- Planning an Experiment
  - Assessing Sample suitability

- Preparing an Experiment
  - Best sample
  - Best buffer

- Performing an Experiment
  - Trouble shooting at the beamline: 5 test cases
The SAXS (small angle X-ray scattering) Experiment

- X-ray beam
- I(s) graph
Signal is amplified
High resolution $d \approx \lambda$

$I(s)$ is isotropic
Concentrates around the primary beam
Low resolution $d \gg \lambda$

No need to grow crystals
No crystallographic packaging forces
Not limited by MW
Physiological conditions
Observe response to changes
Sample requirements

- Amount: 7 μl; 30-50 μl per sample
- Concentration:
- Buffer:
- Sample quality:
Sample requirements

- Amount: 7μl; 30-50 μl per sample
- Concentration: dependent on MW \( (100 \sim MW \times c) \)
- Buffer
- Sample quality

\[ I(0) \approx N (\Delta \rho V)^2 \]
Sample requirements

- Amount: 7μl; 30-50 μl per sample
- Concentration: dependent on MW \((100 \sim MW x c)\)
- Buffer
- Sample quality

\[ I(0) \approx N (\Delta \rho V)^2 \]

PLANNING THE EXPERIMENT
Sample requirements

- Amount: 7μl; 30-50 μl per sample
- Concentration: dependent on MW
- Buffer: as less additives as possible
- Sample quality

\[ I(0) \approx N (\Delta \rho \ V)^2 \]
<table>
<thead>
<tr>
<th>Class of additive</th>
<th>example</th>
<th>concentration</th>
<th>purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salts</td>
<td>NaCl, KCl, (NH₄)₂SO₄</td>
<td>50-150 mM</td>
<td>maintain ionic strength of medium</td>
</tr>
<tr>
<td>Detergents</td>
<td>Deoxycholate, Triton X-100</td>
<td>0.1-1%</td>
<td>solubilization of poorly soluble proteins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As close as</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>possible to cmc!</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td>5-18%</td>
<td>stabilization</td>
</tr>
<tr>
<td>Glucose or sucrose</td>
<td></td>
<td>25 mM</td>
<td>Stabilize lysosomal membranes, reduce protease release</td>
</tr>
<tr>
<td>Metal chelators</td>
<td>EDTA, EGTA</td>
<td>1 mM</td>
<td>reduce oxidation damage, chelate metal ions</td>
</tr>
<tr>
<td>Reducing agents</td>
<td>DTT, DTE 2-Mercaptoethanol</td>
<td>1-10 mM</td>
<td>reduce oxidation damage</td>
</tr>
<tr>
<td>Ligands, metal ions</td>
<td>Mg²⁺, ATP, GTP</td>
<td>1-10 mM</td>
<td>stabilization</td>
</tr>
</tbody>
</table>

https://www.embl.de/pepcore/pepcore_services/protein_purification/extraction_clarification/lysis_buffer_additives

PLANNING THE EXPERIMENT
- Sample requirements
  - Amount: 7 μl; 30-50 μl per sample
  - Concentration: dependent on MW
  - Buffer: as less additives as possible

- Sample quality

Foldon vs Proteasome
Sample preparation strategies

- Sample stable at high concentrations
  - Concentrate
  - Dialyze
  - (Store/Ship)
  - Filter
  - Conc. determination
  - SAXS from dilutions & buffer

- Sample not stable at high concentrations
  - Dialyze
  - (Store/Ship)
  - Filter
  - Step-wise concentration
  - Conc. determination
  - SAXS from different conc. concentration steps & buffer
- Sample Characterization

Native Gel Electrophoresis

Analytical ultracentrifugation

Size Exclusion Chromatography

Dynamic Light Scattering

Static Light Scattering

nativeMS

m/z

percentage
Buffer Preparation
  Method of choice: Dialysis

- diffusion coefficients
- temperature
- time
- concentration of species
- sample volume
- dialysate (buffer) volume (100:1)
- number of dialysate changes (2-3)
- membrane surface area
- membrane thickness
- molecular charges
- dialysate agitation (stirring)

→ standard protocol: 16 to 24 hours
- **Buffer Preparation**
  - **Method of choice: Dialysis**
  - Tubing for ~2mL to 100mL samples
  - Cassette for ~0.5mL to 70mL
  - Cup Devices for ~10μL to 2mL

- **Alternative methods**
  - Spiking
  - Diafiltration
  - Desalting/SEC column
Case 1: the Relaxed Scientist

- Sample: Calmodulin; 100 μl 6.5 mg/ml (UV-Vis)
- Question: confirm monomeric state of protein
Case 1: the Relaxed Scientist

- Sample: Calmodulin; 100 ul 6.5 mg/ml (UV-Vis)
- Question: confirm monomeric state (16.8 kD)
- Result:

$$I(0) \rightarrow 22 \text{ kD}$$
$$\rightarrow (30\% \text{ higher})$$
Case 1: the Relaxed Scientist

- Sample: Calmodulin; 100 ul 6.5 mg/ml (UV-Vis)
- Question: confirm monomeric state of protein
- Result: ambiguous; $\text{MW}_{\text{expected}} = 16.8 \text{ kD}$
  $\text{MW}_{I(0)} = 22 \text{ kD} (30\% \text{ higher})$
- Explanation: unsuitable method for determining c

ADQLTEEQIAEFKEAFSLFDKGDGTITTKELGTVMRSLGQNPTEAELQDMINEV
DADGNGTIDFPEFLTMARKMKDTDSEEIEAREAFRDVFMDGDGNYISAELRHVM
TNLGEKLTDDEEVDEMIREANIDGDGQVNYEEFVQMMTAK $\Rightarrow \varepsilon = 0.17$

protparam: Experience shows that this (no Trp) could result in more than 10% error in the computed extinction coefficient
<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lowry Assay</strong></td>
<td>- Buiret chromophore (copper ion complex with amide bonds)</td>
<td>- Relative sensitive: - 1 to 100 ug</td>
<td>- interfering compounds such as detergents, carbohydrates, glycerol, Tris, EDTA… -- content of Tyr, Trp - time consuming</td>
</tr>
<tr>
<td></td>
<td>- Cu⁺⁺. Tyr, Trp reduce Folin-Ciocalteu reagent (660 nm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BCA Assay</strong></td>
<td>- protein backbone chelates Cu²⁺ ions and reduces them to Cu¹⁺ which shifts color of dye (562 nm)</td>
<td>- less sensitive to the types of amino acids in the protein - suitable for most detergents &amp; denaturants</td>
<td>- Cysteine rich samples (temp) - reducing agents (DTT, 2-ME) - time consuming</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bradford Assay</strong></td>
<td>- Color shift of Coomassie brilliant blue G-250 dye upon binding arg and aromatic residues</td>
<td>- simple, rapid, cheap, sensitive - micro: 1-20 ug - macro: 20-100 ug - copes with reducing agents</td>
<td>-- content of Arg (eg. histones) -- non linear curve (absorbance of free dye) - Choice of standard, pH - “sticky proteins” precipitate</td>
</tr>
<tr>
<td><strong>UV( 280 nm)</strong></td>
<td>Ultraviolet absorbance according to Beer’s law, A ~ c ε</td>
<td>- quick - sample recovery</td>
<td>- sequence dependent - protein complexes, mixtures -- sensitive to pH and ions</td>
</tr>
<tr>
<td><strong>Differential Refractometry</strong></td>
<td>index of refraction according to Snell’s law</td>
<td>- total/ pure protein - quick - sample recovery</td>
<td>- Magic number - Temperature sensitive</td>
</tr>
</tbody>
</table>

**PERFORMING THE EXPERIMENT**
PERFORMING THE EXPERIMENT

Rudolph Research Analytical J357 refractometer

dual cell, deflection design
Assay choice

- Goal: accuracy (compared to precision)
- Consider sample composition (sequence)
- Consider buffer composition (pH, additives)
- Consider a precipitation step to remove buffer
- Sample volume (High-through put)

- Protocol for choosing suitable method,
  eg. Olson, Markwell; Curr Protoc Protein Sci. 2007(3)
Case 1: the Relaxed Scientist

- Question: confirm monomeric state of protein
- Result: ambiguous; \( MW_{\text{expected}} = 16.8 \text{ kD} \)
  \[ MW_{l(0)} = 22 \text{ kD} \text{ (30\% higher)} \]

- Explanation: unsuitable method for determining c

- Solution: use different method \( \rightarrow c = 8.6 \text{ mg/ml} \)
  confirm with other methods \( \rightarrow \text{MW: } 16.6 \)
Case 2: the Lazy Scientist

- Sample: 11 mg/ml Lysozyme in 30 and 90 mM NaCl
- Question: effect of addition of salt

> Conclusion increase in NaCl, unfavorable for the protein
Case 2: the Lazy Scientist

- Sample: 11 mg/ml Lysozyme in 30 and 90 mM NaCl
- Question: effect of adding salt
- Result: ambiguous;  
  \[ R_{g,30mM} = 1.0 \text{ nm} \]
  \[ R_{g,90mM} = 1.2 \text{ nm} \]
  \[ \rightarrow R_{g,\text{expected}} = 1.4 \text{ nm} \]
PERFORMING THE EXPERIMENT
PERFORMING THE EXPERIMENT
Case 2: the Lazy Scientist

- Sample: 11 mg/ml Lysozyme in 30 and 90 mM NaCl
- Question: effect of addition of salt
- Result: ambiguous; \( \text{RG}_{\text{expected}} = 1.4 \text{ nm} \)
  \[ \text{RG}_{30\text{mM}} = 1.0 \rightarrow 1.4 \text{ nm} \]
  \[ \text{RG}_{90\text{mM}} = 1.2 \rightarrow 1.4 \text{ nm} \]
- Explanation: concentration effects
- Solution: measure different concentrations

PERFORMING THE EXPERIMENT
Case 3: the Ambitious (Hasty) Scientist

- Sample: well characterized mutants, different ligands
- Question: understanding the binding mechanism
Case 3: the Ambitious (Hasty) Scientist

- Sample: well characterized mutants, different ligands
- Question: understanding the binding mechanism
- Result: look at automated pipeline
<table>
<thead>
<tr>
<th>Run #</th>
<th>File</th>
<th>Conc., mg/ml</th>
<th>Description</th>
<th>Rg, nm</th>
<th>I(0)</th>
<th>Guinier points</th>
<th>D_max, nm</th>
<th>MM, kDa</th>
<th>Volume, nm³</th>
<th>Quality, %</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>bsa_007.dat</td>
<td>4.4</td>
<td>bsa</td>
<td>3.25±0.9</td>
<td>21.66</td>
<td>33 - 129 (106)</td>
<td>11.1</td>
<td>66</td>
<td>157</td>
<td>86</td>
<td>12:14</td>
</tr>
<tr>
<td>10</td>
<td>ivypw_010.dat</td>
<td>10.0</td>
<td>hdpp3-e451a+ivyp</td>
<td>2.62±1%</td>
<td>20.31</td>
<td>35 - 170 (143)</td>
<td>7.8</td>
<td>62</td>
<td>130</td>
<td>88</td>
<td>14:10</td>
</tr>
<tr>
<td>12</td>
<td>ivypw_012.dat</td>
<td>10.0</td>
<td>hdpp3_2xmut+ivyp</td>
<td>2.73±1%</td>
<td>21.27</td>
<td>30 - 159 (132)</td>
<td>8.1</td>
<td>65</td>
<td>133</td>
<td>87</td>
<td>14:13</td>
</tr>
<tr>
<td>14</td>
<td>ivypw_014.dat</td>
<td>10.0</td>
<td>k570a+ivyp</td>
<td>2.76±1%</td>
<td>16.67</td>
<td>35 - 164 (130)</td>
<td>8.0</td>
<td>51</td>
<td>138</td>
<td>88</td>
<td>14:13</td>
</tr>
<tr>
<td>16</td>
<td>ivypw_016.dat</td>
<td>10.0</td>
<td>r669a+ivyp</td>
<td>2.68±1%</td>
<td>20.04</td>
<td>49 - 171 (123)</td>
<td>8.0</td>
<td>61</td>
<td>156</td>
<td>84</td>
<td>14:17</td>
</tr>
<tr>
<td>19</td>
<td>vypw_019.dat</td>
<td>10.0</td>
<td>hdpp3_e451a+vyp</td>
<td>2.55±1%</td>
<td>19.98</td>
<td>35 - 153 (119)</td>
<td>7.6</td>
<td>61</td>
<td>124</td>
<td>89</td>
<td>14:21</td>
</tr>
<tr>
<td>21</td>
<td>vypw_021.dat</td>
<td>10.0</td>
<td>r669a+vyp</td>
<td>2.65±1%</td>
<td>19.87</td>
<td>29 - 171 (143)</td>
<td>7.5</td>
<td>61</td>
<td>138</td>
<td>86</td>
<td>14:23</td>
</tr>
<tr>
<td>24</td>
<td>vypw_024.dat</td>
<td>10.0</td>
<td>hdpp3_e451a+vyp</td>
<td>2.53±1%</td>
<td>20.15</td>
<td>30 - 177 (143)</td>
<td>7.8</td>
<td>61</td>
<td>128</td>
<td>89</td>
<td>14:27</td>
</tr>
<tr>
<td>26</td>
<td>vypw_026.dat</td>
<td>10.0</td>
<td>hdpp3_2xmut+vyp</td>
<td>2.75±1%</td>
<td>21.45</td>
<td>43 - 164 (122)</td>
<td>8.4</td>
<td>65</td>
<td>142</td>
<td>86</td>
<td>14:29</td>
</tr>
<tr>
<td>28</td>
<td>vypw_028.dat</td>
<td>10.0</td>
<td>k570a+vypw</td>
<td>2.80±1%</td>
<td>15.73</td>
<td>31 - 164 (134)</td>
<td>9.2</td>
<td>51</td>
<td>147</td>
<td>89</td>
<td>14:32</td>
</tr>
<tr>
<td>30</td>
<td>vypw_030.dat</td>
<td>10.0</td>
<td>r669a+vypw</td>
<td>2.69±2%</td>
<td>20.03</td>
<td>39 - 129 (90)</td>
<td>8.0</td>
<td>61</td>
<td>138</td>
<td>86</td>
<td>14:34</td>
</tr>
<tr>
<td>33</td>
<td>valo_033.dat</td>
<td>10.0</td>
<td>hdpp3_e451a+valo</td>
<td>2.59±1%</td>
<td>20.44</td>
<td>33 - 160 (131)</td>
<td>7.5</td>
<td>62</td>
<td>127</td>
<td>89</td>
<td>14:00</td>
</tr>
<tr>
<td>35</td>
<td>valo_035.dat</td>
<td>10.0</td>
<td>hdpp3_2xmut+valo</td>
<td>2.74±1%</td>
<td>21.67</td>
<td>42 - 159 (123)</td>
<td>8.0</td>
<td>66</td>
<td>142</td>
<td>86</td>
<td>14:40</td>
</tr>
<tr>
<td>37</td>
<td>valo_037.dat</td>
<td>10.0</td>
<td>k570a+valo</td>
<td>2.75±1%</td>
<td>15.79</td>
<td>57 - 161 (105)</td>
<td>8.0</td>
<td>51</td>
<td>134</td>
<td>82</td>
<td>14:43</td>
</tr>
<tr>
<td>39</td>
<td>valo_039.dat</td>
<td>10.0</td>
<td>r669a+valo</td>
<td>2.69±1%</td>
<td>20.20</td>
<td>43 - 158 (125)</td>
<td>7.8</td>
<td>62</td>
<td>149</td>
<td>86</td>
<td>14:45</td>
</tr>
<tr>
<td>42</td>
<td>lw_042.dat</td>
<td>10.0</td>
<td>hdpp3_e451a+lw</td>
<td>2.57±1%</td>
<td>20.25</td>
<td>42 - 151 (110)</td>
<td>7.5</td>
<td>62</td>
<td>129</td>
<td>87</td>
<td>14:49</td>
</tr>
<tr>
<td>44</td>
<td>lw_044.dat</td>
<td>10.0</td>
<td>hdpp3_2xmut+lw</td>
<td>2.74±1%</td>
<td>21.44</td>
<td>35 - 157 (132)</td>
<td>8.0</td>
<td>65</td>
<td>140</td>
<td>88</td>
<td>14:51</td>
</tr>
<tr>
<td>46</td>
<td>lw_046.dat</td>
<td>10.0</td>
<td>k570a+lw</td>
<td>2.75±1%</td>
<td>16.63</td>
<td>48 - 158 (121)</td>
<td>8.4</td>
<td>51</td>
<td>136</td>
<td>84</td>
<td>14:53</td>
</tr>
<tr>
<td>48</td>
<td>lw_048.dat</td>
<td>10.0</td>
<td>r669a+lw</td>
<td>2.69±1%</td>
<td>20.79</td>
<td>45 - 171 (127)</td>
<td>7.8</td>
<td>63</td>
<td>148</td>
<td>86</td>
<td>14:56</td>
</tr>
<tr>
<td>51</td>
<td>po_051.dat</td>
<td>10.0</td>
<td>hdpp3_e451a_a0</td>
<td>2.63±1%</td>
<td>20.60</td>
<td>45 - 176 (131)</td>
<td>7.8</td>
<td>63</td>
<td>137</td>
<td>85</td>
<td>14:59</td>
</tr>
<tr>
<td>53</td>
<td>po_053.dat</td>
<td>10.0</td>
<td>hdpp3_2xmut_b0</td>
<td>2.75±1%</td>
<td>21.73</td>
<td>45 - 156 (122)</td>
<td>8.0</td>
<td>66</td>
<td>131</td>
<td>85</td>
<td>15:02</td>
</tr>
<tr>
<td>55</td>
<td>po_055.dat</td>
<td>10.0</td>
<td>k570a_c0</td>
<td>2.79±1%</td>
<td>16.03</td>
<td>44 - 169 (120)</td>
<td>9.1</td>
<td>51</td>
<td>132</td>
<td>85</td>
<td>15:04</td>
</tr>
<tr>
<td>57</td>
<td>po_057.dat</td>
<td>10.0</td>
<td>r669a_d0</td>
<td>2.74±1%</td>
<td>20.35</td>
<td>35 - 159 (135)</td>
<td>8.4</td>
<td>62</td>
<td>149</td>
<td>89</td>
<td>15:06</td>
</tr>
</tbody>
</table>
- Wrong Buffer

**I(s)**

- Under subtraction (Tris)
- Matching buffer (Tris + glycine)
- Over subtraction (Tris + glycerol)

**PLANNING THE EXPERIMENT**
Case 3: the Ambitious (Hasty) Scientist

- Sample: well characterized mutants, different ligands
- Question: understanding the binding mechanism
- Result: ambiguous; $D_{\text{max}}^{\text{unbond}} = 8 \text{ nm}$
  $D_{\text{max}}^{\text{bond}} = 7 \text{ nm}$
  $D_{\text{max}} = 7.5-9.2 \text{ nm}$

- Explanation: incorrect buffer subtraction
- Solution: dialysis, SEC, (centricon)
Case 4: the Super Scientist

- Sample: excellently prepared, fully analyzed
- Question: pH dependent oligomerization
Case 4: the Super Scientist

- Sample: excellently prepared, well analyzed sample
- Question: pH dependent oligomerization
- Result:

[Graphs showing data analysis and results]
Case 4: the Super Scientist

- Sample: excellently prepared, well analyzed sample
- Question: pH dependent oligomerization
- Result: polydisperse sample

- Explanation: time/storage/transport dependent alterations
- Solution: re-characterize the sample, ‘first aid’ at the beamline
- **Removal of aggregates**
- **Time/ storage/ transport**
  - **Stability**
    - pH
    - Concentration
    - Additives
    - Proteases
  - **Freezing/ Thawing**
  - Tube size, concentration
Table 1. Comparison of Protein Storage Conditions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Storage Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solution at 4°C</td>
</tr>
<tr>
<td>Typical shelf life</td>
<td>1 month</td>
</tr>
<tr>
<td>Requires sterile conditions or addition of antibacterial agent</td>
<td>Yes</td>
</tr>
<tr>
<td>Number of times a sample may be removed for use</td>
<td>Many</td>
</tr>
</tbody>
</table>

Protein Concentration:

Dilute protein solutions (< 1 mg/ml) are more prone to inactivation and loss as a result of low-level binding to the storage vessel. Therefore, it is common practice to add “carrier” or “filler” protein, such as purified bovine serum albumin (BSA) to 1-5 mg/ml (0.1-0.5%), to dilute protein solutions to protect against such degradation and loss.
- Time/ storage/ transport
  - Stability
  - Freezing/ Thawing
    - Speed
    - Additives
    - Dried ice
  - Tube size, concentration

**PERFORMING THE EXPERIMENT**
Case 5: the Brilliant Scientist

- Sample: dilution series of Ribonuclease
- Question: *ab initio* Model
- Result: Radiation Damage

### Data Table

<table>
<thead>
<tr>
<th>Run#</th>
<th>Description</th>
<th>Code</th>
<th>Conc. mg/ml</th>
<th>Log plot</th>
<th>Kratky plot</th>
<th>Guinier points</th>
<th>Quality</th>
<th>$R_g$ nm</th>
<th>$D_{max}$ nm</th>
<th>$V_{prot}$ nm$^3$</th>
<th>$V_{BM}$ nm$^3$</th>
<th>$MW_{(0)}$ kDa</th>
<th>$MW_{prot}$ kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>238</td>
<td>ribo_10</td>
<td>r_h_2</td>
<td>10.0</td>
<td></td>
<td></td>
<td>66</td>
<td>76%</td>
<td>1.7 ± 0.3</td>
<td>6</td>
<td>17</td>
<td>16</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>ribo_10</td>
<td>r_h_2</td>
<td>10.0</td>
<td></td>
<td></td>
<td>67</td>
<td>70%</td>
<td>1.7 ± 0.3</td>
<td>6</td>
<td>17</td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>242</td>
<td>ribo_10</td>
<td>r_h_2</td>
<td>10.0</td>
<td></td>
<td></td>
<td>43</td>
<td>50%</td>
<td>1.7 ± 0.3</td>
<td>6</td>
<td>18</td>
<td>16</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>
PERFORMING THE EXPERIMENT

Frame 4

RG = 6.4 nm
Porod V = 153.52 nm³
Dmax = 22.52 nm

Frame 1

RG = 1.7 nm
Porod V = 17.39 nm³
Dmax = 5.32 nm
Data collection strategy

- Increase sample flow
  - Requires more material
- Attenuate the beam
  - Influences signal to noise
Scavengers

- DTT (b)
- Ascorbic Acid (c)
- Glycerol (d)

PERFORMING THE EXPERIMENT
PERFORMING THE EXPERIMENT
- Scavengers

  - DTT
    - Short shelf-life
    - Reduces disulfide bridges
  - Ascorbic Acid
    - Only works in some cases
  - Glycerol
    - Decrease in contrast
    - Difficult to pipette
Batch Mode

- Attenuate beam
- Add glycerol or ascorbate or DTT
- Decrease exposure time

Flow

- Standard Operation: Sufficient sample amount (> 30 μl)
- Collect data

Static

- Non-standard operation: Limited sample amount (< 30 μl)*
- Decrease exposure time
- Attenuate beam

*It is very difficult to accurately add additives to sample volumes < 30 μl
Case 5: the Brilliant Scientist

- Sample: dilution series of Ribonuclease
- Question: *ab initio* Model
- Result: Radiation Damage

- Solution: modifications of buffer and/or data collection strategy
Not so-ideal samples

- Case 1: wrong concentration
  - determine best suitable method depending on proteins

- Case 2: concentration effect (repulsion, interparticle interference)
  - measure concentration series

- Case 3: incorrect buffer subtraction
  - dialysis, SEC

- Case 4: Polydisperse sample
  - Biophysical, biochemical characterization

- Case 5: Radiation damage
  - Alter data collection strategy and buffer composition
Take home message

- Don’t be too RELAXED
- Don’t be too LAZY
- Don’t work too HASTY (plan your experiments well)
- Be prepared for the unforeseen
- Remember (just like you) the Beam is Brilliant, so be prepared

- Know your sample!
- Know your question!
Books
Small Angle X-Ray and Neutron Scattering from Solutions of Biological Macromolecules
Oxford University Press

Protocol:
Synchrotron-based small-angle X-ray scattering of proteins in solution
Skou, Gillilan & Ando;

Preparing monodisperse macromolecular samples for successful biological small-angle X-ray and neutron-scattering experiments
Jeffries et al.;