Time-Resolved Scattering from Solutions (Liquidography)♪

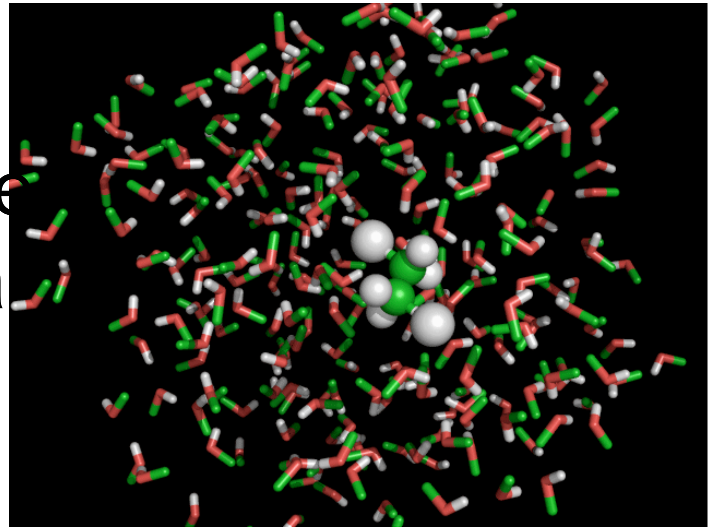
Hyotcherl (Harry) Ihee
Dept. Chemistry, KAIST

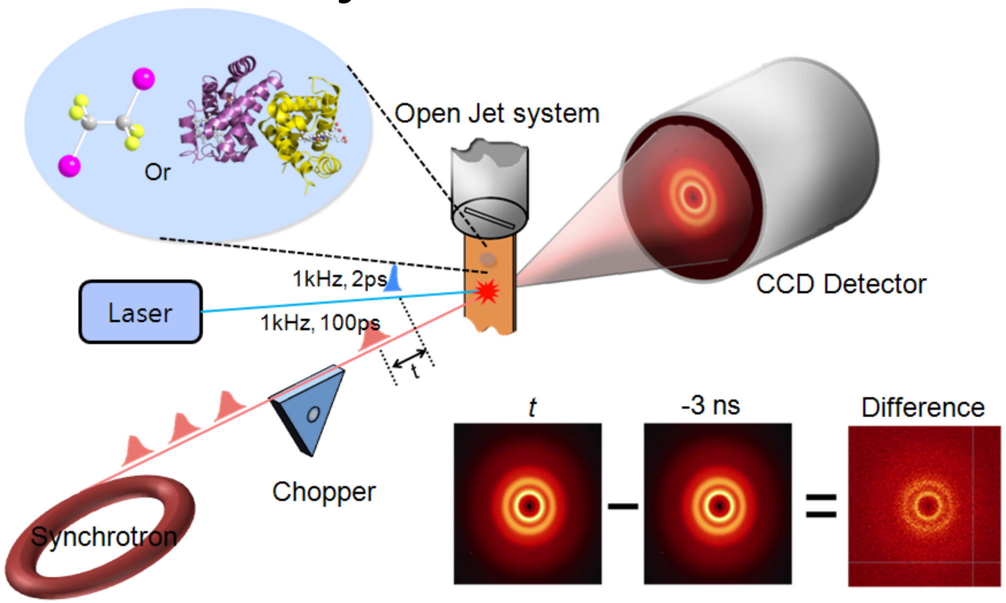
3rd European XFEL Meeting
Hamburg, Germeny
2009-1-28

Solution-phase reactions

- Most reactions related with Chemistry & Biology occur in solution phase.

- Time-resolved solution scatte ring (diffraction) to probe rea ction dynamics

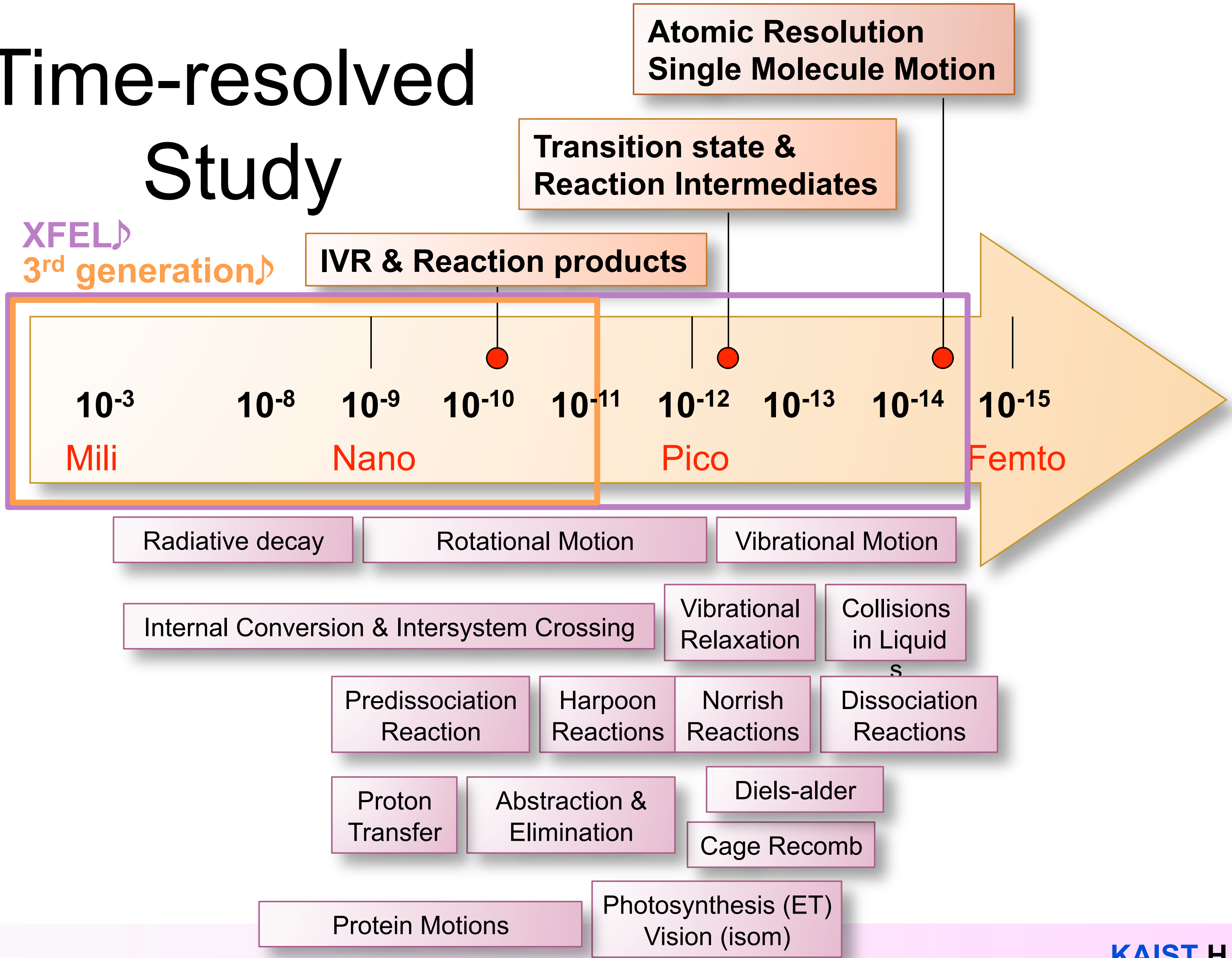


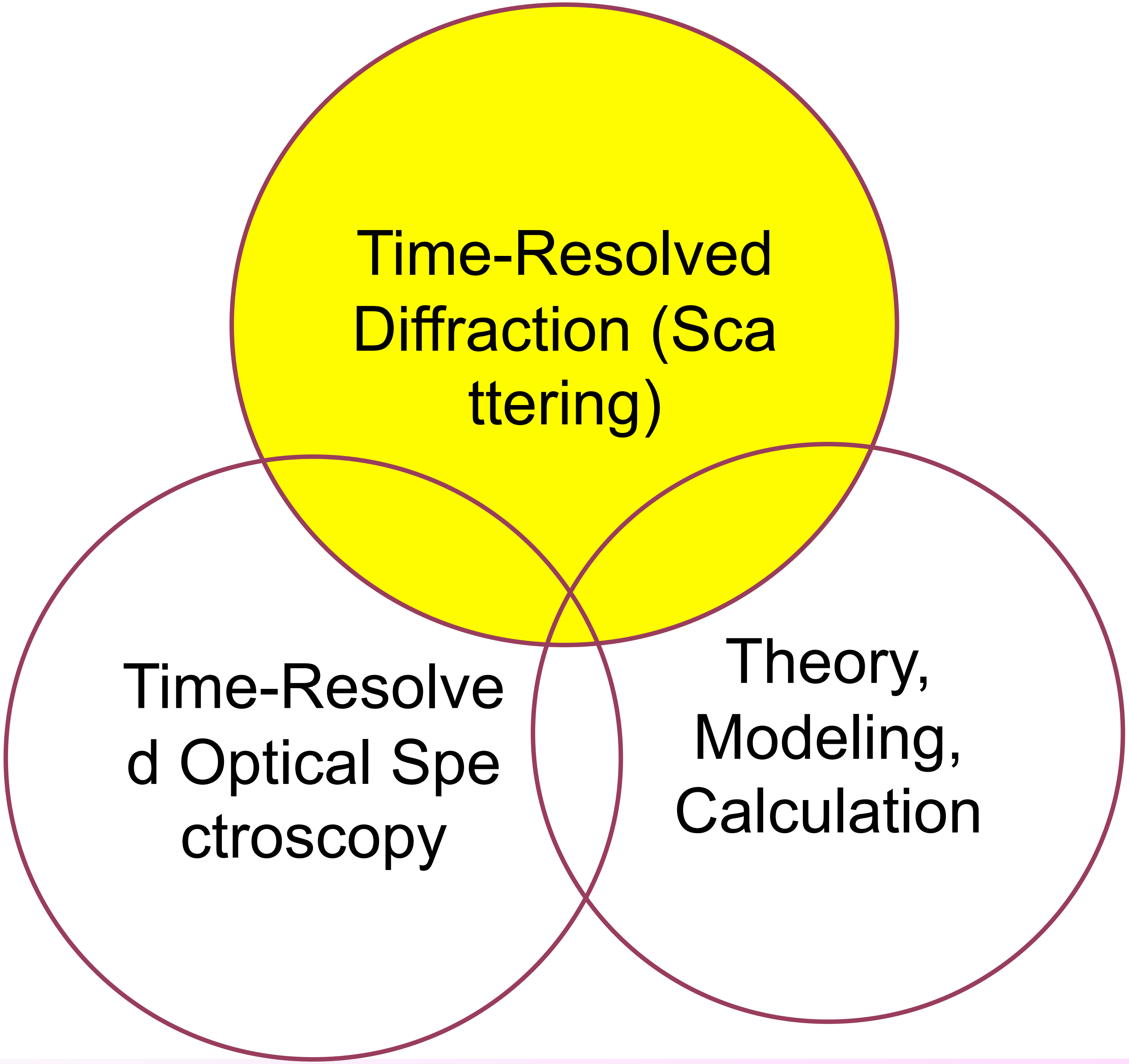


X-ray Pulse Parameters

	3^{rd} Gen. Synchrotron	XFEL
▪ Temporal pulse width:♪	~100 ps♪	< ~0.1 ps♪
▪ Intensity: ♪	<10^9 ph/pulse♪	>10^{12} ph/pulse♪
▪ Repetition Rate:♪	~1000 Hz♪	~100-5000 Hz♪
▪ Bandwidth ($\Delta E/E$):♪	~3%♪	~0.1%♪

Time-resolved Study




Time-Resolved Diffraction (Scattering)
Time-Resolved Optical Spectroscopy
Theory, Modeling, Calculation

Outline

Background & Introduction

- Optical Spectroscopy vs Scattering (Diffraction)♪
- Crystal Diffraction vs Liquid Scattering♪

Data Analysis

Application Examples (Small Molecules)

- Optical Spectroscopy vs Scattering (Diffraction)♪
- Fingerprinting the reaction intermediates, Structural Sensitivity♪
- Gas phase vs Solution phase♪
- Pushing the time resolution♪

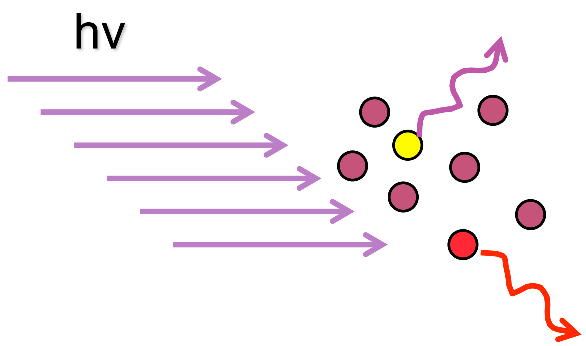
TR Protein Solution Scattering

- Quaternary and Tertiary structural transition of Hemoglobin, Myoglobin♪
- Protein folding dynamics of cytochrome-c♪
- Photocycle of photoactive yellow protein (PYP)♪

Optical Spectroscopy versus Diffraction

Optical Spectroscopy	Diffraction
• Optical Resonances	• Elastic Scattering
• Change in populations of energy states	• Change in molecular structures
• Signal does not have direct relationship with molecular structure	• Signal is directly related with molecular structure
• Signals are not easily predictable by a simple equation, and often not accurate enough	• **Scattering patterns are easily and accurately predictable from a structure model by a scattering equation**

Optical Spectroscopy

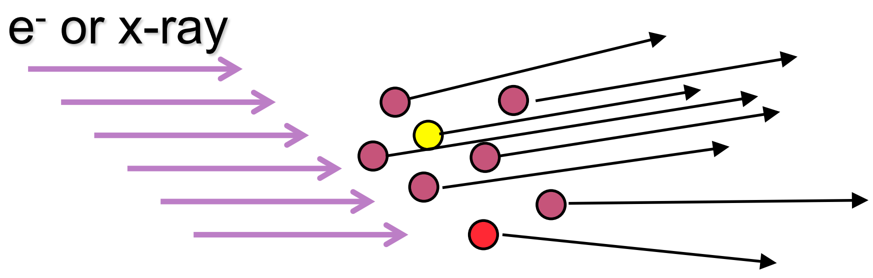


- Sensitive only to specific energy state or species

- Signals from different species can be well resolved in the wavelength space

→ **high sensitivity**

Diffraction



- Sensitive to **all species and energy states**

- Signals from different species are mixed in all diffraction angles

→ low sensitivity

Optical Spectroscopy

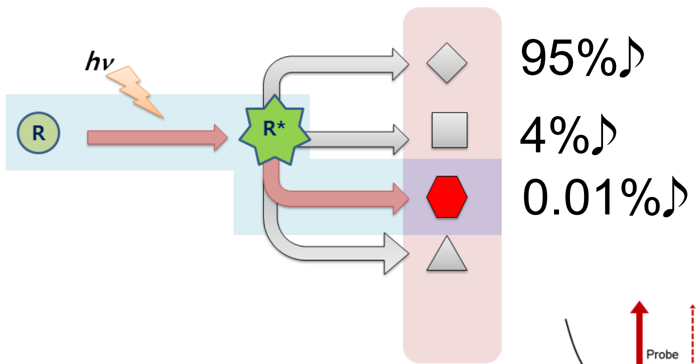
- Sensitive only to specific energy state or species
- Can be sensitive to only specific location in the potential energy space
- Scale is not maintained

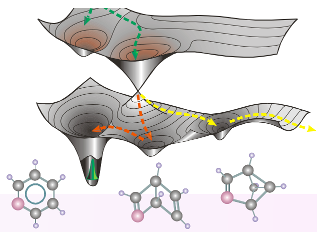
→ high sensitivity but, may not be global

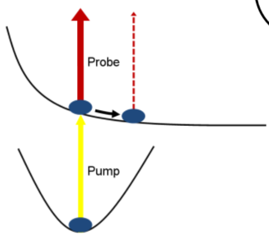
Diffraction

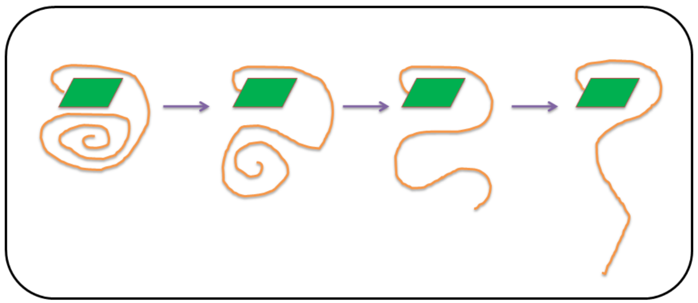
- Sensitive to all species and energy states
- Sensitive to major channel
- **Scale is maintained (Branching ratio)**

→ low sensitivity but, **global**



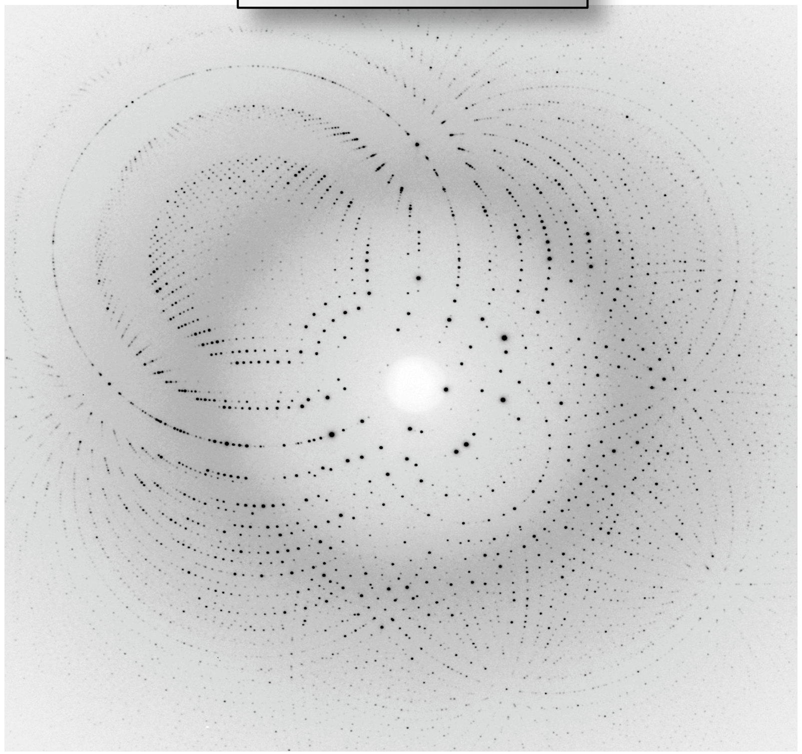




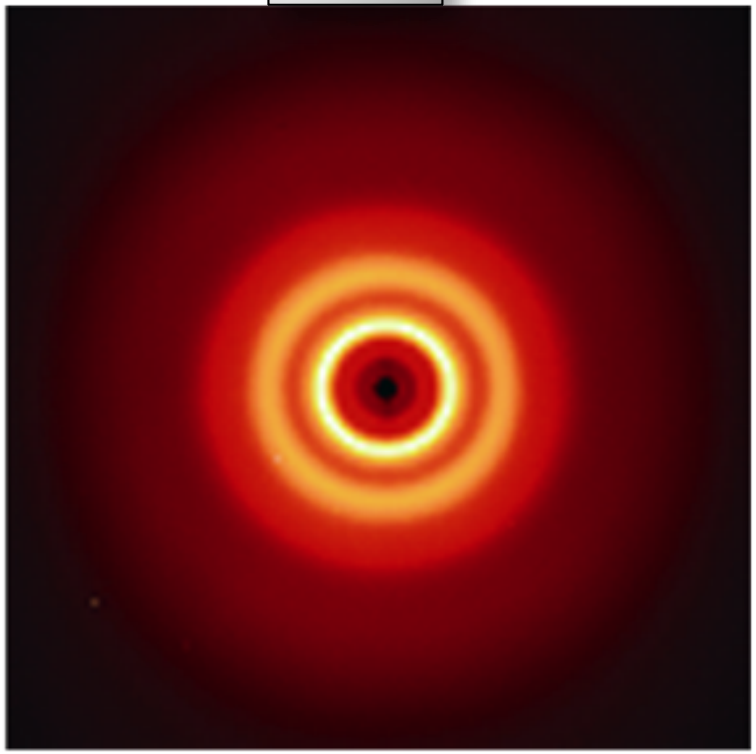


Crystal Diffraction vs Liquid Scattering

Single Crystal



Liquid



- Laue Conditions:
  Only hkl♪

$$\rho(xyz) = \frac{1}{V}\sum_h\sum_k\sum_l \overrightarrow{F(hkl)}\exp(-2\pi i(hx+ky+lz))$$

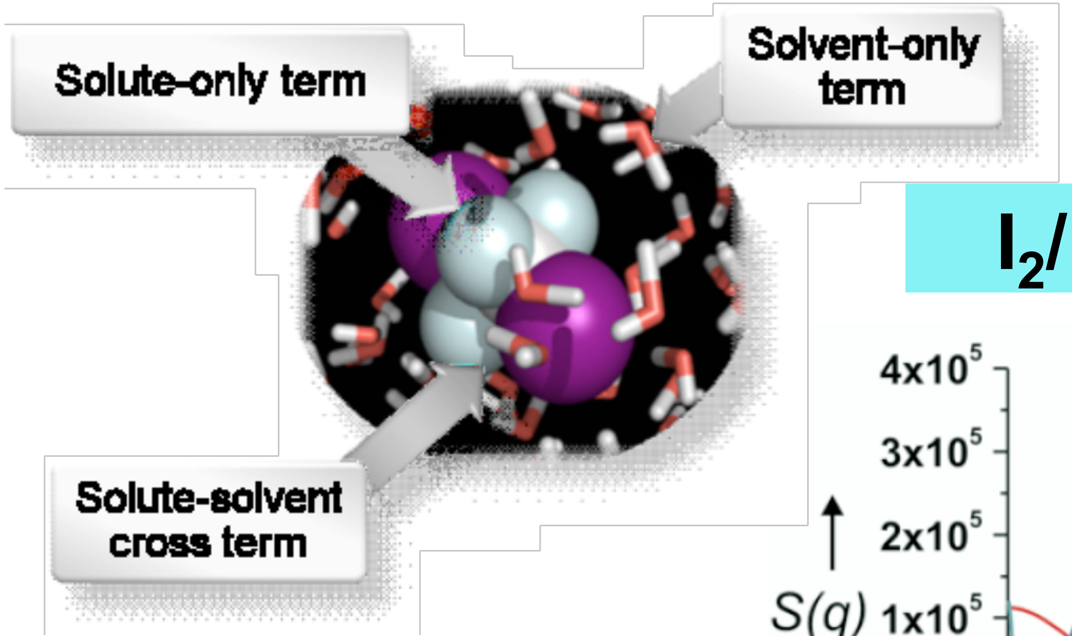
$$= \frac{1}{V}\sum_h\sum_k\sum_l \left|\overrightarrow{F(hkl)}\right|\exp(i\alpha(hkl))\exp(-2\pi i(hx+ky+lz))$$

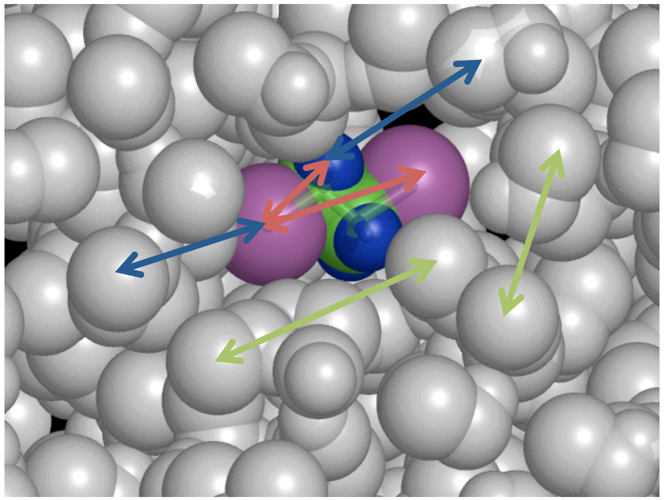
- Isotropic Averaging:
  All q range is important
- Huge background♪

Photon-hungry♪

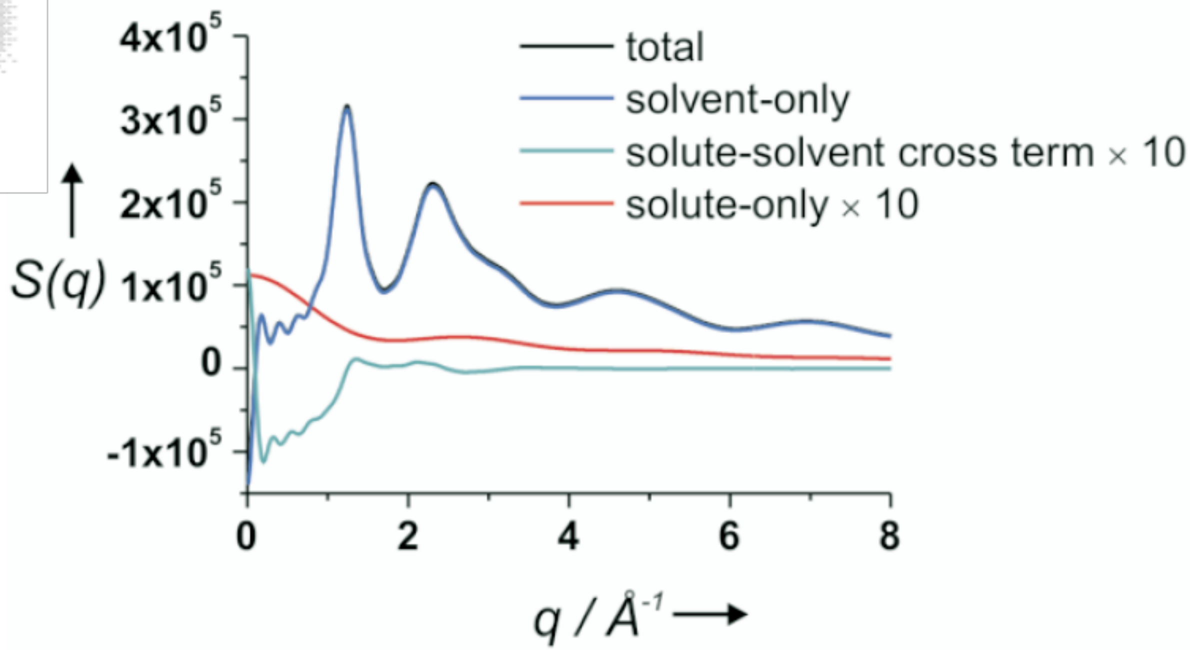
$$4\pi r^2\rho_0[g(r)-1] = \frac{2r}{\pi}\int_0^\infty q\left(\frac{I(q)-I_{atom}(q)}{f^2(q)}\right)\sin qr\,dq \qquad q = \frac{4\pi}{\lambda}\sin\theta$$

Scattering from Solutions

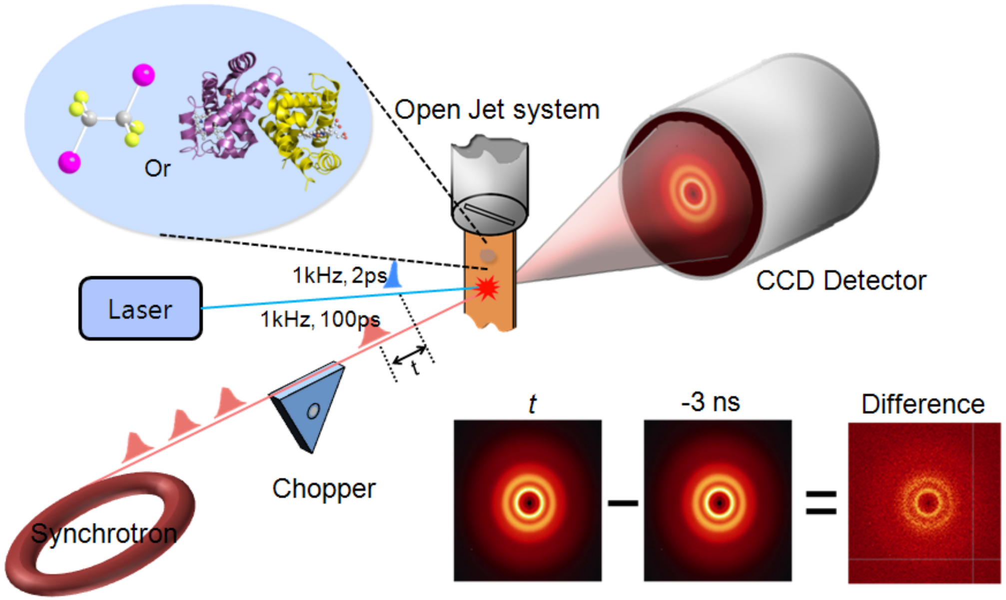


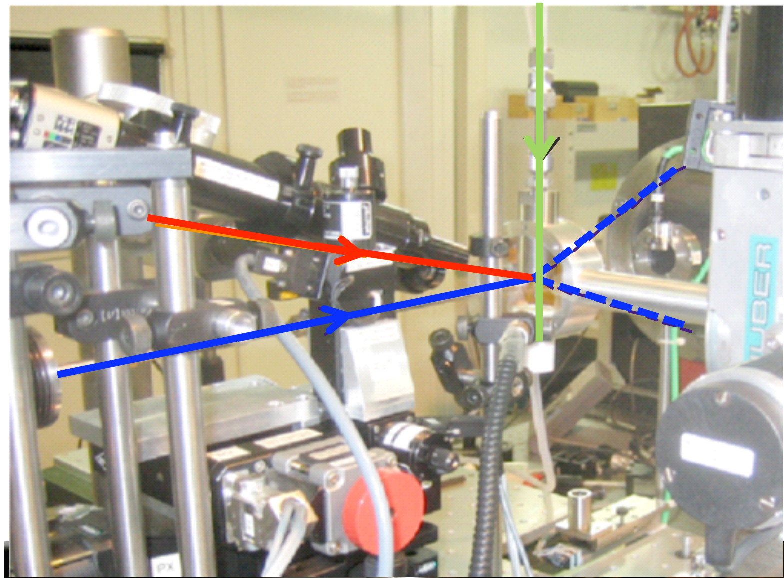


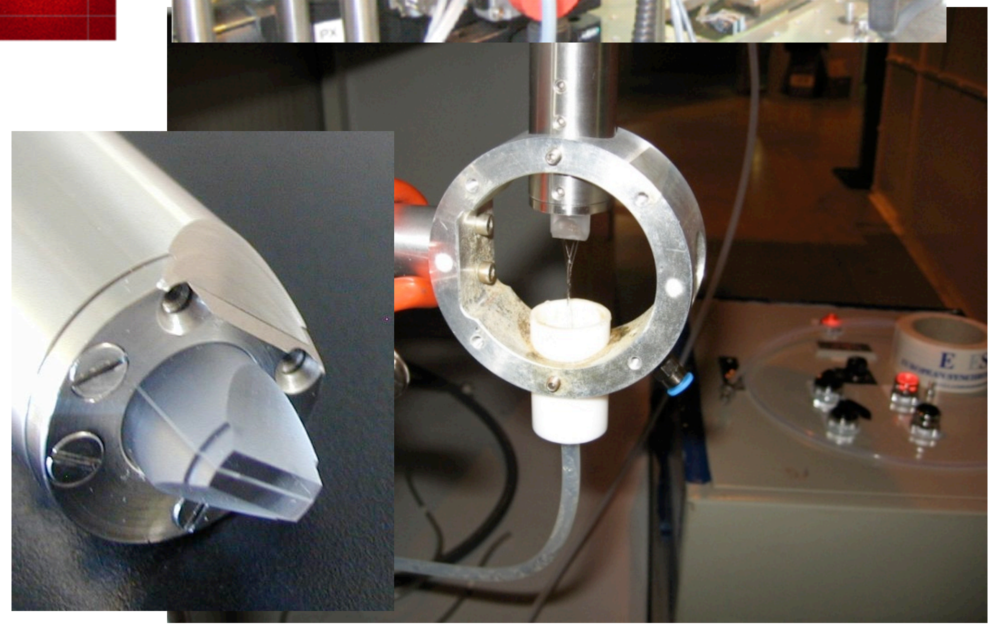
I_2/methanol solution

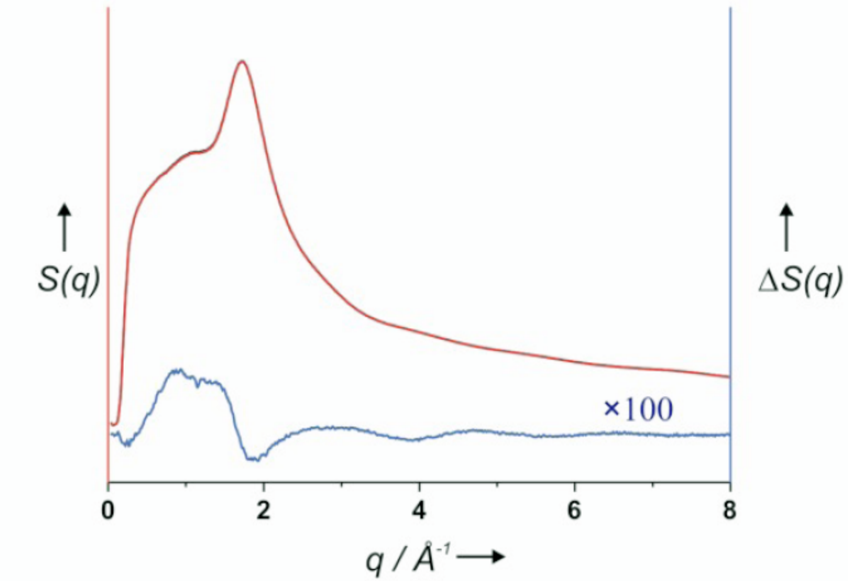


Net Signal (Difference) is even smaller

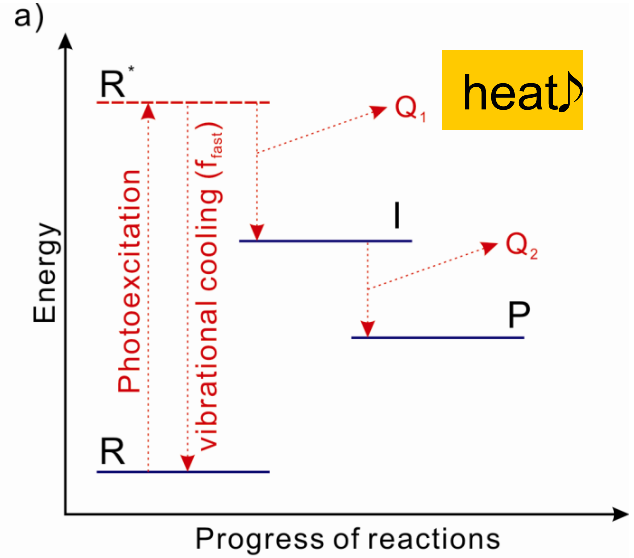


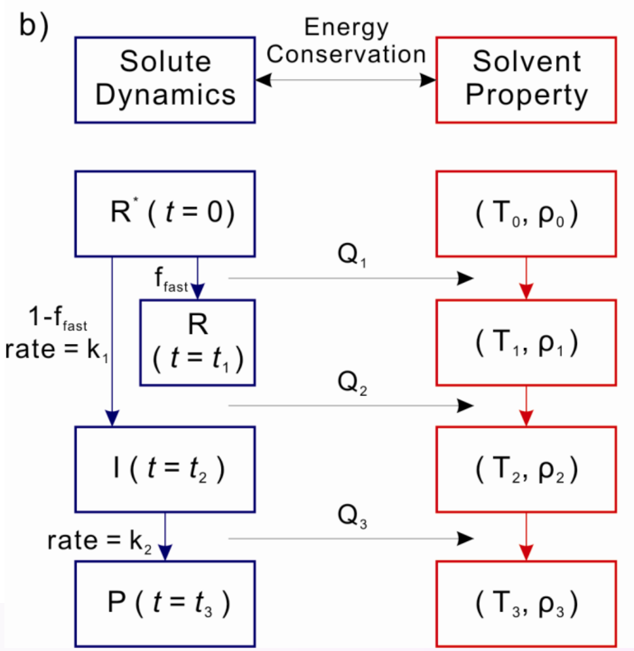






Scattering from Solutions

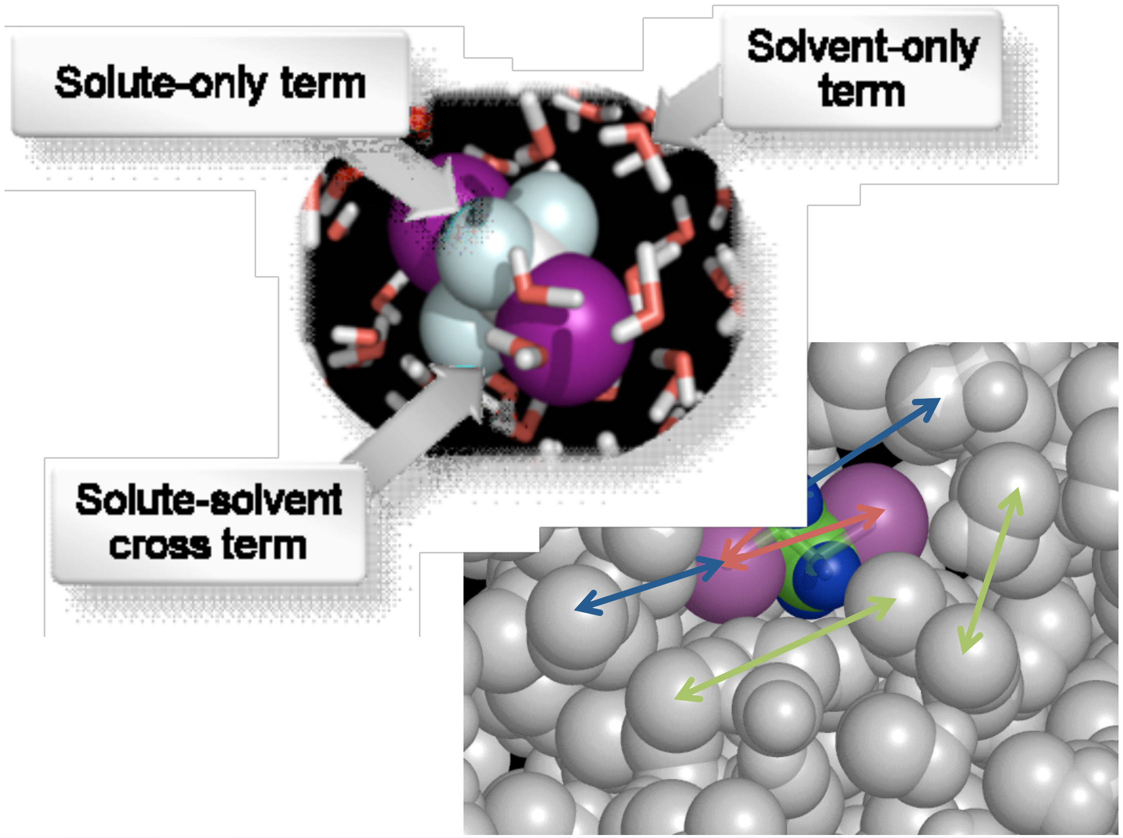




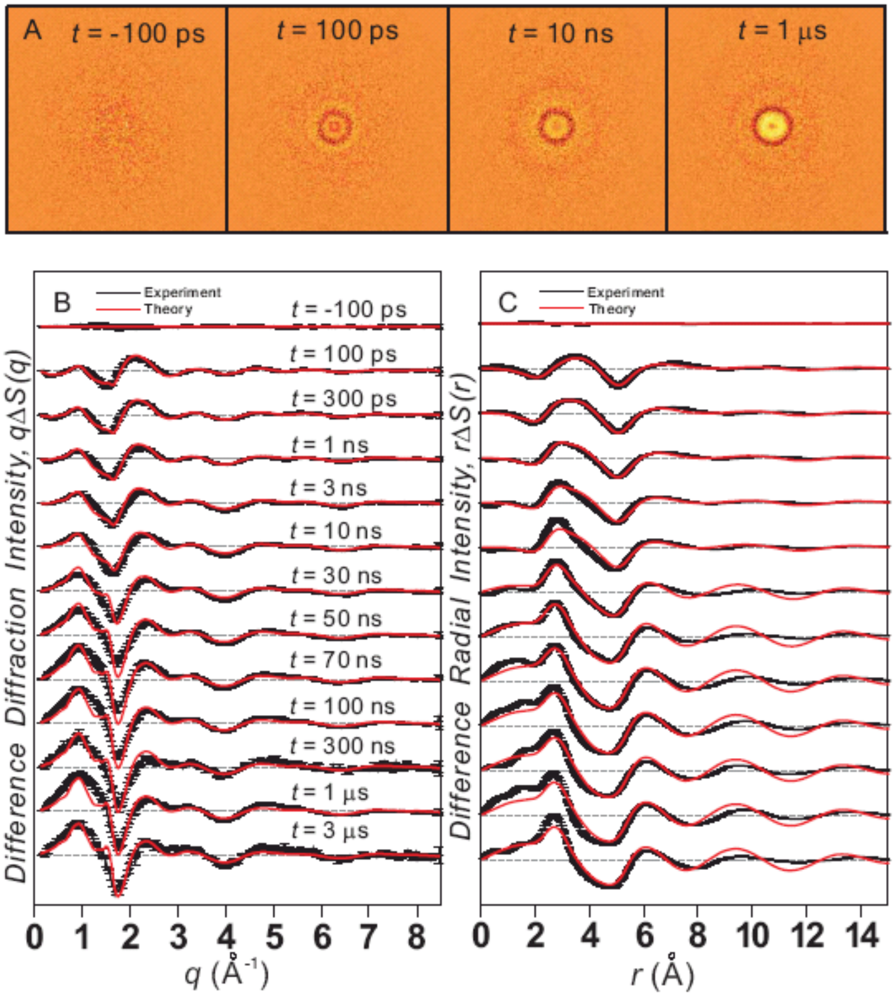
$$\Delta S_{the}(q,t)$$

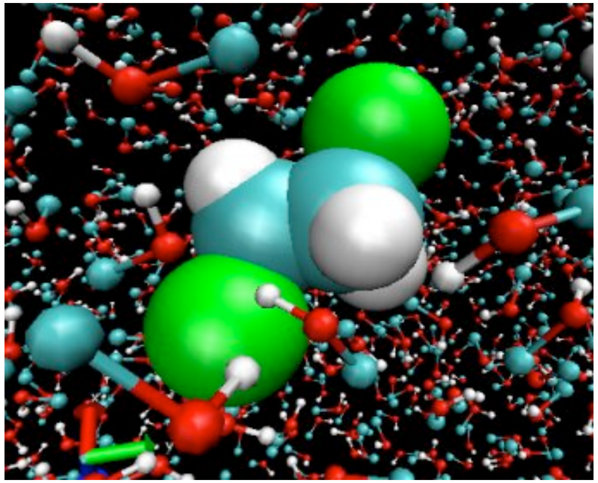
$$= \Delta S_{solute-only}(q,t) + \Delta S_{solute-solvent}(q,t) + \Delta S_{solvent-only}(q,t)$$

$$= \underbrace{\left[\sum_k N_k(t) S_k(q) - S_g(q) \sum_k N_k(0) \right]}_{solute-only\ term\ +\ solute-solvent\ cross\ term} + \underbrace{\left(\frac{\partial S}{\partial T} \right)_\rho \Delta T(t) + \left(\frac{\partial S}{\partial \rho} \right)_T \Delta \rho(t)}_{solvent-only\ term}$$



Time-Resolved X-ray Liquidography: Results





$$\Delta S(q,t) =$$

$$\left.\begin{array}{l} \Delta S(q,t)_{solute} \\ + \Delta S(q,t)_{solute\text{-}solvent} \end{array}\right\} \text{solute}$$

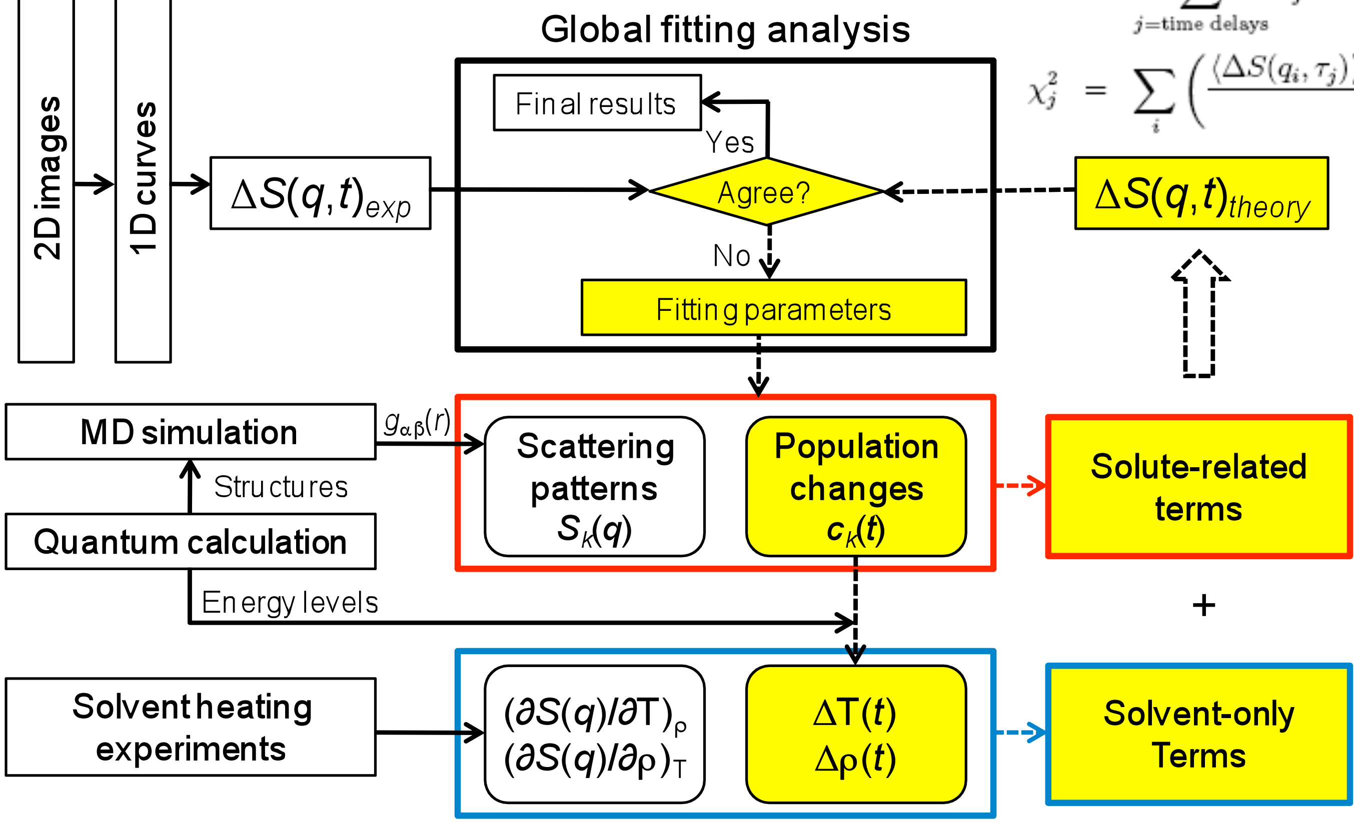
$$+ \Delta S(q,t)_{solvent} \quad \} \text{solvent}$$

$$r\,\Delta S(r) = \frac{1}{2\pi^2}\int_0^\infty q\,\Delta S(q)\sin(q\,r)\exp(-q^2\alpha)\,dq$$

Data Analysis (Global Fitting Analysis)

$$\chi^2 = \sum_{j=\text{time delays}} \chi_j^2$$

$$\chi_j^2 = \sum_i \left(\frac{\langle \Delta S(q_i, \tau_j) \rangle - \Delta S_{exp}(q_i, \tau_j)}{\sigma_{i,j}} \right)^2$$

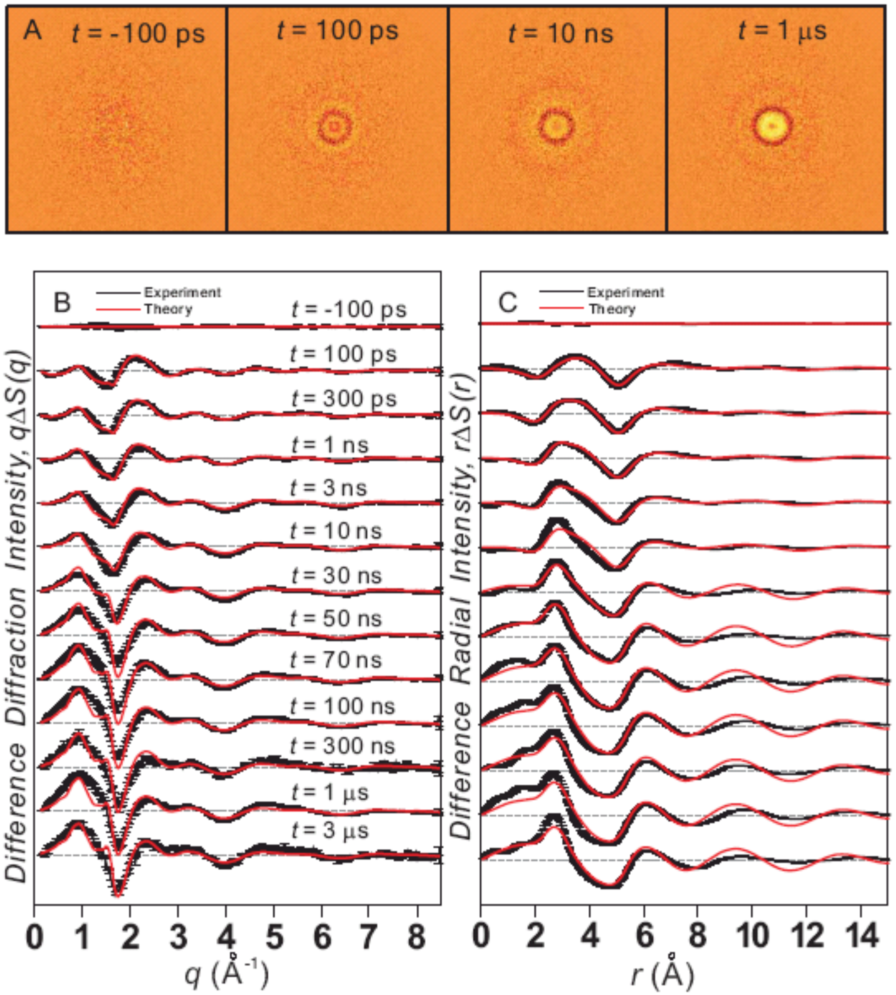


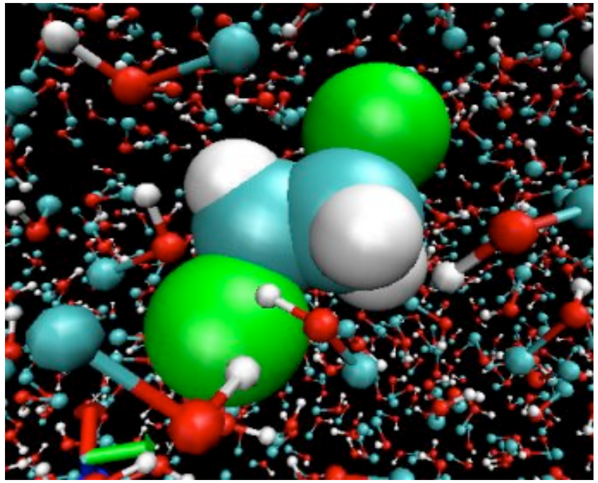
$$\Delta S(q,t)^{theory} = \Delta S(q,t)_{solute-only} + \Delta S(q,t)_{solute-solvent} + \Delta S(q,t)_{solvent-only}$$

$$= \Delta S(q,t)_{solute-related} + \Delta S(q,t)_{solvent-only}$$

$$= \frac{1}{R}\left[\sum_k c_k(t) S_k(q) - S_g(q) \sum_k c_k(0) \right] + \left(\partial S/\partial T\right)_\rho \Delta T(t) + \left(\partial S/\partial \rho\right)_T \Delta\rho(t)$$

Time-Resolved X-ray Liquidography: Results





$$\Delta S(q,t) =$$

$$\left.\begin{array}{l}\Delta S(q,t)_{solute} \\ +\ \Delta S(q,t)_{solute\text{-}solvent}\end{array}\right\}\text{solute}$$

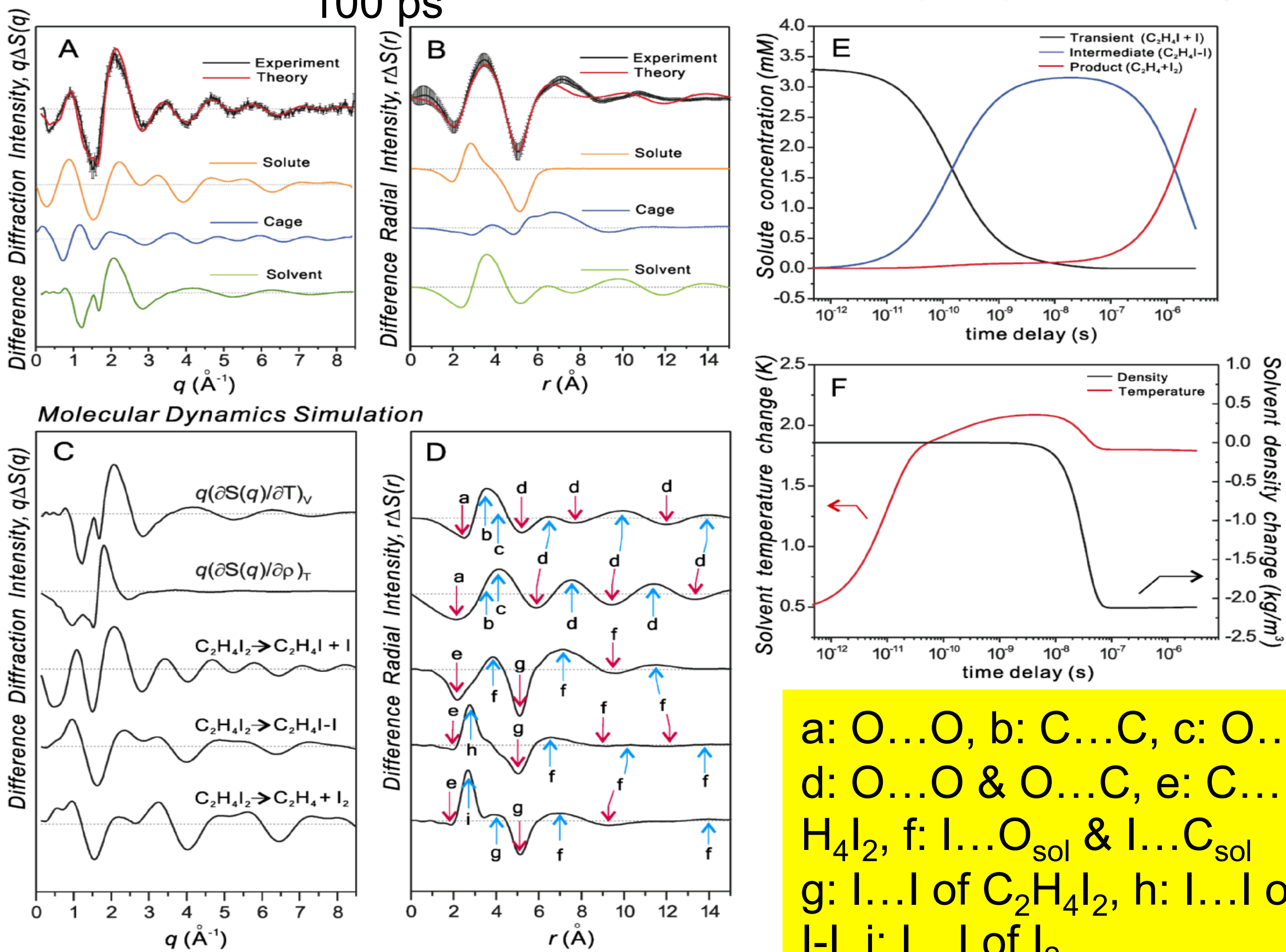
$$+\ \Delta S(q,t)_{solvent}\ \}\text{solvent}$$

$$r\,\Delta S(r) = \frac{1}{2\pi^2}\int_0^\infty q\,\Delta S(q)\sin(q\,r)\exp(-q^2\alpha)\,dq$$

Time-Resolved X-ray Liquidography: Analysis

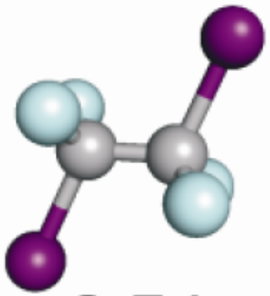
100 ps

Science, 309, 1223-1227 (2005)

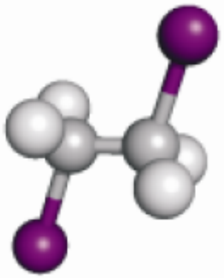


a: O…O, b: C…C, c: O…C,
d: O…O & O…C, e: C…I of $C_2H_4I_2$, f: I…O_{sol} & I…C_{sol}
g: I…I of $C_2H_4I_2$, h: I…I of C_2H_4 I-I, i: I…I of I_2

Molecule Gallery

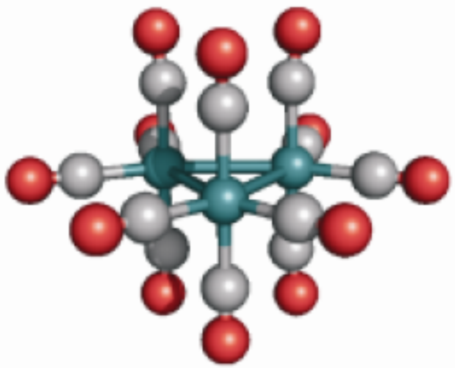


$C_2F_4I_2$

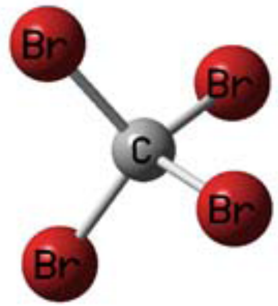


$C_2H_4I_2$

I_2

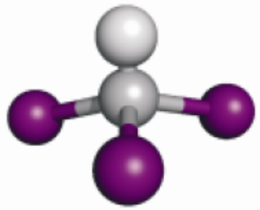


$Ru_3(CO)_{12}$

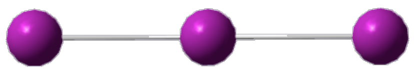


CBr_4♪

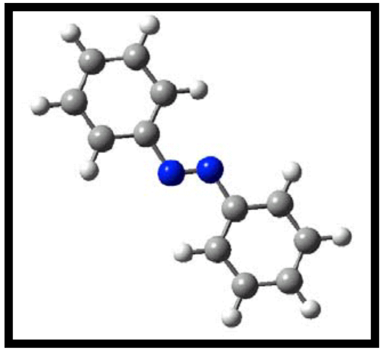
HgI_2



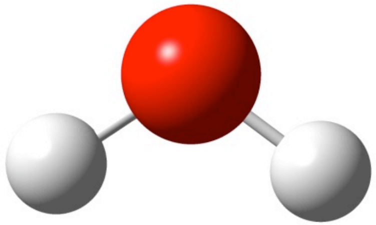
CHI_3



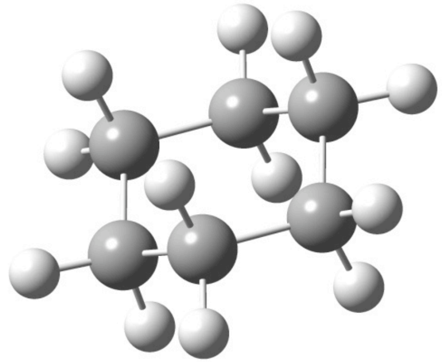
I_3^-♪



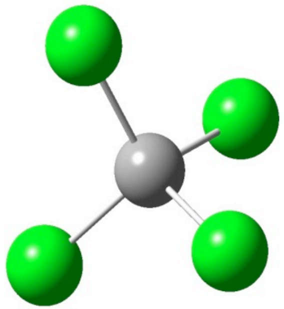
$C_{12}H_{10}N_2$
(Azobenzene)♪



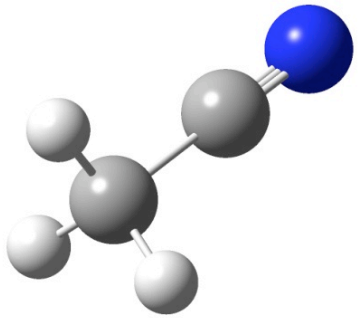
Water (H_2O)♪



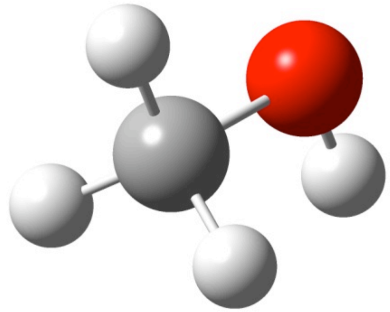
Cyclohexane (C_6H_{12})♪



Tetralchloro Methane(CCl_4) ♪



Acetonitrile (CH_3CN)♪



Methanol (CH_3OH)♪

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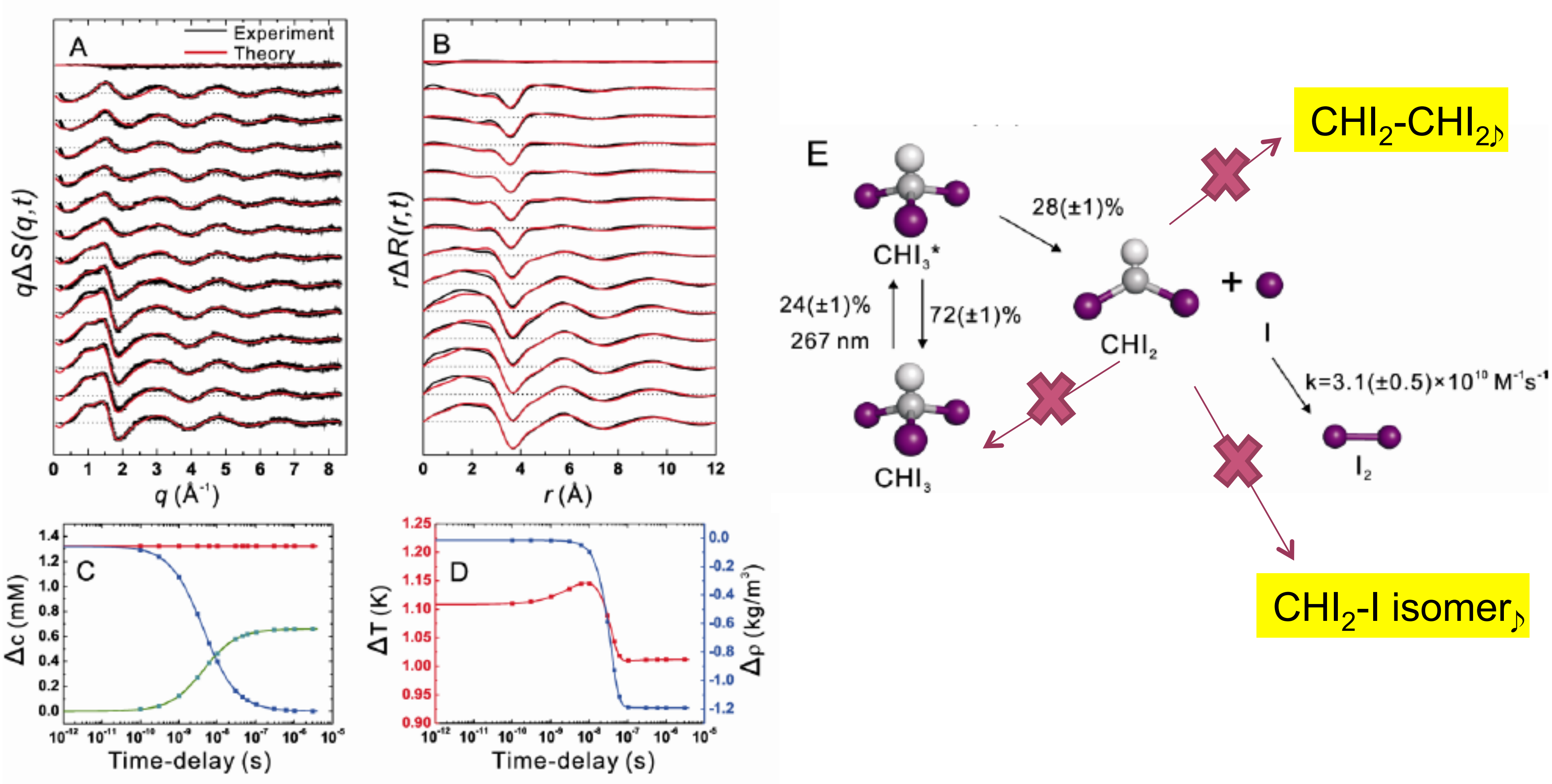
- Pushing the time resolution♪

TR Protein Solution Scattering

- Quaternary and Tertiary structural transition of Hemoglobin, Myoglobin♪
- Protein folding dynamics of cytochrome-c♪
- Photocycle of photoactive yellow protein (PYP)♪

Photochemistry of CHI_3 (Iodoform) in methanol



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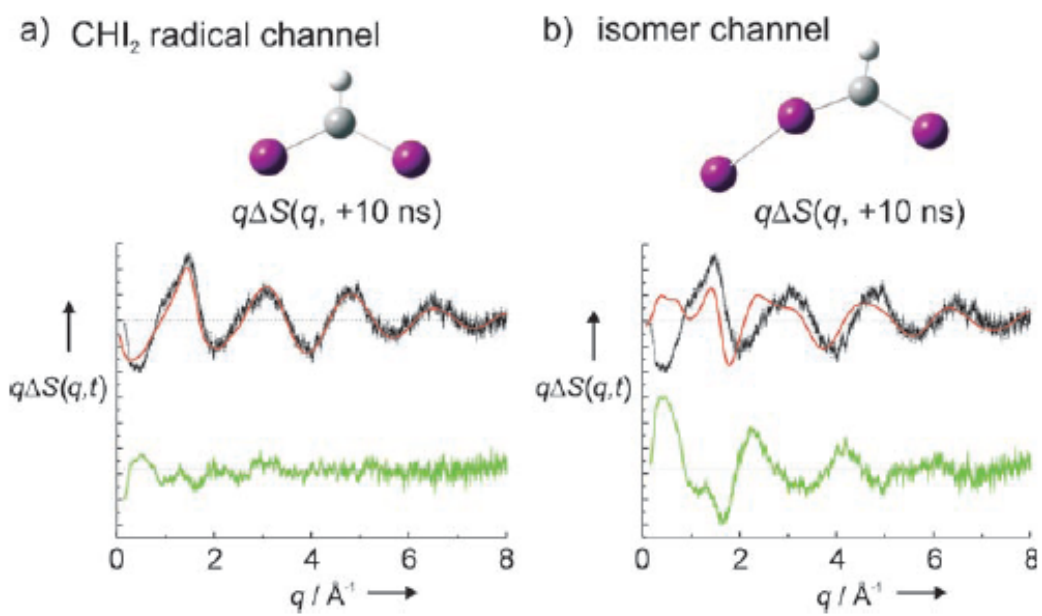
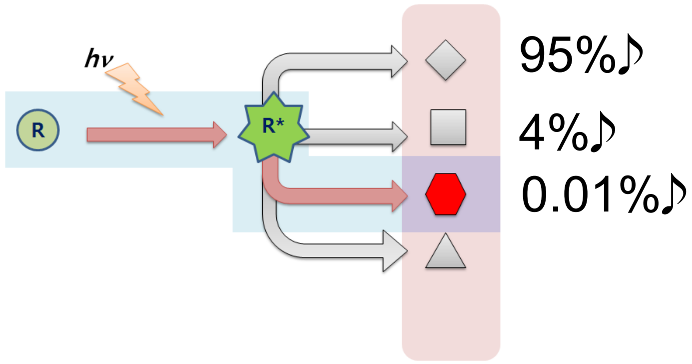
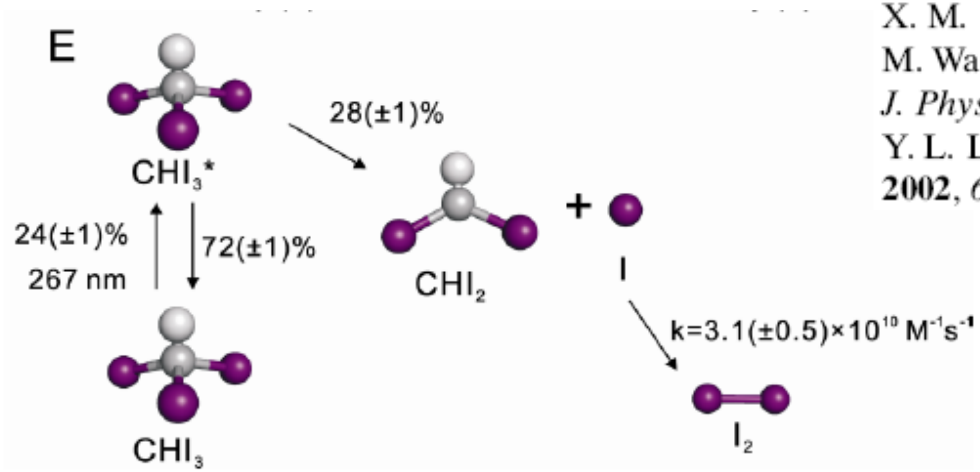
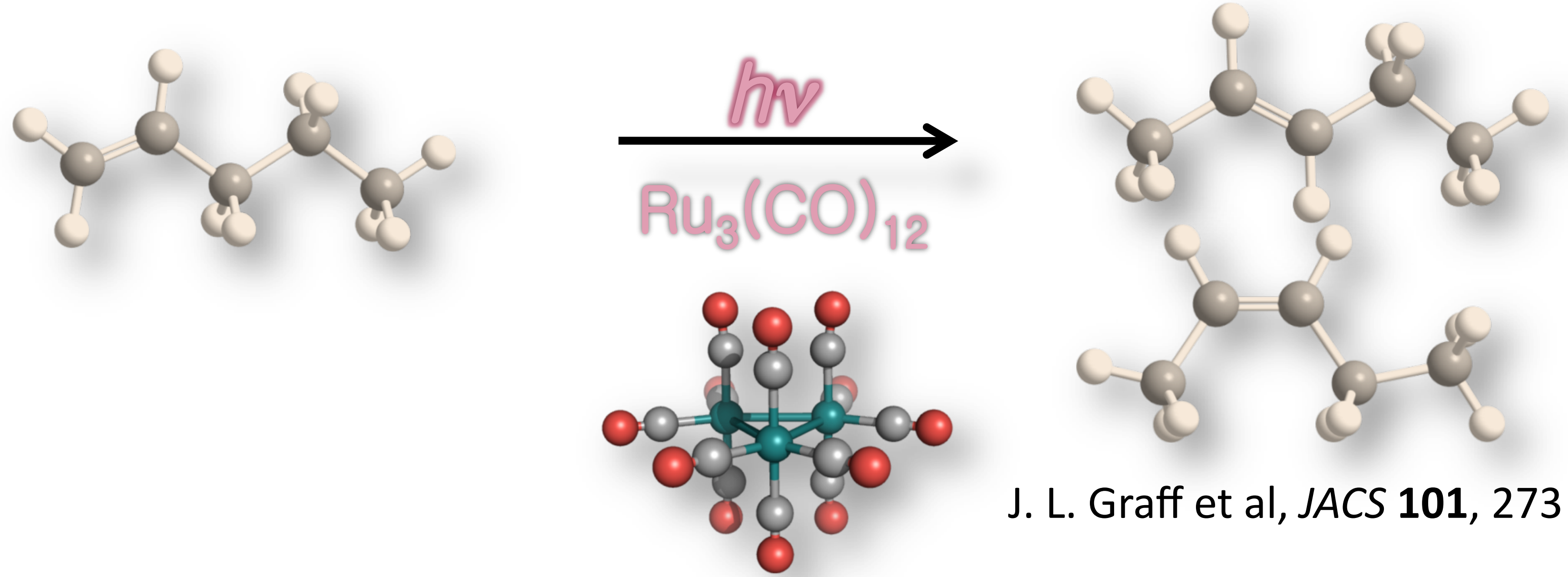






X. M. Zheng, D. L. Phillips, *Chem. Phys. Lett.* **2000**, *324*, 175.
M. Wall, A. N. Tarnovsky, T. Pascher, V. Sundstrom, E. Akesson, *J. Phys. Chem. A* **2003**, *107*, 211.
Y. L. Li, D. M. Chen, D. Q. Wang, D. L. Phillips, *J. Org. Chem.* **2002**, *67*, 4228.

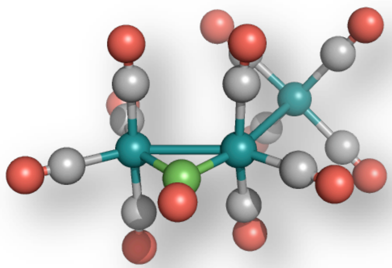
ACIE, 47, 1047-1050 (2008)



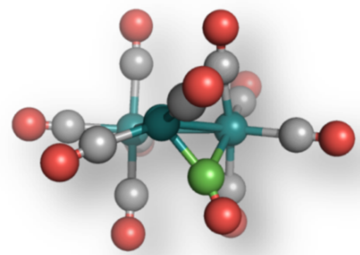
J. L. Graff et al, *JACS* **101**, 273 (1979)♪

Previous Spectroscopic Studies

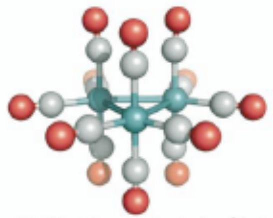
E.A. Glascoe et al, *Organomet.* **25**, 775 (2006)♪



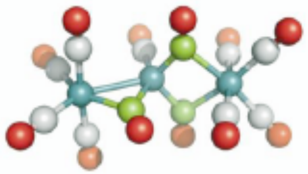
$Ru_3(CO)_{11}(\mu\text{-}CO)$♪
Intermediate 1♪



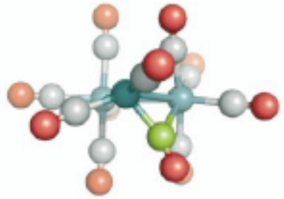
$Ru_3(CO)_{10}(\mu\text{-}CO)$♪
Intermediate 2♪



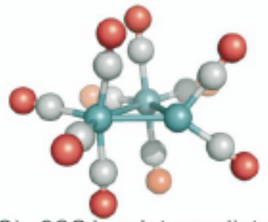
$Ru_3(CO)_{12}$ Ground state, $^1A_1'$



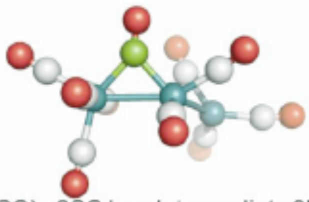
$Ru_3(CO)_{12}$ Intermediate 1A, ^1A



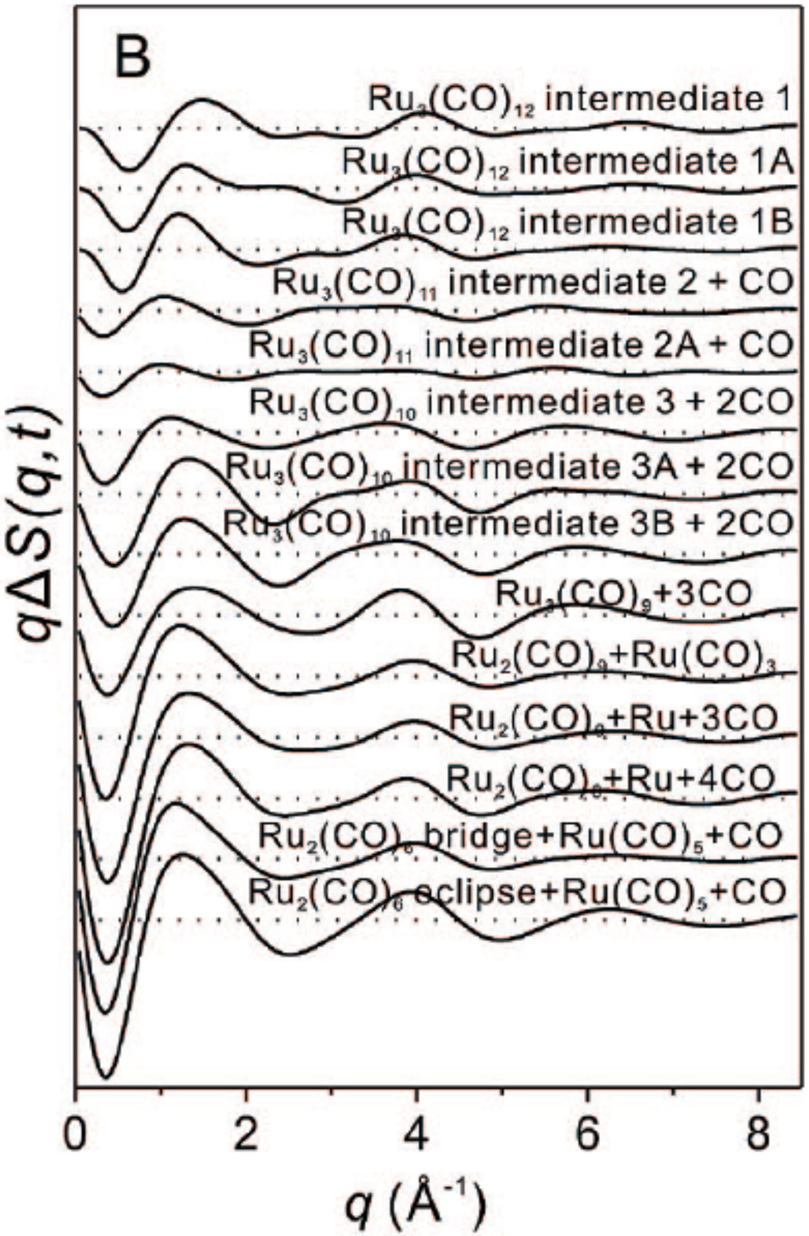
$Ru_3(CO)_{11}$ axial CO loss Intermediate 2, ^1A

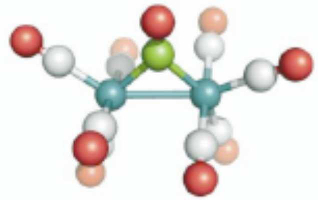


$Ru_3(CO)_{10}$ 2CO loss Intermediate 3, ^1A

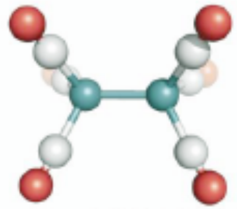


$Ru_3(CO)_{10}$ 2CO loss Intermediate 3B, ^1A

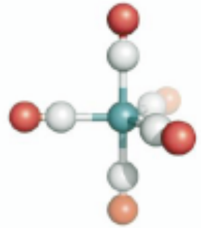




$Ru_2(CO)_9$, ^1A



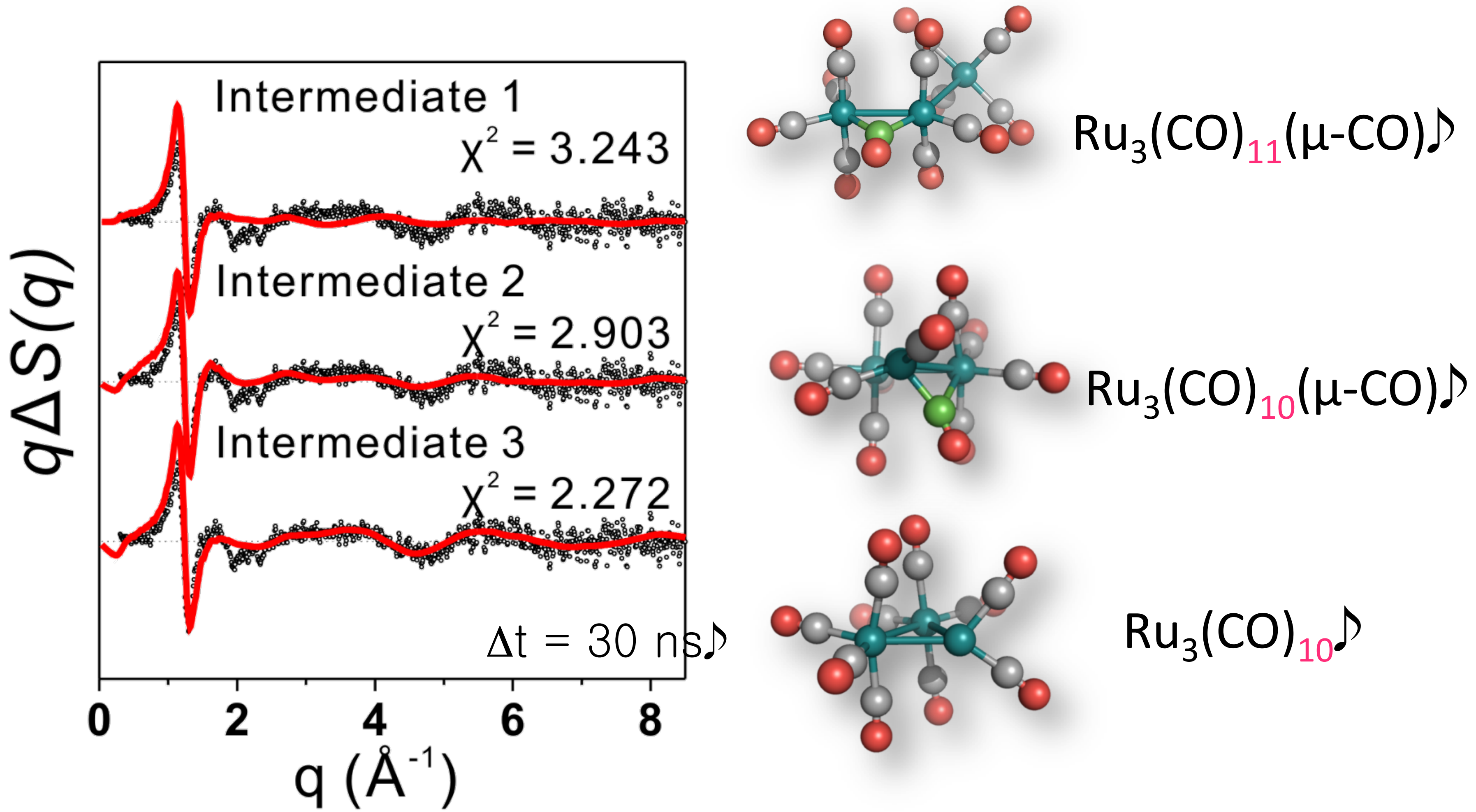
$Ru_2(CO)_6$ Eclipsed



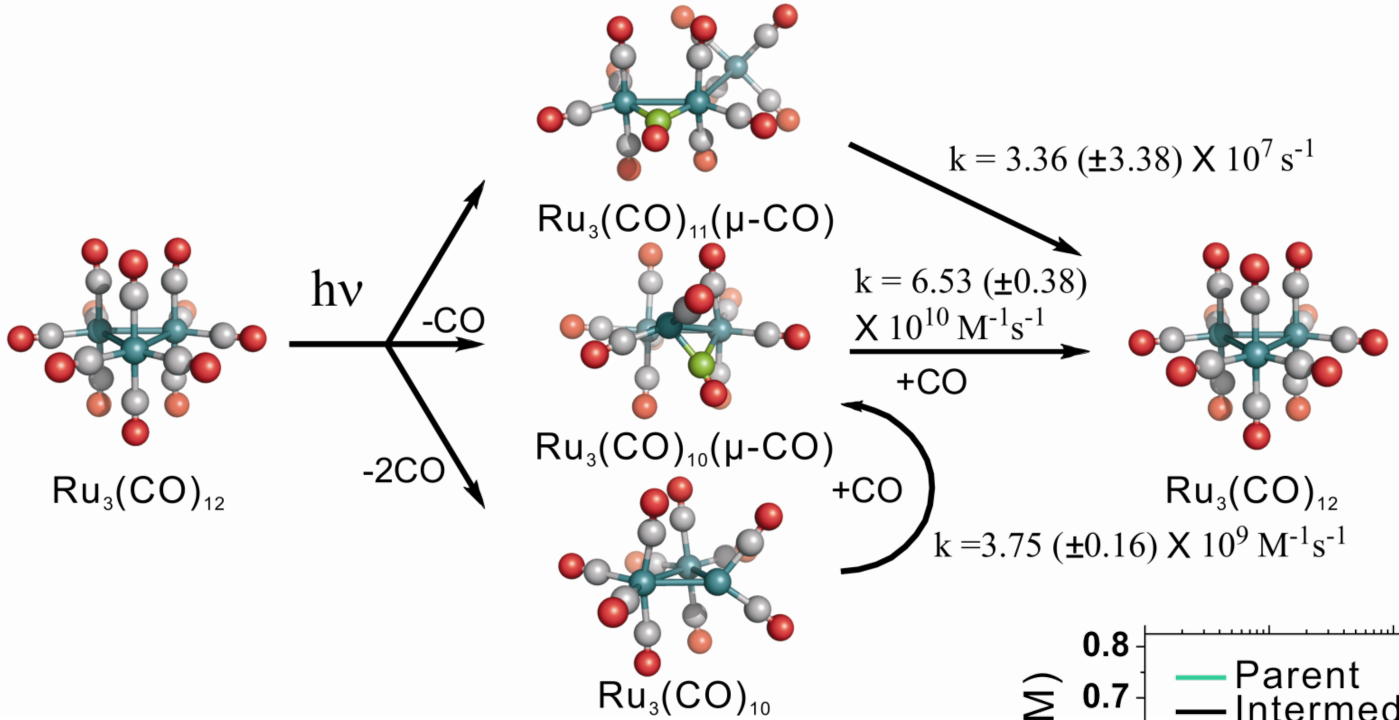
$Ru(CO)_5$, ^1A

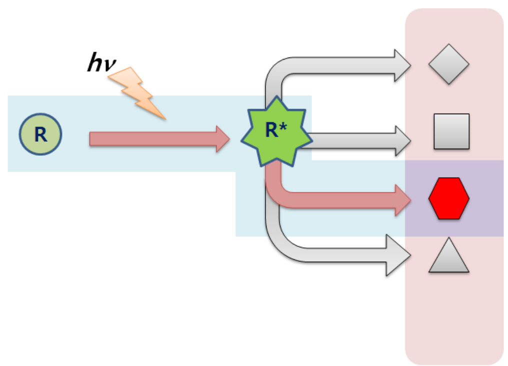
$Ru(CO)_3$, $^3A_1$

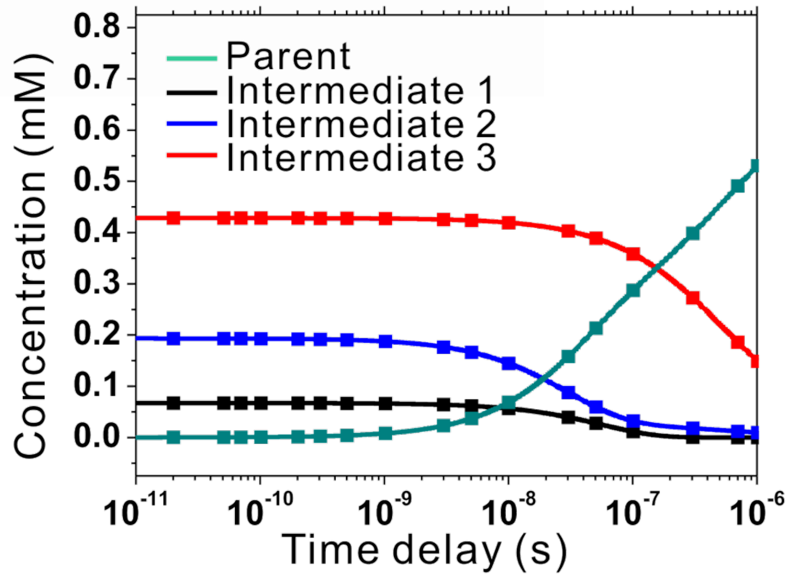
Determination of the Photoproduct



Photochemical Reaction Pathway







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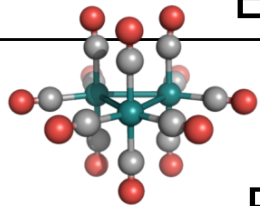
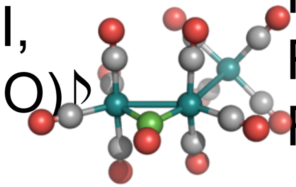
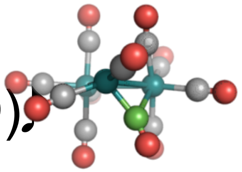
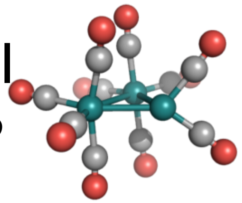
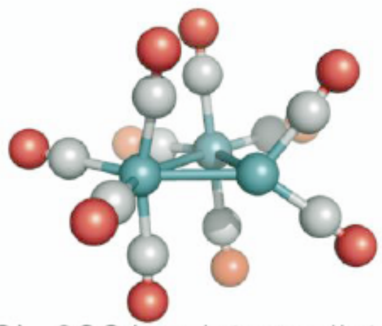
Structure Refinement

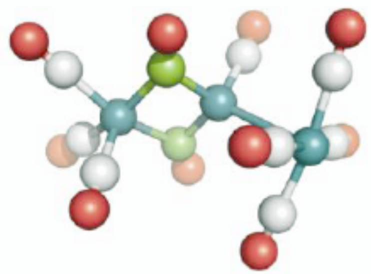
Species		Experiment (Å)	DFT (Å)
$Ru_3(CO)_{12}$		Ru-Ru: 2.88	2.93
Intermediate I, $Ru_3(CO)_{11}(\mu\text{-}CO)$		Ru1-Ru2: 2.86 Ru1-Ru3: 5.05 Ru2-Ru3: 3.12	2.91 5.14 3.17
Intermediate II, $Ru_3(CO)_{10}(\mu\text{-}CO)$		Ru1-Ru2: 2.76 Ru1-Ru3: 2.89 Ru2-Ru3: 2.79	2.81 2.94 2.84
Intermediate III ($Ru_3(CO)_{10}$)		Ru1-Ru2: 2.66 Ru1-Ru3: 2.89 Ru2-Ru3: 2.68	2.71 2.94 2.73

X 0.983

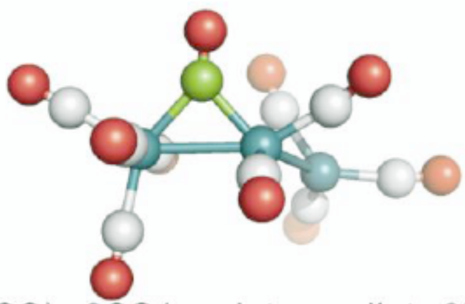
***Angew Chemie Int Ed*, 47, 5550-5553 (2008)**



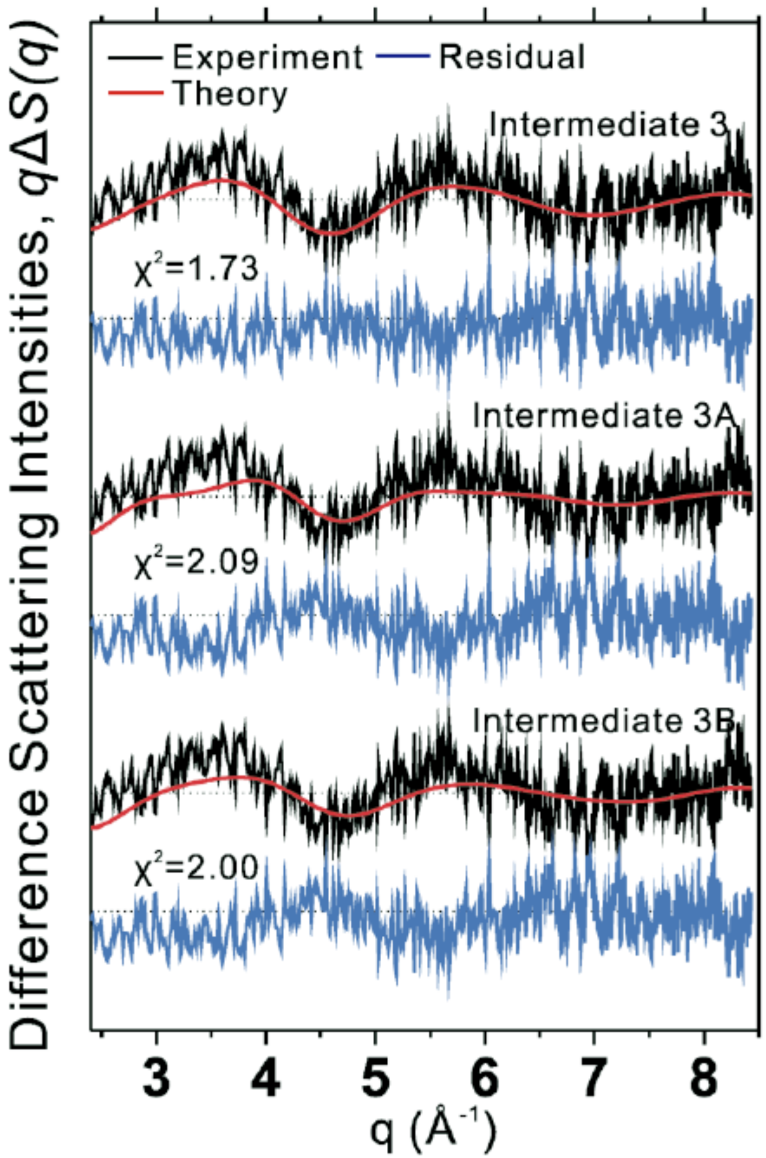
$Ru_3(CO)_{10}$ 2CO loss Intermediate 3, ^1A

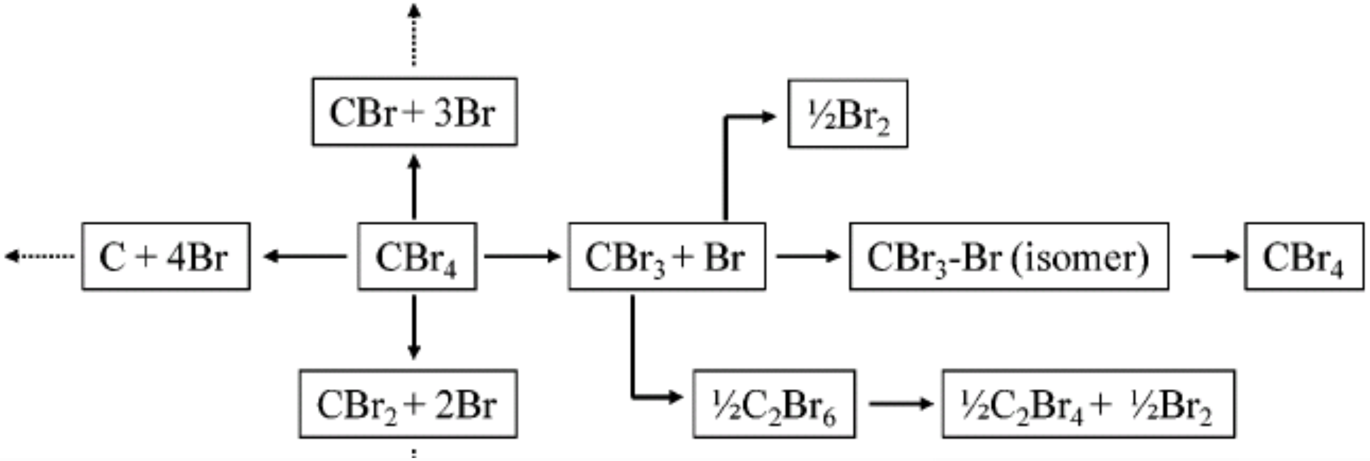


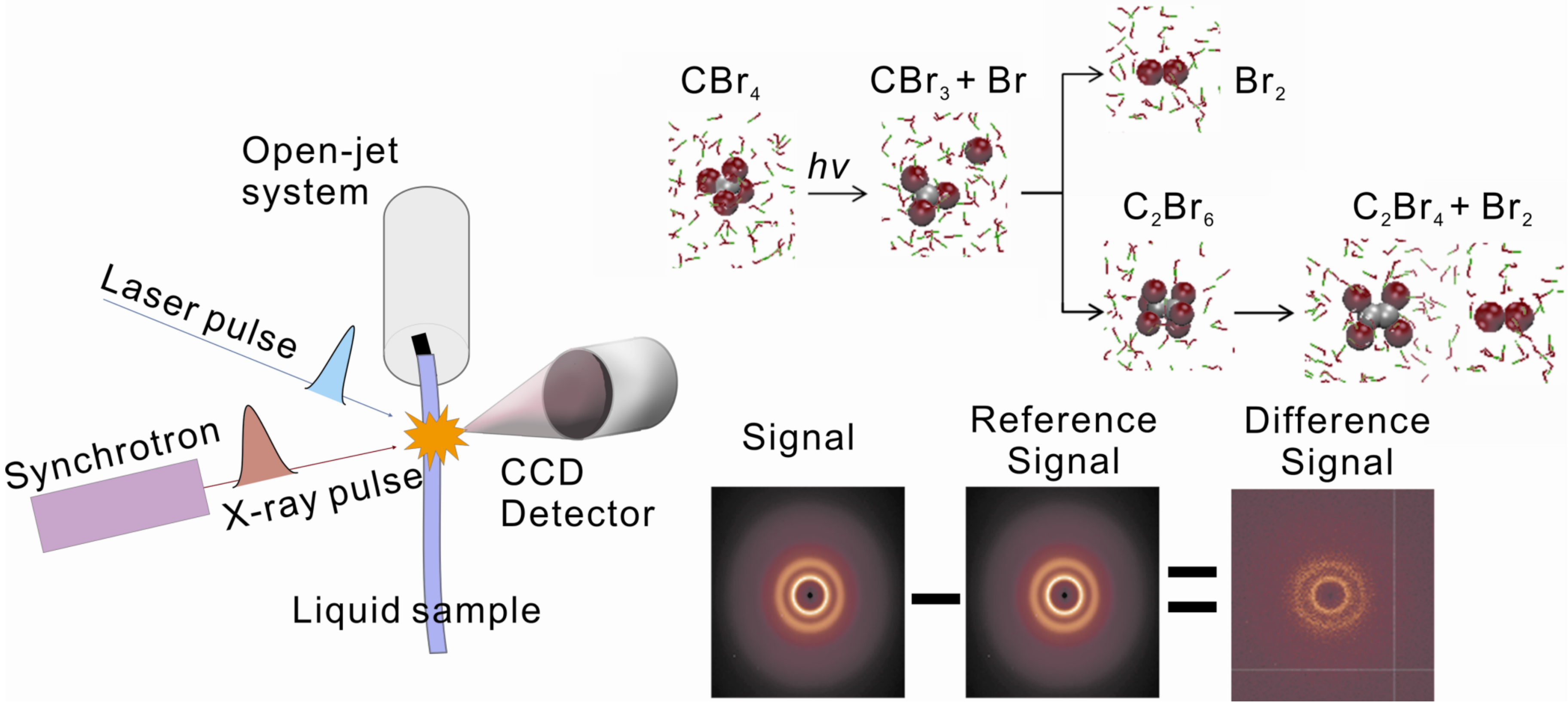
$Ru_3(CO)_{10}$ 2CO loss Intermediate 3A, ^1A

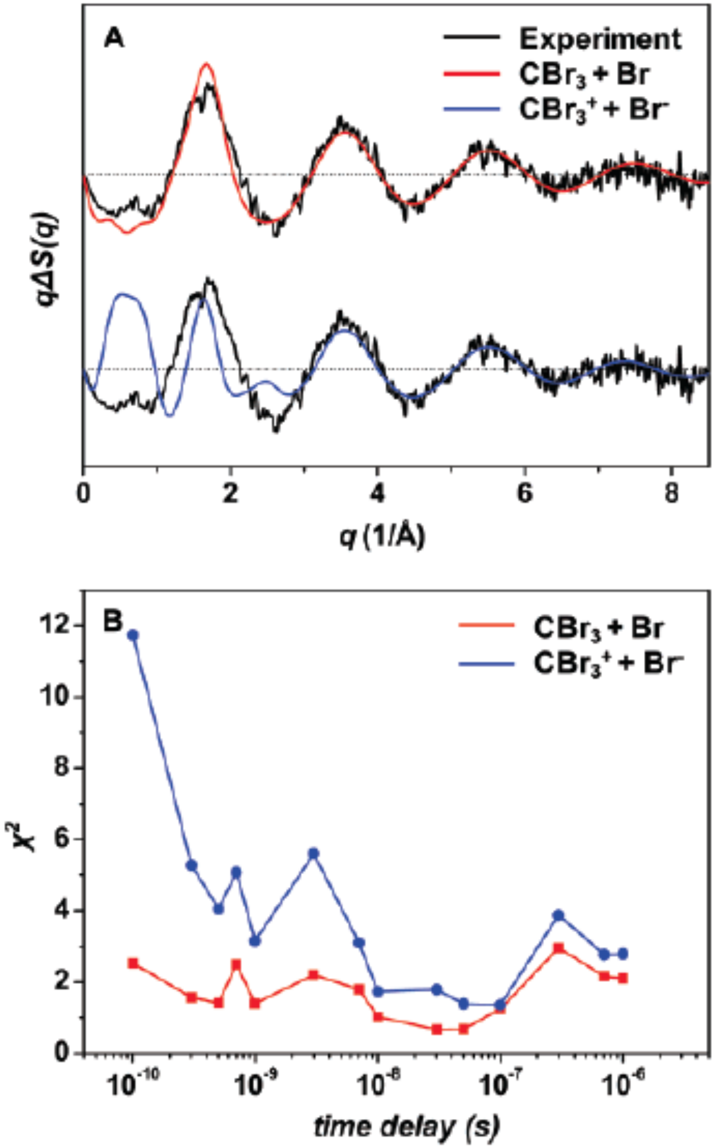


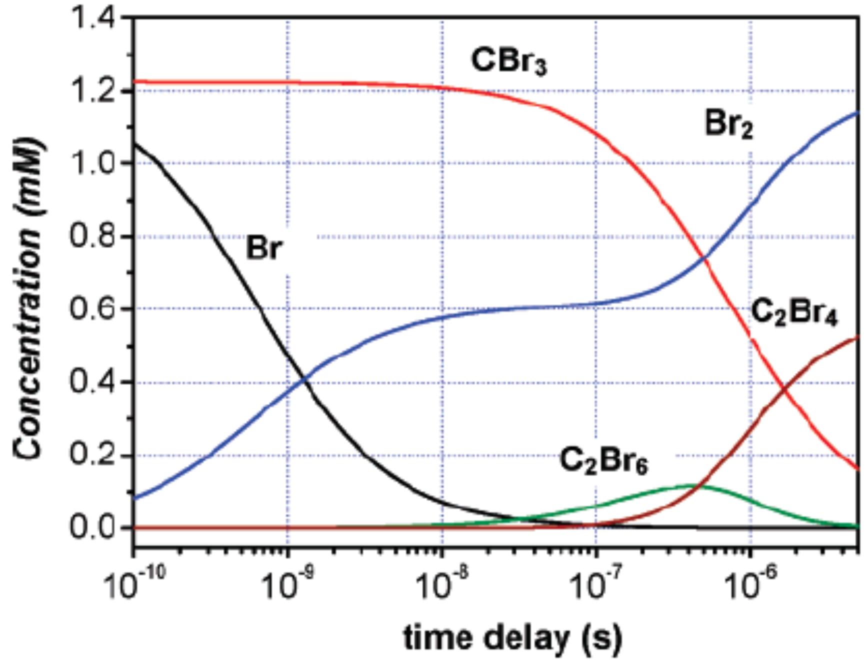
$Ru_3(CO)_{10}$ 2CO loss Intermediate 3B, ^1A



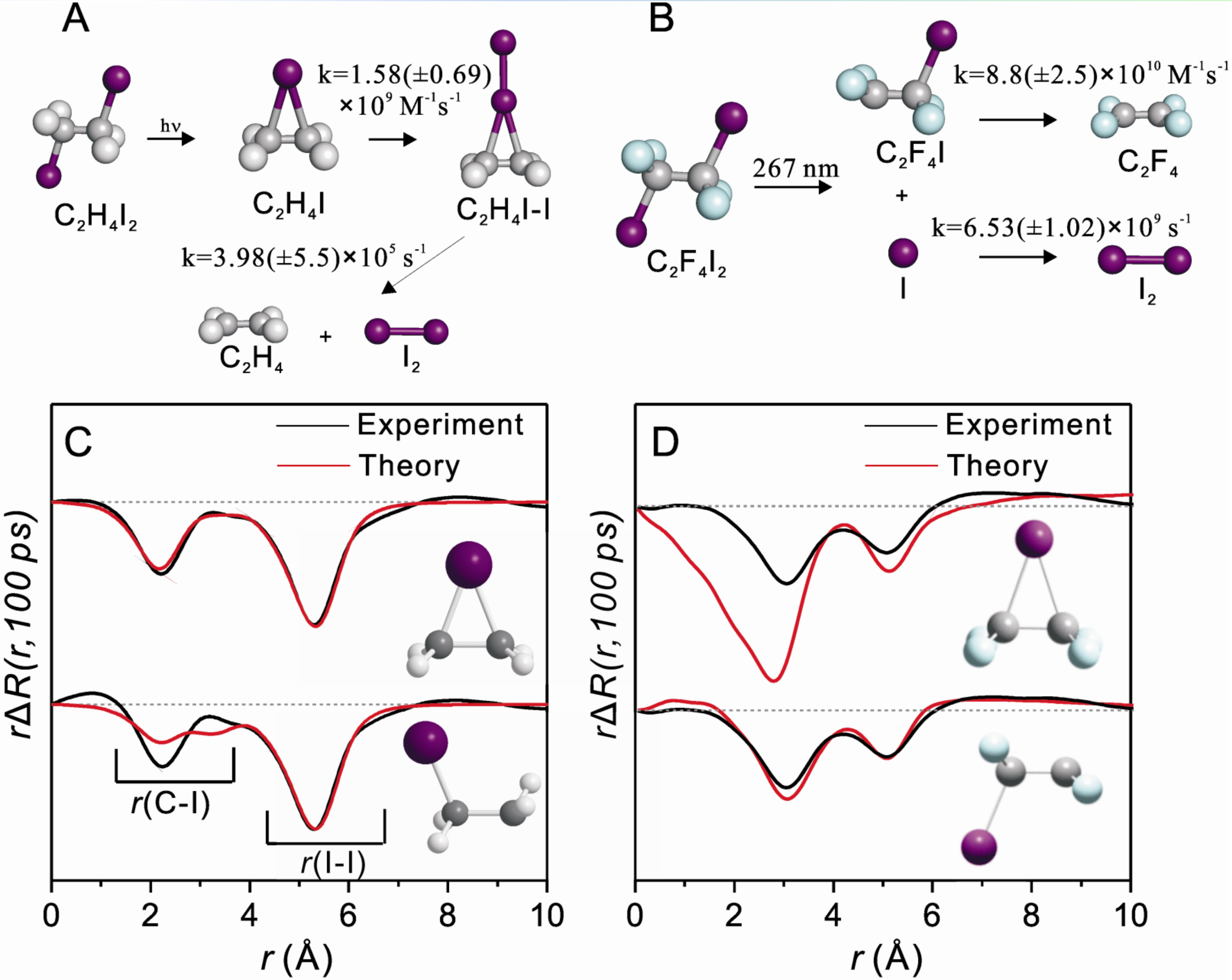

CBr + 3Br
½Br$_2$
C + 4Br
CBr$_4$
CBr$_3$ + Br
CBr$_3$-Br (isomer)
CBr$_4$
CBr$_2$ + 2Br
½C$_2$Br$_6$
½C$_2$Br$_4$ + ½Br$_2$


CBr$_4$
CBr$_3$ + Br
Br$_2$
hv
C$_2$Br$_6$
C$_2$Br$_4$ + Br$_2$
Open-jet system
Laser pulse
Synchrotron
X-ray pulse
CCD Detector
Liquid sample
Signal
Reference Signal
Difference Signal





JACS, 129, 13584–13591 (2007
)♪



***Science*, 309, 1223-1227 (2005)**

***JACS*, 130, 5834-5835 (2008)**

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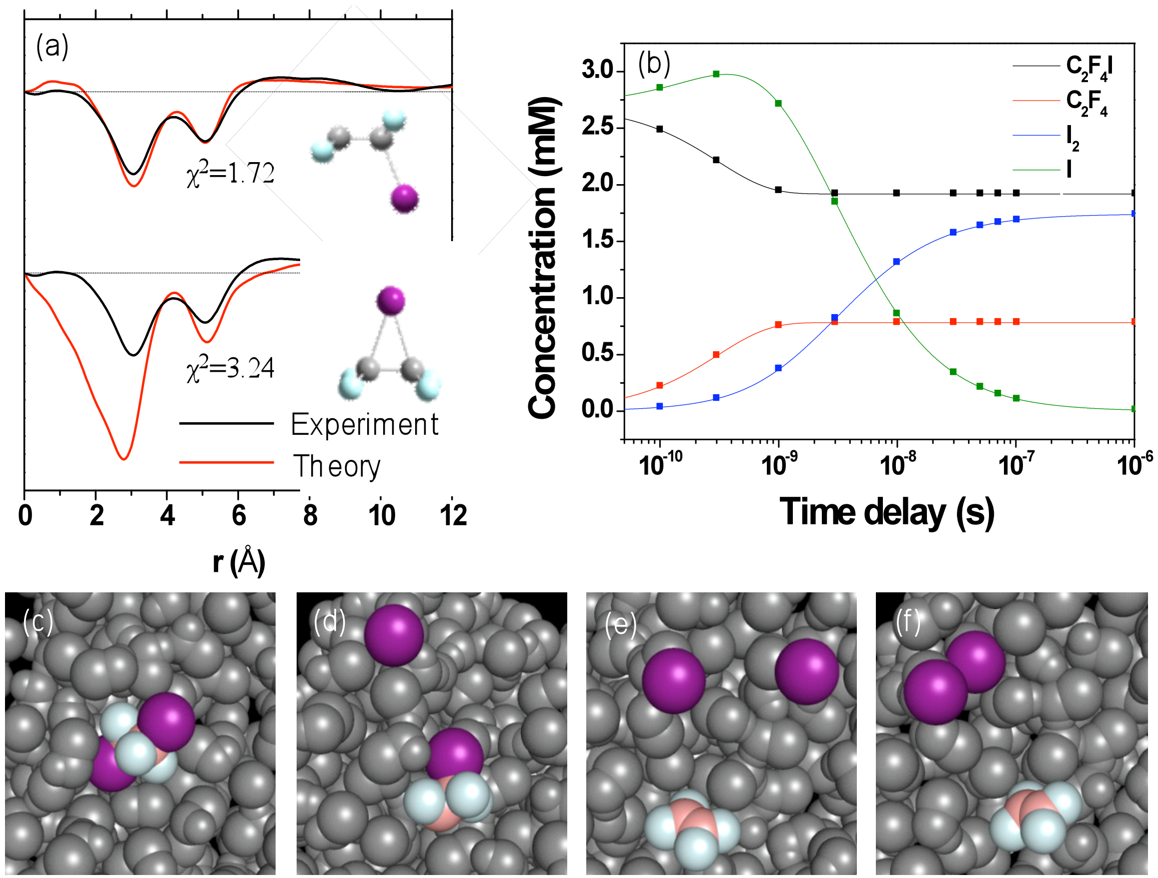
Dynamics of $C_2F_4I_2$ in solution

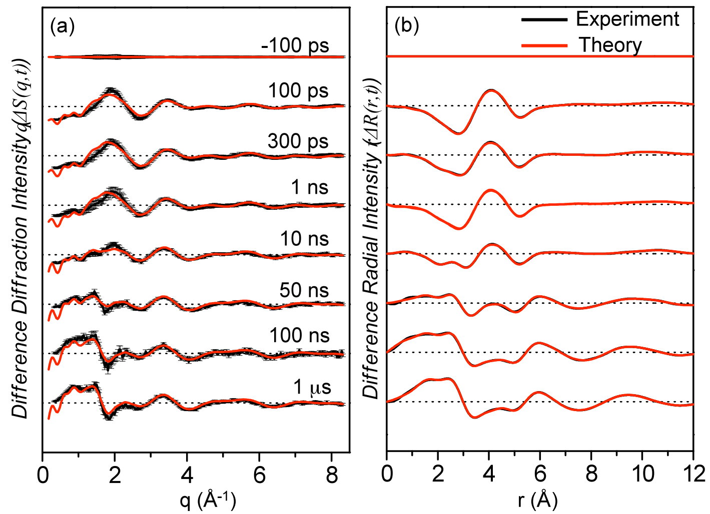
- Contrast with $C_2H_4I_2$

- Comparison with $C_2F_4I_2$ in gas phase (Ultrafast electron diffraction):

~300 ps vs. ~23 ps
~20% vs. 55%
for $C_2F_4I \rightarrow C_2F_4 + I$



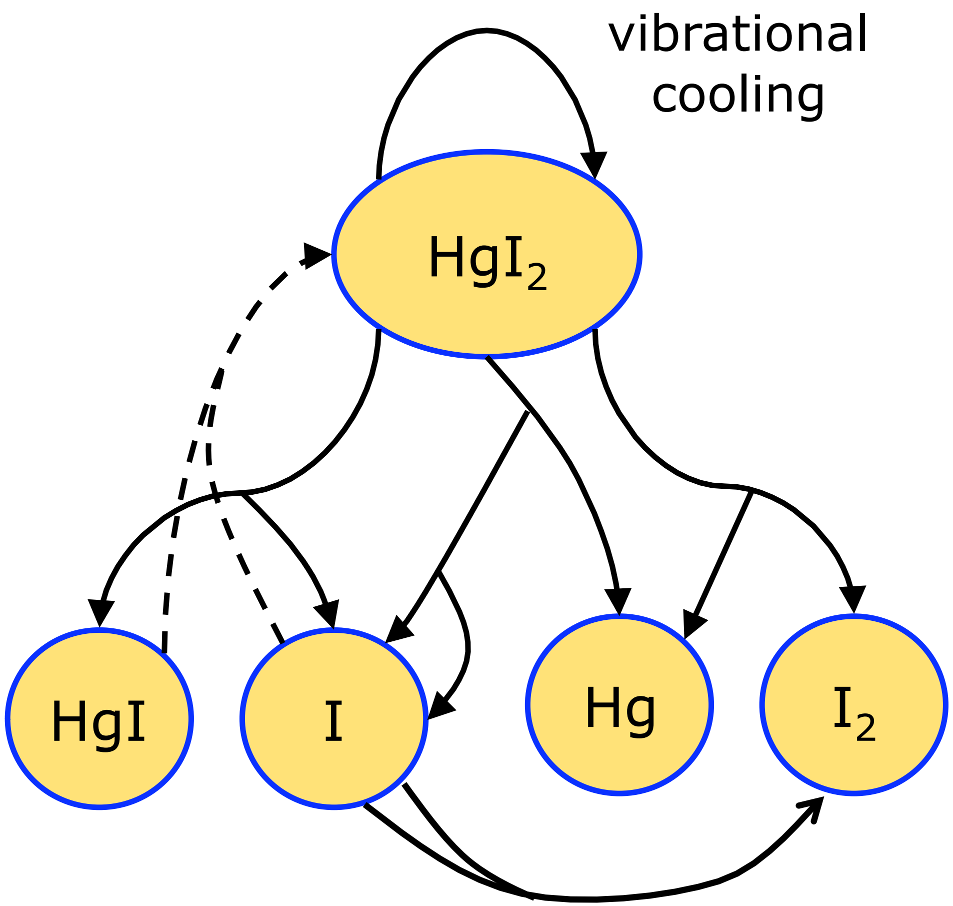
$C_2F_4I_2$ $C_2F_4I + I$ $C_2F_4 + 2I$ $C_2F_4 + I_2$



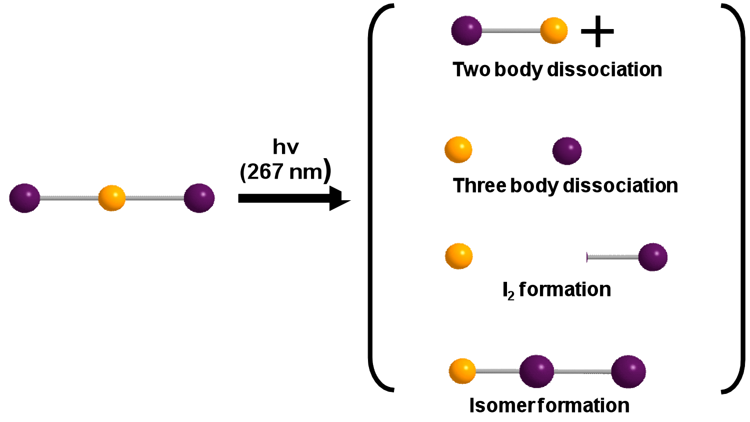
JACS, 130, 5834–5835 (2008)

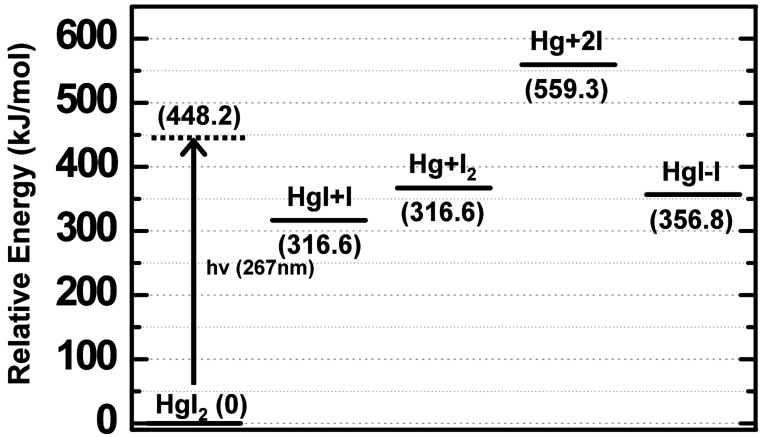
Gas Phase♪	Solution Phase♪
for $C_2F_4I \rightarrow C_2F_4 + I$	for $C_2F_4I \rightarrow C_2F_4 + I$
Ultrafast electron diffraction	Time-Resolved X-ray Solution Scattering
Time Scale: ~23 ps	Time Scale: ~300 ps
Fraction: 55%	Fraction: 20%

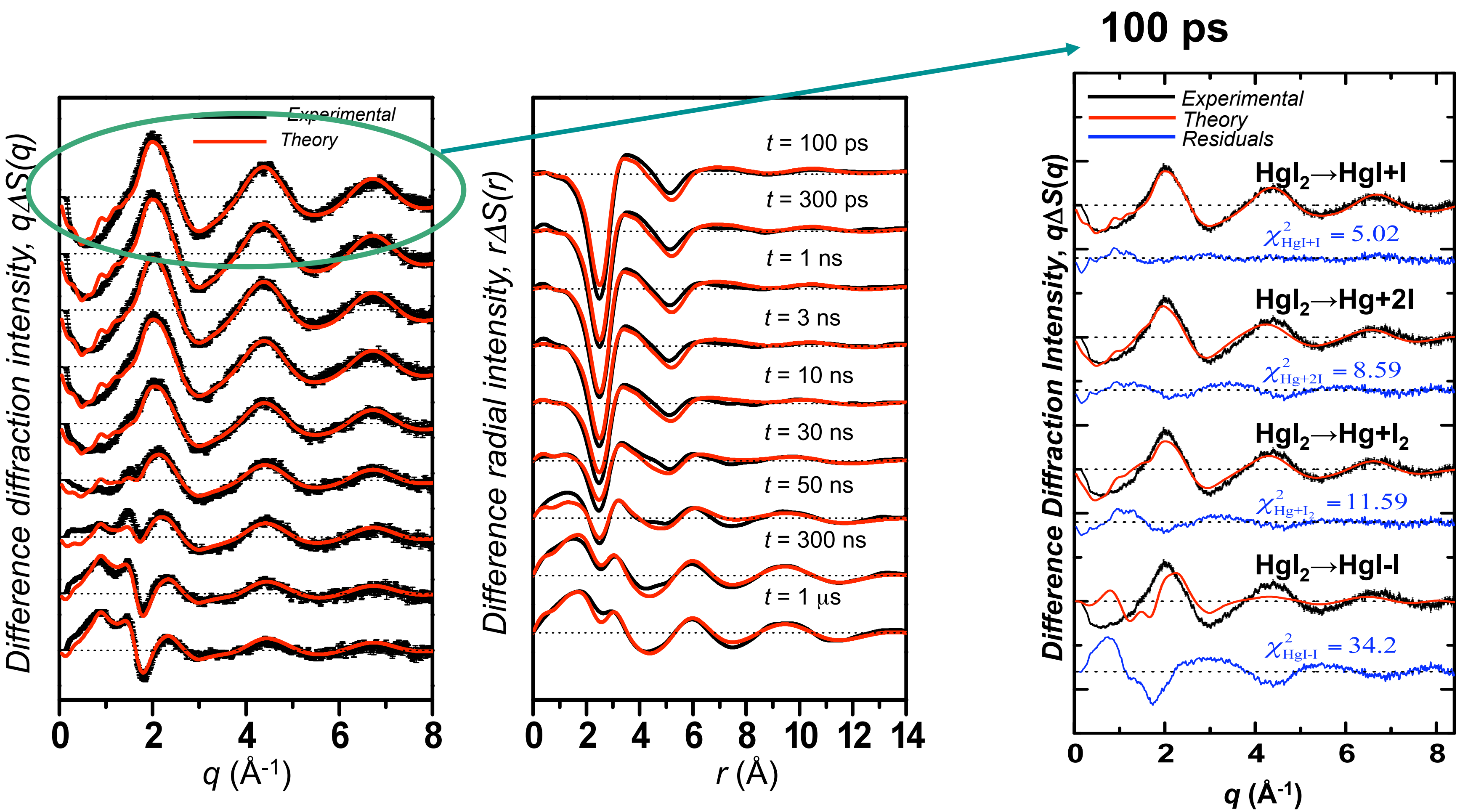
Structural Dynamics of HgI_2



In the gas phase, both HgI+I and Hg + 2I are observed.♪





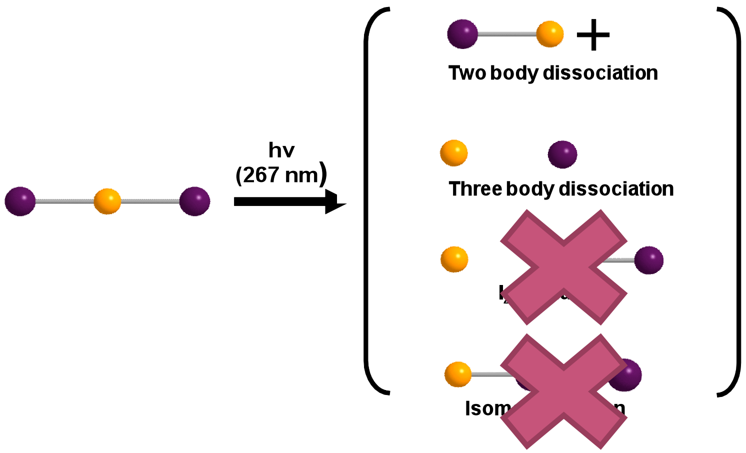


PNAS, 103, 9410-9415 (2006)♪

Gas Phase♪

Photolysis of HgI_2 at 267 nm

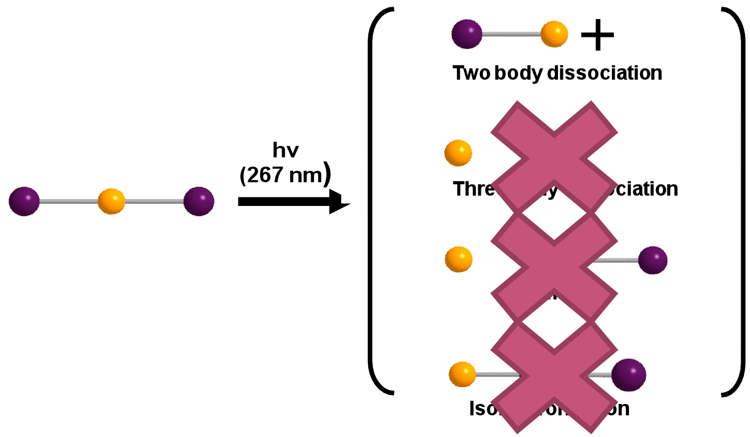
Femtosecond Mass spectrometry



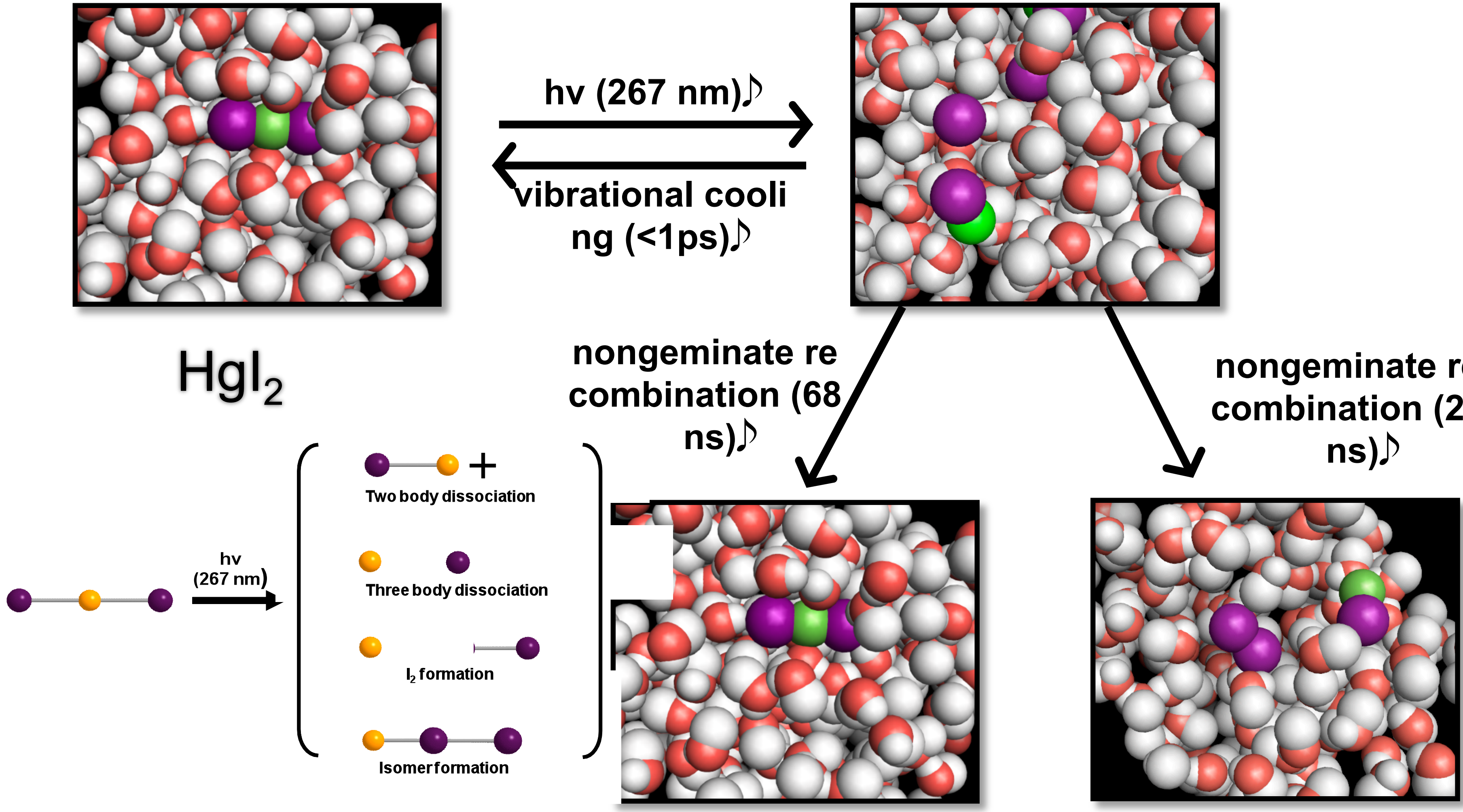
Solution Phase♪

Photolysis of HgI_2 at 267 nm

Time-Resolved X-ray Solution Scattering



Reaction in the solvent & Non-geminate recombination



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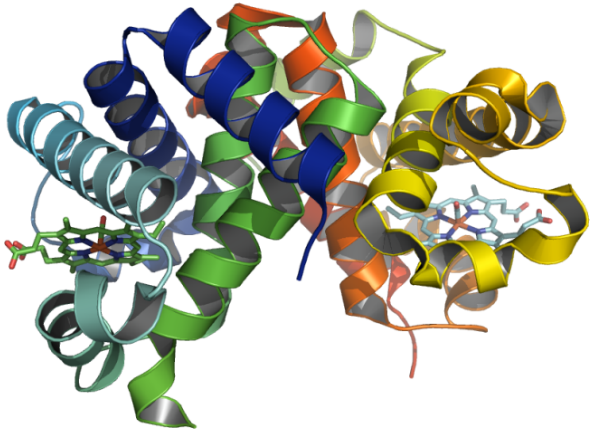
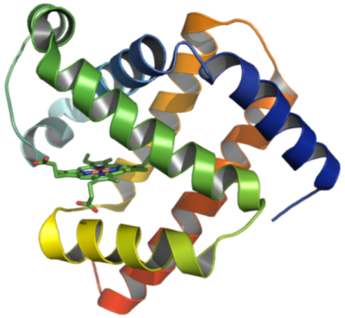
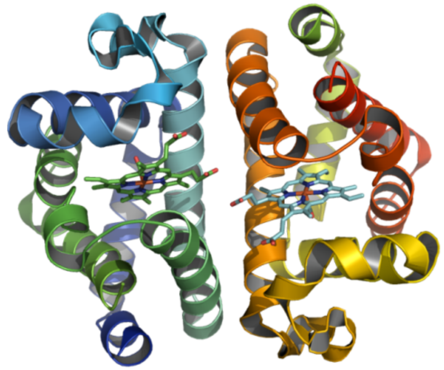
Protein Gallery



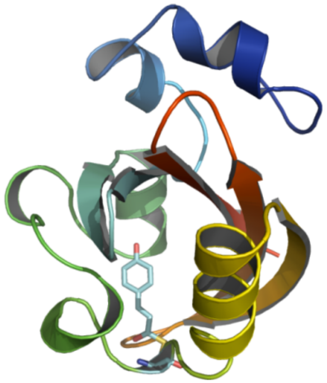
Hemoglobin (Hb)



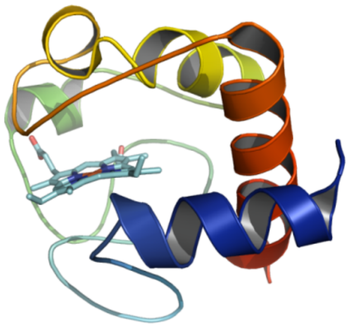
Myoglobin (Mb)



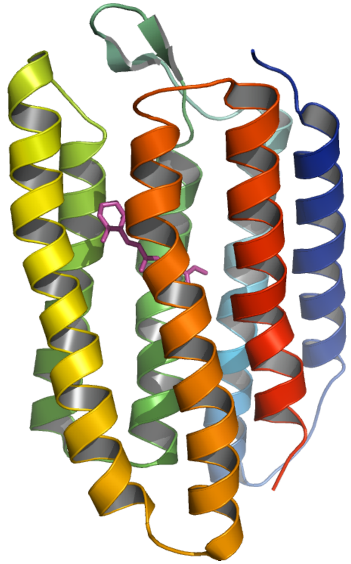
Dimeric Hemoglobin (HbI
)



Photoactive Yellow Protein
(PYP)



Cytochrome C (CytC)

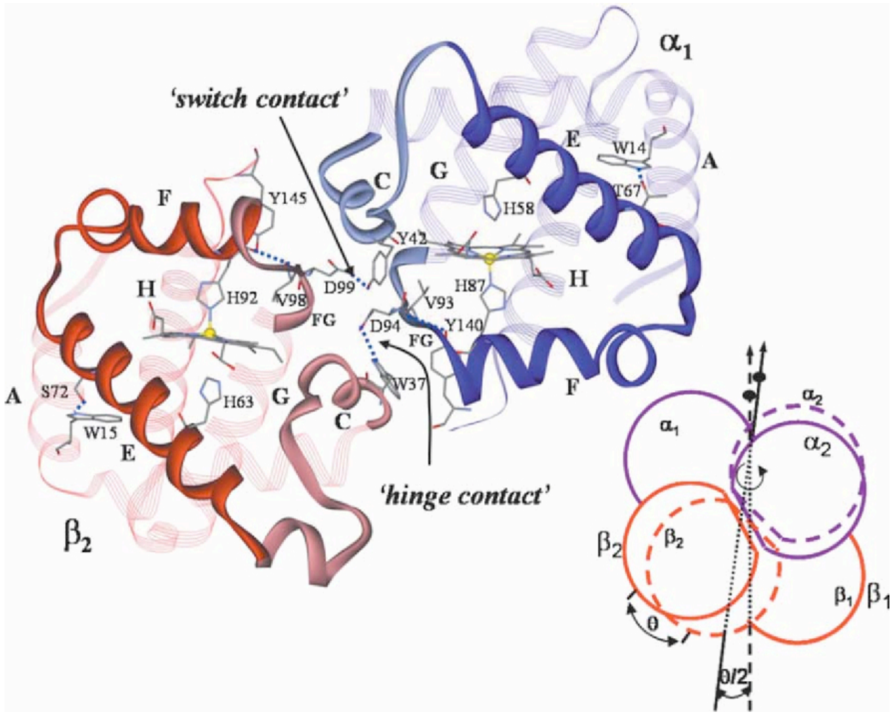


Bacteriorhodopsin (bR
)

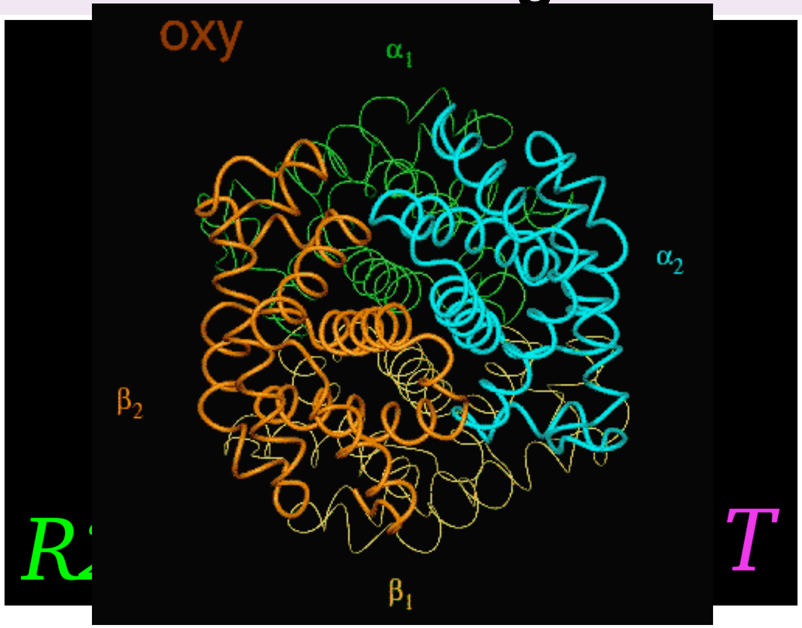
Hemoglobin (Hb) is a tetrameric liand-binding heme

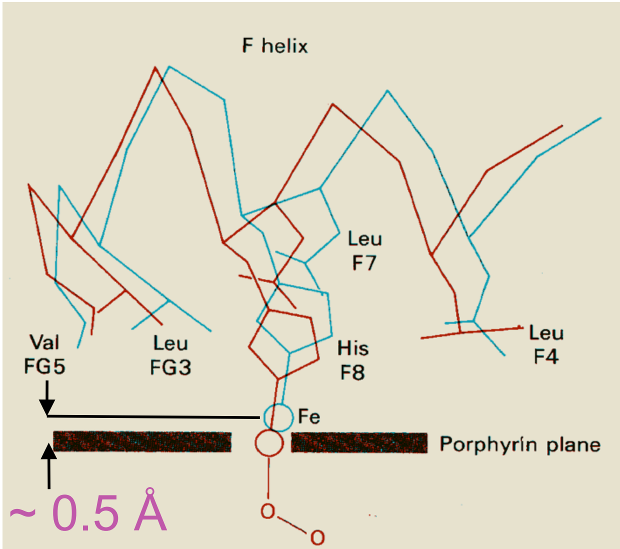
Oxy and Deoxy states have a different qu aternary conformation.

Displacement of Fe from plane of heme (~ 0.5 Å) triggers change of quaternary co nformation: ab *dimers rotate ~ 15° and tra nslate ~ 0.8 Å*

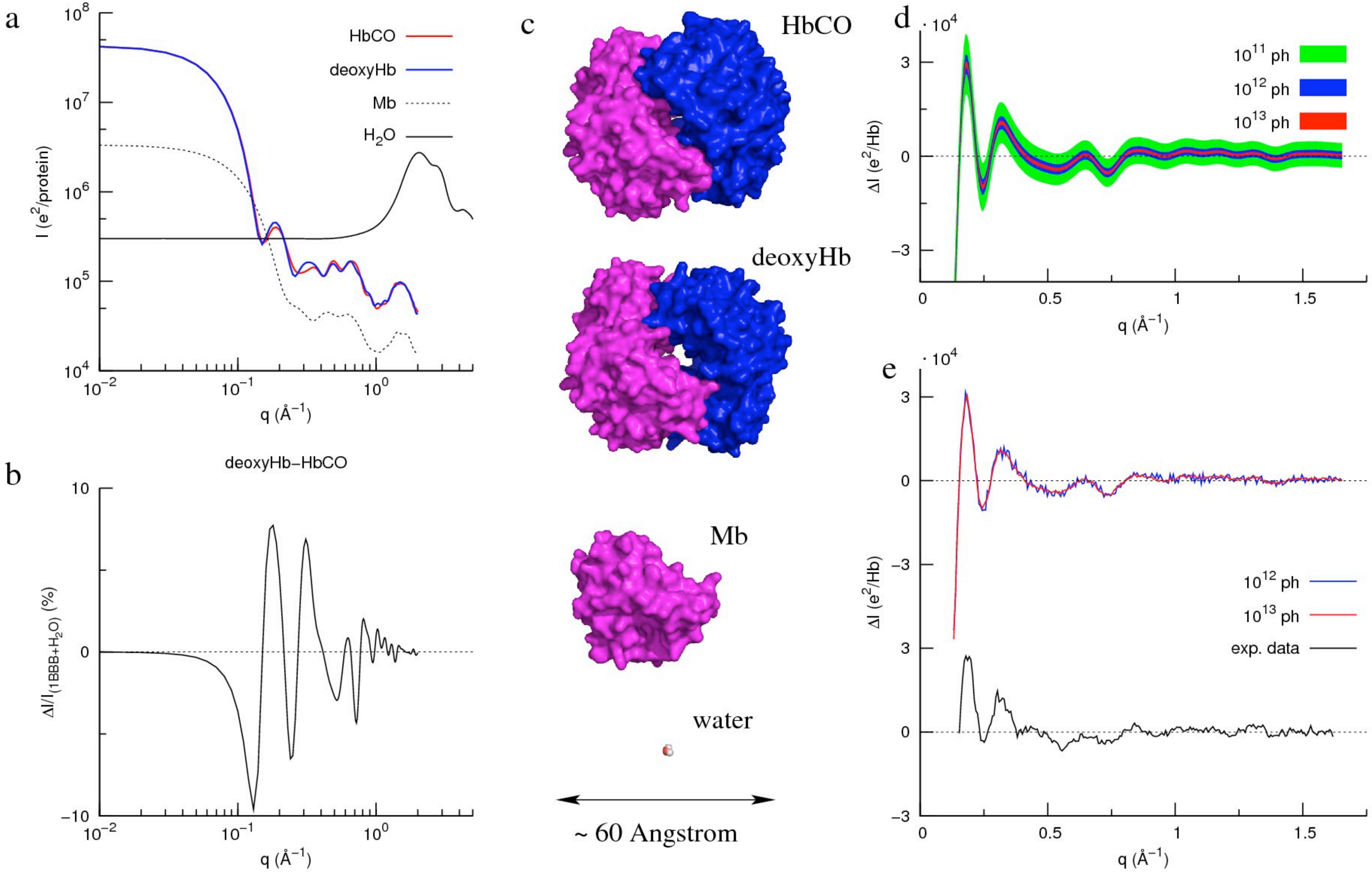


From Balakrishnan et al. J Mol Biol (2004) 340

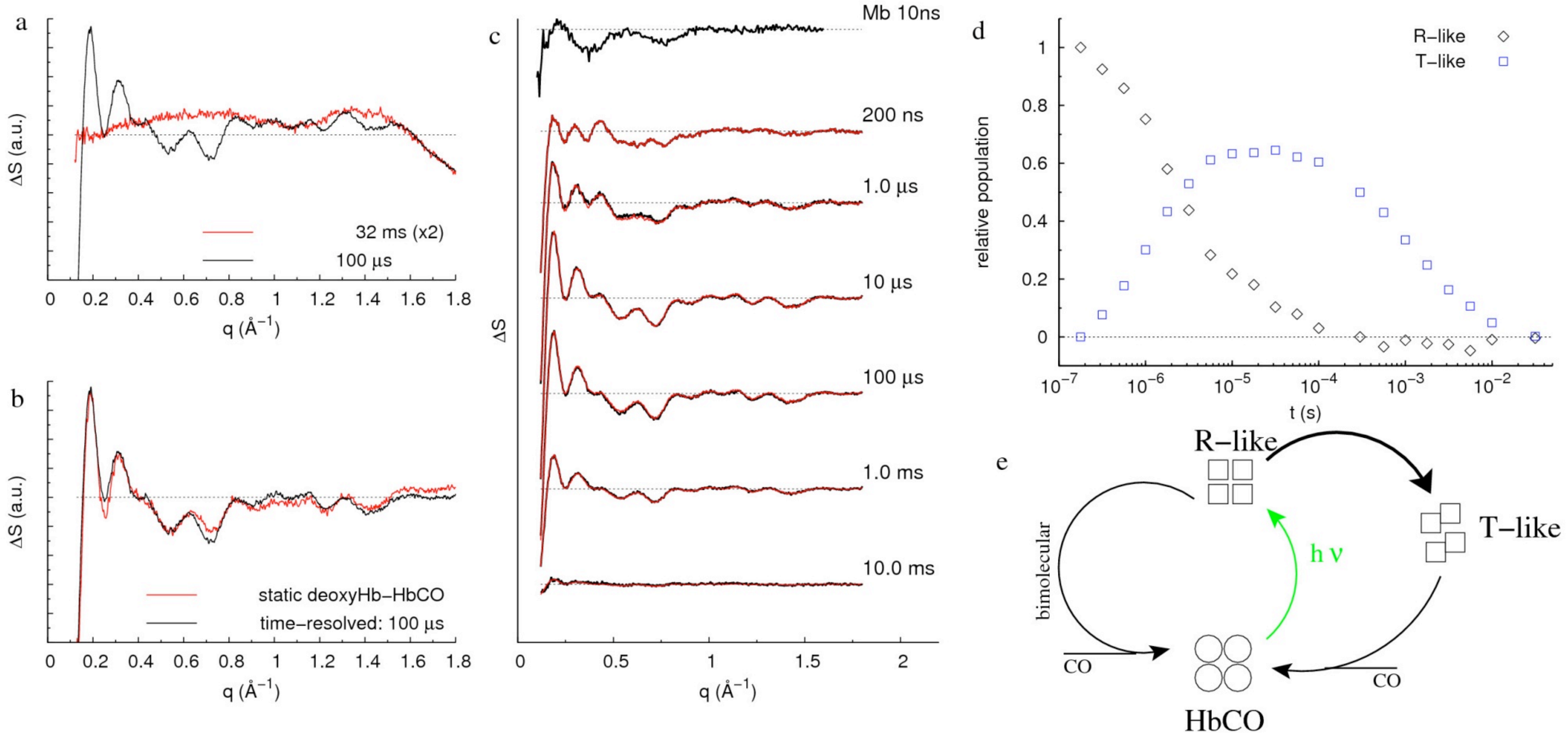




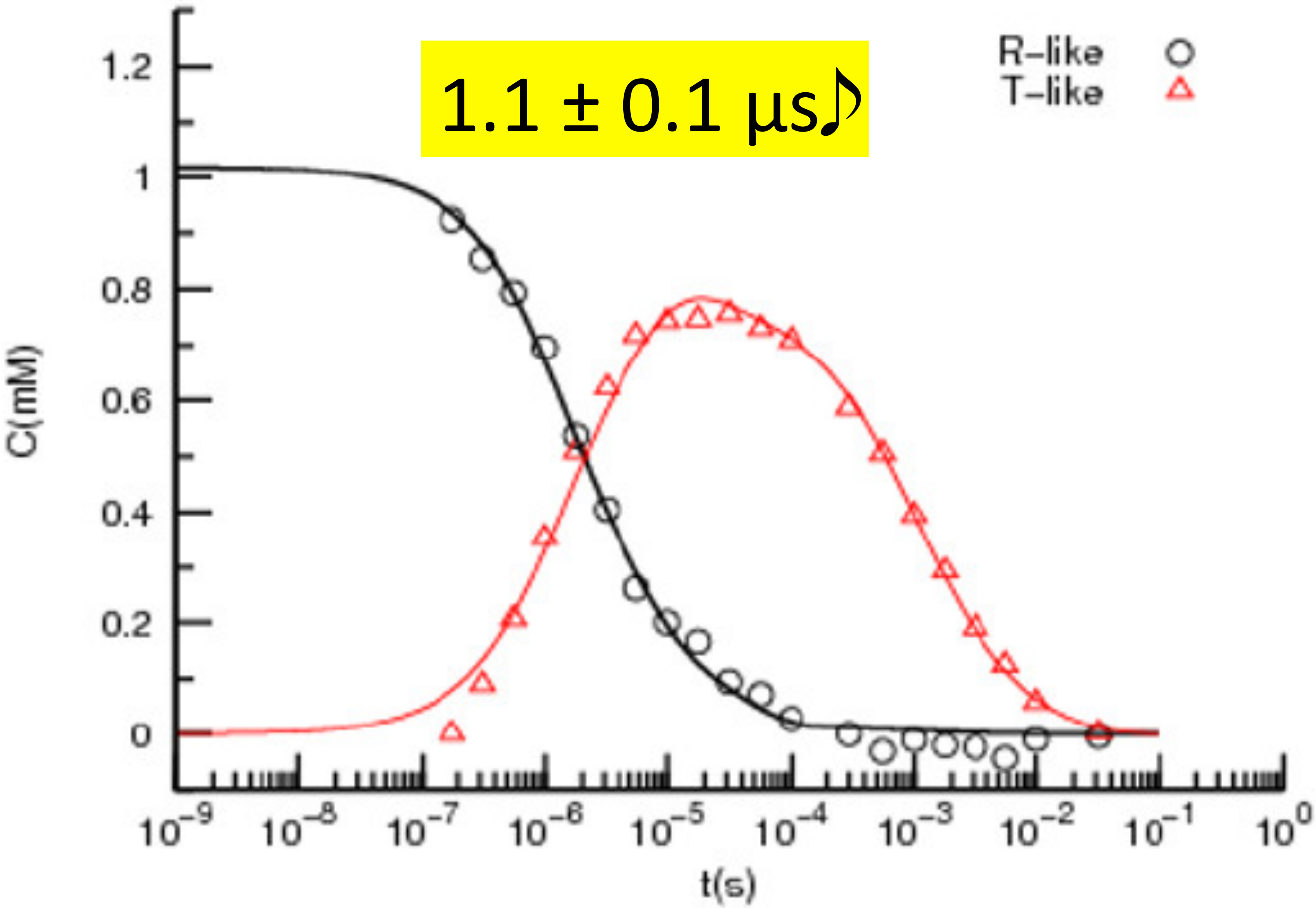
Plausibility of TR-WAXS



TR-WAXS of Hemolgobin



***Nature Methods*, 5, 881-887 (2008) (COVER in the October issue)**

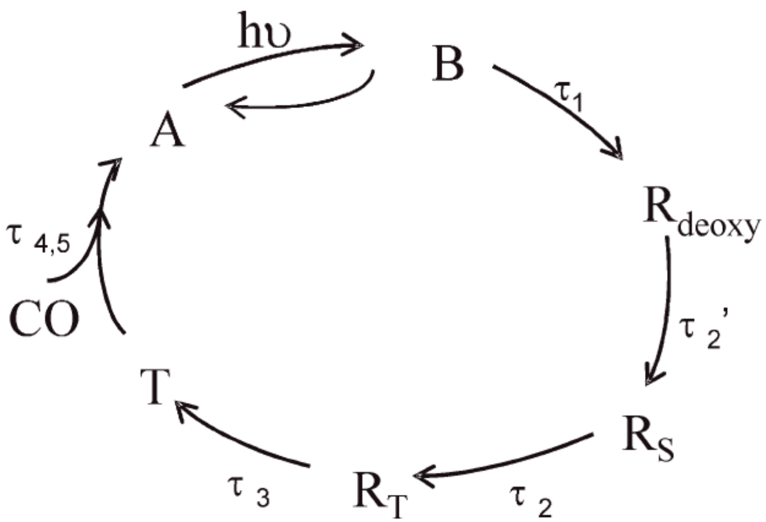


***Nature Methods*, (2008), in press (COVER in the October issue)**

Previous Optical Spectroscopy

TR Raman Spectroscopy

Balakrishnan et al. J Mol Biol (2004) 340



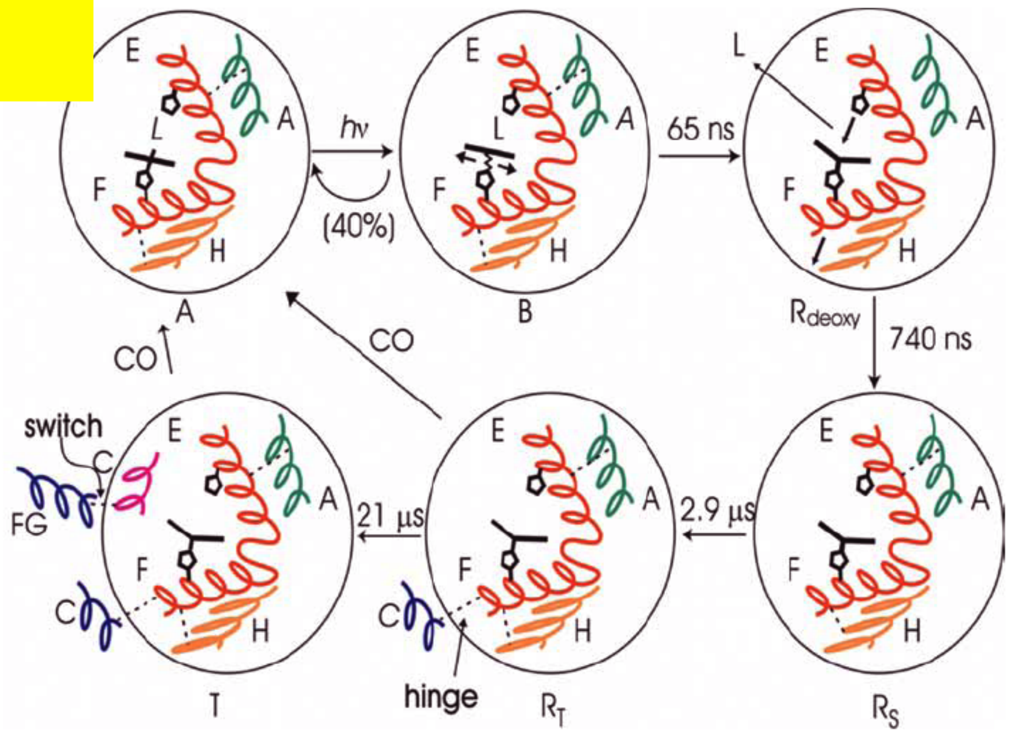
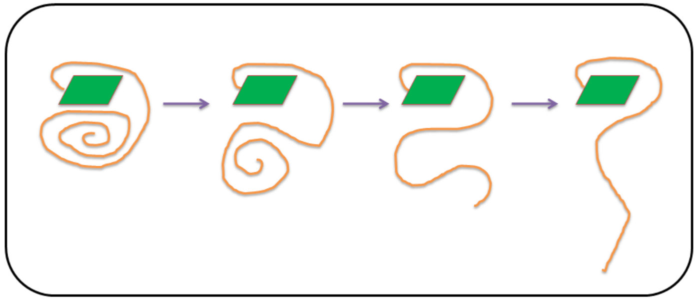
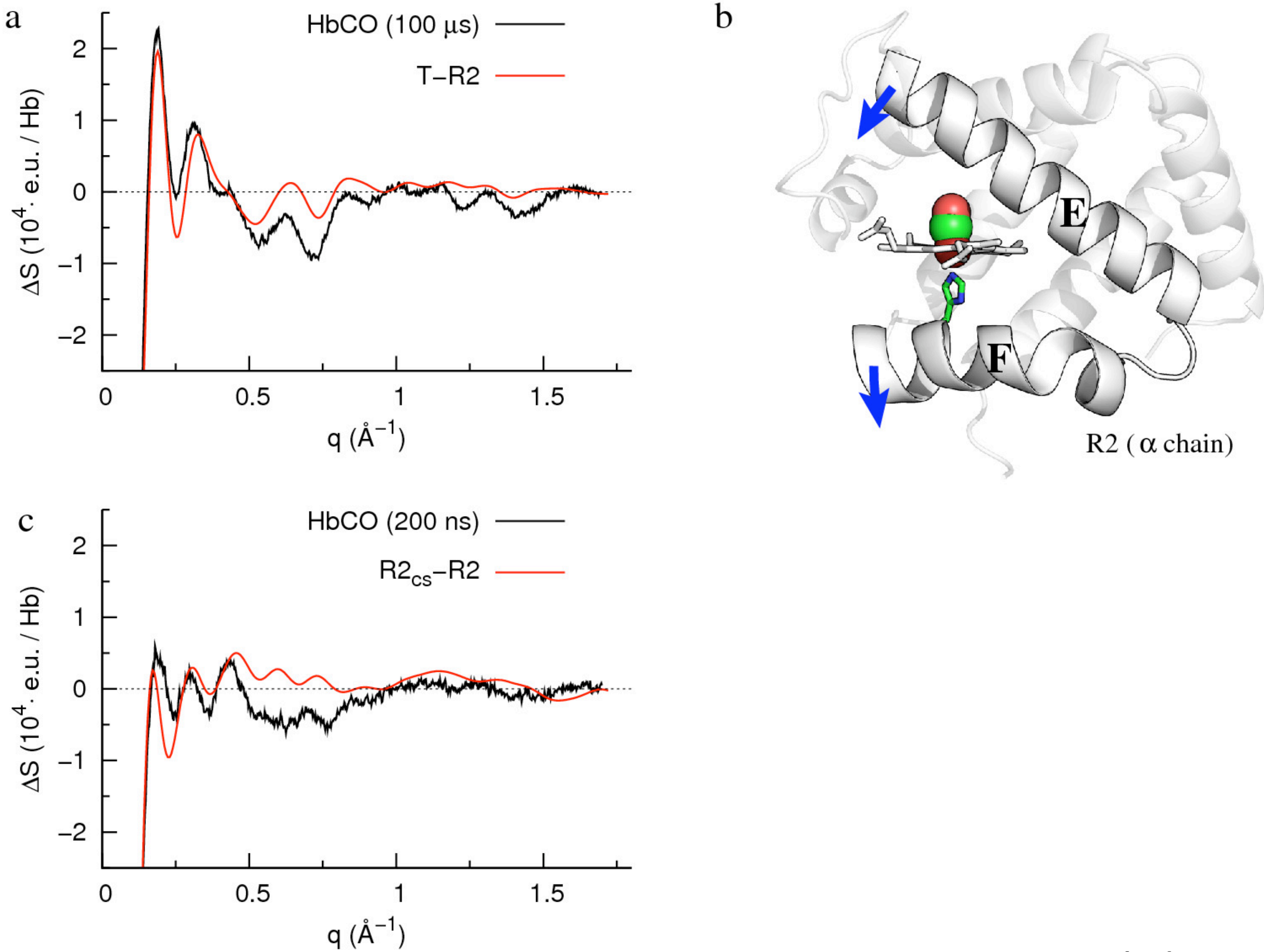


Table 1. Time constants, τ, of successive exponential phases fit to Soret absorption changes upon HbCO photolysis

Transient Absorption Spectroscopy

Study	$\tau_{1'}$ (ns)	τ_1 (ns)	τ_2 (μs)	τ_3 (μs)	τ_4 (μs)	τ_5 (ms)
Balakrishnan et al.		85	1.3	53	276	1.5
Goldbeck *et al.*[45]	22	109	2.1	44	183	3.9
Hofrichter *et al.*[48]		40	0.83	20	190	3.8





***Nature Methods*, 5, 881-887 (2008)**

Summary

- **TR X-ray Solution Scattering Resolves Structural Dynamics: Molecular structures of short-lived intermediates and the rebinding kinetics of such intermediates can be visualized by Time-Resolved X-ray Diffraction in Solution for various small molecules and proteins.**

- **TR X-ray Solution Scattering: A versatile tool to investigate solution-phase reaction dynamics, complementary to spectroscopy.**

"Visualizing Solution-Phase Reaction Dynamics with Time-Resolved X-ray Liquidography", Hyotcherl Ihee, *Acc. Chem. Res.* Publication Date (Web): December 31 (**2008**).♪

Acknowledgement

Collaborators (Experiment)

Michael Wulff (ESRF)
Marco Cammarata (ESRF)
Qingyu Kong (SOLEIL)
Anton Plech (U Konstanz)
Philip Anfinrud (NIH)
Friedrich Schotte (NIH)
Antonio Cupane (U Palermo)
Matteo Levantino (U Palermo)
Friederike Ewald (ESRF)
Maciej Lorenc (U Rennes)
Michel Koch (EMBL)
Emanuele Pontecorvo
Manuela Lo Russo (ESRF)
T. Narayanan (ESRF)
Shin-ichi Adachi (KEK)
Shin-ya Koshihara (TIT)
Jasper Van Thor (Imperial)

Collaborators (Theory)

Savo Bratos (UPMC)
Rudolphe Vuilleumier (UPMC)
Fabien Mirloup (UPMC)

Korean Members

Jae Hyuk Lee (KAIST)
Tae Kyu Kim (PNU)
Kyung Hwan Kim (KAIST)
Yang Ouk Jung (KAIST)
Sena Ahn (KAIST)
Jungkweon Choi (KAIST)
Joonghan Kim (KAIST)
Jangbae Kim (KAIST)
Youngmin Kim (KAIST)
Srinivasan Muniyappan (KAIST)
Cheol Hee Yang (KAIST)
Sunhong Jun (KAIST)
G. Prabhakar
Sehyung Eum (KAIST)
Youhong Lee (KAIST)

Financial Support

National Creative Research Initiatives (Center for Time-Resolved Diffraction) of MEST/KOSEF

Thank you