Joint use of AUC and SAS

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- AUC: background and principles
- How AUC experiments are performed
- Data analysis
- Hydrodynamic bead modelling
- Example: oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands
- Example: *E. coli* virulence inhibitor drug targets
- DMD & EOM: modelling flexible systems
Outline

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Questions that can be answered by AUC

- Is the sample homogeneous or heterogeneous?
  - If heterogeneous, is it in molecular weight, shape, or both?
  - If heterogeneous, does heterogeneity depend on pH, salt, buffer, etc?
- Is the sample pure enough for X-ray crystallography, SAXS, SANS or NMR?
- Does the sample:
  - self-associate?
  - aggregate?
- What is the molecular weight of the sample, or a mixture of samples?
- Does the sample bind to a ligand?
- What is the stoichiometry of binding?
- What is the $K_d$?
- Is the association state/conformation of the sample affected by tagging?
More questions that can be answered by AUC

- What is the sedimentation and diffusion coefficient of the sample?
  - Is it globular or unfolded/disordered?
  - Is the conformation dependent on salt, pH, ligand concentration, deuteration, etc?
- Do mutations affect $K_d$, conformation, stoichiometry, etc?
- Is the sample affected by crowding?
The analytical ultracentrifuge (AUC) was invented by Theodor (The) Svedberg

Nobel Prize in Chemistry 1926 awarded to The Svedberg "for his work on disperse systems"
In the 1960’s – 1980’s the AUC was a core biochemical/biophysical technology

- Advice from the Beckman *Model E* AUC 1964 manual:
- “The *Model E*, like a woman, performs best when you care. But you needn’t pamper it - just give it the understanding it deserves.”

The modern AUC: a high speed preparative UC with optics
Inside an AUC

vacuum chamber

rotor

Rayleigh interference optics

sample cell (minus casing)

UV-vis optics

Inside an AUC
The most difficult part of an AUC experiment: assembling the sample holders

image from Beckman AUC manual
http://www.beckmancoulter.com/resourcecenter/labresources/resource_xla_xli.asp
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2 modes of operation - several data types

- Sedimentation velocity (SV)
- Sedimentation equilibrium (SE)
  - In solution
  - Non-destructive
  - Self-cleaning
  - Absolute
Sedimentation velocity (SV): shape and homogeneity data

- Absorbance vs. radius at different times:
  - t=0
  - t=1 h
  - t=3 h

Heterogeneity determination:
- Sedimentation (s) & diffusion (D) coefficients (shape)
- Association/dissociation constant ($K_a/K_d$)
- Stoichiometry
Sedimentation equilibrium (SE): mass and self-association

- $t=0$
- $t=1$ h
- $t=3$ h
- $t\approx 24$ h+

M
association/dissociation constant ($K_a/K_d$)
stoichiometry
non-ideality (B)
Sample requirements

- **Sample volume**
  - **SV**
    - 360 µl (up to 480 µl) in 12 mm pathlength
    - 90 µl (up to 120 µl) in 3 mm pathlength
  - **SE**
    - 20 µl (8-channel centrepiece - interference optics only)
    - 80 µl (2- or 6-channel centrepiece)

- **Sample concentration**
  - Absorbance optics: $A_l \approx 0.1-1.0$ in 12 mm pathlength cell
    - $l = 180-800$ nm
  - Interference optics: typically 0.05-30 mg/ml

- **Sample reference**
  - Absorbance optics: can be column eluant or dialysate better
  - Interference optics: must be dialysate

- **Typical multiplexing: 3 or 7 sample holders (“cells”) / run**
  - Up to 28 samples per run
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SV: radial movement recorded as function of time
Many methods & programs for AUC data analysis

- Too many for comprehensive review here.
- Model independent:
  - \( \frac{dC}{dt} \) (Stafford, SedAnal)
    - Eliminates time invariant noise. Resultant curves can be fitted with Gaussians to reveal species content and sedimentation coefficients.
  - \( c(s) \) (Schuck, Sedfit)
    - Good for “first look” at data to get an idea of number of species. Not a proper fit to data.
  - van Holde-Weischet (Demeler, UltraScan III)
    - Diffusion corrected s distribution. Good for detection of aggregates and identification of underlying model.
- Model dependent:
  - Non-interacting discrete species (Schuck, Sedfit)
    - Up to 4 separate species can be fitted.
  - Self-association (Stafford, SedAnal; Demeler, UltraScan III)
    - Determination of \( K_d, k_{on}, k_{off} \), stoichiometry
Almost all AUC data analysis software is freely available

- The RASMB website
  - “Reversible Associations in Structural and Molecular Biology”
  - http://www.rasmb.bbri.org/
  - Access to freely available software
  - Subscription to AUC-related discussion group
- Schuck lab (SEDFIT, SEDPHAT)
  - http://www.analyticalultracentrifugation.com/default.htm
- Demeler lab (UltraScan III (including SOMO))
  - http://www.ultrascan.uthscsa.edu/
SEDNTERP: Calculation of $\rho$, $\eta$ and partial specific volume

http://www.rasmb.bbri.org/software/PC/sednterp-philo/
c(s) analysis: how many species + s of species
1: Load SV data

http://www.analyticalultracentrifugation.com/default.htm
2: Specify parameters

- Specify parameters
- http://www.analyticalultracentrifugation.com/default.htm
3: Set meniscus, cell base and analysis limits
4: Run

http://www.analyticalultracentrifugation.com/default.htm
5: Subtract time and radial invariant noise
6: Fit (with solutions to the Lamm equation)
7: Integrate to obtain estimate of concentration of species and weight-average values
Sum of Lamm equations $0 \leq s \leq 20$ S discretised by 200
Sum of Lamm equations $0 \leq s \leq 15$ S discretised by 200
Sum of Lamm equations $0 \leq s \leq 12$ S discretised by 200
SE: 6-hole centrepiece data recorded until no change
SE data: the sum of exponentials for self-association

\[ A_r = \exp[\ln A_0 + H.M(r^2 - r_0^2)] + \exp[n_2 \ln A_0 + \ln Ka_2 + n_2 H.M(r^2 - r_0^2)] + \exp[n_3 \ln A_0 + \ln Ka_3 + n_3 H.M(r^2 - r_0^2)] + \exp[n_4 \ln A_0 + \ln Ka_4 + n_4 H.M(r^2 - r_0^2)] + E \]
Self-association: “deconvolution” into individual components

Experimental data = sum of species

- Monomer
- Dimer
- Tetramer
SE: best model revealed by residuals

2-4

1-4
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s = deviation from sphericity + hydrodynamic hydration

\[ s = \frac{M(1 - \bar{v}\rho)}{N_A f} \]
Sedimentation coefficient is a constraint for SAS modelling

- For one sphere \( f_0 = 6\pi\eta R_0 \)
- For an assembly of spheres an approximate solution is

\[
f_t = \frac{\sum_{i=1}^{N} \xi_i}{1 + (6\pi\eta_0 \sum_{i=1}^{N} \xi_i)^{-1} \sum_{i \neq j}^{N} \sum_{j=1}^{N} \xi_i \xi_j r_{ij}^{-1}}
\]

- where \( \xi_i = 6\pi\eta_0 \sigma_i \)
Several freely available programs for HBM

- A more exact expression for $f_t$ together with expressions for other hydrodynamic and related parameters are encoded in HBM software.

- José García de la Torre et al (Universidad Murcia, Spain)
  - http://leonardo.inf.um.es/macromol/programs/programs.htm
  - HYDRO
    - Computes hydrodynamic & other parameters for any bead model
  - HYDROPRO
    - Computes hydrodynamic & other parameters for models constructed from pdb files
  - And many other programs....

- Mattia Rocco, Emre Brookes, Borries Demeler
  - http://www.ultrascan.uthscsa.edu/SOMO
    - Generates HBMs from pdb files, computes hydrodynamic & other parameters with realistic hydration

e.g. parameters computed by SOMO (1)
e.g. parameters computed by SOMO (2)
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Oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

- $\alpha_5\beta_1$ ligands used to immobilise cells on surfaces via
  - 9th type III FN domain synergy site (PHSRN)
  - 10th type III FN domain RGD site
- $\alpha_5\beta_1$ ligand oligomers facilitate increased binding
- Oligomerisation accomplished via 5 heptad repeats based on GCN4 leucine zipper
  - I/L placed variously @ a and d positions to promote di-, tri- & tetramerisation
- Thiol-linked immobilisation to surface achieved via C-terminal Cys

- Question: do the ligands oligomerise as designed?
Construction of hydrodynamic bead models

- From vector (including His-tag) – too short for e.g. SWISSMODEL
- FN III 9-10 domain pair homology model (SWISSMODEL)
- Coiled-coil (42 a.a.) – SWISSMODELs generated for underlined segment
- Synthesised “missing beads”
Oligomer models generated

linear monomer
$s = 1.7 \, S$

Bent monomer
$s = 1.8 \, S$

linear dimer
$s = 2.7 \, S$

Bent dimer
$s = 2.5 \, S$

Kreiner et al., (2009) Biophysical Chemistry 142 34-39
Oligomer models generated

**linear trimer**
$s = 3.9 \, S$

**bent trimer**
$s = 3.1 \, S$

Kreiner et al., (2009) Biophysical Chemistry 142 34-39
Oligomer models generated

linear tetramer
$s = 4.7 \, S$

bent tetramer
$s = 3.7 \, S$

linear hexamer
$s = 5.0 \, S$

disulphide bridge

Kreiner et al., (2009) Biophysical Chemistry 142 34-39
AUC SV no DTT: c(s) analysis reveals complex composition

“dimer”

“trimer”

“tetramer”

Kreiner et al., (2009) Biophysical Chemistry 142 34-39
AUC SV + DTT: c(s) analysis reveals simplified composition

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Acknowledgements

- Kate Beckham, Andy Roe
- Mads Gabrielsen
  - University of Glasgow
- Emre Brookes
  - University of Texas Health Science Center, San Antonio
- Mattia Rocco
  - Istituto Nazionale per la Ricerca sul Cancro, Genoa
Salicylidene acylhydrazides inhibit virulence of *E. coli* O157

Compound immobilised on beads

Tandem MS-ID’d: 16 proteins

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Tree et al., 2009 Infection and Immunity **77**, 4209-4220
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Andrew Roe
Tree et al., 2009 Infection and Immunity **77**, 4209-4220
FolX is a tetramer in crystal

Andrew Roe, Kate Beckham, Mads Gabrielsen
SV & SE: FolX is an octamer in solution

- $s_{\text{exp}} = 6.09$ S
- $s_{\text{SOMO,8}} = 5.97$ S
- $s_{\text{SOMO,4}} = 3.62$ S
- $K_{d4-8} = 0.887$ μM

Andrew Roe, Kate Beckham, Mads Gabrielsen
Octameric structure superimposes well with SAXS envelope

Andrew Roe, Kate Beckham, Mads Gabrielsen
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Tree et al., 2009 Infection and Immunity **77**, 4209-4220
AUC: FkIβ is a dimer

- $S_{20,w} = 3.04 \text{ S}$
- $K_d = 7.6 \mu\text{M}$
There is no crystal structure of FklB

- Homology model based on another PPIases
SAXS: solution structure of FklB

- Homology model compared with the SAXS envelope
N-terminus of FkIb is not in the homology model
Also, likely to be significant flexibility in other regions.
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</tbody>
</table>

Andrew Roe
Tree et al., 2009 Infection and Immunity **77**, 4209-4220
Tpx: an atypical 2-Cys peroxiredoxin involved in oxidative stress recovery

Andrew Roe, Kate Beckham

AUC & SAXS: Tpx biological unit is a dimer

- Solved crystal structure of oxidised, reduced and inactive mutant (C61S)

\[ s = 3.04 \text{ S} \]

Andrew Roe, Kate Beckham
Tpx N-termini are absent from crystal structure

• Missing C-alphas added by modelling SAXS data using EOM
• Side chains added using WHAT IF
• Hydrodynamics computed with SOMO
SOMO - construction of “intelligently” hydrated bead models from atomic coordinates

- Water of hydration included in each bead
- Bead overlaps removed heirarchically
  - Reducing radii + translating bead centres outwards
- Beads overlapping by > preset threshold are fused (“popped”)
  - Buried beads excluded from hydrodynamic calculations
    - Reduces cpu time

SOMO is a subprogram of UltraScan III

Olwyn Byron/ Nithin Rai/ Marcelo Nöllmann/ Mattia Rocco/ Borries Demeler/ Emre Brooks
Rai et al. (2005) Structure 13 723-34
http://www.ultrascan.uthscsa.edu/
Step 1: open SOMO from US III Simulation menu
Step 2: Load pdb file
Step 3: Create bead model
Step 4: Compute hydrodynamics for bead model
### Step 5: Set up DMD simulation

<table>
<thead>
<tr>
<th>PDB file</th>
<th>Active</th>
<th>Relax temp kcal/molkB</th>
<th>Relax time * 50fs</th>
<th>Relax PDB output timestep</th>
<th>Relax PDB output count</th>
<th>Run temp kcal/molkB</th>
<th>Run time * 50fs</th>
<th>Run PDB output timestep</th>
<th>Run PDB output count</th>
<th>Static range</th>
<th>Static range (native)</th>
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</thead>
<tbody>
<tr>
<td>Tpx_dimer_sc_fixed_2.pdb</td>
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<td>.7</td>
<td>100</td>
<td>50</td>
<td>2</td>
<td>1</td>
<td>100336</td>
<td>2000</td>
<td>56</td>
<td>59 A:34-200, B:34-200</td>
<td>1.34-200, 2.34-200</td>
</tr>
<tr>
<td>Tpx_dimer_sc_fixed_2.pdb</td>
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<td>.7</td>
<td>100</td>
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<td>1</td>
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<td>4000</td>
<td>59</td>
<td>59 A:34-200, B:34-200</td>
<td>1.34-200, 2.34-200</td>
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<tr>
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<td>.7</td>
<td>100</td>
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<td>2</td>
<td>1</td>
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<td>6000</td>
<td>59</td>
<td>59 A:34-200, B:34-200</td>
<td>1.34-200, 2.34-200</td>
</tr>
</tbody>
</table>

**THIS WINDOW IS UNDER DEVELOPMENT.**
Step 6: Create a job package for remote cluster
Step 7: Log on to cluster and select server
Step 8: Submit job to cluster
Step 9: Obsessively check job status

THIS WINDOW IS UNDER DEVELOPMENT.
Step 10: join resultant 50 pdbs into 1 file
Step 11: Use batch system to compute hydrodynamics (and SAS curves)
Step 12: Compare with experimental data
Outline

- AUC: background and principles
- How AUC experiments are performed
- Systems and data
- Hydrodynamic modelling
- Examples: *E. coli* virulence inhibitor drug targets
- DMD: generating models of flexible systems
Discrete molecular dynamics modelling in SOMO

- $T = 50000$ means $0.25 \text{ ns}$
- $t = 0.5 \text{ kcal/mol/kB} / (1.9866 \times 10^3 \text{ kcal/mol/kB/K}) \approx 251 \text{ K (-22°C)}$
- $t = 1.0 \text{ kcal/mol/kB} / (1.9866 \times 10^3 \text{ kcal/mol/kB/K}) \approx 503 \text{ K (230°C)}$
Tpx SAXS data poorly described by dimer or dimer plus “tails”
TpX: No static residues, run temp = 0.5, run time = 10000
Tpx: static residues A:34-200, B:34-200
run temp = 0.1, run time = 50000
Tpx: static residues A:34-200, B:34-200
run temp = 0.5, run time = 10000
Tpx: static residues A:34-200, B:34-200  
run temp = 1.0, run time = 50000
TpX: static residues A:34-200, B:34-200
run temp = 1.0, run time = 100000
What about the hydrodynamics?

- Experimental
  - \( s = 3.04 \) S

- Crystal structure dimer without N-terminal tails
  - \( s = 3.06 \) S

- Crystal structure dimer with N-terminal tails
  - \( s = 3.15 \) S

- Average of 50 structures (\( T=0.1, t=50000 \))
  - \( s = 3.25 \pm 0.01 \) S

- Average of 50 structures (\( T=0.5, t=10000 \))
  - \( s = 3.15 \pm 0.02 \) S

- Average of 50 structures (\( T=1.0, t=50000 \))
  - \( s = 2.96 \pm 0.09 \) S

- Average of 50 structures (\( T=1.0, t=100000 \))
  - \( s = 2.73 \pm 0.21 \) S

- Average of 50 structures (no static residues, \( T=0.5, t=10000 \))
  - \( s = 3.08 \pm 0.02 \) S
EOM modelling

\[ R_g = 2.54 \pm 0.09 \text{ nm} \]

\[ D_{\text{max}} = 8.2 \text{ nm} \]
Rg distribution of best ensemble

Ensemble Average Radius of gyration = 25.70
P(r) distribution of best ensemble

Ensemble Average Radius of gyration = 90.85

Size_distr_002_1.dat
Fit to data by best ensemble

CYCLE: 35  CHI: 0.413  GENER.:1000  ENSEMBLES: 50  CURVES: 22

Log{1}

profiles_002_1.fit
The structures of the best ensemble
What is the average sedimentation coefficient of the ensemble?

<table>
<thead>
<tr>
<th>Model name</th>
<th>s [S]</th>
<th>Rg [nm] (from bead model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00876tpx_039_047_1-so</td>
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<td>2.45</td>
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<td>01084tpx_039_047_1-so</td>
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<td>2.36</td>
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<td>01664tpx_039_047_1-so</td>
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<td>2.39</td>
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<td>01888tpx_039_047_1-so</td>
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<tr>
<td>07464tpx_039_047_1-so</td>
<td>2.87</td>
<td>3.14</td>
</tr>
</tbody>
</table>

Average: 00876tpx_039_047_1-so, 01084tpx_039_047_1-so, 01664tpx_039_047_1-so, 01888tpx_039_047_1-so, 04780tpx_039_047_1-so, 06067tpx_039_047_1-so, 07108tpx_039_047_1-so, 07464tpx_039_047_1-so

Standard deviation: 00876tpx_039_047_1-so, 01084tpx_039_047_1-so, 01664tpx_039_047_1-so, 01888tpx_039_047_1-so, 04780tpx_039_047_1-so, 06067tpx_039_047_1-so, 07108tpx_039_047_1-so, 07464tpx_039_047_1-so

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Average</td>
<td>3.23</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.23</td>
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</tbody>
</table>
Hydrodynamics and I(s) of ensemble agrees with experimental data

\[ s = 3.04 \text{ S} \]

\[ R_g = 2.54 \pm 0.09 \text{ nm} \]