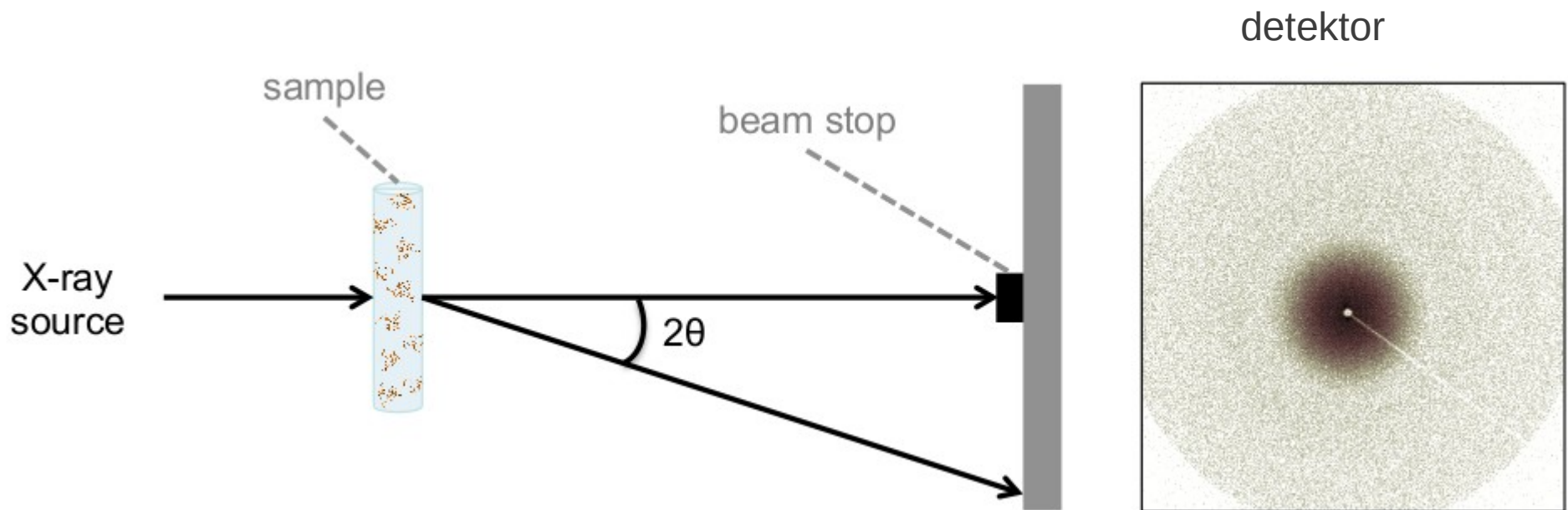
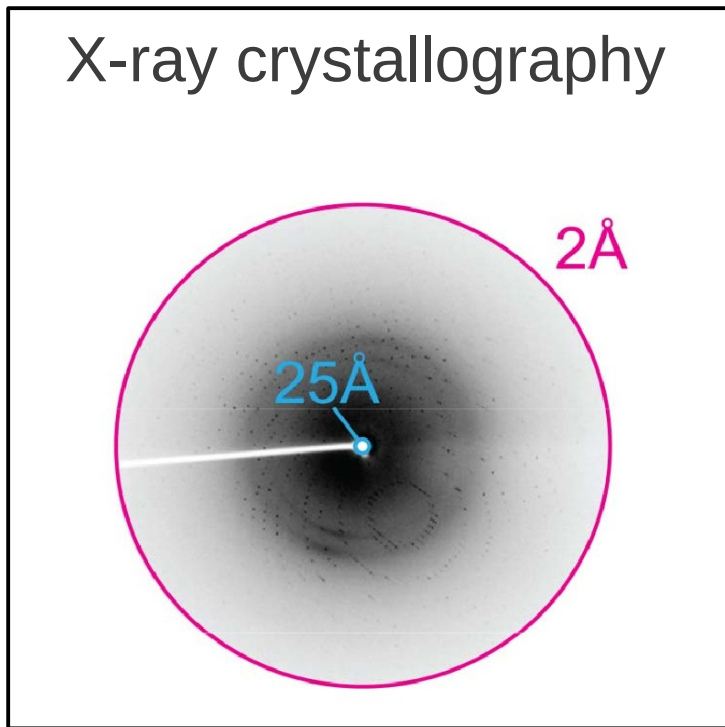
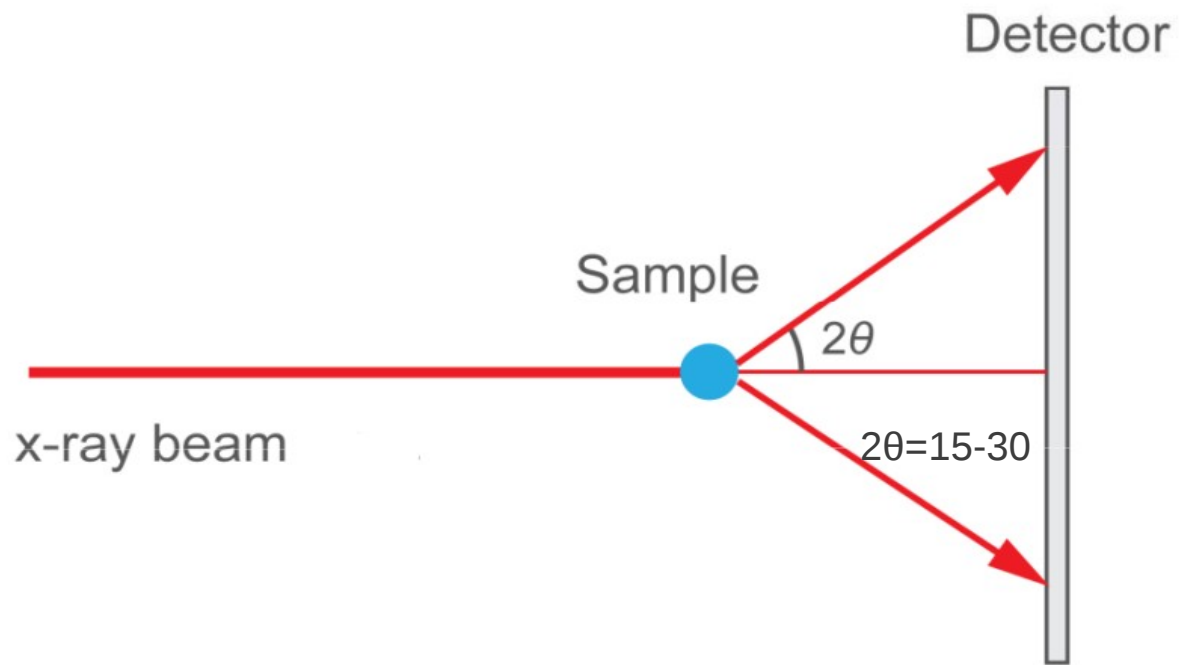


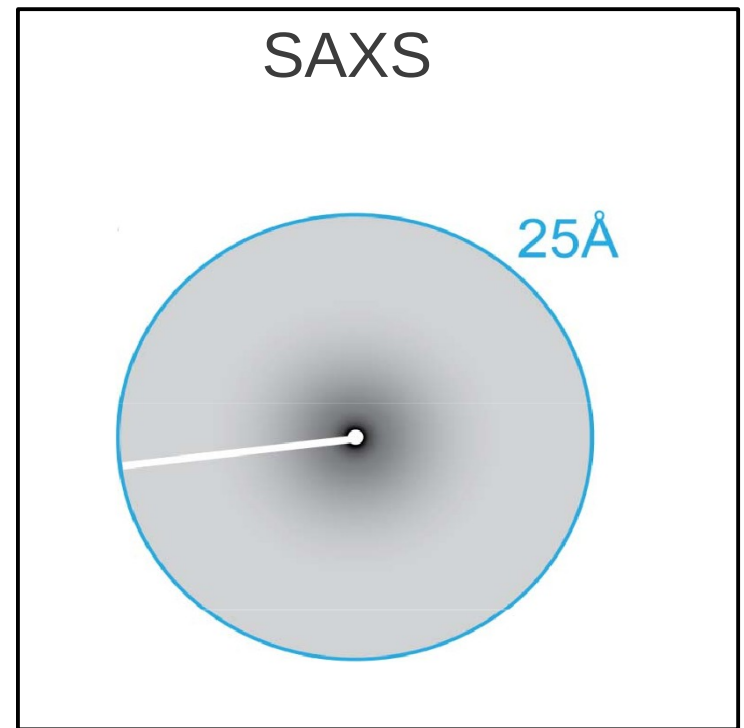
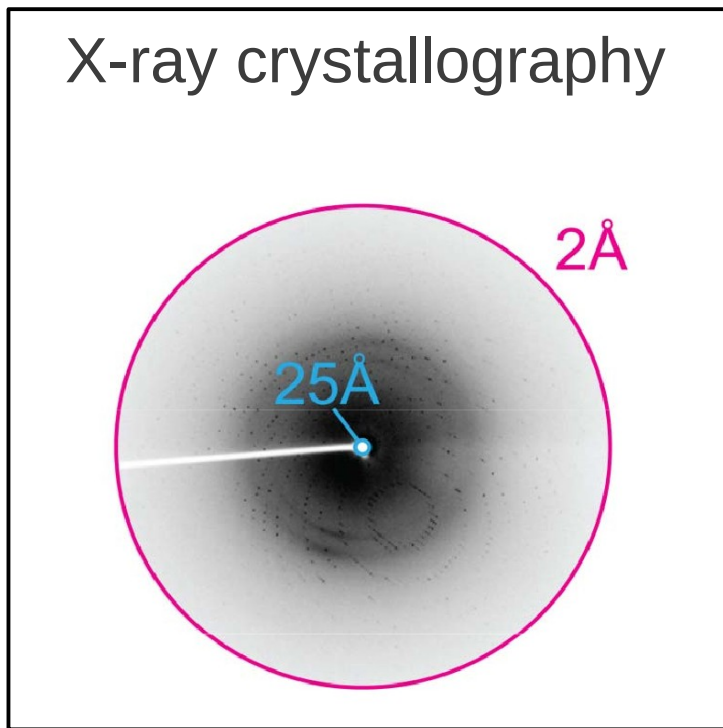
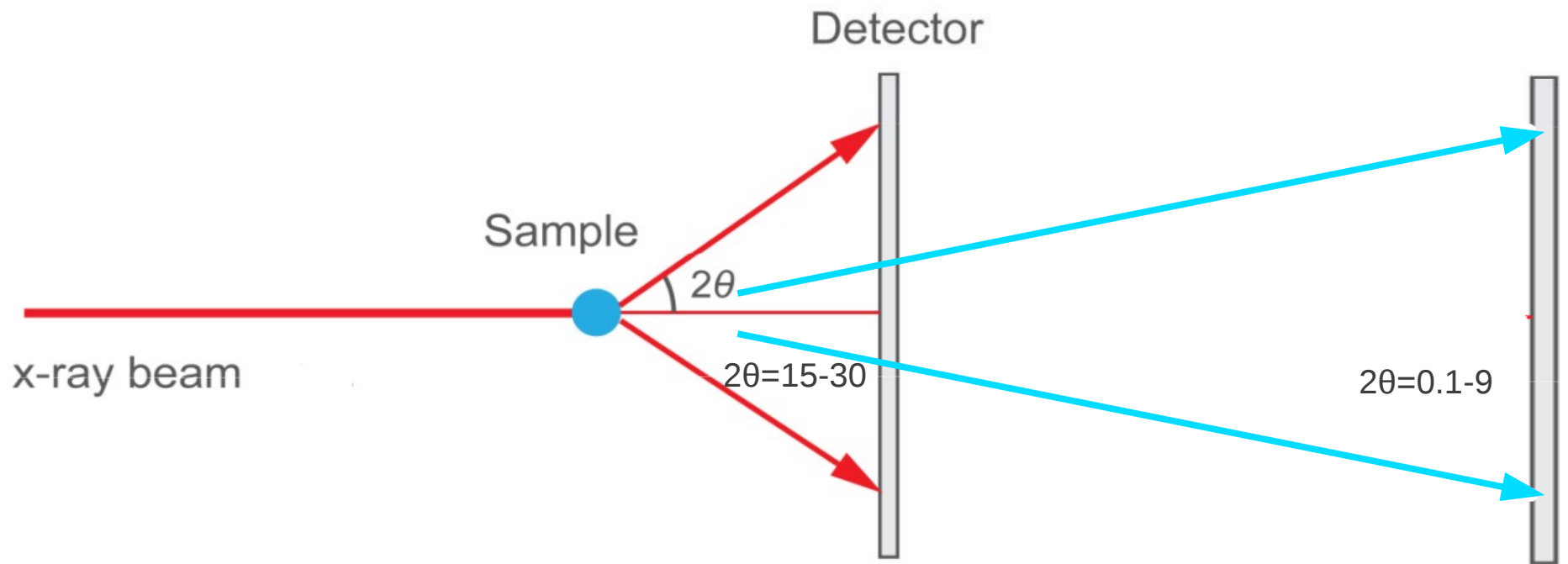
SAXS

Small angle X-ray scattering

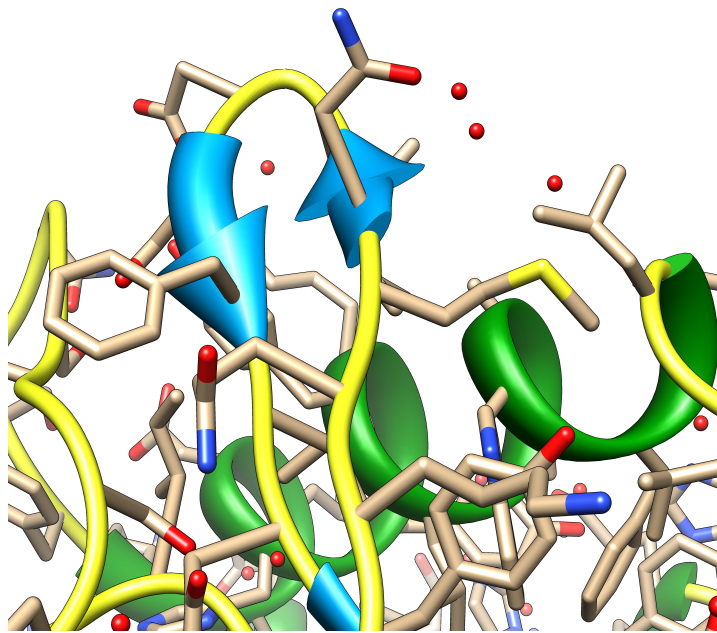
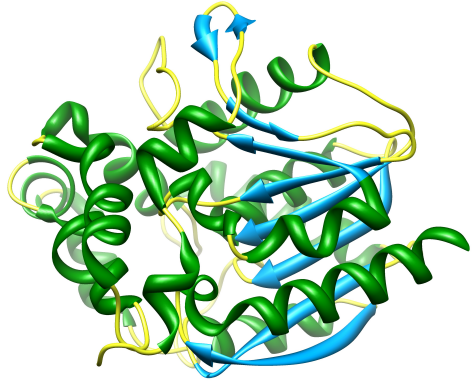
- rozptyl rentgenového záření v malých úhlech



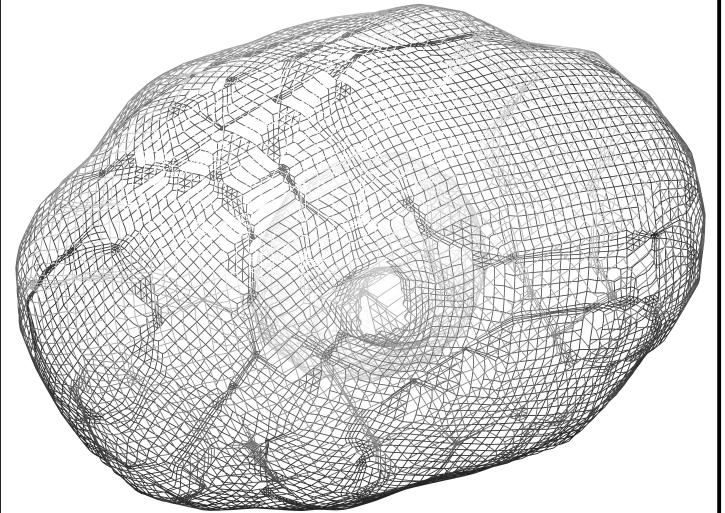




X-ray crystallography



SAXS



SAXS

Vzorek - pevná látka, tekutiny, vrstvy
- koloidní roztoky
- částečně uspořádané systémy

Informace - velikost a tvar částic, distribuce pórů,

Aplikace - kovy, plasty, oleje, nanočástice
- potraviny, farmaceutický průmysl

Biologické aplikace

Vzorek: protein, nukleové kys., komplexy v roztoku

1. Obecné strukturní parametry
2. Tvar částic
3. Homogenní vs. agregovaný vzorek
4. Složený vs. nesložený protein
5. Oligomerizace, interakce, kvarterní struktura
6. Modelování molekulární flexibility

Nanočásticové aplikace

Vzorek: koloidní roztok nanočástic

1. Obecné strukturní parametry
2. Tvar částic
3. Homogenní vs. agregovaný vzorek
4. Distribuce velikostí (polydisperzní vzorky)
5. Core-shell struktura
6. Distribuce a velikost pórů

SAXS - **výhody**

1. snadná příprava vzorku
2. sleduje chování molekul v roztoku
3. užitečný před i po vyřešení molekulární struktury
4. rychlost experimentu
5. není limitován velikostí molekul

SAXS - **nevýhody**

1. rozlišení $\sim 10\text{-}20\text{\AA}$
2. nejednoznačná interpretace
3. neexistuje R_{faktor}

Rozlišení SAXS modelů

1. Braggovo

$$q_{\min} = 0.006 \text{Å}^{-1} \sim d = 1000 \text{Å}$$

$$q_{\max} = 0.6 \text{Å}^{-1} \sim d = 10 \text{Å}$$

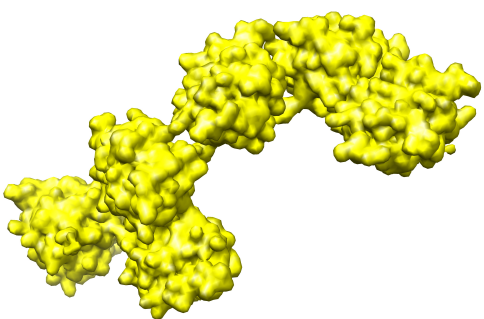
$$d = \frac{2\pi}{q}$$

2. Efektivní

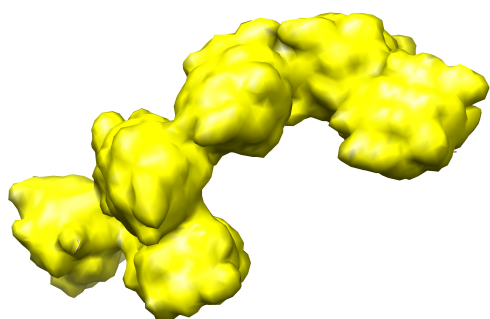
“low resolution structure” ~ bez číselné hodnoty
odpovídá mapě el. hustoty s rozlišením cca 20Å

Rozlišení

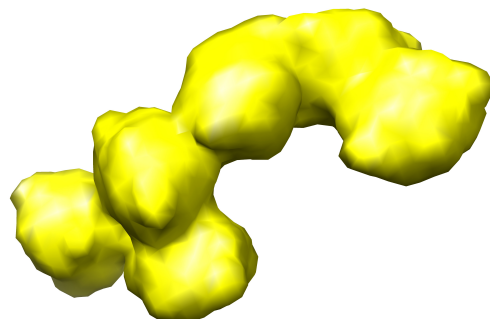
SAXS 10-20Å



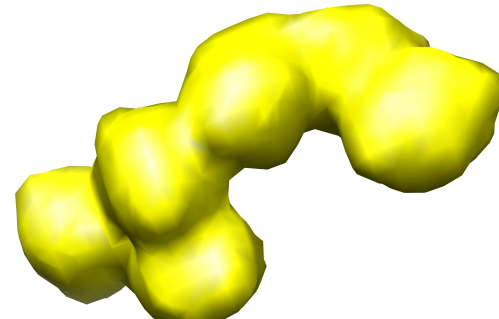
5Å



10Å



15Å

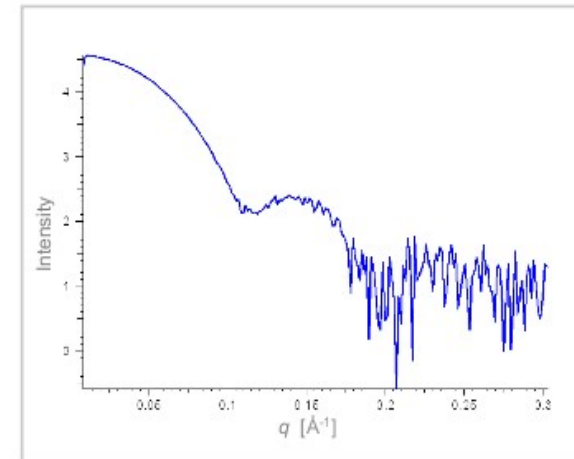
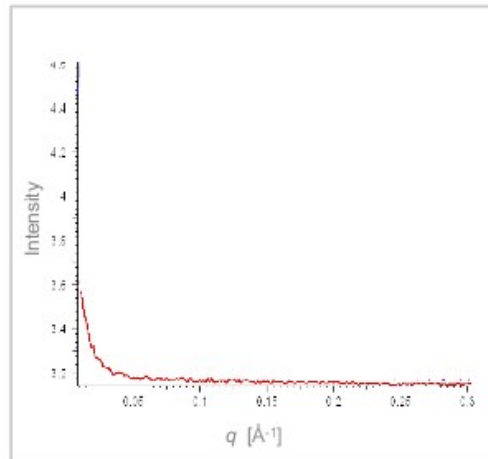
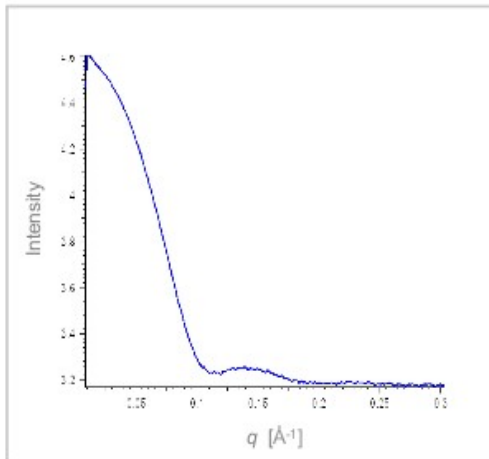
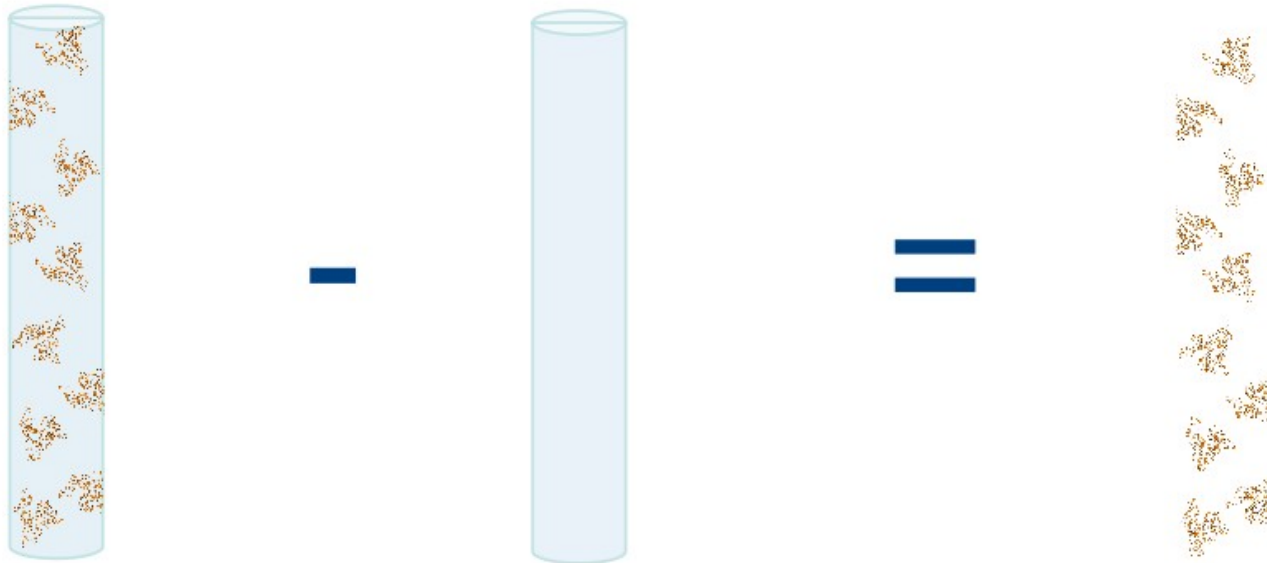


20Å

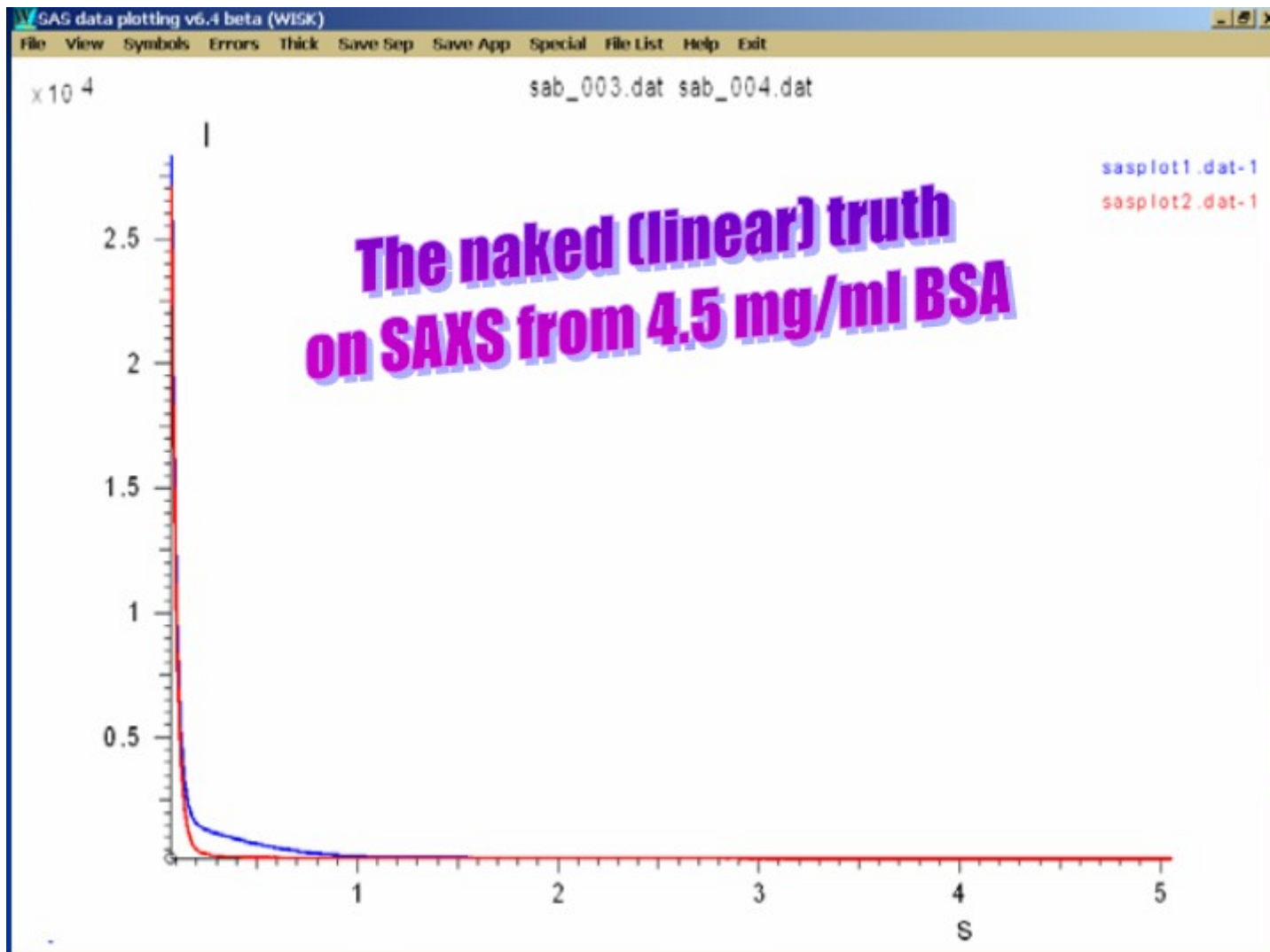
50Å

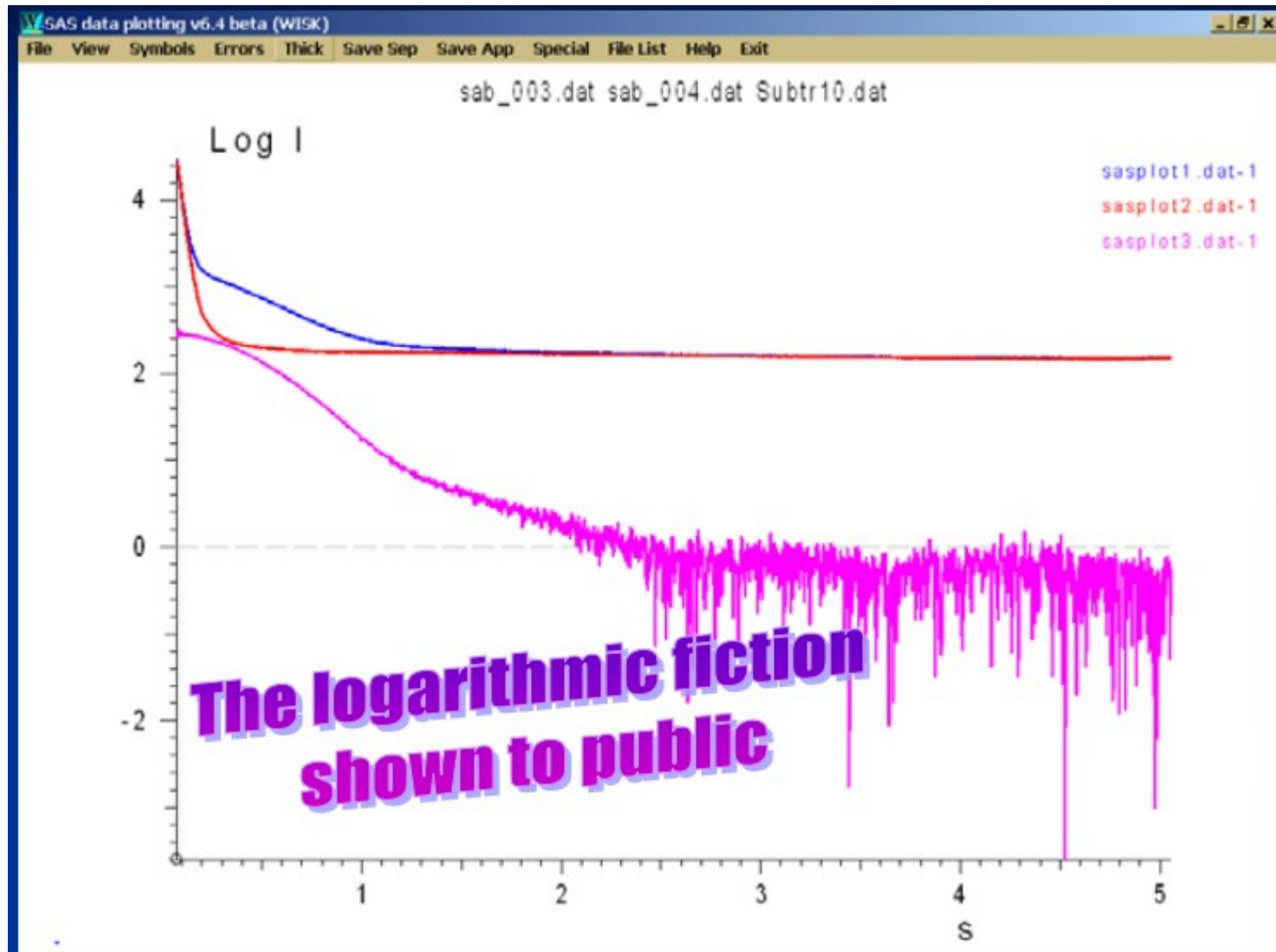
RTT complex ~7000atoms

Odečtení příspěvku solventu



Looking At Nothing





SAXS strukturní informace

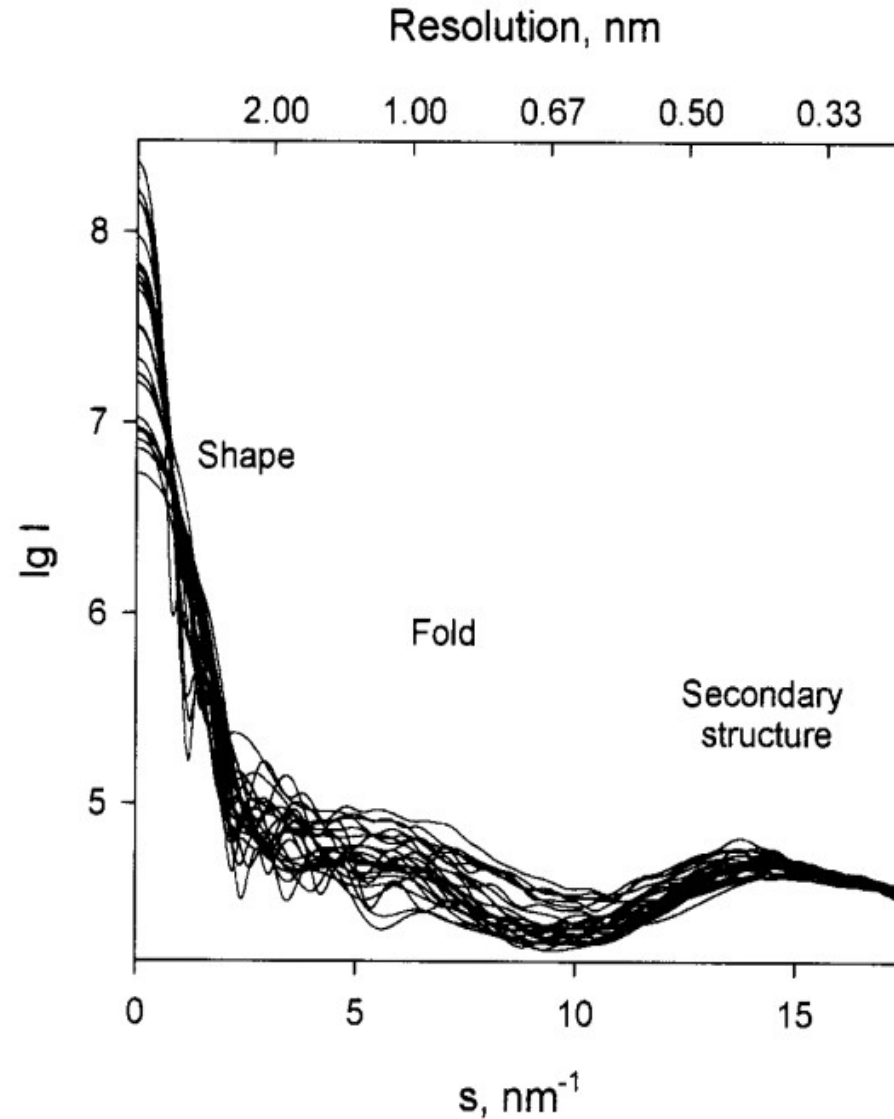
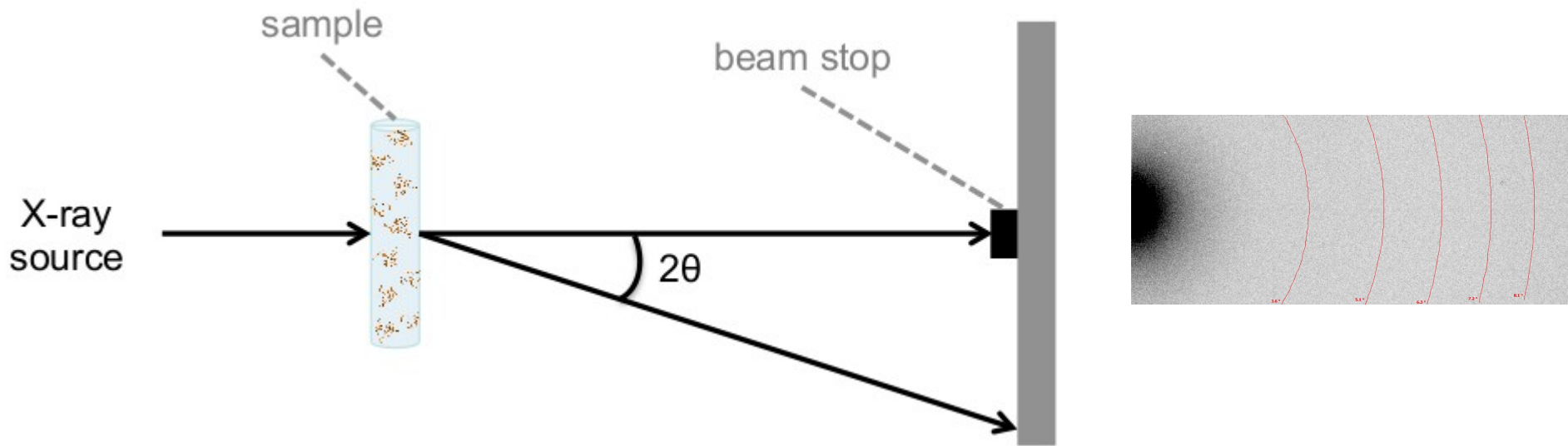
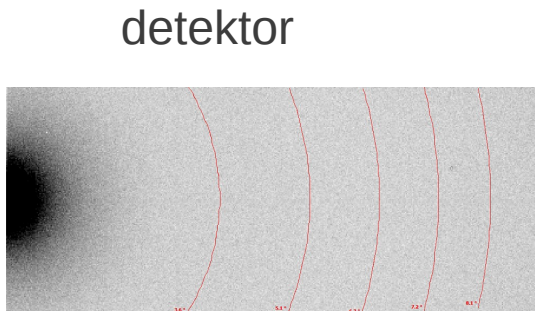


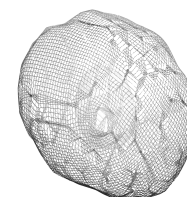
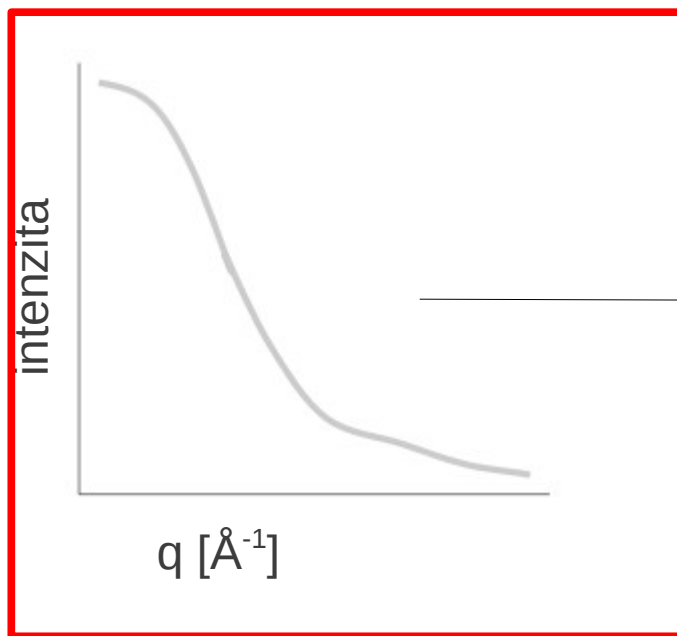
FIGURE 1 Theoretical x-ray solution scattering curves computed from atomic models of 25 different proteins. The upper axis displays the spatial resolution $\Delta = 2\pi/s$ and the text labels indicate the levels of structure organization characteristic of this resolution.

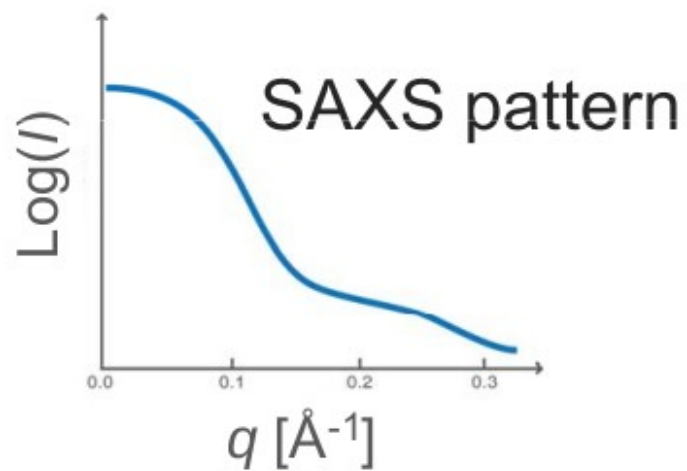


$$I(q) = \left| \int \int \int q(r) e^{-iqr} dr \right|^2$$

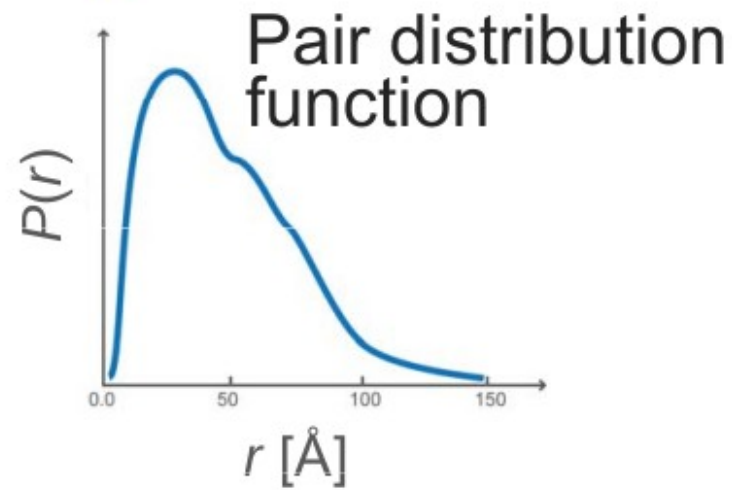
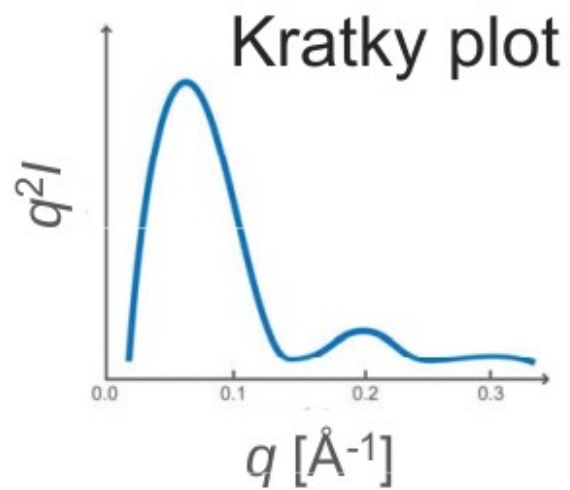
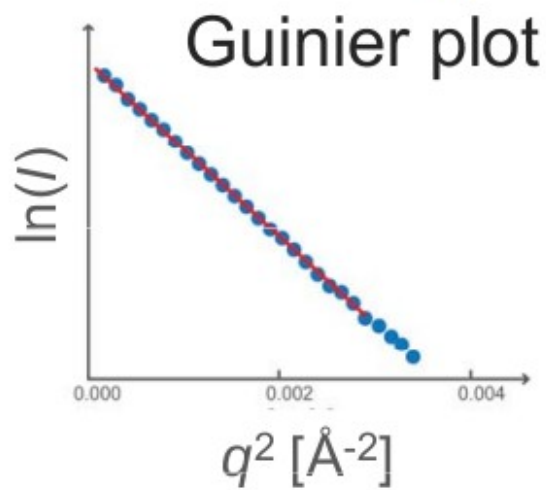


$$q = \frac{4\pi \sin\theta}{\lambda}$$



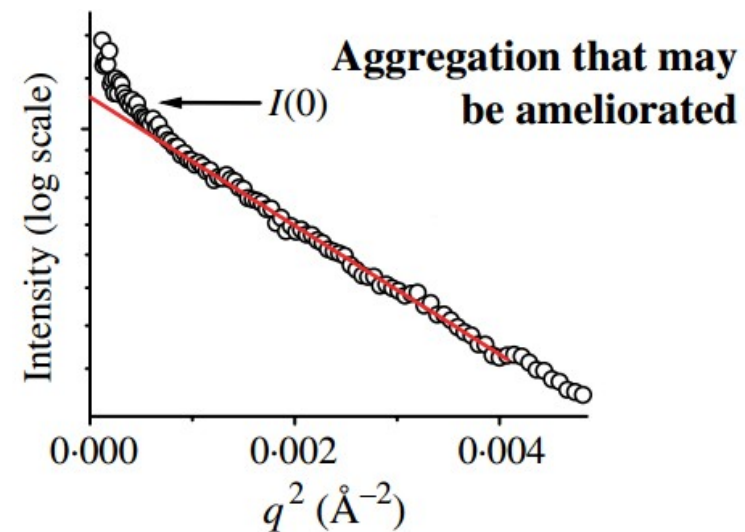
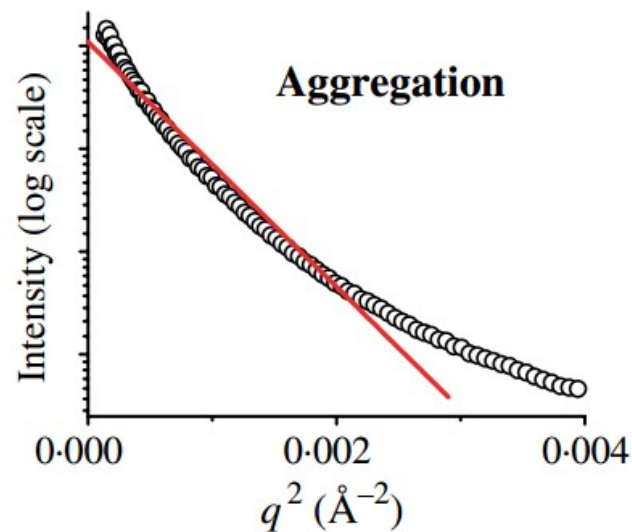
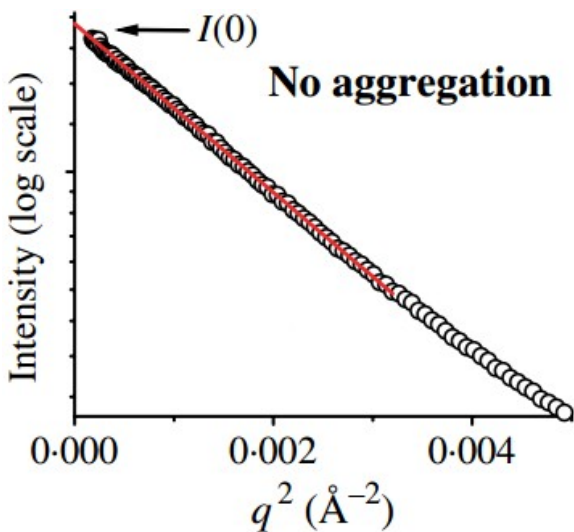


Fourier Transform



Guinier plot

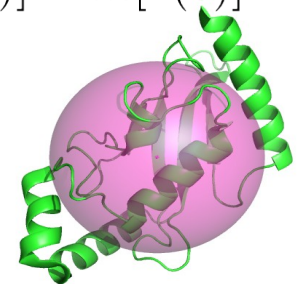
Analýza rozptylové křivky ve velmi malých úhlech



1. **Odchyly od linearity** – agregace (nepříznivě ovlivňuje sběr a interpretaci dat)

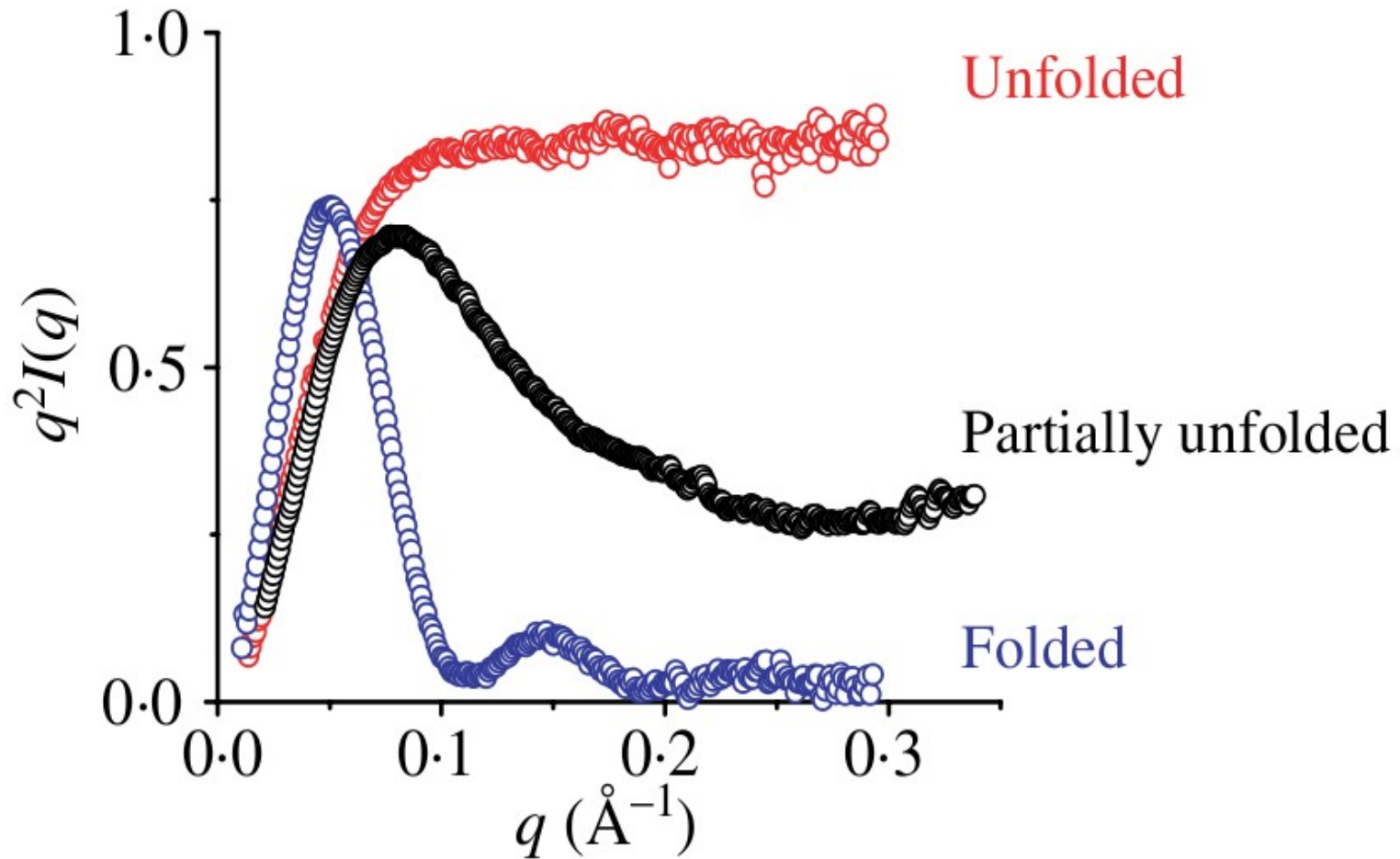
2. **Poloměr gyrace**

$$\ln[I(q)] = \ln[I(0)] - \frac{q^2 R^2}{3}$$

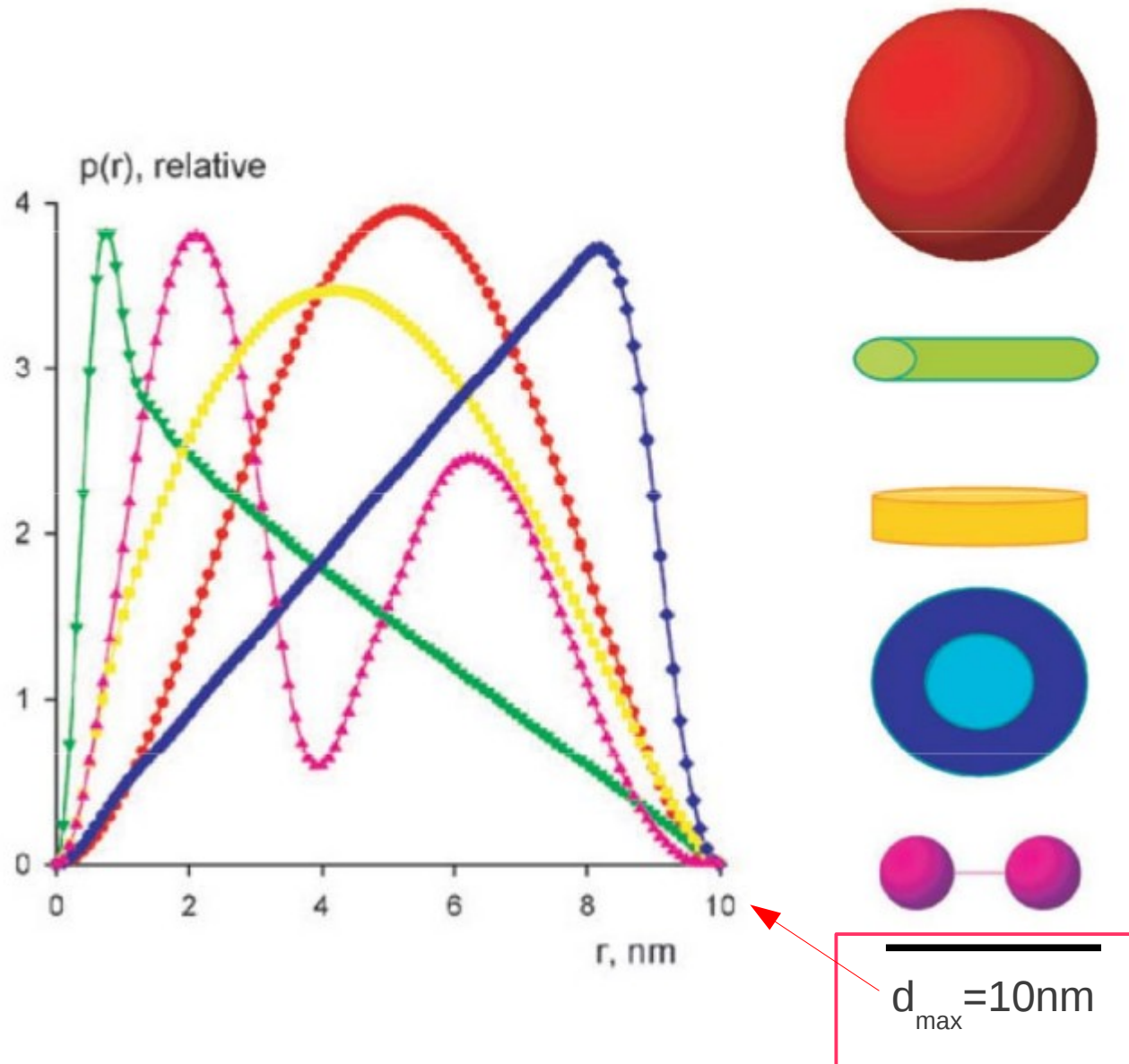


Kratky plot

Kompaktnost molekuly

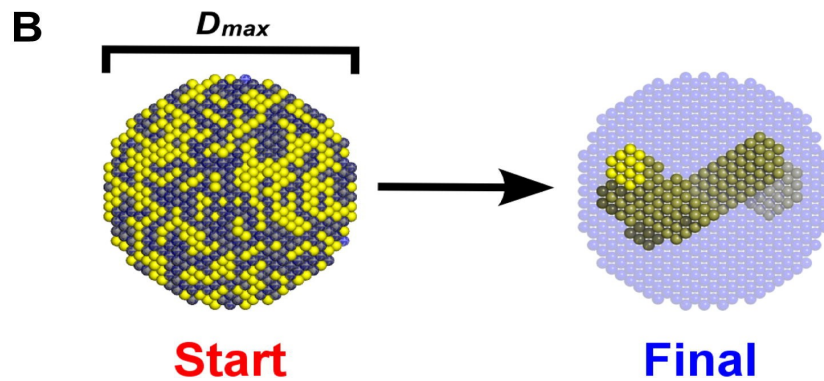
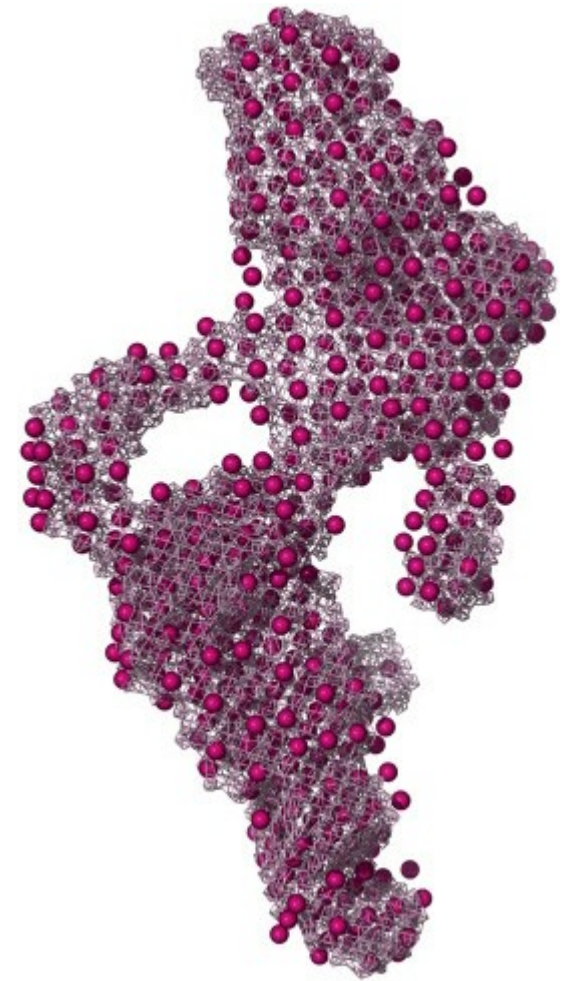
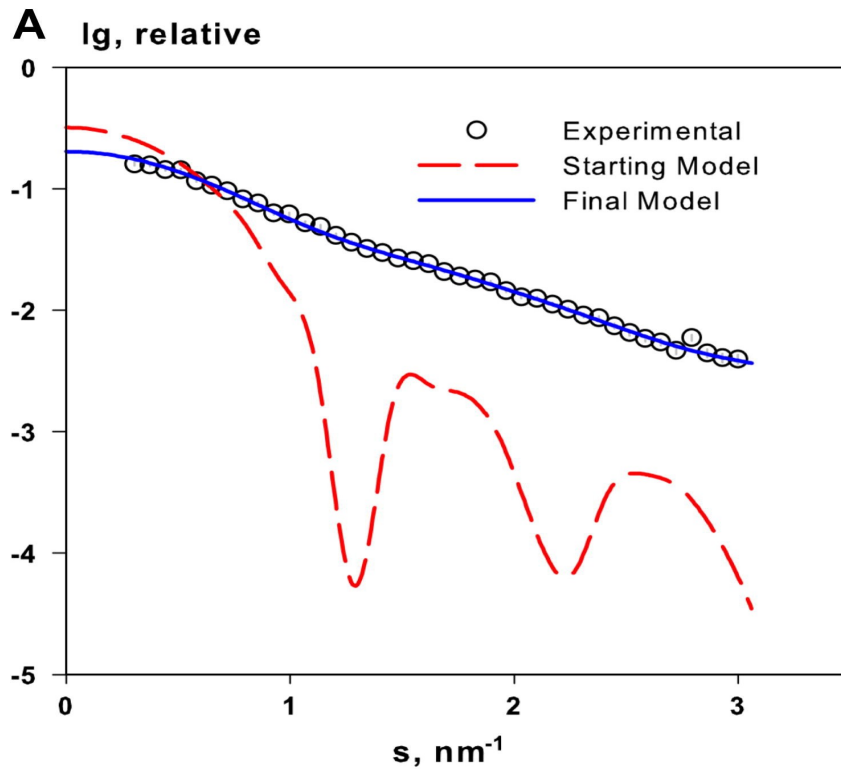


Pair distribution function

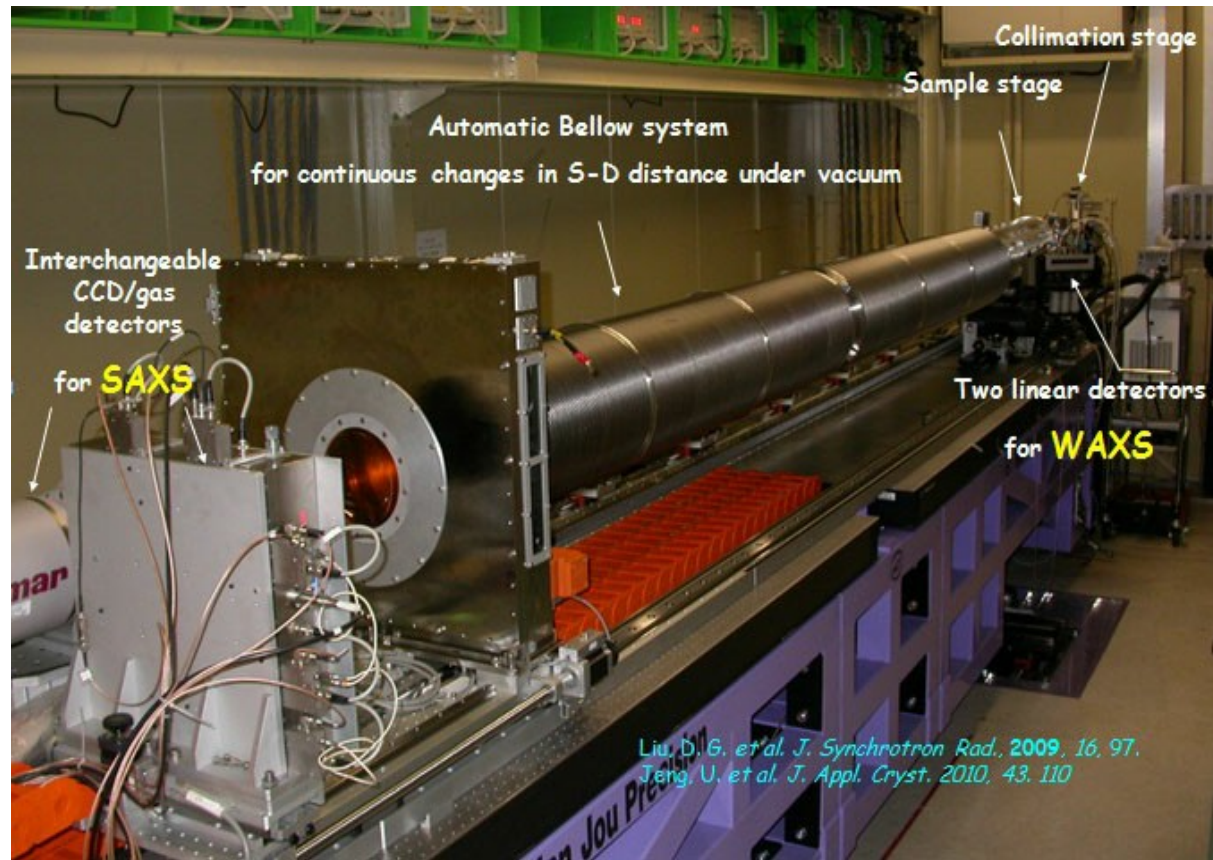
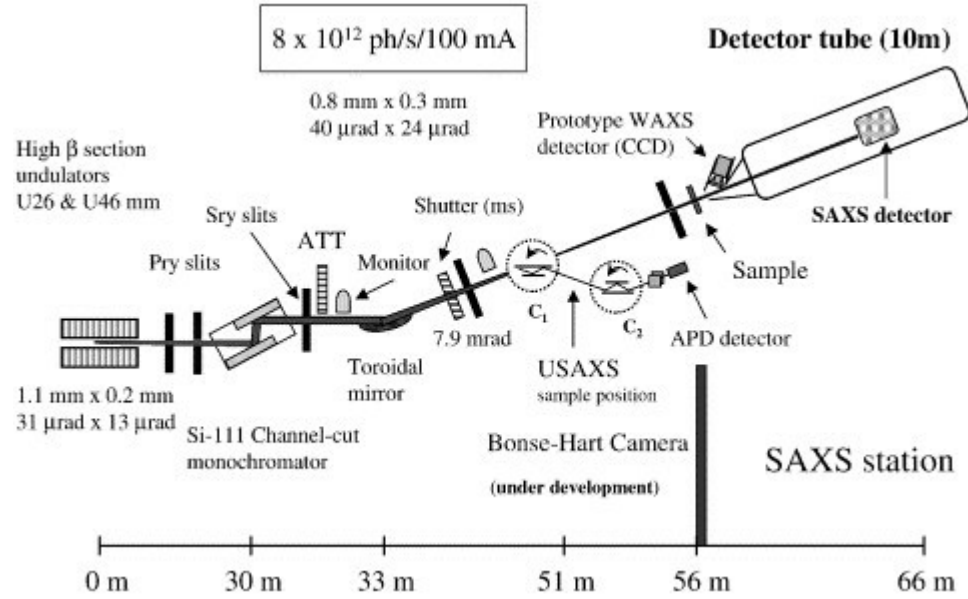


SAXS modelování tvaru

Program DAMMIN



SAXS experiment-synchrotron

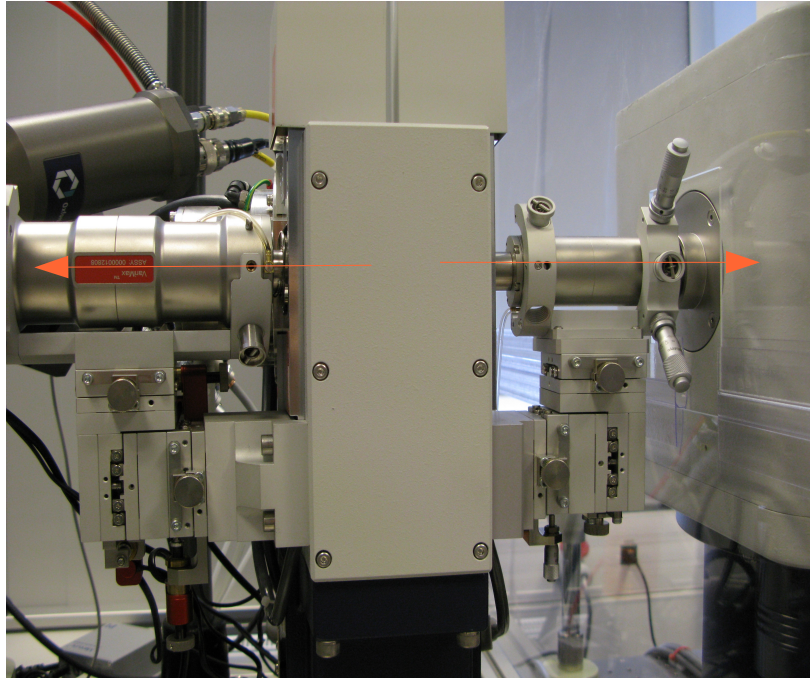


SAXS experiment-domáci zdroj



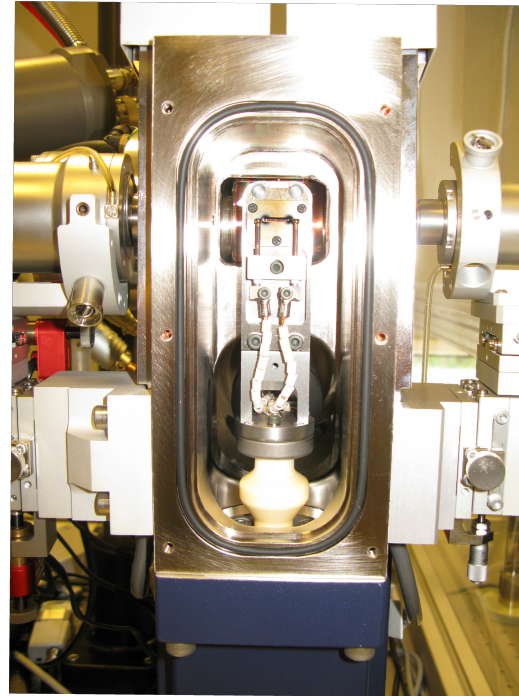


Rotating Anode: MicroMax-007 HF



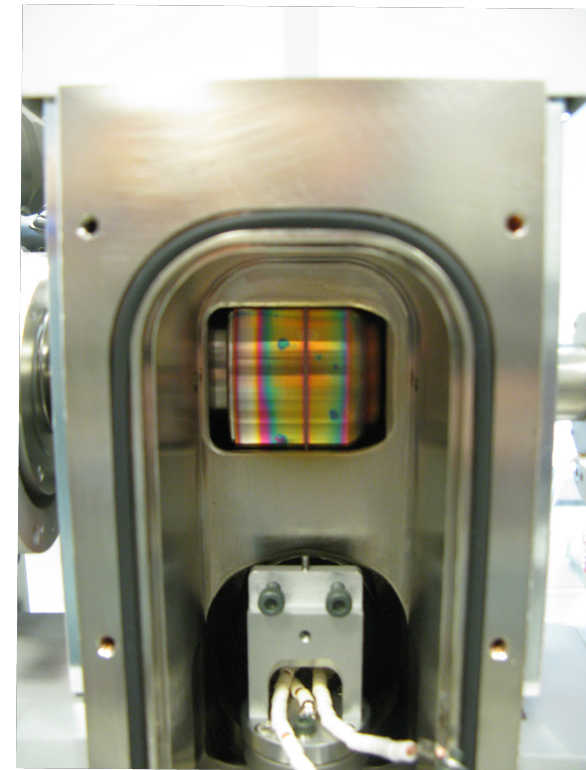
Tube tower

- 2 X-ray ports
- 0.2mm Be windows
- integrated vacuum pump
- water-cooled



Filament

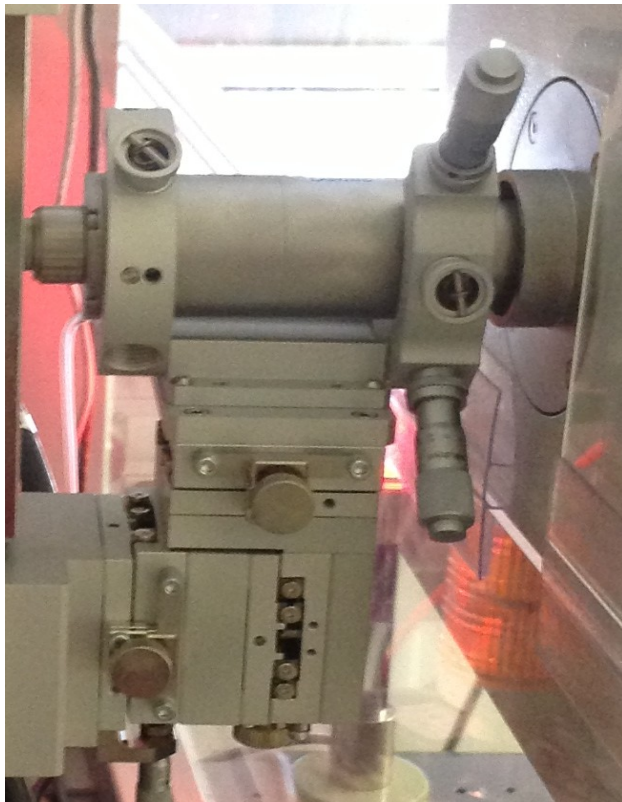
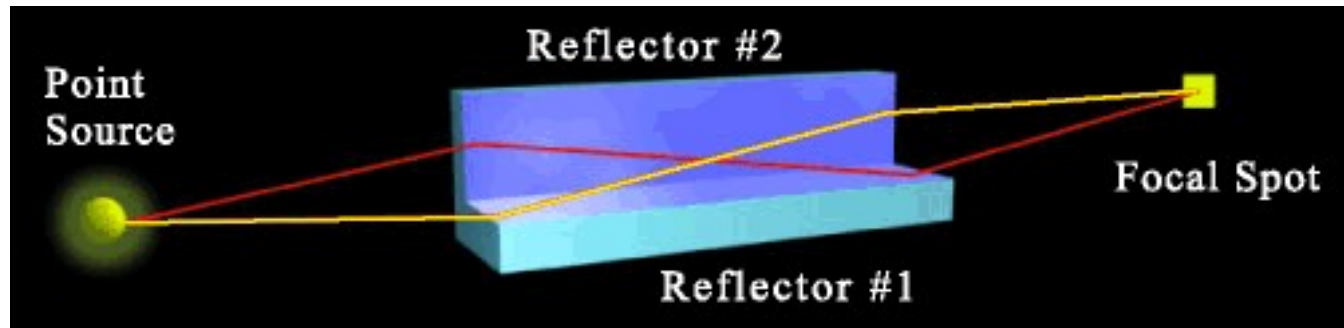
- Tungsten (W)
- 70 μ m focal spot



Anode

- 99mm diameter
- Cu-target
- 9000 RPM
- water-cooled

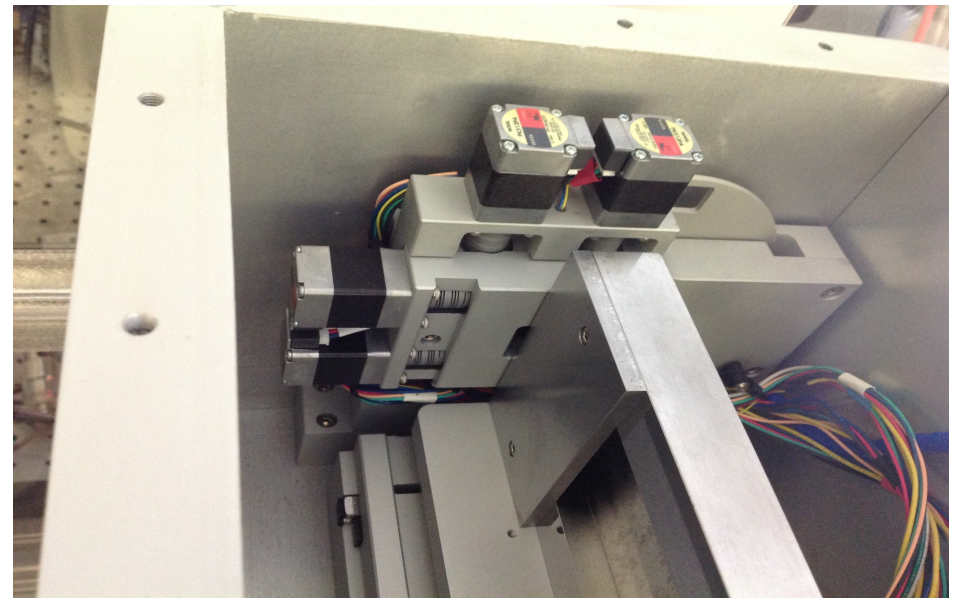
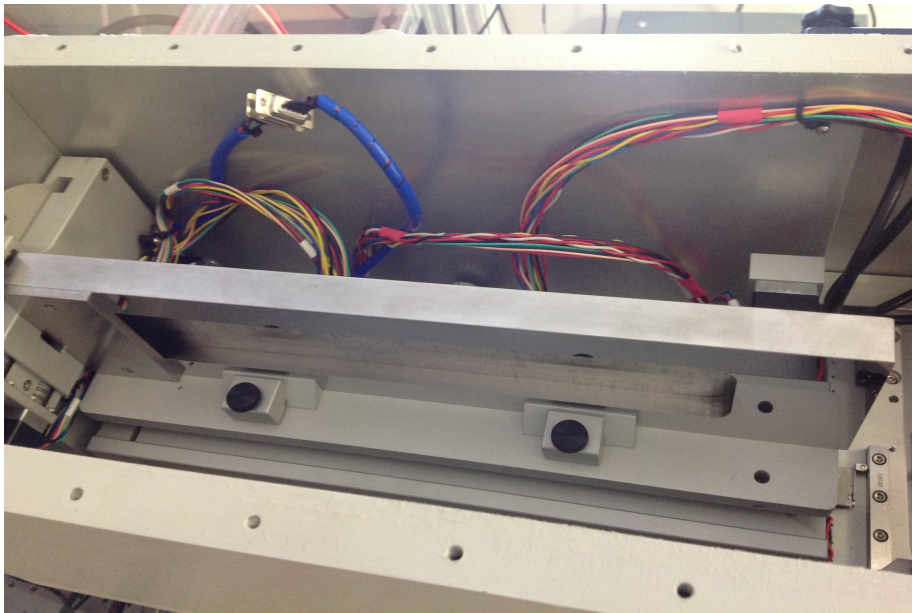
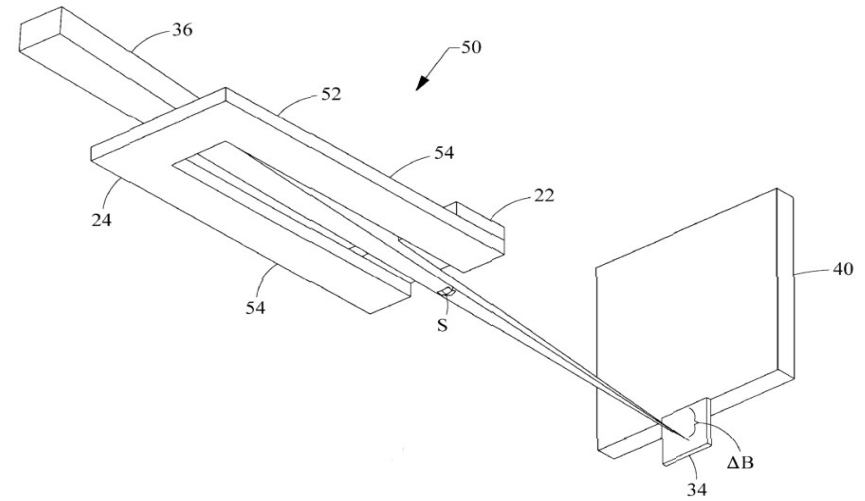
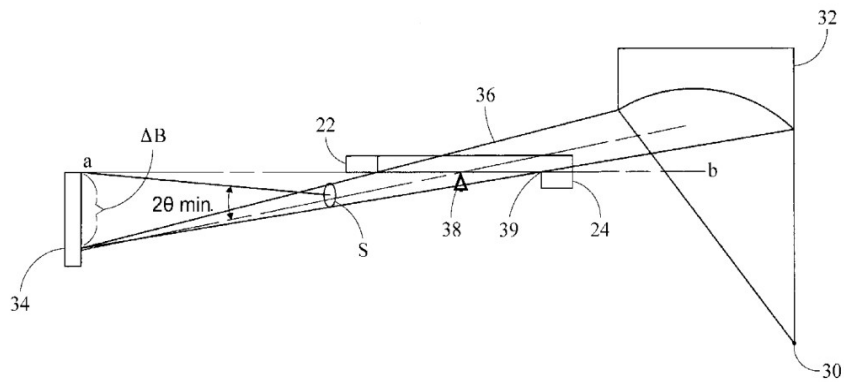
Confocal Max-Flux Optics



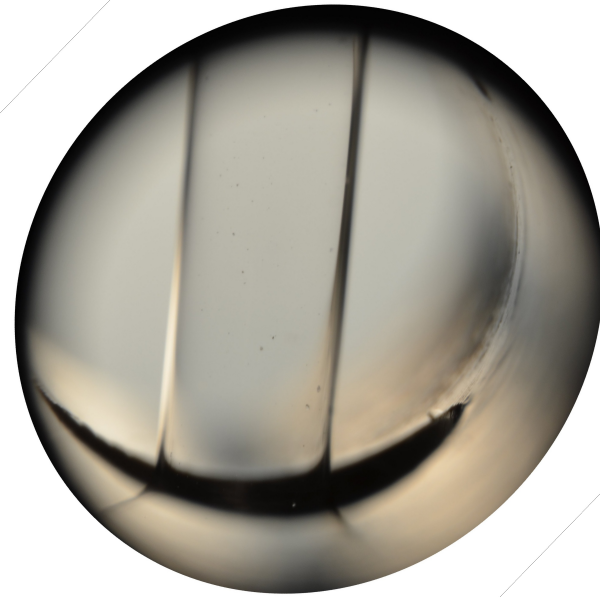
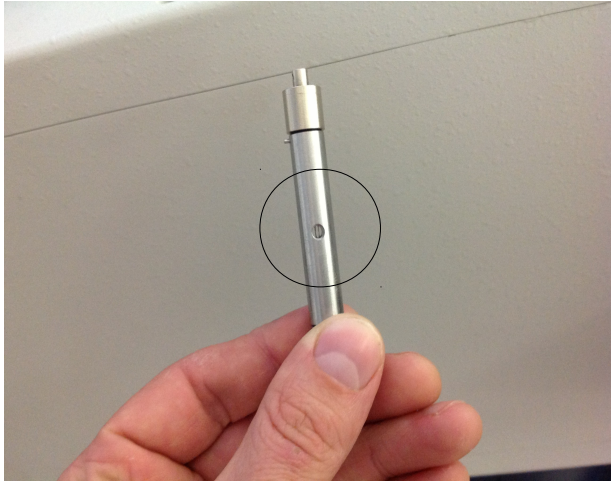
- 2 perpendicular multilayers
- flux maximalization
- spectral purity
- spatial definition

Kratky block

- pair of colimating blocks
- blocks are parallel and rotatable
- produce symmetric beam
- detector in fixed position



Sample Capillary

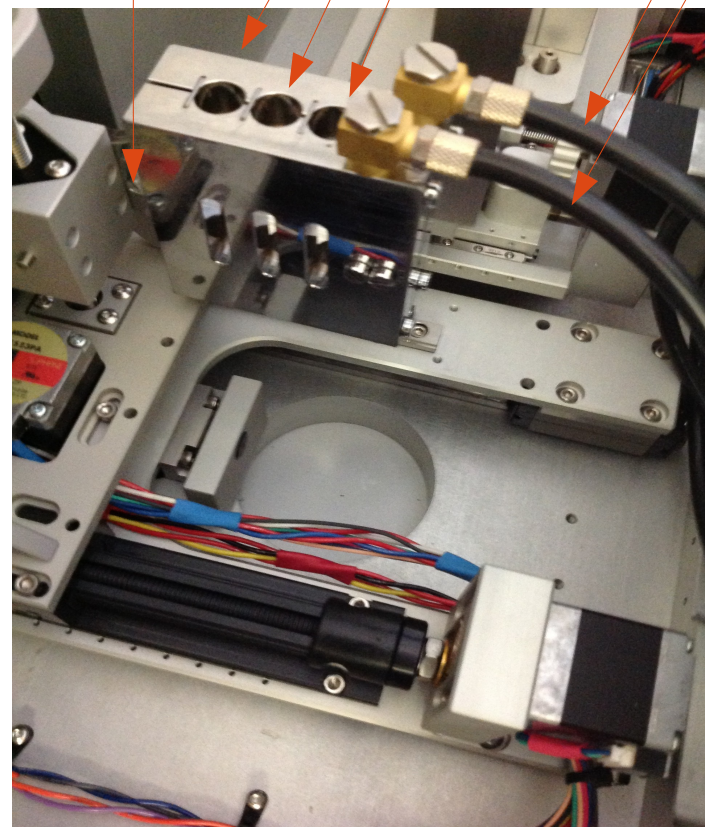


- Sample volume 15 μ L (30-40 μ L)
- Glass capillary in steel holder
- Manual handling

Motorized Sample Holder

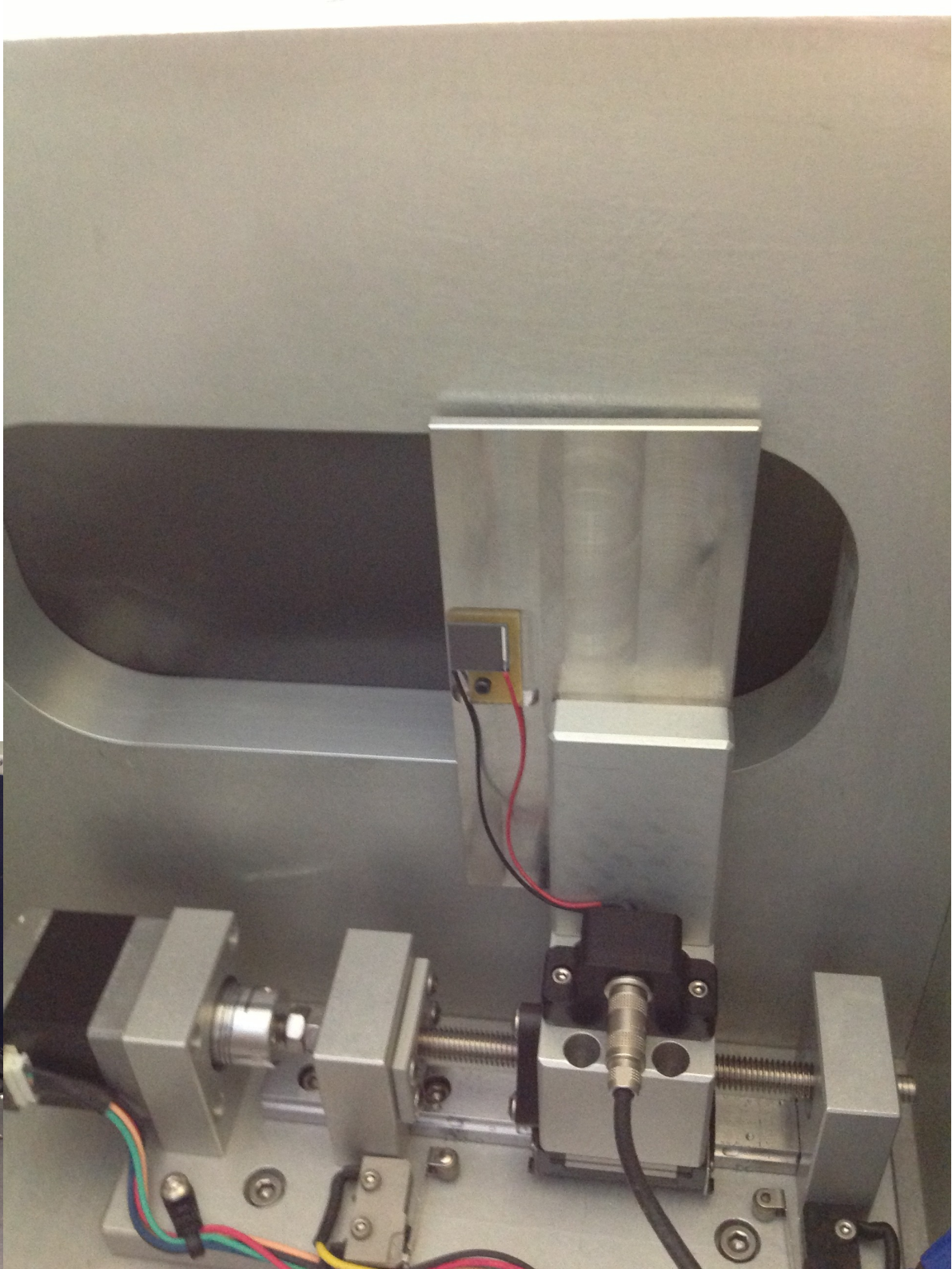
1 powder standard 3 sample positions

-30 / +60°C
temperature control



Beamstop

- Photodiode beamstop
- Intensity measurements for:
 - Sample absorption correction
 - Sample positioning
 - Kratky block alignment



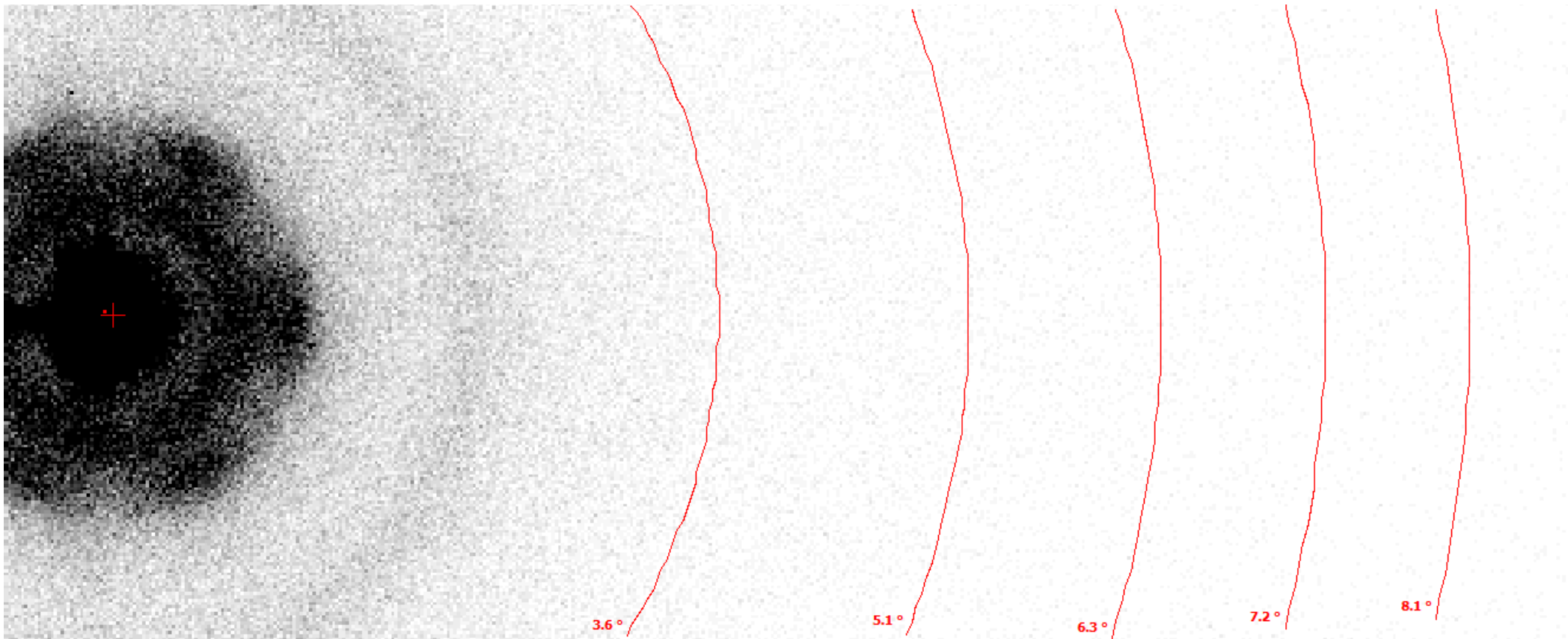
Pilatus 100K



- CMOS hybrid-pixel technology
- Single-photon-counting mode
- Pixel size $172 \times 172 \text{ } [\mu\text{m}^2]$
- Counting rate per pixel $2 \times 10^6 \text{ [photons/s]}$
- Area $83.8 \times 33.5 \text{ [mm}^2]$



Direct beam



Direct beam image from BioSAXS-1000, aquisition time 3s

SAXSLab2.0.1b2

SAXSLab 2.0.1b2

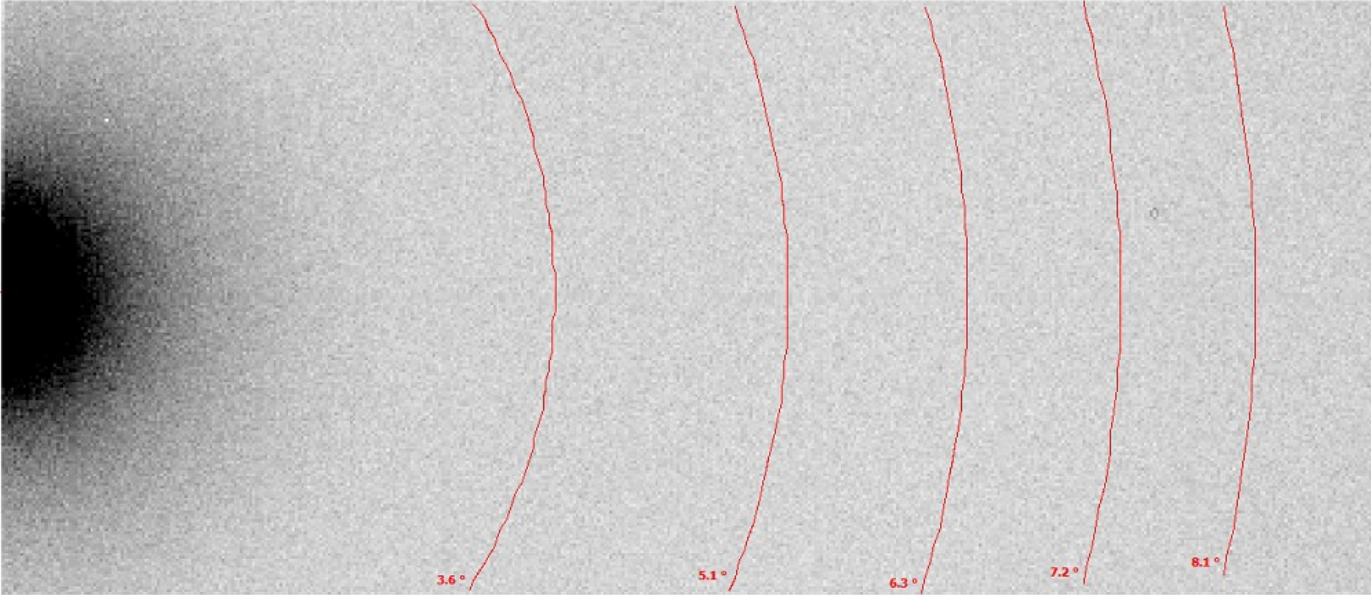
File Edit View Instrument Process Help

Setup Collect Calibrate Processing ImageViewer: C:\Data\KUBICEK\20130712\c3_RTT6E2_3p0_0003.img

Beamstop: 7.90
Beam Intensity: 7.79351 nA
Sample: X: 12.25 mm
Y: 2.70 mm
Temperature: 17.0 °C
Kratky block: X: 30.68 mm
Z: 1.234 °
Slt: ?
Attenuator: 0
Shutter: Open

1109 of 2400

Current Image: c1_buffer_0001.img
Image Finish: Mon Sep 09 03:40 PM
Scan Finish: Mon Sep 09 03:40 PM
Schedule Finish: Mon Sep 09 05:00 PM



Sample (X: 12.25 mm, Y: 2.70 mm)

X: 12.96
Y: 2.70

Move Sample
Move to Load/Unload Position

Existing file will be overwritten.

Directory: C:\Data\ALIGN
Filename: direct_0001 .img
Exposure Time: 10.00 sec min h
Collect Image

Sample Temperature: 17.0 °C

Change Temperature
Turn off controller

Shutter (Open)

Timed 0.0 sec
Close Shutter

1109 of 2400

Beam Intensity

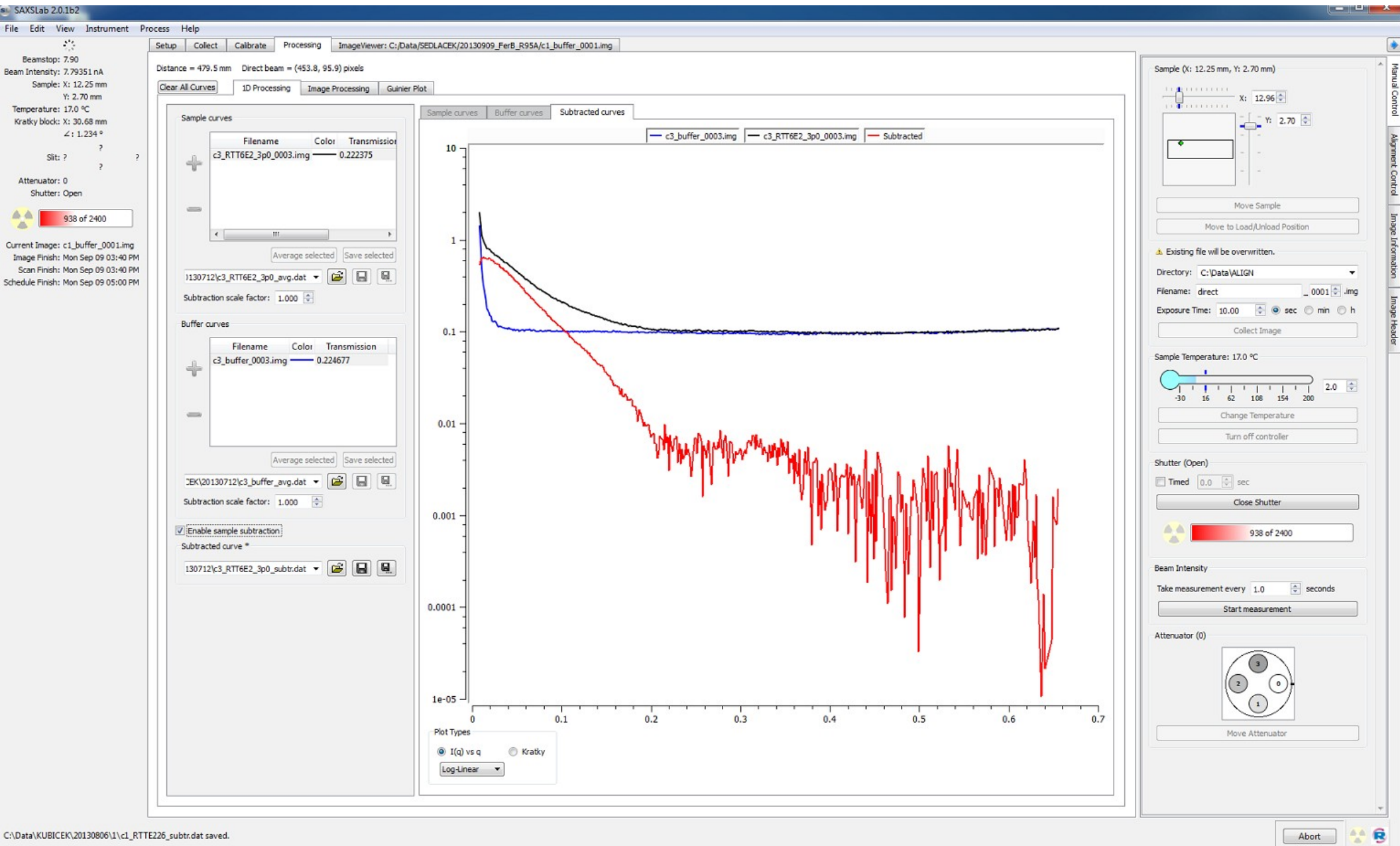
Take measurement every 1.0 seconds
Start measurement

Attenuator (0)

Move Attenuator

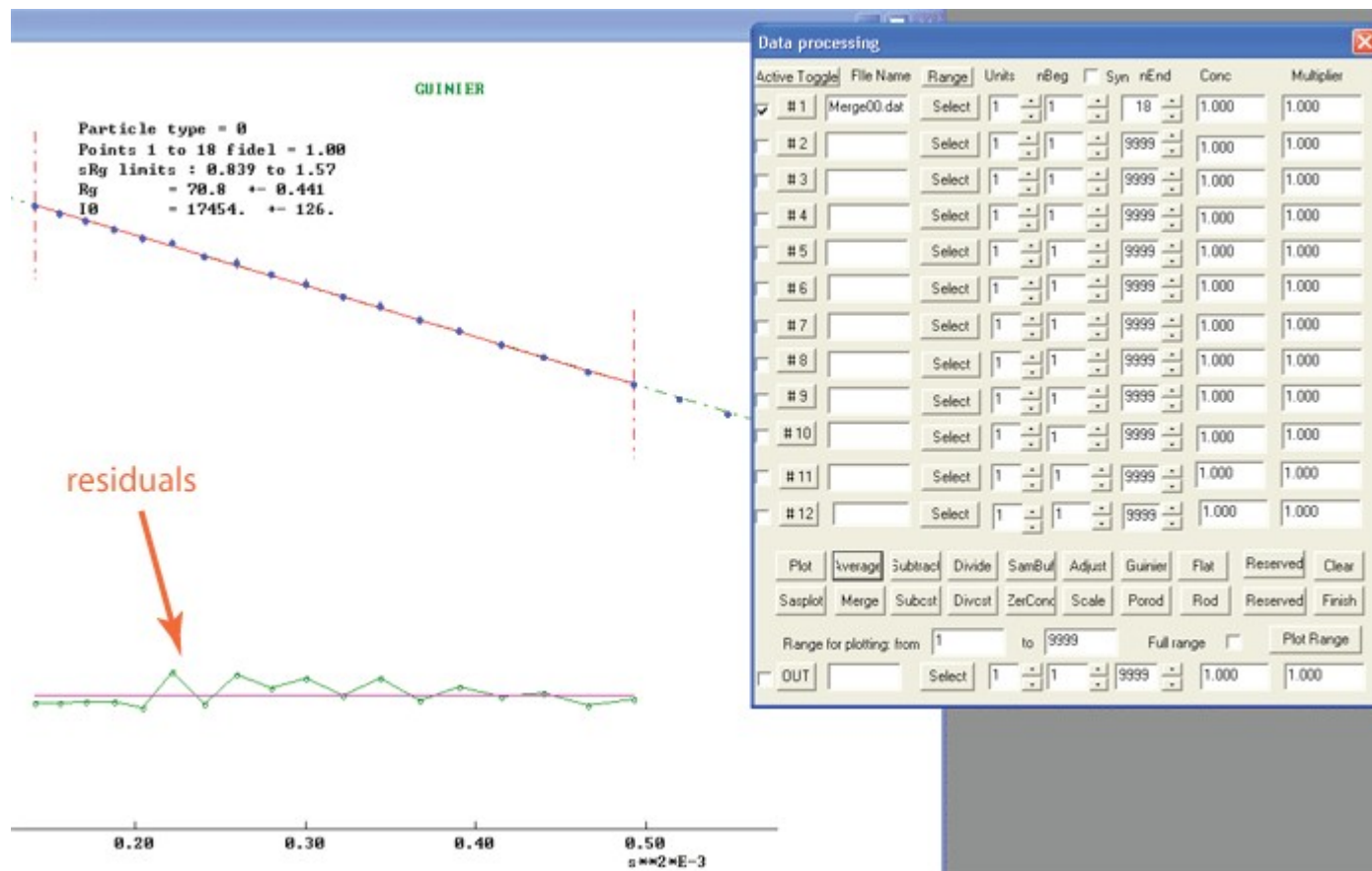
Abort

Odečtení příspěvku solventu



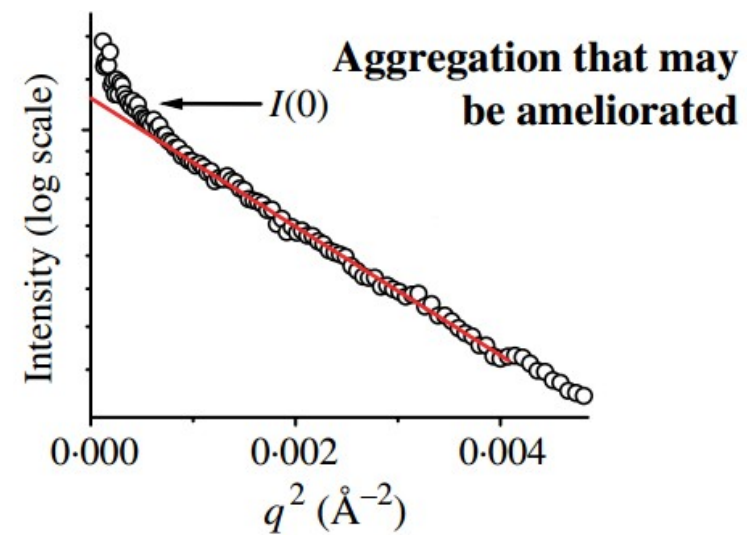
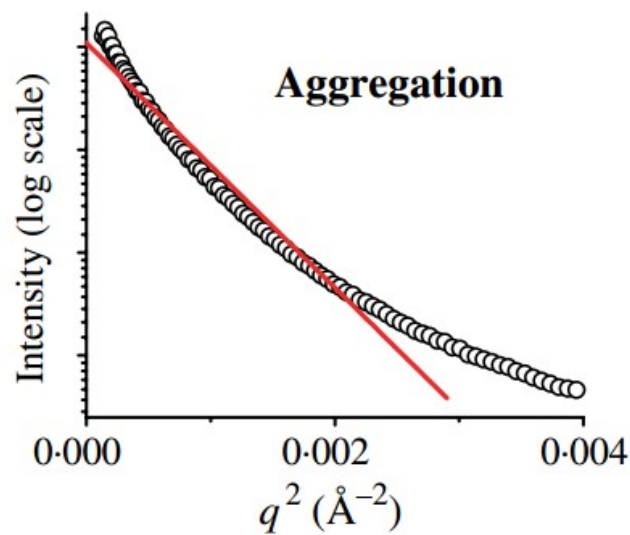
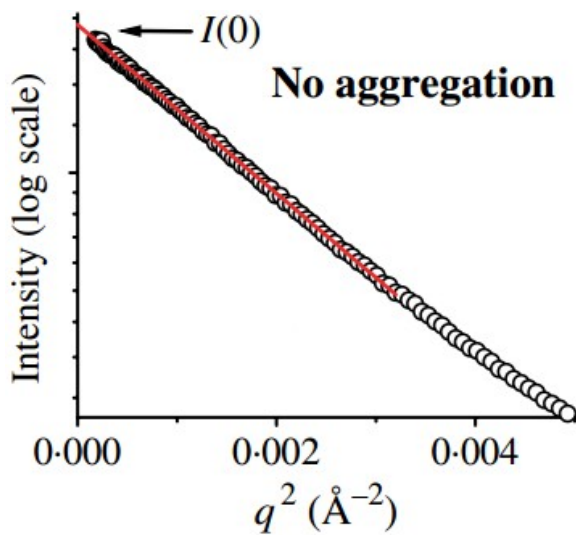
SAXS experiment-Guinierova analýza

Program PRIMUS aproximace $I(0)$, R_g , agregace



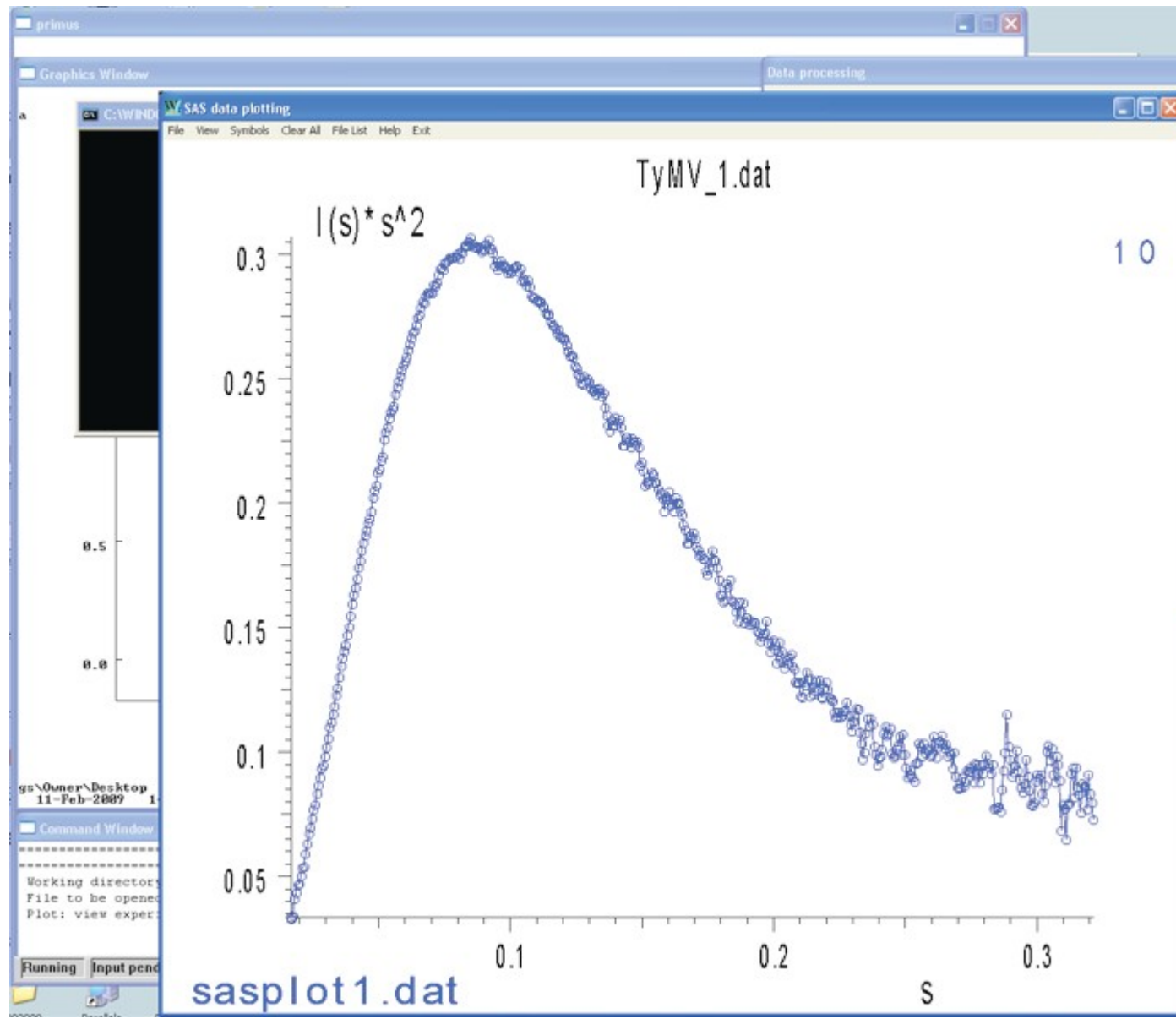
SAXS experiment-Guinierova analýza

aproximace $I(0)$, R_g , agregace



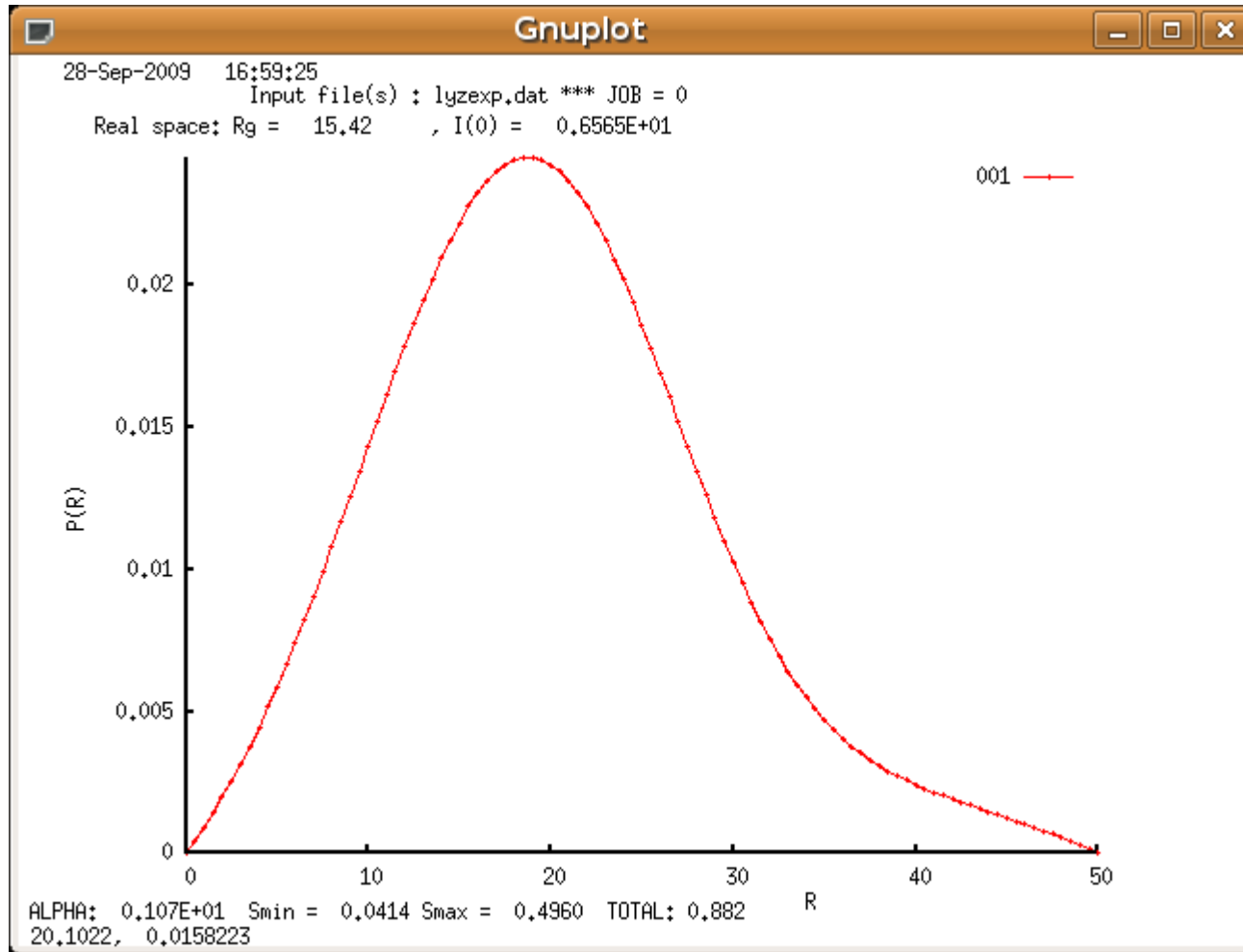
SAXS experiment-Kratkého analýza

Program PRIMUS - Folding, Porodův objem



SAXS experiment - P(r)

Program GNOM - Tvar, d_{\max}

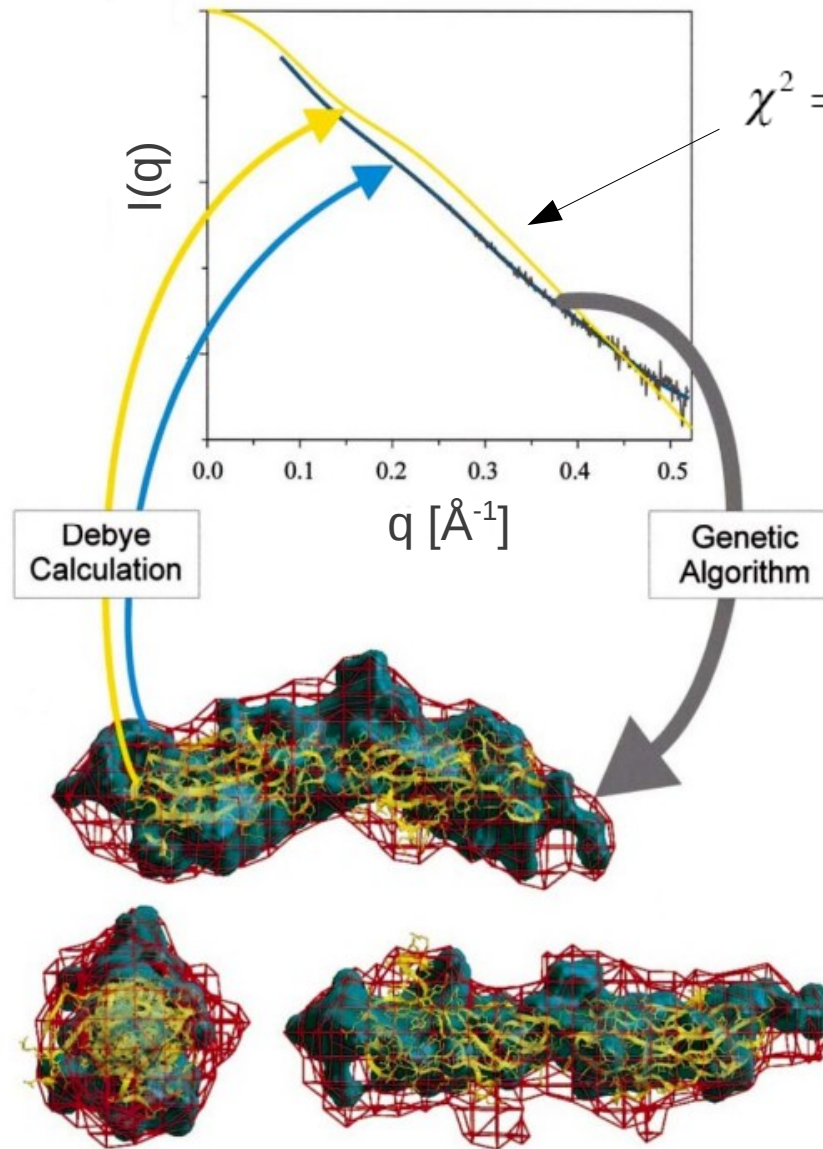


SAXS modelování tvaru *ab initio*

test shody:

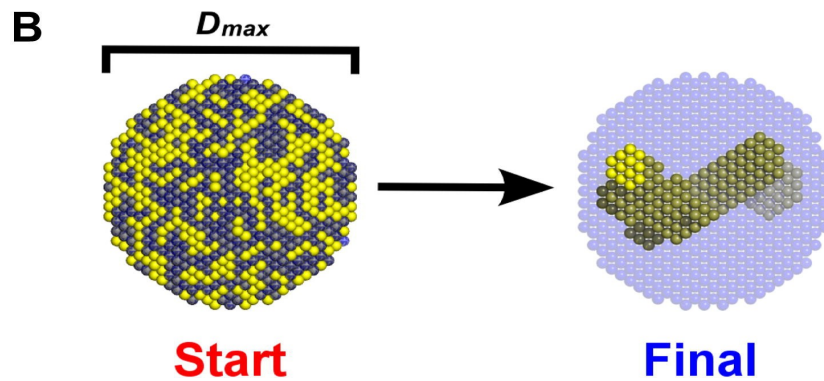
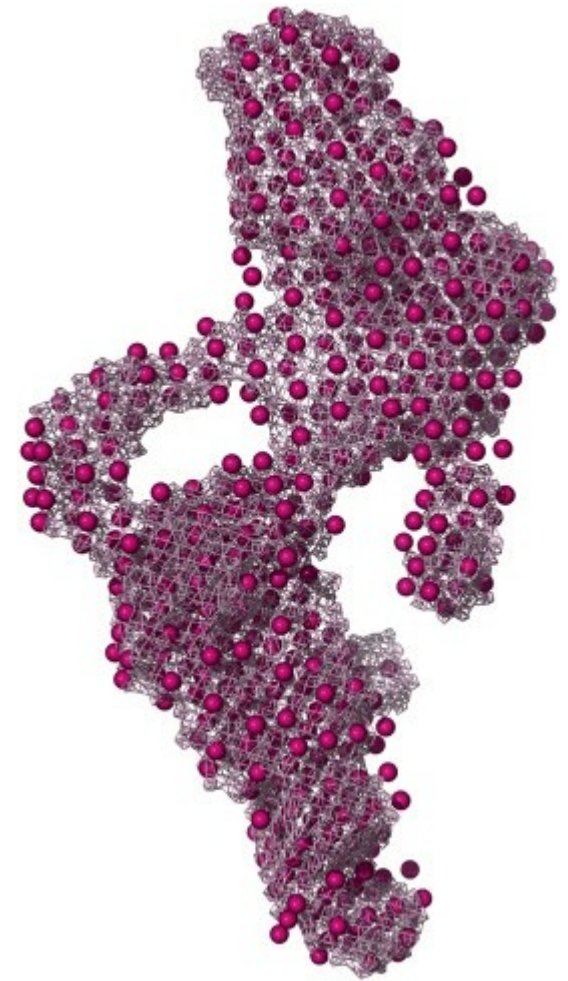
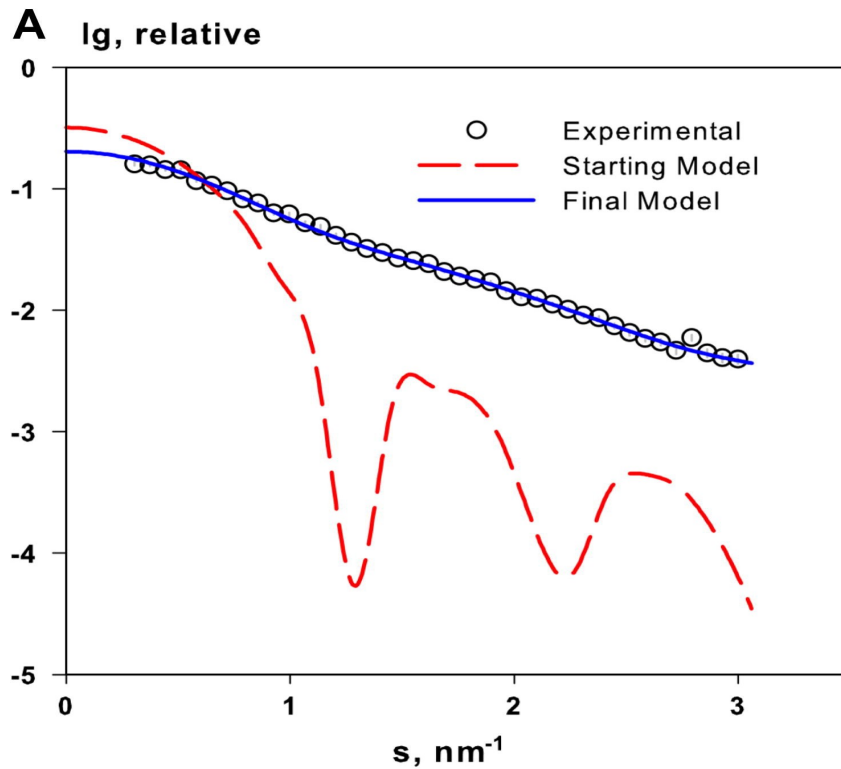
$$\chi^2 = \sum_{k=1}^Q \left(\frac{I_{\text{exp}}(q_k) - c \cdot I_{\text{m}}(q_k)}{\sigma_{\text{exp}}(q_k)} \right)^2$$

$$I_{\text{m}}(q) = \sum_{j=1}^{N_A} \sum_{i=1}^{N_A} f_i(q) f_j(q) \frac{\sin(qd_{ij})}{qd_{ij}}$$



SAXS modelování tvaru

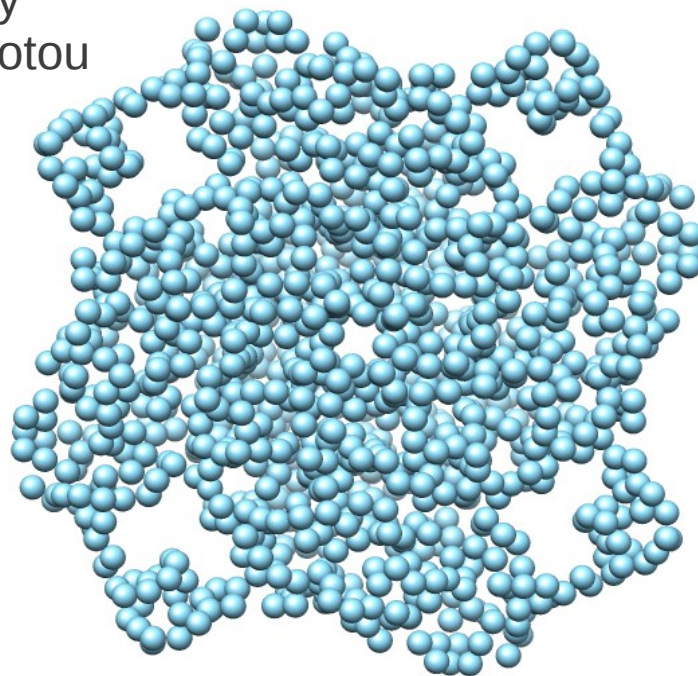
Program DAMMIN



SAXS modelování tvaru

Program GASBOR

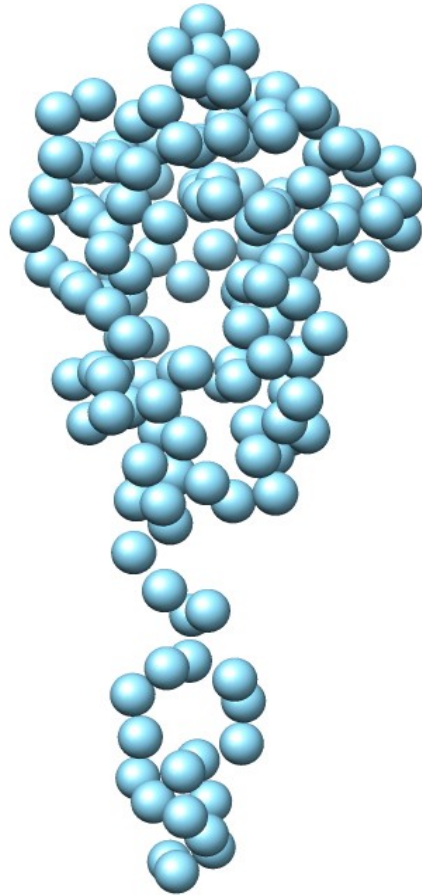
Chain-like GASBOR model složen
z univerzálních residuí (Dummy
residues) s průměrnou el. hustotou
a rozestupy ~ 0.38 nm



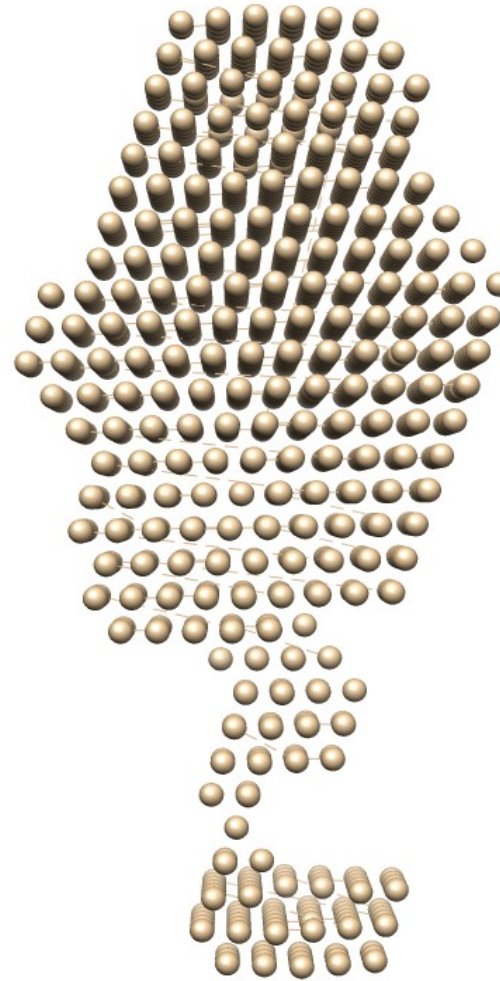
Pozice středu kuliček odpovídá C^α
Počet kuliček odpovídá počtu aminokyselin
v proteinu

SAXS modelování tvaru

Srovnání GASBOR vs. DAMMIN

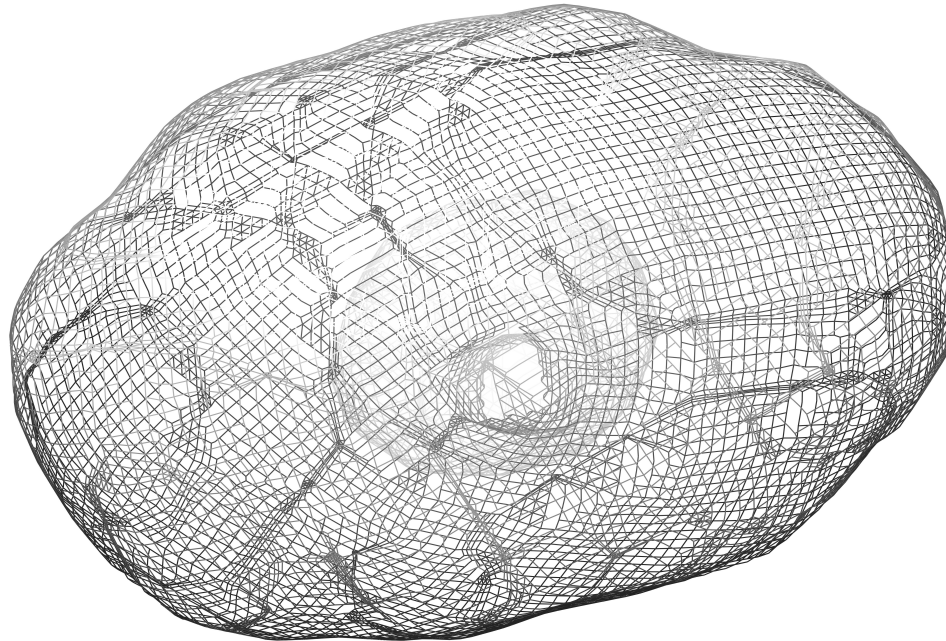


Program GASBOR



Program DAMMIN

SAXS experiment - envelope



10Å

Rigid-body modeling

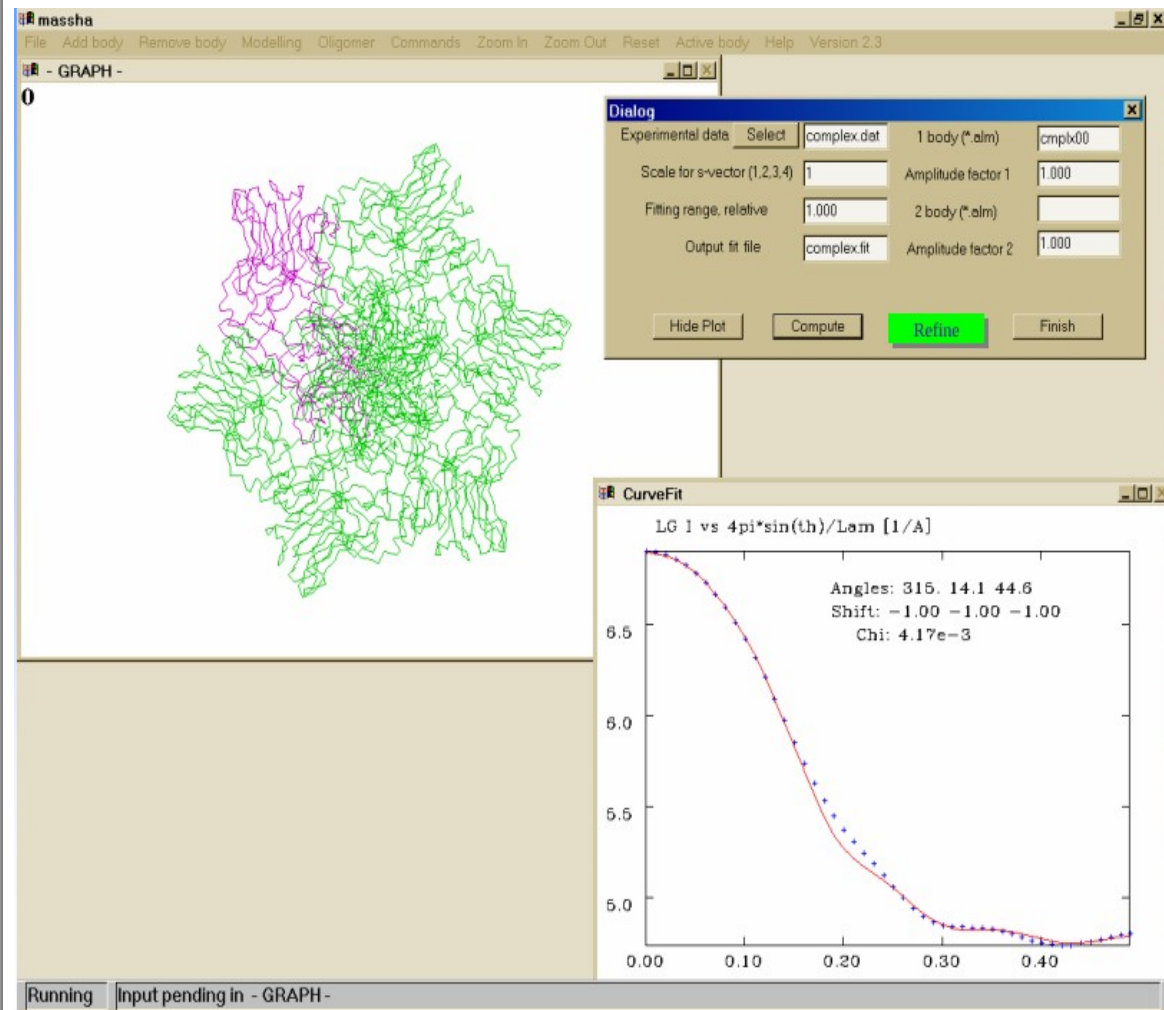
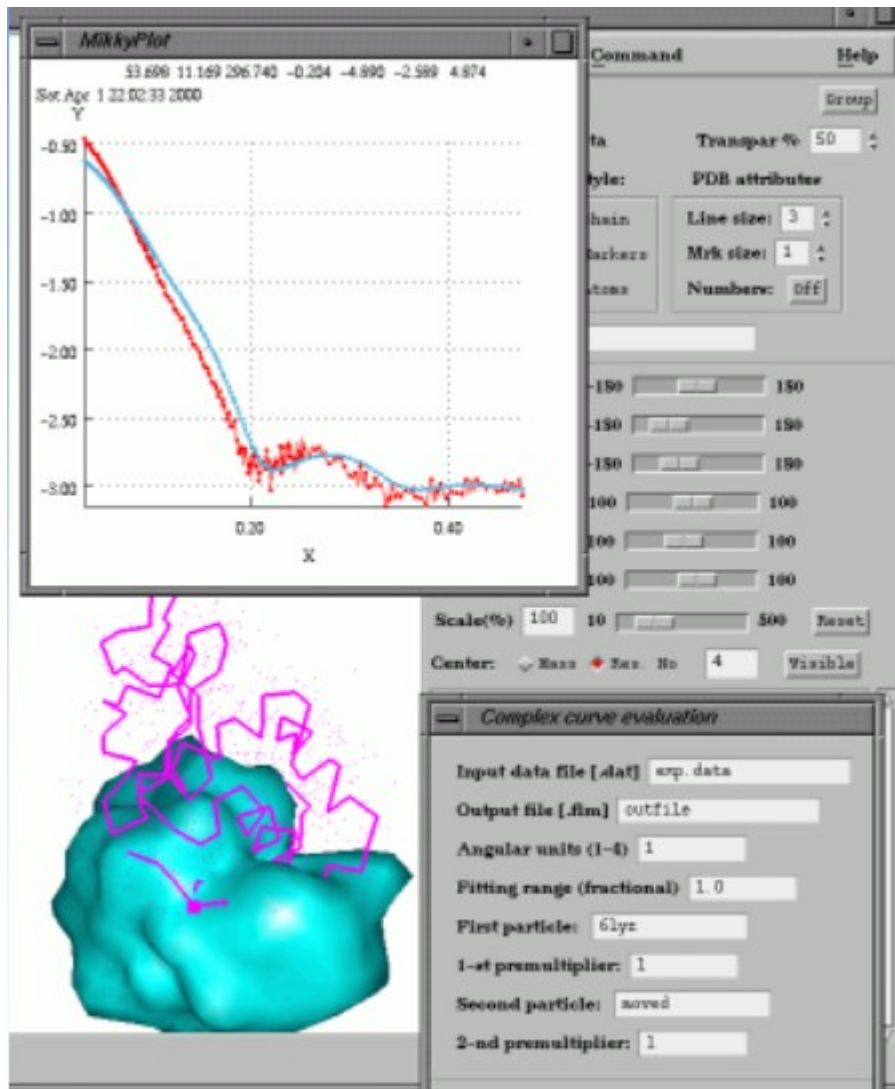
- Jsou známy X-ray struktury podjednotek
- Není známa kvartérní struktura

- Automatické hledání
- Interaktivní hledání
- Doplnování chybějících komponent (ab initio)

Rigid-body modeling

ASSA: manual refinement

MASSHA: automatic refinement



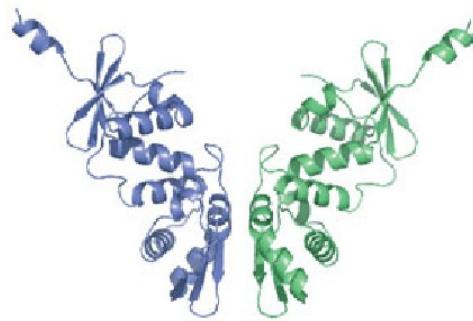
SAXS experiment - CRY SOL

teoretický rozptyl vs. experimentální data

oligomerní stav v roztoku při znalosti krystalové struktury



Dimer 1, ($B/2 = 755 \text{ \AA}^2$)
 $R_G = 26.08 \text{ \AA}$ $D_{\max} = 80 \text{ \AA}$



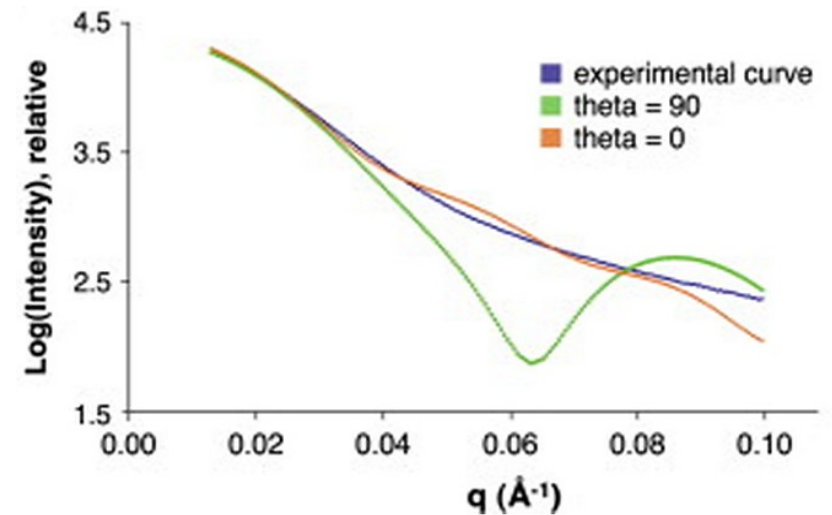
Dimer 3, ($B/2 = 406 \text{ \AA}^2$)
 $R_G = 28.3 \text{ \AA}$ $D_{\max} = 90 \text{ \AA}$



Dimer 2, ($B/2 = 923 \text{ \AA}^2$)
 $R_G = 34.04 \text{ \AA}$ $D_{\max} = 120 \text{ \AA}$



Dimer 4, ($B/2 = 255 \text{ \AA}^2$)
 $R_G = 30.4 \text{ \AA}$ $D_{\max} = 100 \text{ \AA}$

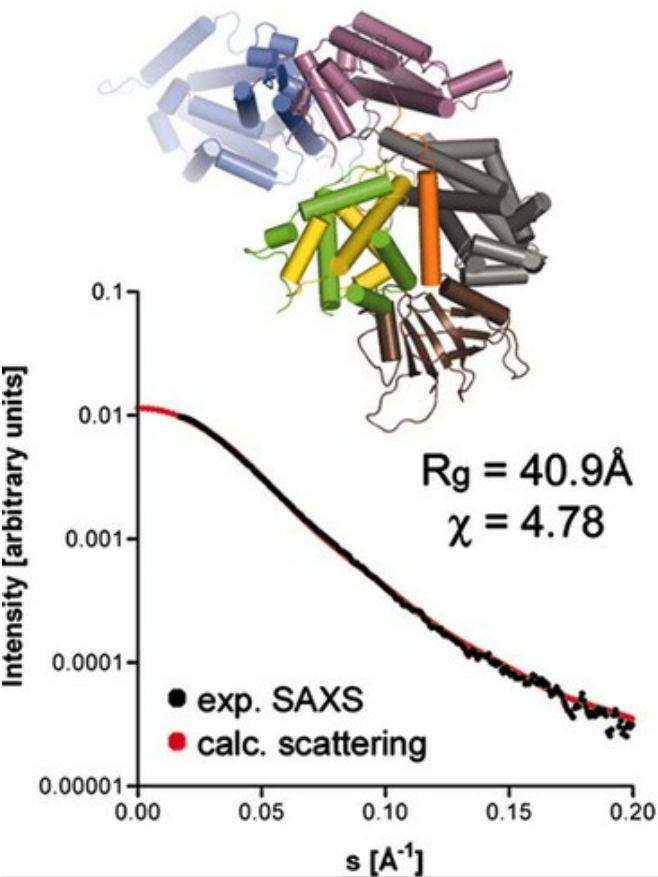


SAXS experiment - CRYSOLE

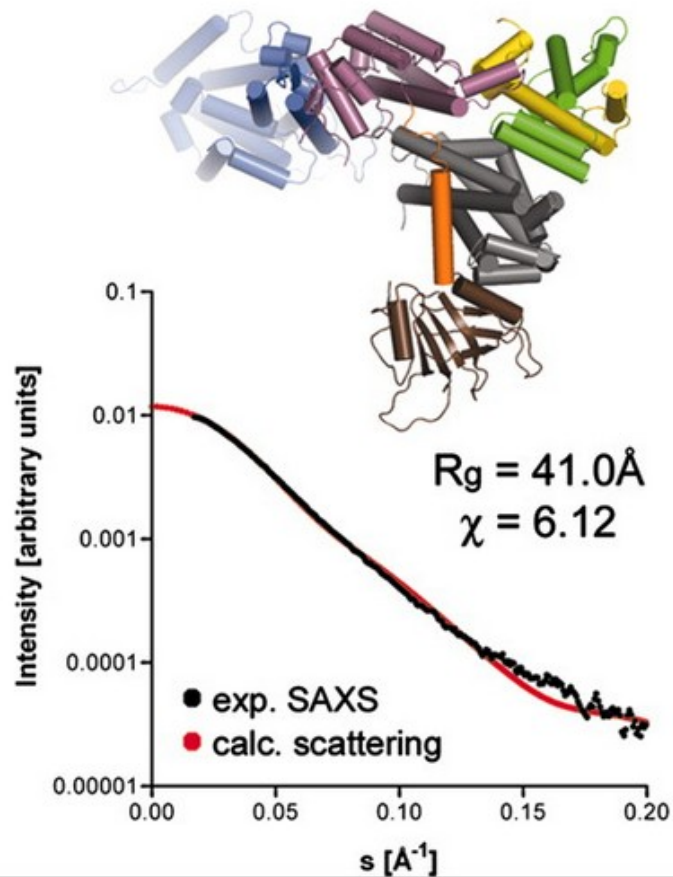
teoretický rozptyl vs. experimentální data

vyhodnocení výsledků dockingu

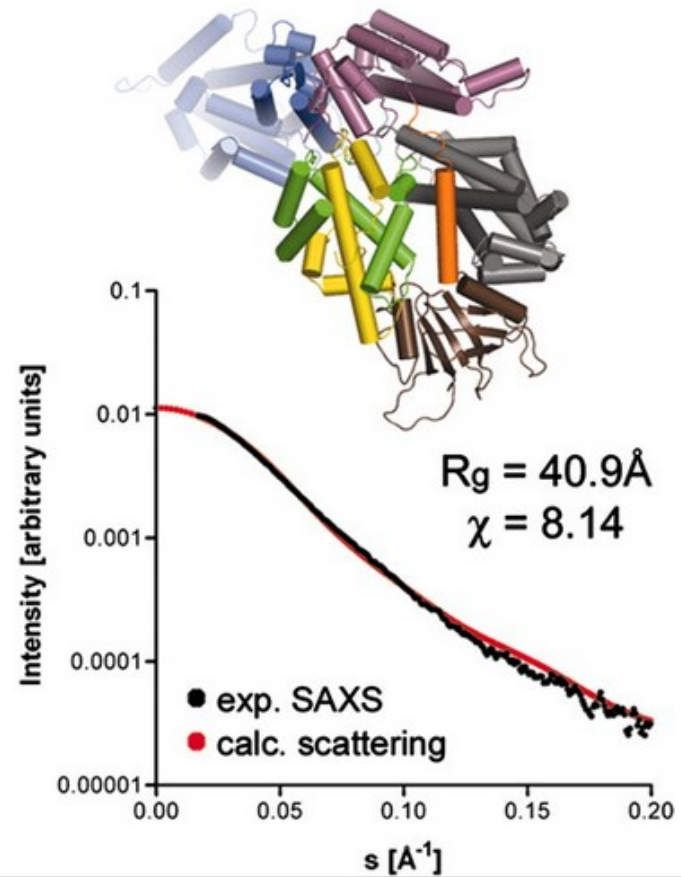
Model 1: SOS^{Histone-DH-PH-cat}



Model 3: SOS^{Histone-DH-PH-cat}



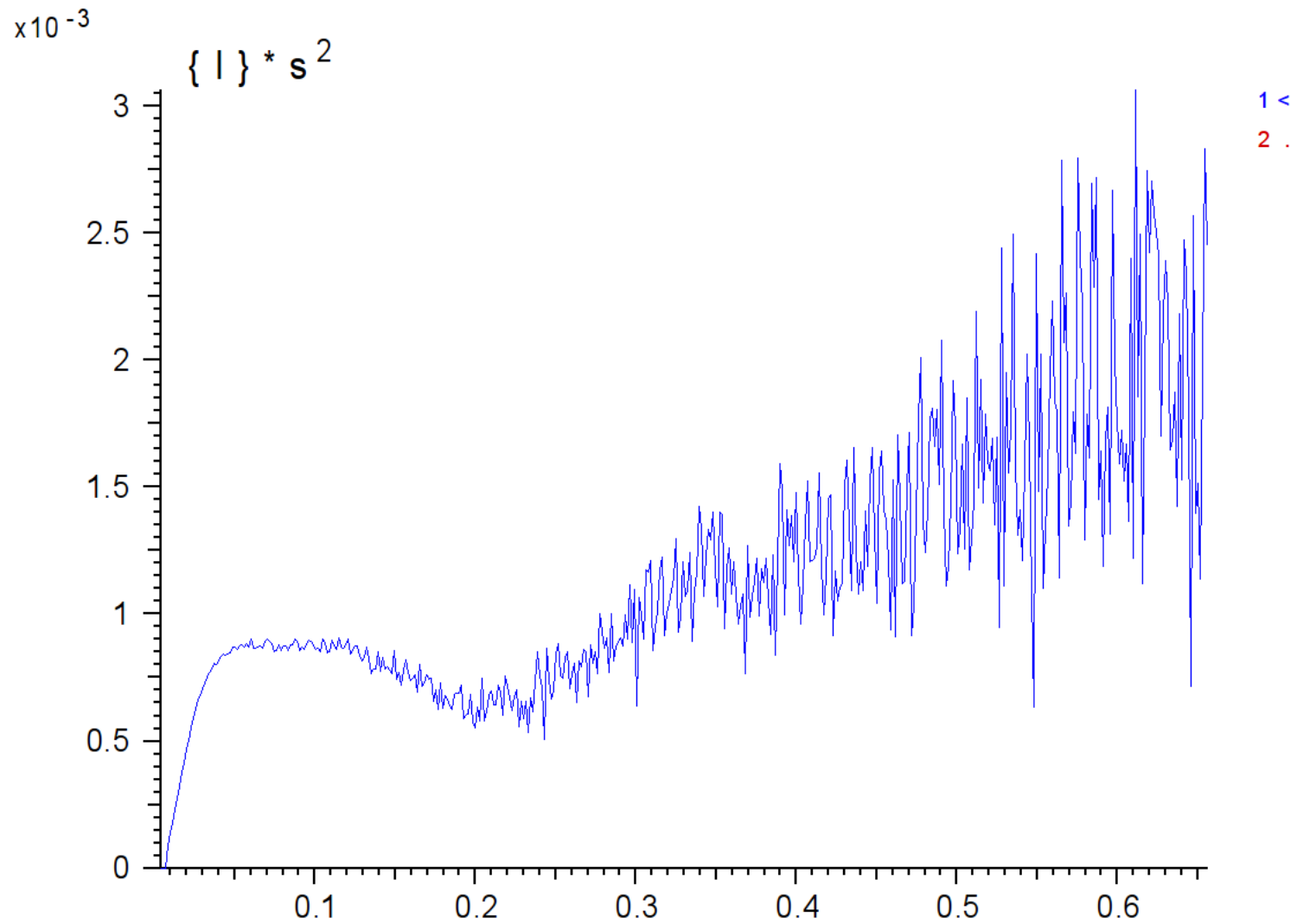
Model 9: SOS^{Histone-DH-PH-cat}



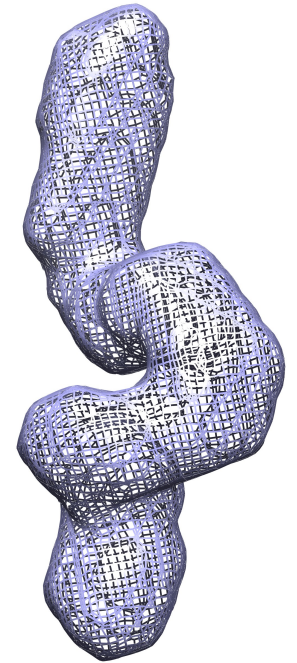
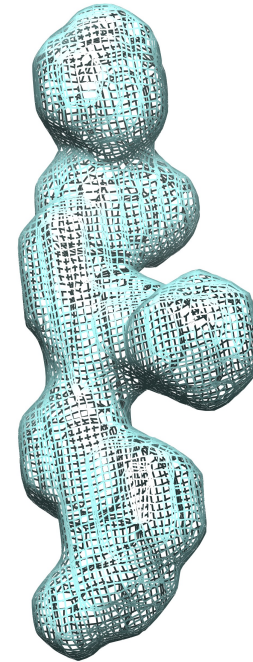
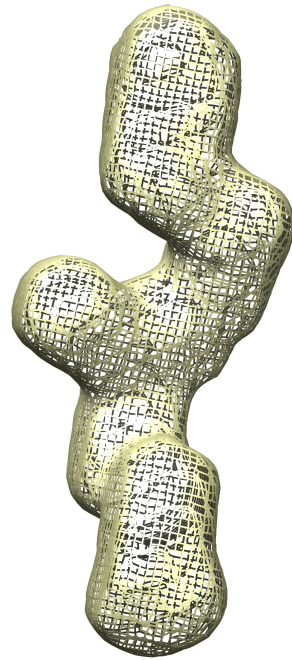
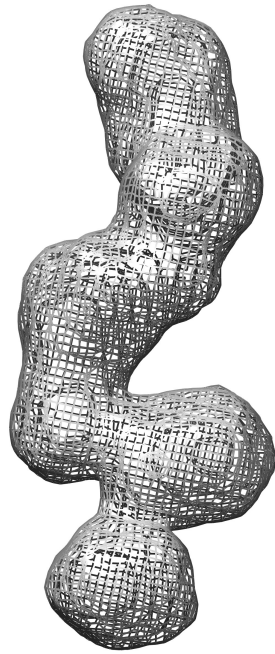
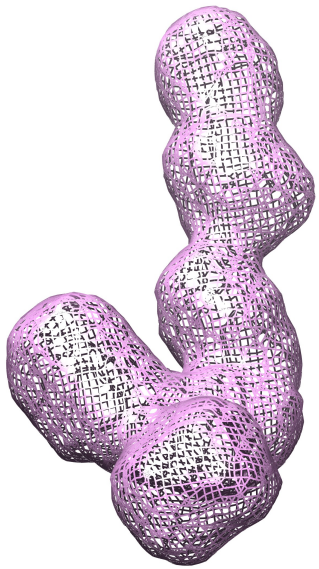
Molekulární flexibilita

- vibrace [fs]
- rotamery bočních řetězců [ps]
- ohýbání β -řetězců a α -helixů [zlomky ns]
- rotace molekul v roztoku [ns]
- pohyby domén [ns]

Flexibilní systémy



Flexibilní systémy

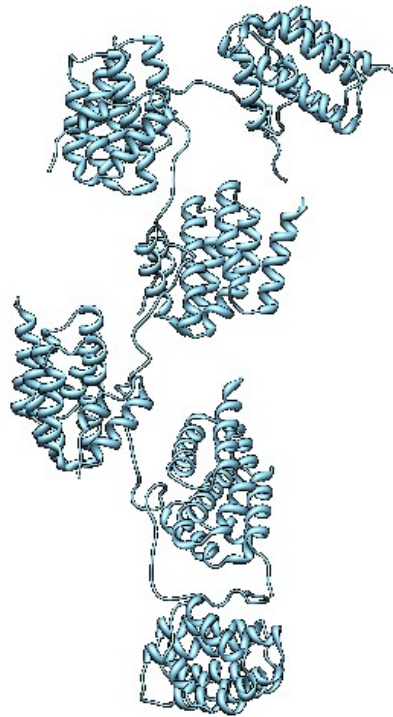


Flexibilní systémy

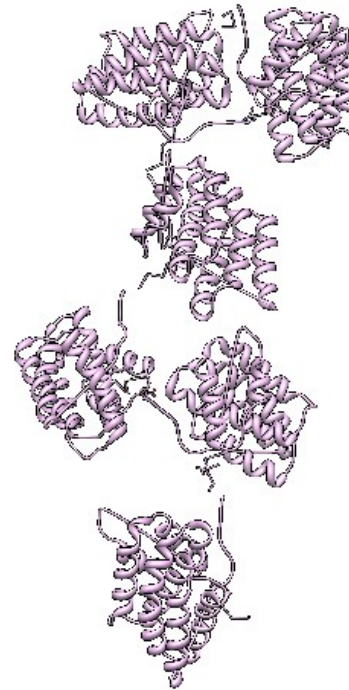
EOM - kombinace rigid body + *ab initio* modelingu



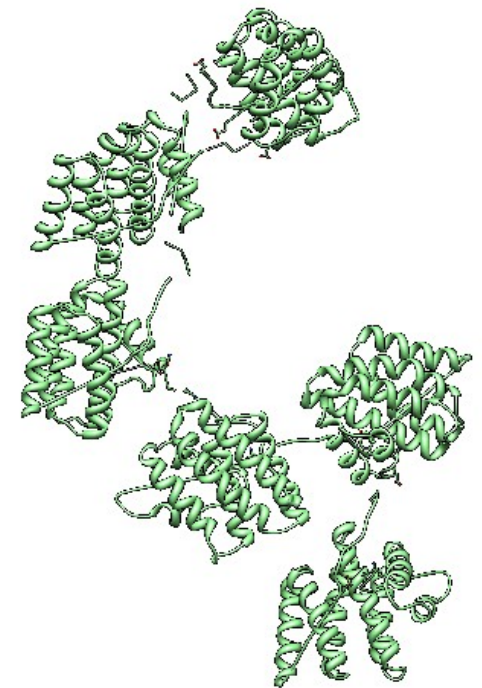
18%



11%



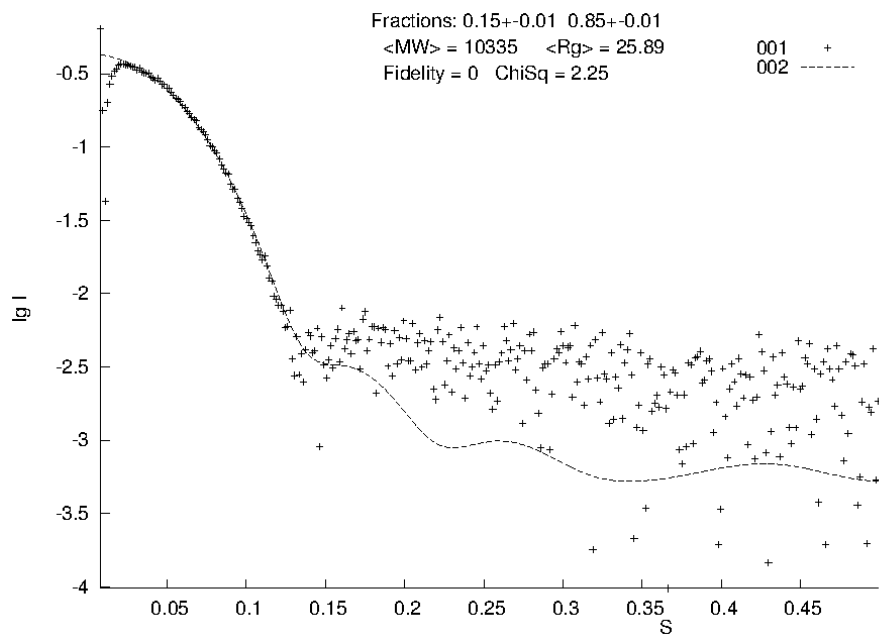
10%



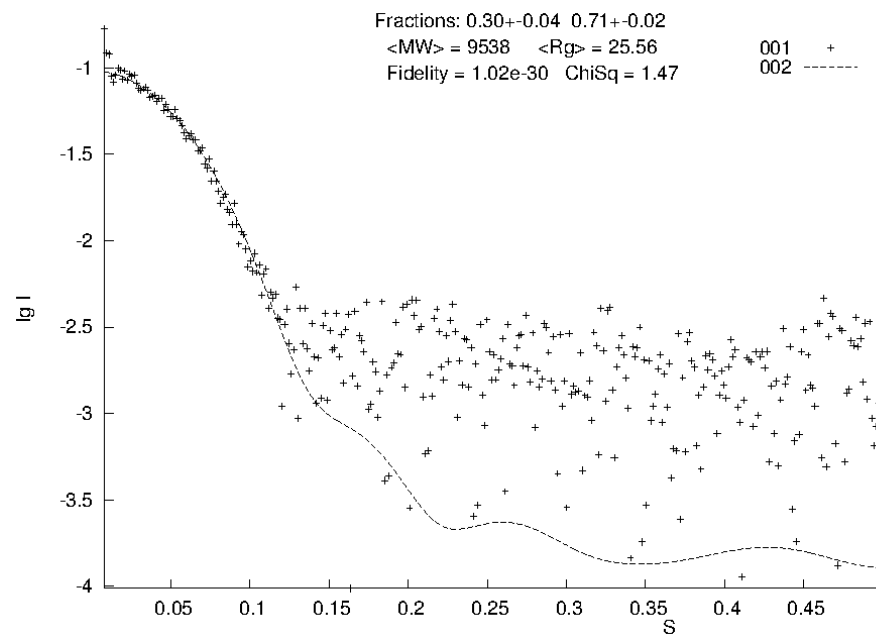
5%

Oligomerní stav v roztoku

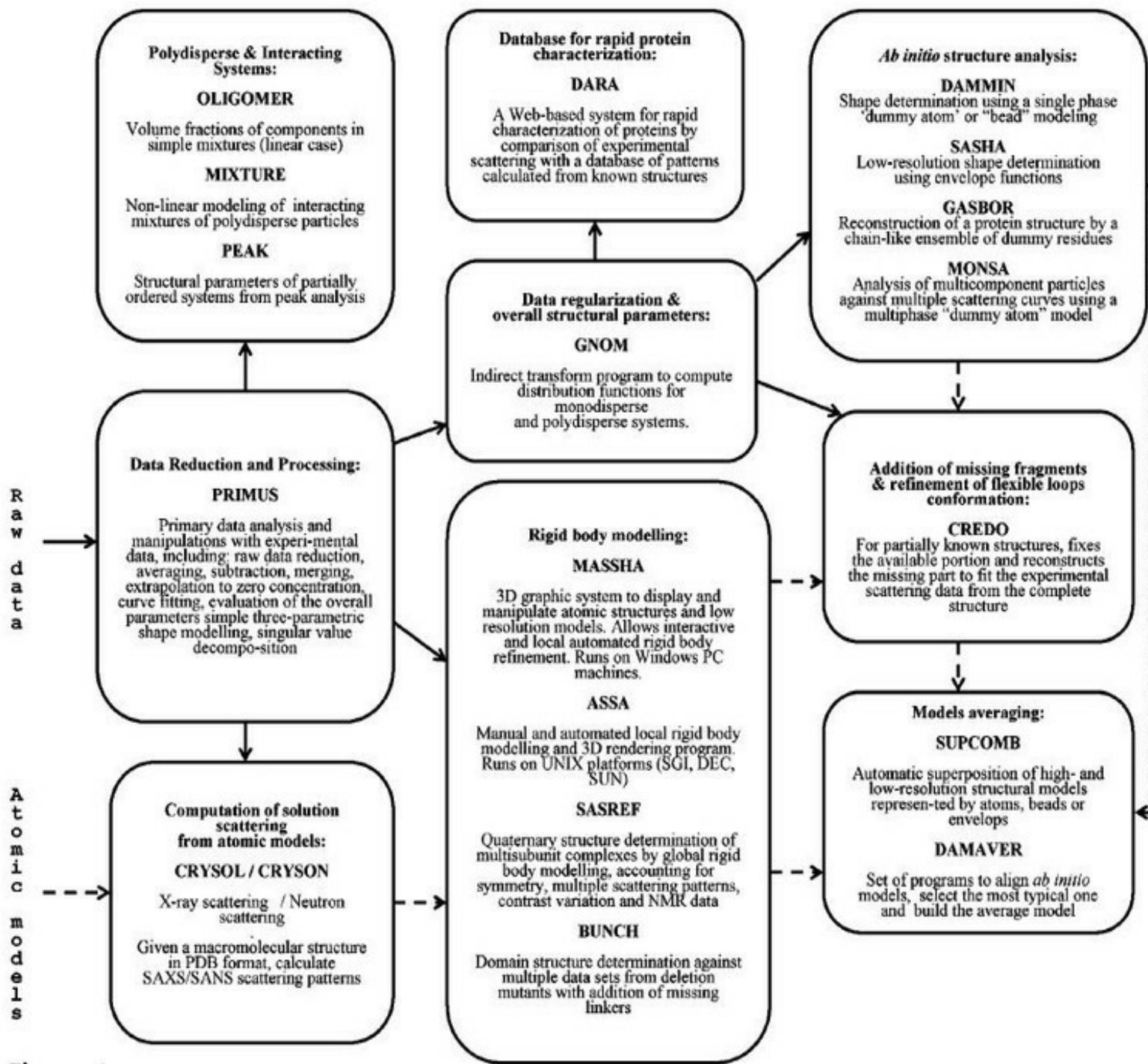
OLIGOMER - lineární kombinace teor. profilů



c=2.6mg/ml



c=0.9mg/ml



Teoretické informace pro uživatele

- obecné strukturní parametry
- homogenní vs. agregovaný vzorek
- složený vs. nesložený protein/DNA
- oligomerní stav v roztoku
- kvartérní struktura a interakce
- SAXS struktura s nízkým rozlišením

Praktické informace pro uživatele

- Sample volume: **15 μ L** (30 μ L); **pure and monodisperse** :)
- Exposure time: **40min** per sample ($q=0.008$; $c>1\text{mg/ml}$)
- q_{range} : 0.008(0.005) - 0.65 \AA^{-1} ; $d_{\text{range}} = 785/(\mathbf{1231}) - 10$ (20) \AA

- Reservation system:

http://www.sci.muni.cz/~necas/new/index_saxs.html

- Local contact:

klumpler@sci.muni.cz

marek@chemi.muni.cz

Závěr

- Jak se provádí SAXS experiment a co je měřeno?
- Jak veliké částice lze pozorovat SAXS?
- Jaké informace o vzorku lze zjistit nepřímo?