Joint use of AUC and SAS

Olwyn Byron

School of Life Sciences
College of Medical, Veterinary and Life Sciences

University of Glasgow, Scotland UK
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
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
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 are the equilibrium and rate constants for the binding?
- 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 the strength of binding, self-association, conformation, stoichiometry, etc?
• Is the sample affected by crowding?
Questions that can be answered by SAS

• What is the solution shape of the molecule?
• Does its shape change when it binds a ligand?
• What is the shape of the complex it makes with other molecules?
• Where are the individual components within the complex?
• What is the range of flexibility?
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

Beckman Coulter ProteomeLab XL-A/XL-I; €250-350 k
Inside an AUC

- Vacuum chamber
- Rotor
- Rayleigh interference optics
- Sample cell (minus casing)
- UV-vis optics

Diagram showing the components of an AUC.
Inside the rotor chamber

- absorbance slit assembly
- drive spindle
- condenser lens for interference optics
- radiometer
- monochromator mount

Absorbance optics: the AUC is like a spinning double-beam spectrophotometer

Top view of an AUC cell

image from Beckman AUC manual
http://www.beckmancoulter.com/resourcecenter/labresources/resource_xla_xli.asp
Interference optics acquire refractive index data rapidly, independent of chromophores.

Image from Beckman AUC manual
http://www.beckmancoulter.com/resourcecenter/labresources/resource_xla_xli.asp
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
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
- Radius

- 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

- absorbance vs. radius
- $t=0$
- $t=1 \text{ h}$
- $t=3 \text{ h}$
- $t\approx 24 \text{ h}^+$

M association/dissociation constant ($K_a/K_d$)
stoichiometry
non-ideality ($B$)
SV versus SE

- SV: observe movement of sedimentation boundary
- Change in (sometimes complex) boundary over time is due to
  - Sedimentation
  - Diffusion
- SE: rotor spun more slowly so diffusion can balance sedimentation - system reaches thermodynamic equilibrium
- Observe no change in boundary over time
  - Unless sample is degrading or changing in some other way
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: $A_{\lambda} \approx 0.1-1.0$ in 12 mm pathlength cell
    - $\lambda = 180-800$ 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
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
2 important equations

\[ s = \frac{u}{\omega^2 r} = \frac{M(1 - \bar{v}\rho)}{N_A f} \]

Svedberg equation

\[ D = \frac{sRT}{M(1 - \bar{v}\rho)} \]
SV: radial movement recorded as function of time
SV: species can resolve into separate boundaries
SV: the c(s) distribution reveals less obvious species
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
Self-association: “deconvolution” into individual components

experimental data = sum of species

- monomer
- dimer
- tetramer
SE data: the sum of exponentials for self-association

\[ A_r = \exp[\ln A_0 + H.M(r^2 - r_0^2)] + \exp[n_2 \ln A_0 + \ln K_a + n_2 \cdot H.M(r^2 - r_0^2)] + \exp[n_3 \ln A_0 + \ln K_a + n_3 \cdot H.M(r^2 - r_0^2)] + \exp[n_4 \ln A_0 + \ln K_a + n_4 \cdot H.M(r^2 - r_0^2)] + E \]
SE: best model revealed by residuals
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
Hydrodynamic bead modelling

- Frictional properties of sphere and assemblies of spheres exactly known
- $s$ for molecule represented as sphere assembly (bead model) can be accurately computed
- If $s_{\text{comp}} \approx s_{\text{exp}}$ model is one plausible solution conformation for the molecule
- $s$ and $D$ are constraints for modelling SAS data
SOMO: computation of $s$ from atomic coordinates

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/
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
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

Andrew Roe
Tree et al., 2009 Infection and Immunity 77, 4209-4220
Salicylidene acylhydrazides inhibit virulence of *E. coli* O157

Compound immobilised on beads

Tandem MS-ID’d: 16 proteins

<table>
<thead>
<tr>
<th>Band (Fig. 1B)</th>
<th>Genbank ID</th>
<th>MOWSE score</th>
<th># peptides matched</th>
<th>Gene</th>
<th>Protein function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gi</td>
<td>15802850</td>
<td>250</td>
<td>9</td>
<td>fotX</td>
</tr>
<tr>
<td>2</td>
<td>gi</td>
<td>15802098</td>
<td>232</td>
<td>12</td>
<td>Z2714</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>15803192</td>
<td>137</td>
<td>8</td>
<td>Z3974</td>
</tr>
<tr>
<td>4</td>
<td>gi</td>
<td>15001846</td>
<td>197</td>
<td>6</td>
<td>txp</td>
</tr>
<tr>
<td>5</td>
<td>gi</td>
<td>12515926</td>
<td>179</td>
<td>13</td>
<td>yecD</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>1537048</td>
<td>382</td>
<td>20</td>
<td>fktB</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>15800925</td>
<td>322</td>
<td>16</td>
<td>wtaA</td>
</tr>
<tr>
<td>7</td>
<td>gi</td>
<td>147379</td>
<td>415</td>
<td>19</td>
<td>prs</td>
</tr>
<tr>
<td>8</td>
<td>gi</td>
<td>15804160</td>
<td>328</td>
<td>20</td>
<td>tdh</td>
</tr>
<tr>
<td>9</td>
<td>gi</td>
<td>42497</td>
<td>344</td>
<td>28</td>
<td>prob</td>
</tr>
<tr>
<td>9</td>
<td>gi</td>
<td>15802064</td>
<td>125</td>
<td>12</td>
<td>nemA</td>
</tr>
<tr>
<td>9</td>
<td>gi</td>
<td>396292</td>
<td>117</td>
<td>6</td>
<td>gldA</td>
</tr>
<tr>
<td>10</td>
<td>gi</td>
<td>15799738</td>
<td>334</td>
<td>19</td>
<td>surA</td>
</tr>
<tr>
<td>11</td>
<td>gi</td>
<td>15804455</td>
<td>265</td>
<td>26</td>
<td>glnA</td>
</tr>
<tr>
<td>12</td>
<td>gi</td>
<td>15801467</td>
<td>522</td>
<td>41</td>
<td>adhE</td>
</tr>
<tr>
<td>13</td>
<td>gi</td>
<td>15833452</td>
<td>110</td>
<td>9</td>
<td>fkpA</td>
</tr>
<tr>
<td>14</td>
<td>gi</td>
<td>15801691</td>
<td>116</td>
<td>8</td>
<td>yncE</td>
</tr>
<tr>
<td>15</td>
<td>gi</td>
<td>1942721</td>
<td>264</td>
<td>26</td>
<td>Ef-Tu</td>
</tr>
<tr>
<td>16</td>
<td>gi</td>
<td>52078252</td>
<td>100</td>
<td>15</td>
<td>stcE</td>
</tr>
</tbody>
</table>
FolX is a tetramer in crystal

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

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

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

Andrew Roe, Kate Beckham, Mads Gabrielsen
Salicylidene acylhydrazides inhibit virulence of E. coli O157

Compound immobilised on beads

Tandem MS-ID'd: 16 proteins

<table>
<thead>
<tr>
<th>Band (Fig. 1B)</th>
<th>Genbank ID</th>
<th>MOWSE score</th>
<th># peptides matched</th>
<th>Gene</th>
<th>Protein function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gi</td>
<td>15802850</td>
<td>250</td>
<td>9</td>
<td>fotX</td>
</tr>
<tr>
<td>2</td>
<td>gi</td>
<td>15802098</td>
<td>232</td>
<td>12</td>
<td>Z2714</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>15803192</td>
<td>137</td>
<td>8</td>
<td>Z3974</td>
</tr>
<tr>
<td>4</td>
<td>gi</td>
<td>15601846</td>
<td>179</td>
<td>6</td>
<td>yscD</td>
</tr>
<tr>
<td>5</td>
<td>gi</td>
<td>12515916</td>
<td>179</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>537048</td>
<td>382</td>
<td>20</td>
<td>fkbB</td>
</tr>
<tr>
<td>7</td>
<td>gi</td>
<td>15800925</td>
<td>322</td>
<td>16</td>
<td>wrbA</td>
</tr>
<tr>
<td>8</td>
<td>gi</td>
<td>147379</td>
<td>415</td>
<td>19</td>
<td>prs</td>
</tr>
<tr>
<td>9</td>
<td>gi</td>
<td>15804164</td>
<td>328</td>
<td>20</td>
<td>ldh</td>
</tr>
<tr>
<td>10</td>
<td>gi</td>
<td>42497</td>
<td>344</td>
<td>28</td>
<td>proB</td>
</tr>
<tr>
<td>11</td>
<td>gi</td>
<td>15802064</td>
<td>125</td>
<td>12</td>
<td>nemA</td>
</tr>
<tr>
<td>12</td>
<td>gi</td>
<td>396922</td>
<td>117</td>
<td>6</td>
<td>gldA</td>
</tr>
<tr>
<td>13</td>
<td>gi</td>
<td>5799738</td>
<td>334</td>
<td>19</td>
<td>surA</td>
</tr>
<tr>
<td>14</td>
<td>gi</td>
<td>1580455</td>
<td>265</td>
<td>26</td>
<td>gltA</td>
</tr>
<tr>
<td>15</td>
<td>gi</td>
<td>15801467</td>
<td>522</td>
<td>41</td>
<td>adhE</td>
</tr>
<tr>
<td>16</td>
<td>gi</td>
<td>15833452</td>
<td>110</td>
<td>9</td>
<td>fkpA</td>
</tr>
<tr>
<td>17</td>
<td>gi</td>
<td>15801691</td>
<td>116</td>
<td>8</td>
<td>yscE</td>
</tr>
<tr>
<td>18</td>
<td>gi</td>
<td>1942721</td>
<td>264</td>
<td>26</td>
<td>E-f Tu</td>
</tr>
<tr>
<td>19</td>
<td>gi</td>
<td>52078252</td>
<td>100</td>
<td>15</td>
<td>stcE</td>
</tr>
</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)

Andrew Roe, Kate Beckham
N termini are absent from crystal structure: effect on s hidden by mass effects cancelling friction effects

- SOMO model of Tpx crystal dimer
- Computed s (3.06 S) is close to experimental value (3.04 S)
- But model does not include 2 x 36 amino acid N-termini
Tpx N-termini are absent from crystal structure

- Missing C-alphas added by modelling SAXS data using EOM
- Side chains added using WHAT IF
SAXS data poorly described by dimer or dimer plus “tails”
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 ns
- \( t = 0.5 \text{ kcal/mol/k}_B / (1.9866 \times 10^3 \text{ kcal/mol/k}_B/K) \approx 251 \text{ K} (-22^\circ \text{C})
- \( t = 1.0 \text{ kcal/mol/k}_B / (1.9866 \times 10^3 \text{ kcal/mol/k}_B/K) \approx 503 \text{ K} (230^\circ \text{C})

Tpx: No static residues, run temp = 0.5, run time = 10000
50 computed SAXS curves overlaid with expt’al data
But single model does not portray dynamics – average of ensembles more meaningful
Tpx: static residues A:34-200, B:34-200
run temp = 0.1, run time = 50000
A low Andersen thermostat temperature (T) provides very little conformational variability.
Tpx: static residues A:34-200, B:34-200
run temp = 0.5, run time = 10000
Increase in Andersen thermostat temperature results in more variation (even when offset by reduced run time)
Average of 50 models
TpX: static residues A:34-200, B:34-200
run temp = 1.0, run time = 50000
Further increase in Andersen thermostat temperature plus longer simulation interval results in even more variation.
Average of 50 models
This average model describes the data better than the single starting model.
What about the hydrodynamics?

- **Experimental**
  - $s = 3.04 \text{ S}$

- **Crystal structure dimer without N-terminal tails**
  - $s = 3.06 \text{ S}$

- **Crystal structure dimer with N-terminal tails**
  - $s = 3.15 \text{ S}$

- **Average of 50 structures (T=0.1, t=50000)**
  - $s = 3.25 \pm 0.01 \text{ S}$

- **Average of 50 structures (T=0.5, t=10000)**
  - $s = 3.15 \pm 0.02 \text{ S}$

- **Average of 50 structures (T=1.0, t=50000)**
  - $s = 2.96 \pm 0.09 \text{ S}$

- **Average of 50 structures (no static residues, T=0.5, t=10000)**
  - $s = 3.08 \pm 0.02 \text{ S}$
So this is the likely conformational ensemble in solution
Salicylidene acylhydrazides inhibit virulence of *E. coli* O157

Compound immobilised on beads

Tandem MS-ID’d: 16 proteins

<table>
<thead>
<tr>
<th>Band</th>
<th>Genbank ID</th>
<th>MOWSE score</th>
<th># peptides matched</th>
<th>Gene</th>
<th>Protein function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gi</td>
<td>15802850</td>
<td>250</td>
<td>9</td>
<td>fotX</td>
</tr>
<tr>
<td>2</td>
<td>gi</td>
<td>15802098</td>
<td>232</td>
<td>12</td>
<td>Z3714</td>
</tr>
<tr>
<td>3</td>
<td>gi</td>
<td>15803192</td>
<td>137</td>
<td>8</td>
<td>Z3974</td>
</tr>
<tr>
<td>4</td>
<td>gi</td>
<td>15801846</td>
<td>197</td>
<td>6</td>
<td>lpx</td>
</tr>
<tr>
<td>5</td>
<td>gi</td>
<td>12515962</td>
<td>179</td>
<td>13</td>
<td>yedD</td>
</tr>
<tr>
<td>6</td>
<td>gi</td>
<td>537048</td>
<td>382</td>
<td>20</td>
<td>yecI</td>
</tr>
<tr>
<td>7</td>
<td>gi</td>
<td>15800925</td>
<td>322</td>
<td>16</td>
<td>wrbA</td>
</tr>
<tr>
<td>8</td>
<td>gi</td>
<td>147379</td>
<td>415</td>
<td>19</td>
<td>prs</td>
</tr>
<tr>
<td>9</td>
<td>gi</td>
<td>15804160</td>
<td>328</td>
<td>20</td>
<td>ldh</td>
</tr>
<tr>
<td>10</td>
<td>gi</td>
<td>42497</td>
<td>344</td>
<td>28</td>
<td>prob</td>
</tr>
<tr>
<td>11</td>
<td>gi</td>
<td>15802064</td>
<td>125</td>
<td>12</td>
<td>nemA</td>
</tr>
<tr>
<td>12</td>
<td>gi</td>
<td>396292</td>
<td>117</td>
<td>6</td>
<td>gdiA</td>
</tr>
<tr>
<td>13</td>
<td>gi</td>
<td>15789738</td>
<td>334</td>
<td>19</td>
<td>surA</td>
</tr>
<tr>
<td>14</td>
<td>gi</td>
<td>15804455</td>
<td>265</td>
<td>26</td>
<td>glmA</td>
</tr>
<tr>
<td>15</td>
<td>gi</td>
<td>15801467</td>
<td>522</td>
<td>41</td>
<td>adhE</td>
</tr>
<tr>
<td>16</td>
<td>gi</td>
<td>15833452</td>
<td>110</td>
<td>9</td>
<td>fkpA</td>
</tr>
<tr>
<td>17</td>
<td>gi</td>
<td>15801691</td>
<td>116</td>
<td>8</td>
<td>yncE</td>
</tr>
<tr>
<td>18</td>
<td>gi</td>
<td>1942721</td>
<td>264</td>
<td>26</td>
<td>Ef-Tu</td>
</tr>
<tr>
<td>19</td>
<td>gi</td>
<td>52078252</td>
<td>100</td>
<td>15</td>
<td>stcE</td>
</tr>
</tbody>
</table>

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

\[ S_{20,w} = 3.04 \, S \]
\[ K_d = 7.6 \, \mu M \]
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

Kate Beckham
N-terminus of Fklb is not in the homology model
SAXS data not well reproduced by dimer with or without N-terminal tails
T = 0.1, t = 50000 50 curves overlaid with expt’al data
$T = 0.5$, $t = 10000$ 50 curves overlaid with expt’al data
$T = 1.0$, $t = 50000$ 50 curves overlaid with expt’al data
T = 0.5, t = 10000 no static residues
50 curves overlaid with expt’al data
DMD of tail really doesn’t make much difference to improving the fit to SAXS data: more EOM needed!