# Sample and buffer preparation

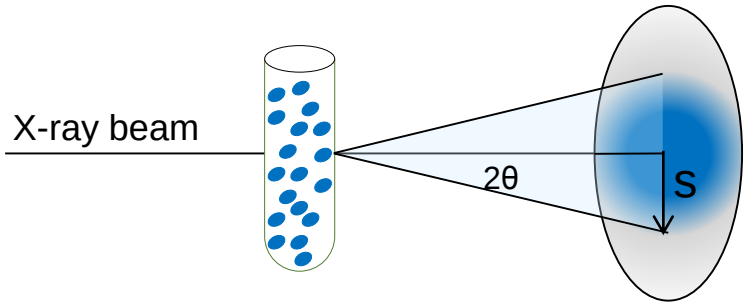
Melissa Graewert

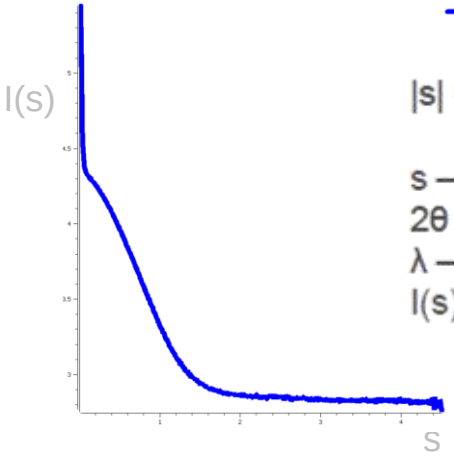
Monday, November 19th

EMBL

- 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

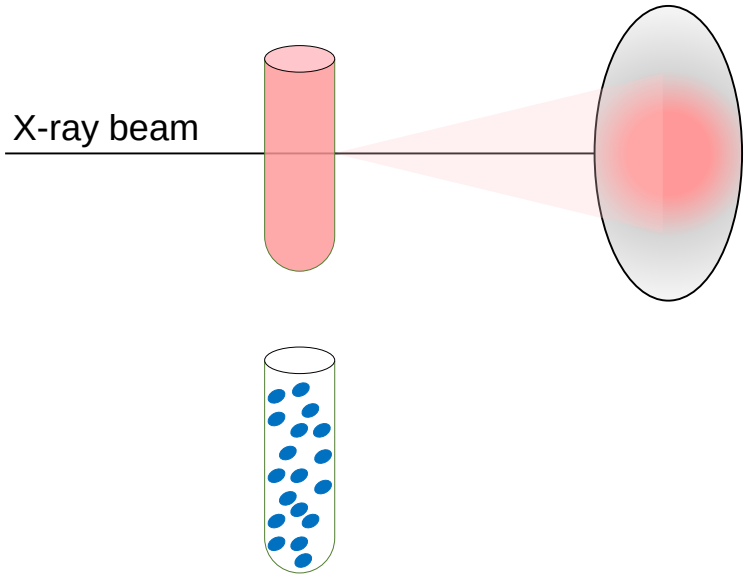


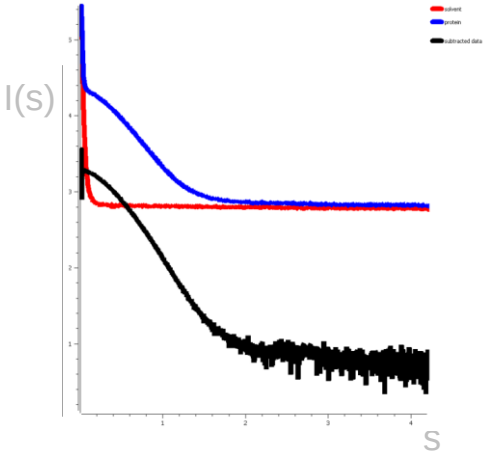



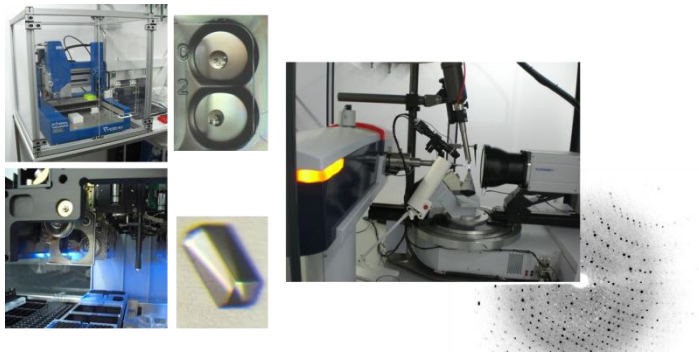

$|s| = 4\pi \sin\theta/\lambda$

s – scattering vector
2θ – scattering angle
λ – wavelength
I(s) – intensity

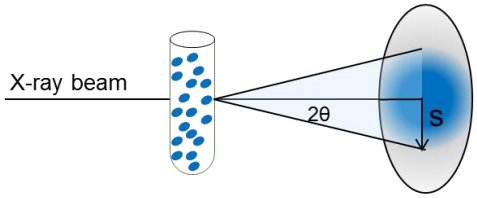
- The SAXS (small angle X-ray scattering) Experiment

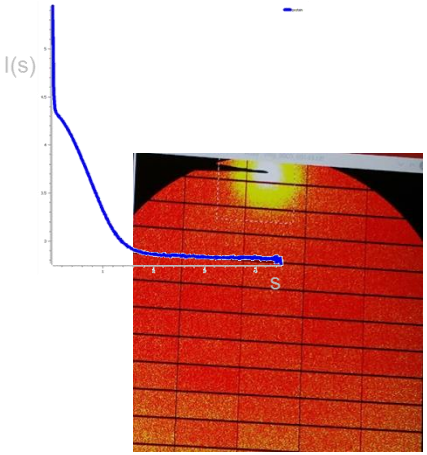




Signal is amplified
High resolution d ≈ λ




I(s) is isotropic
Concentrates around the primary beam
Low resolution d >> λ

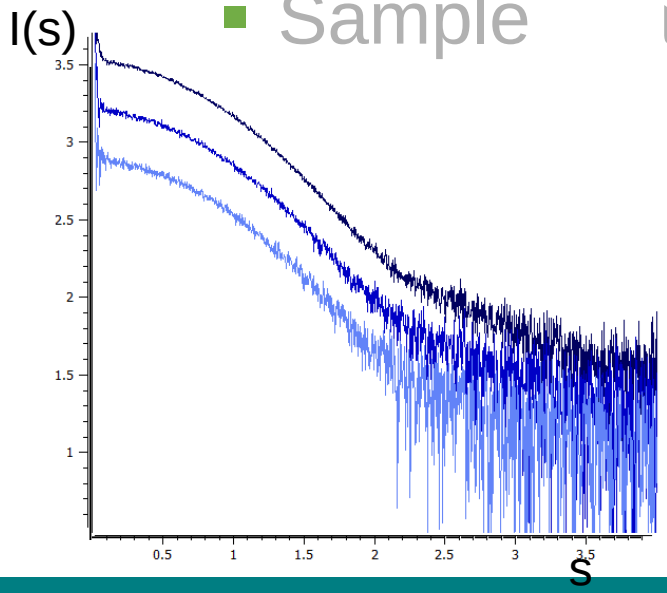
No need to grow crystals
No crystallographic packaging forces
Not limited by MW
Physiological conditions
Observe response to changes

# PLANNING THE EXPERIMENT

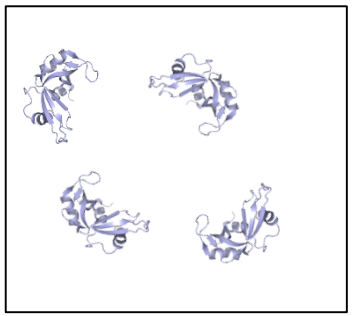
EMBL

- Sample requirements
  - Amount: 10µl (30 µl); 40 µl per sample
  - Concentration:
  - Buffer:
  - Sample quality:

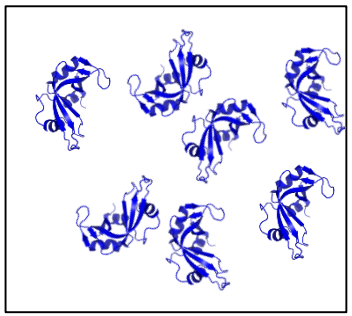
- Sample requirements
  - Amount: 7µl; 30-50 µl per sample
  - Concentration: more particles → stronger signal
  - Buffer
  - Sample lity



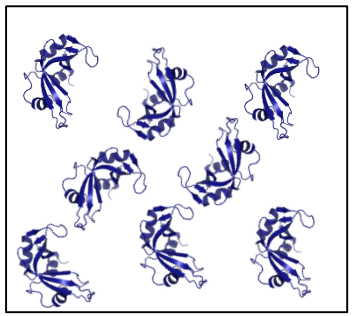

$$I(0) \approx N\,(\Delta\rho\,V)^2$$



3.7 mg/ml

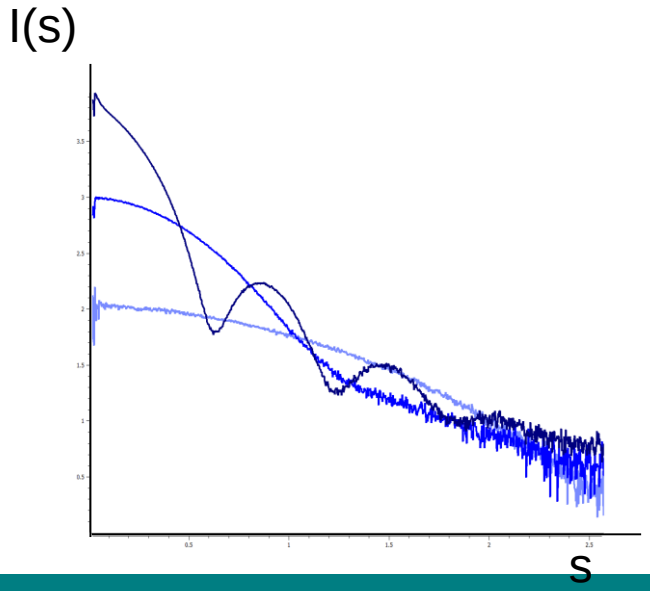


7.5 mg/ml



15 mg/ml

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

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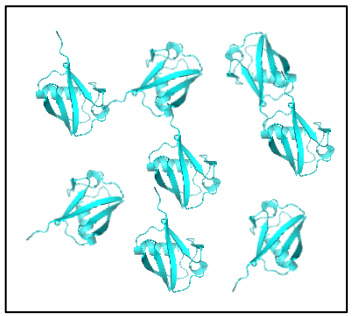
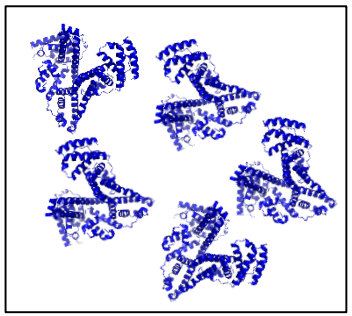
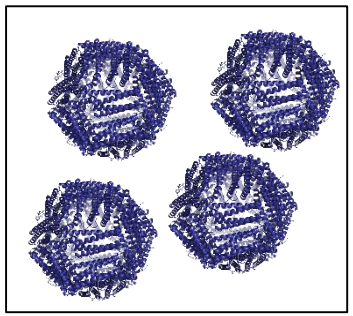


< 20 kD

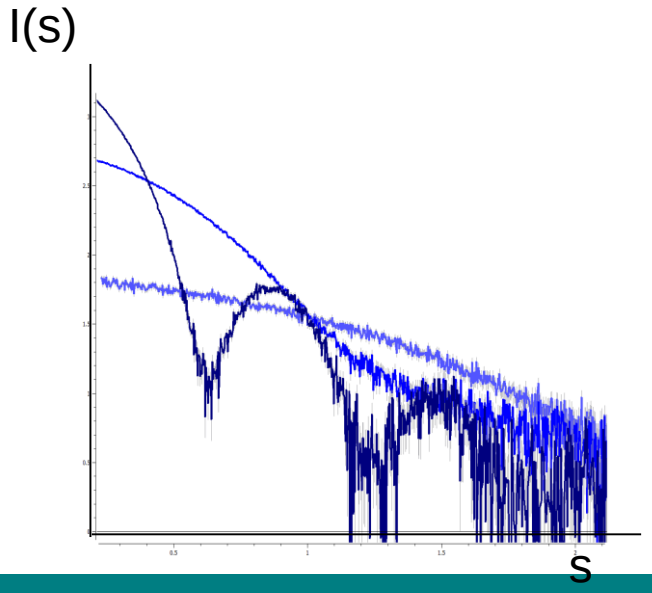


~ 70 kD



> 400 kD

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

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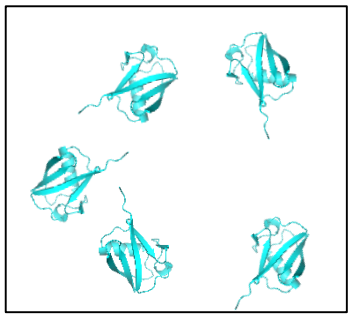
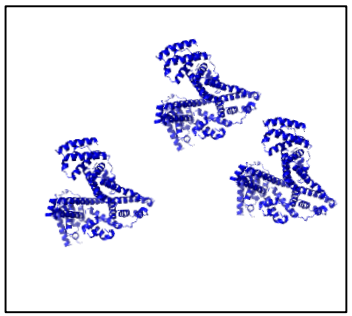
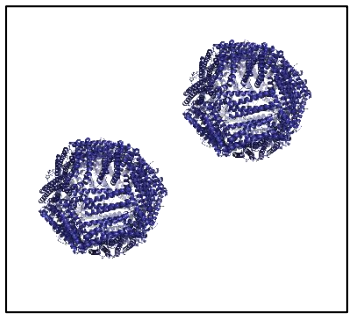


< 20 kD



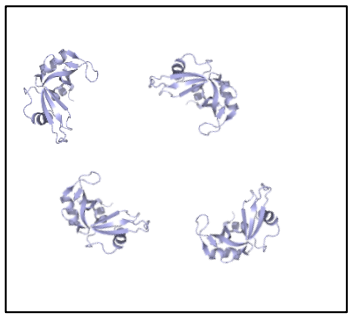
~ 70 kD



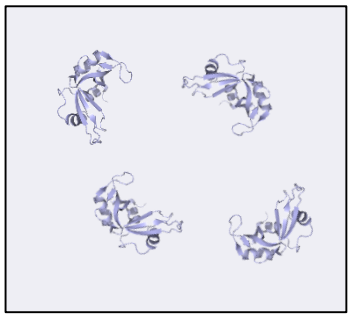
> 400 kD

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

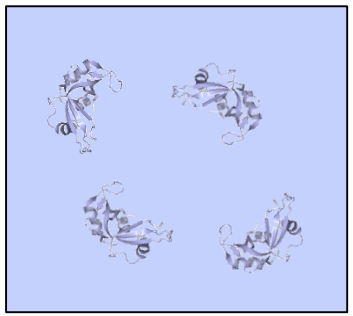
$$I(0) \approx N\,(\Delta\rho\ V)^2$$



No additives



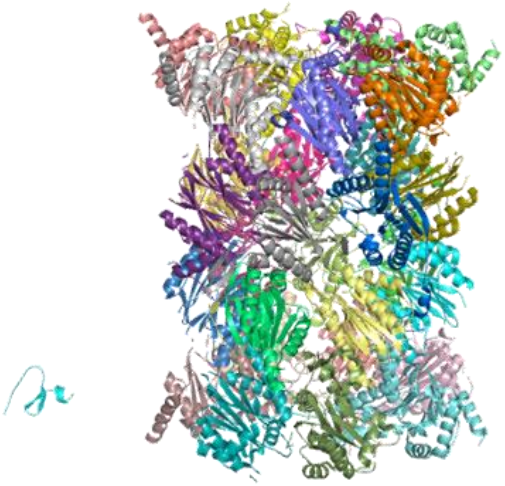
small amount

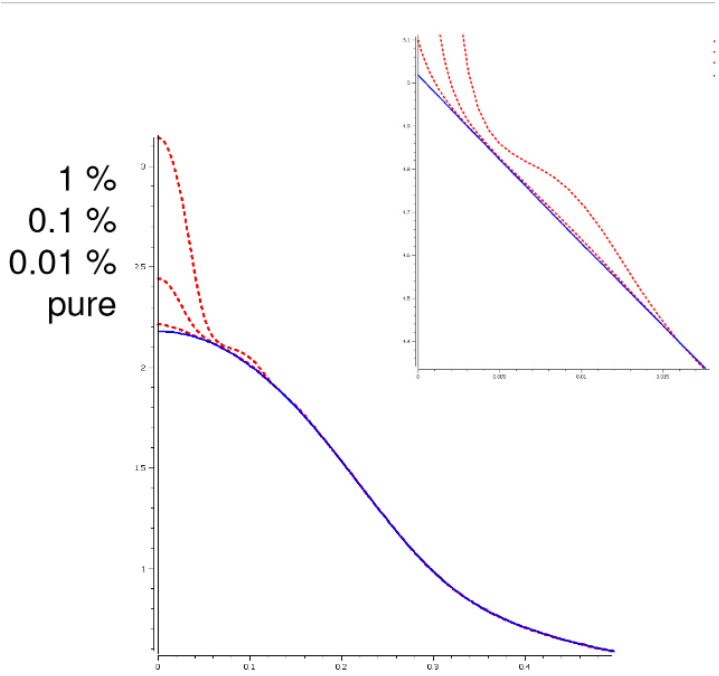


high amount

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

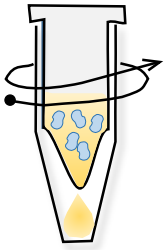
Foldon vs Proteasome



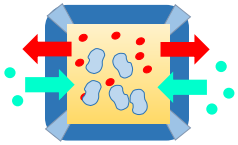


# Sample preparation strategies

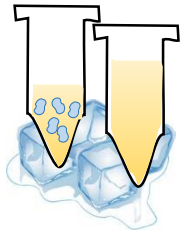
- Sample stable at high concentrations



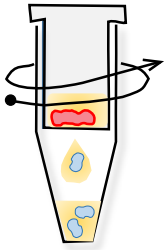
Concentrate



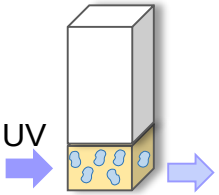
Dialyze



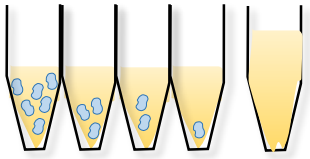
(Store/Ship)



Filter

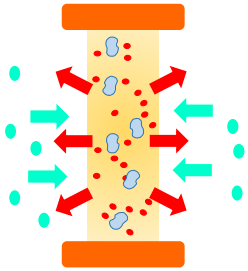



Conc. determination

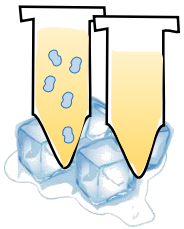


SAXS from dilutions & buffer

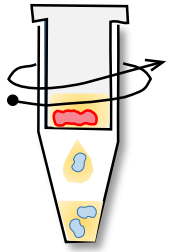
- Sample not stable at high concentrations



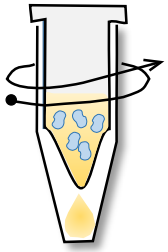
Dialyze



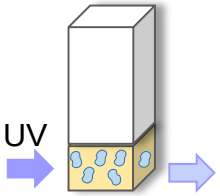
(Store/Ship)



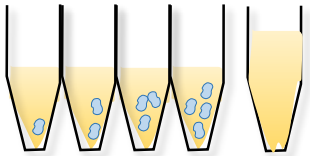
Filter



Step-wise concentration



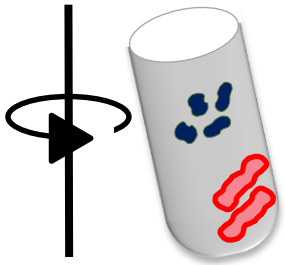

Conc. determination



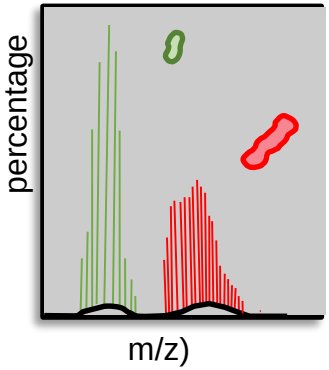
SAXS from different conc. centration steps & buffer

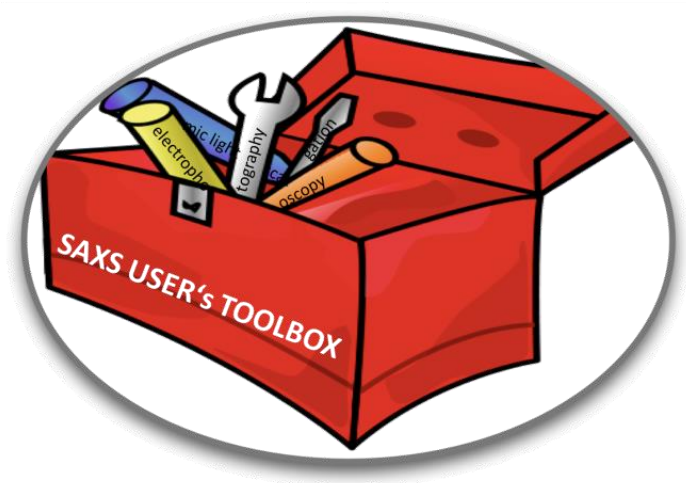
- Sample Characterization

Analytical ultracentrifugation

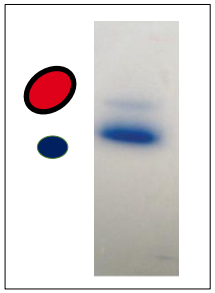


nativeMS

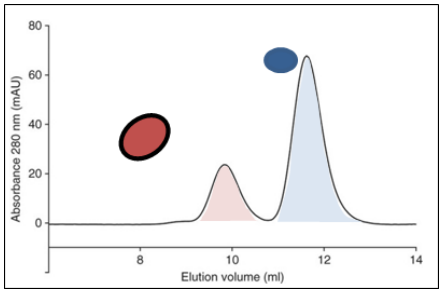


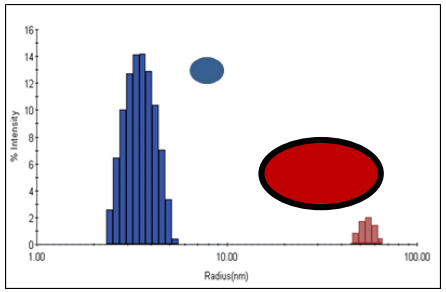

Native Gel Electrophoresis



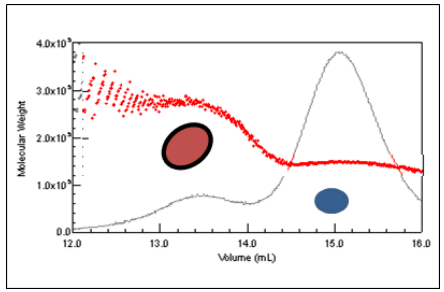
Size Exclusion Chromatography




Dynamic Light Scattering



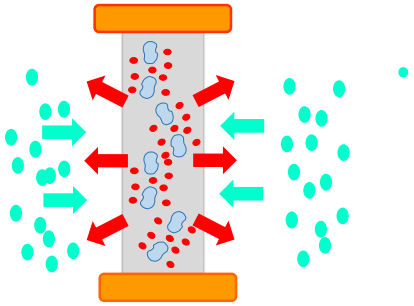

Static Light Scattering






EMBL

- Buffer Preparation
  - Method of choice: Dialysis

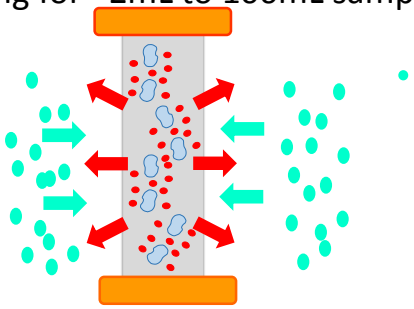


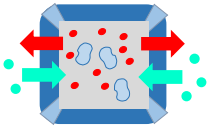
- diffusion coefficients
-temperature
- time
- concentration of species
- sample volume
- dialysate (buffer) volume (100:1)
- number of dialysate changes (2-3)
- membrane surface area
- membrane thickness
- molecular charges
- dialysate agitation (stirring)

→ standard protocol: 16 to 24 hours

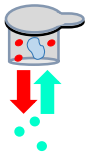
- Buffer Preparation
  - Method of choice: Dialysis

Tubing for ~2mL to 100mL samples





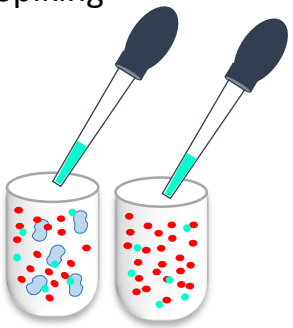
Cassettes for ~0.5mL to 70mL



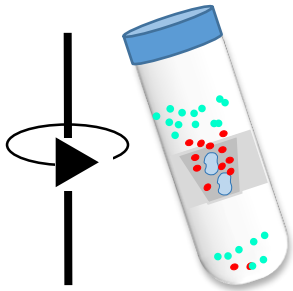
Cup Devices for ~10µL to 2mL

  - Alternative methods

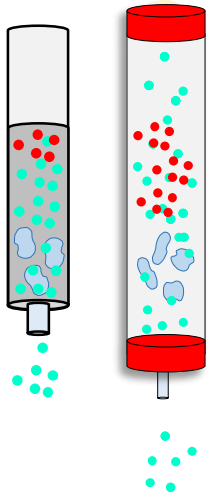
Spiking



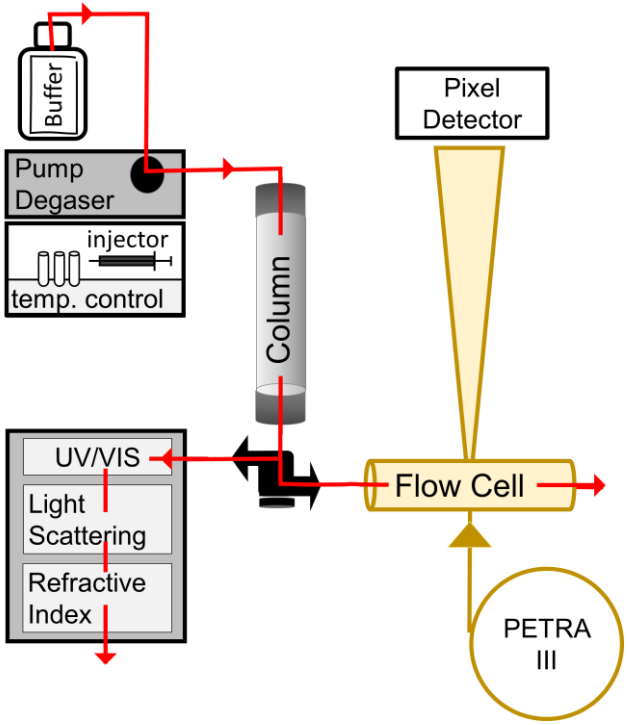
Diafiltration

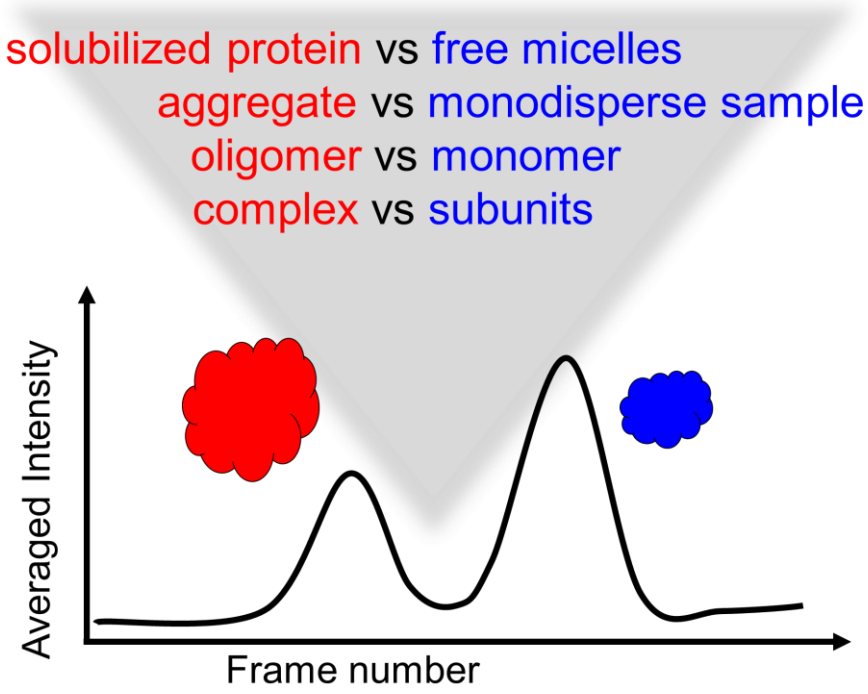


Desalting/SEC column



- Batch mode or SEC-SAXS mode



- Batch mode or SEC-SAXS mode
  - Remember

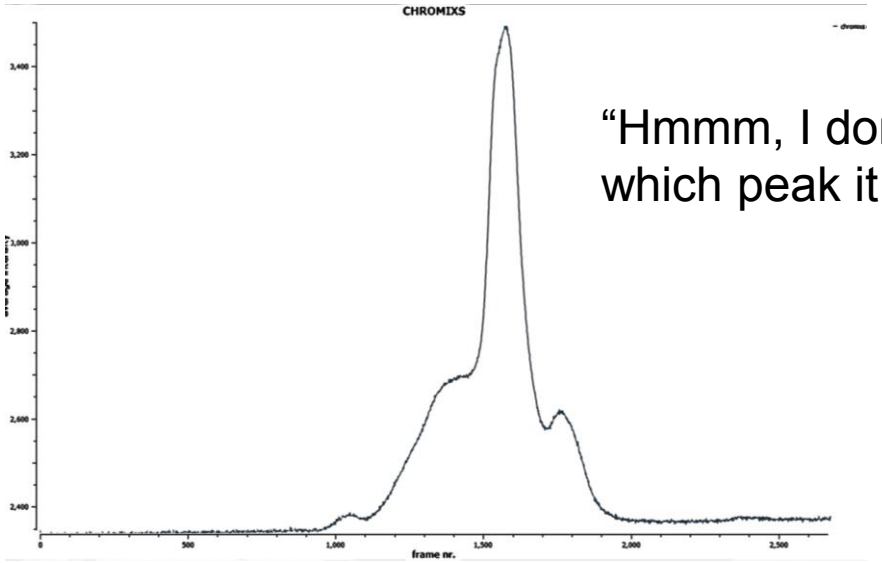
Heavy cleaning in the lab

final “polishing” at the beamline

- Batch mode or SEC-SAXS mode
  - Keep in mind:

❑ “ideal sample”

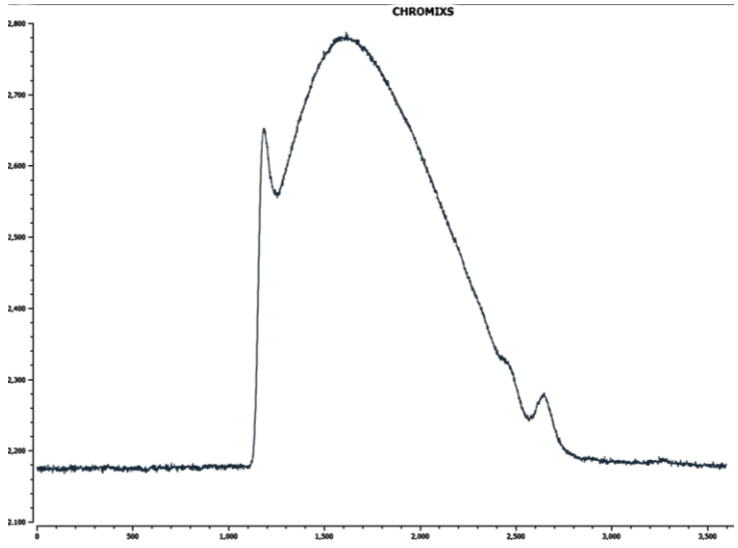
➢ Pre-analysis of sample is very important



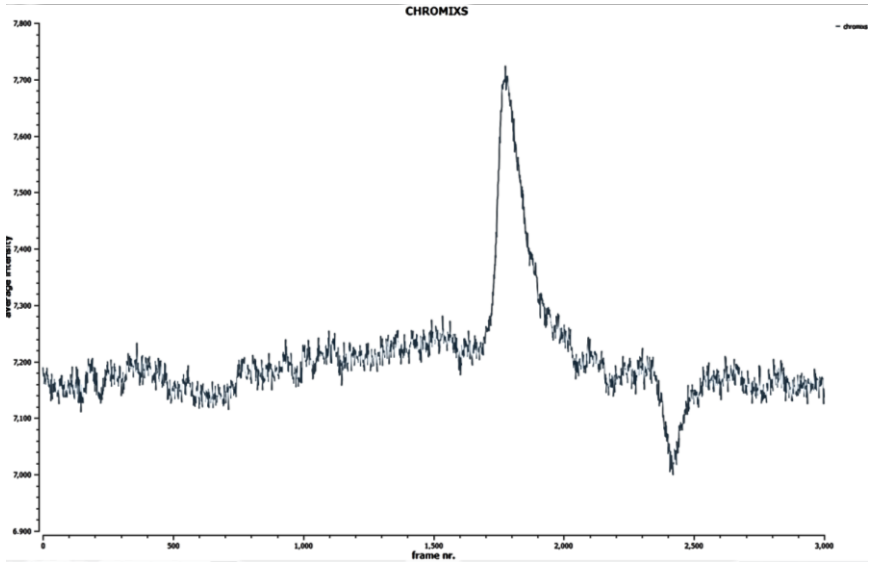

“Hmmm, I don’t know which peak it is?”

- Batch mode or SEC-SAXS mode
  - Keep in mind:

1. Trip:



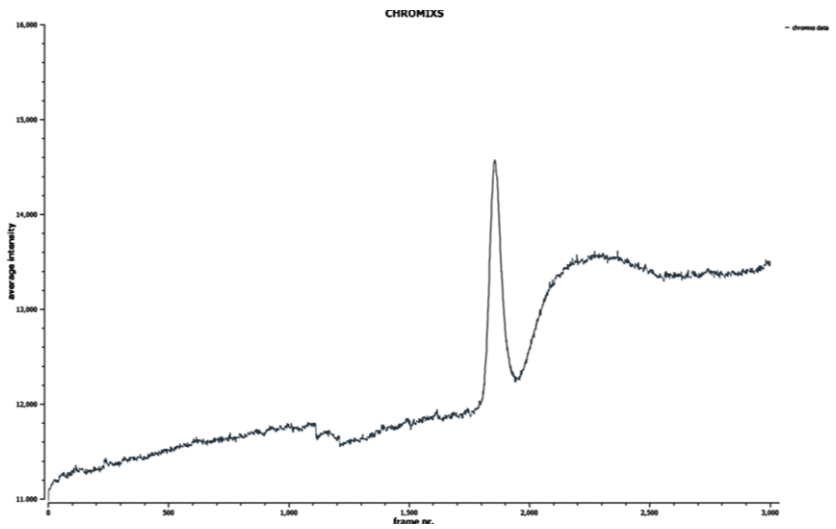

2. Trip:



- Batch mode or SEC-SAXS mode
  - Keep in mind:

- “ideal sample”
  - Pre-analysis of sample is very important
- not quite pure sample
  - SEC-SAXS is analytical! Not preparative!
- radiation damage can be a issue
  - Measure batch sample as well, add scavengers

- Batch mode or SEC-SAXS mode
  - Keep in mind:




- radiation damage can be a issue
  - Measure batch sample as well, add scavengers

- Batch mode or SEC-SAXS mode
  - Keep in mind:

- “ideal sample”
  - Pre-analysis of sample is very important
- not quite pure sample
  - SEC-SAXS is analytical! Not preparative!
- radiation damage can be a issue
  - Measure batch sample as well, add scavengers
- Sample stability, low affinity complexes
  - Sample can be altered with column interaction

What is **SEC-SAXS**/SLS, why does one need this?

- Alternative strategy to study (moderatly) polydisperse samples

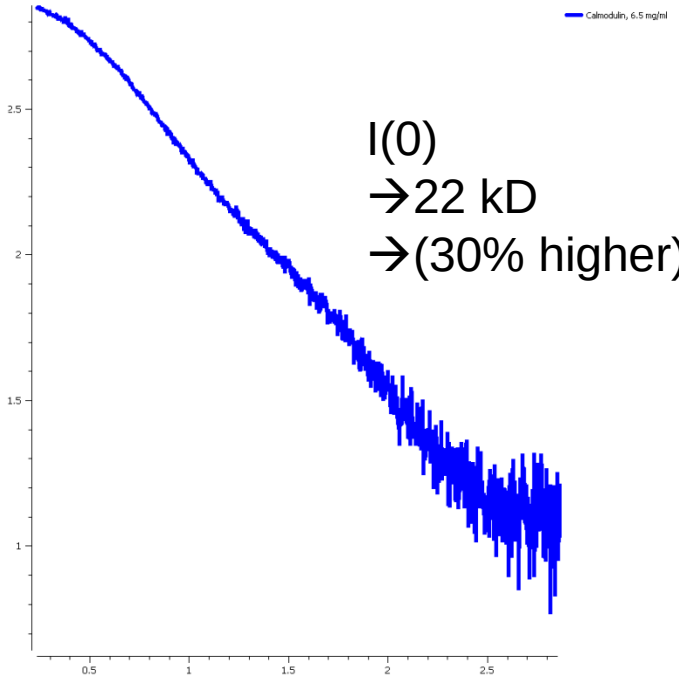
How is SEC-SAXS done

- Required sample amounts: at least 50 ul of > 5mg/ml
- Sufficient buffer
- Optimize your SEC run
- If possible collect batch sample as well

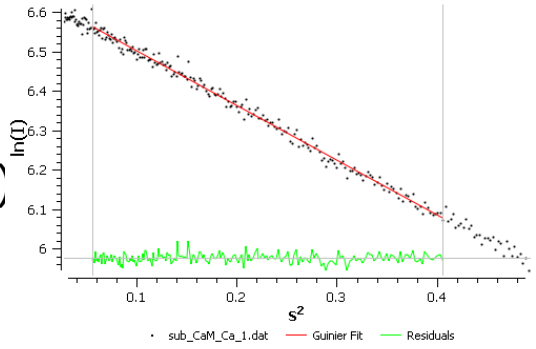
- Check for radiation damage, add 3% glycerol (if feasible)

- 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)
→22 kD
→(30% higher)




$$\mathrm{MM_p} = I(0)_\mathrm{p}/c_\mathrm{p}\,\frac{\mathrm{MM_{st}}}{I(0)_\mathrm{st}/c_\mathrm{st}}$$



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- Case 1: the Relaxed Scientist

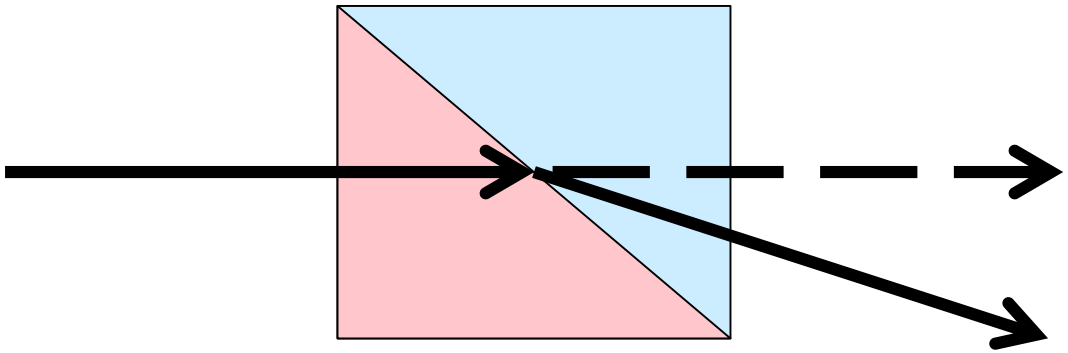
  - Sample:Calmodulin; 100 ul 6.5 mg/ml (UV-Vis)
  - Question: confirm monomeric state of protein
  - Result: ambigious; $MW_{expected}$ = 16.8 kD

    $MW_{I(0)}$ = 22 kD (30% higher)
  - Explanation: unsuitable method for determining c

ADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEV
DADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVM
TNLGEKLTDEEVDEMIREANIDGDGQVNYEEFVQMMTAK → $\varepsilon = 0.17$

protparam: Experience shows that this (no Trp) could result in more than 10% error in the computed extinction coefficient

| Method | Principle | Advantages | Limitations |
|---|---|---|---|
| **Lowry Assay** | - Buiret chormophore (copper ion complex with amide bonds)<br>- $Cu^{+I,}$ Tyr, Trp reduce Folin-Ciocalteu reagent (660 nm) | - Relative sensitive:<br>- 1 to 100 ug | -interfering compounds such as detergents, carbohydrates, glycerol, Tris, EDTA…<br>-- content of Tyr, Trp<br>- time consuming |
| **BCA Assay**<br>bicinchoninic acid | - protein backbone chelates $Cu^{2+}$ ions and reduces them to $Cu^{1+}$ which shifts color of dye (562 nm) | -less sensitive to the types of amino acids in the protein<br>- suitable for most detergents & denaturants | - Cysteine rich samples (temp)<br>- reducing agents (DTT, 2-ME)<br>-time consuming |
| **Bradford Assay** | - Color shift of Coomassie brilliant blue G-250 dye upon binding arg and aromatic residues | -simple, rapid, cheap, sensitive<br>- micro: 1-20 ug<br>- macro: 20-100 ug<br>- copes with reducing agents | -- content of Arg (eg. histones)<br>-- non linear curve (absorbance of free dye)<br>- Choice of standard, pH<br>- “sticky proteins” precipitate |
| **UV( 280 nm)** | Ultraviolet absorbance according to Beer’s law, A~c ε | - quick<br>- sample recovery | - sequence dependent<br>- protein complexes, mixtures<br>-- sensitive to pH and ions |
| **Differential Refractometry** | index of refraction according to Snell's law | - total/ pure protein<br>- quick<br>- sample recovery | -Magic number<br>-Temperature sensitive |

dual cell, deflection design

Rudolph Research Analytical J357 refractometer

- 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

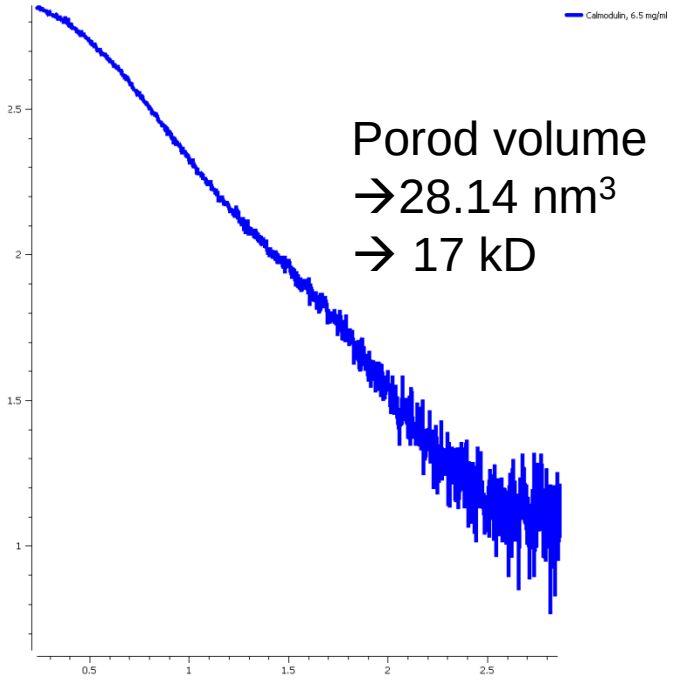
  - Question: confirm monomeric state of protein
  - Result: ambigious; $MW_{expected}$ = 16.8 kD
    $MW_{I(0)}$ = 22 kD (30% higher)

  - Explanation: unsuitable method for determining c

  - Solution: use different method → c= 8.6 mg/ml
    confirm with other methods → MW: 16.6

- Case 1: the Relaxed Scientist

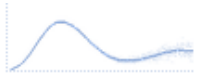
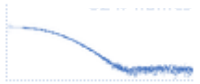
  - Sample:Calmodulin; 100 ul 6.5 mg/ml (UV-Vis)
  - Question: confirm monomeric state (16.8 kD)
  - Result:




Porod volume
→28.14 $nm^3$
→ 17 kD

- Case 2: the Lazy Scientist

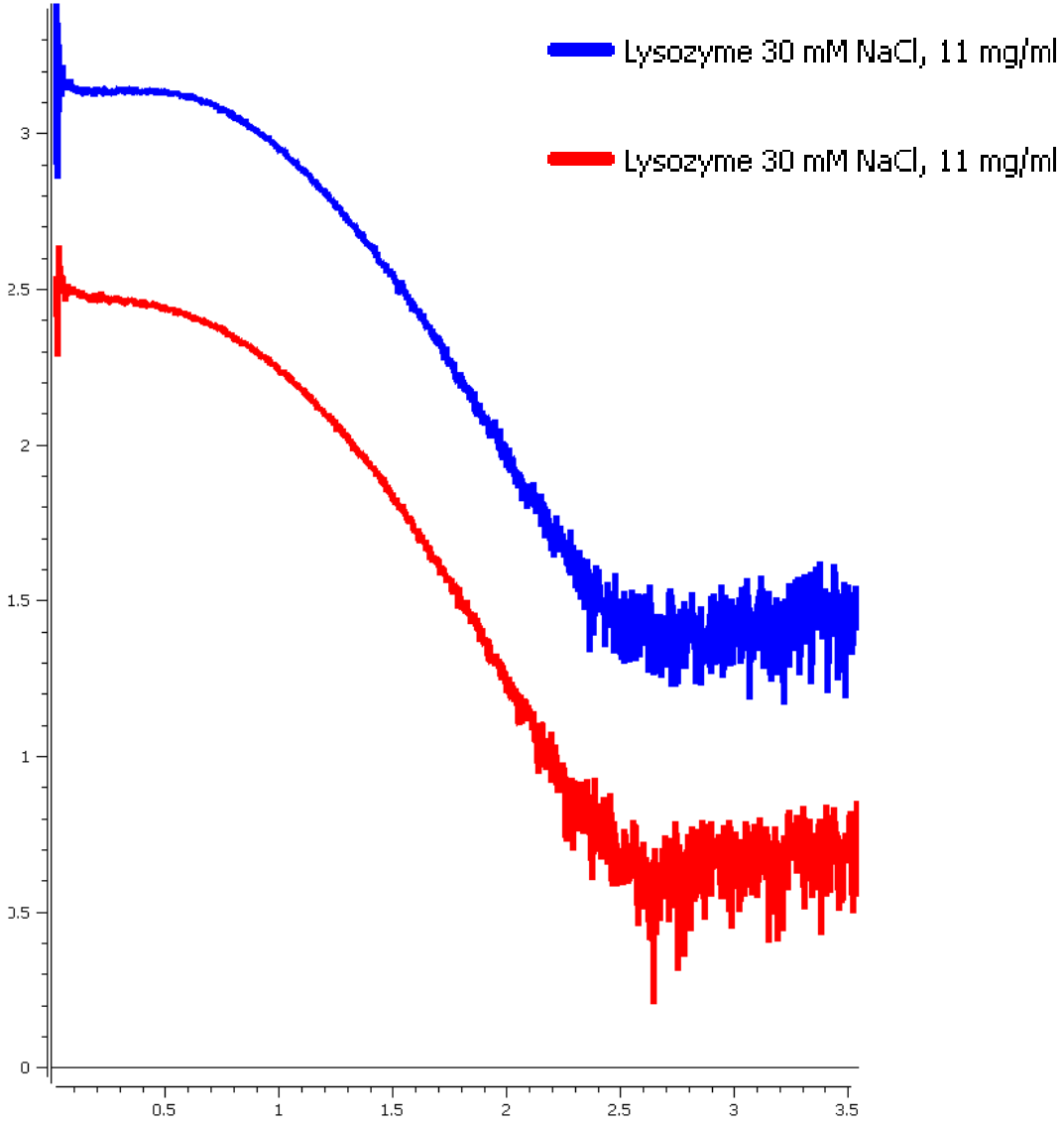
  - Sample: 11 mg/ml Lysozyme in 30 and 90 mM NaCl
  - Question: effect of addition of salt

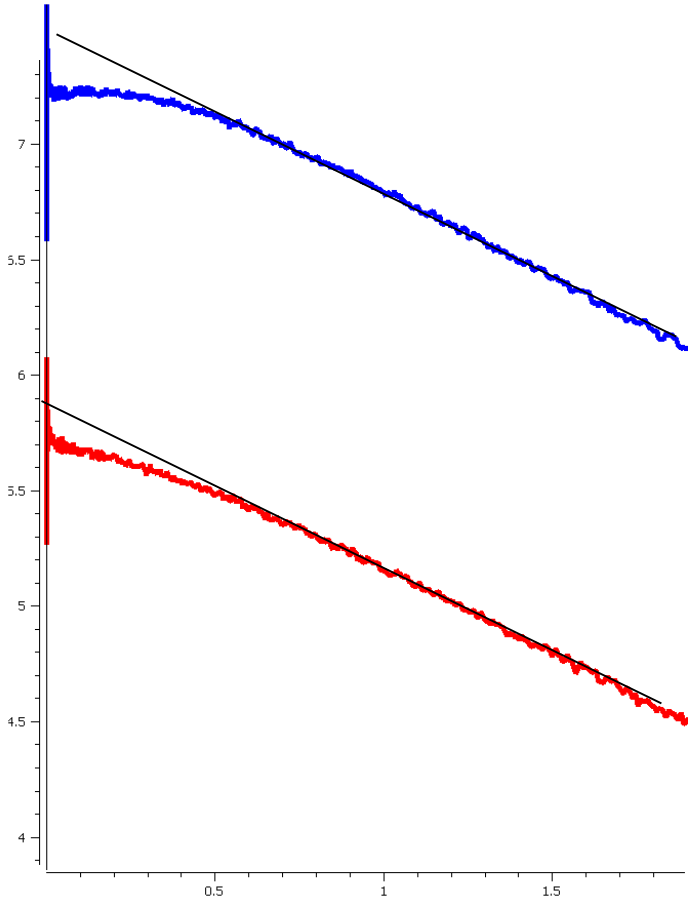
| Code | Conc. | Log plot | Kratky plot | Guinier points | | Quality | $R_g$ | | $D_{max}$ | $V_{Porod}$ | $V_{DAM}$ | $MW_{I(0)}$ | $MW_{Porod}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/ml | | | | | | nm | | nm | $nm^3$ | $nm^3$ | kDa | kDa |
| b_30 | 11.0 |  |  | 153 | 266 | 68% | 1.0 ±0.5 | | 3 | 19 | 21 | 12 | 12 |
| d_90 | 11.0 |  |  | 118 | 282 | 77% | 1.2 ±0.2 | | 4 | 19 | 22 | 12 | 12 |

→Conclusion increase in NaCl,
unfavorable for the protein

- Case 2: the Lazy Scientist

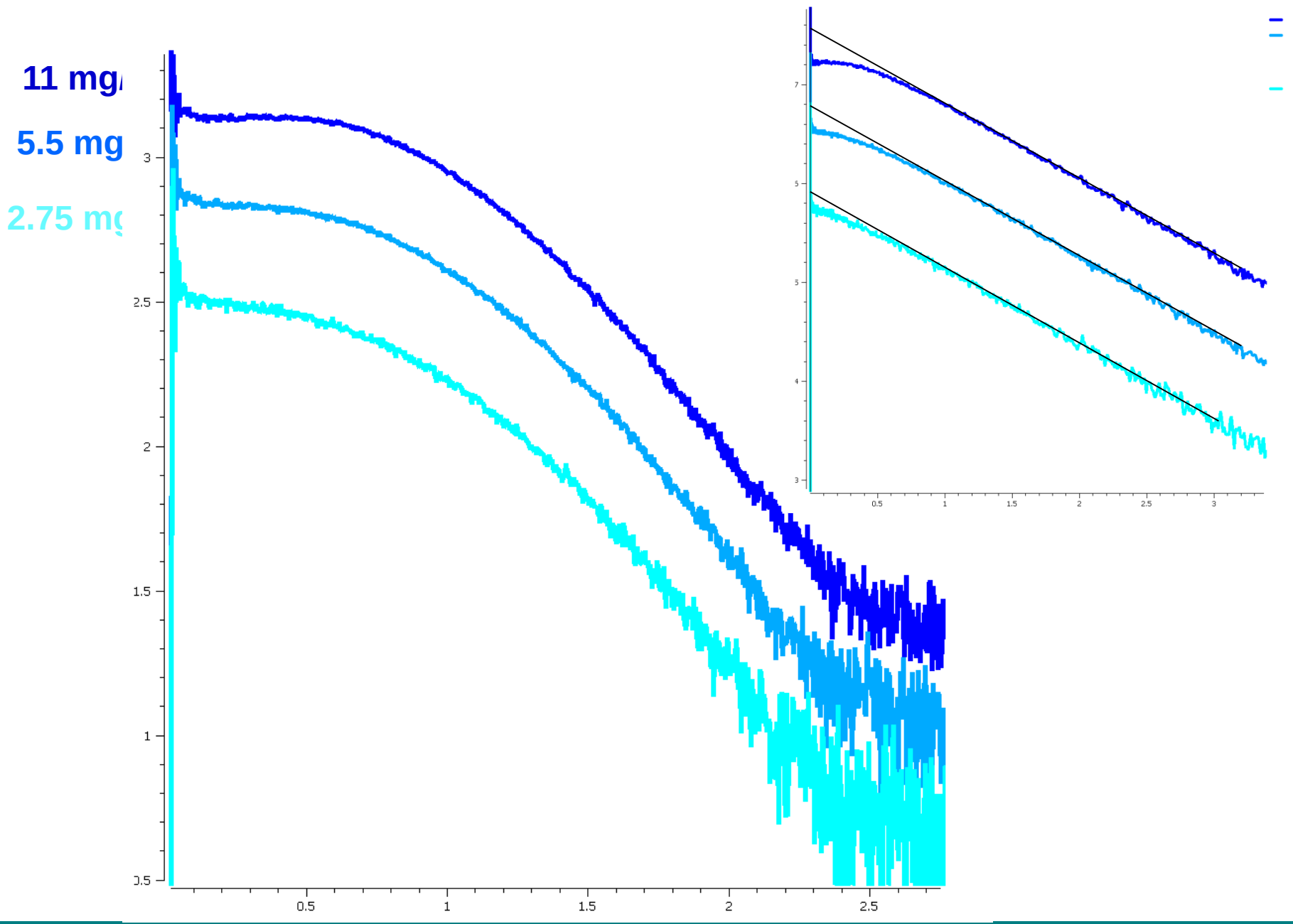
  - Sample: 11 mg/ml Lysozyme in 30 and 90 mM NaCl
  - Question: effect of adding salt
  - Result: ambigious; $Rg_{30mM}$ = 1.0 nm
    $Rg_{90mM}$ = 1.2 nm
    → $Rg_{expected}$ = 1.4 nm




# PERFORMING THE EXPERIMENT

EMBL




# PERFORMING THE EXPERIMENT

EMBL

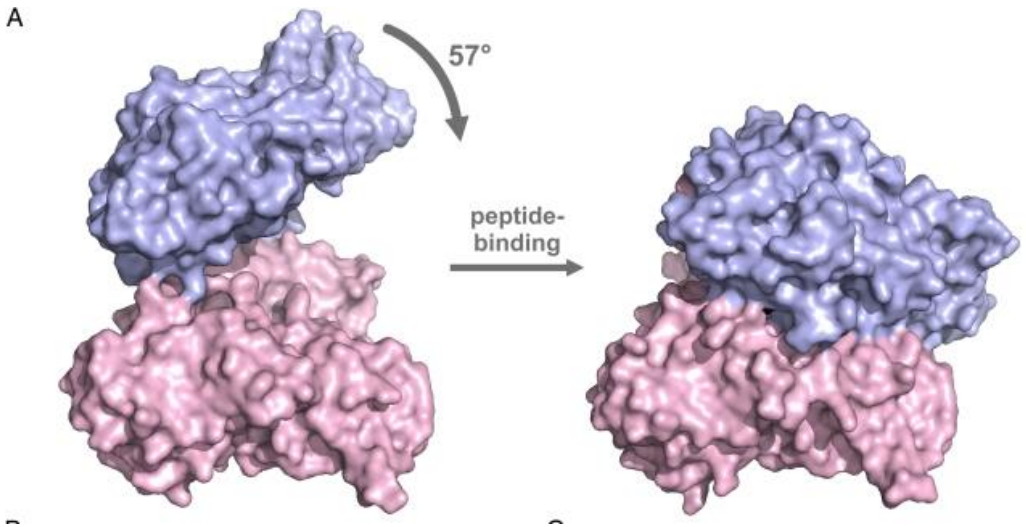
- Case 2: the Lazy Scientist

  - Sample: 11 mg/ml Lysozyme in 30 and 90 mM NaCl
  - Question: effect of addition of salt
  - Result: ambigious; $RG_{expected} = 1.4$ nm
    $RG_{30mM} = 1.0 \rightarrow 1.4$ nm
    $RG_{90mM} = 1.2 \rightarrow 1.4$ nm

    - Explanation: concentration effects
    - Solution: measure different concentrations

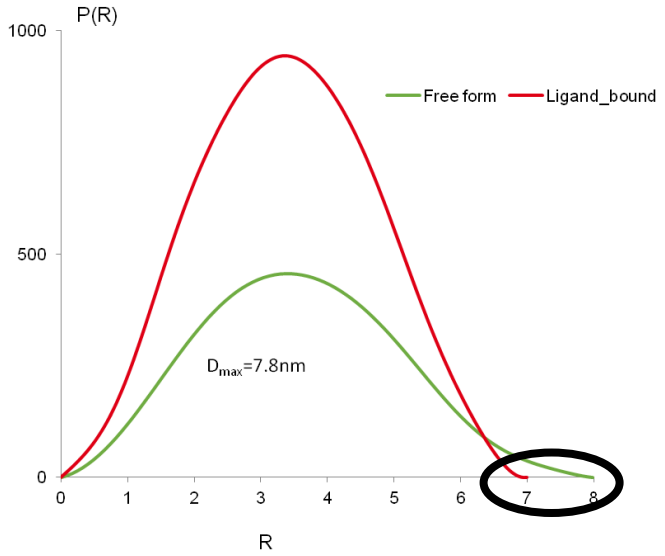
- Case 3: the Ambitious (Hasty) Scientist

  - 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



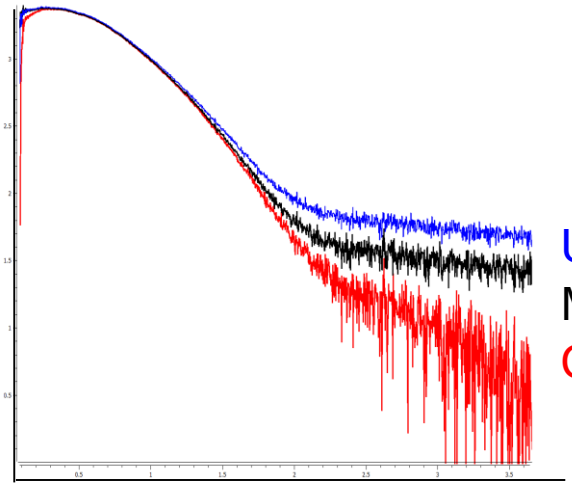
| Run # | File | Conc., mg/ml | Description | $R_g$, nm | I(0) | Guinier points | $D_{max}$, nm | MM, kDa | Volume, $nm^3$ | Quality, % | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | bsa_007.dat | 4.4 | bsa | 3.25±3% | 21.66 | 33 - 138 (106) | 11.4 | 66 | 157 | 86 | 12:46 |
| 10 | ivypw_010.dat | 10.0 | hdpp3_e451a+ivy | 2.62±1% | 20.31 | 36 - 178 (143) | 7.8 | 62 | 130 | 88 | 14:10 |
| 12 | ivypw_012.dat | 10.0 | hdpp3_2xmut+ivy | 2.73±1% | 21.27 | 38 - 169 (132) | 8.1 | 65 | 133 | 87 | 14:13 |
| 14 | ivypw_014.dat | 10.0 | k670a+ivyp | 2.76±1% | 16.67 | 35 - 164 (130) | 8.6 | 51 | 138 | 88 | 14:15 |
| 16 | ivypw_016.dat | 10.0 | r669a+ivyp | 2.68±1% | 20.04 | 49 - 171 (123) | 8.0 | 61 | 136 | 84 | 14:17 |
| 19 | vvypw_019.dat | 10.0 | hdpp3_e451a+vvy | 2.55±1% | 19.98 | 35 - 153 (119) | 7.6 | 61 | 124 | 89 | 14:21 |
| 21 | vvypw_021.dat | 10.0 | r669a+vvyp | 2.65±1% | 19.87 | 29 - 171 (143) | 7.5 | 61 | 138 | 90 | 14:23 |
| 24 | vypw_024.dat | 10.0 | hdpp3_e451a+vyp | 2.63±1% | 20.15 | 30 - 177 (148) | 7.8 | 61 | 128 | 90 | 14:27 |
| 26 | vypw_026.dat | 10.0 | hdpp3_2xmut+vyp | 2.76±1% | 21.45 | 43 - 164 (122) | 8.4 | 65 | 142 | 86 | 14:29 |
| 28 | vypw_028.dat | 10.0 | k670a+vypw | 2.80±1% | 16.73 | 31 - 164 (134) | 9.2 | 51 | 147 | 89 | 14:32 |
| 30 | vypw_030.dat | 10.0 | r669a+vypw | 2.69±2% | 20.03 | 39 - 128 (90) | 8.0 | 61 | 138 | 86 | 14:34 |
| 33 | valo_033.dat | 10.0 | hdpp3_e451a+val | 2.59±2% | 20.44 | 33 - 163 (131) | 7.5 | 62 | 127 | 89 | 14:38 |
| 35 | valo_035.dat | 10.0 | hdpp3_2xmut+val | 2.74±1% | 21.67 | 42 - 169 (128) | 8.8 | 66 | 142 | 86 | 14:40 |
| 37 | valo_037.dat | 10.0 | k670a+valo | 2.75±1% | 16.79 | 57 - 161 (105) | 8.0 | 51 | 134 | 82 | 14:43 |
| 39 | valo_039.dat | 10.0 | r669a+valo | 2.69±1% | 20.20 | 43 - 168 (126) | 7.8 | 62 | 149 | 86 | 14:45 |
| 42 | lw_042.dat | 10.0 | hdpp3_e451a+lw | 2.57±1% | 20.26 | 42 - 151 (110) | 7.5 | 62 | 129 | 87 | 14:49 |
| 44 | lw_044.dat | 10.0 | hdpp3_2xmut+lw | 2.74±1% | 21.44 | 36 - 167 (132) | 8.6 | 65 | 140 | 88 | 14:51 |
| 46 | lw_046.dat | 10.0 | k670a+lw | 2.75±1% | 16.63 | 48 - 168 (121) | 8.4 | 51 | 136 | 84 | 14:53 |
| 48 | lw_048.dat | 10.0 | r669a+lw | 2.69±1% | 20.79 | 45 - 171 (127) | 7.8 | 63 | 148 | 86 | 14:56 |
| 51 | p0_051.dat | 10.0 | hdpp3_e451a_a0 | 2.63±1% | 20.60 | 46 - 176 (131) | 7.6 | 63 | 137 | 85 | 14:59 |
| 53 | p0_053.dat | 10.0 | hdpp3_2xmut_b0 | 2.75±1% | 21.73 | 45 - 166 (122) | 8.8 | 66 | 131 | 85 | 15:02 |
| 55 | p0_055.dat | 10.0 | k670a_c0 | 2.79±1% | 16.89 | 44 - 163 (120) | 9.1 | 51 | 132 | 85 | 15:04 |
| 57 | p0_057.dat | 10.0 | r669a_d0 | 2.74±1% | 20.35 | 35 - 169 (135) | 8.4 | 62 | 149 | 88 | 15:06 |
| Run # | File | Conc., mg/ml | Description | $R_g$, nm | I(0) | Guinier points | $D_{max}$, nm | MM, kDa | Volume, $nm^3$ | Quality, % | Comments |

# PERFORMING THE EXPERIMENT

EMBL

- Wrong Buffer

I(s)



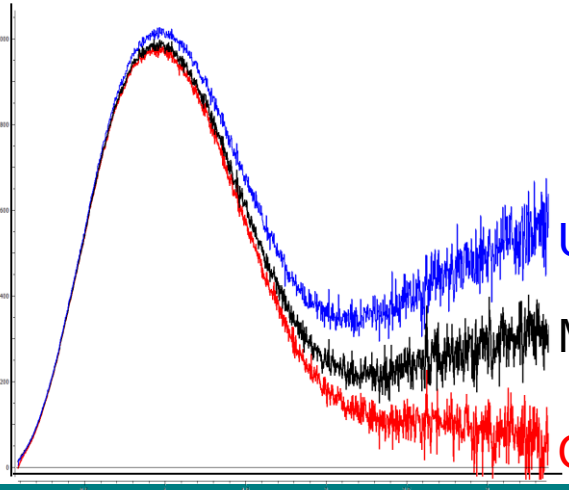
Kratky

s

Under subtraction (Tris)

Matching buffer (Tris + glycine)

Over subtraction (Tris + glycerol)



Under subtraction (Tris)

Matching buffer (Tris + glycine)

Over subtraction (Tris + glycerol)

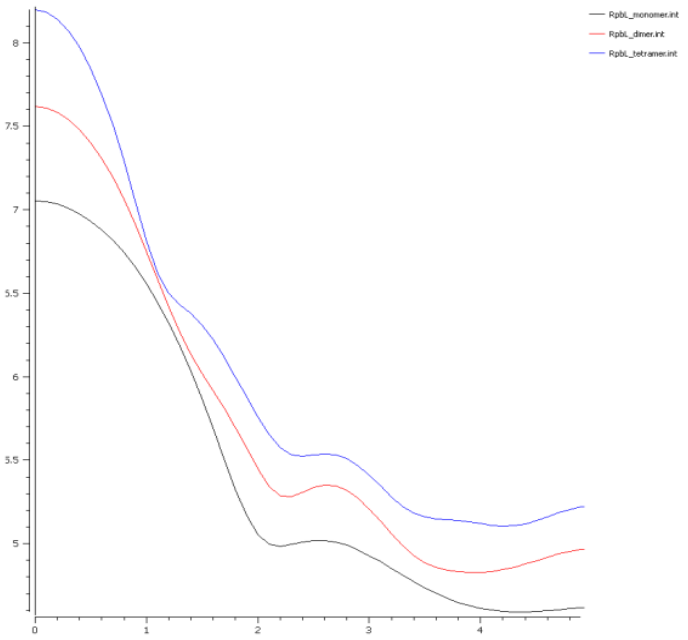
- Case 3: the Ambitious (Hasty) Scientist

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

    - Explanation: incorrect buffer subtraction
    - Solution: dialysis, SEC, (centricon)

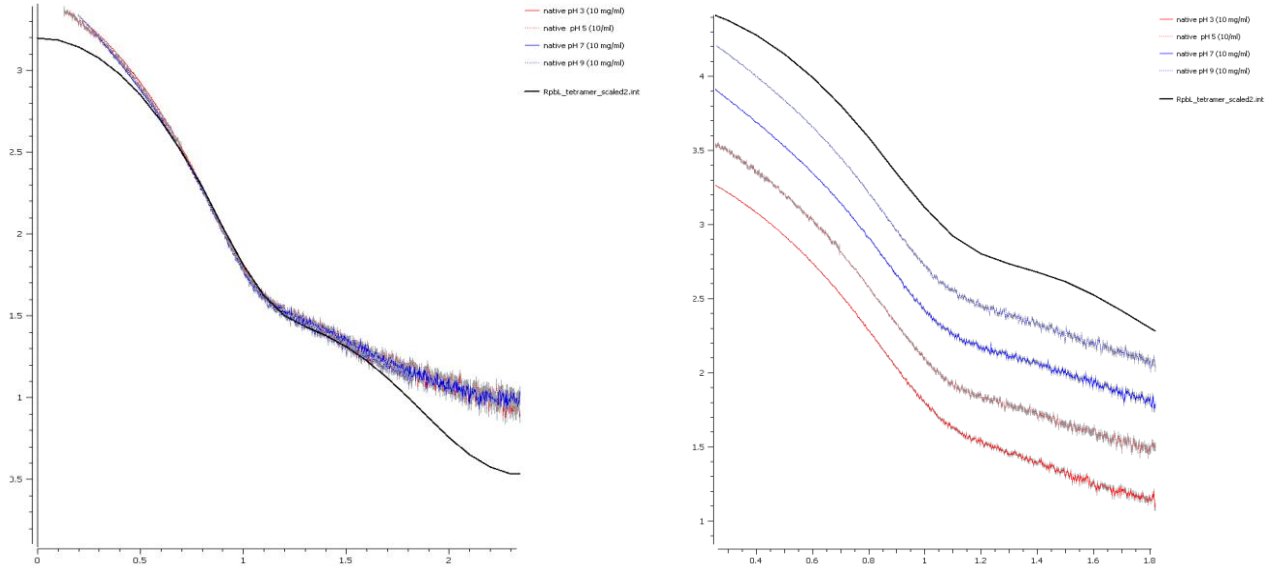
- Case 4: the Super Scientist

  - Sample: excellently prepared, fully analyzed
  - Question: pH dependent oligomerization




- Case 4: the Super Scientist

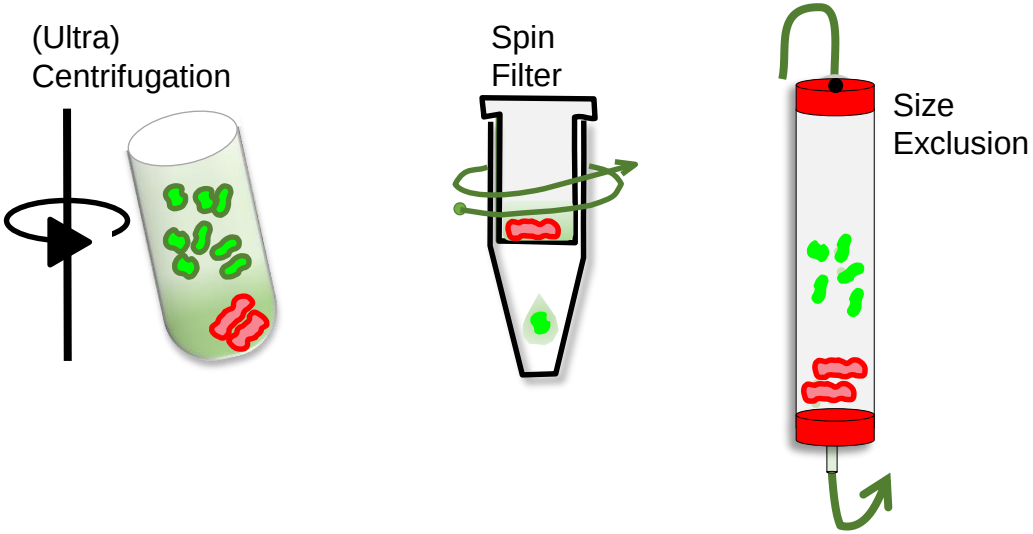
  - Sample: excellently prepared, well analyzed sample
  - Question: pH dependent oligomerization
  - Result:






EMBL

- Removal of aggregates



- Time/ storage/ transport

  - Stability
    - pH
    - Concentration
    - Additives
    - Proteases
  - Freezing/ Thawing
  - Tube size, concentration

**Only use additives when it is really necessary!**

| Class of additive | example | concentration | purpose |
|---|---|---|---|
| Salts | NaCl, KCl, $(NH_4)_2SO_4$ | 50-150 mM | maintain ionic strength of medium |
| Detergents | Deoxycholate, Triton X-100 | 0.1-1% As close as possible to cmc! | solubilization of poorly soluble proteins |
| Glycerol | | 5-~~10%~~ | stabilization |
| Glucose or sucrose | | 25 mM contrast! | Stabilize lysosymal membranes, reduce protease release |
| Metal chelators | EDTA, EGTA | 1 mM | reduce oxidation damage, chelate metal ions |
| Reducing agents | DTT, DTE 2-Mercaptoethanol | 1-10 mM 0.05% | reduce oxidation damage low shelf-life! |
| Ligands, metal ions | $Mg^{2+}$, ATP, GTP | 1-10 mM | stabilization absorption |

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

**Table 1.** Comparison of Protein Storage Conditions

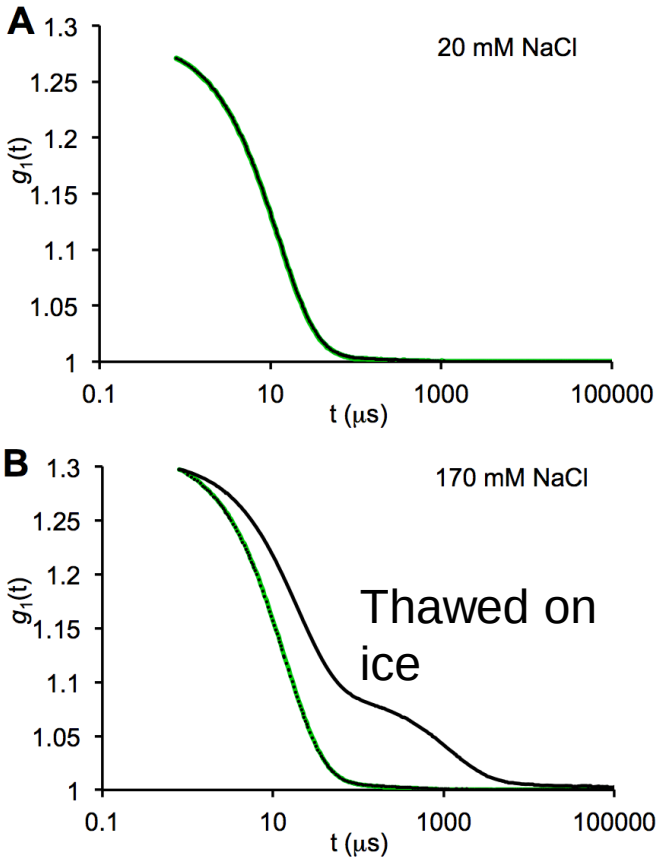
| | Storage Condition | | | |
|---|---|---|---|---|
| **Characteristic** | Solution at 4°C | Solution in 25-50% glycerol or ethylene glycol at -20°C | Frozen at -20° to -80°C or in liquid nitrogen | Lyophilized (usually also frozen) |
| **Typical shelf life** | 1 month | 1 year | Years | Years |
| **Requires sterile conditions or addition of antibacterial agent** | Yes | Usually | No | No |
| **Number of times a sample may be removed for use** | Many | Many | Once; repeated freeze-thaw cycles generally degrade proteins | Once; it is impractical to lyophilize a sample multiple times |

**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.

@ Pierce

- Time/ storage/ transport
  - Stability
  - Freezing/ Thawing
    - Speed
    - Additives
    - Dried ice
  - Tube size, concentration



- Case 4: the Super Scientist

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

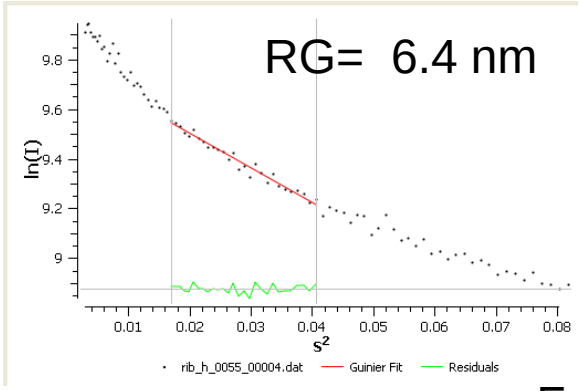
    - Explanation: time/storage/transport dependent alterations
    - Solution: re-characterize the sample,
      ‘first aid’ at the beamline

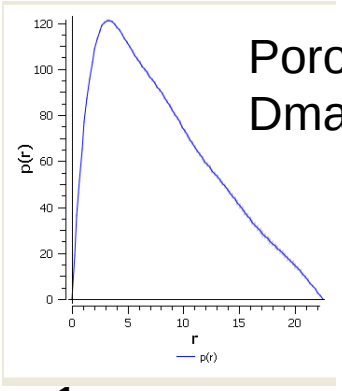
- Case 5: the Brilliant Scientist

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

| Run# | Description | Code | Conc. | Log plot | Kratky plot | Guinier points | | Quality | $R_g$ | | $D_{max}$ | $V_{Porod}$ | $V_{DAM}$ | $MW_{I(0)}$ | $MW_{Porod}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mg/ml | | | | | | nm | | nm | $nm^3$ | $nm^3$ | kDa | kDa |
| 238 | ribo_10 | r_h_2 | 10.0 | Only one frame | | 66 | 271 | 76% | 1.7 ±0.3 | | 6 | 17 | | 16 | 11 |
| 240 | ribo_10 | r_h_2 | 10.0 | Only one frame | | 87 | 288 | 70% | 1.7 ±0.3 | | 6 | 17 | | 16 | 10 |
| 242 | ribo_10 | r_h_2 | 10.0 | Only one frame | | 43 | 288 | 80% | 1.7 ±0.3 | | 6 | 18 | | 16 | 11 |

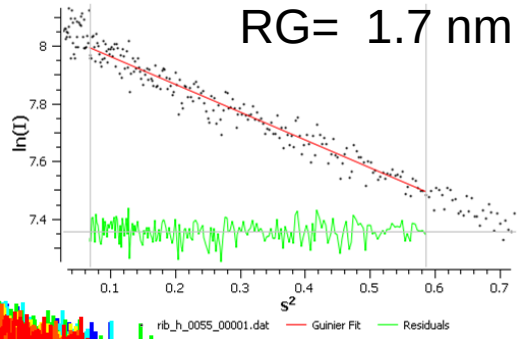
Frame 4

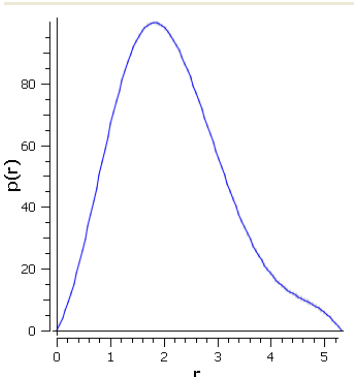



Porod V = 153.52 nm$^3$
Dmax = 22.52 nm

Frame 1



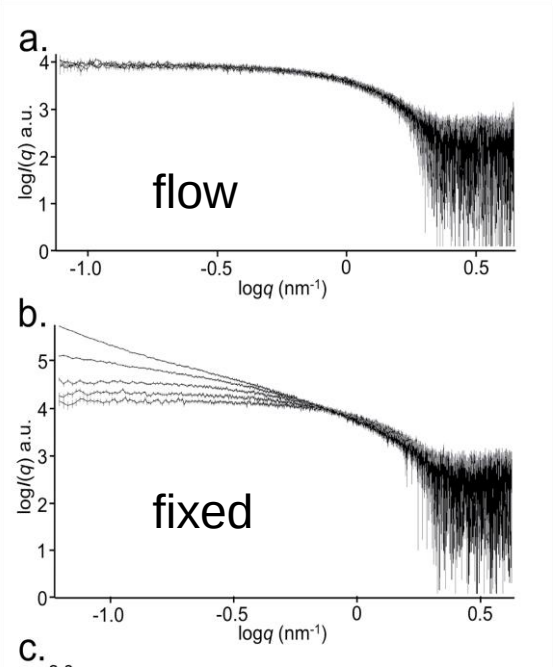




Porod V = 17.39 nm$^3$
Dmax = 5.32 nm

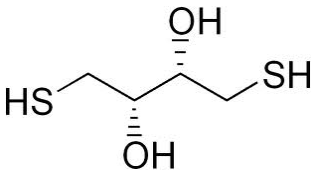
# PERFORMING THE EXPERIMENT

EMBL

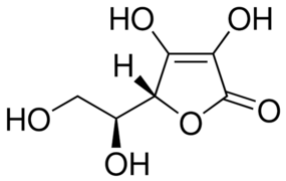
- Data collection strategy
  - Increase sample flow
    - Requires more material
  - Attenuate the beam
    - Influences signal to noise



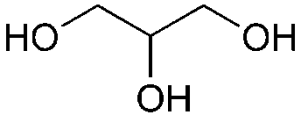
- Scavengers
  - DTT (b)

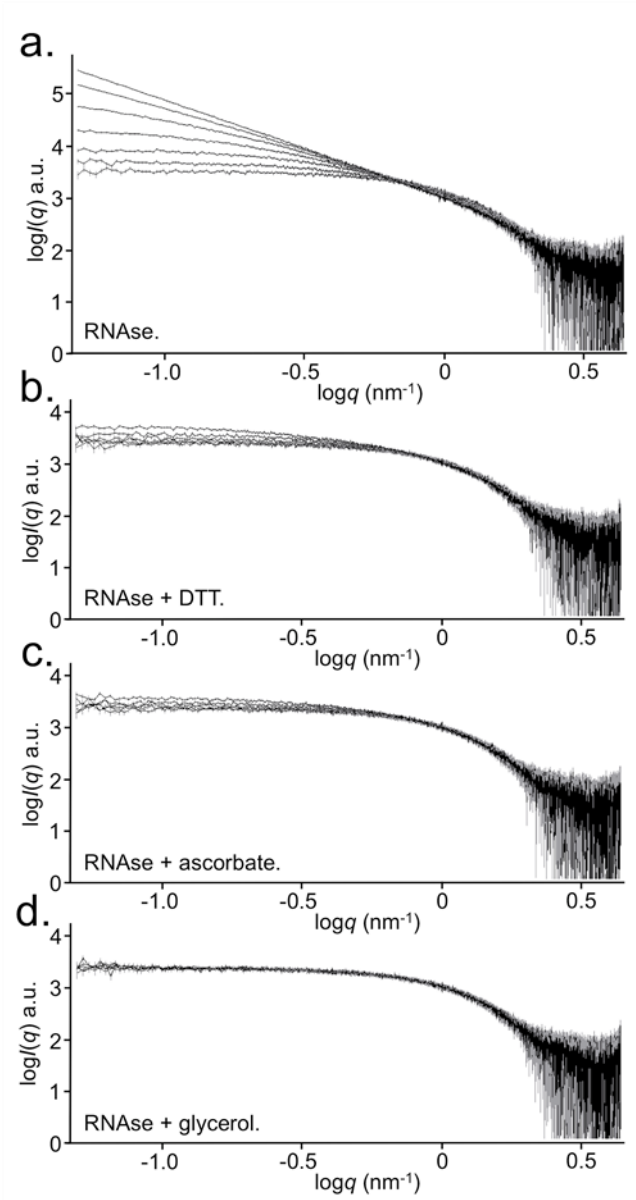



  - Ascorbic Acid (c)



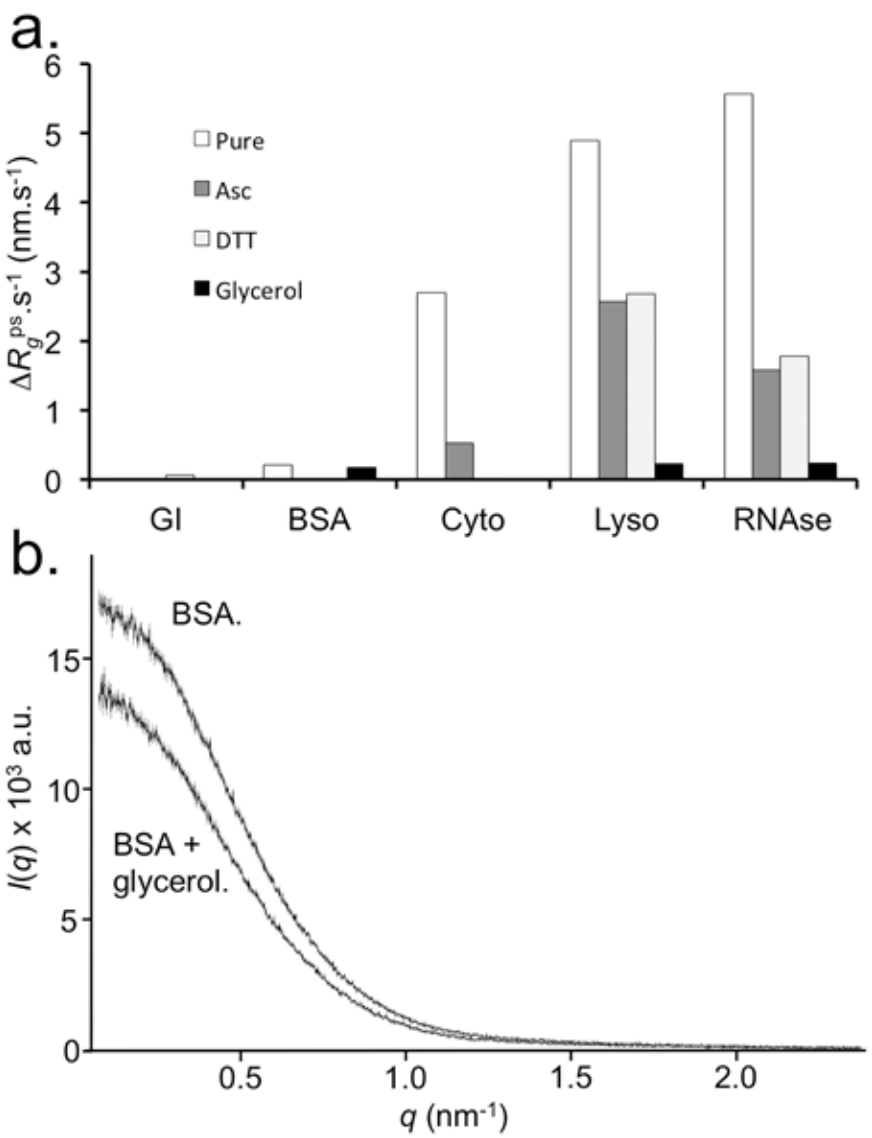

  - Glycerol (d)





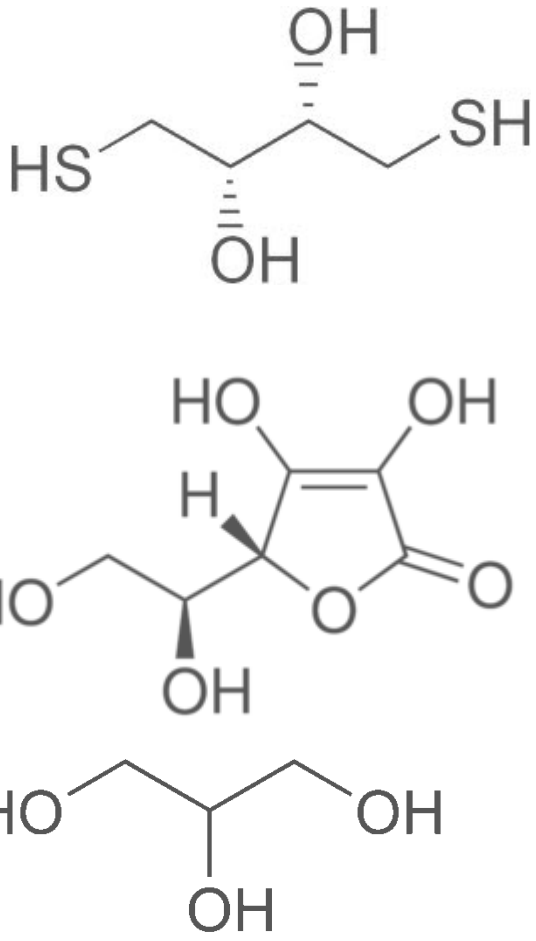

EMBL

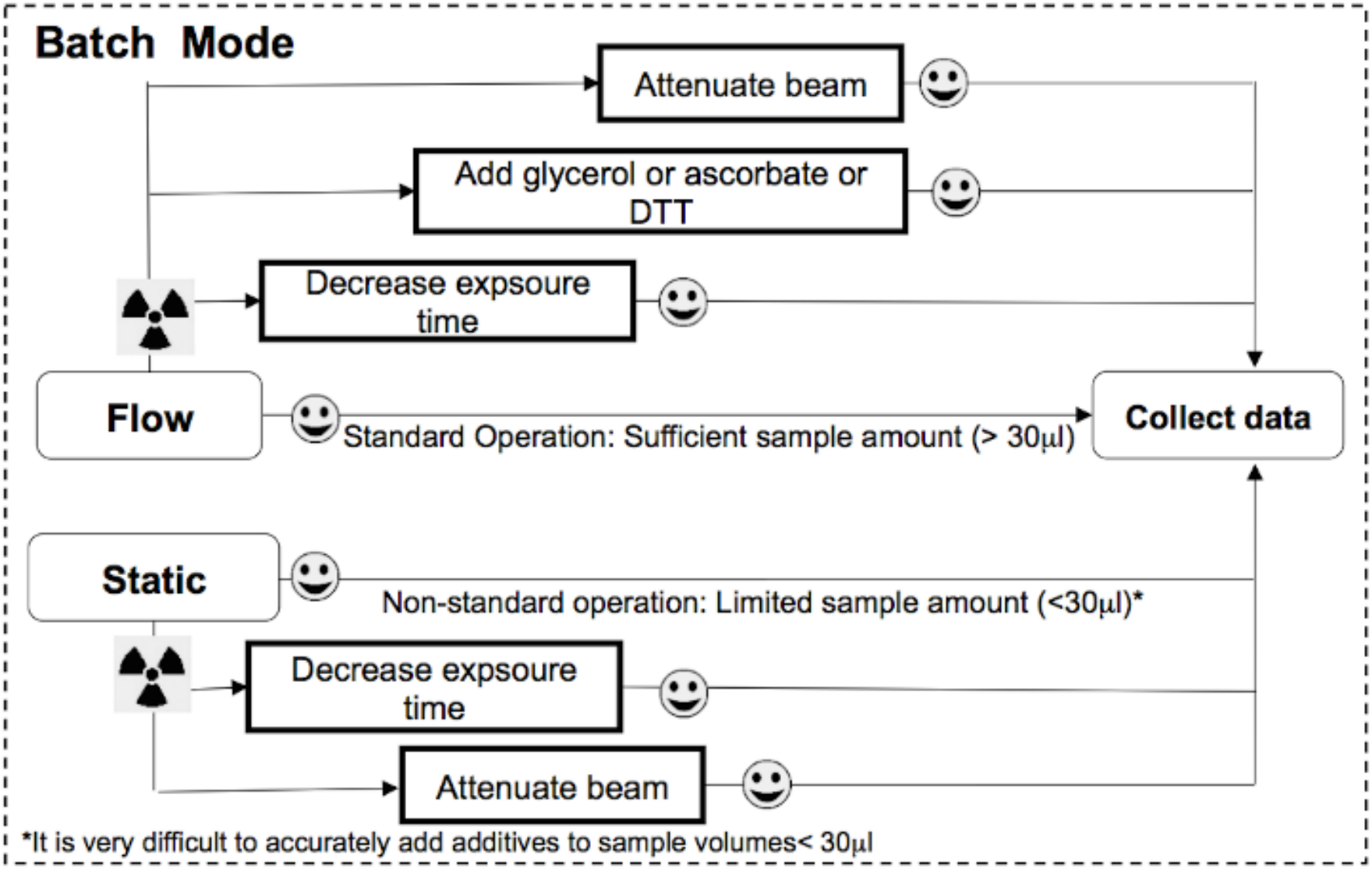



# PERFORMING THE EXPERIMENT

EMBL

- 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 expsoure time
Flow
Standard Operation: Sufficient sample amount (> 30µl)
Collect data
Static
Non-standard operation: Limited sample amount (<30µl)*
Decrease expsoure time
Attenuate beam
*It is very difficult to accurately add additives to sample volumes< 30µl

- 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!

**Reading:**

Svergun, D.I., Koch, M.H.J., Timmins, P.A., May, R.P. (2013)
Small Angle X-Ray and Neutron Scattering from Solutions of Biological Macromolecules
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Graewert MA, Jeffries CM. (2017)
Sample and Buffer Preparation for SAXS.
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Skou, Gillilan & Ando;
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