiwires

- Problem #1: slow readout; long dead time between image frames
- Problem #2: dynamic range and DQE are limited

- Despite many attractive features, there is little prospect for further development of this technology

Fiberoptically-coupled CCD Detectors

- Integrating detector; exhibits dark image
- Spatial resolution better than IPs or MWs (FWHM 0.05 mm, vs 0.15 mm for IPs)
- Very dense pixel rasters; can be made modular
- Resolving power of multi-modular CCD system surpasses IPs
- Fast readout (<1s) permits fine phi slicing
- Higher efficiency than phosphor plates

- Problem #1: Expensive to make very large
- Problem#2: Limited dynamic range
- Problem #3: Fast, but not fast enough
- Problem #4: Image degraded by fibers (chickenwire, vignetting, blemishes, etc.)

Amorphous silicon TFT Arrays

- Integrating detector; exhibits dark image
- Can easily be made very large
- Sharp spatial resolution: no “tails”
- Current embodiment has low (12-bit) dynamic range; technology ***could*** have higher dynamic range.
- DQE limited by very high read noise: engineering needed.
- Can be made inexpensively
- Reasonable readout speed (2-10 seconds)

- **Problem#1: converting X-ray image to electronic image: needs a photoconducting converter, not a phosphor**
- **Problem#2: very noisy medium**

Lens-coupled CCD Detectors

- Uses one single CCD
- Integrating detector; exhibits dark image
- Can be made very large
- Dynamic range and DQE equivalent to modular CCDs
- Fast readout; short dead time between image frames, permits fine phi slicing
- Easier to calibrate and correct than modular systems
- Inexpensive

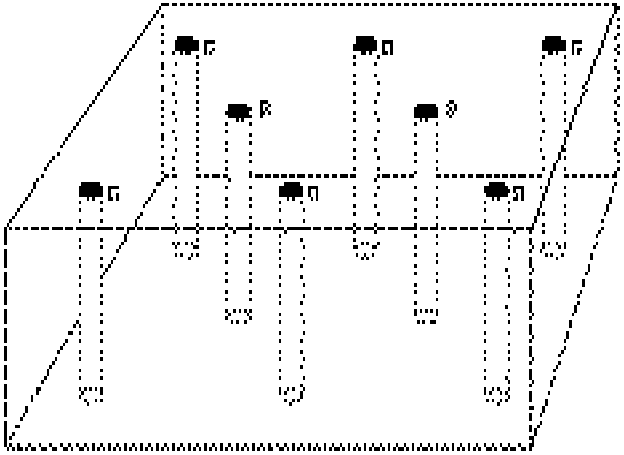
- **Problem#1: needs custom-designed, very-high numerical aperture lens**
- **Problem#2: needs careful engineering to "get it right"**

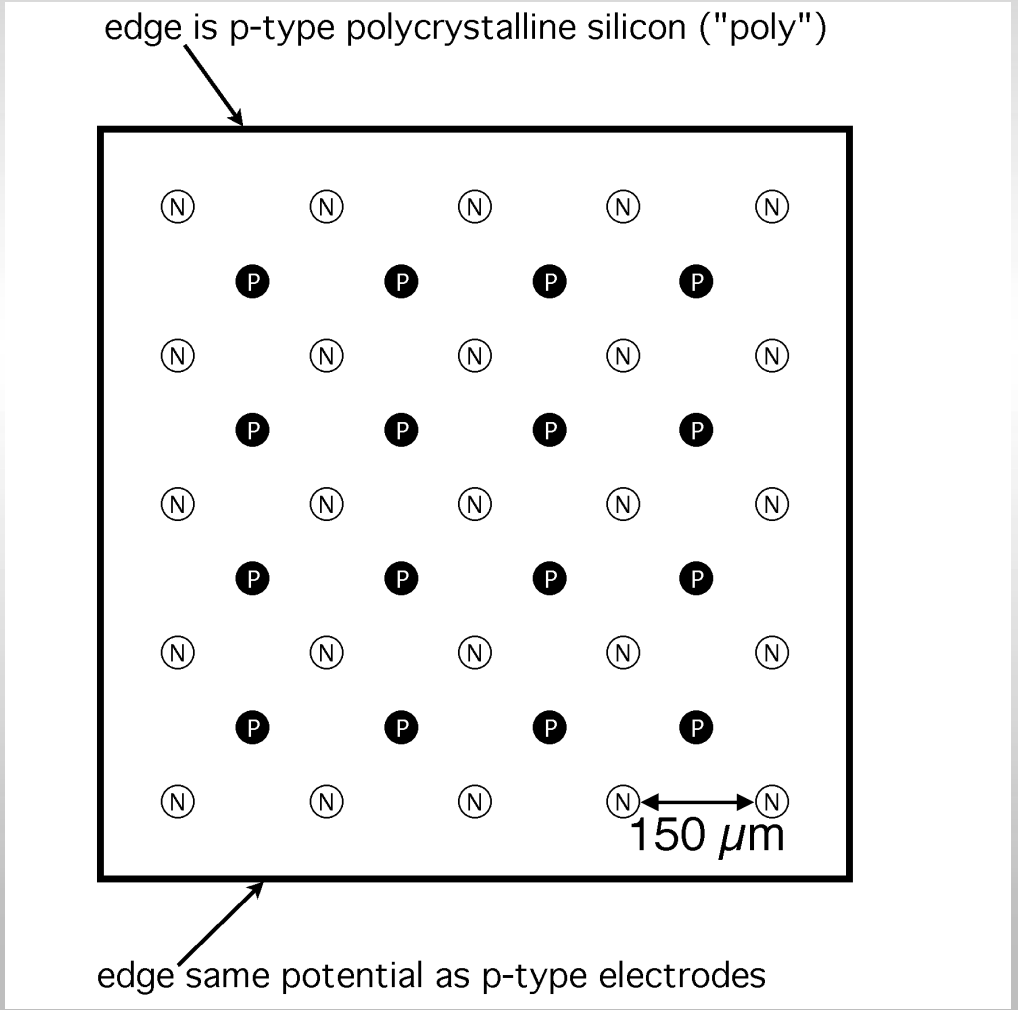
Pixel Array Detectors

- Counting detector system: Poisson statistics, no dark image
- Efficient even at high count rates
- Sharp spatial resolution: no “tails”
- Large detectors must be modular
- Expensive to develop; ***may*** be inexpensive to produce

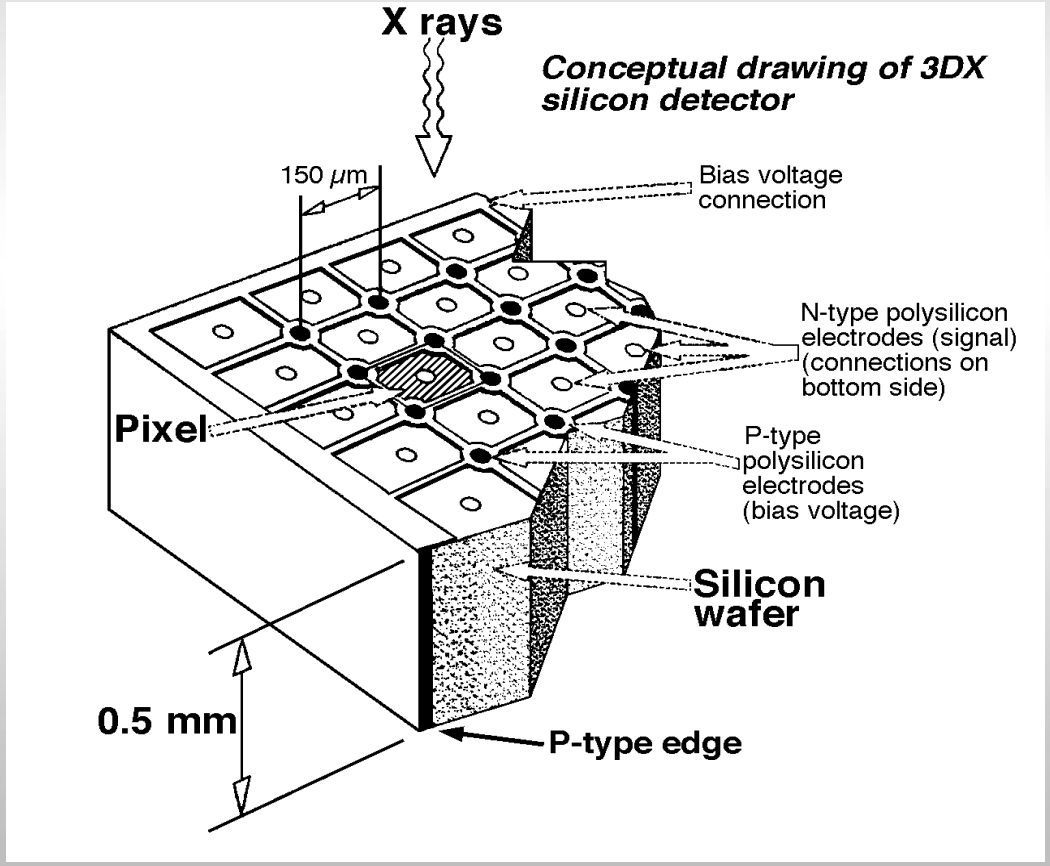
Silicon “3DX” Concept

- **Micromachined silicon detection wafer:**

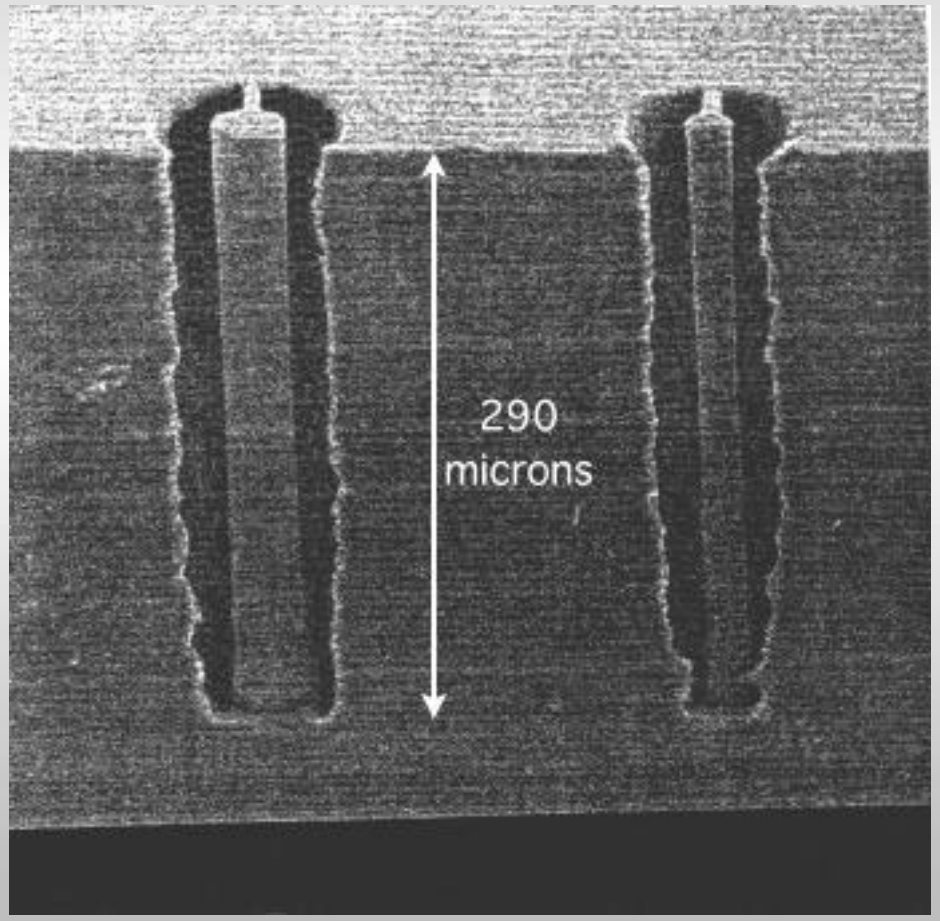




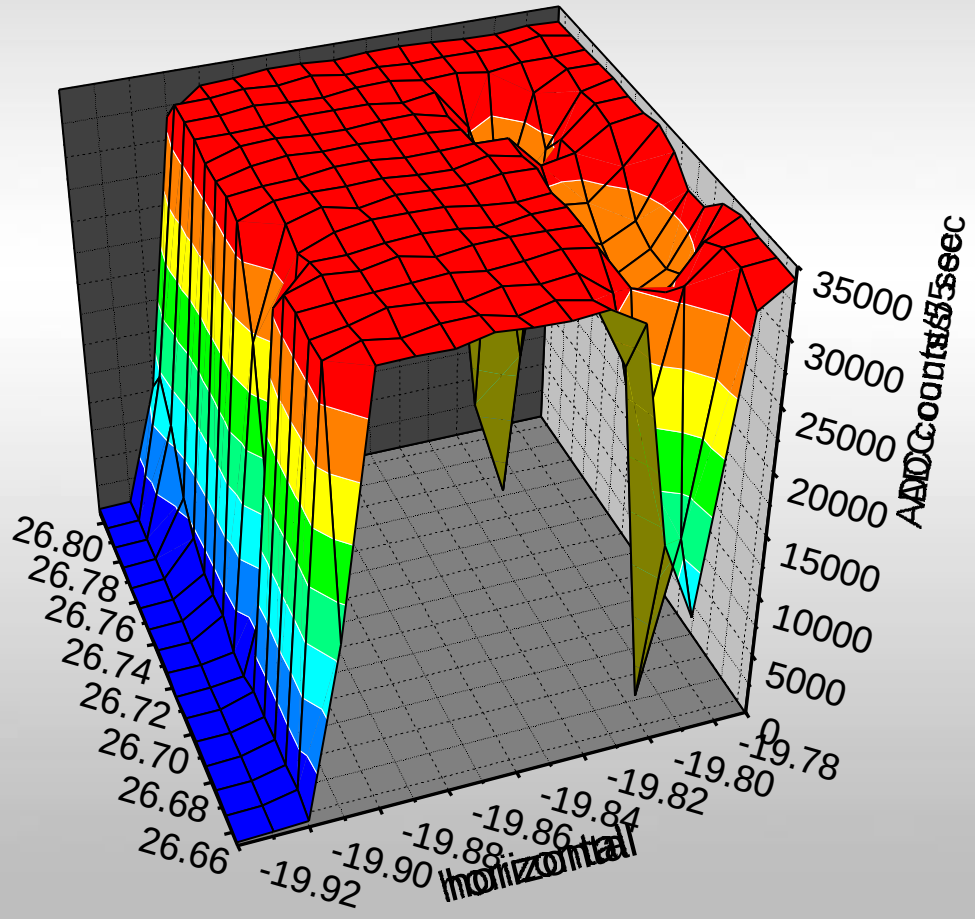
Detector Wafer Processed into Pixels



Micromachining Silicon with High Aspect Ratios

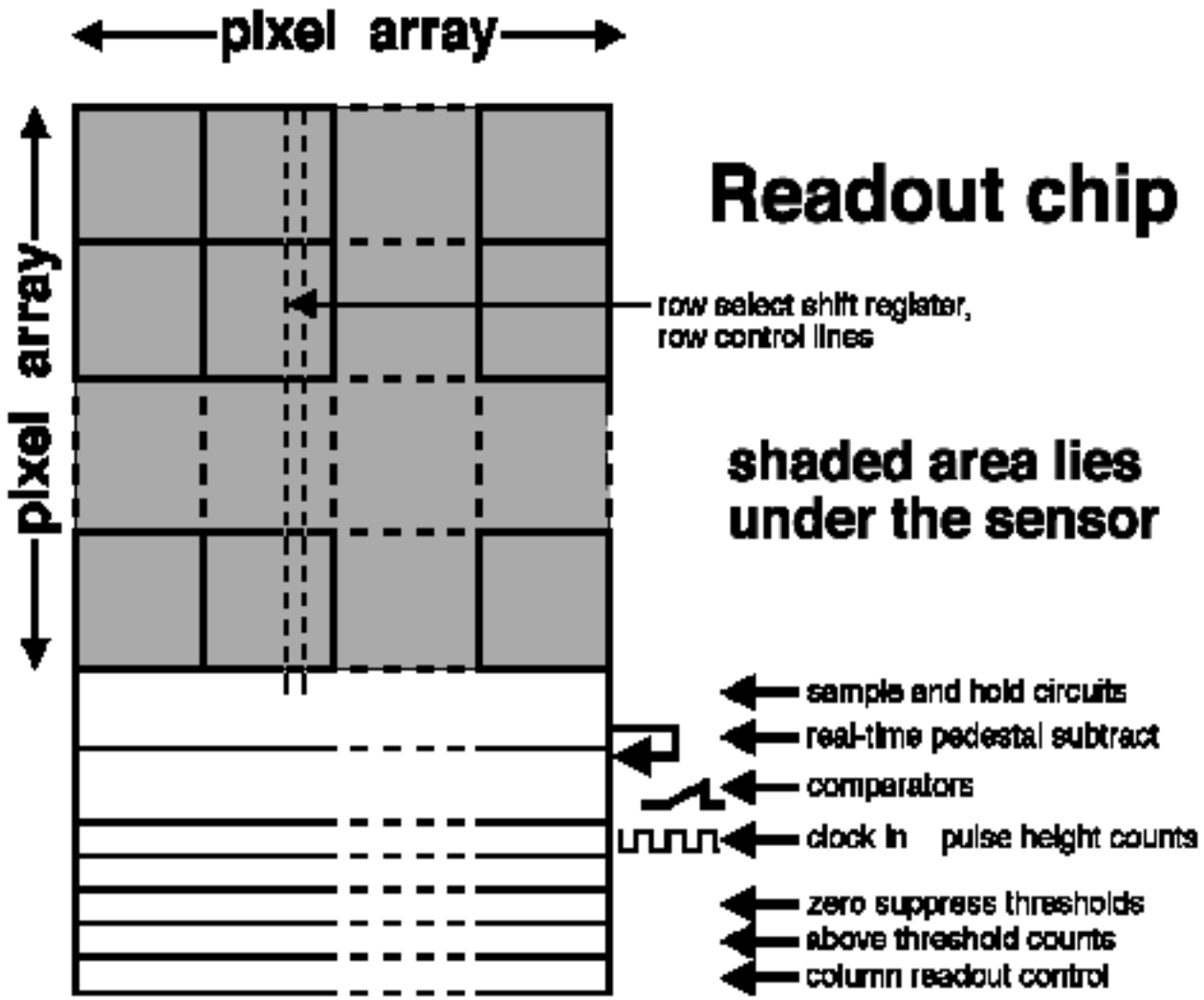


Excellent response uniformity (except at electrodes!)

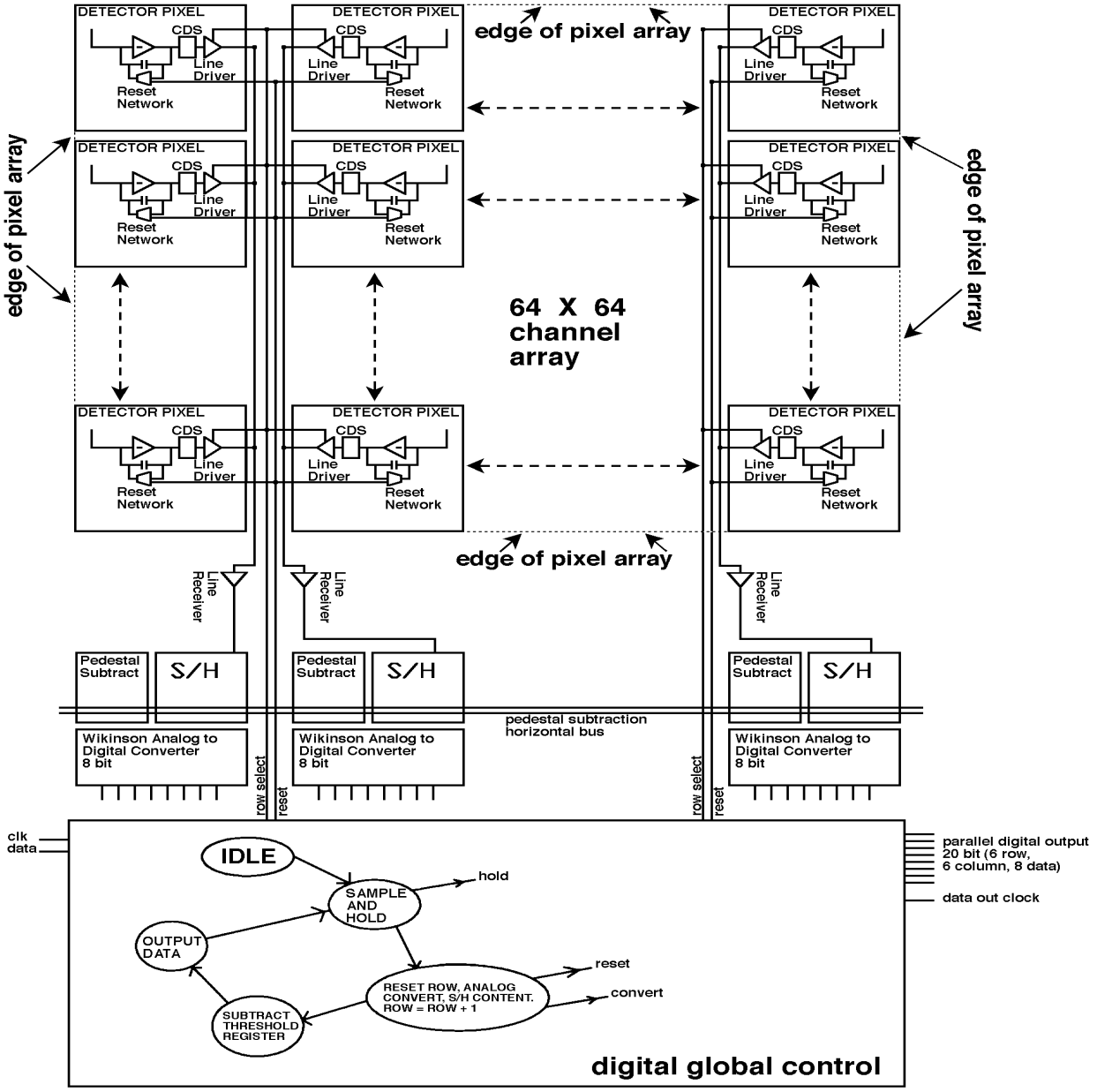


Hybrid Technology: Separate Sensor & Readout Chips

"Deep sub-micron" CMOS circuit design for readout wafer



64 x 64 array
150 µm x 150 µm pixels



S/H : Sample and Hold
CDS: Correlated Double Sampling

3DX READOUT
EMANUELE MANDELLI Dec 1, 2000
LAWRENCE BERKELEY NATIONAL LABORATORY

Concept for Tiling Large Areas

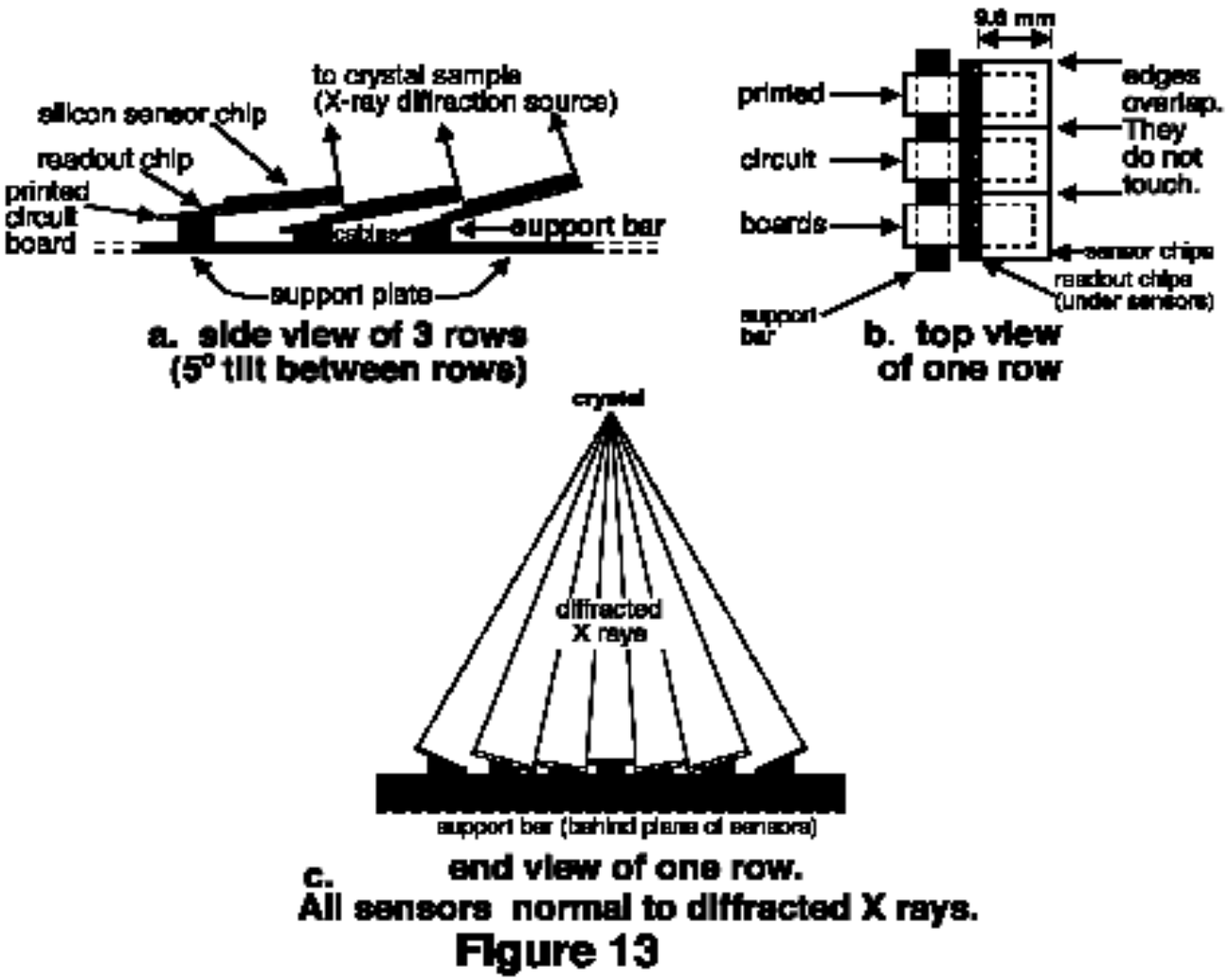


Figure 13