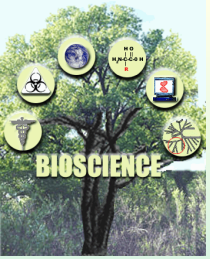
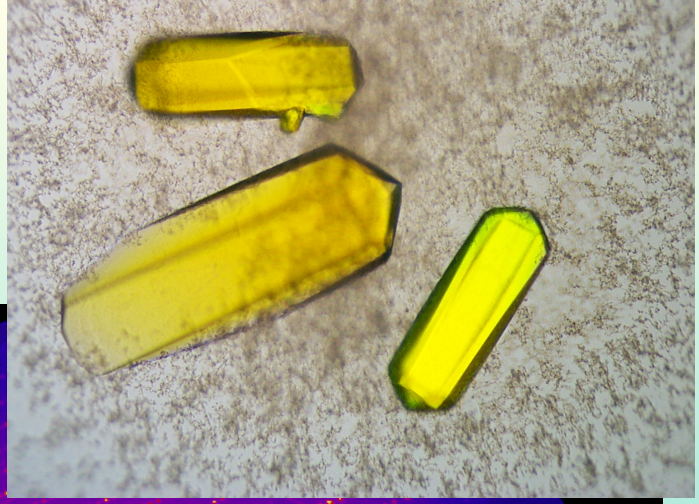
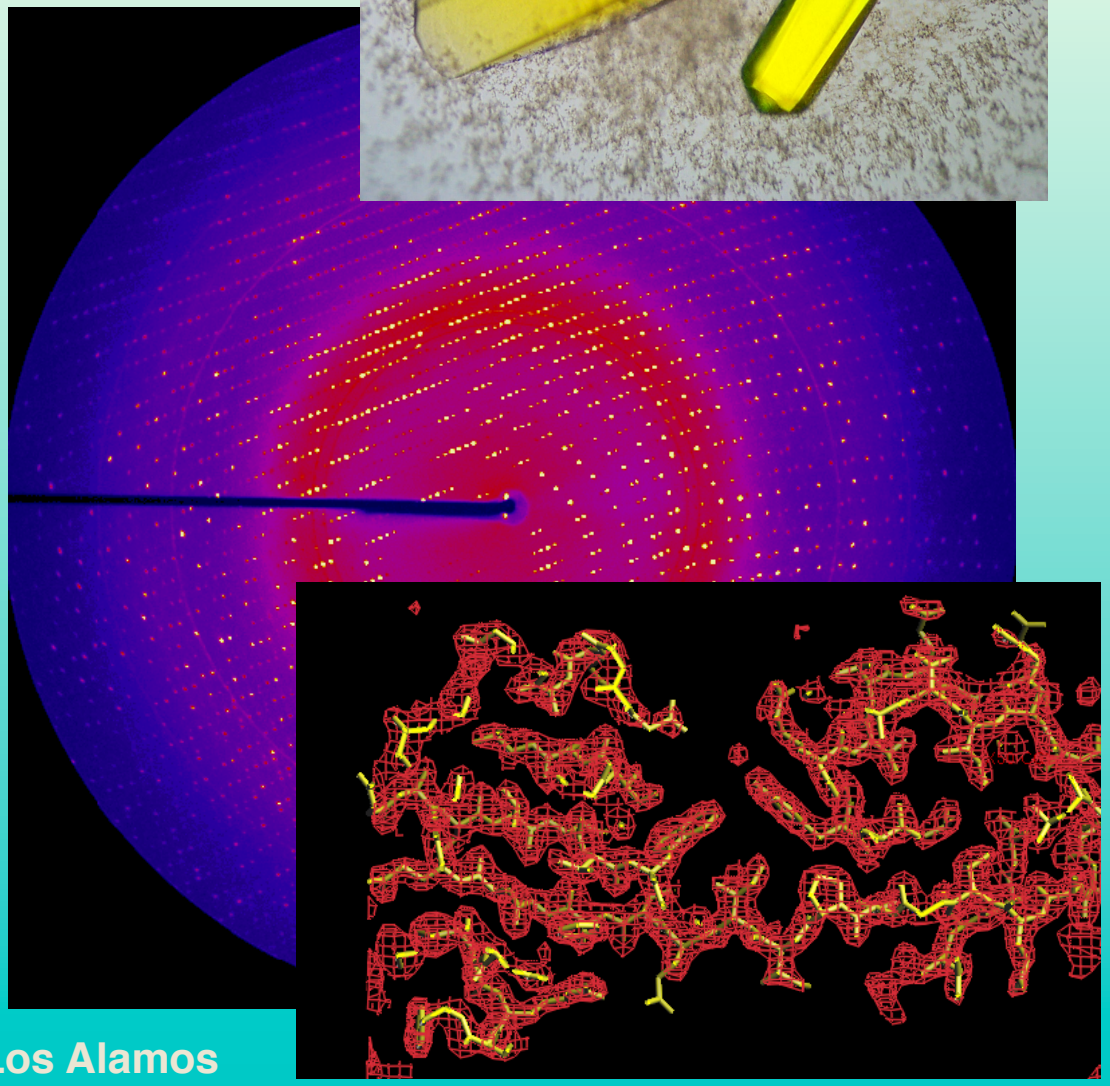




A brief introduction to X-ray crystallography



Growing protein crystals

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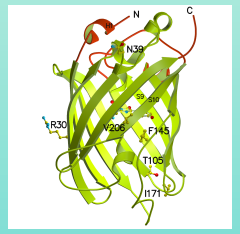
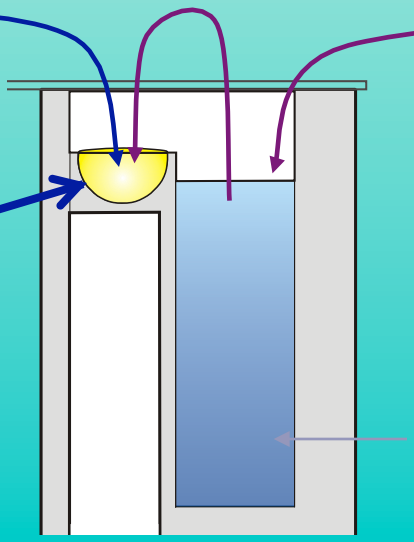
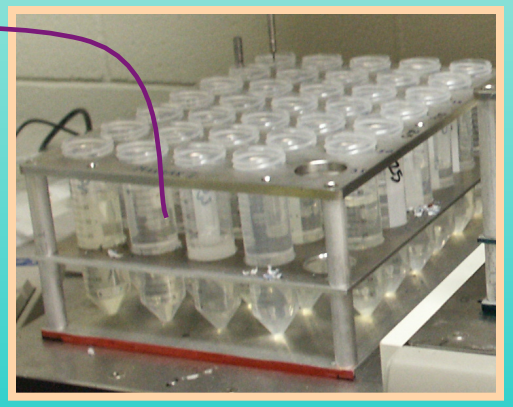
Growing protein crystals



Protein

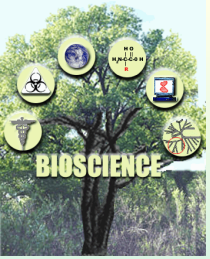
Protein +alcohol=Crystals





Salt, alcohol,
poly ethylene glycol…

Reservoir



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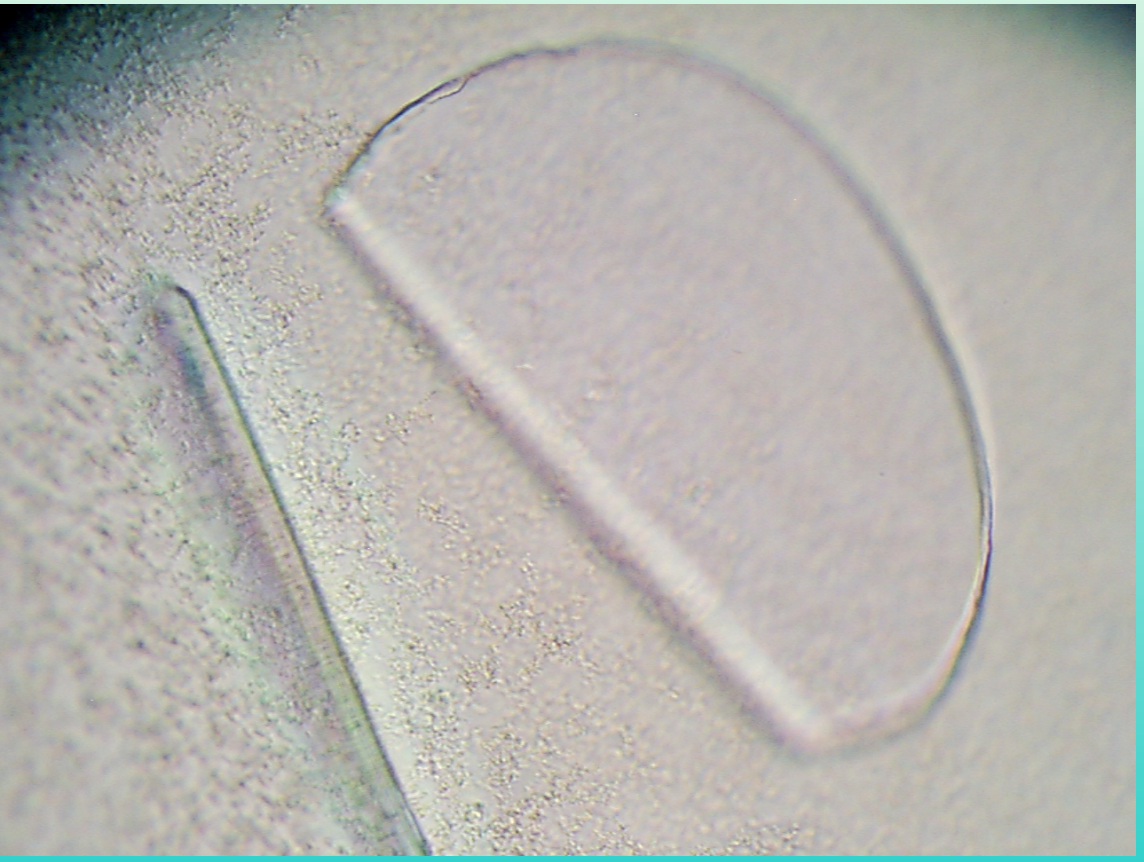

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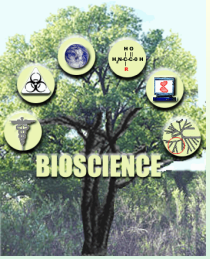

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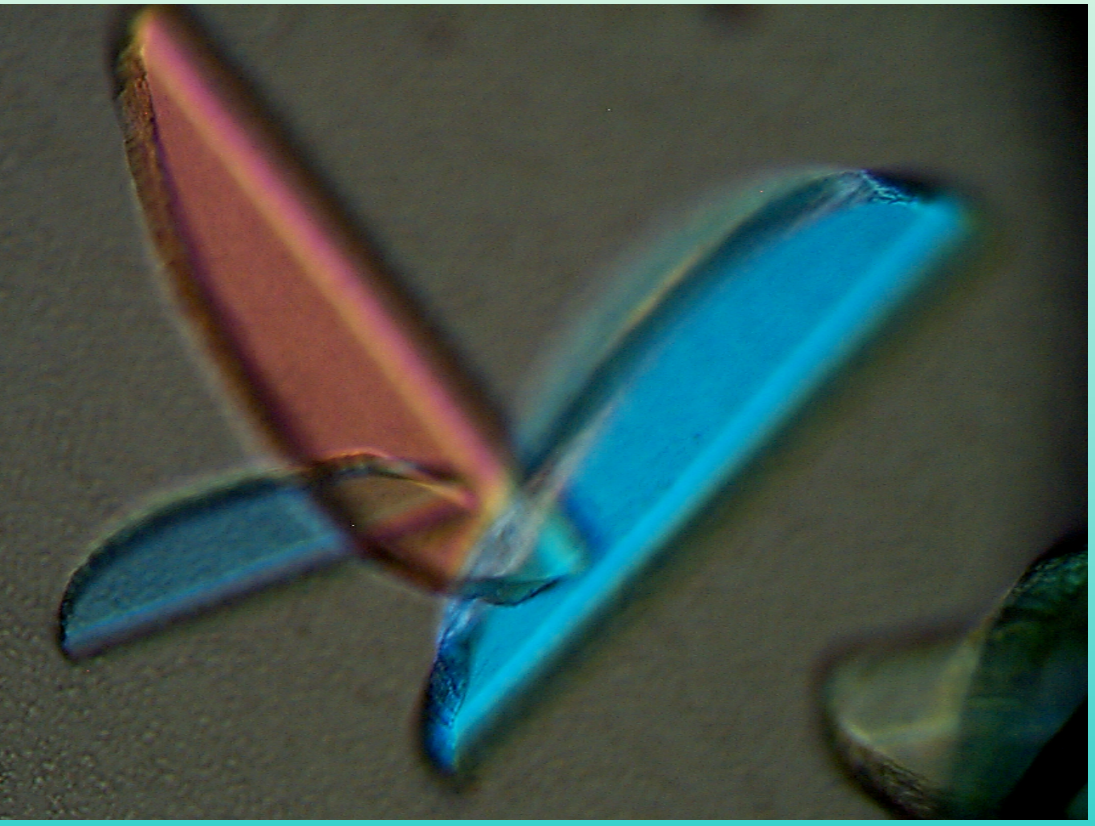

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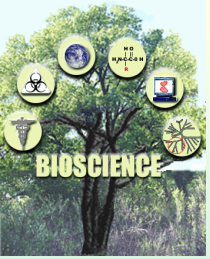

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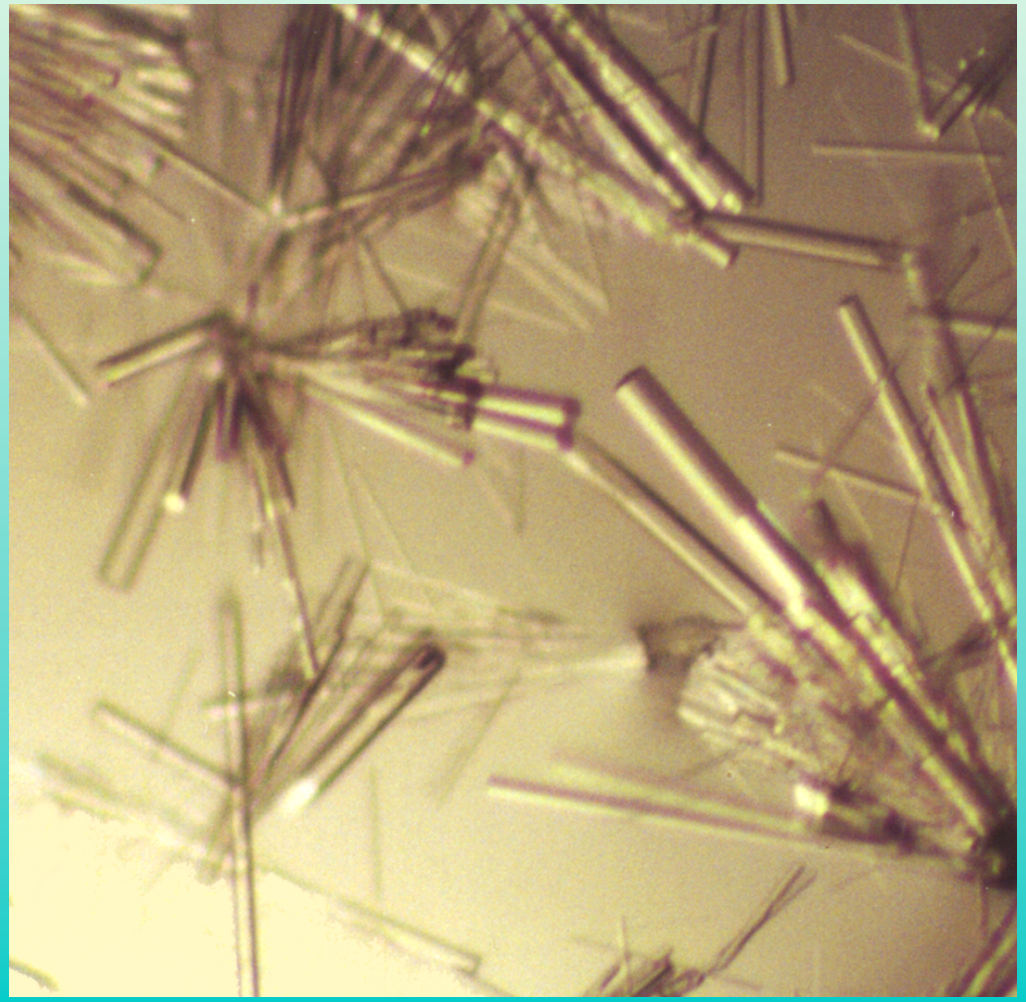

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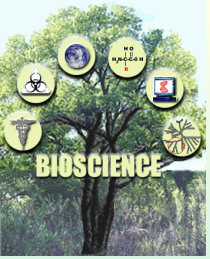

A brief introduction to X-ray crystallography

1.5 mm

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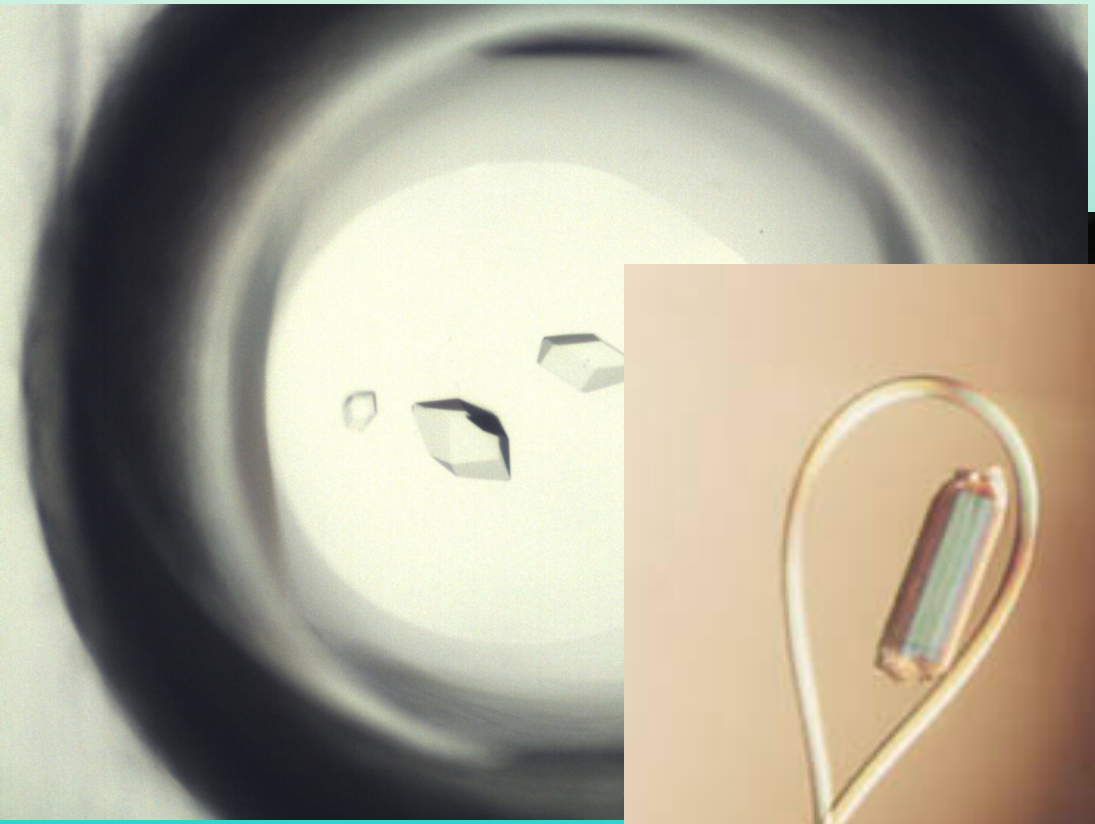
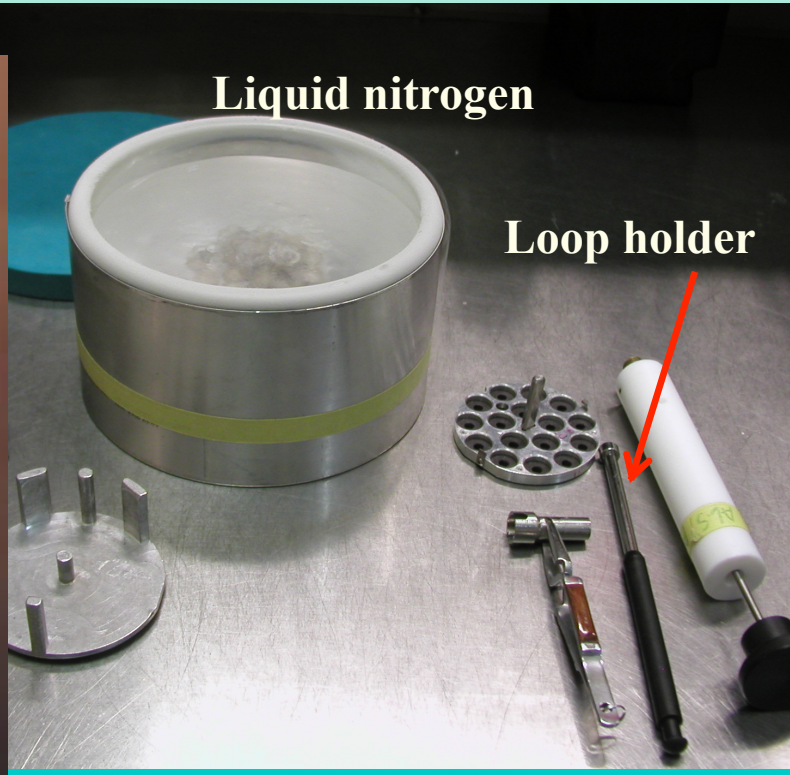
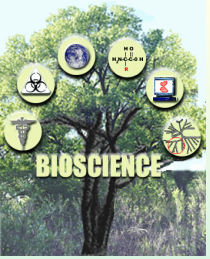
Mounting crystals in nylon loops and cryo-cooling them





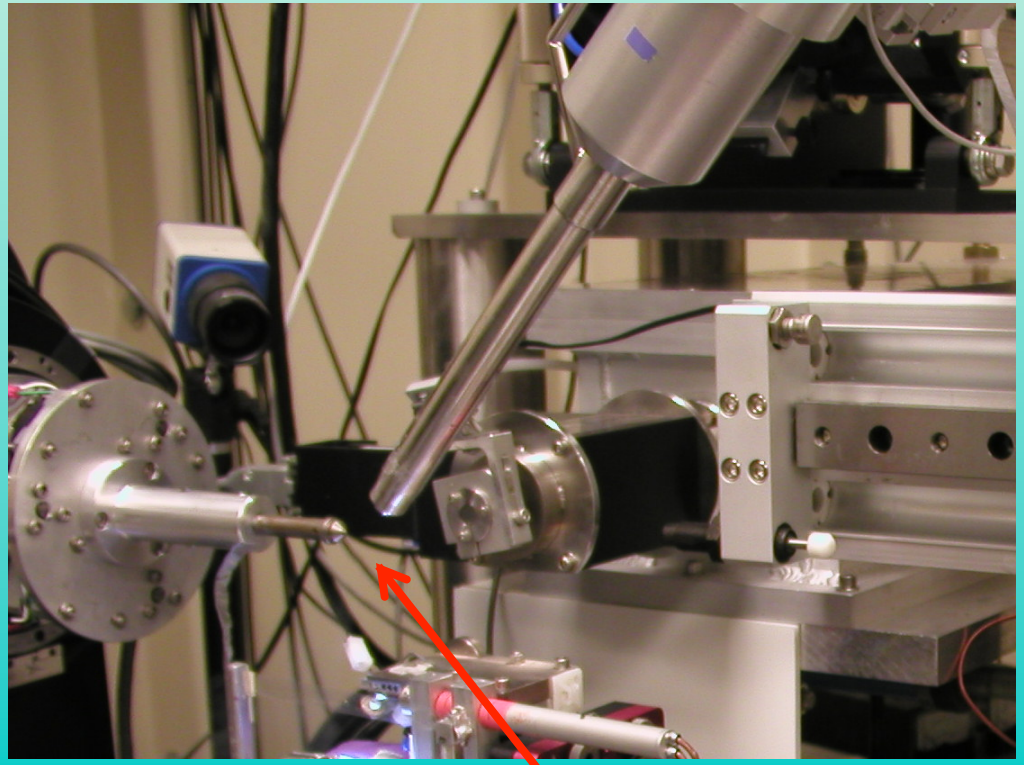
Los Alamos
Bioscience Division

Innovation for Health and Security



Advanced Light Source, Berkeley, CA

A brief introduction to X-ray crystallography



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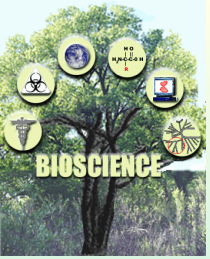

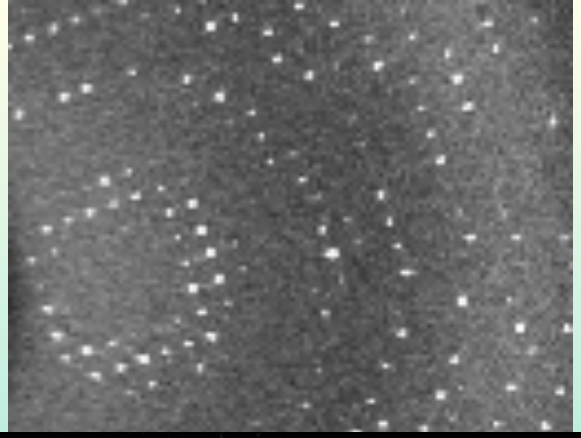

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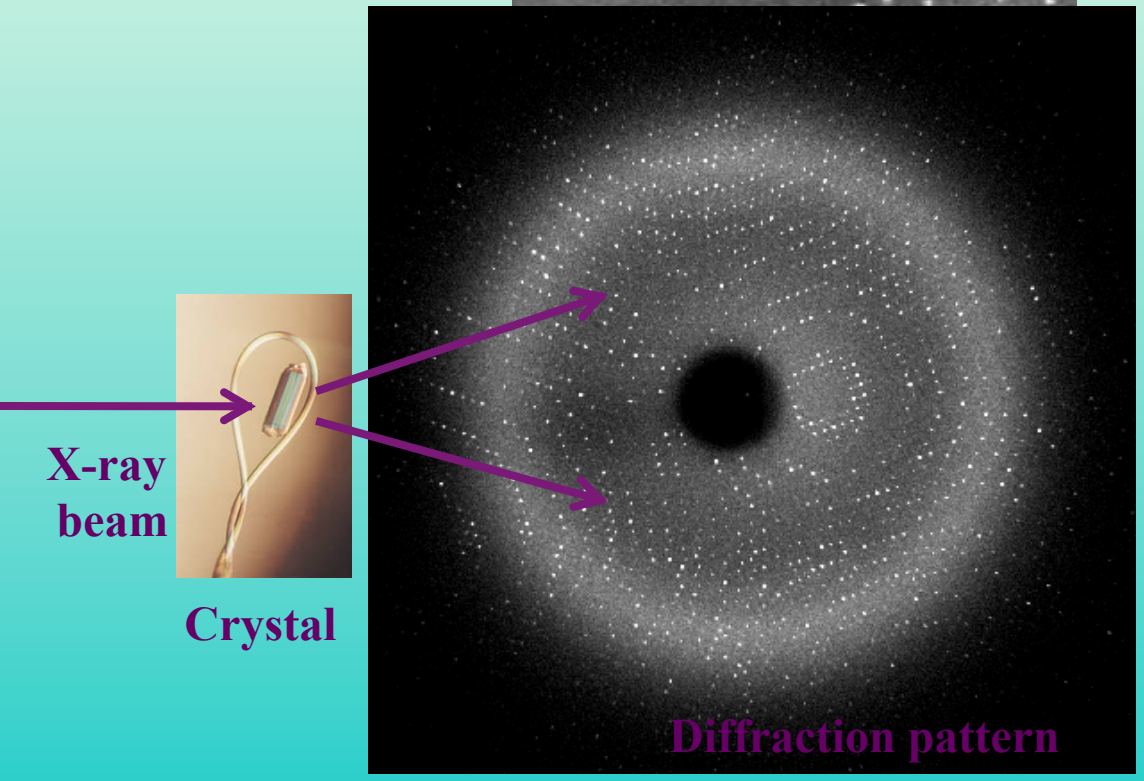

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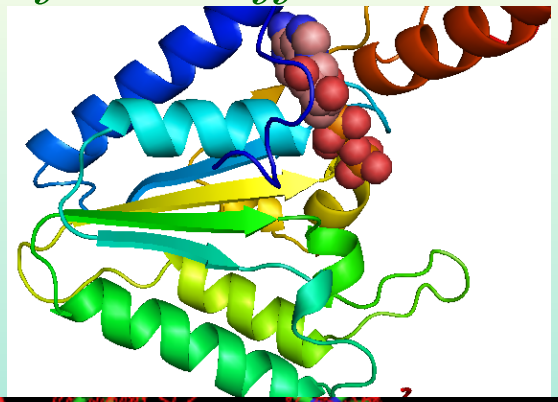
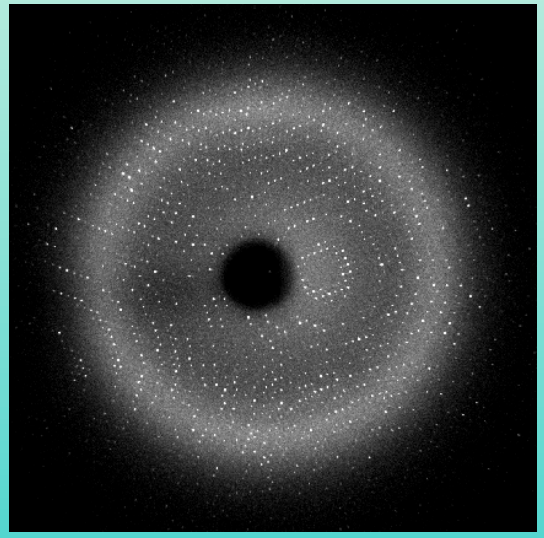
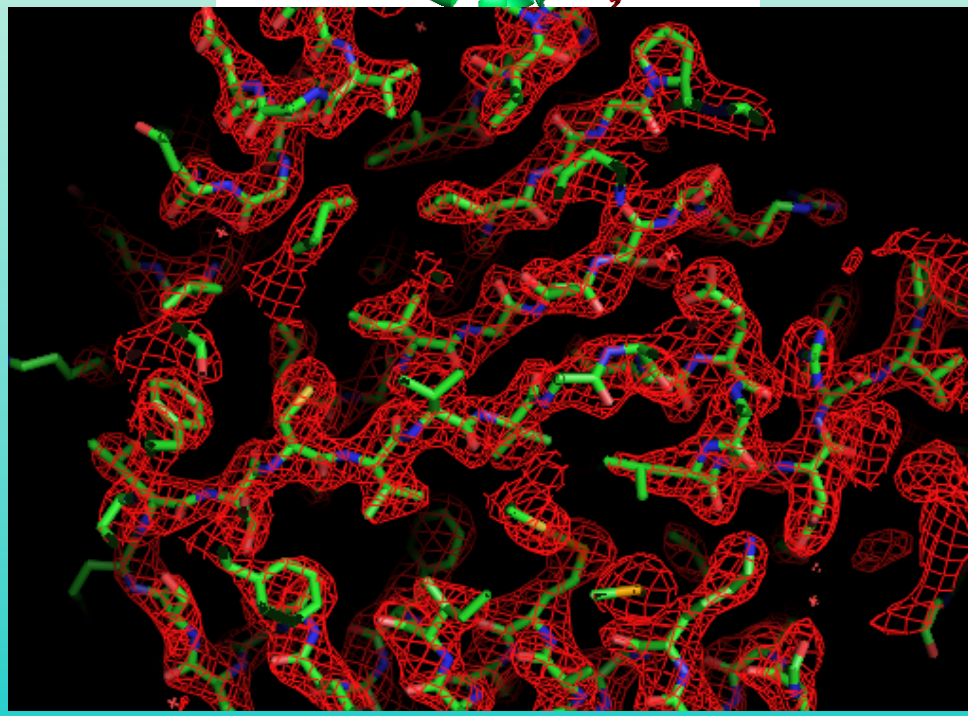




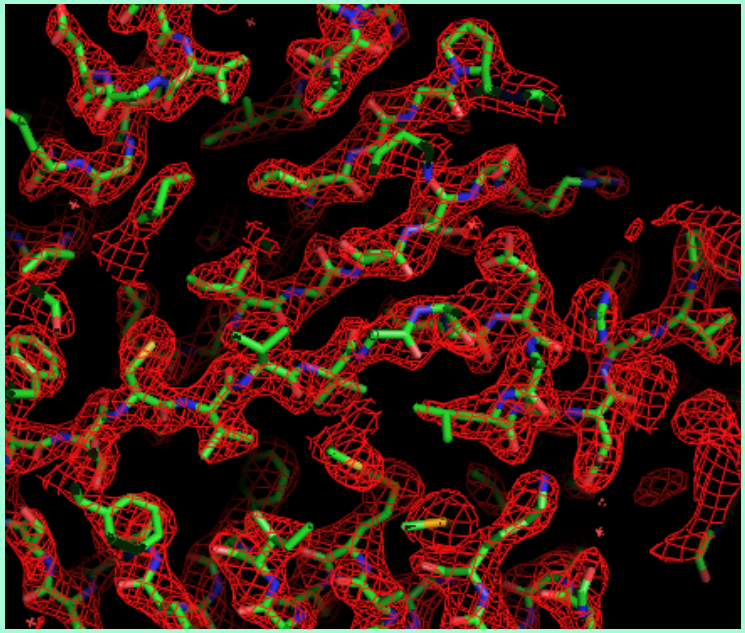
Diffraction pattern

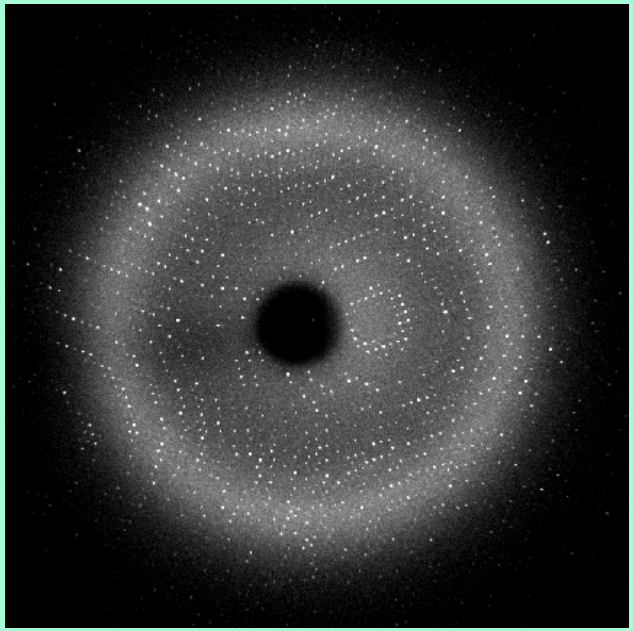
Analysis of diffraction spots

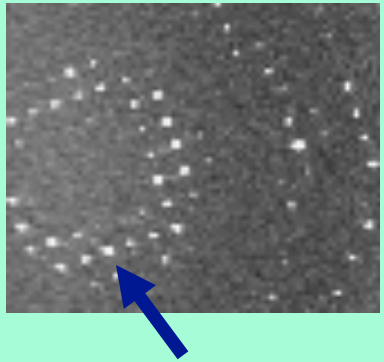


Picture and model of macromolecule

The intensities of X-ray diffraction spots depend on what is in the crystal







I_h

Electrons in a protein crystal (ρ is high where there are many electrons)

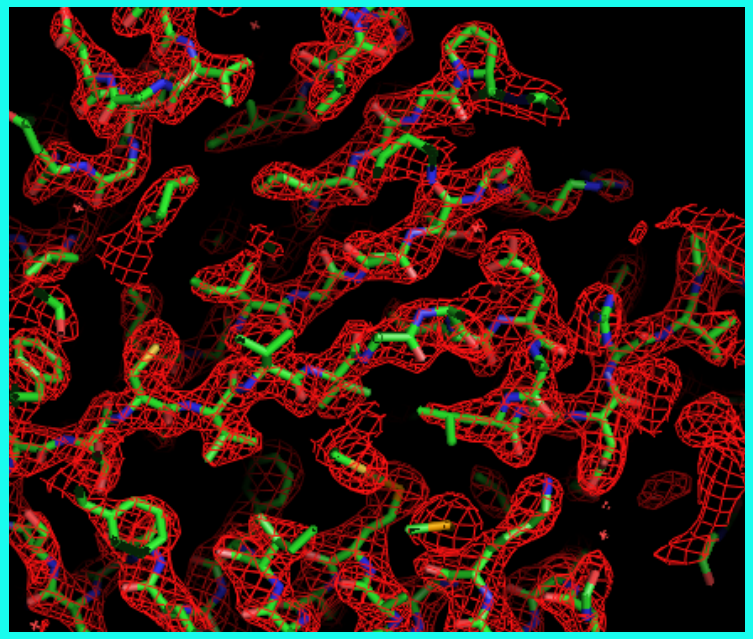
Diffraction pattern (I_h is intensity of spot “h”)

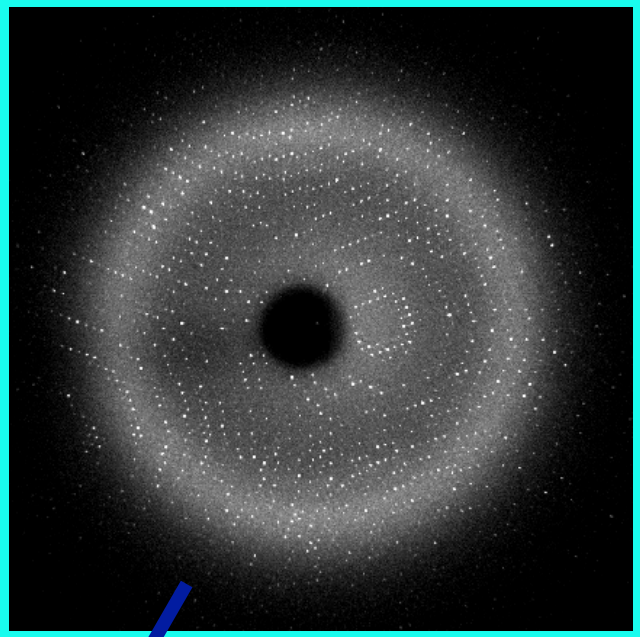
$$\rho(x) = \sum_h F_h e^{i\phi_h} e^{-2\pi i h x}$$

$$F_h e^{i\phi_h} = \int \rho(x) e^{2\pi i h x} dx$$

$$I_h = F_h^2$$

We can almost calculate a picture of where the atoms are from the diffraction pattern (but are missing the phases of diffraction spots)





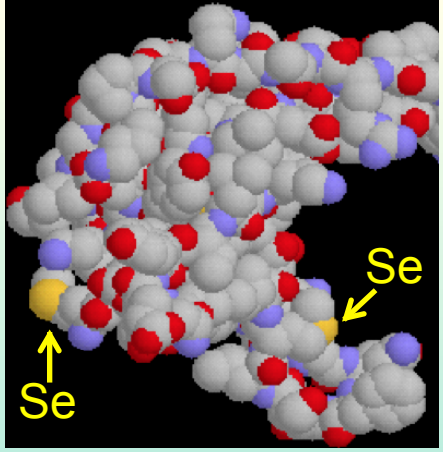
$\rho(x)$

(Where the atoms are)

F_h is square root of measured intensity of spot *h*

$$\rho(x) = \sum_h F_h e^{i\phi_h} e^{-2\pi i h x}$$

We do not know the phase (ϕ_h)



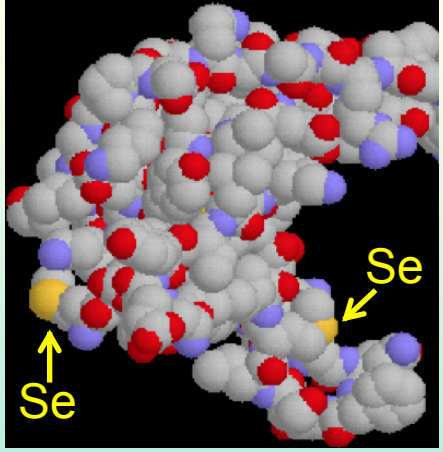
Estimating crystallographic phases: example with multiwavelength X-ray data

- Measure diffraction (I_h , I'_h) at two X-ray wavelengths near absorption edge of selenium

- Differences in diffraction are due to changes in scattering from the Se atoms (ΔF_h)

First figure out where the Se atoms are located

Then use the Se atoms and the diffraction intensities to draw a picture of all the atoms

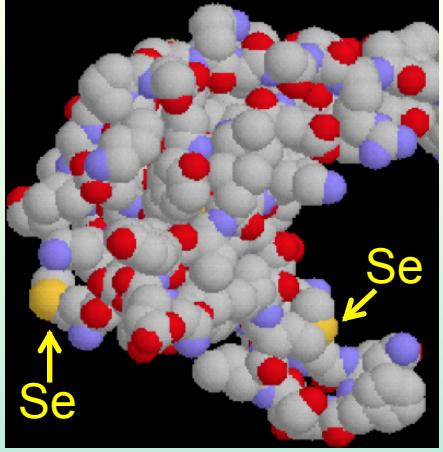


Estimating crystallographic phases with multiwavelength X-ray data

- Measure diffraction (I_h , I'_h) at two X-ray wavelengths near absorption edge of selenium

- Differences in diffraction are due to changes in scattering from the Se atoms ($\Delta \mathbf{F_h}$)

Wavelength	*Scattering density*	*Structure Factor*	*Intensity of diffraction spot*
λ_1	$\rho(x)$	$\mathbf{F_h} = F_h e^{i\phi_h} = \int \rho(x) e^{2\pi i h x} dx$	$I_h = \lvert \mathbf{F_h} \rvert^2$



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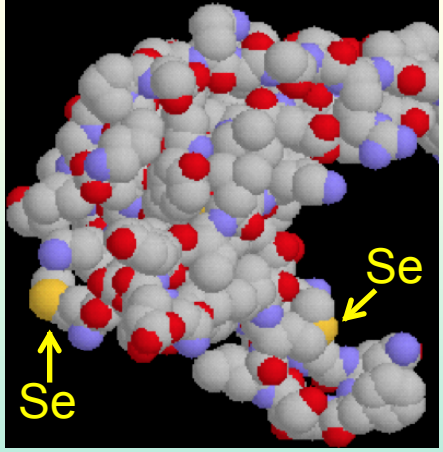
λ_2	$\rho'(x) = \rho(x) + \Delta\rho(x)$	$\mathbf{F'_h} = \mathbf{F_h} + \Delta\mathbf{F_h}$	$I'_h = \lvert\mathbf{F_h} + \Delta\mathbf{F_h}\rvert^2$



Estimating crystallographic phases with multiwavelength X-ray data

•Measure diffraction (I_h , I'_h) at two X-ray wavelengths near absorption edge of selenium

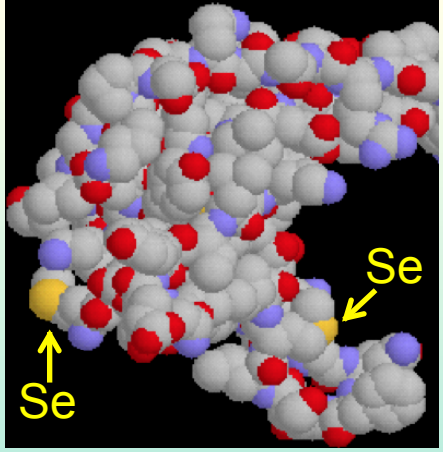
•Differences in diffraction are due to changes in scattering from the Se atoms ($\Delta\mathbf{F_h}$)

How to figure out where the Se atoms are:

Assume that structure factors for Se are similar to changes between wavelengths:

$$|\Delta\mathbf{F_h}| \approx |F'_h - F_h|$$

Then use techniques from small-molecule crystallography to find the Se atoms (guess locations, compare calculated and observed $\Delta\mathbf{F_h}$, update guess)



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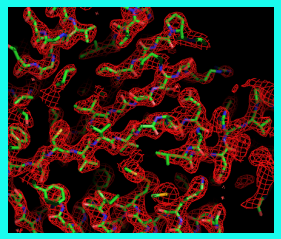
If we know where the Se atoms are ...

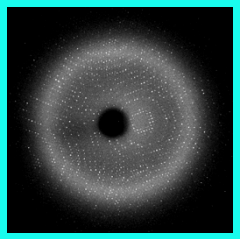
we know: $\Delta\rho(x)$

...so we can calculate: $\Delta\mathbf{F_h}$

and the phase (ϕ_h) must satisfy: $I'_h = \lvert I_h^{1/2} e^{i\phi_h} + \Delta\mathbf{F_h}\rvert^2$

Many ways to find the phases





Method	Source of phasing information
SIR – single isomorphous replacement	A few heavy atoms (e.g., Hg, Au) in "derivative" contribute to differences from "native"
SAD – single-wavelength anomalous diffraction	A few atoms (e.g., Se, I, Hg atoms) contribute to "anomalous" differences in diffraction between spot *h* and spot *-h*
MAD – multiple-wavelength anomalous diffraction	A few atoms contribute to anomalous and wavelength-dependent "dispersive" differences
SIRAS, MIR	Combinations of SIR and SAD
Molecular replacement	Molecular location and phases are found using a related molecule as a template
Direct methods	Guess where atoms are, good guesses match the measured structure factors

The PHENIX Project

Lawrence Berkeley Laboratory

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Los Alamos National Laboratory

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Cambridge University

Randy Read, Airlie McCoy, Gabor Bunkoczi, Rob Oeffner

Duke University

Jane & David Richardson, Vincent Chen, Chris Williams, Bryan Arendall, Laura Murray

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