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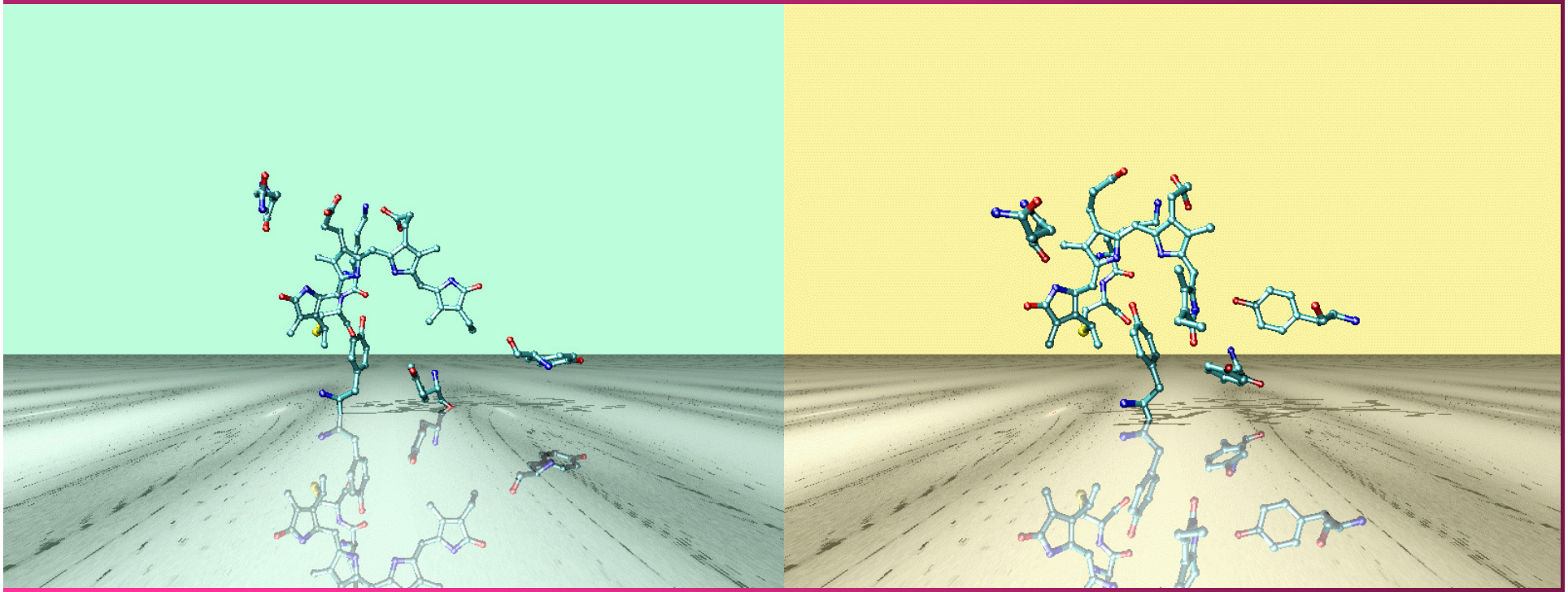
Analysis of Time-Resolved Macromolecular X-ray Data Kinetics and Atomic Structures of Transient States

Marius Schmidt



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The goal: structure and kinetic mechanism from TR-xray data

1.) visual inspection
- **signal yes/no**
- **where?**

2.) integration of difference electron density features
- **small/large volumes**
- **fit with trial functions**

3.) component analysis
- **global**
- **effective noise reduction (SVD-flattening)**
- **kinetics AND structures of the intermediates**

4.) outlook: kinetic on very fast time-scales

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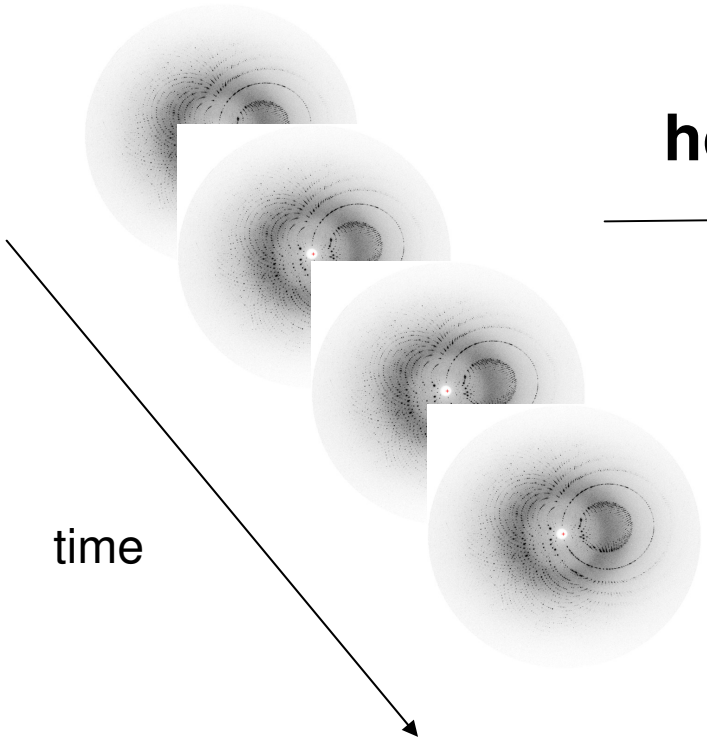
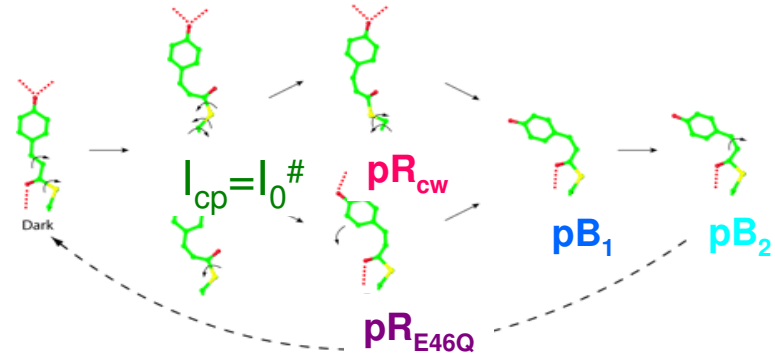
Laue data

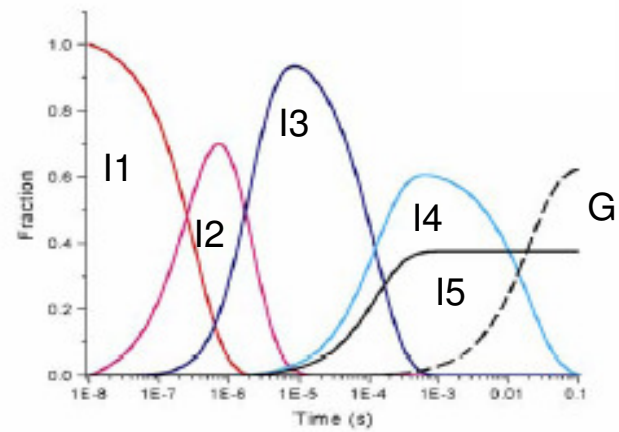


how ?

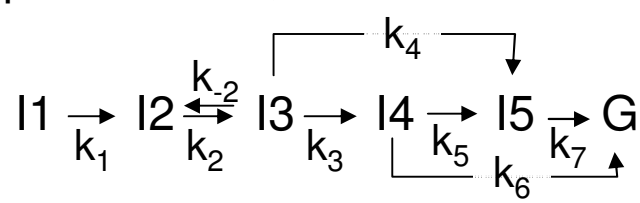
Structures of the Intermediates
(time-independent)



Concentrations (time-dependent)



Unique chemical, kinetic mechanism



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Difference structure factors:

noise is substantial

signal is distributed over all ΔF (small quantity per ΔF)

Structure Factors not linearly dependent on occupancy ≡ concentration

Difference electron density

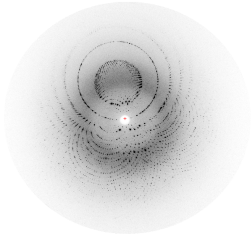
noise is randomly distributed,

signal is confined to small but spatially contiguous volumes

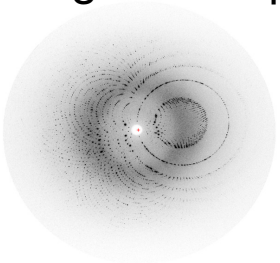
$\Rightarrow$ Power of the Fourier synthesis

Difference Electron density is ideal subject to be analyzed

time resolved scattering patterns



corresponding dark exposures



raw data

data reduction

LaueView, Precognition/Epinorm, Prow

time dependent data set

$|F(h,k,l,t)|$

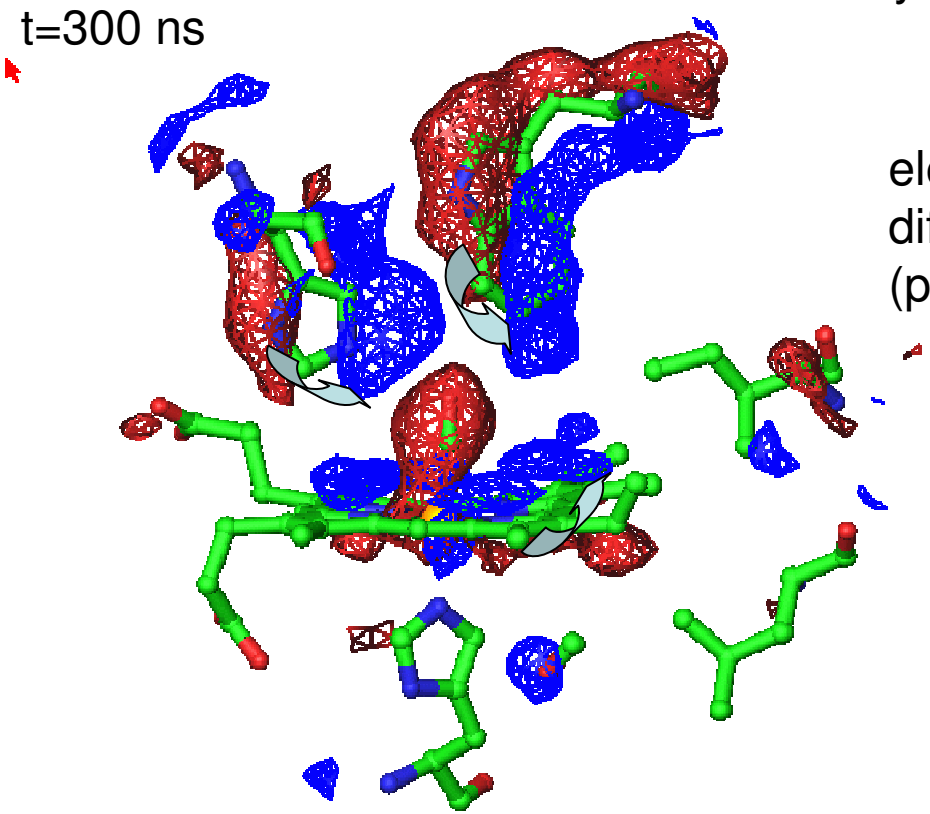
corresponding dark data set

$|F^D(h,k,l)|; \varphi^D_{hkl}$

$$\Delta F(h,k,l,t) = |F(h,k,l,t)| - |F_D(h,k,l)|; \varphi^D_{hkl}$$

difference electron density maps $\Delta\rho(x,y,z,t)$

t=300 ns

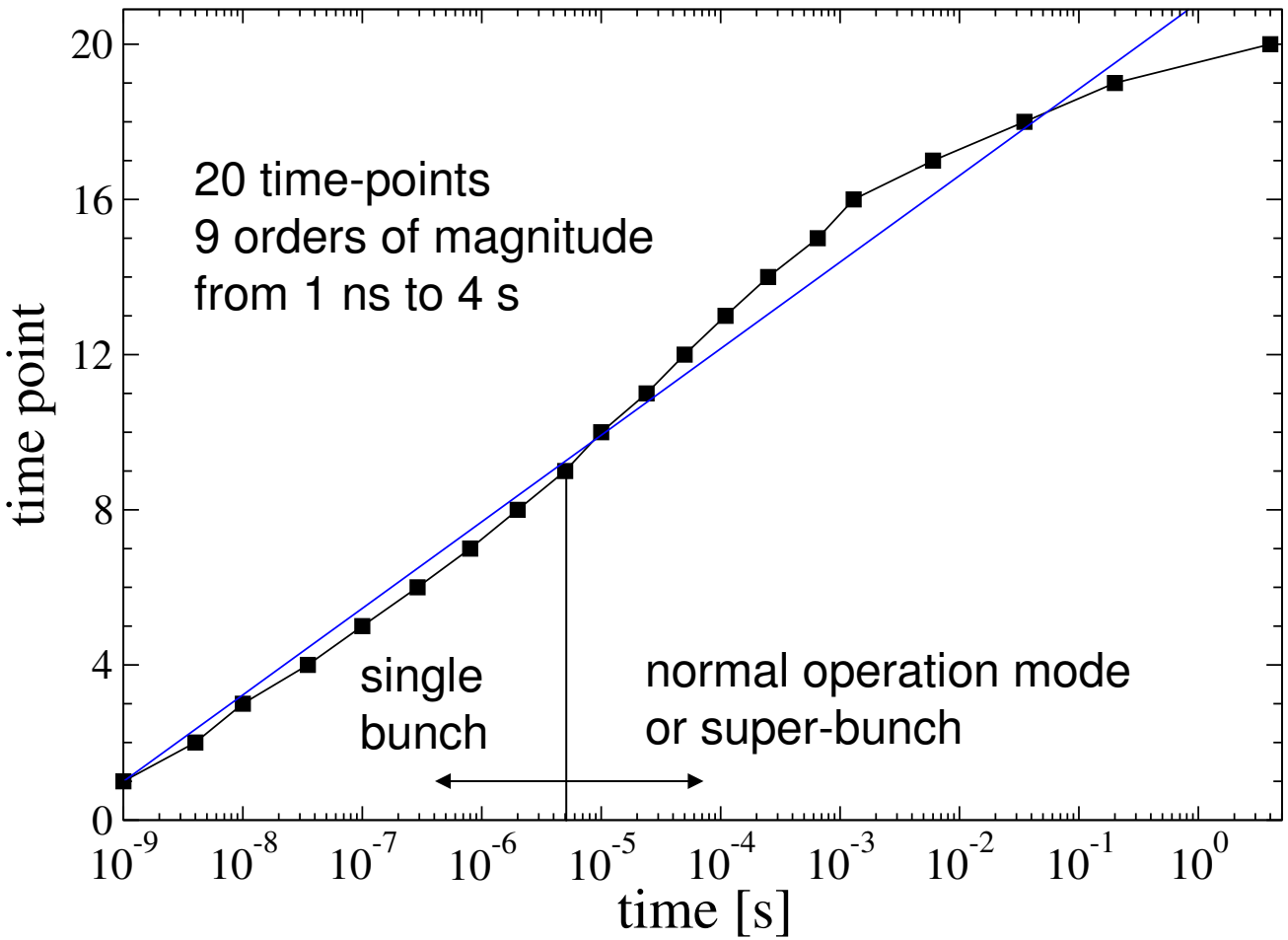


electrons moved from the red (negative) difference electron density to the blue (positive) difference electron density

Strategy

Follow the entire reaction from the beginning to the end after reaction initiation

collect data points equidistantly (linearly) in logarithmic time



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1.) Visual Inspection

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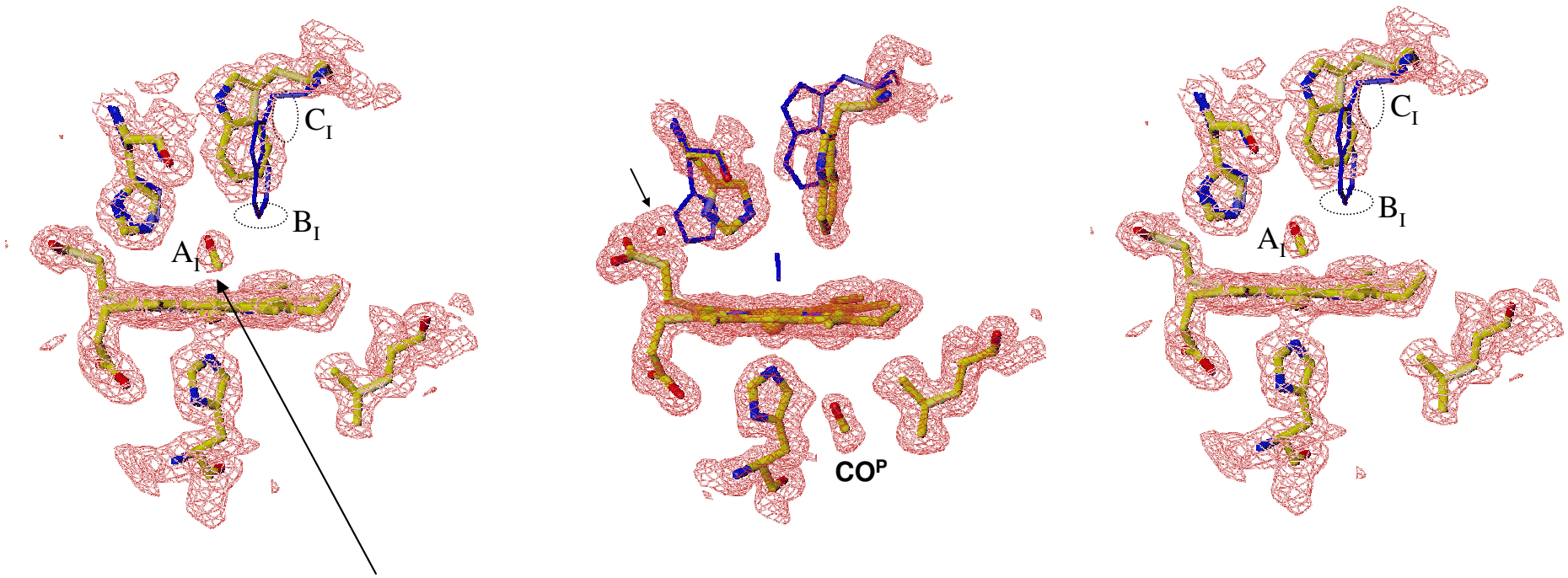
Photoflash Experiments on L29W Myoglobin

MbCO → Mb-deoxy + CO → MbCO

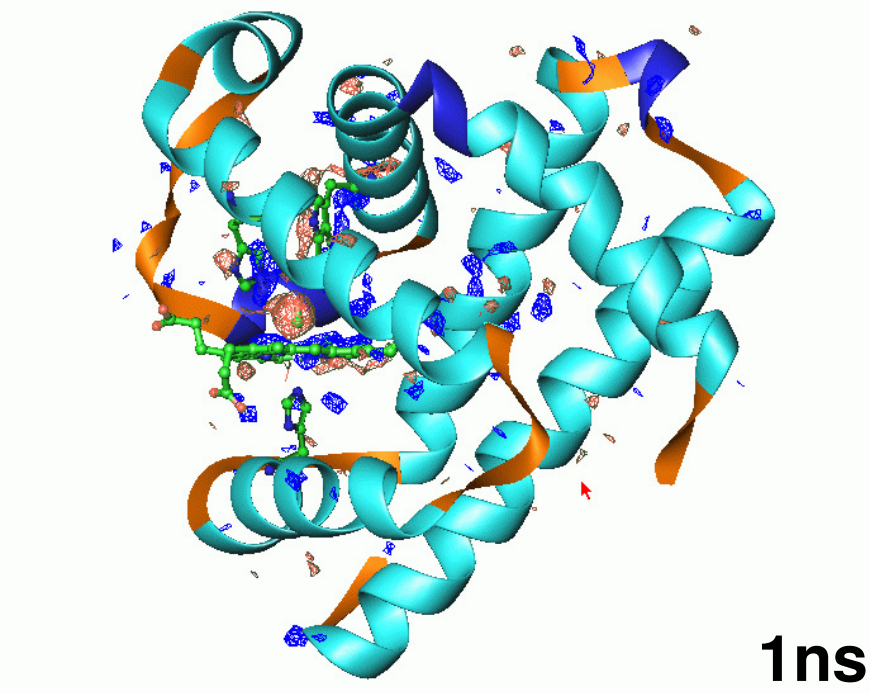
Dissociation
10 fs to 100 ns

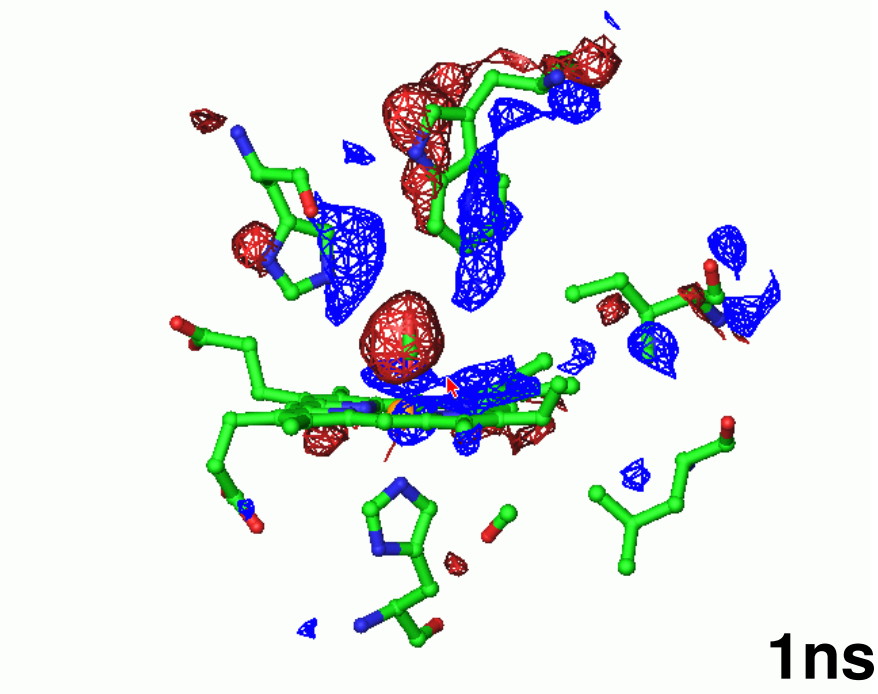
Rebinding
µs to ms (30 ms in L29W)



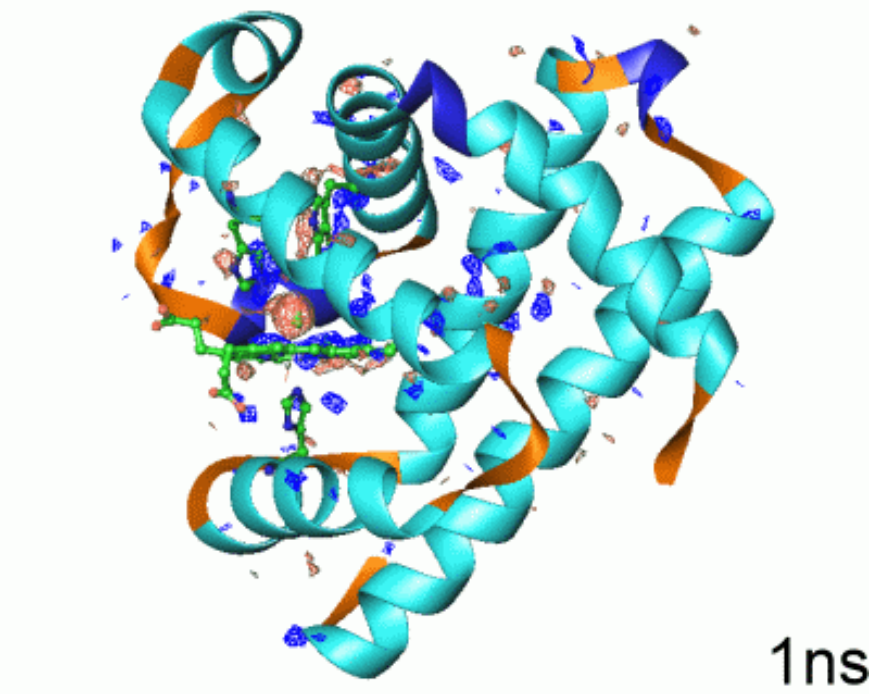
bond brakes once irradiated
by Laser light **(518 nm)**

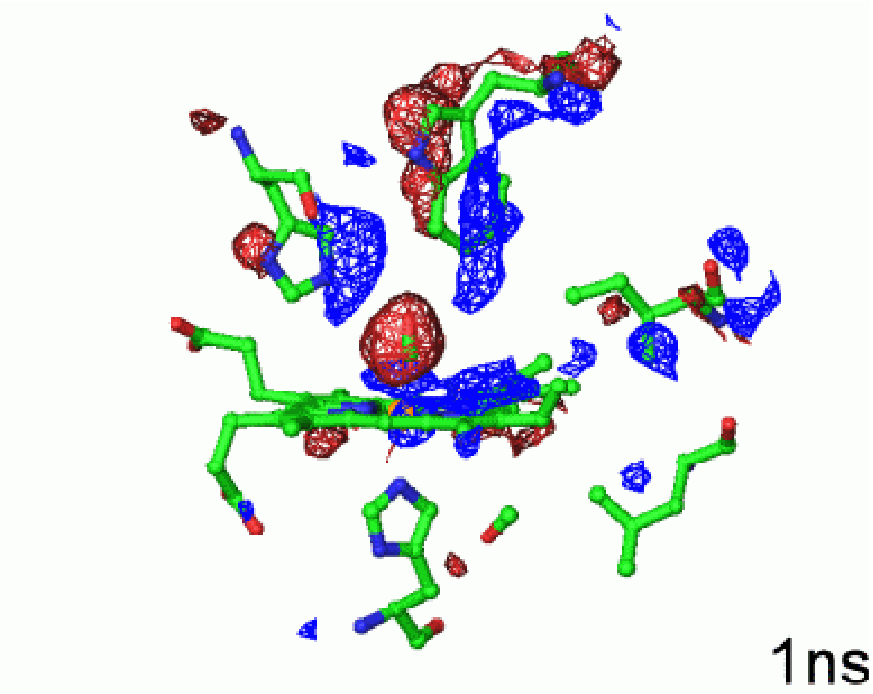
Movies, extreme slow-motion





Movies, extreme slow-motion





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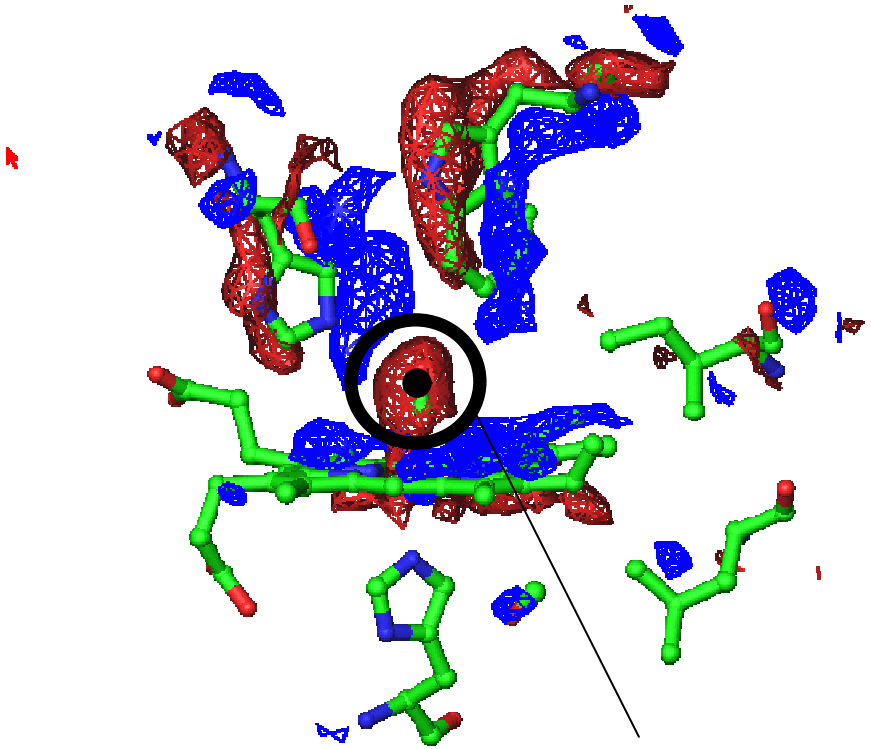
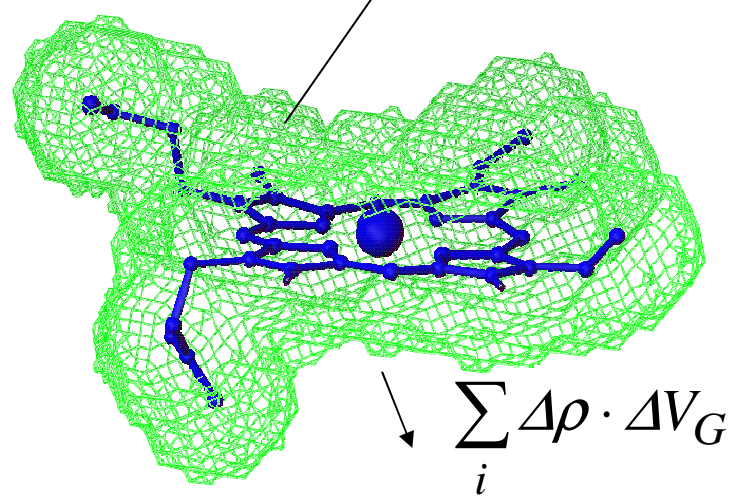
2.) Quantification by Integration
Fit of trial functions

Integration of difference electron density within a mask



integrate spherical volumes

mask out entire moieties

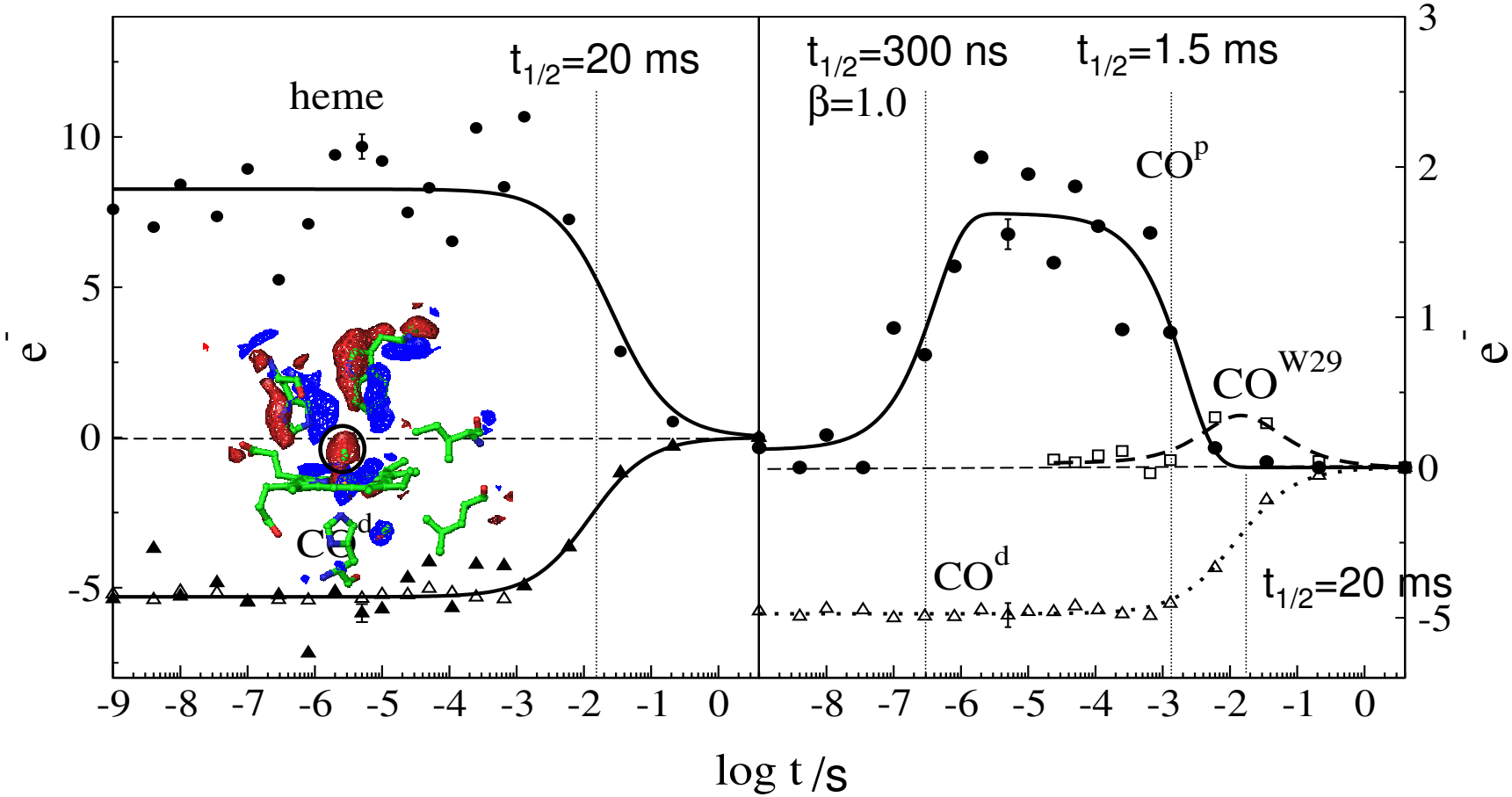


$$\sum_i \Delta\rho \cdot \Delta V_G$$

number of
displaced electrons

Programs for users:
Probe_v6: Srajer et al., 2001
Probe and Mask (PROMSK): Schmidt et al., 2005, Knapp et al., 2006

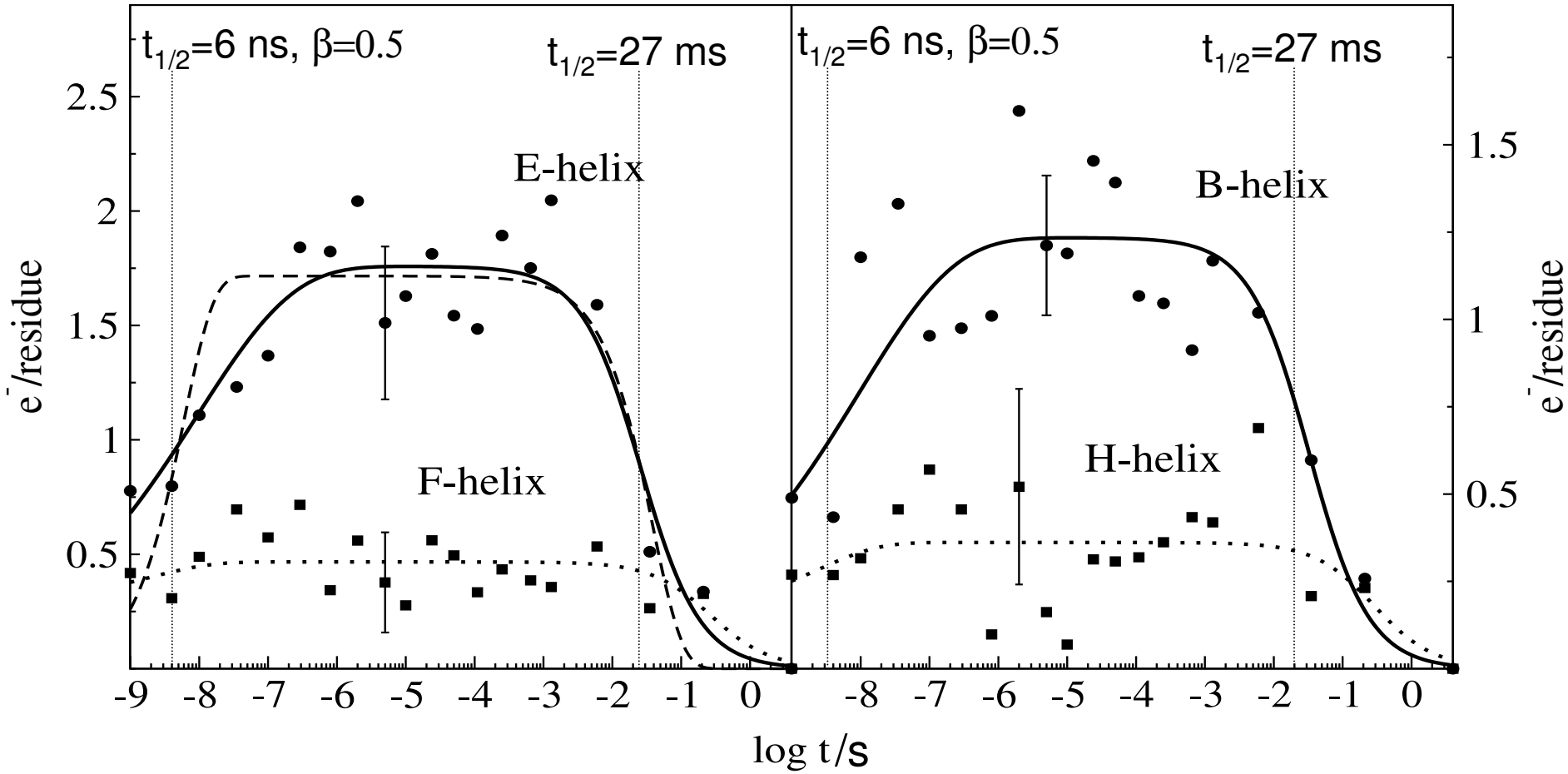
The small molecules in L29W MbCO: CO and Heme

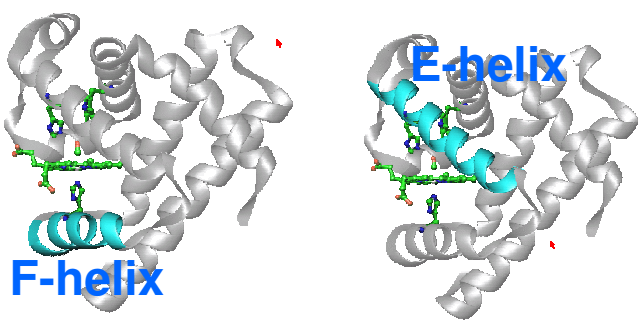


Trial Function:

$$\Delta\rho(\mathrm{t})_{\mathrm{integrated}} = \underbrace{-A_1 e^{-\left(\frac{t}{\tau}\right)^{\beta}}}_{\text{streched, if } 0<\beta<1} + \underbrace{\frac{A_2}{\lambda' \cdot t + 1}}_{\text{bimolecular rebinding}}$$

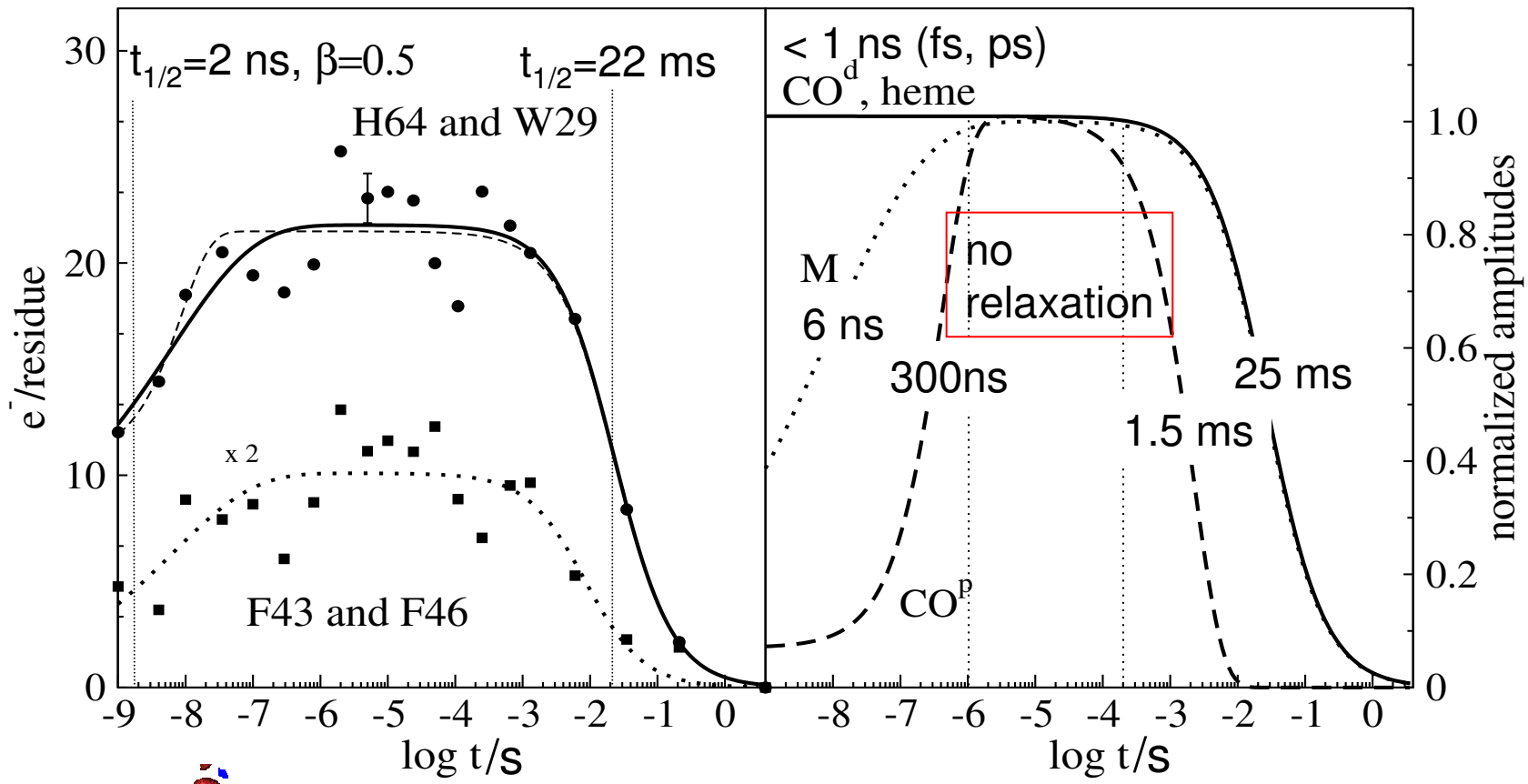
Visco-elastic moieties in myoglobin: the helices

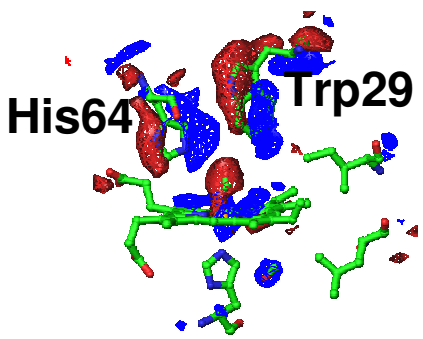




$$\Delta\rho(\mathrm{t})_{\mathrm{integrated}} = \underbrace{-A_1 e^{-\left(\frac{t}{\tau}\right)^{\beta}}}_{\text{streched, if } 0<\beta<1} + \underbrace{\frac{A_2}{\lambda' \cdot t + 1}}_{\text{bimolecular rebinding}}$$

side chains coupled to helices **kaleidoscope of relaxations**

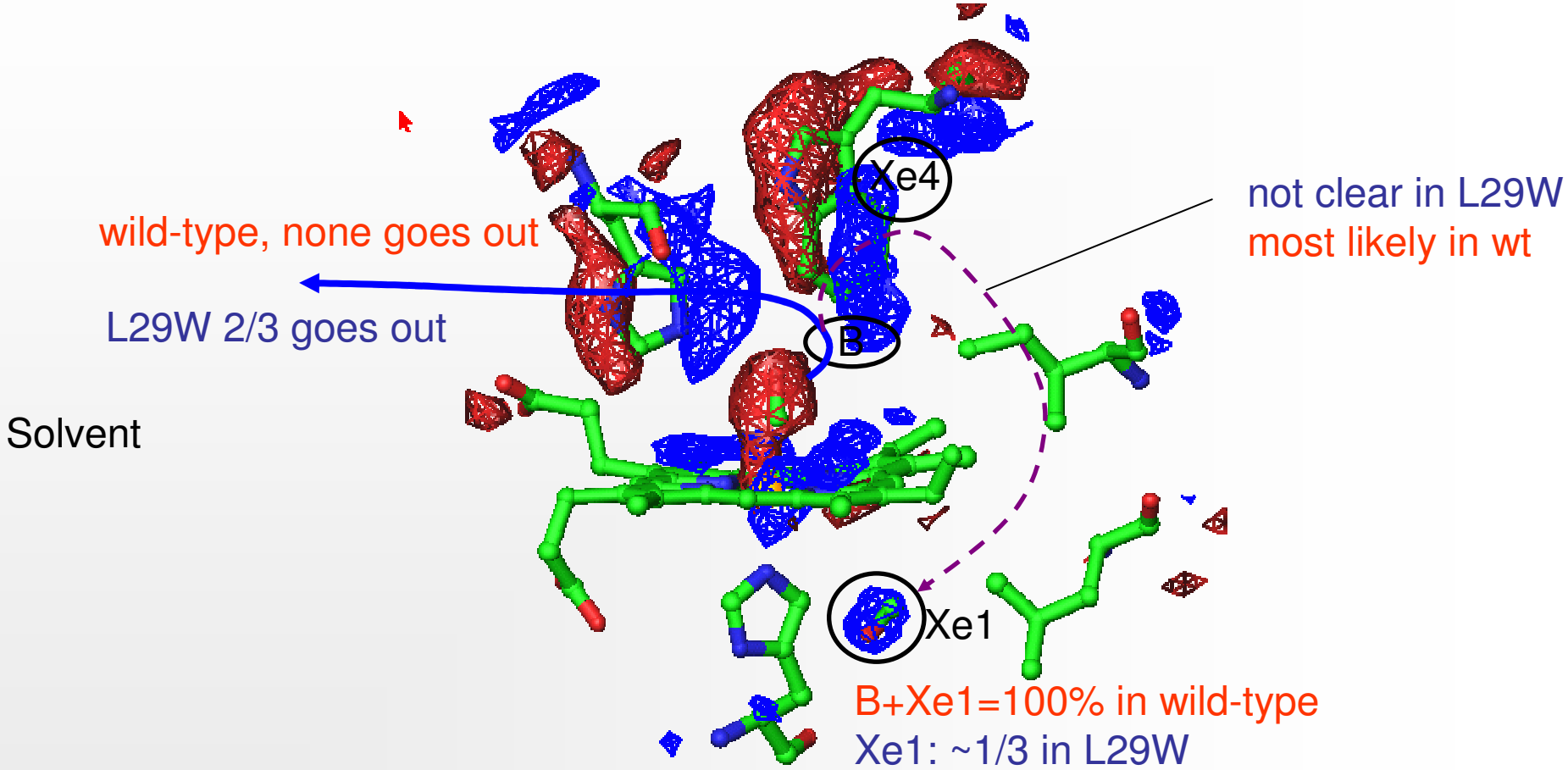




$$\Delta\rho(\mathrm{t})_{\mathrm{int}} = \underbrace{-A_1 e^{-\left(\frac{t}{\tau}\right)^{\beta}}}_{\text{streched, if } 0<\beta<1} + \underbrace{\frac{A_2}{\lambda' \cdot t + 1}}_{\text{bimolecular rebinding}}$$

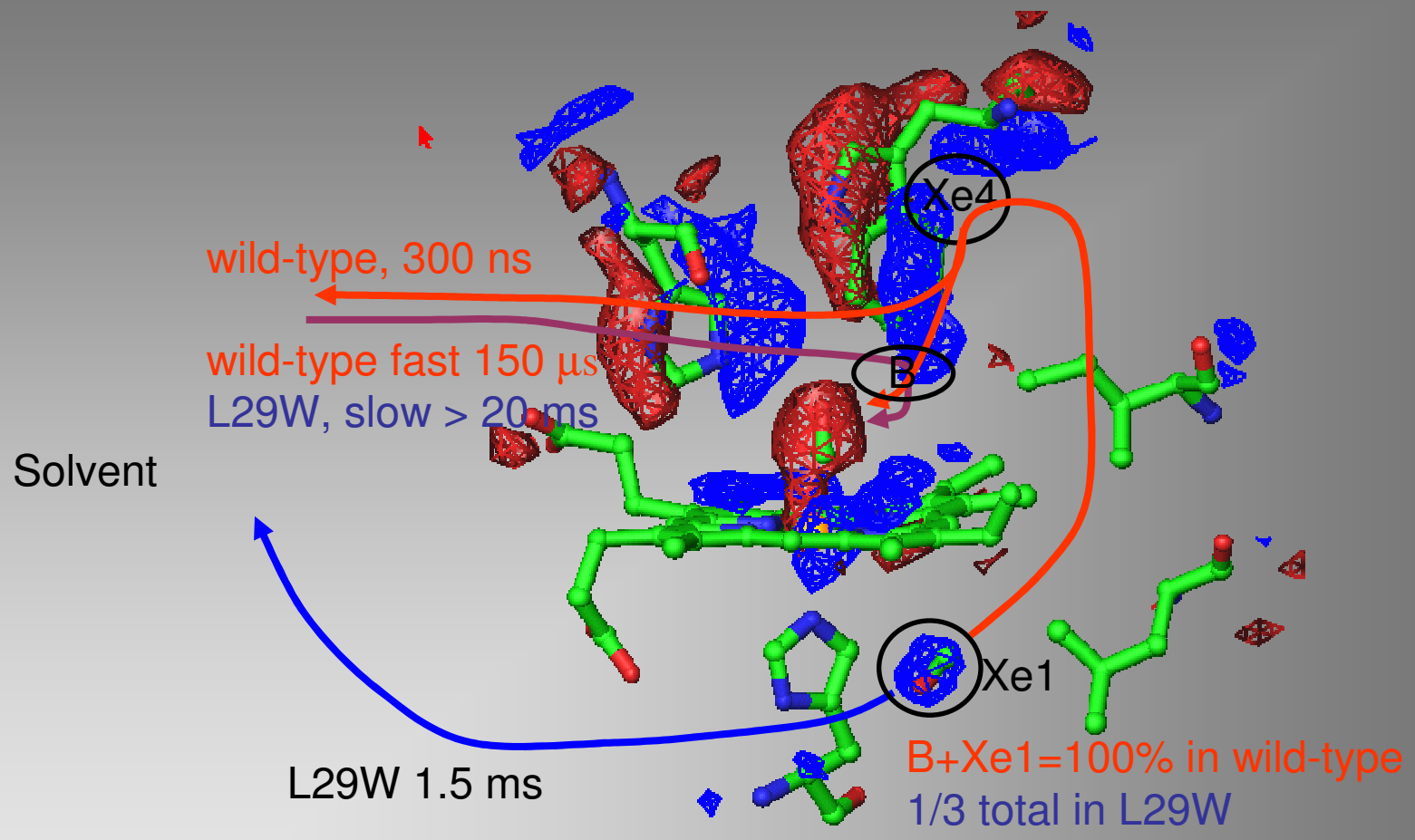
Initial CO migration

In L29W MbCO: fast Trp29 relaxation blocks way to Xe1, most CO in solvent
In WT-MbCO: all CO in B and Xe1



Return of CO to the iron

In L29W MbCO: Trp29 blocks access, inefficient leaking on proximal side
In WT-MbCO: 2 orders of magnitude faster, migration to distal side



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Results are appealing
-relaxation times
-stretched at fastest times, simple at slower times
-period of time with no relaxations

HOWEVER:
-no separation of heavily overlapping intermediate states

3. Analysis of TR-crystallographic data with a component analysis
Structures and Kinetics at the same Time

Schmidt, Rajagopal, Ren, Moffat, BJ 2003

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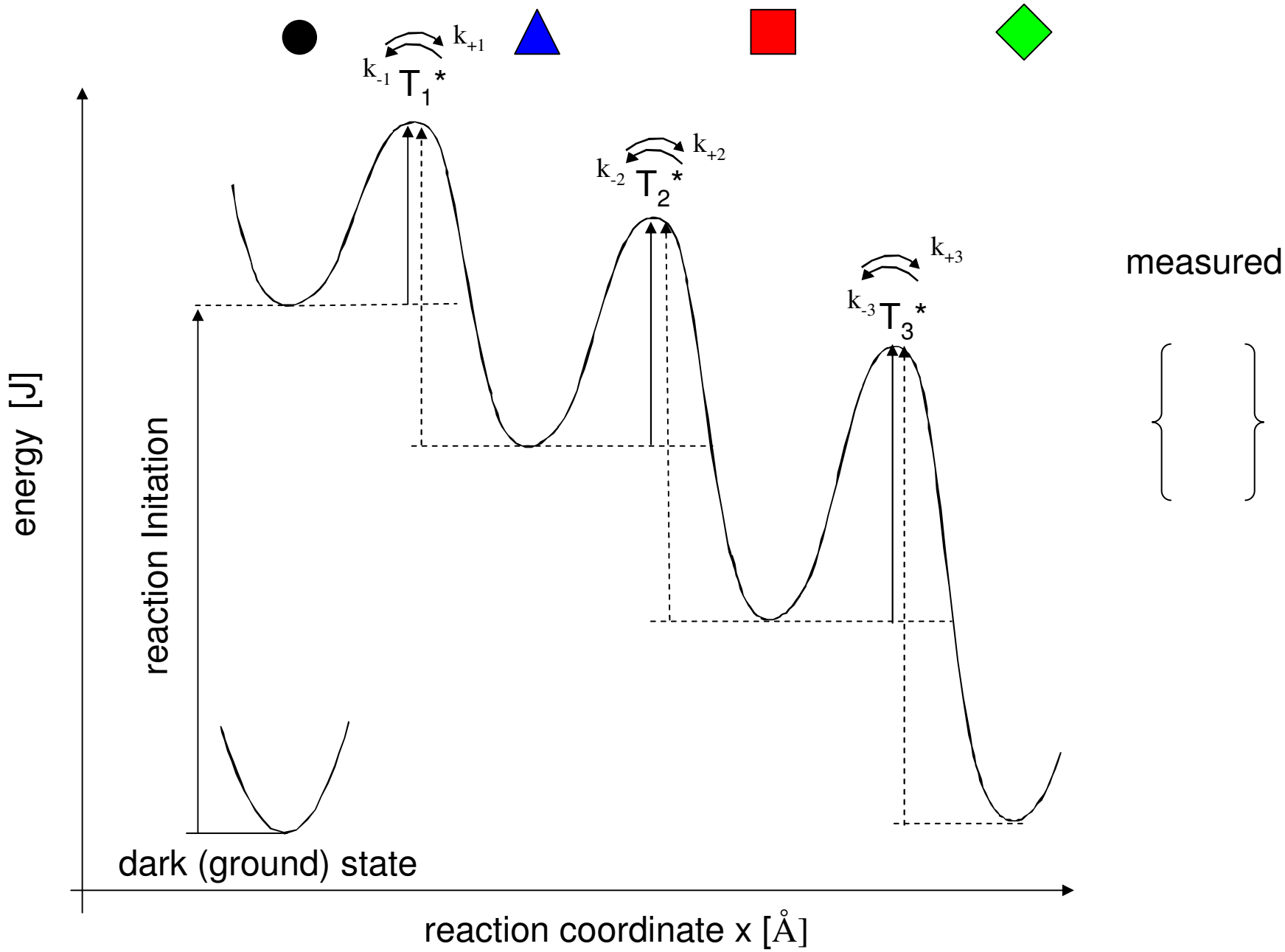
Chemical Kinetics:

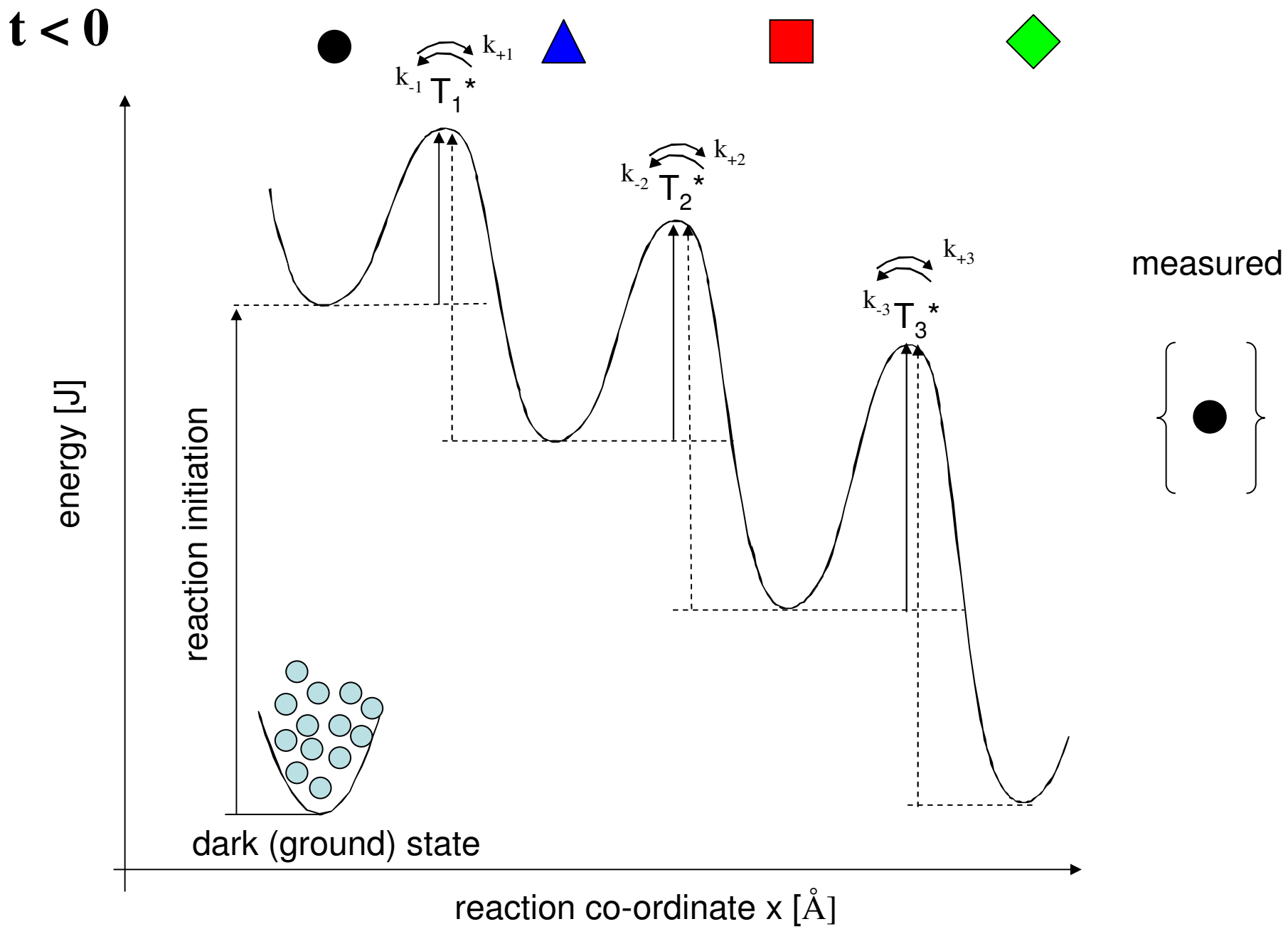
Chemical reactions in general (nomenclature)

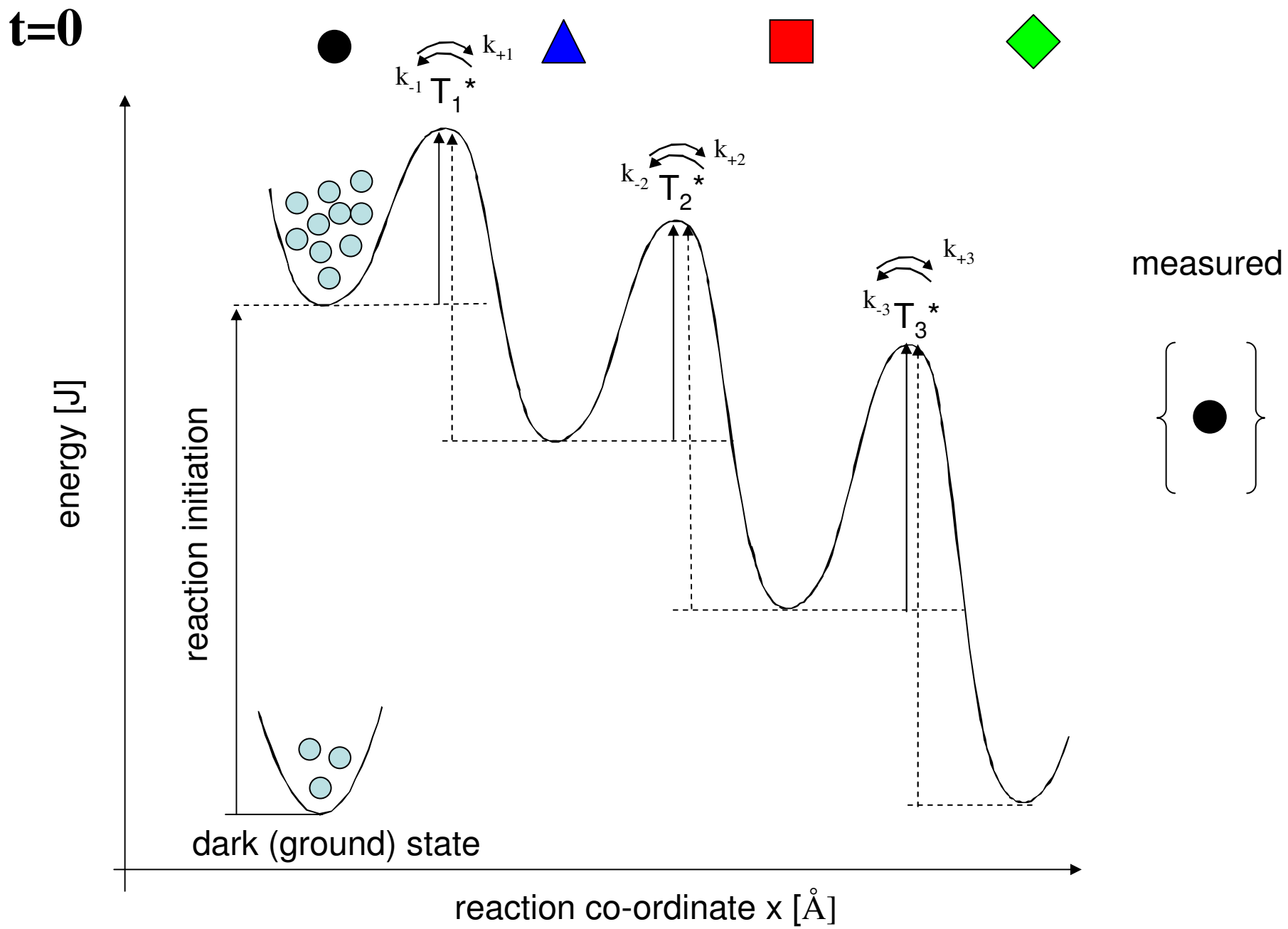
-proceed through intermediate states
-intermediate states are shortly (=transiently) occupied by real molecules
-intermediate states are energy minima in multidimensional conformational space
-intermediate states are connected by a chemical kinetic mechanism

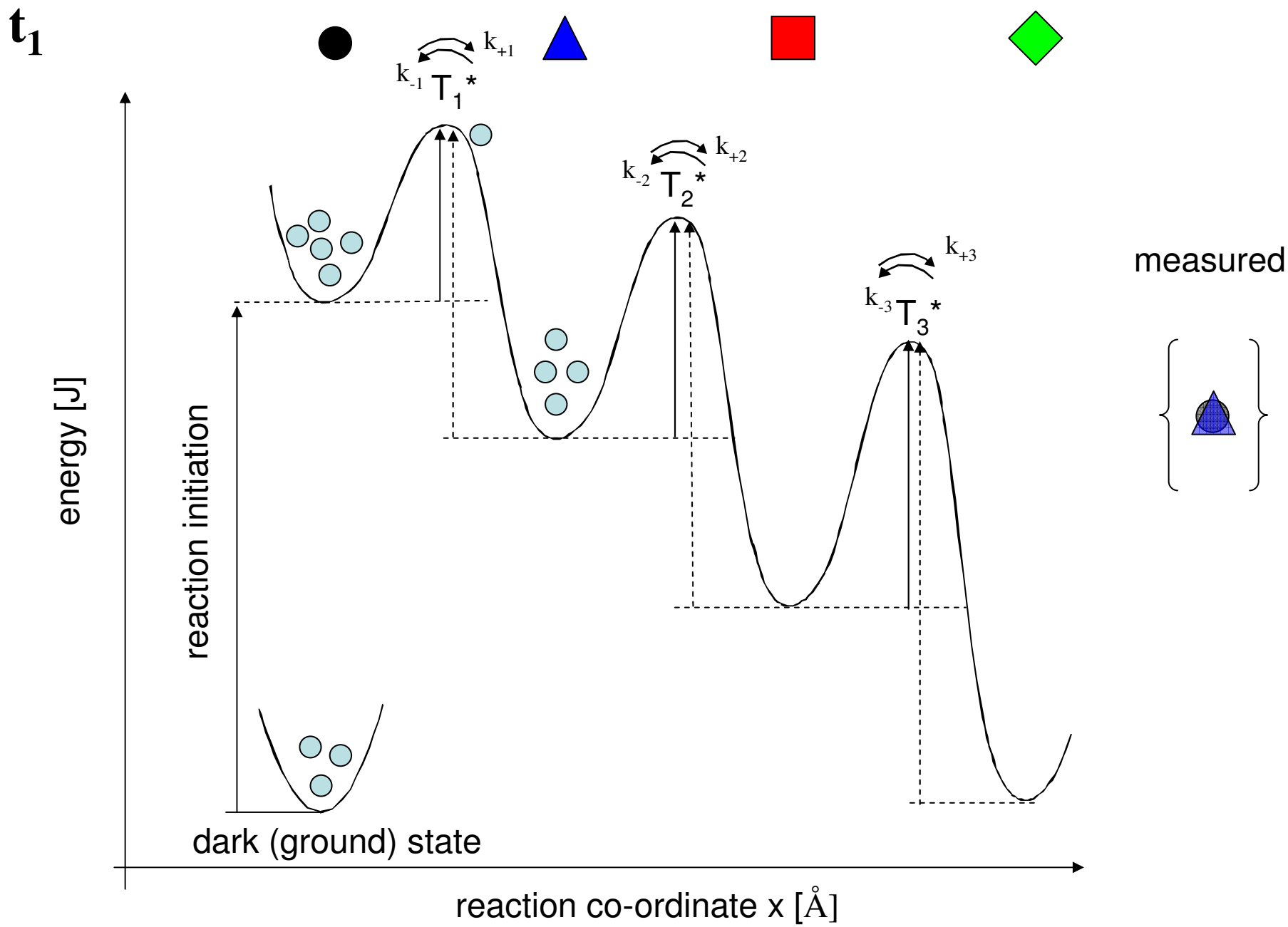
Transient state kinetics
-allows the determination of the physical properties (structure) of the intermediate states together with the kinetics

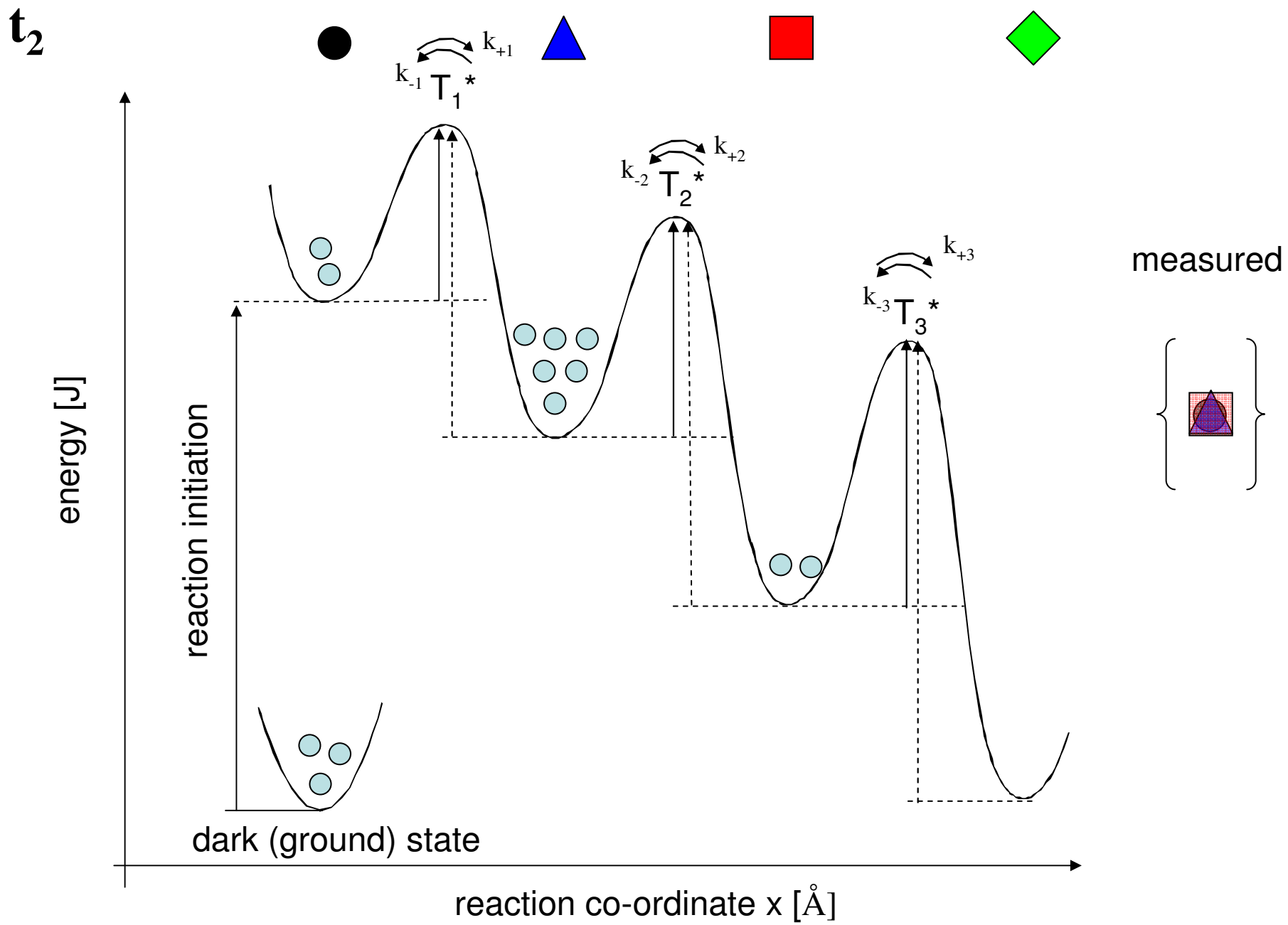
Note: a transient state is a short lived intermediate state but NOT a transition state

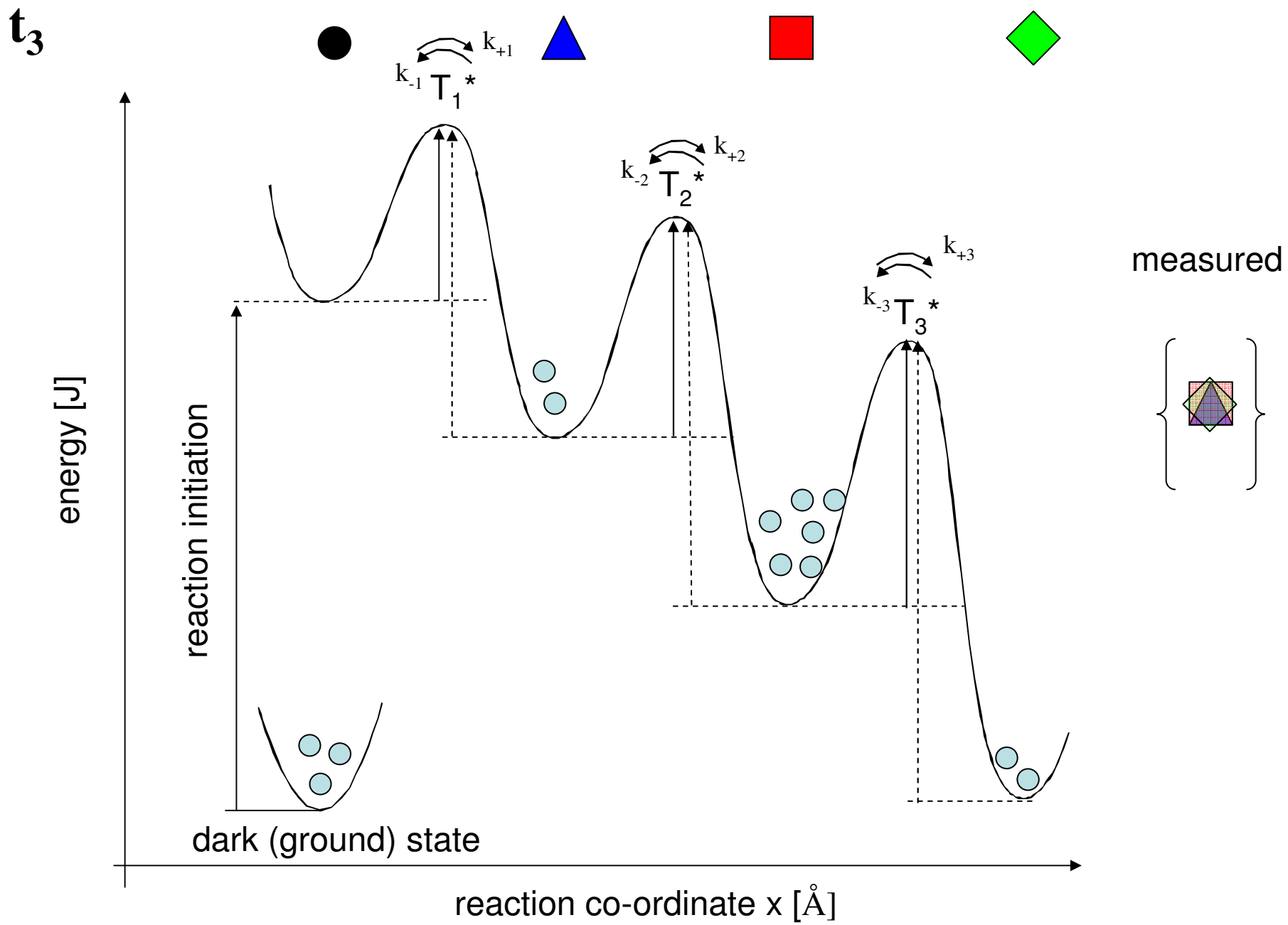

k+1
k-1
T1*
k+2
k-2
T2*
k+3
k-3
T3*
measured
energy [J]
reaction Initation
dark (ground) state
reaction coordinate x [Å]

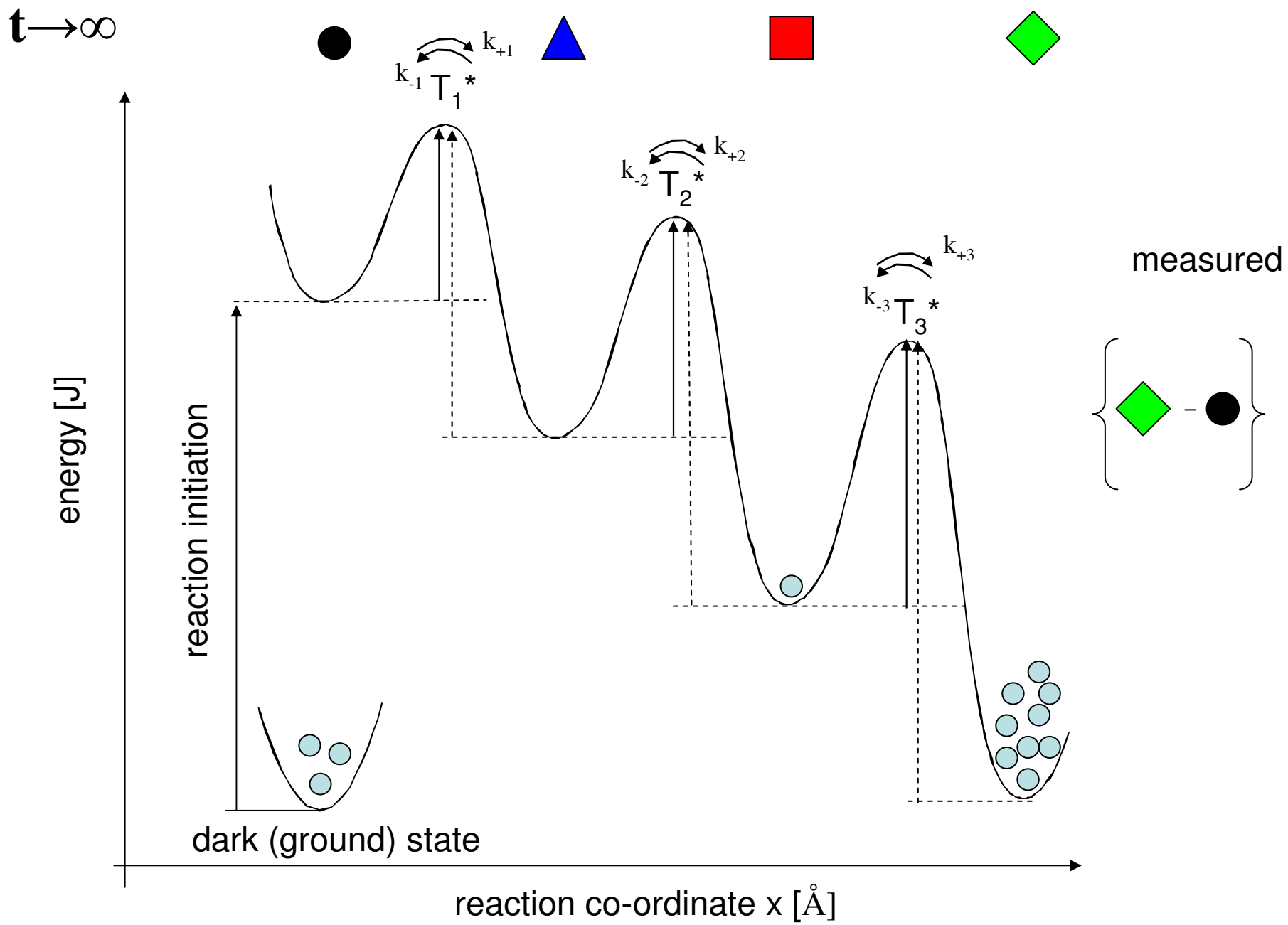

t < 0
k+1
k-1
T1*
k+2
k-2
T2*
k+3
k-3
T3*
measured
energy [J]
reaction initiation
dark (ground) state
reaction co-ordinate x [Å]


t=0
k+1
k-1
T1*
k+2
k-2
T2*
k+3
k-3
T3*
measured
energy [J]
reaction initiation
dark (ground) state
reaction co-ordinate x [Å]

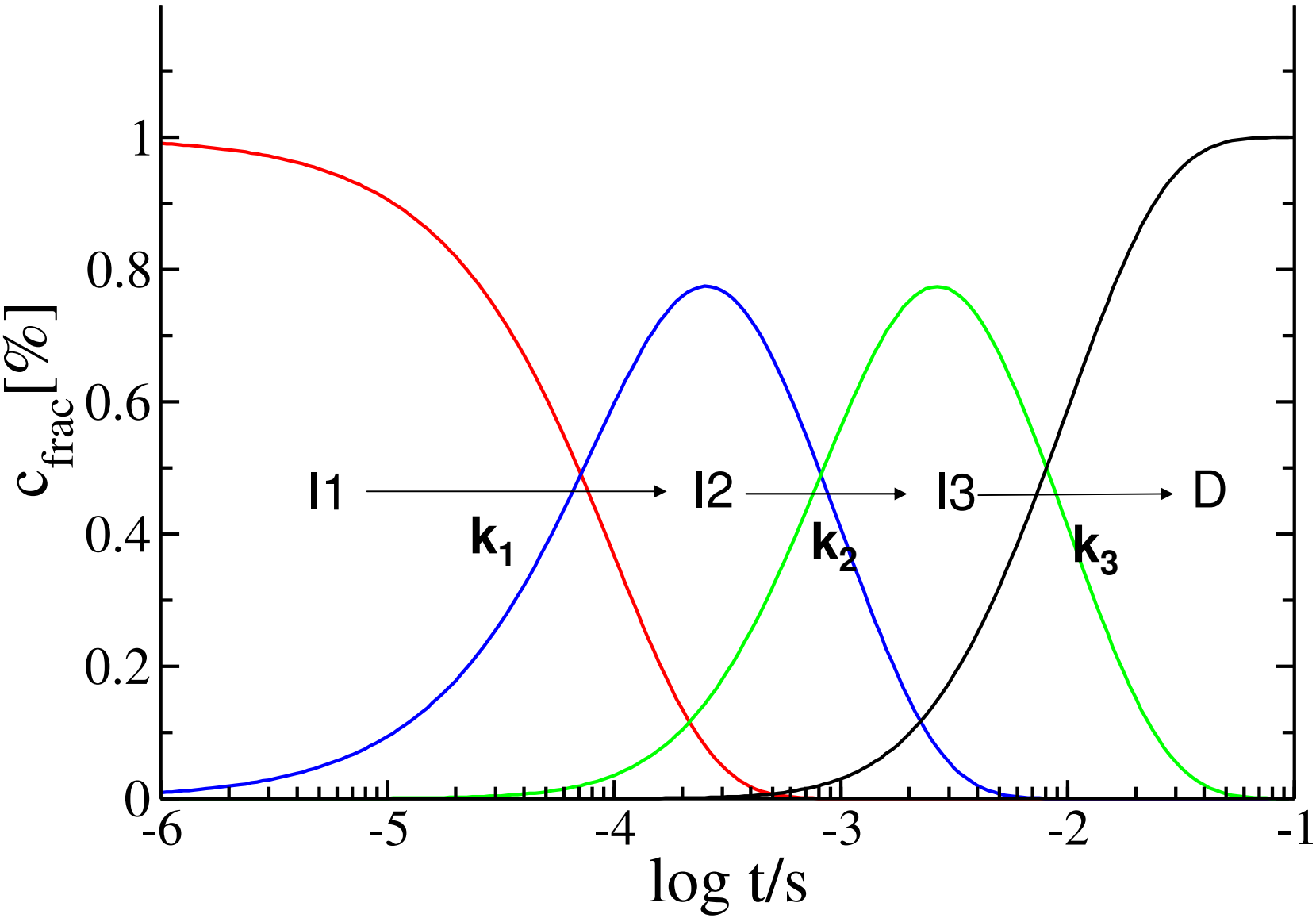

t1
k+1
k-1
T1*
k+2
k-2
T2*
k+3
k-3
T3*
measured
energy [J]
reaction initiation
dark (ground) state
reaction co-ordinate x [Å]


t2
k+1
k-1
T1*
k+2
k-2
T2*
k+3
k-3
T3*
measured
energy [J]
reaction initiation
dark (ground) state
reaction co-ordinate x [Å]

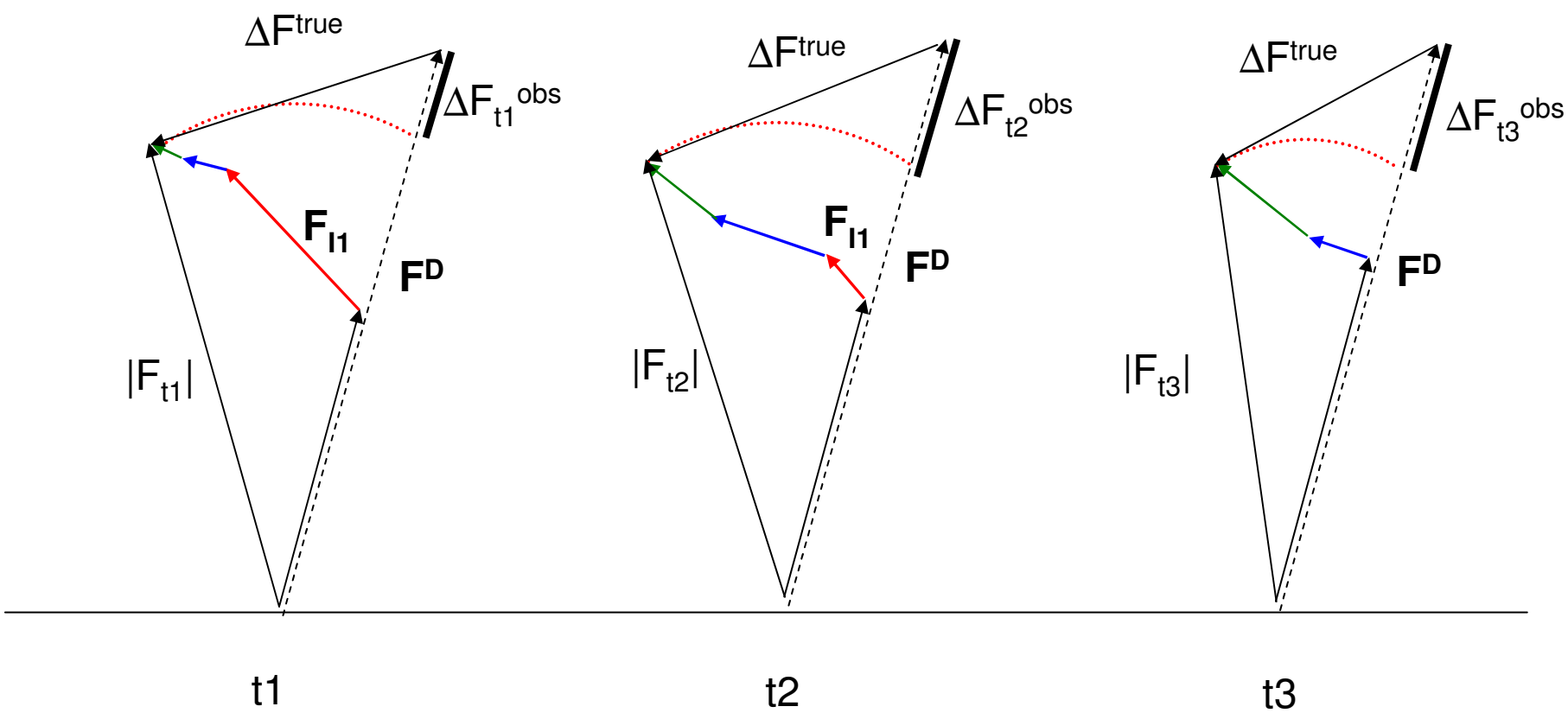

t3
k+1
k-1
T1*
k+2
k-2
T2*
k+3
k-3
T3*
measured
energy [J]
reaction initiation
dark (ground) state
reaction co-ordinate x [Å]


t→∞
k+1
k-1
T1*
k+2
k-2
T2*
k+3
k-3
T3*
measured
energy [J]
reaction initiation
dark (ground) state
reaction co-ordinate x [Å]

Example: Reaction with 3 intermediate states plus the DARK state (D)
connected by an irreversible, sequential chemical kinetic mechanism

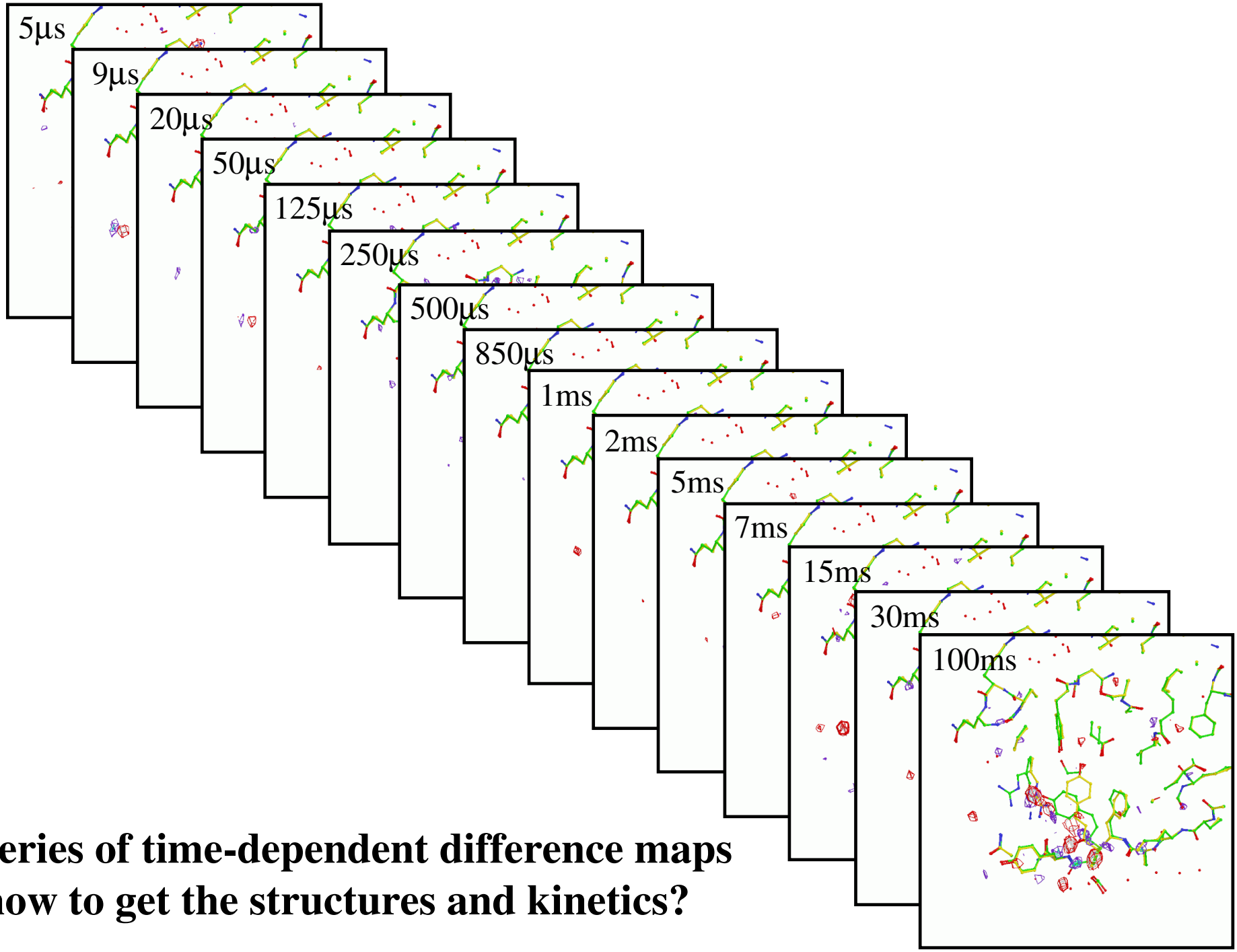


Argand-diagrams for 3 time points, 3 intermediates plus dark are involved



Goal: to determine structure factors F_{I1}…F_{I3} with amplitude and phase
Problem: non-linearity
Solution: use difference maps, perfect maps on half the absolute scale

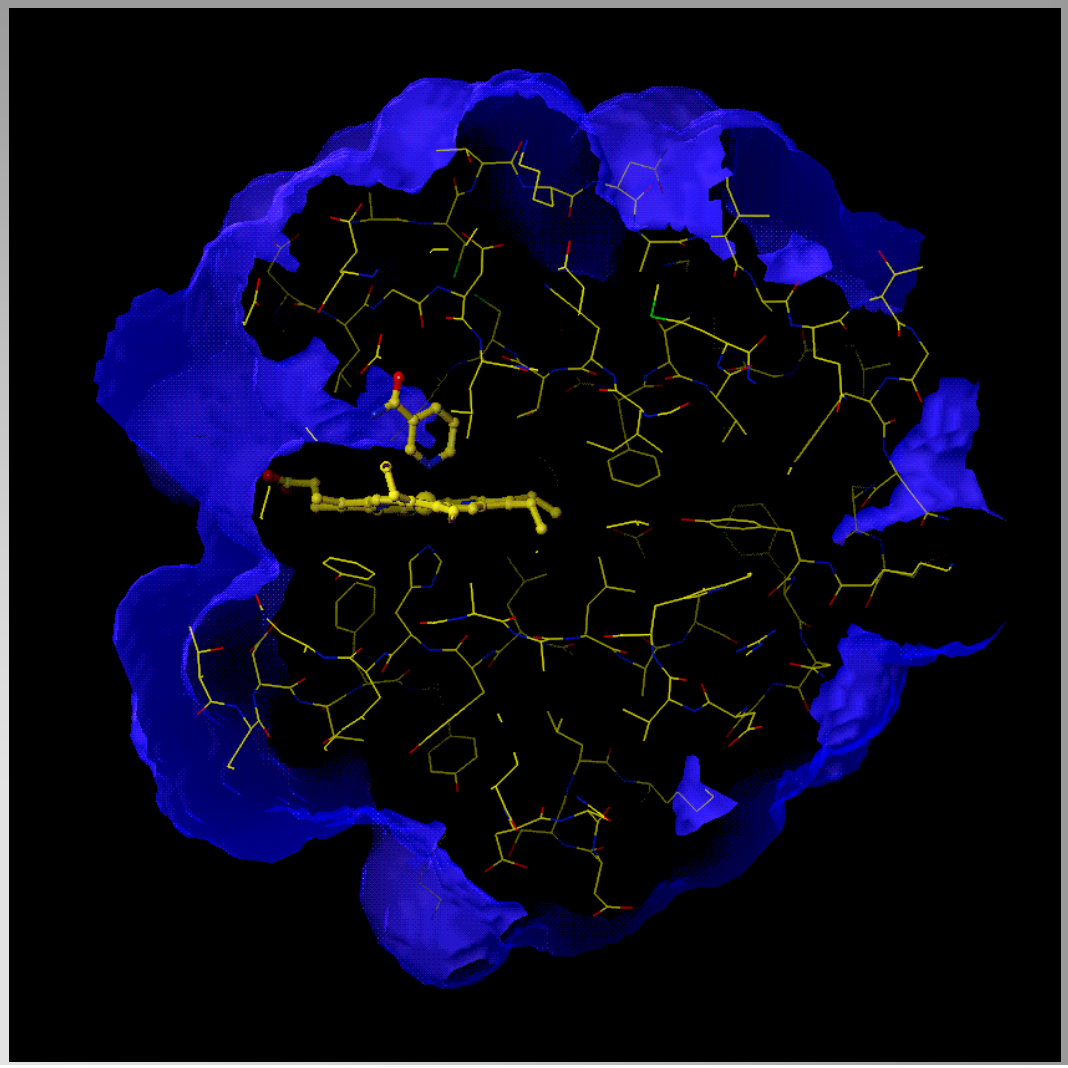
(Henderson and Moffat, 1971, textbook: Drenth)



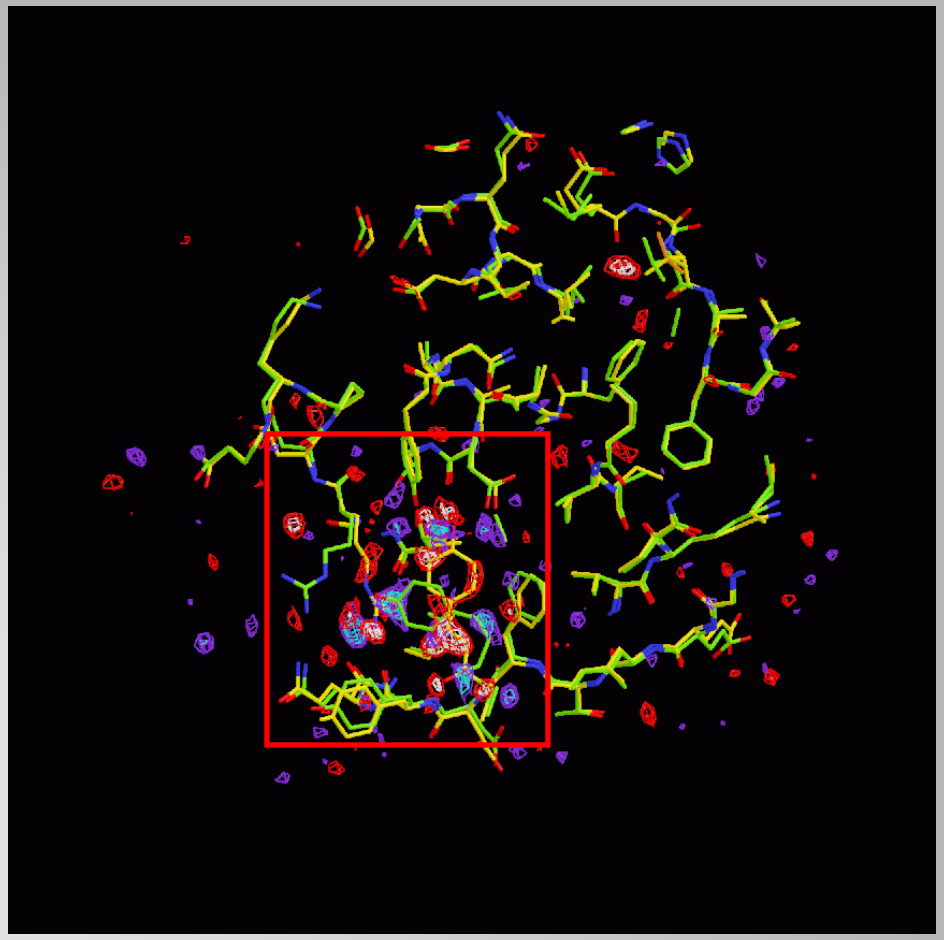
series of time-dependent difference maps
how to get the structures and kinetics?

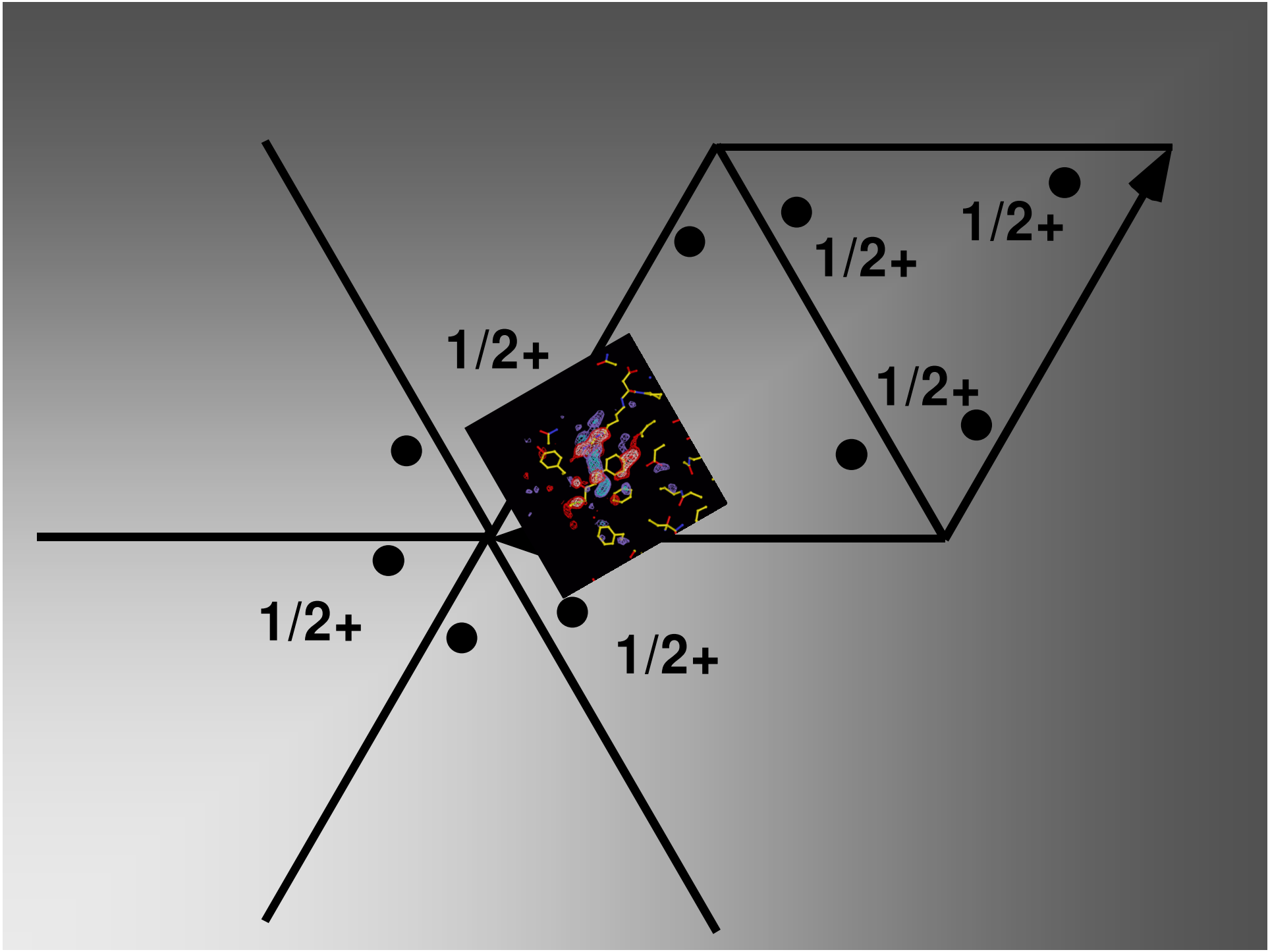
The Art of Vectorizing a (difference) Electron Density MAP

i.) We ONLY need volume occupied by THE MOLECULES IN THE asymmetric unit
ii.) Difference maps are represented on a 3D grid (voxels)
We ONLY need voxels that contain SIGNAL



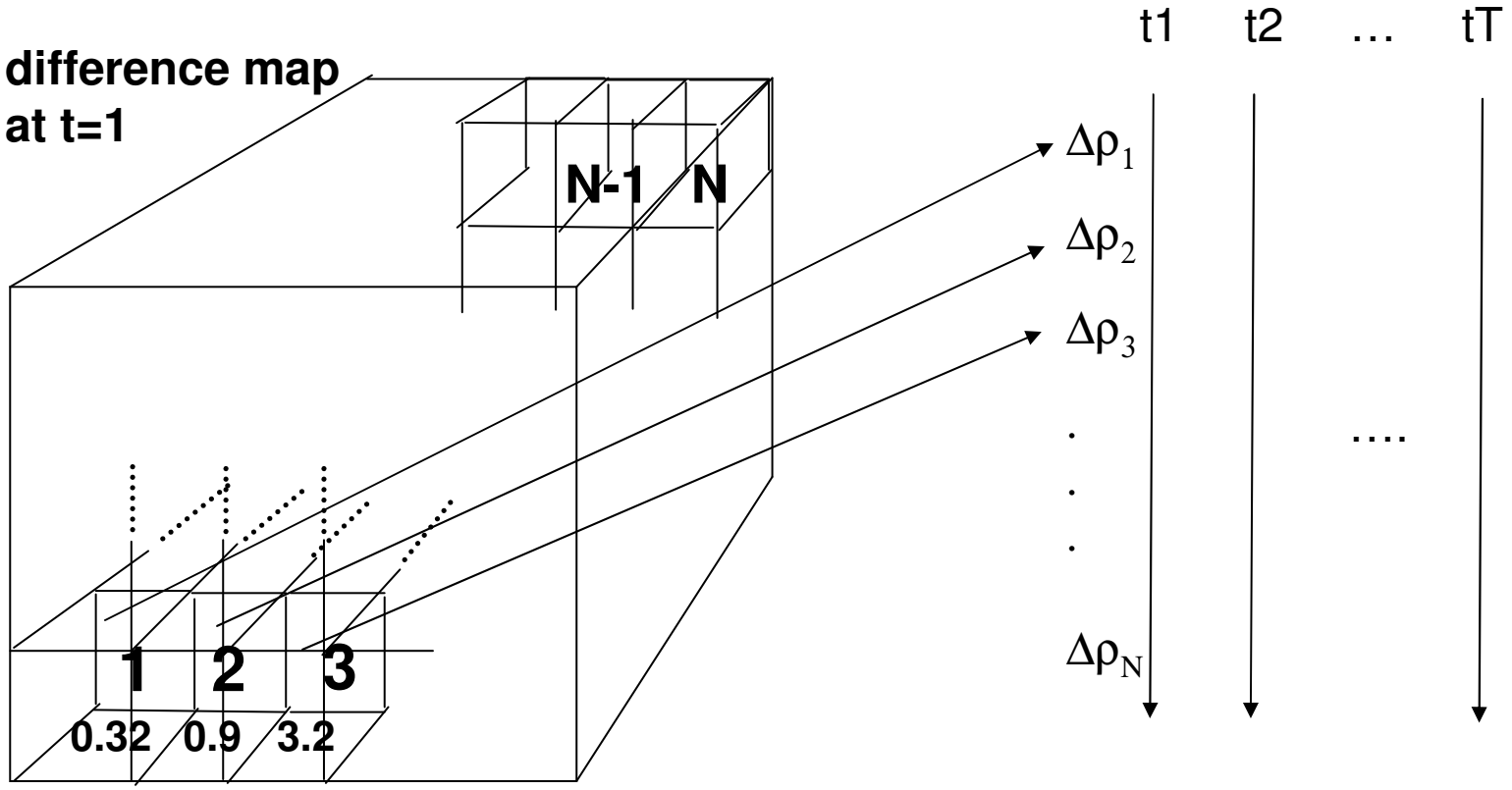
-Search all masked out volumes in the time series
-Determine those voxels that contain (abs)density > certain threshold (2σ)
-Mark these voxels

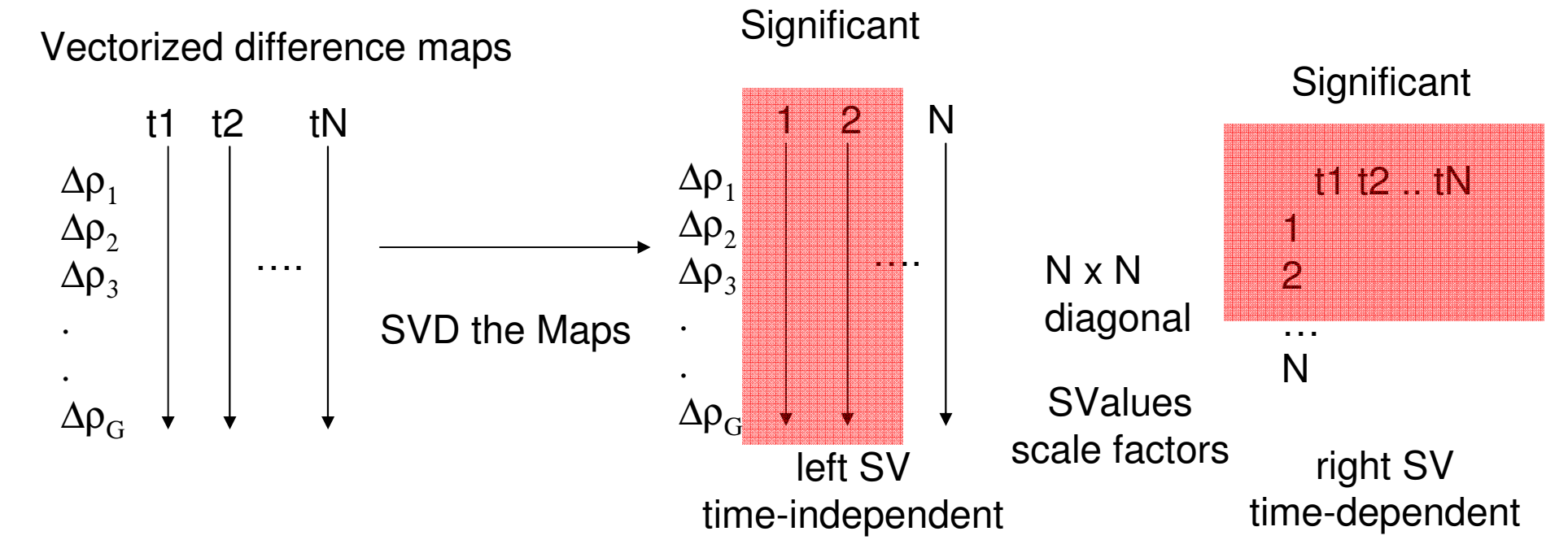



1/2+
1/2+
1/2+
1/2+
1/2+
1/2+

Vectorize the maps:

- 1…N voxels, each for t=1…T maps



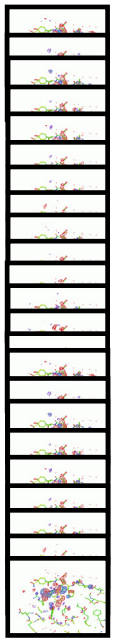


Program for users: SVD4TX
select significant SVs
re-construct A

$$U^S \quad S^S \quad V^{T,S} = A'$$

noise reduced !

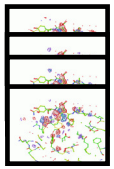
time-dependent
difference maps (4D)



decomposition

SVD

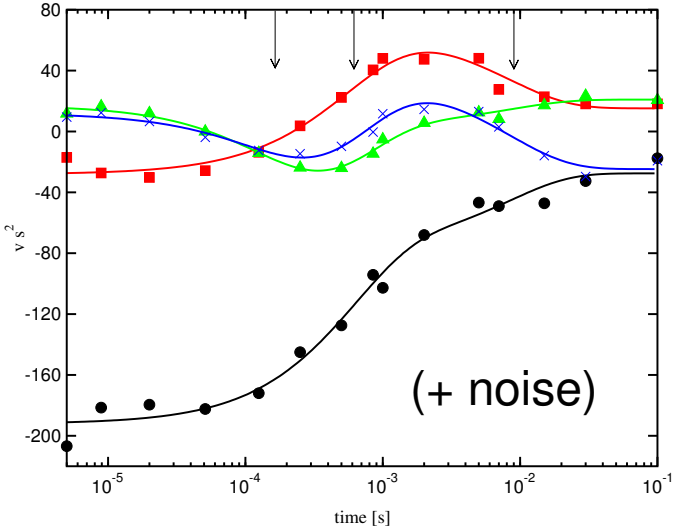
significant
singular maps (3D)



singular
values >> 0

(+ noise)

significant time-courses



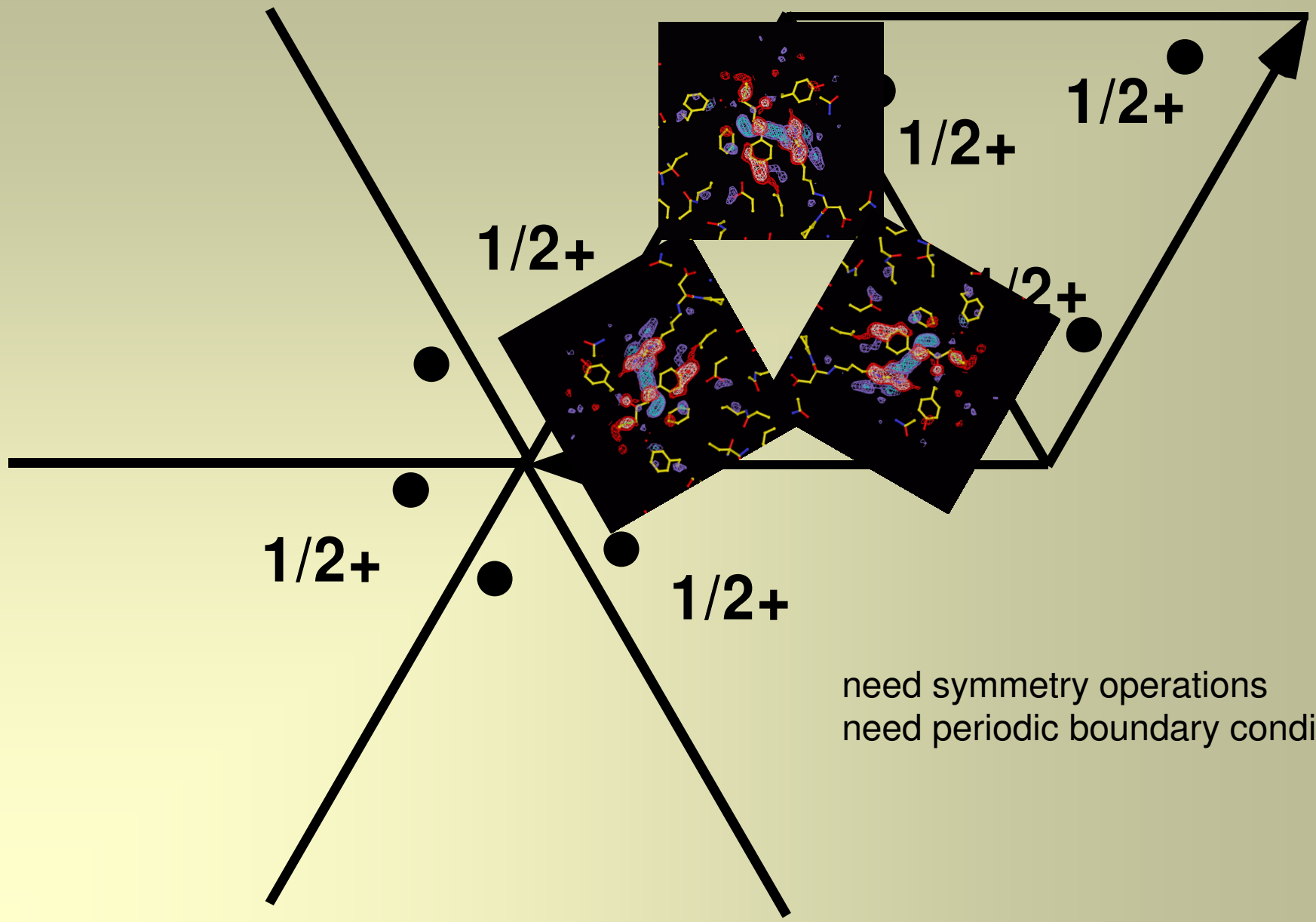
$$A = U \quad S \quad V^T$$

(noisy)

$$U^s \quad S^s \quad V^{T,s} = A'$$

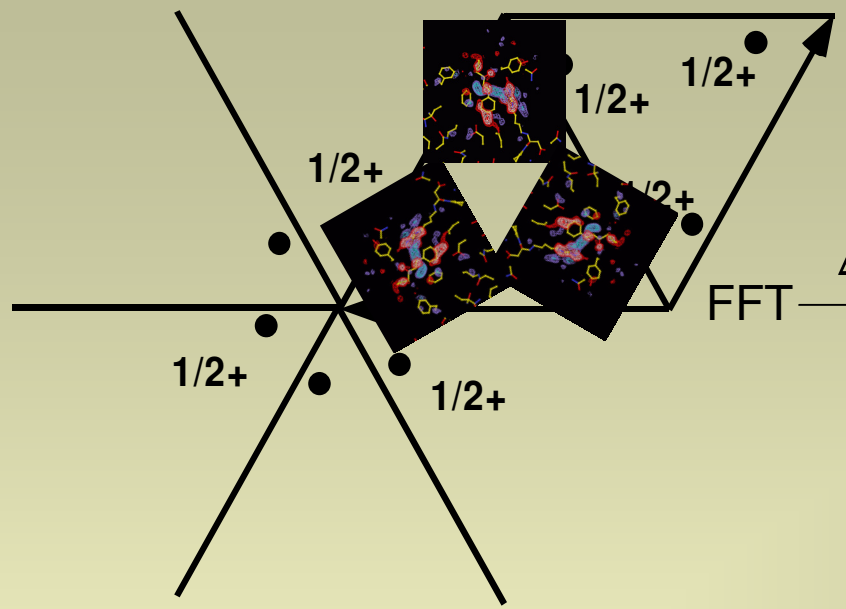
noise reduced

$$U^s \; S^s \; V^{T,s} = A'$$



need symmetry operations
need periodic boundary conditions

(i) SVD-flattening



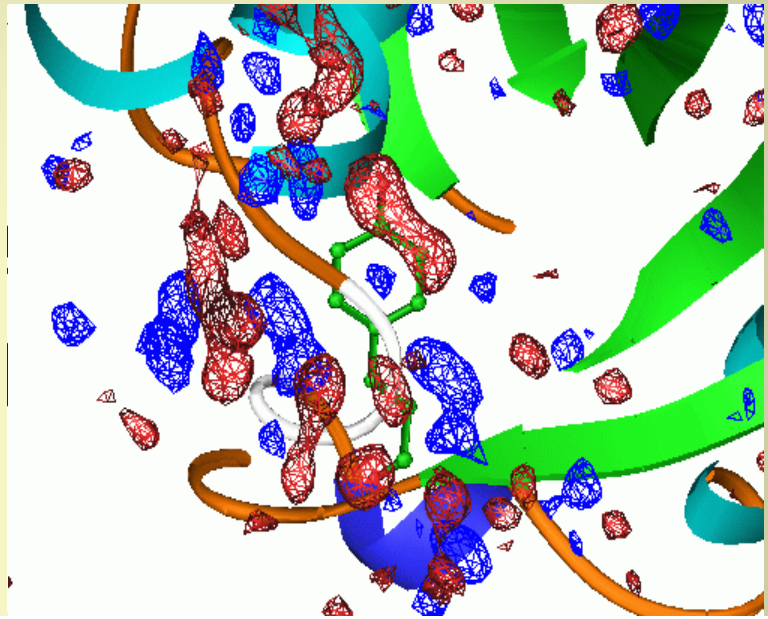
FFT ΔF^{SVD} →

measured ΔF, F_t, F_D
calculated F_D

↓

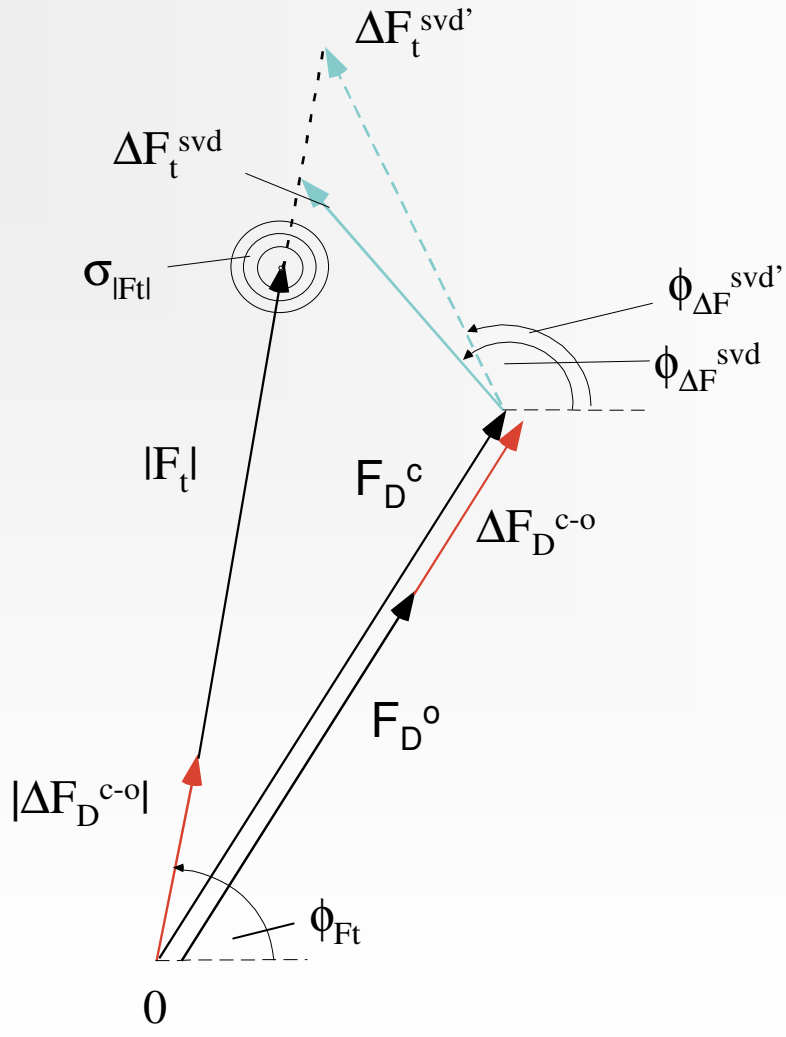
phase and amplitude-combination

↓ SVD4TX



new difference maps
noise free, phase improved
SVD again

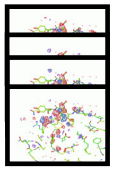
Phase- and Amplitude Recombination in SVD-flattening



time-dependent, SVD-flattened
difference maps (4D)

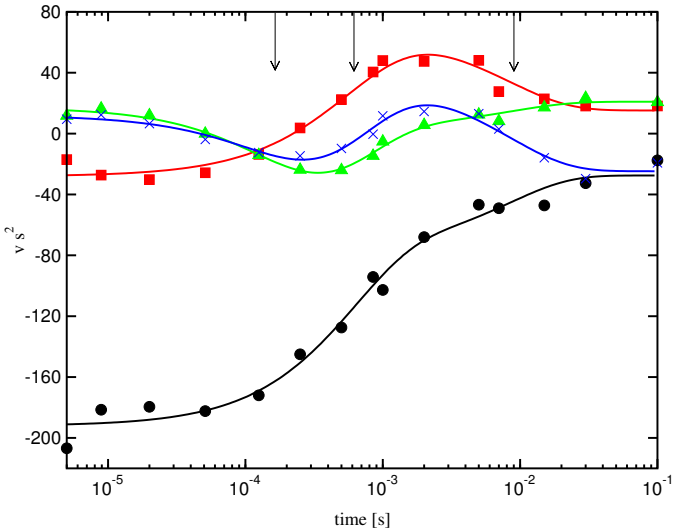
decomposition

significant
singular maps (3D)



few singular
values >> 0

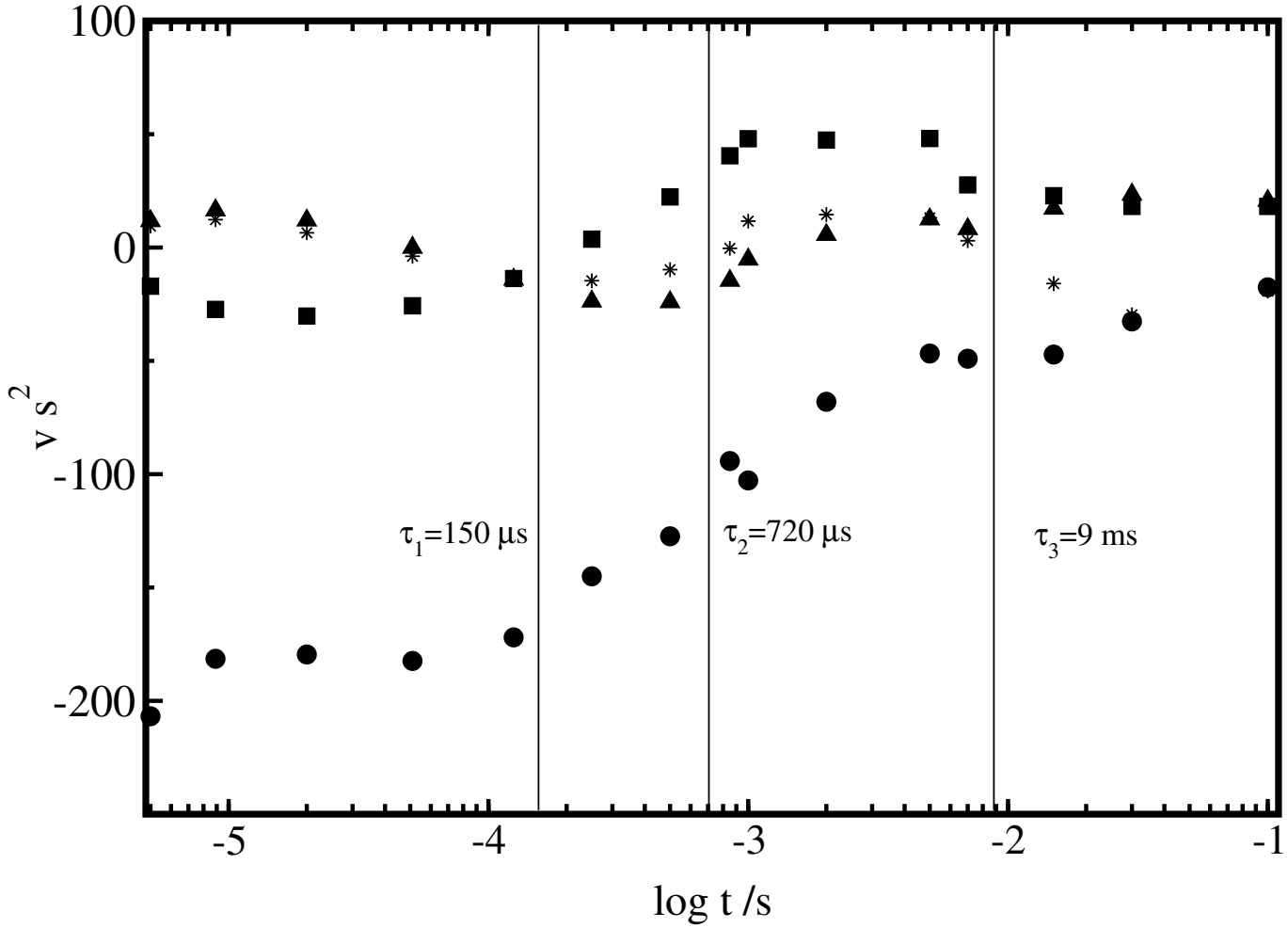
significant time-courses



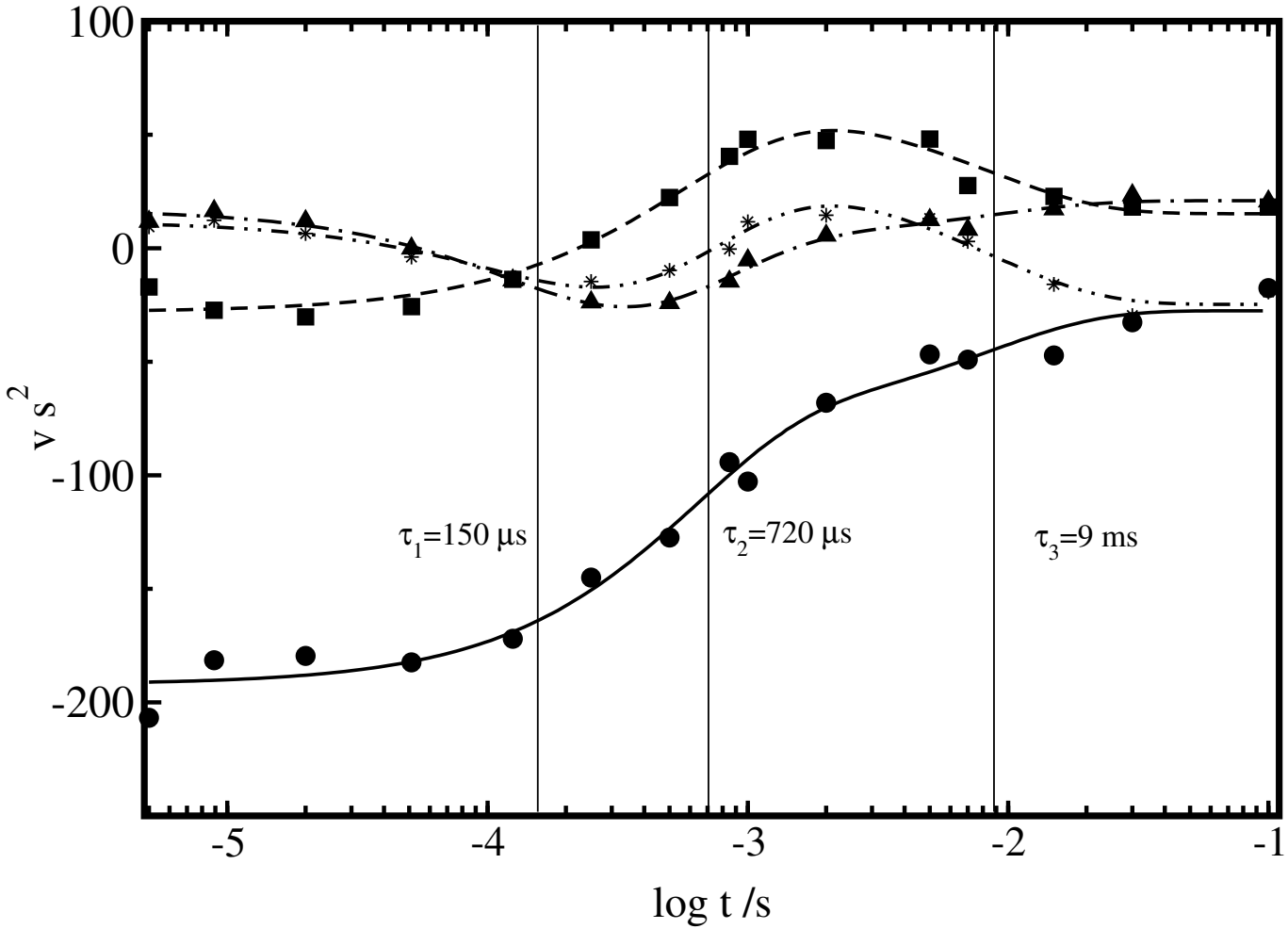
A‘ = U S V^T

(less noisy)

(ii) Relaxation Times



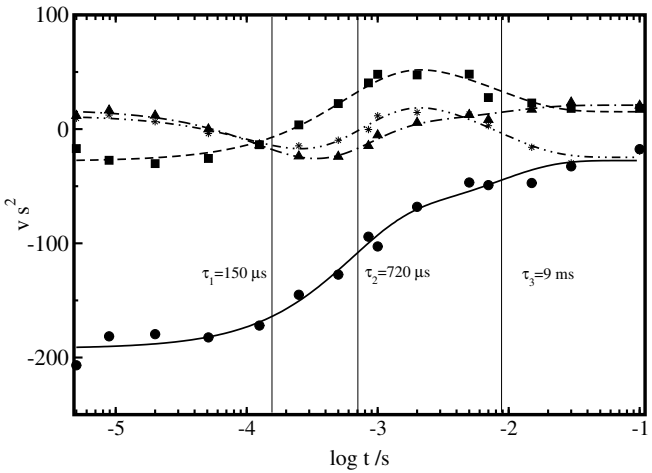
(ii) Relaxation Times

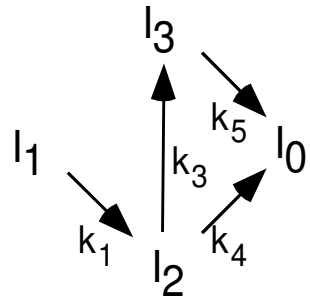


$$v_i^{fit} = s_i^2 \left(D_{i0} + \sum_{q=1}^{3} D_{iq} \cdot e^{-\frac{1}{\tau_q} t} \right)$$

iii) Chemical kinetics is described by chemical kinetic mechanism

3 relaxation times



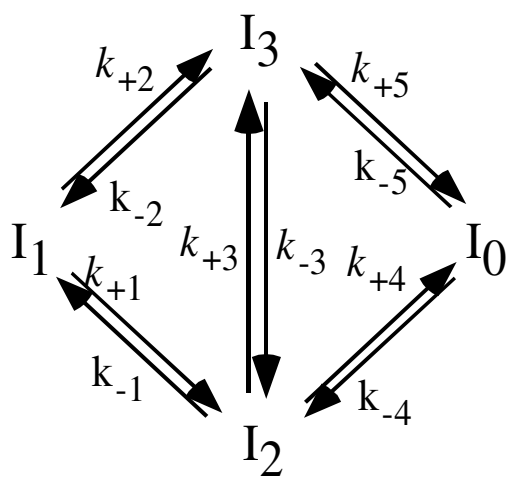


candidate mechanism

small dt in log time (SVD4TX)

$$\frac{d[I_1]}{dt} = -k_{+1}[I_1]$$
$$\frac{d[I_2]}{dt} = k_{+1}[I_1] - (k_{+3} + k_{+4})[I_2]$$
$$\frac{d[I_3]}{dt} = k_{+3}[I_2] - k_{+5}[I_3]$$

general mechanism



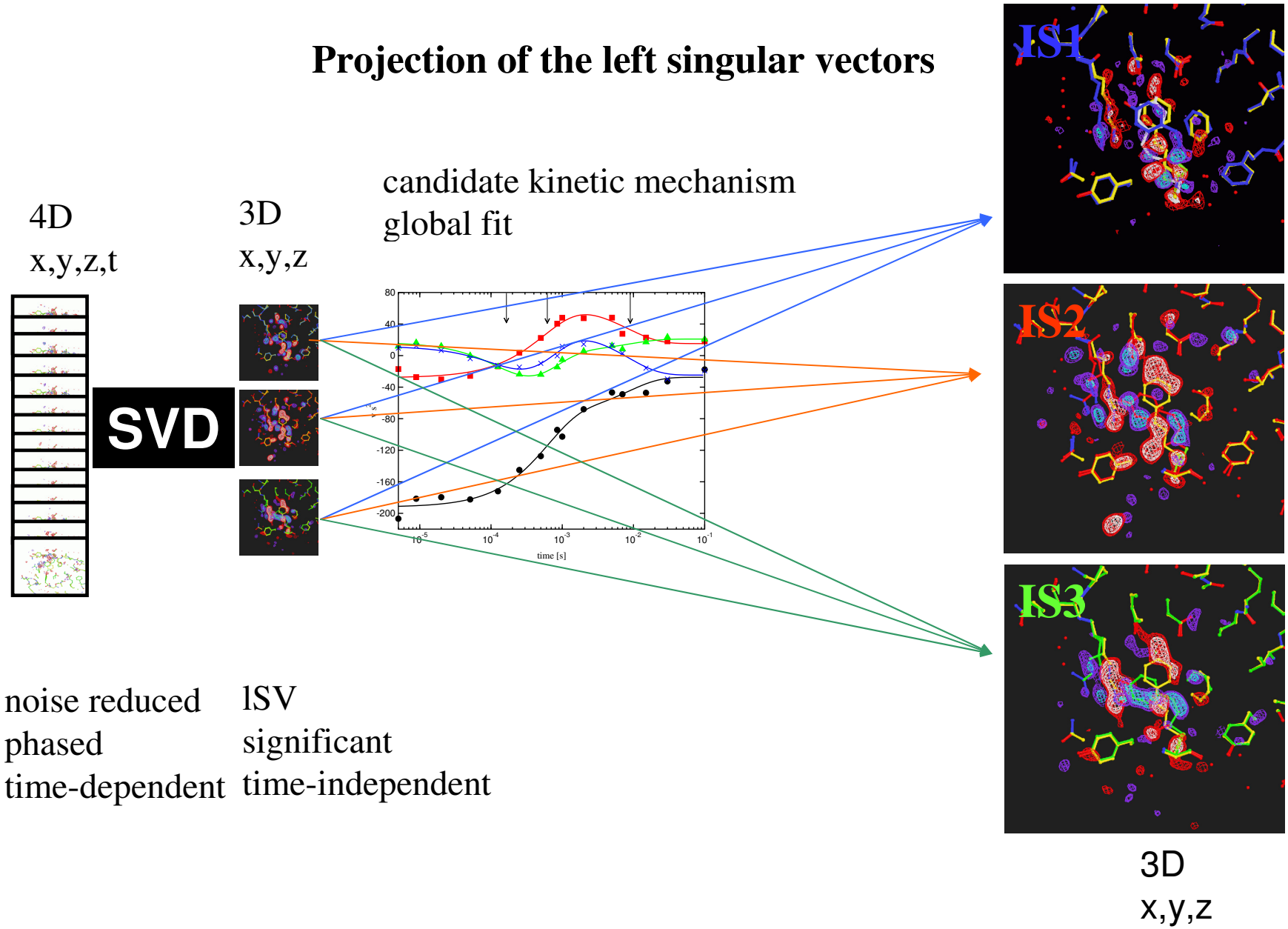
better approach: diagonalize matrix

$$\begin{pmatrix} \frac{d[I_1]}{dt} \\ \frac{d[I_2]}{dt} \\ \frac{d[I_3]}{dt} \end{pmatrix} = \underbrace{\begin{pmatrix} -k_{+1} & \varepsilon & \varepsilon \\ k_{+1} & -(k_{+3}+k_{+4}) & \varepsilon \\ \varepsilon & k_{+3} & -k_{+5} \end{pmatrix}}_{\text{coefficient matrix } K} \begin{pmatrix} [I_1] \\ [I_2] \\ [I_3] \end{pmatrix}$$

$\varepsilon \sim 0$

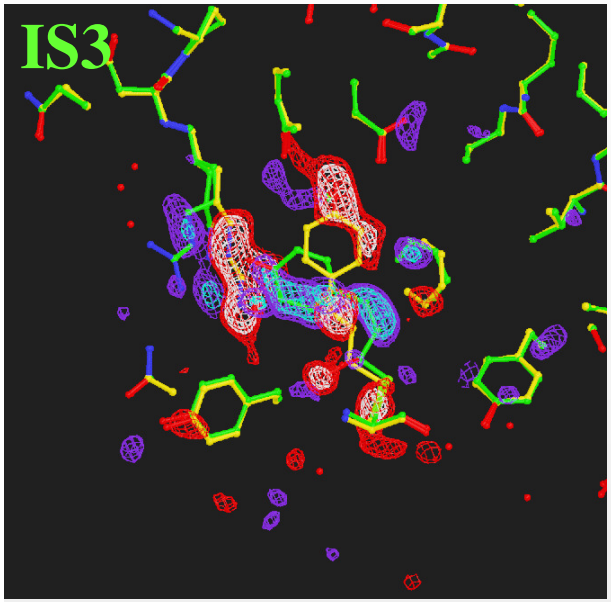
Determine tentative intermediate difference electron density
by fitting a preliminary mechanism to the right singular vectors

Projection of the left singular vectors



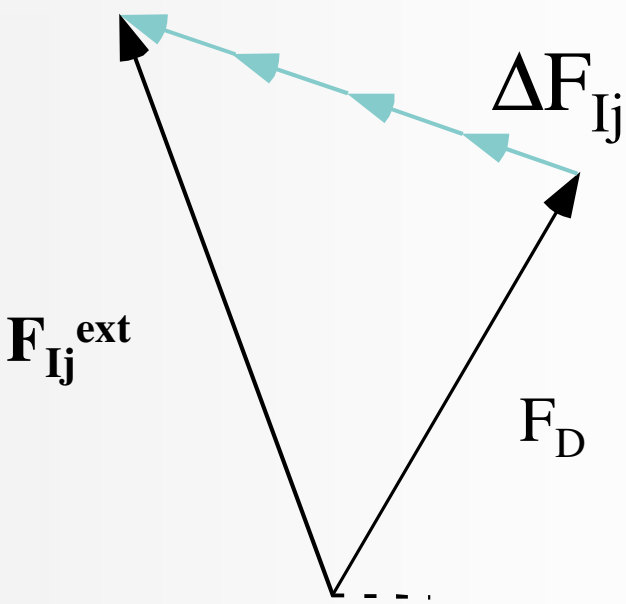
Modeling of structure:

-Extrapolated, Conventional Maps
-Generated from Difference Maps of the Intermediates

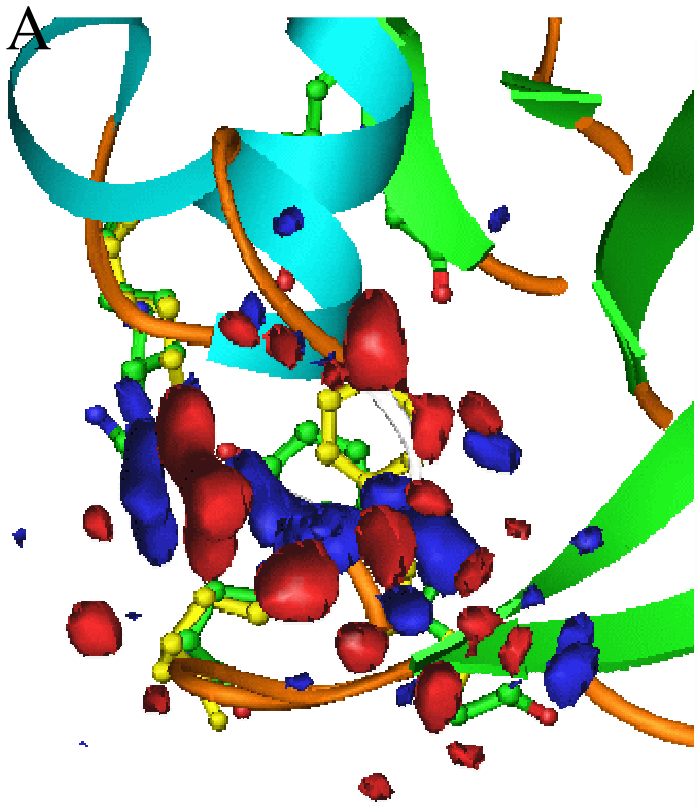


FFT →

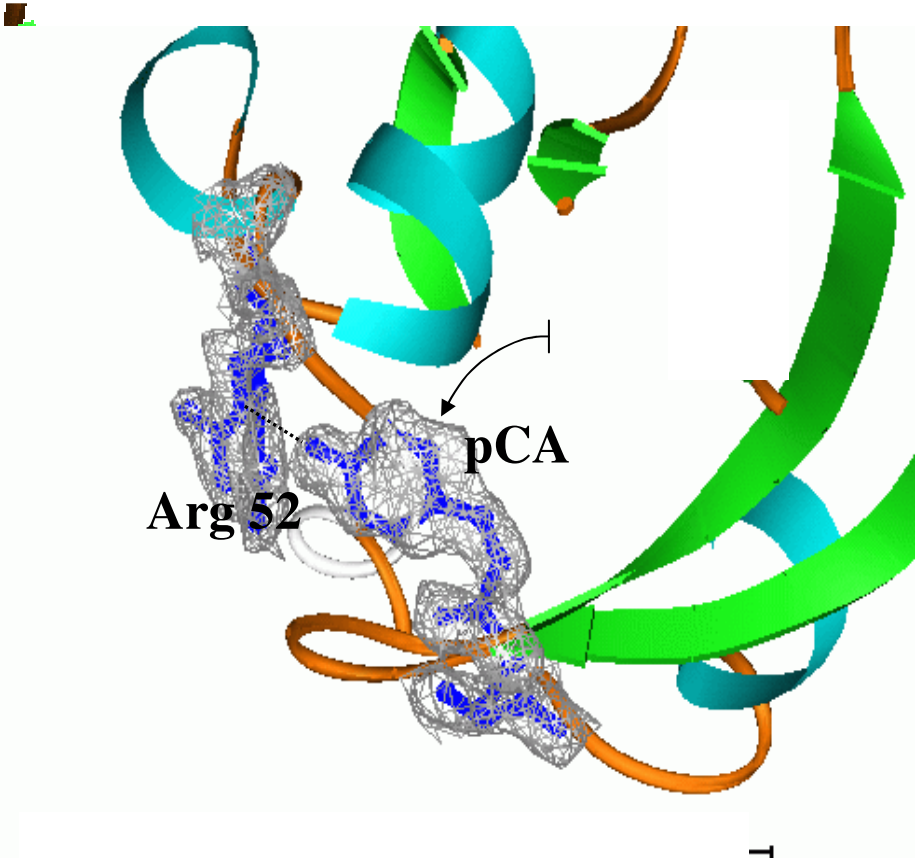
ΔF_{Ij} **(Amplitude AND Phase)**



UNIVERSITY of WISCONSIN
UWMILWAUKEE
Schmidt



Time-Independent Difference Map
- Extracted by SVD



Extrapolated Conventional Map
- Used to model structure

Goal reached?
-ultimate goal: unique, unambiguous mechanism
-structural information: "**posterior analysis**"
-Program for users: **GetMech** (get mechanism)

Structures of the intermediates and the ground state

Structure factors
Difference structure factors by subtracting the ground state

calculated difference electron densities $\Delta\rho_j$

candidate mechanism
reaction coefficients k
concentrations $C_{j,t}$

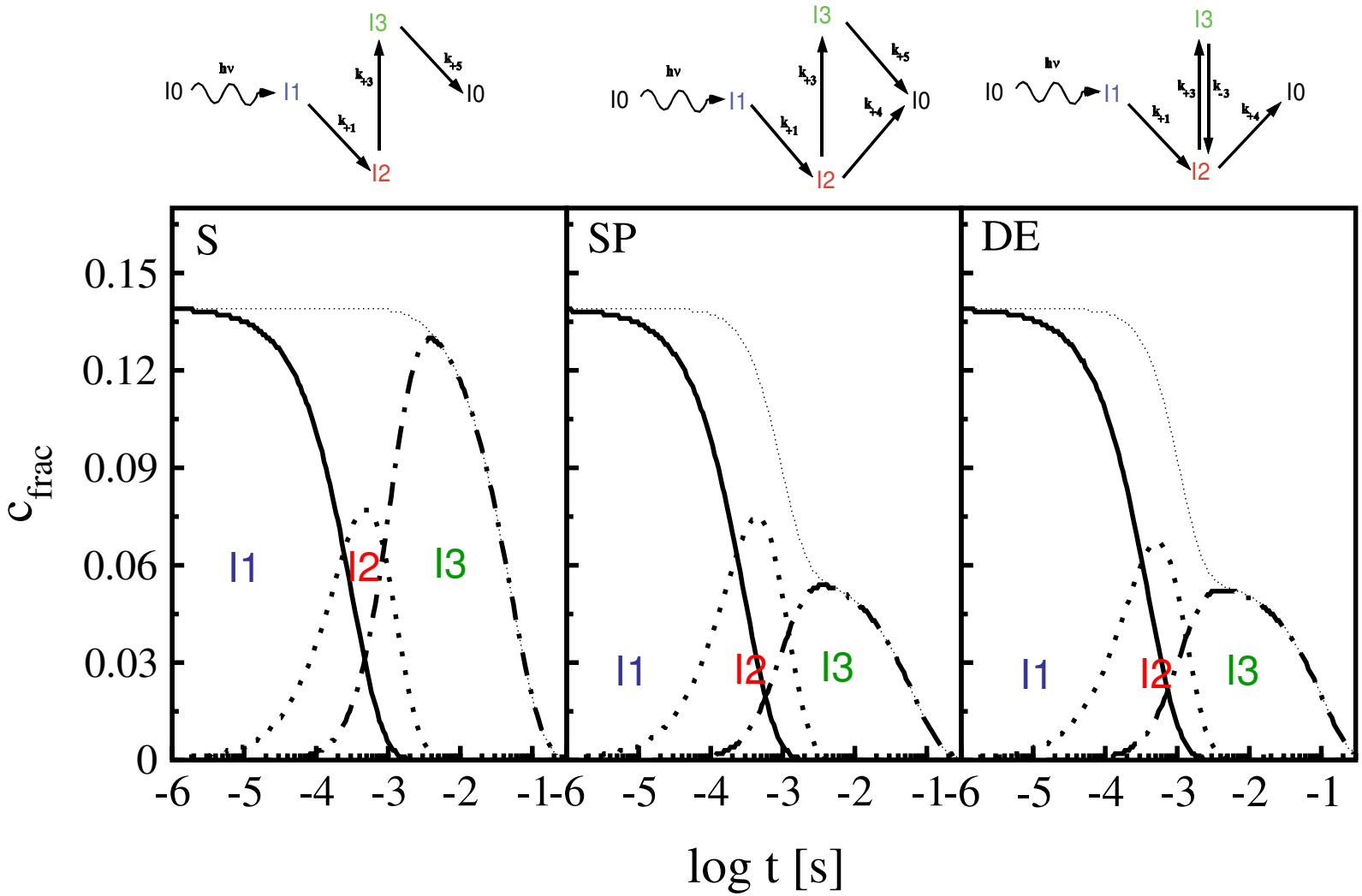
$$\Delta\rho_t(k) = \sum_{j=1}^{N} C_{j,t}(k) \cdot \Delta\rho_j$$

calculated, time-dependent difference maps $\Delta\rho_t^{calc}(k)$

goodness of fit

$$\sum_{t=1}^{N} \left(\Delta\rho_t^{obs} - sc \cdot \Delta\rho_t^{calc}(k)\right)^2 \longrightarrow \min$$

observed, measured, time-dependent difference maps $\Delta\rho_t^{obs}$

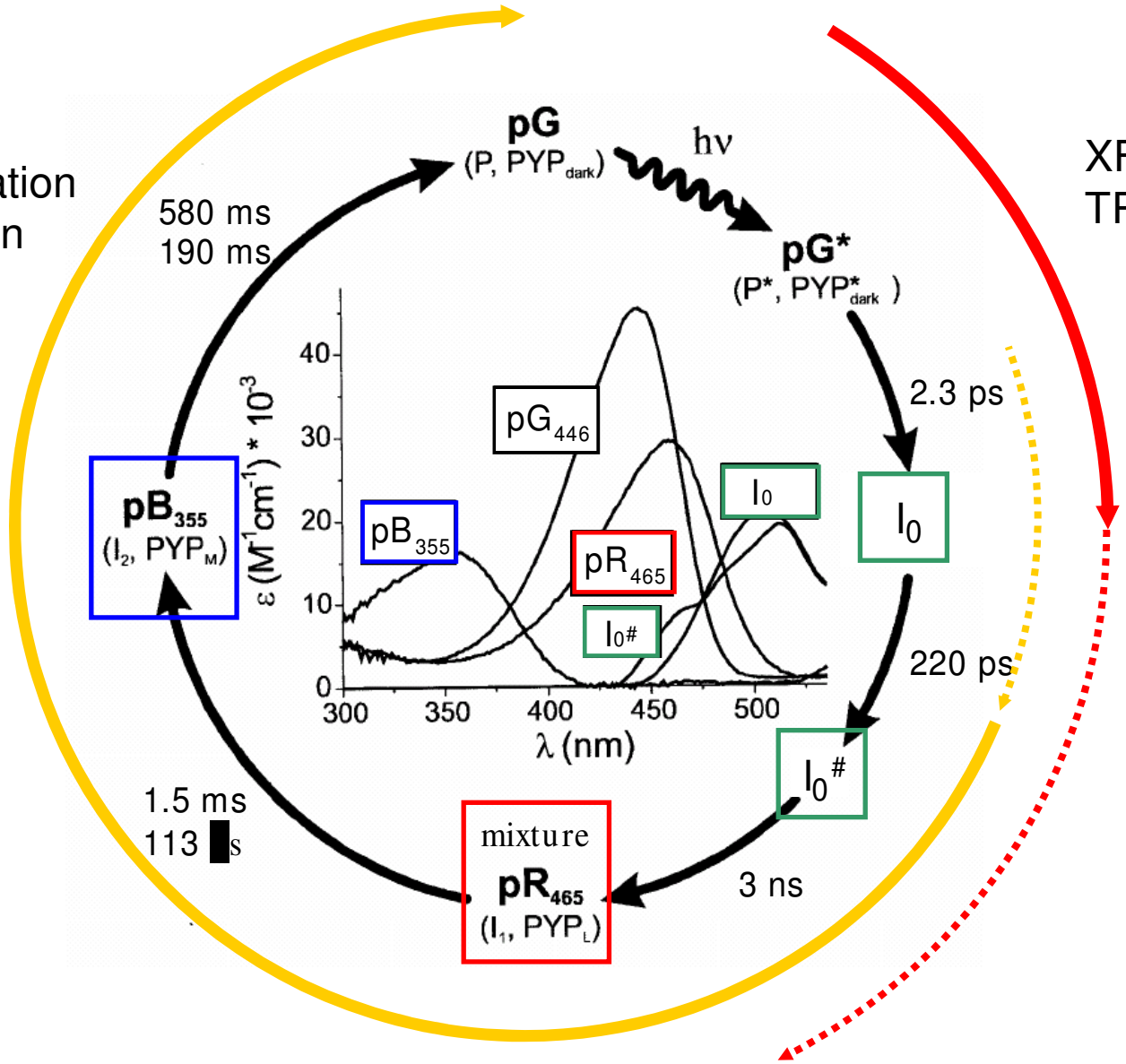

I0
hν
I1
I2
I3
S
SP
DE
0.15
0.12
0.09
0.06
0.03
0
-6
-5
-4
-3
-2
-1
log t [s]
c_frac

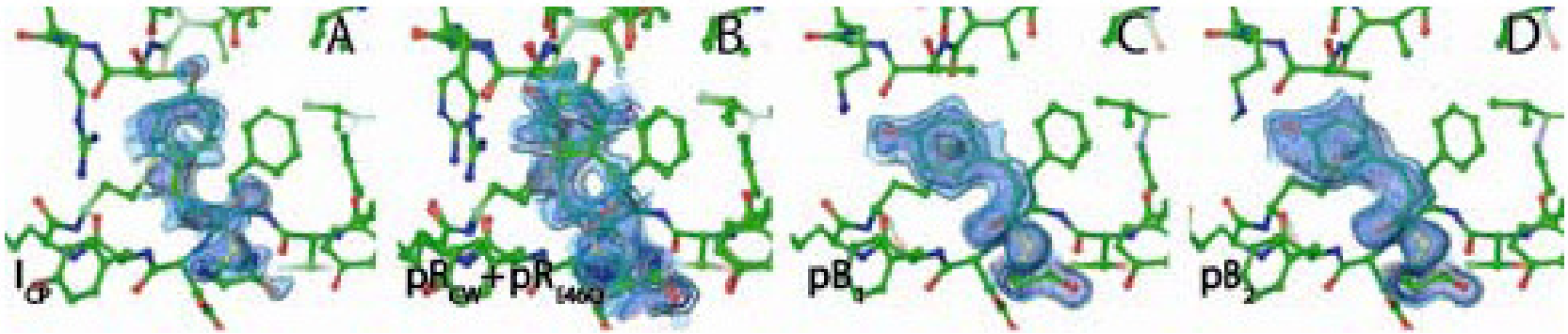
Example: The PYP photocycle (Moffat group)

Schmidt et al, 2004, Rajagopal et al., 2005, Ihee et al., 2005

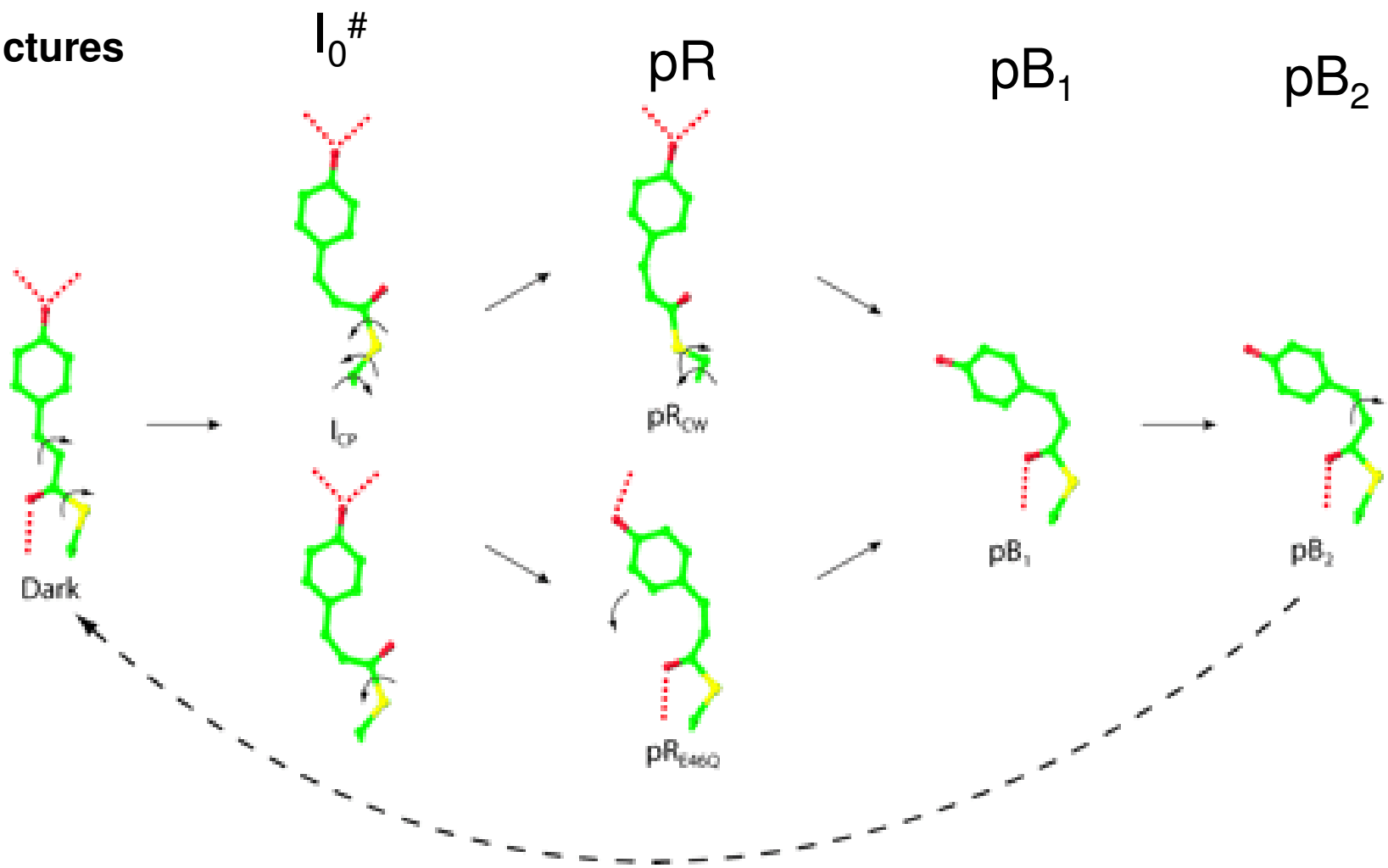
3rd generation synchrotron

XFEL TR-experiments

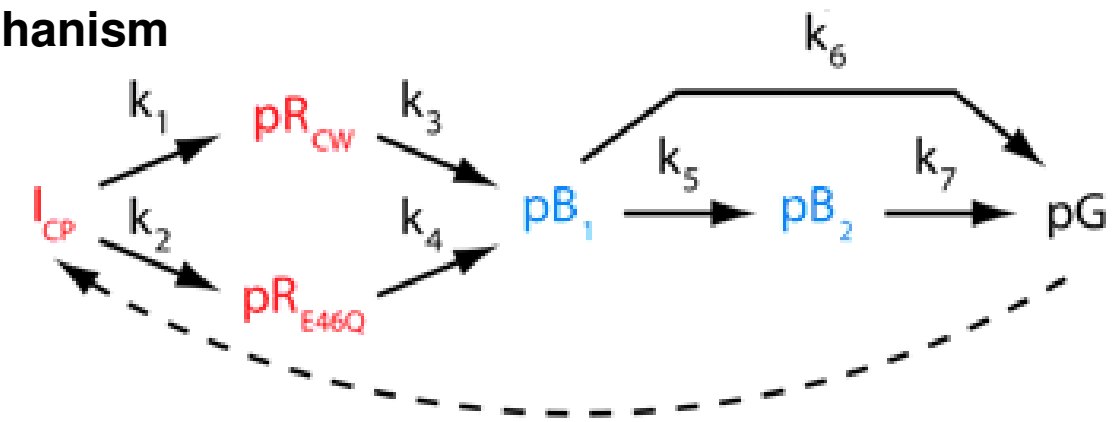



A
B
C
D
I_{CP}
$pR_{CW}+pR_{E46Q}$
pB_1
pB_2

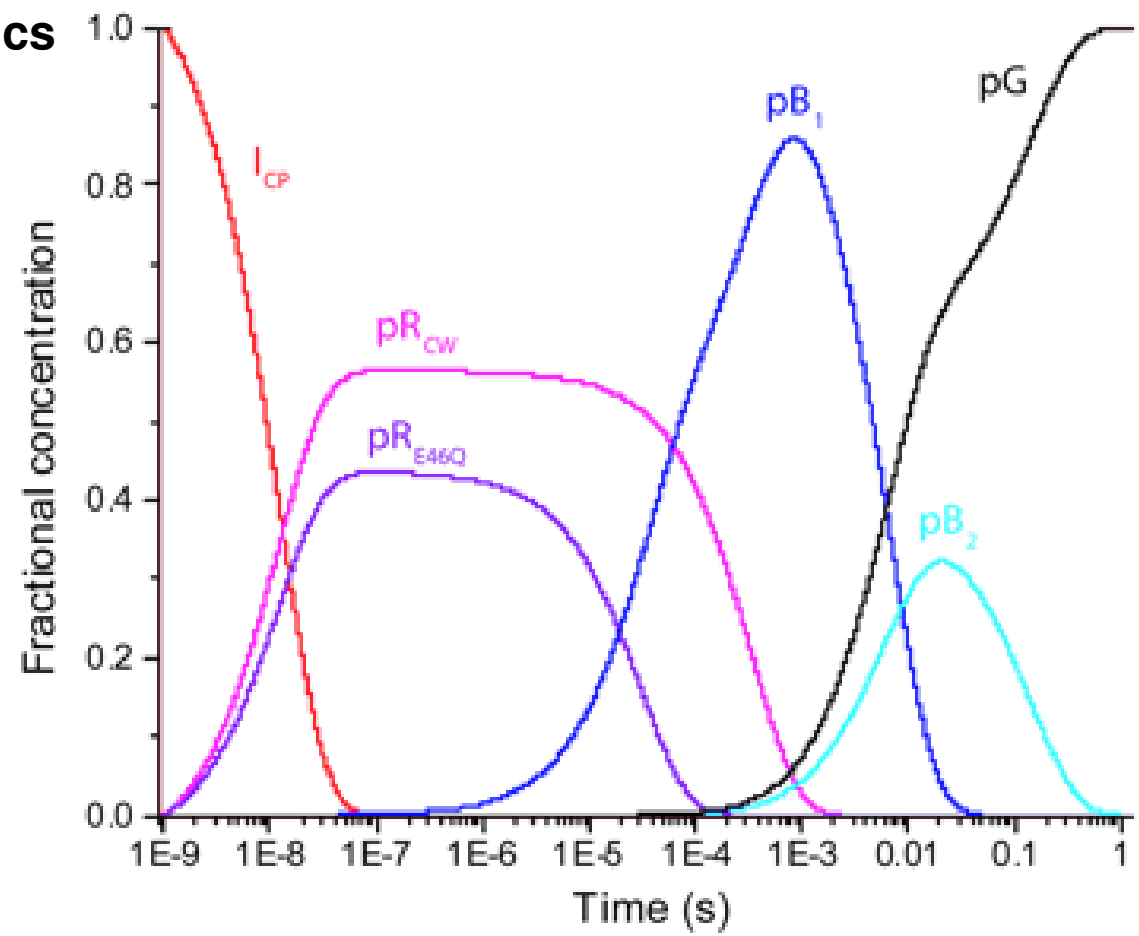
Structures


$I_0^{\#}$
pR
pB_1
pB_2
Dark
I_{CP}
pR_{CW}
pR_{E46Q}
pB_1
pB_2

Kinetic Mechanism

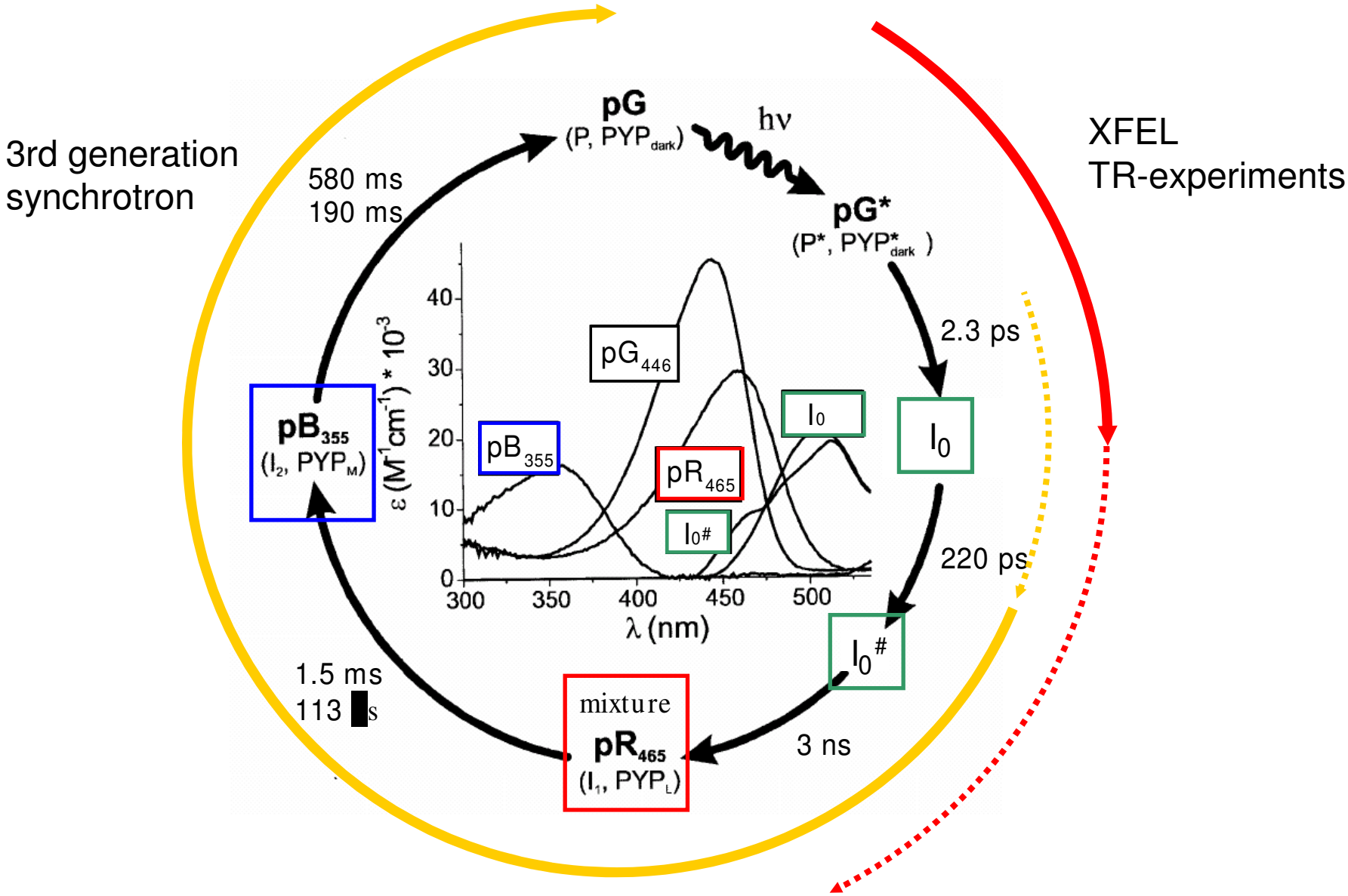


The Kinetics

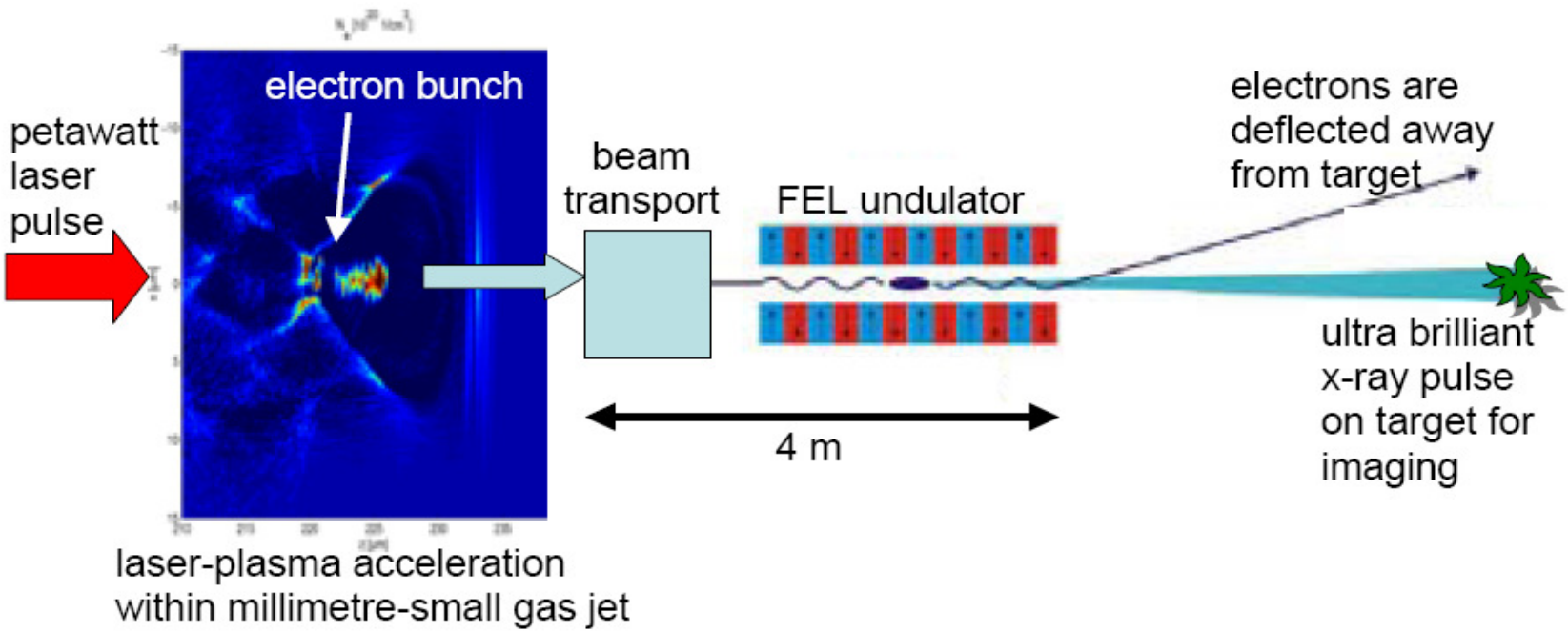


5. Outlook

analysis (not data reduction) of pico and femto-second time-resolved data



A table top hard X-ray free electron laser
(TT-XFEL)



X-ray pulse: 10 fs
# of photons: 10^{13} per pulse

ps	fs/as
•physical kinetics replaces chemical kinetics •Langevin type of relaxation employing friction/diffusion in restricted space •not well defined states •inhomogeneous ensemble •main directions of structural changes (maybe curved) instead of isotropic B-values	•protein RIGID •collective motions in the crystal: phonons •protein is container of small molecule •relaxation of small molecule (bond braking: within some ten fs, iron-out-of plane: a few 100 fs) •electronic relaxation ⇒ theory …

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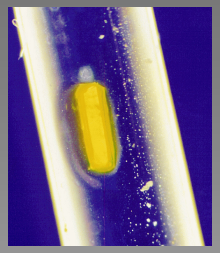
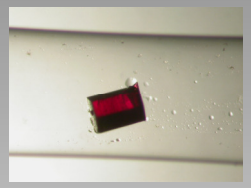
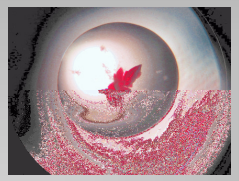
Tim Graber
Rob Henning

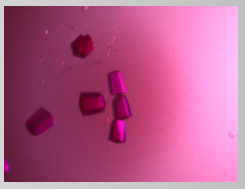
NIH
Phil Anfinrud
Friedrich Schotte

crystals









Money:

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