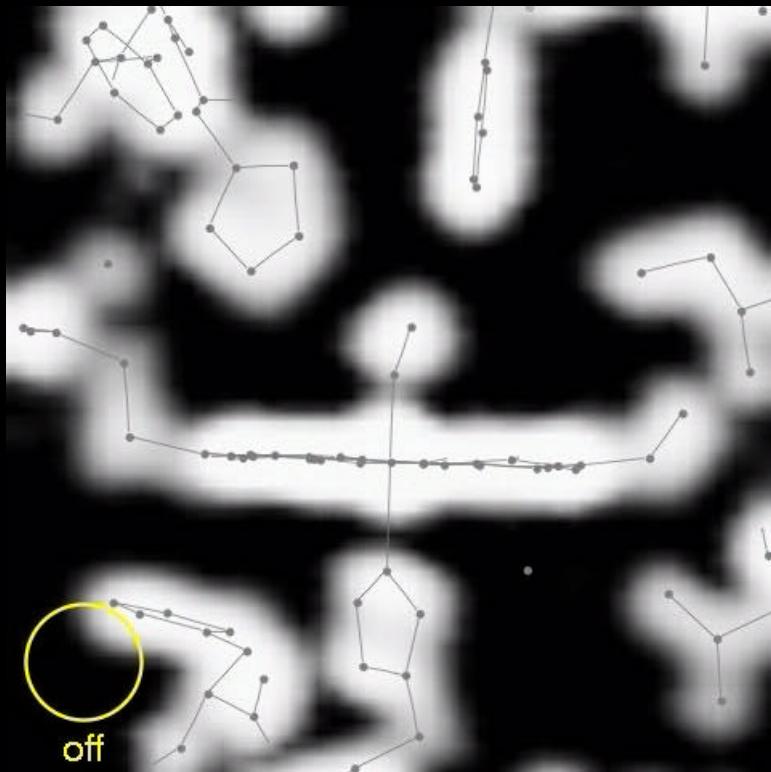


Watching proteins function with picosecond time-resolved X-ray crystallography



Philip Anfinrud

Laboratory of Chemical Physics, NIDDK
National Institutes of Health, Bethesda, MD USA

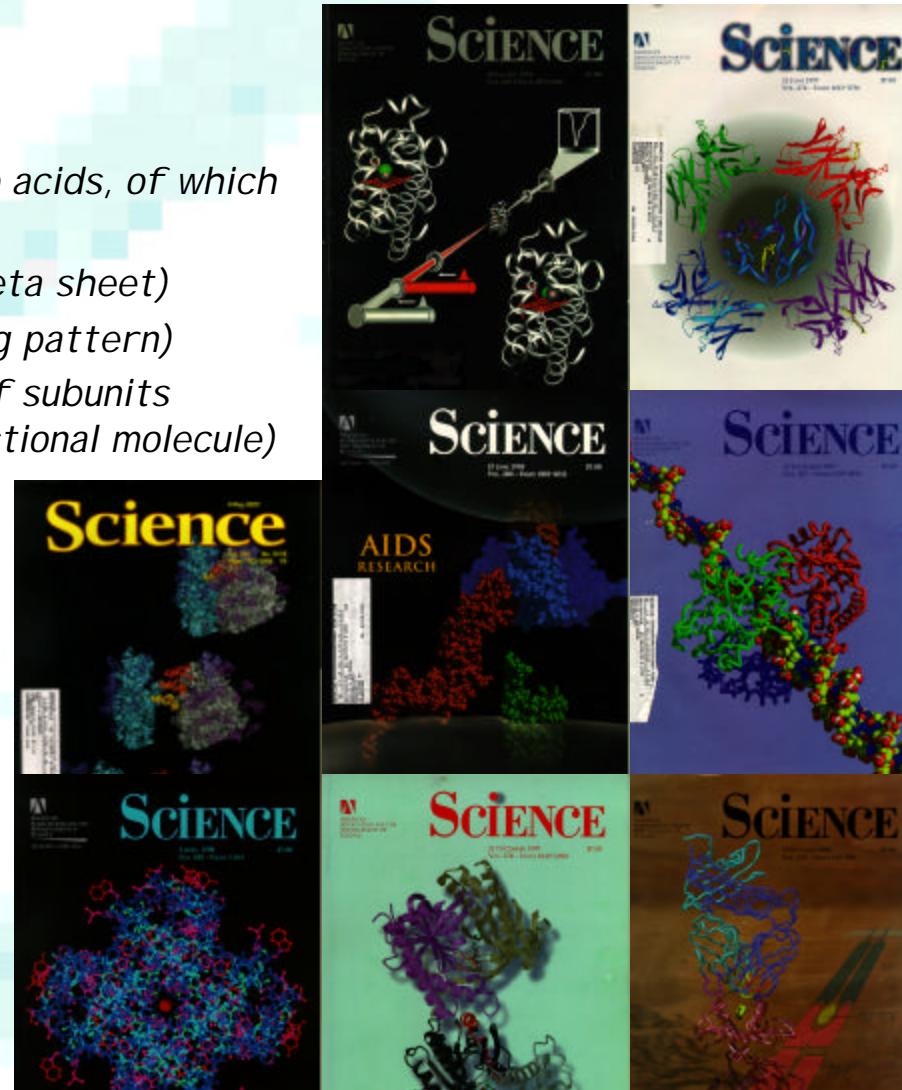


Lake Geneva , August 2004



Proteins: Molecules of “Life”

- **Highly organized structure**
 - primary (sequence of amino acids, of which there are 20)
 - secondary (alpha helical; beta sheet)
 - tertiary (unique 3-D folding pattern)
 - quaternary (spatial array of subunits assembled into larger, functional molecule)
- **Physico-chemical function**
 - macro structures
 - harvest light energy
 - enzymatic catalysis
 - biomechanics
 - ligand transport
 - etc., etc.
- **Functional characteristics**
 - high selectivity
 - high efficiency



Myoglobin

distal histidine His-64

proximal histidine His-93

CO

heme

$C_{823} H_{1045} Fe N_{222} O_{221} S_3$

2305 atoms, 153 amino acids

Heme group

Science Times
The New York Times
TUESDAY, NOVEMBER 11, 2003

Deep Underwater, the Breath of Life
Researchers Observe Diving Mammals for Clues to Improving Human Health

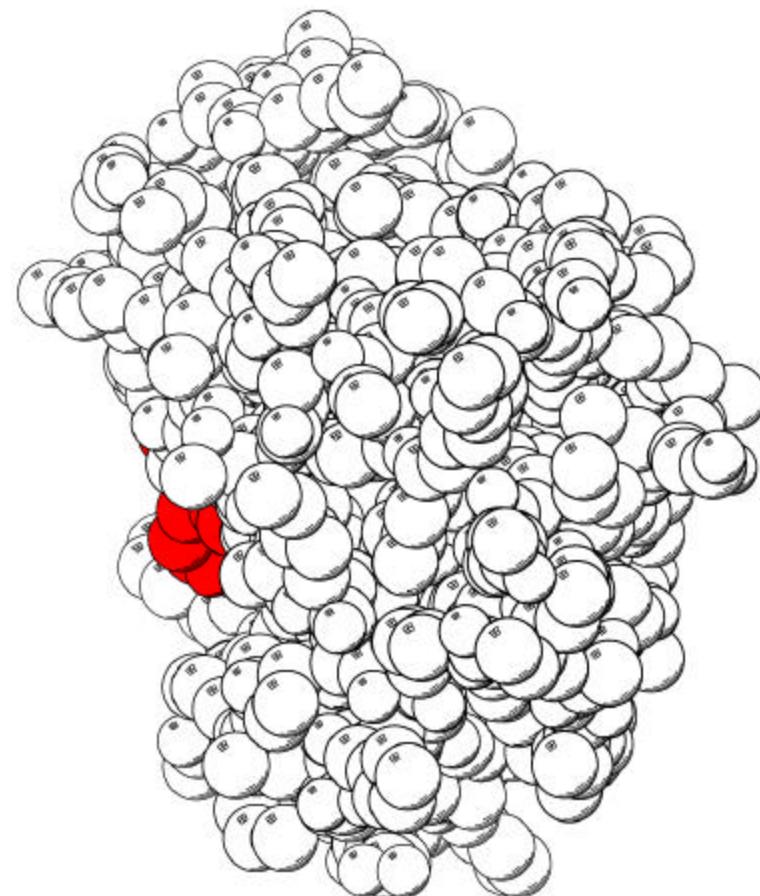
What Seals Can Teach Divers and Doctors

Undiscovered Bach? No, a Computer Wrote It

To People the World, Start With 500



Space-Filling Model of Myoglobin

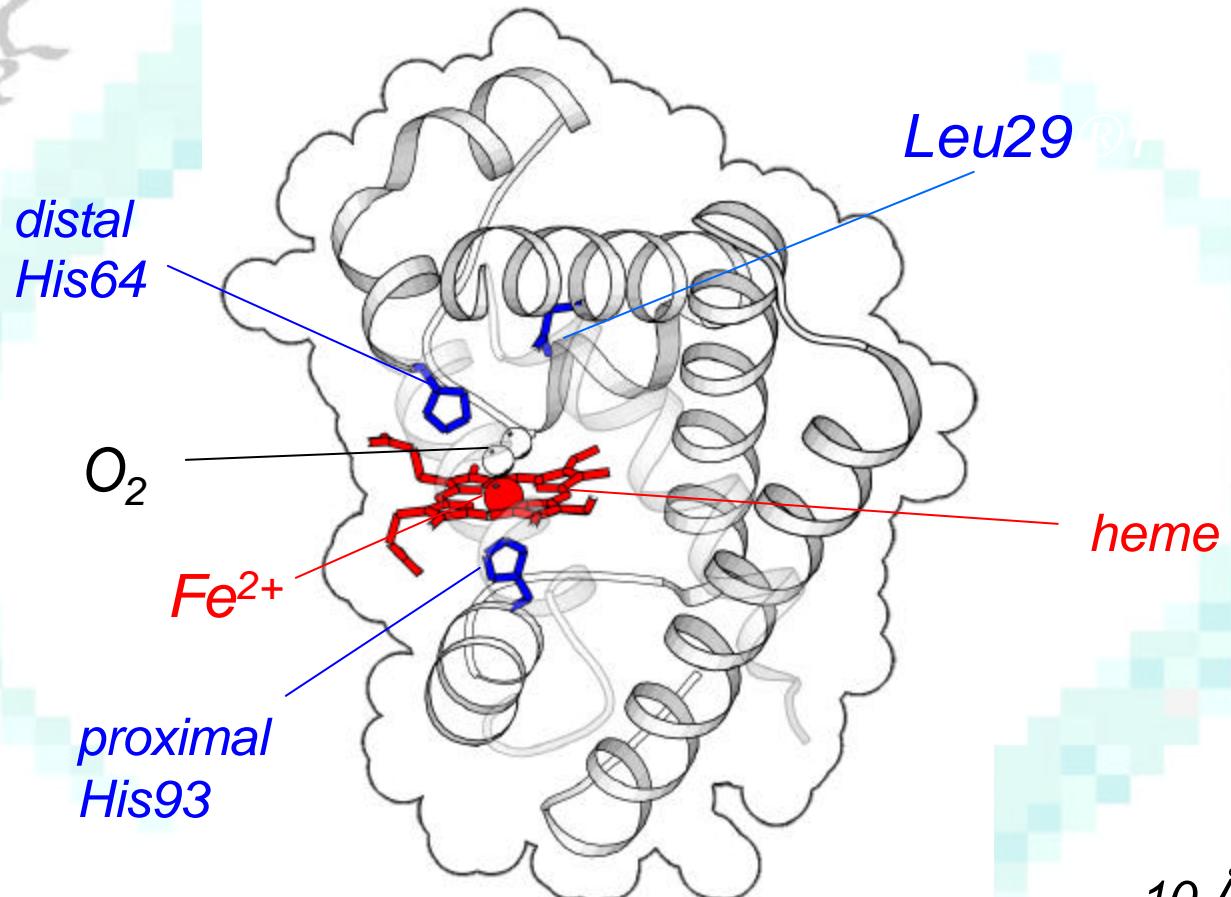


10 Å

C₈₂₃ H₁₀₄₅ Fe N₂₂₂ O₂₂₁ S₃, 2305 atoms, 153 amino acids



Wild-type Myoglobin



10 Å

$C_{823} H_{1045} Fe N_{222} O_{221} S_3$, 2305 atoms, 153 amino acids



L29F Myoglobin

*distal
His64*

O₂

Fe²⁺

*proximal
His93*

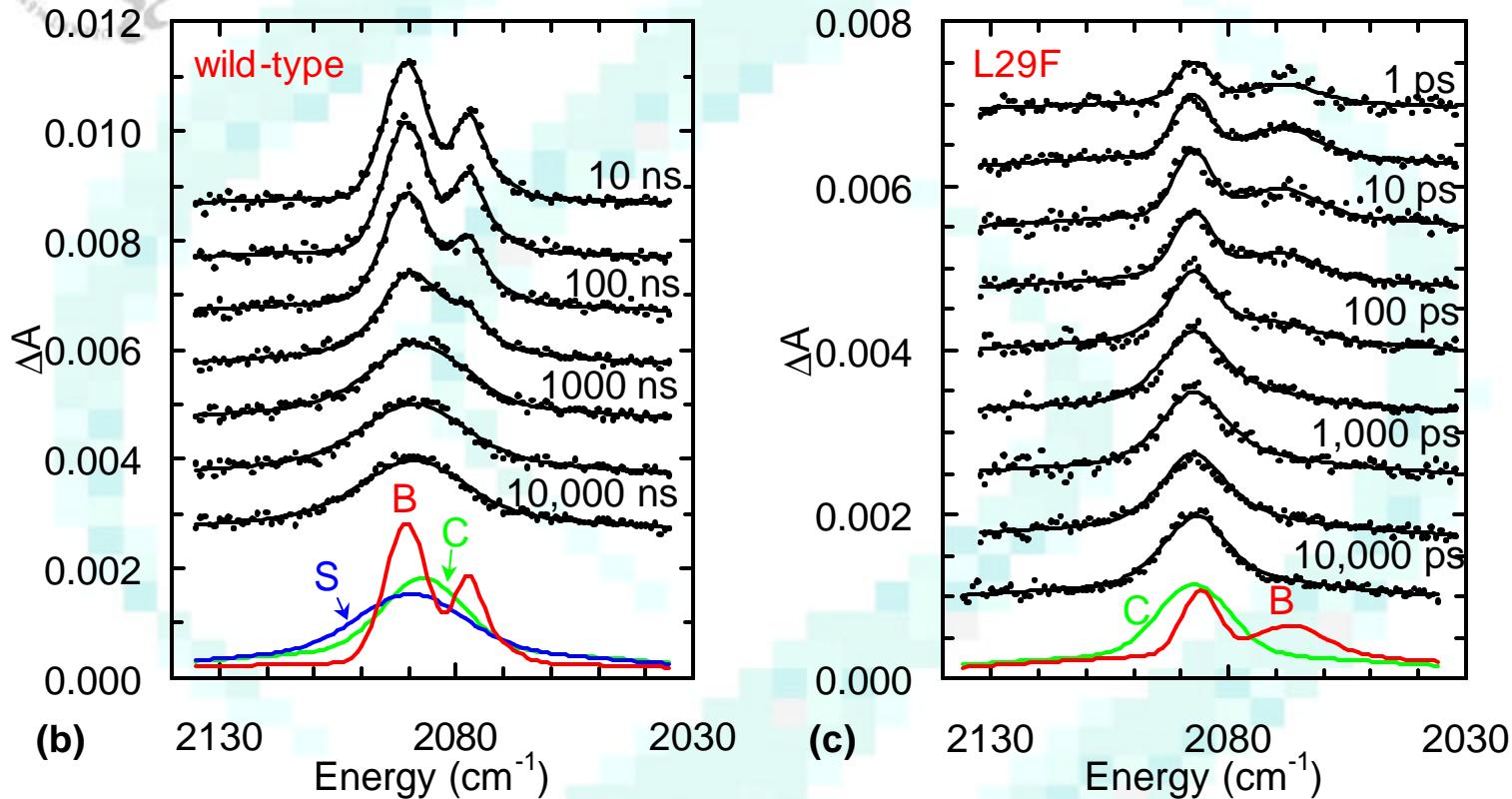
Leu29®Phe

heme

10 Å

C₈₂₃ H₁₀₄₅ Fe N₂₂₂ O₂₂₁ S₃, 2305 atoms, 153 amino acids

Ligand Escape Dynamics for **WT** and **L29F Mb** (in D₂O at ~ 10 °C)
(Time-resolved Mid-IR spectra of Photolyzed WT- and L29F- MbCO)



- Mutants generously provided by Prof. John Olson, Rice University
- From Fig. 3 in Schotte et. al., Science **300**, 1944 (2003)



European Synchrotron and Radiation Facility, Grenoble, FRANCE

ESRF



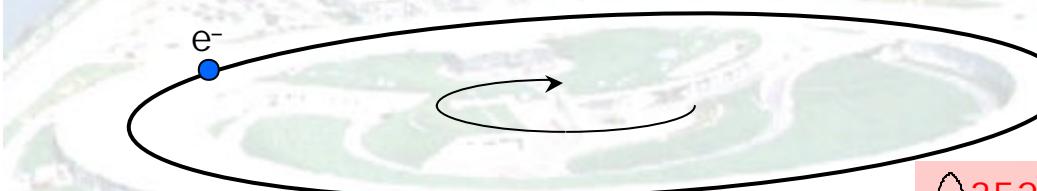
- Time-Resolved X-ray (TReX) Studies



Source of Picosecond X-ray Radiation: Synchrotron

Synchrotron energy: 6 GeV
e⁻ rest energy ($m_e c^2$): 0.511 MeV

$$g = \frac{6 \text{ GeV}}{0.511 \text{ MeV}} = \frac{1}{\sqrt{1-u^2/c^2}}$$
$$\Rightarrow u = 0.999999995 \text{ } c$$



844 m circumference:

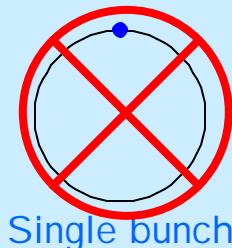
$$\ell/c = 2.82 \mu\text{s}$$

RF at 992 harmonic (ESRF):

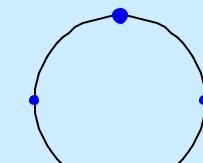
$$992/2.82 \mu\text{s} = 352 \text{ MHz}$$

Storage ring half life
(~ 2×10^{10} e⁻/mA):

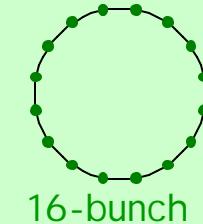
- 16 mA in single bunch: ~6 hours; ~175 ps
- 40 mA in 4-bunch: ~6 hours; ~150 ps
- 90 mA in 16-bunch: ~10 hours; ~115 ps
- 200 mA in 1/3-filling: ~40 hours; ~50 ps
7 mA isolated pulse



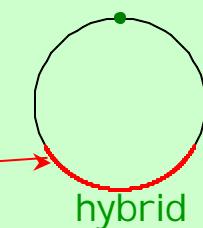
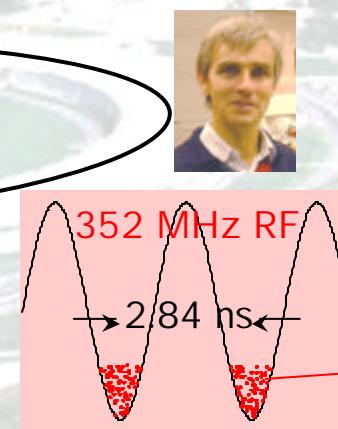
Single bunch



4-bunch

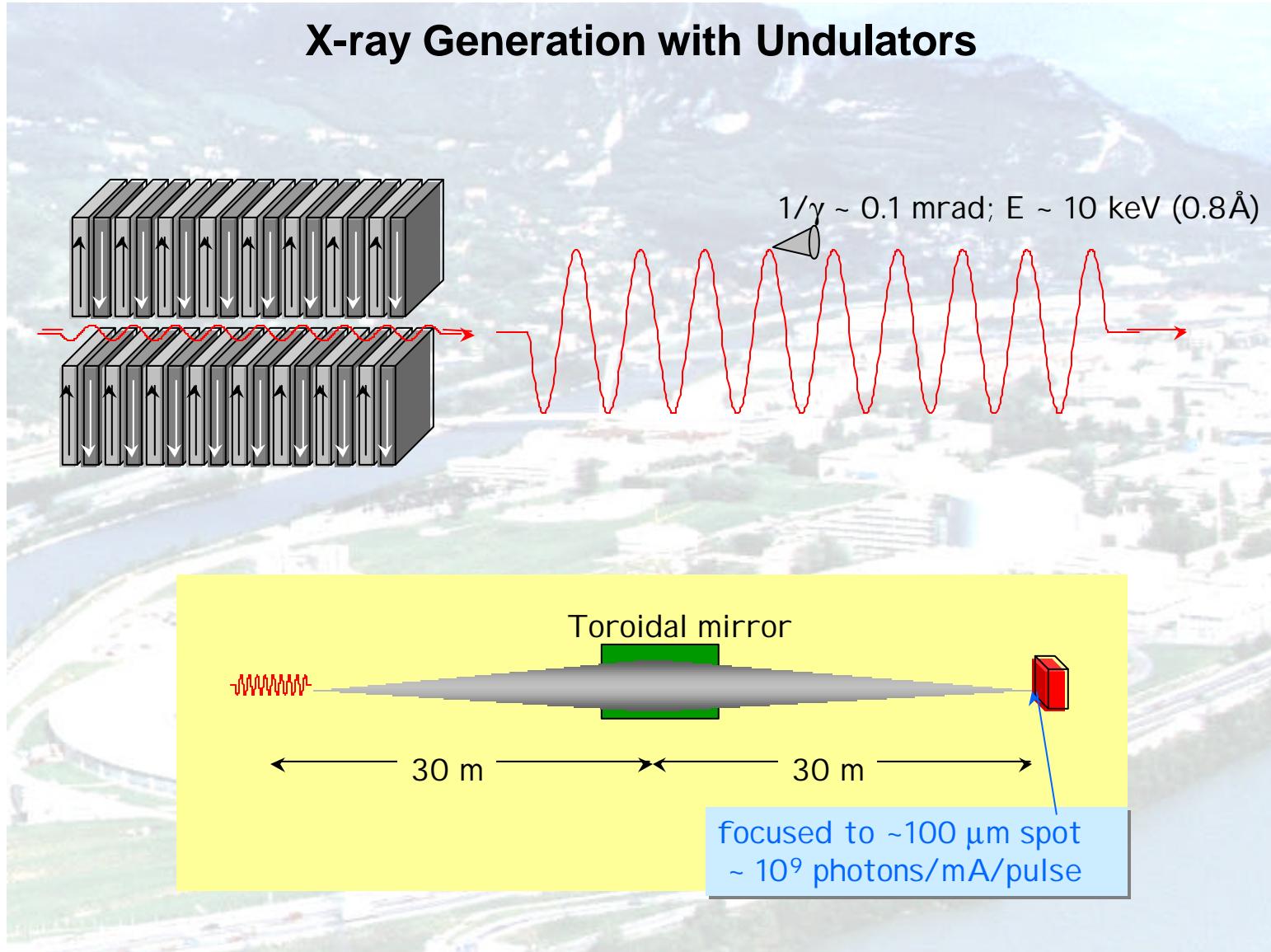


16-bunch

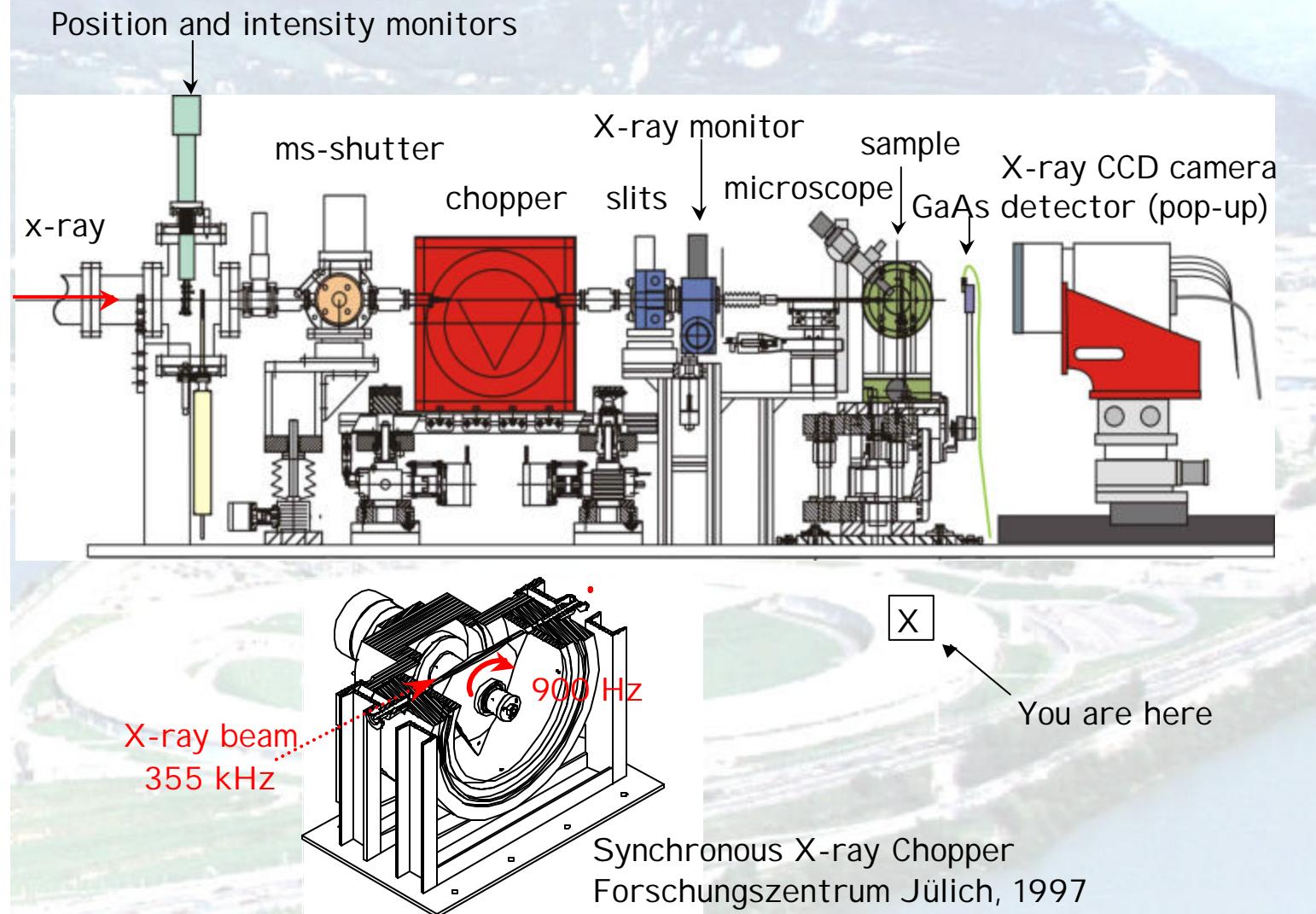


hybrid

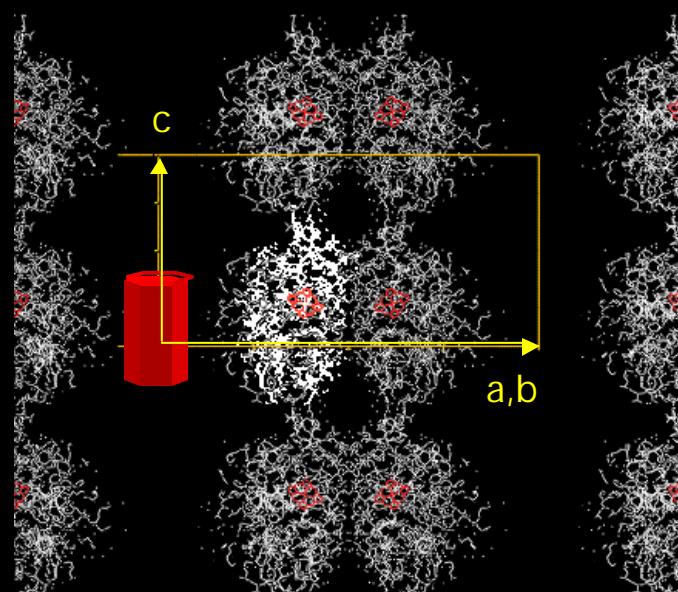
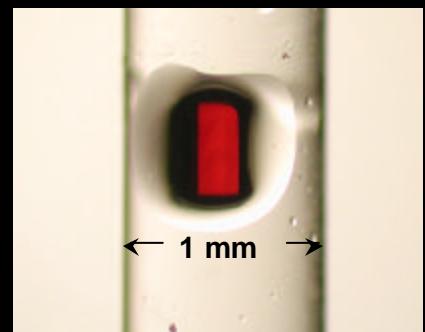
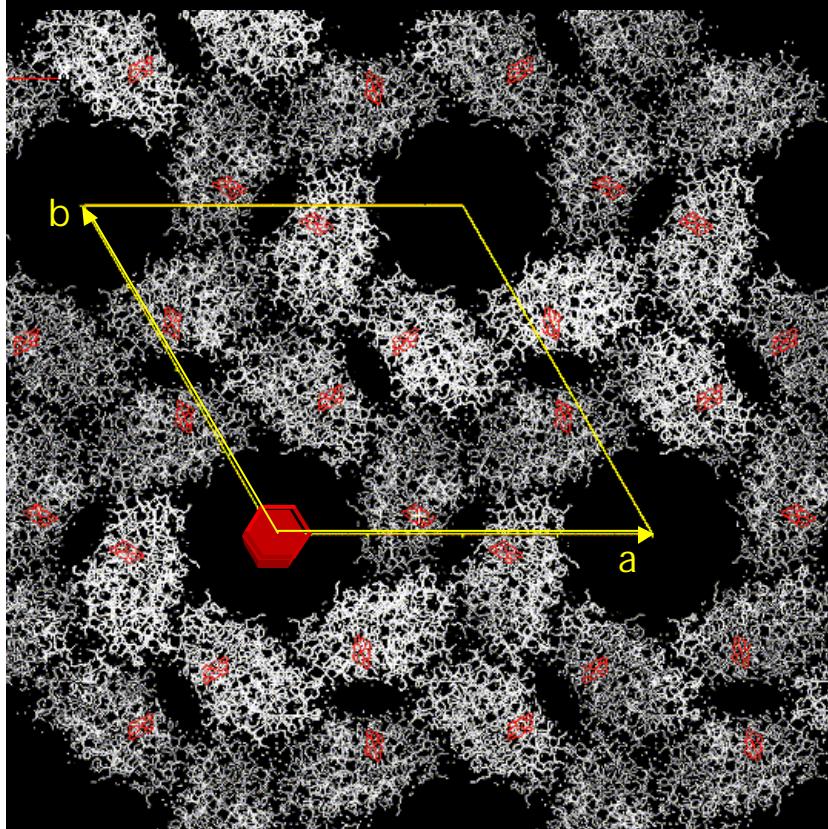
X-ray Generation with Undulators



X-ray Diffractometer at ID9 End Station (ESRF)

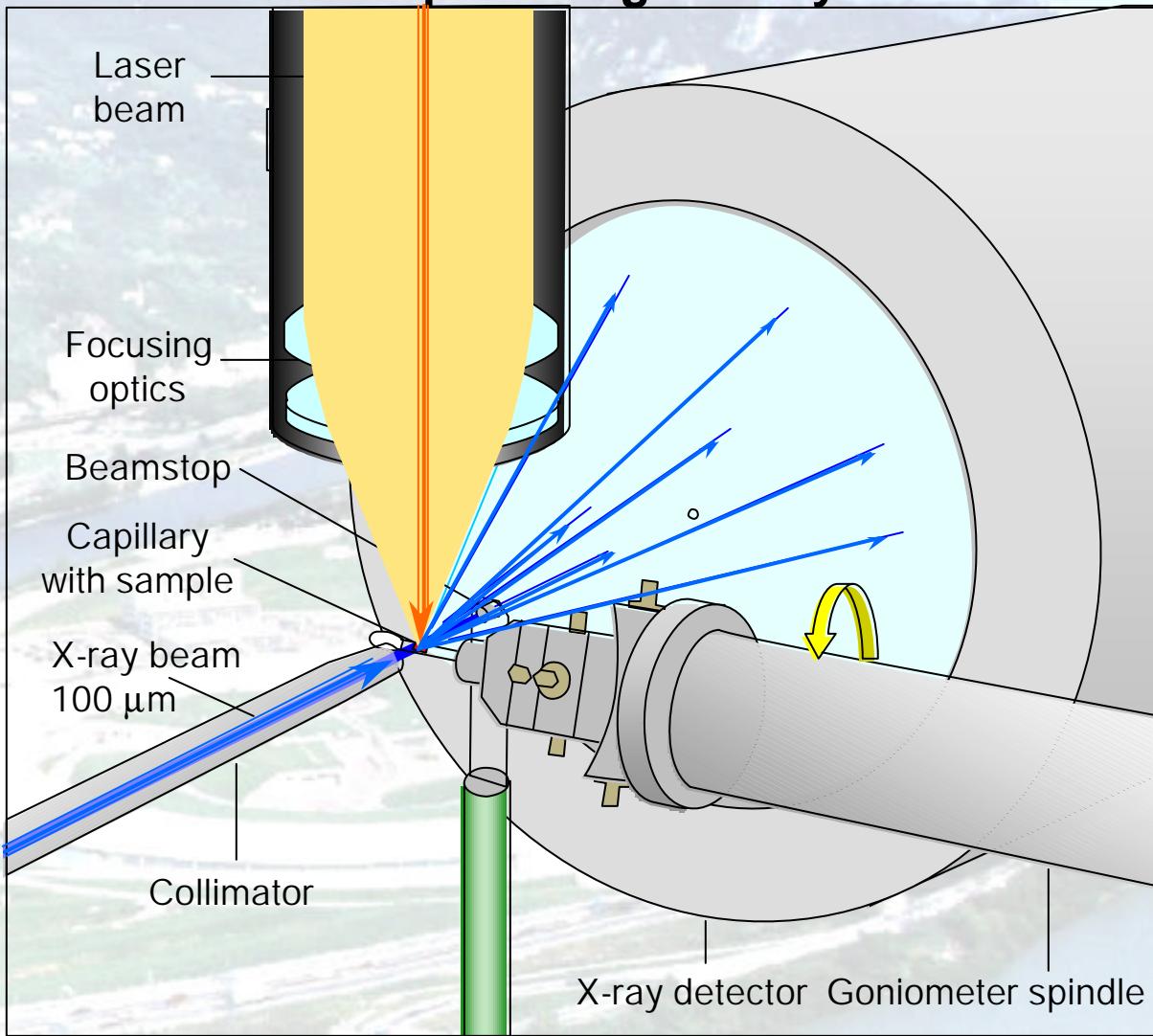


Packing of P6 Myoglobin Crystal



a = b = 91.20 Å, c = 45.87 Å, $\alpha = \beta = 90^\circ, \gamma = 120^\circ$; heme plane to a,b plane $\delta = 55^\circ$

Pump-Probe geometry



Laue diffraction pattern from L29F MbCO 100 ps after photolysis

32 x-ray shots at 3.3 Hz
(April 14, 2002)

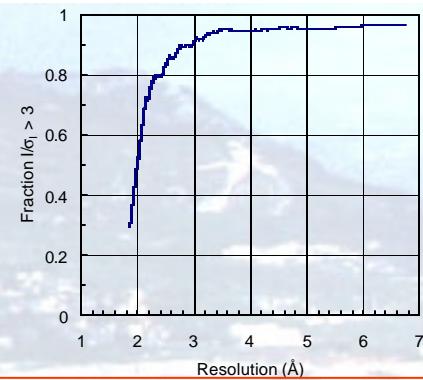
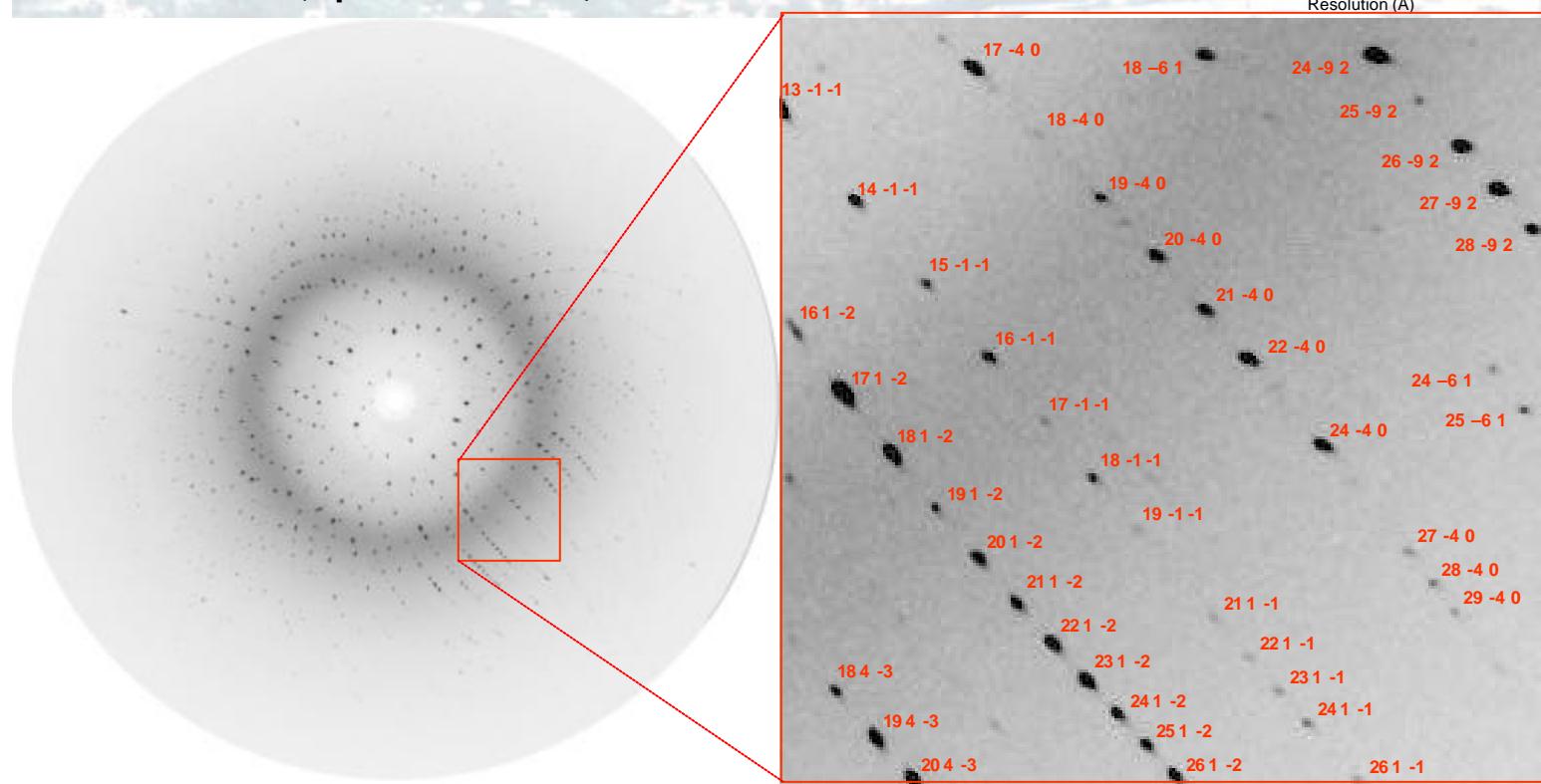
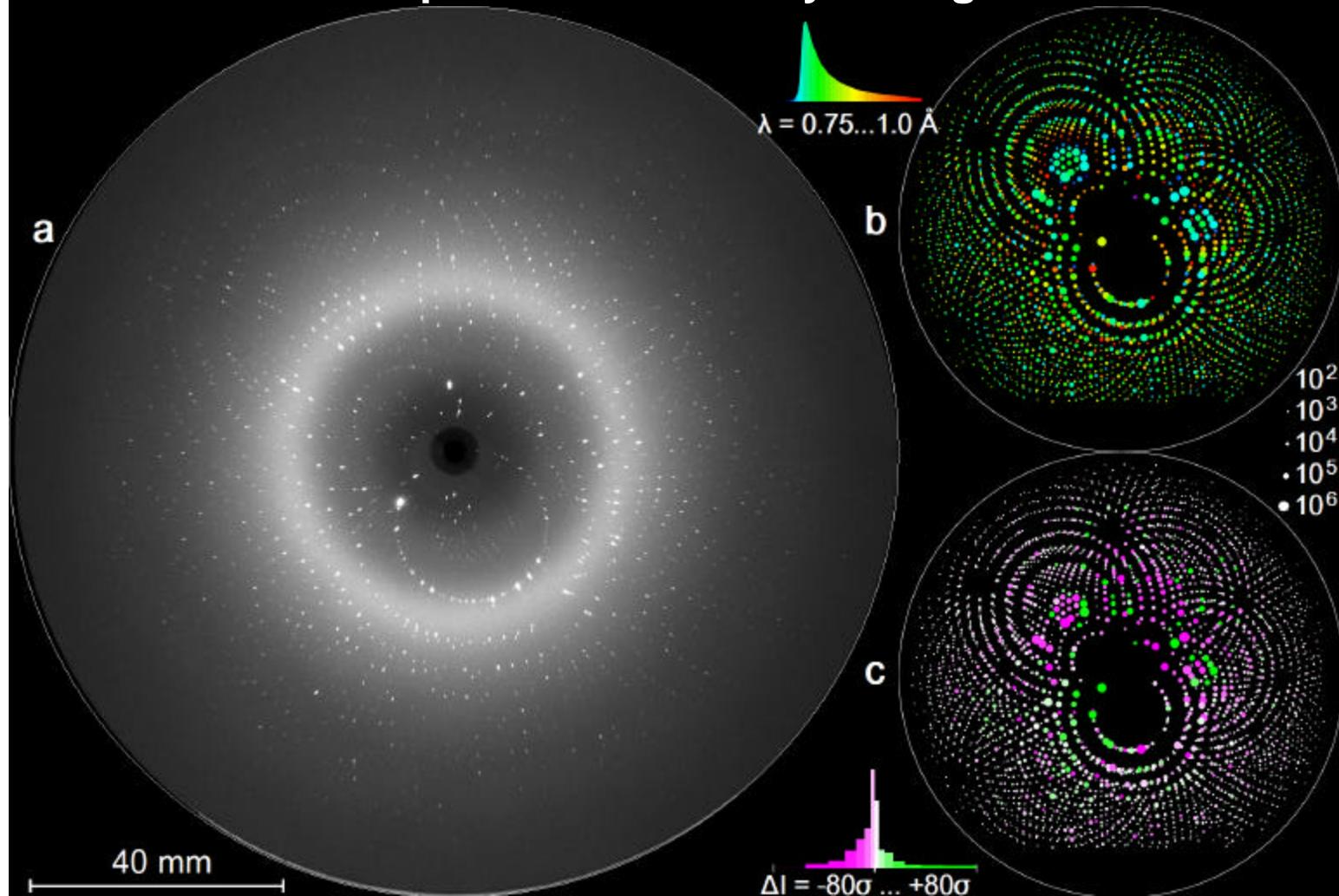
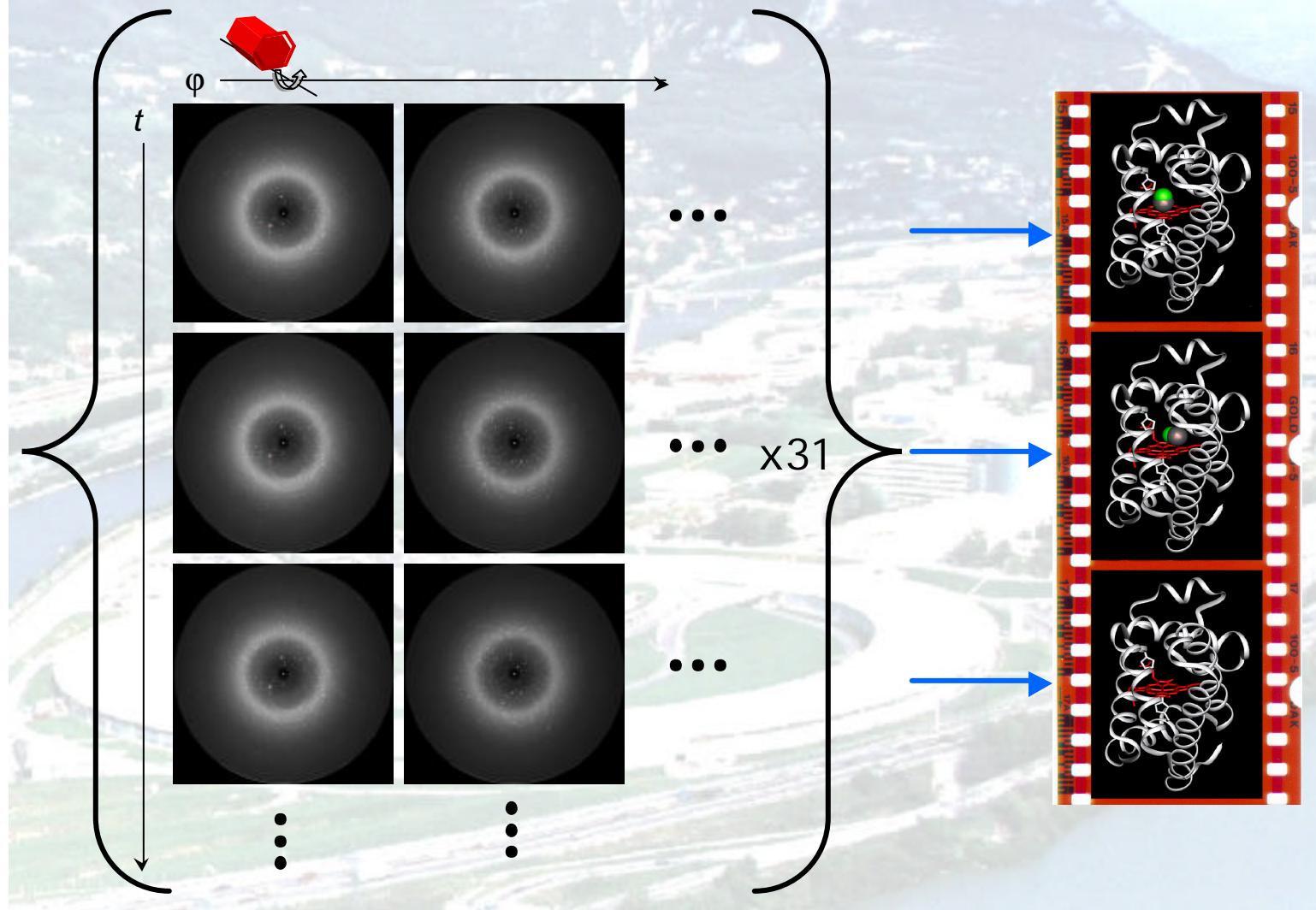


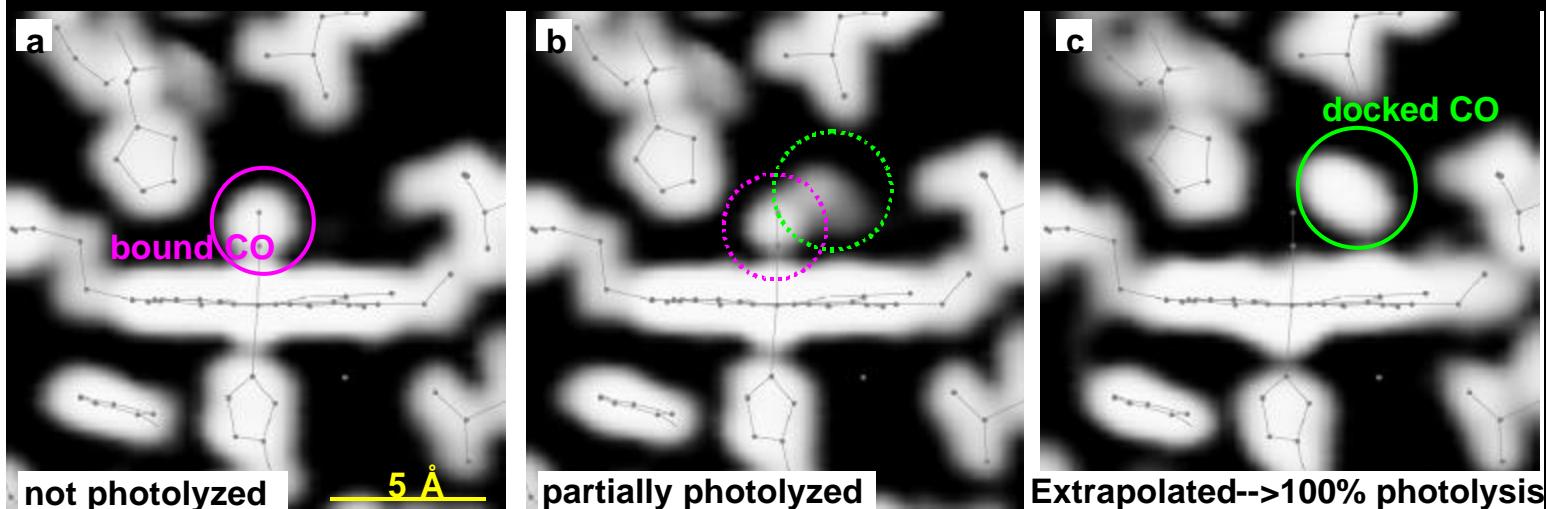
Image Analysis: X-ray Wavelength and Pump-induced Intensity Changes



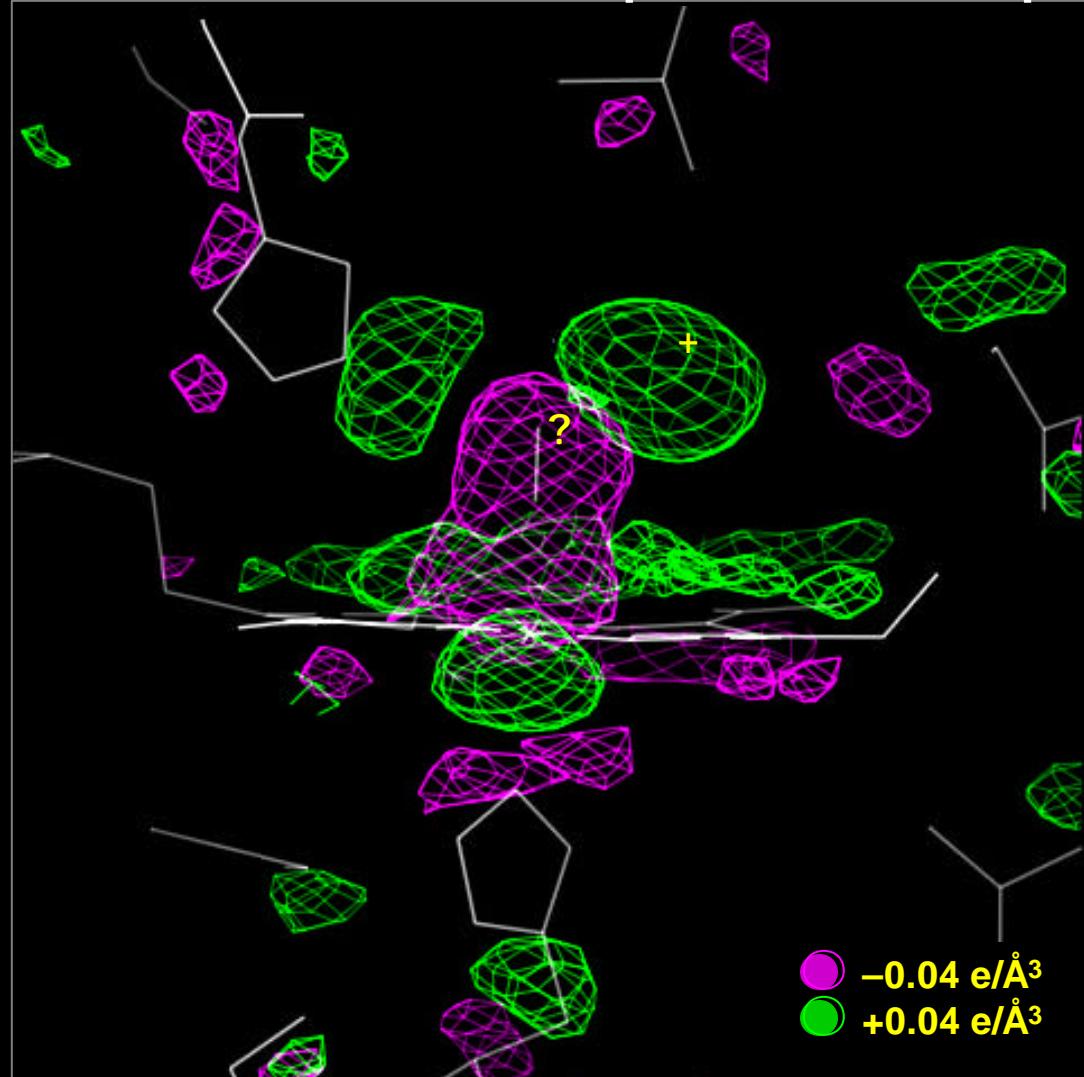
Generate “film strip” from diffraction data



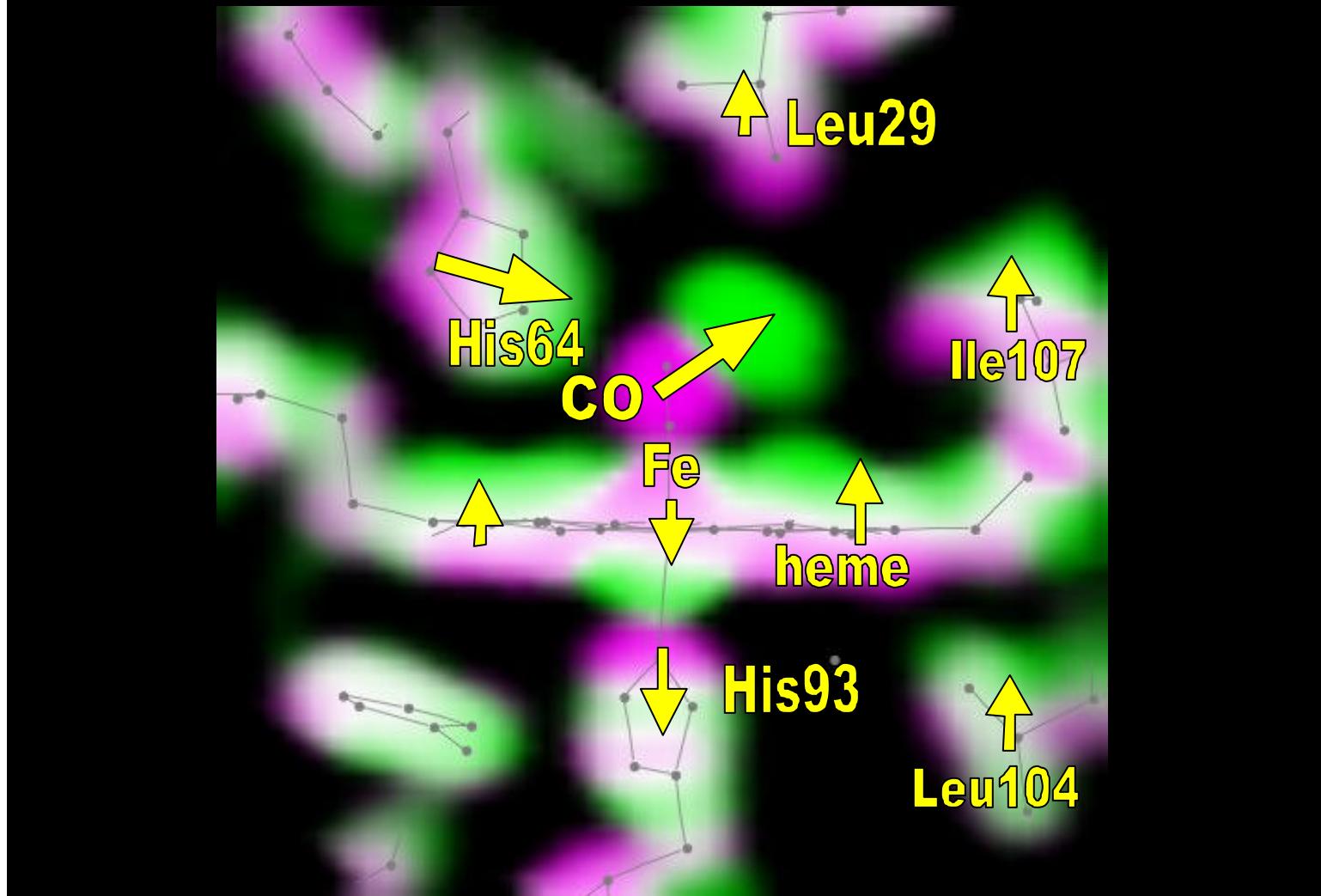
Extrapolation to Complete Photolysis : MbCO at 100 ps



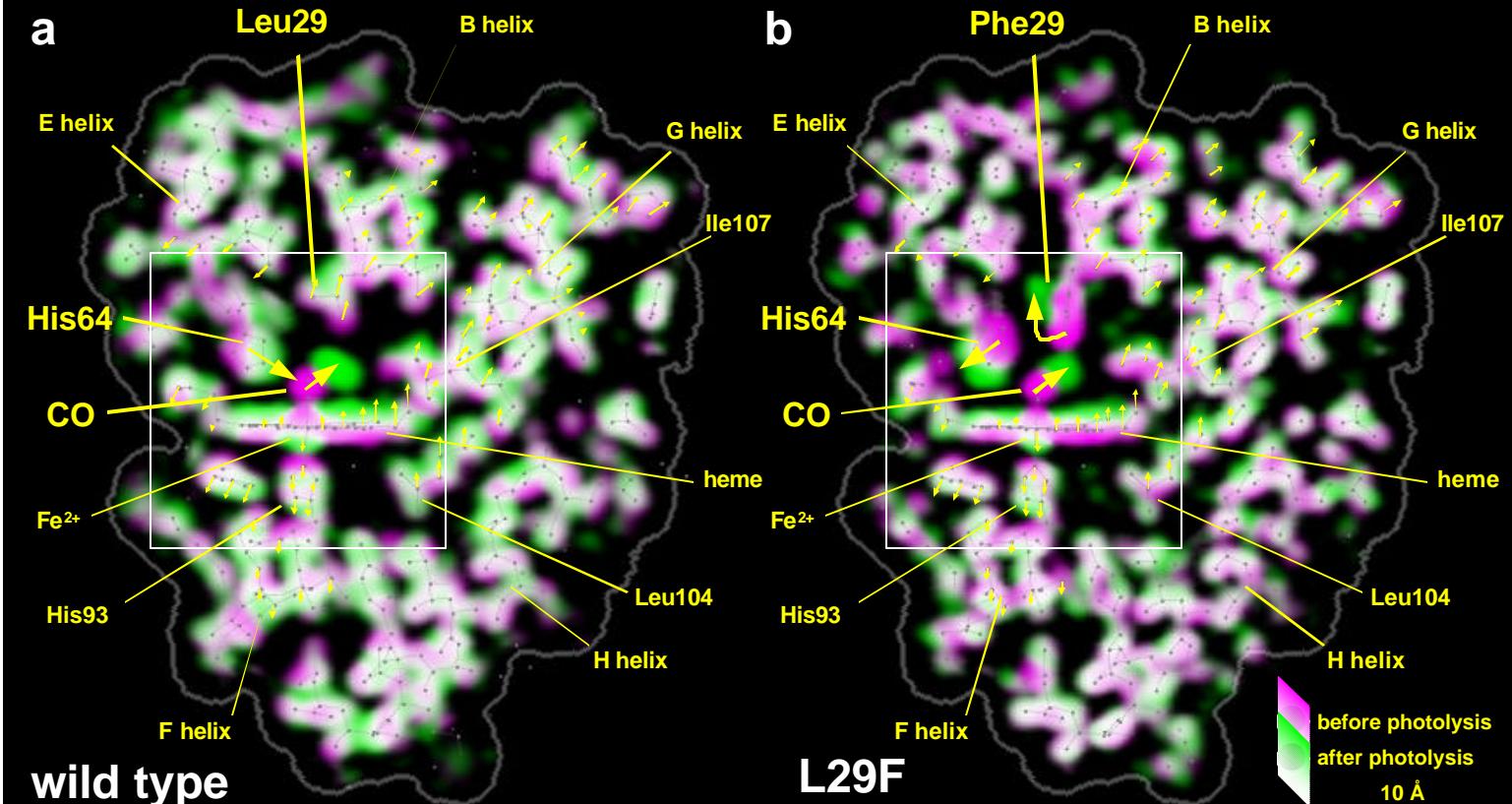
Contoured difference map : MbCO at 100 ps



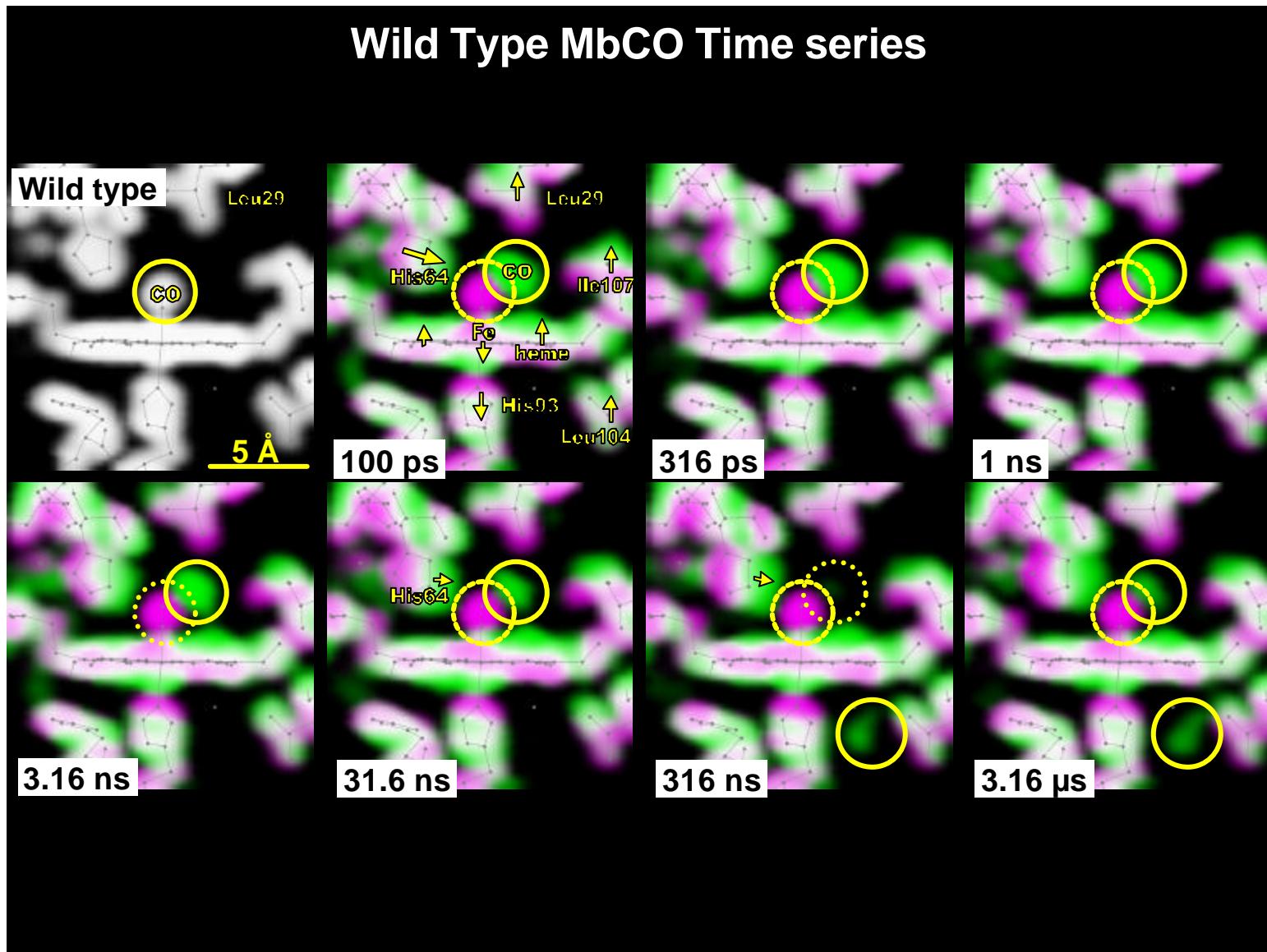
Color-coded maps superimposed: MbCO at 100 ps



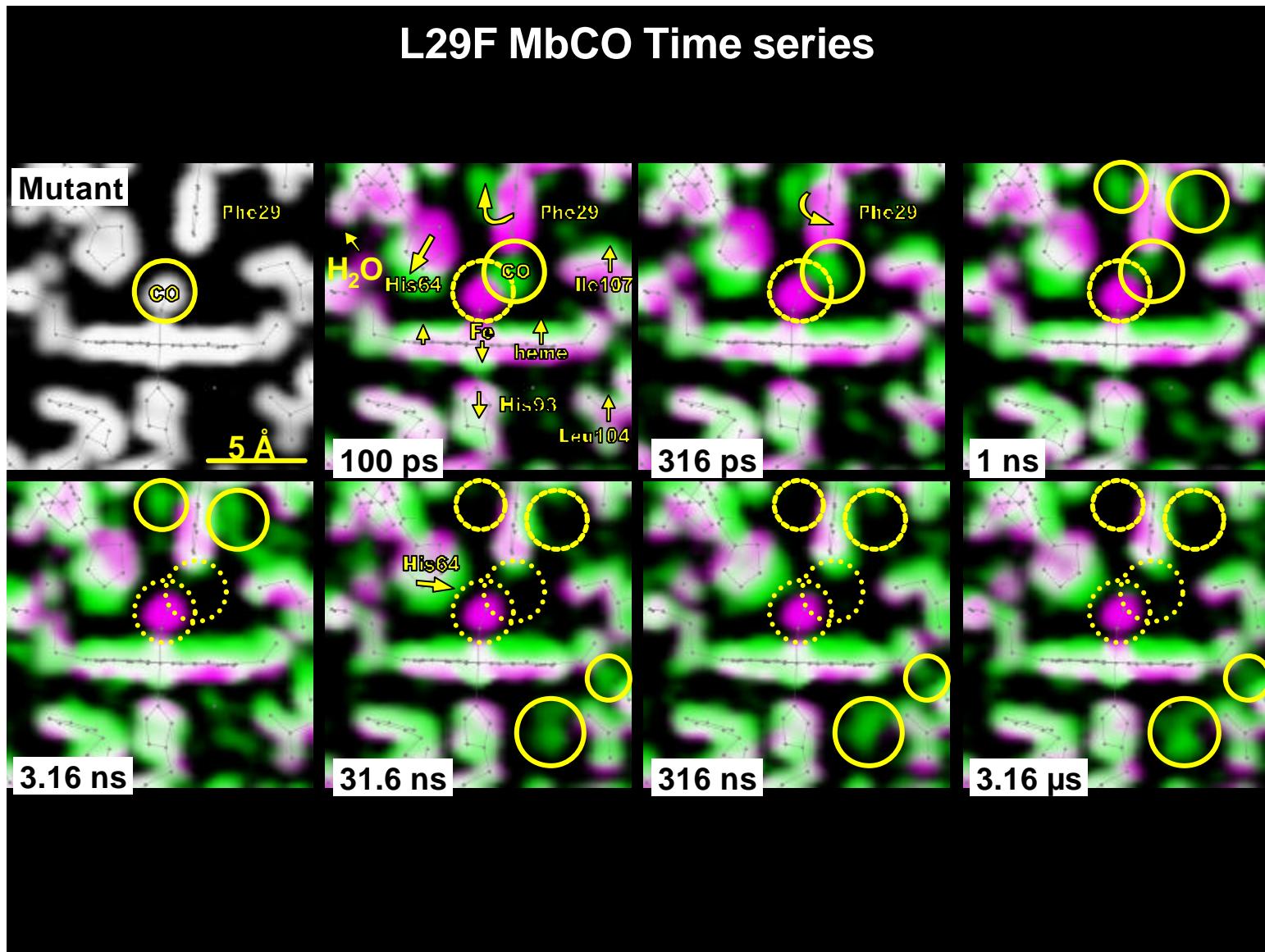
Wild Type vs. L29F MbCO at 100 ps



Wild Type MbCO Time series



L29F MbCO Time series



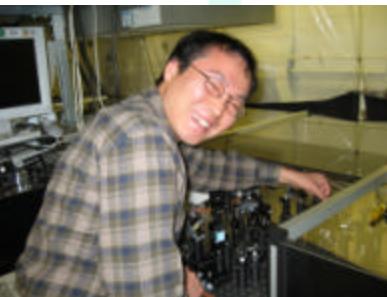


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LCP, NIH
MD simulations




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LCP, NIH
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