Time resolved scattering studies

Clement Blanchet
Time resolved study

• Collect data at different time point to study sample whose structure are evolving in time

• A tool to study kinetics
Kinetic experiment

• Perturb a system

• Monitor the return to equilibrium
Perturbation

• Different techniques:
  – Mixing
  – T, P jump
  – Light triggered reaction, ....

• Homogeneous perturbation

• Fast perturbation for fast kinetic
Monitor the reaction

• Fast reaction $\rightarrow$ short collection time

• But one need enough photons to collect a proper SAXS data $\rightarrow$ High flux
High flux

• Third generation synchrotron

• Multilayer monochromator
Dead time

• Time between the beginning of the reaction and the first data point

• Depends on:
  – How fast the reaction is triggered
  – How fast the first point can be collected

• Short dead time needed to study fast kinetic
Time scale of biological processes

[Diagram showing various biological processes and their time scales, including helix/coil transition, beta-hairpin formation, hydrophobic collapse, proline isomerization, protein rotation, loop closure, side-chain contacts formation, and native state formation.]
Examples

• “Slow Kinetics”
  – Fibril formation
• Sub-Second kinetics
  – Stopped-flow
• Millisecond kinetics
  – Continuous flow
  – Caged compound
• Ultrafast kinetics
“slow” kinetics

Subsecond kinetics

- Stopped-flow (dead time: 1-10 ms)
Stopped flow - Example

Characterization of Transient Intermediates in Lysozyme Folding with Time-resolved Small-angle X-ray Scattering

Segel et al. 
JMB, 1999, Volume 288 (3), 489-499
Lysozyme Folding

1 x
Lysozyme 3.6M GdmCl

5 x
Buffer Without GdmCl

Lysozyme 0.6M GdmCl
Lysozyme Folding

• Evolution of $R_g$ in time
Singular value decomposition

(Wildegger & Kiefhaber, 1997)
Interrupted refolding experiment

• Double mixing step monitored by fluorescence
Reconstruction of the scattering profile

\[ I(s, t) = \nu_C(t)I_C(s) + \nu_I(t)I_I(s) + \nu_N(t)I_N(s) \]

\[ I(S') = \sum_k \nu_k I_k(S) \]
Continuous flow

Time resolved scattering studies - C. Blanchet
Continuous flow

• Continuous flow $\rightarrow$ high sample consumption
  – Microfluidic continuous flow system

• Space $\leftrightarrow$ time
  – low flux OK
  – time resolution $\leftrightarrow$ flow rate and size of the beam

• Dead time $\approx 150$ microseconds

Time resolved scattering studies - C. Blanchet

12/4/2012
Example continuous flow

Conformational landscape of cytochrome c folding studied by microsecond-resolved small-angle x-ray scattering. Akiyama et al. PNAS 2002
Continuous flow

Mylar Film (22 µm Width)

Image intensifier & CCD

Syringe Drive

Mixing Plate (400 µm Width)

Distance (Time)

X-ray

200 x 400 µm

100 µm

33 x 400 µm

Mixing Point

12/4/2012

Time resolved scattering studies - C. Blanchet
Radius of gyration
Kratky plots
Singular value decomposition
Conformational landscape of Cyto C

![Graph showing the conformational landscape of Cyto C]

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Component I</th>
<th>Component II</th>
<th>Component N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_g$, Å</td>
<td>$D_{max}$, Å</td>
<td>$R_g$, Å</td>
</tr>
<tr>
<td>U ↔ I → II → N*</td>
<td>20.5</td>
<td>66</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>13.9</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

12/4/2012

Time resolved scattering studies - C.
Blanchet
Caged compound release by flash photolysis

• DM-nitrophen
Calmodulin


Mastoparan
Equilibrium measurement
Kinetics

(A)

$R_g^2$ (Å$^2$)

Time (msec)
12/4/2012

Time resolved scattering studies - C. Blanchet

0.5 ms

With mastoparan

Without mastoparan
Model
Ultra-fast time resolved
Ultra short collection time

- Beamline ID09B, ESRF, Grenoble
- Using the pulsed structure of the synchrotron

- About 5000000 bunch/sec
Isolate one bunch

• Isolate one bunch (ms shutter + fast chopper)
Single bunch experiment

• High flux needed

• Repetition of the measurements
Pump and probe experiment

Trigger with Laser pulse

Probe with X-ray

Bunch length ≈ 100 ps

Resolution: up to 100 ps
What is 100ps

100 psec $\rightarrow$ second
Second $\rightarrow$ 315 years

Light travels 3 cm in 100ps
Too fast for SAXS
TR WAXS

T and R states of hemoglobin

Looking at the unbinding of oxygen by hemoglobin

12/4/2012

Time resolved scattering studies - C. Blanchet
Experimental setup
Structural change in hemoglobin

(b) Graph showing intensity ($I$) vs. wavevector ($q$) for hemoglobin before and 31.6 µs after photolysis.

(c) Graph showing changes in intensity ($\Delta I$) vs. wavevector ($q$).

(d) Snapshots showing structural changes at different time points: <150 ns, 3 µs, 1-10 ms.
Conclusion

• SAS can be used to study kinetic

• For fast reaction:
  – Special setup required to triggered the reaction
  – High flux is needed: third generation source
    (impossible with lab source and neutrons)