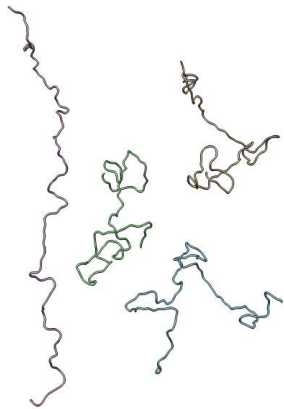
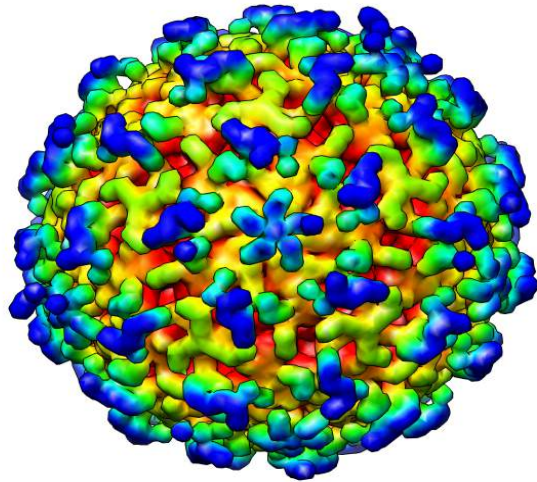


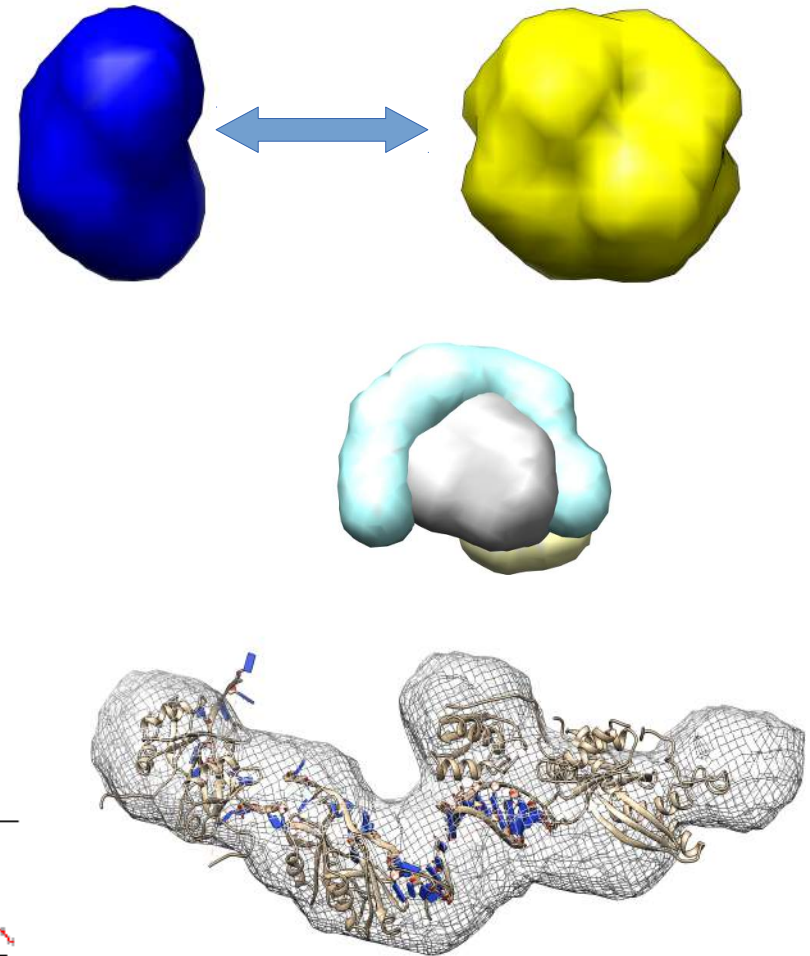
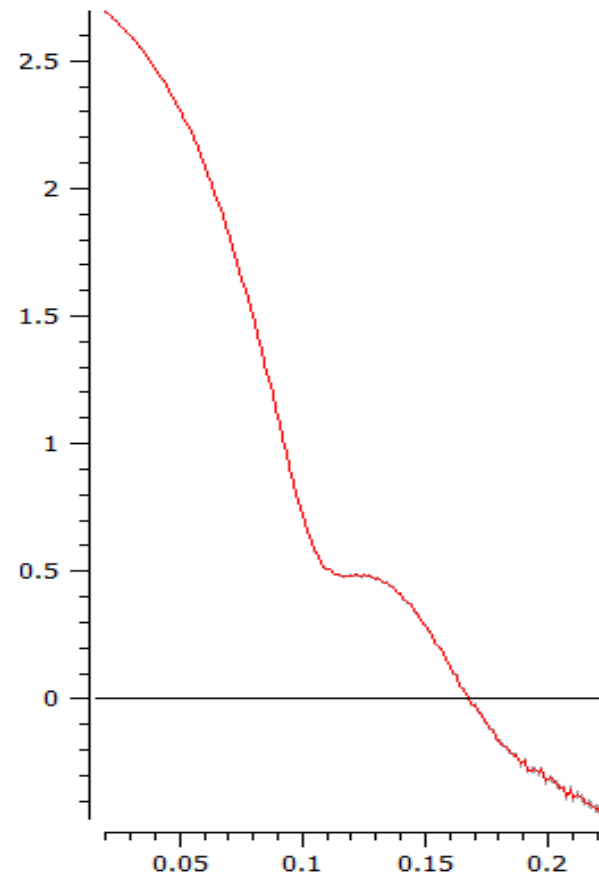
# Biologický Small-Angle X-ray Scattering

v

CF RTG difrakce a bioSAXS,  
CEITEC-MU, Brno



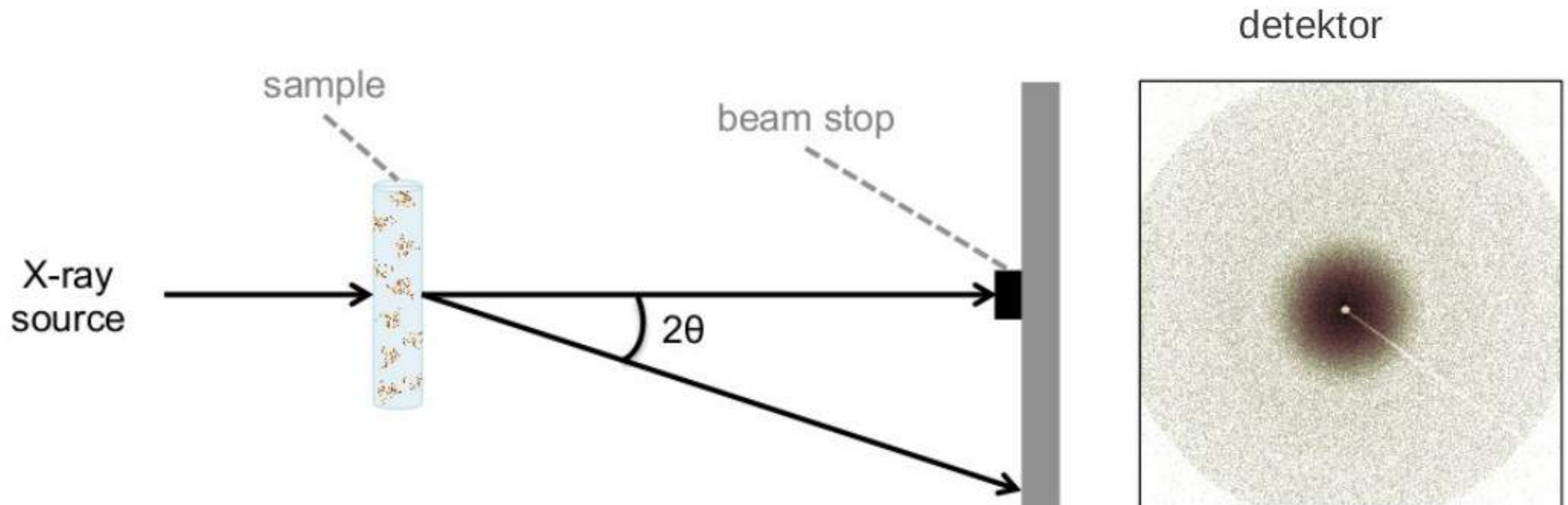
Tomáš Klumpler

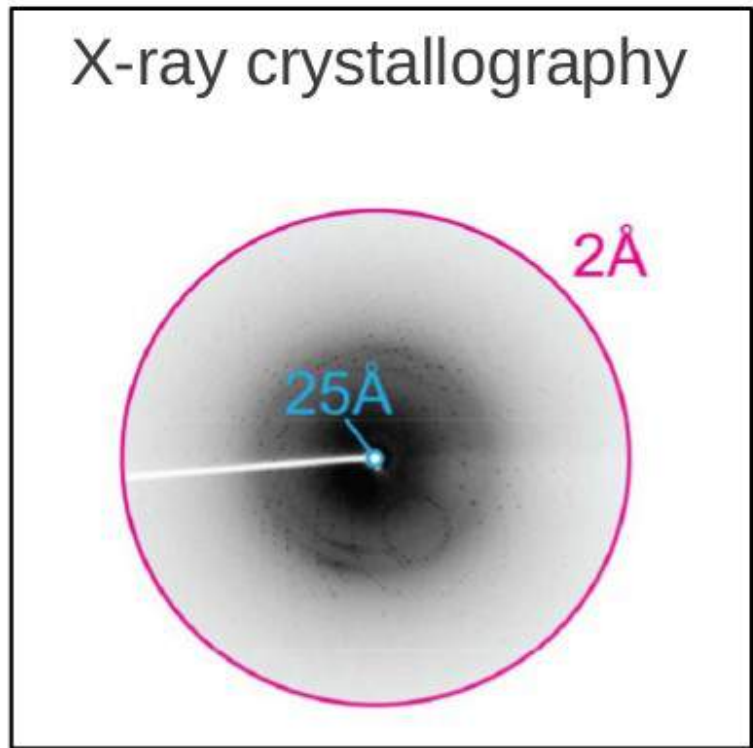
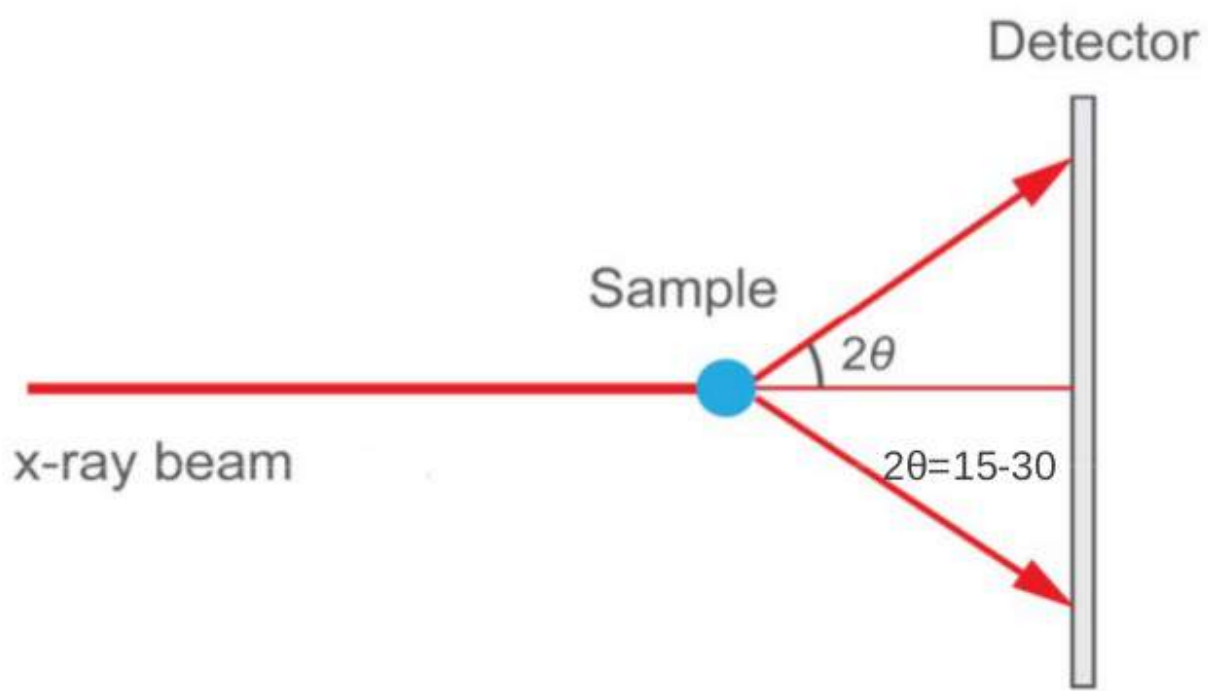


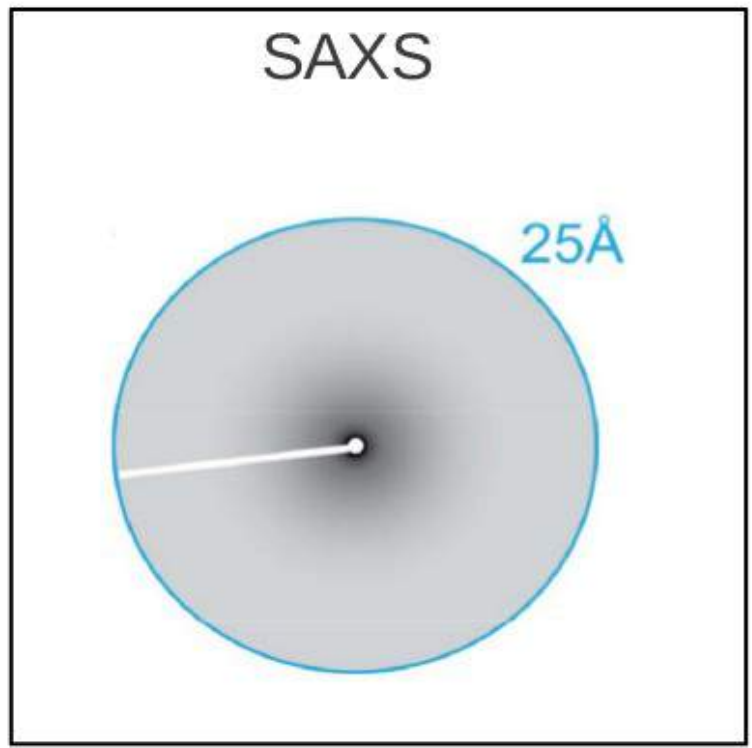
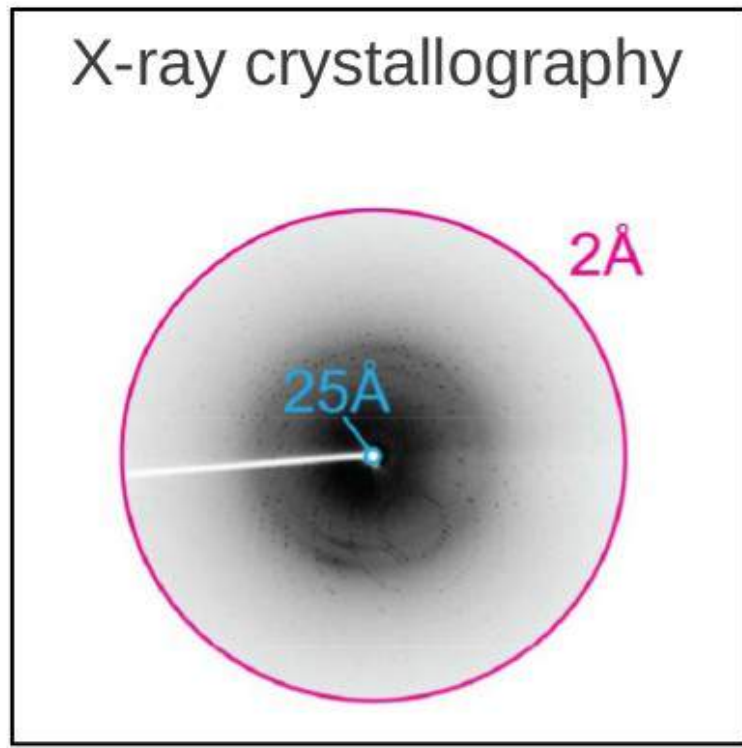
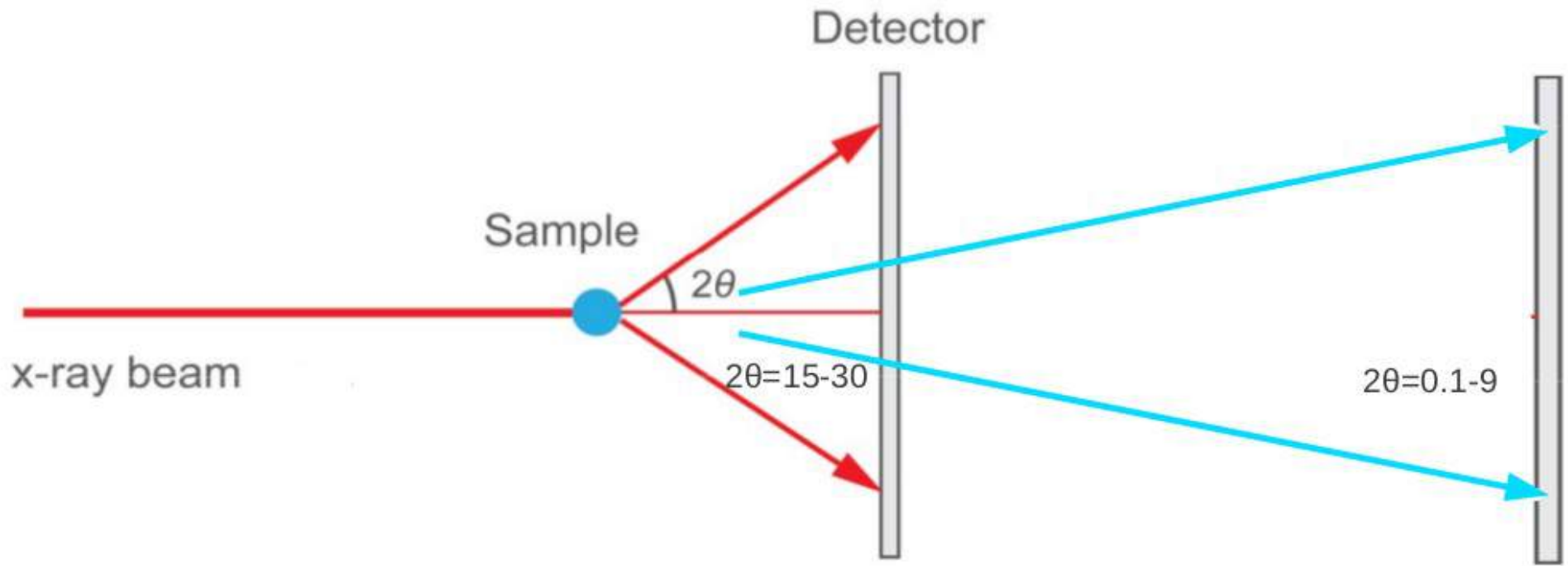
# SAXS

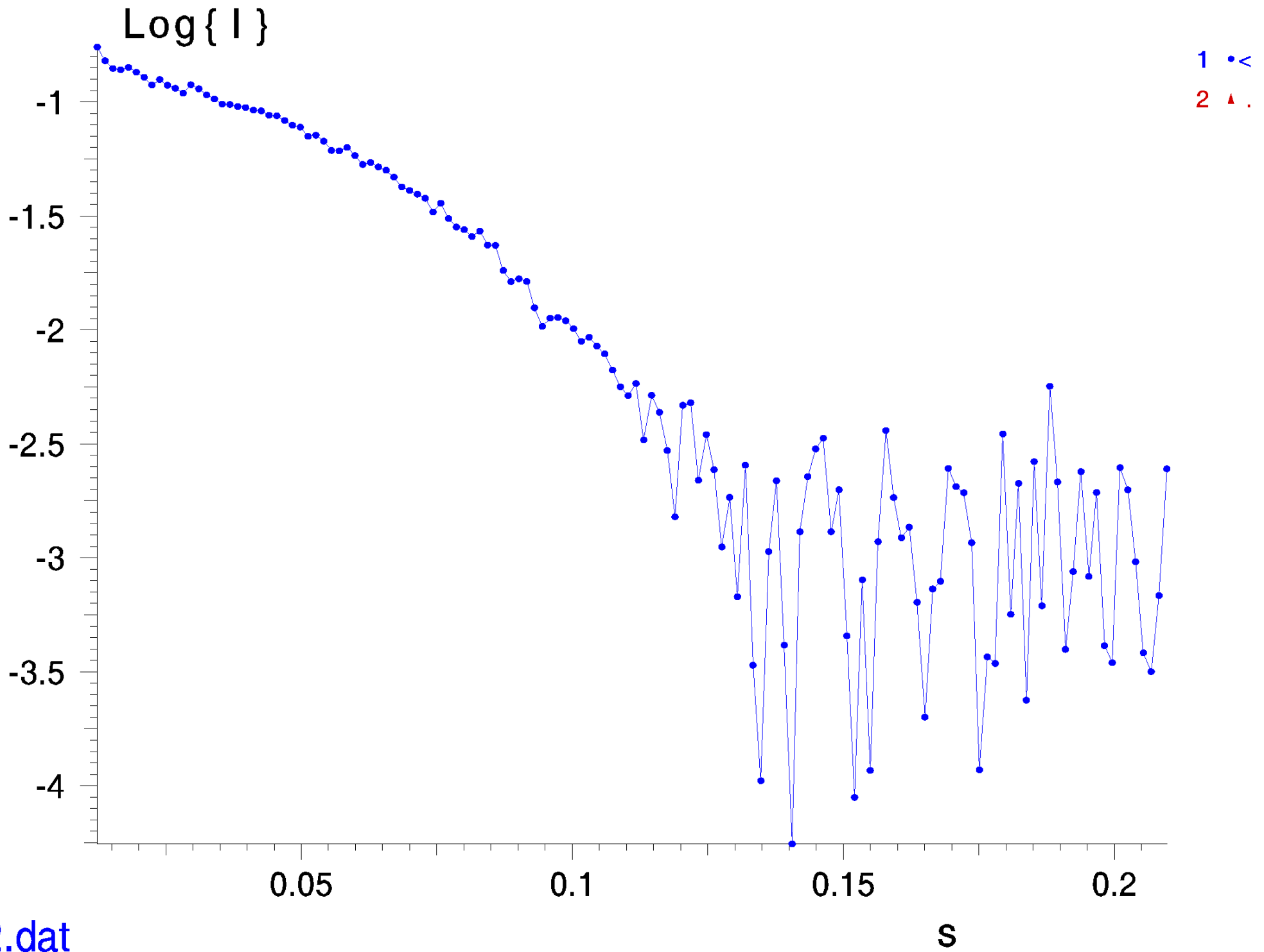
Small angle X-ray scattering

- rozptyl rentgenového záření v malých úhlech



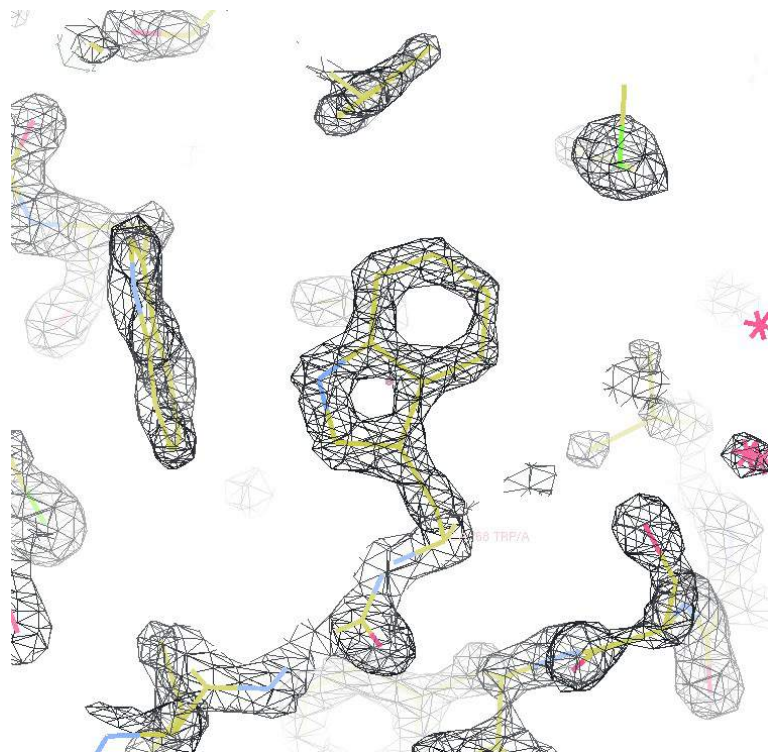






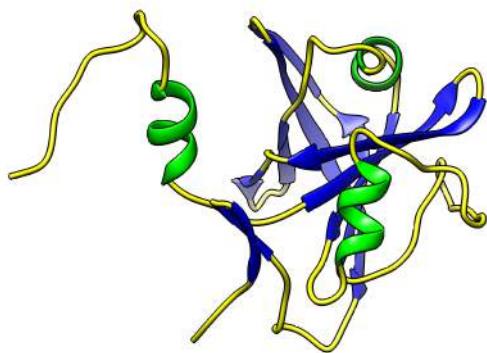
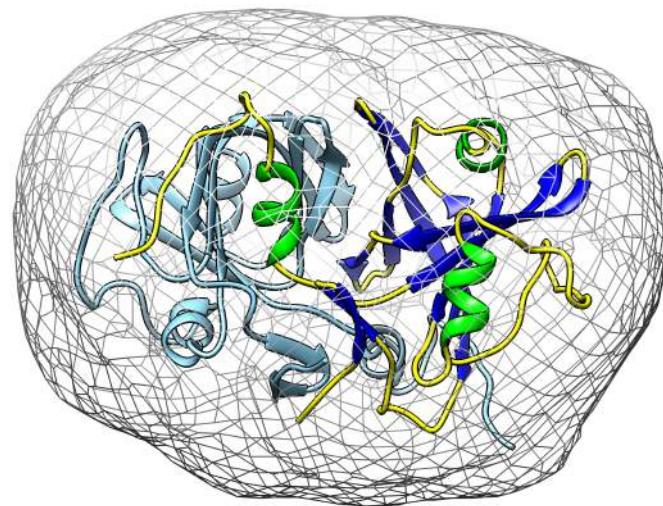
c2.dat

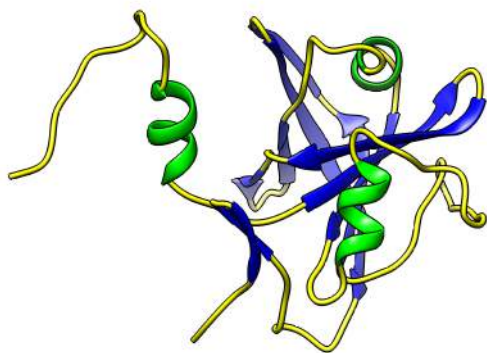
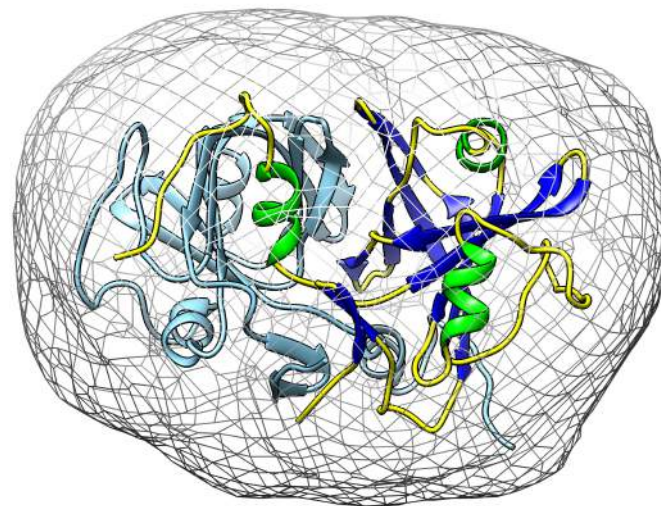
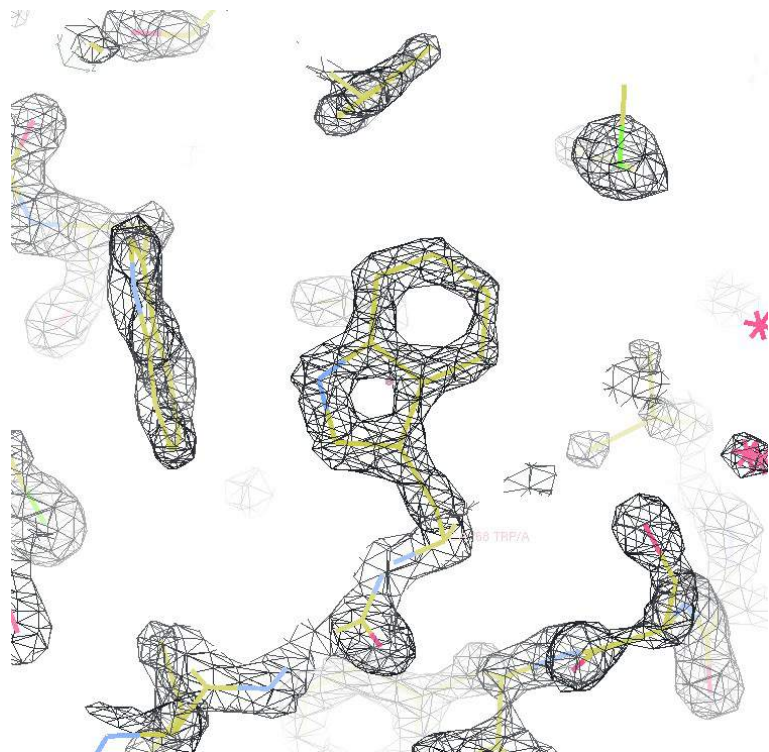




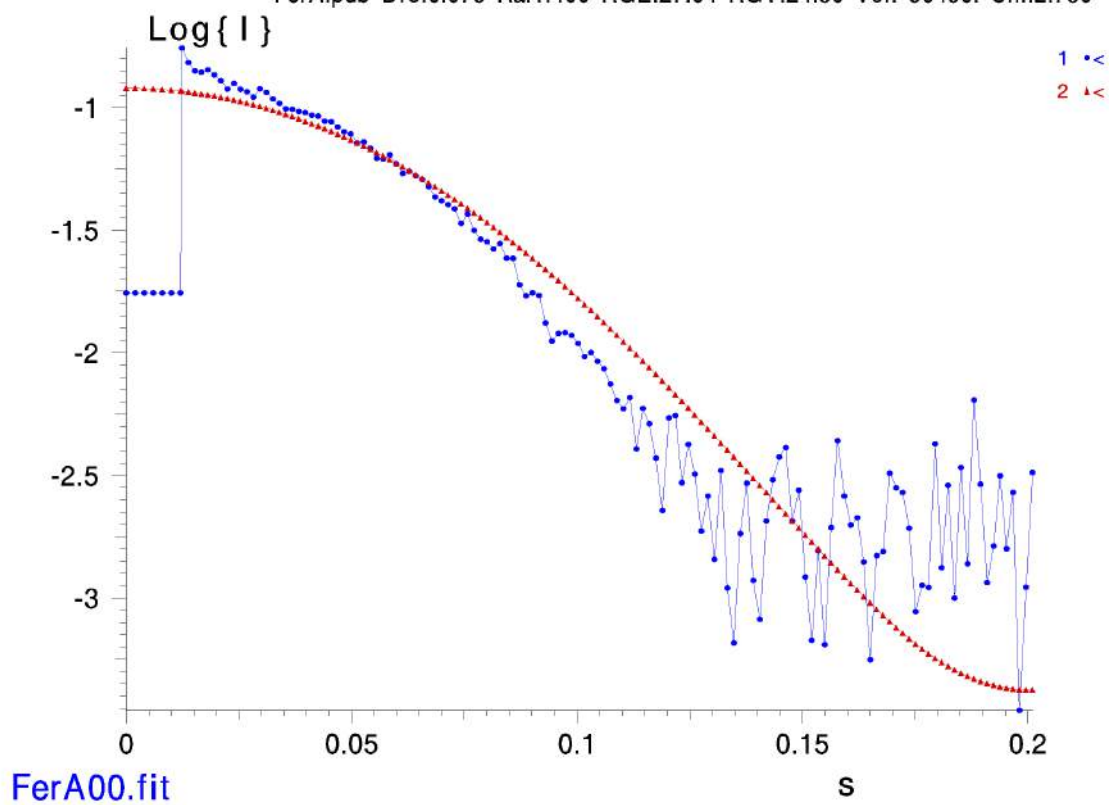
5A

40A

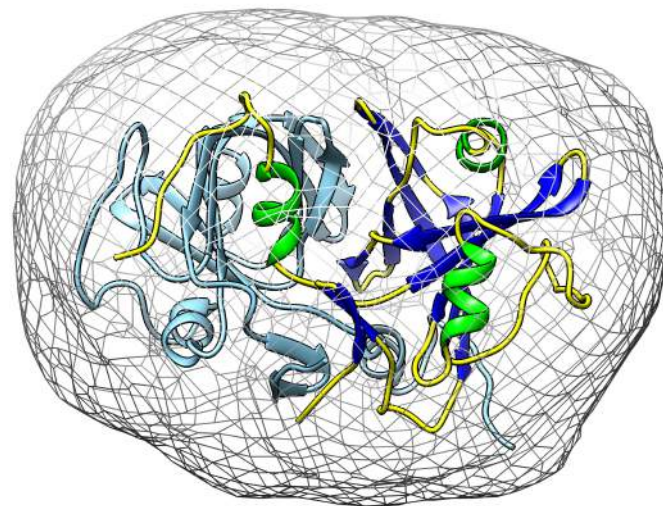
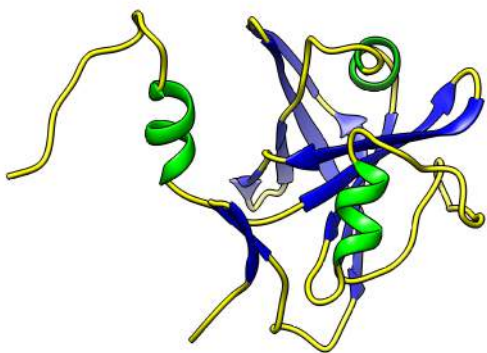
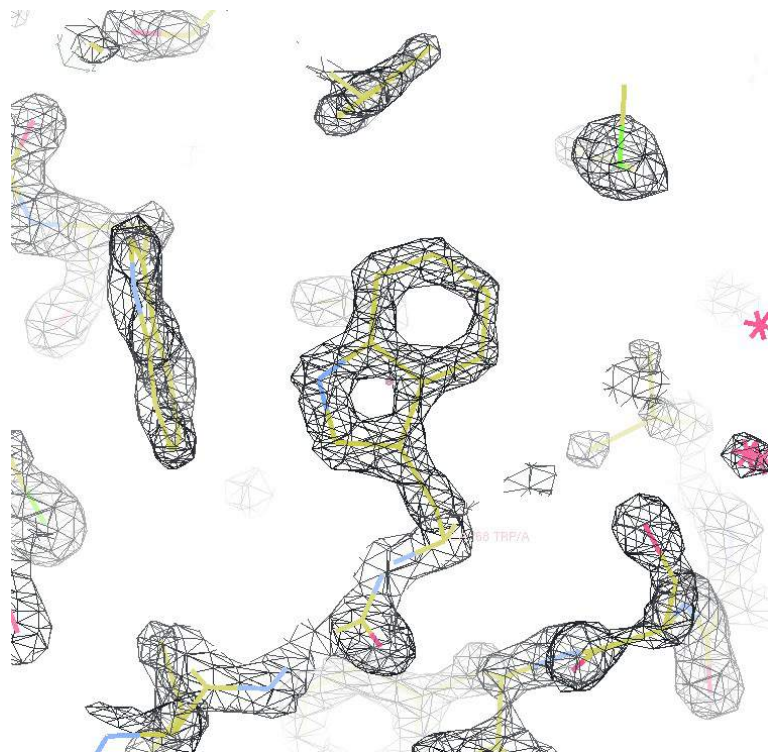




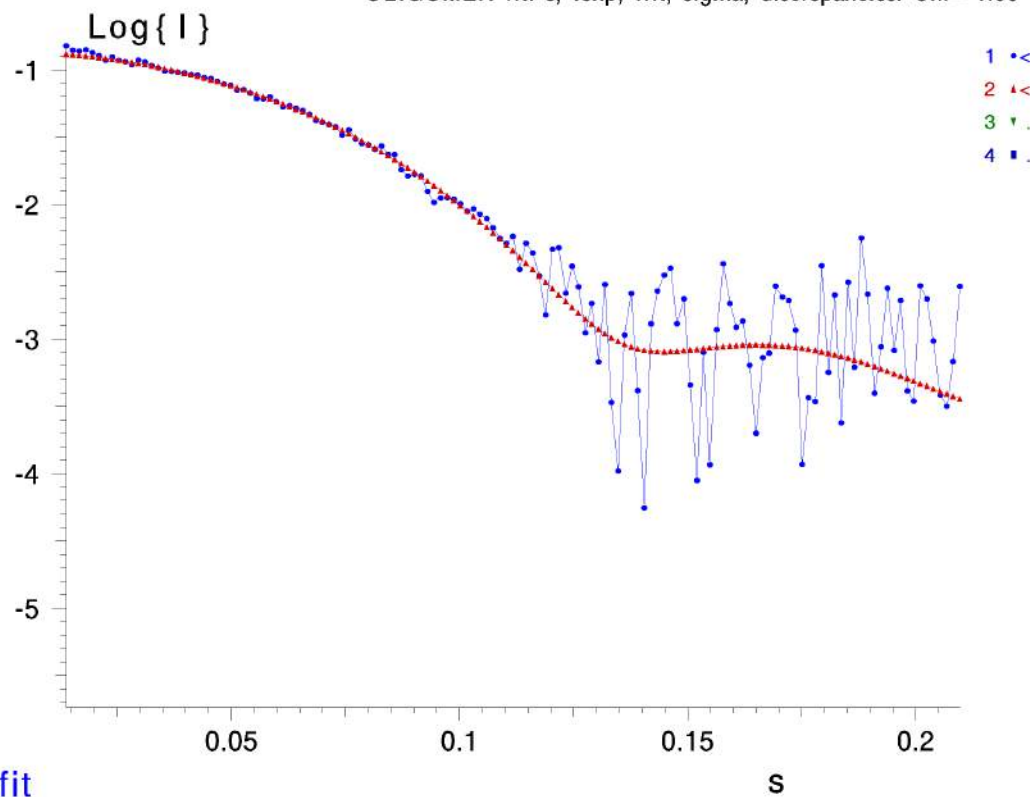
FerA.pdb Dro:0.075 Ra:1.400 RGE:27.64 RGT:24.30 Vol: 56456. Chi:2.730





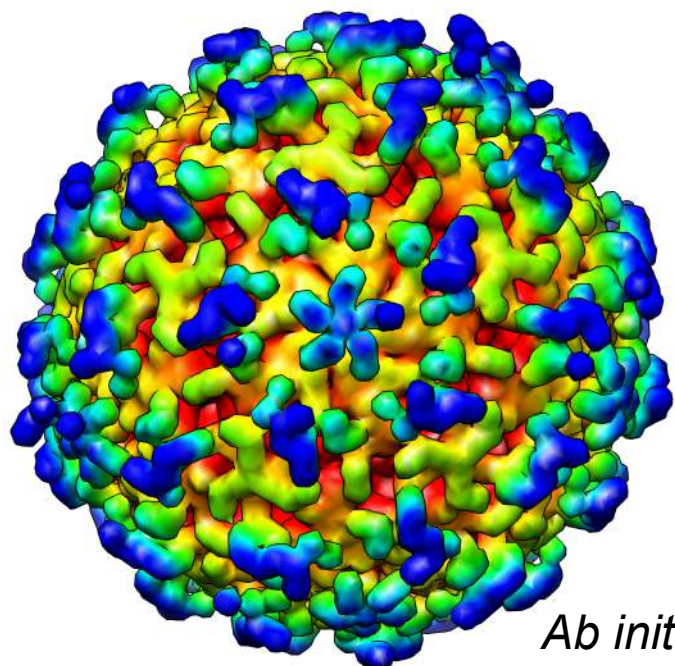


OLIGOMER fit: s, l<sub>exp</sub>, l<sub>fit</sub>, sigma, discrepancies. Chi= 1.06

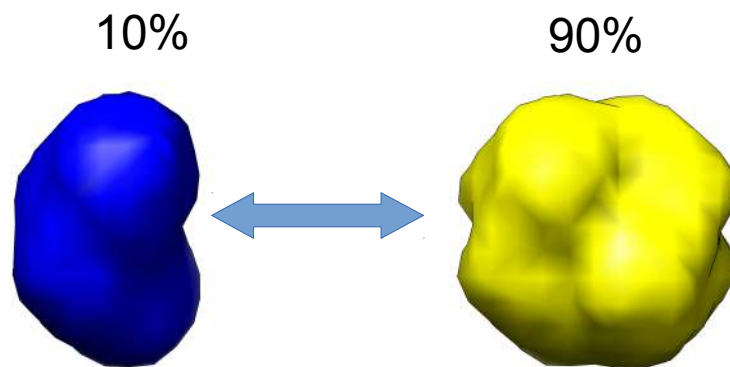


c2.fit

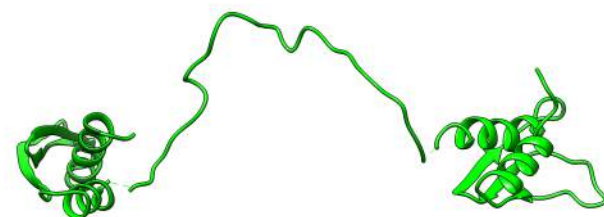




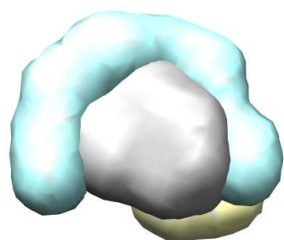
*Ab initio*



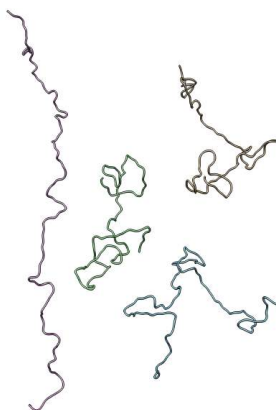
oligomeric state in solution/hierarchical structures



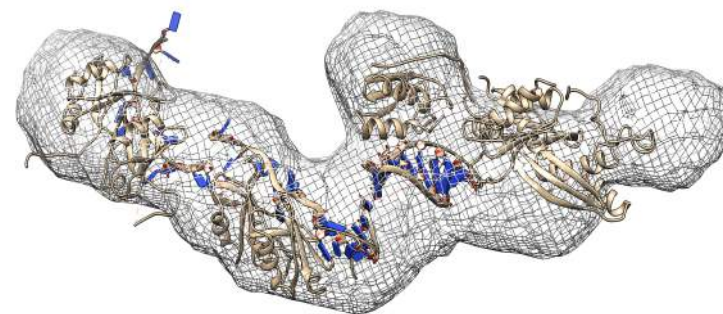
missing fragments



*Ab initio*: multi-phase



flexible systems



rigid body modeling

# 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  $R_{\text{faktor}}$



# Rozlišení SAXS modelů

## 1. Braggovo

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

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

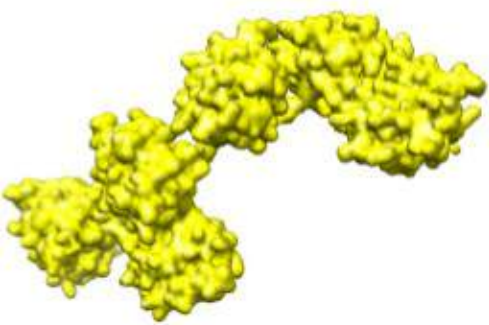
$$d = \frac{2\pi}{q}$$

## 2. Efektivní

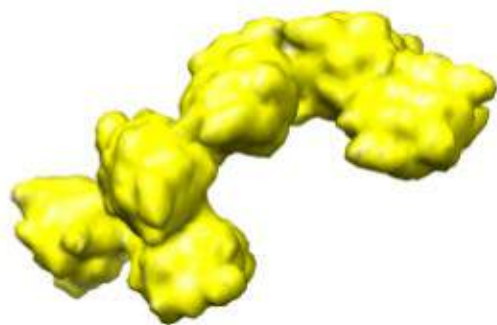
“low resolution structure” ~ bez číselné hodnoty  
odpovídá mapě el. hustoty s rozlišením cca 20Å

# Rozlišení

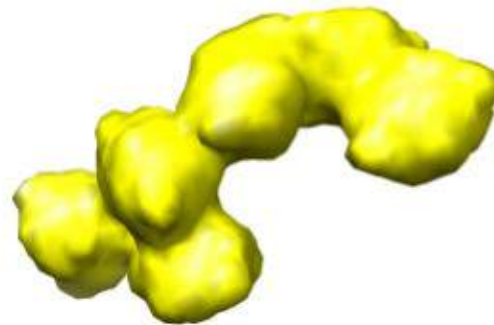
SAXS 10-20Å



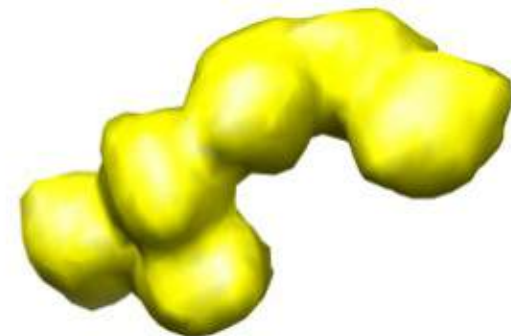
5Å



10Å



15Å

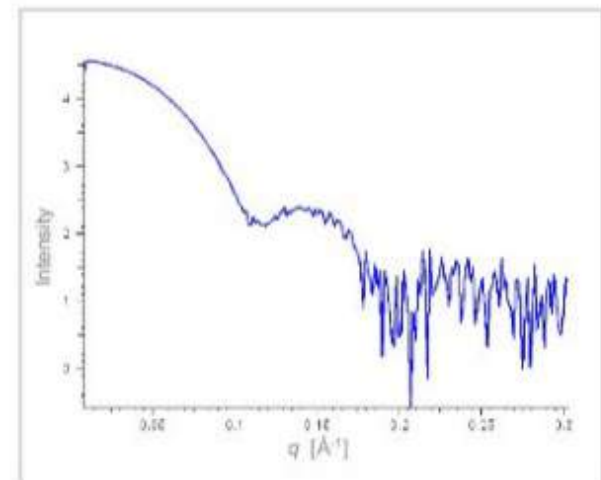
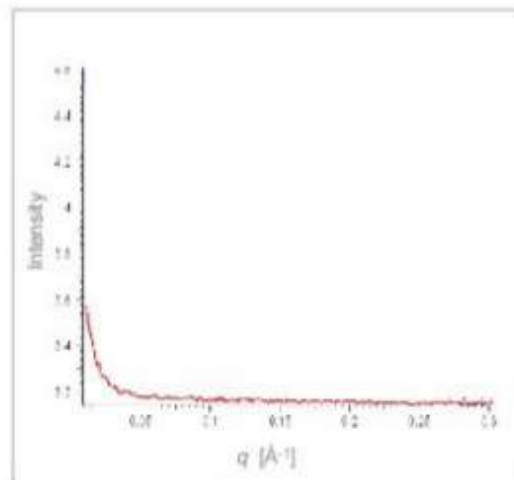
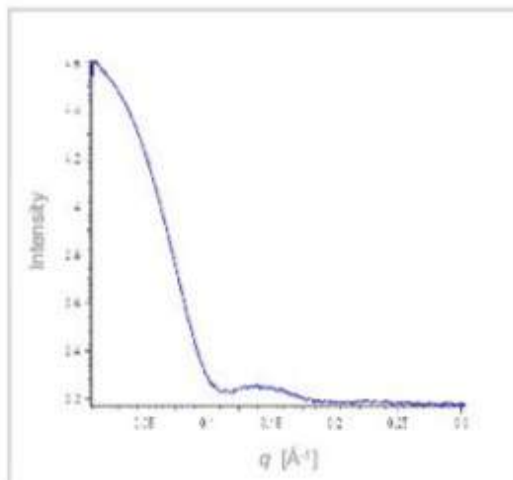
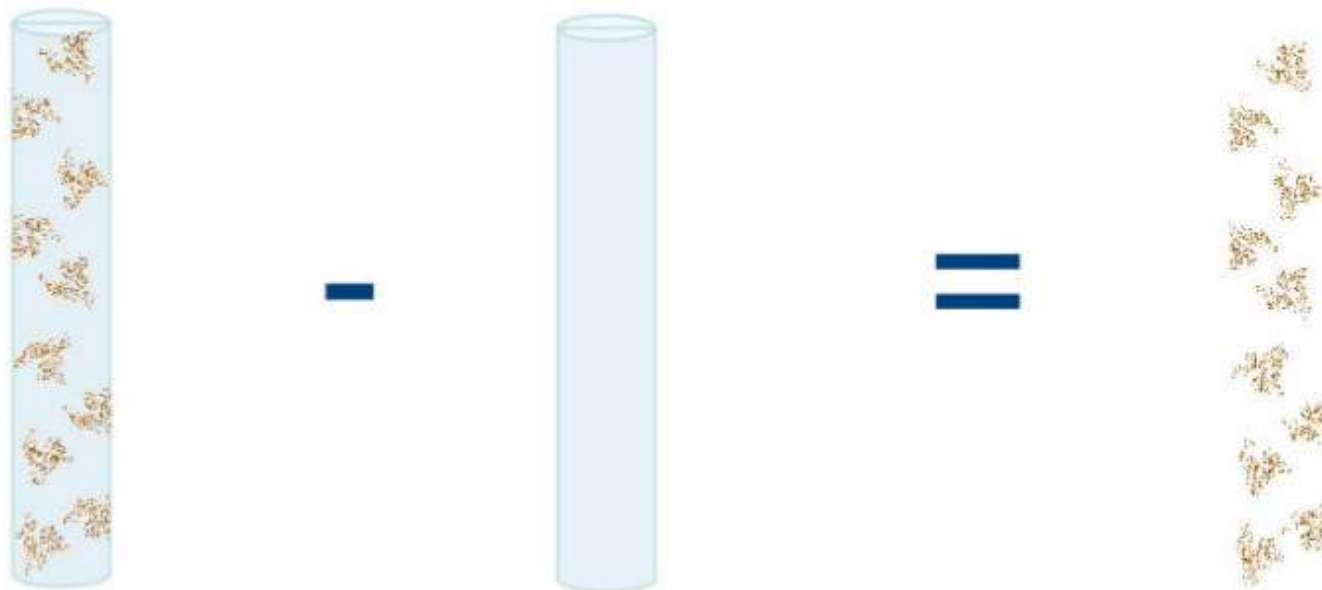


20Å

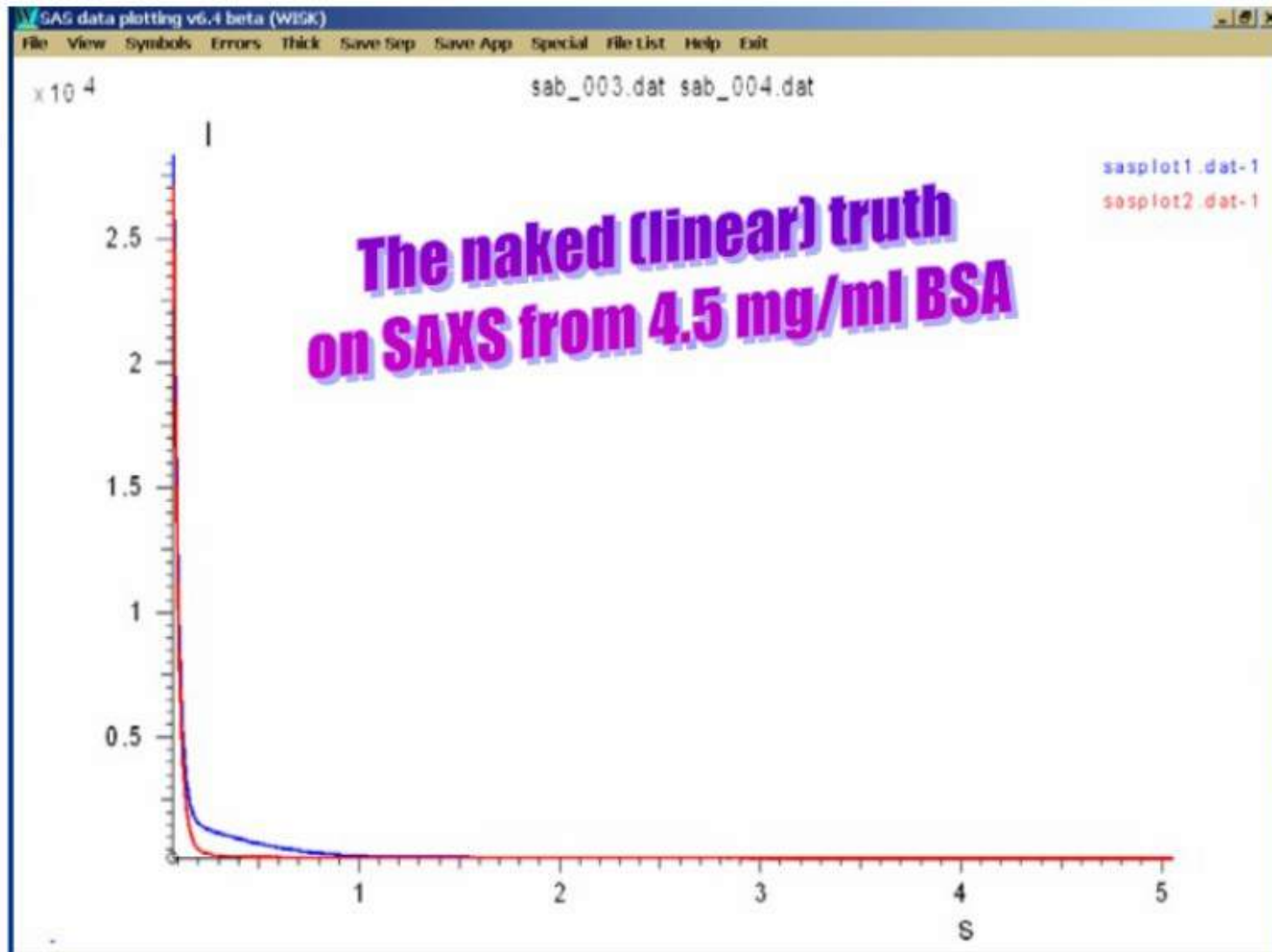
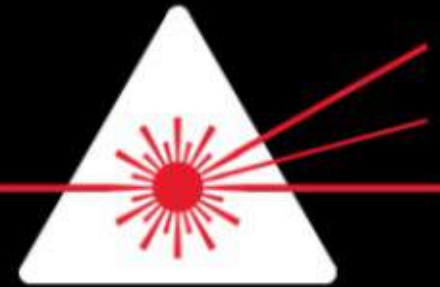
50Å

RTT complex ~7000atoms

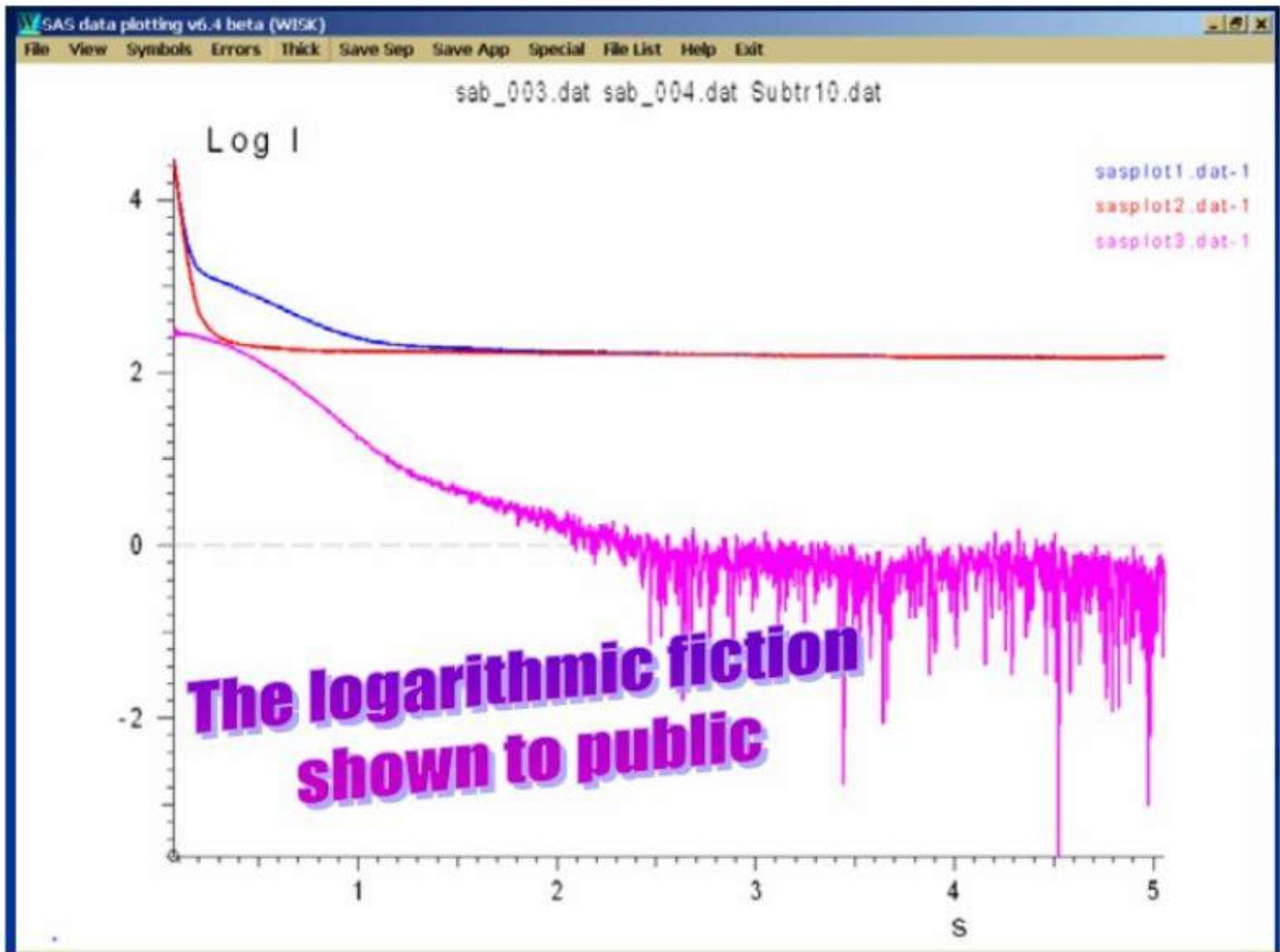
# Odečtení příspěvku solventu

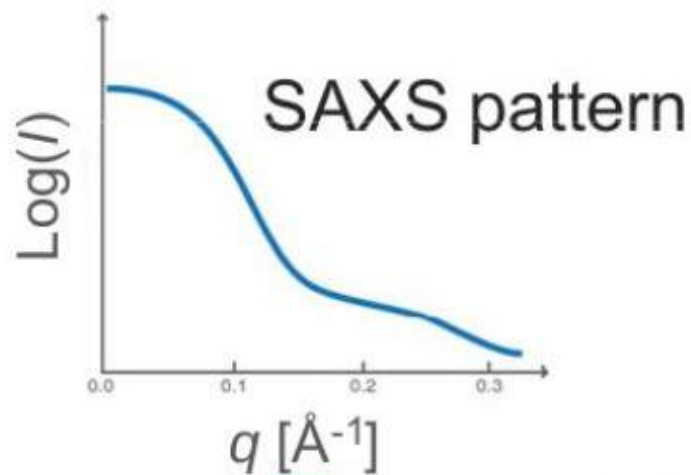


# Looking At Nothing

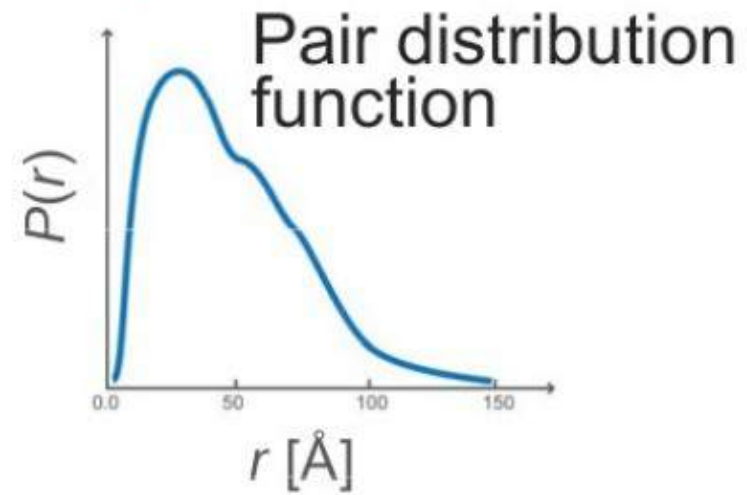
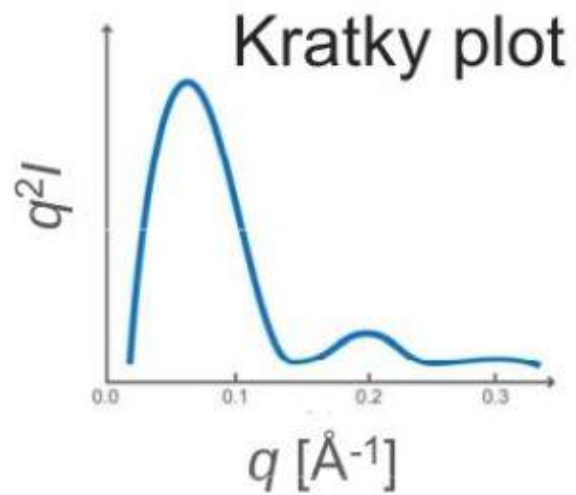
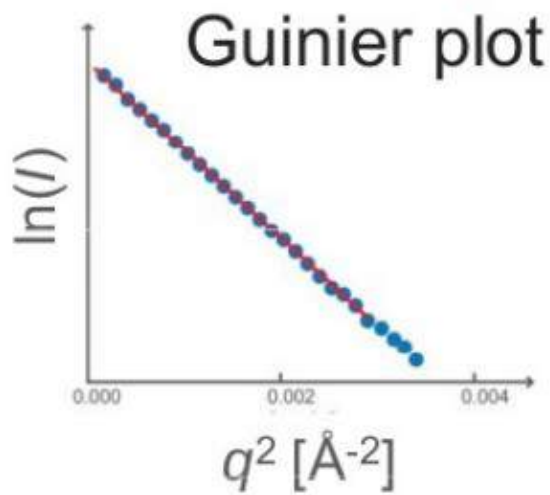






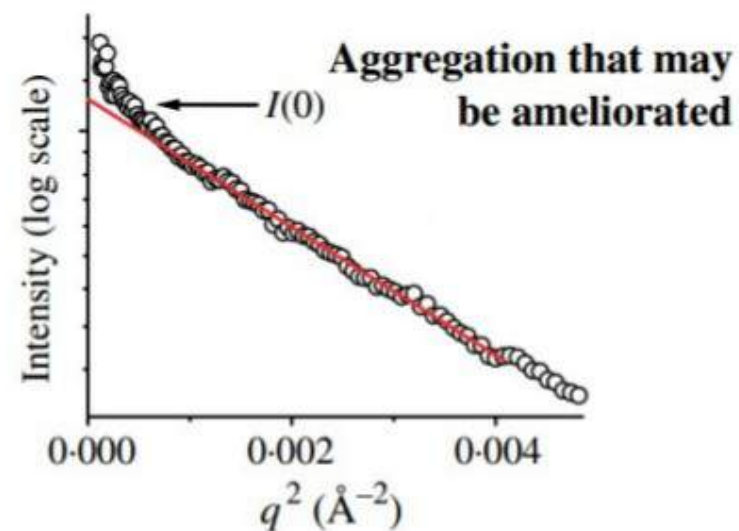
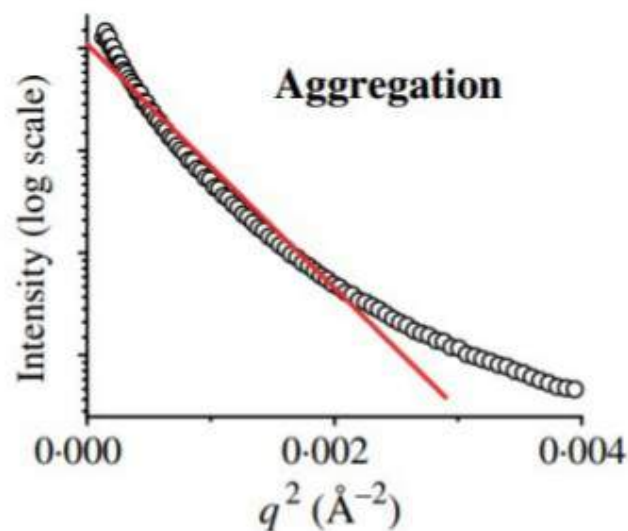
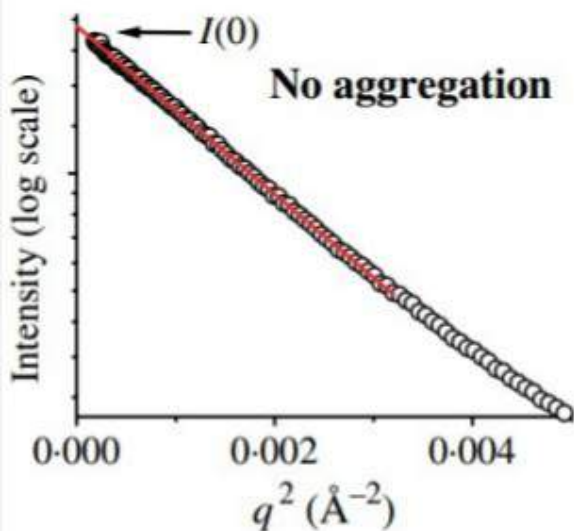


Fourier Transform



# Guinier plot

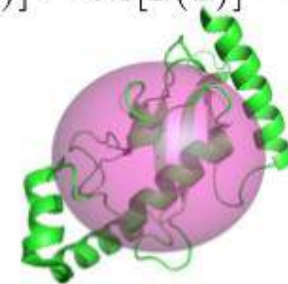
Analýza rozptylové křivky ve velmi malých úhlech



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

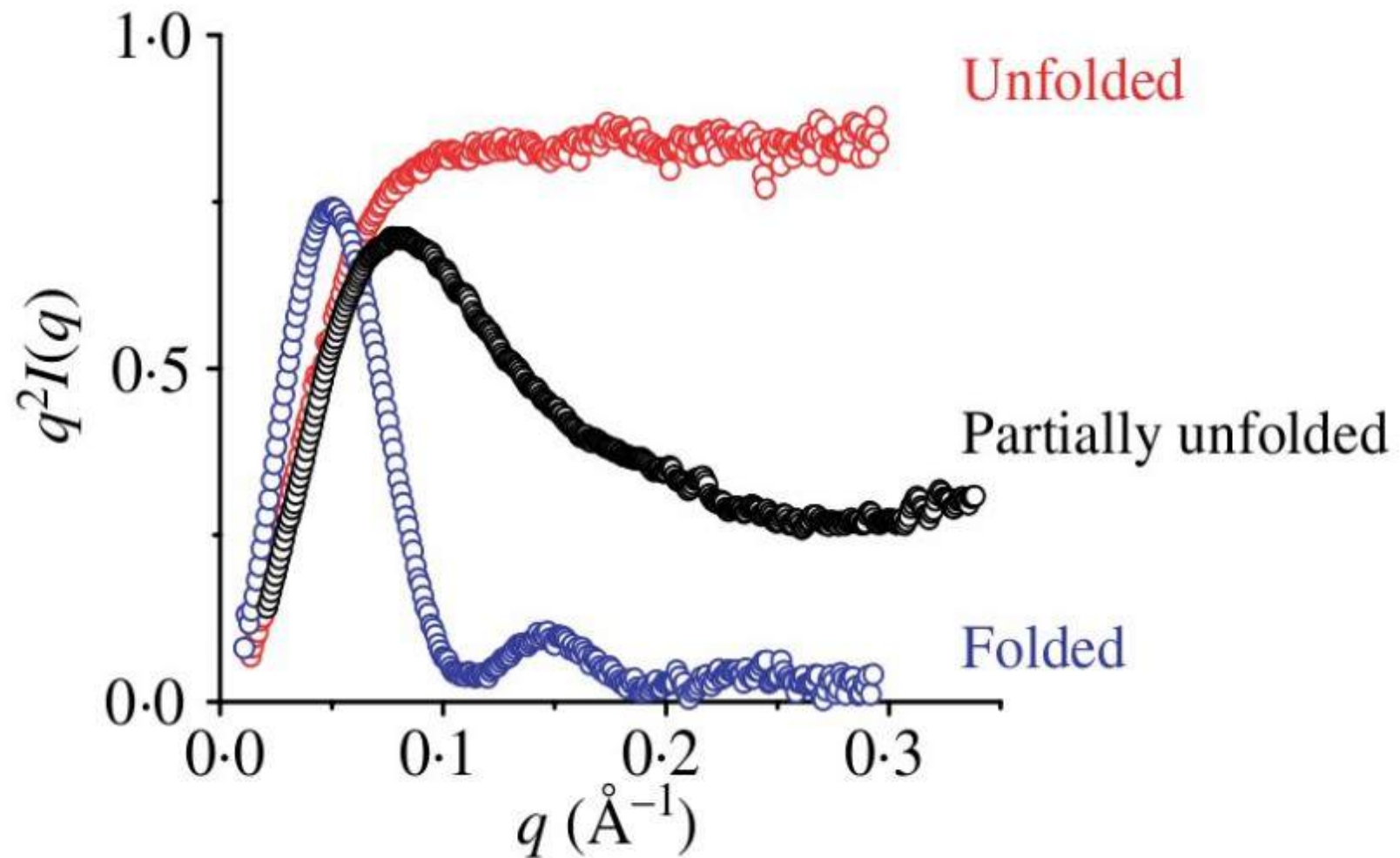
2. **Poloměr gyrace**

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



# Kratky plot

Kompaktnost molekuly



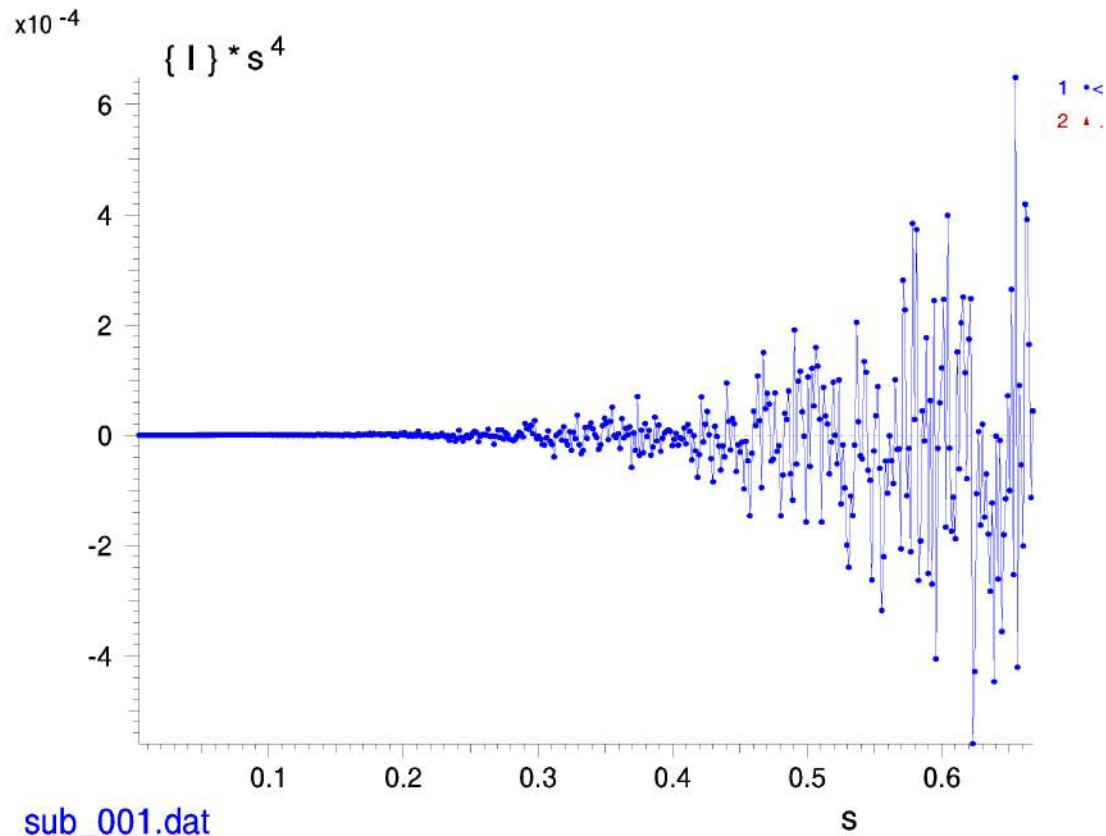


# Porod volume

$$Q = \int_0^{\infty} q^2 I(q) dq = 2\pi^2 \Delta\rho^2 V$$

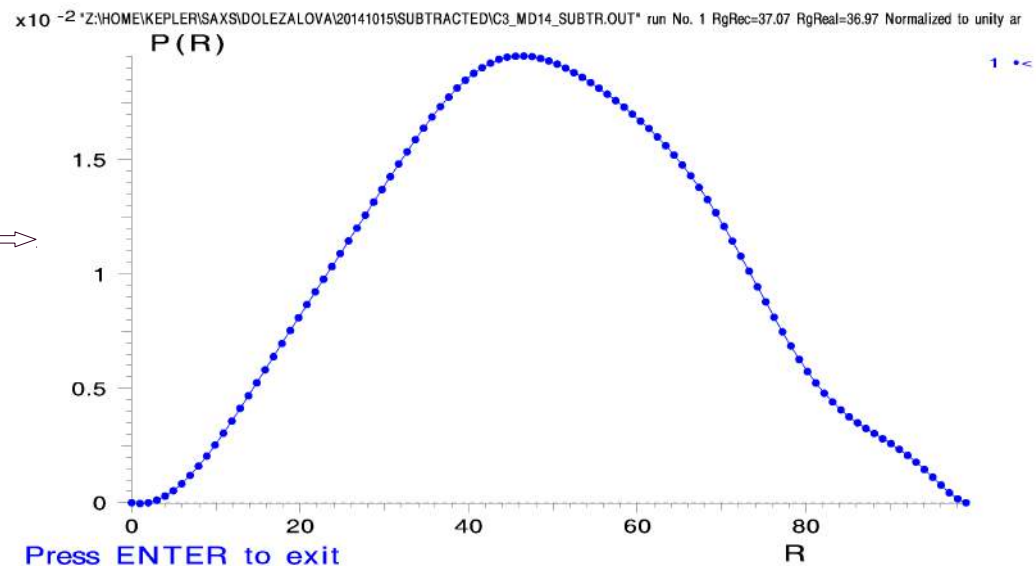
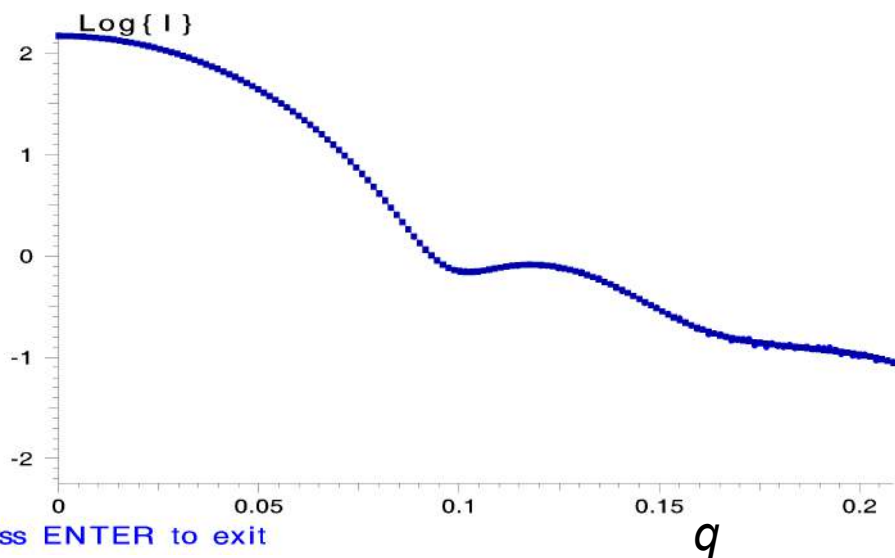
$$I(0) = (\Delta\rho V)^2$$

$$V = 2\pi^2 \frac{I(0)}{Q}$$

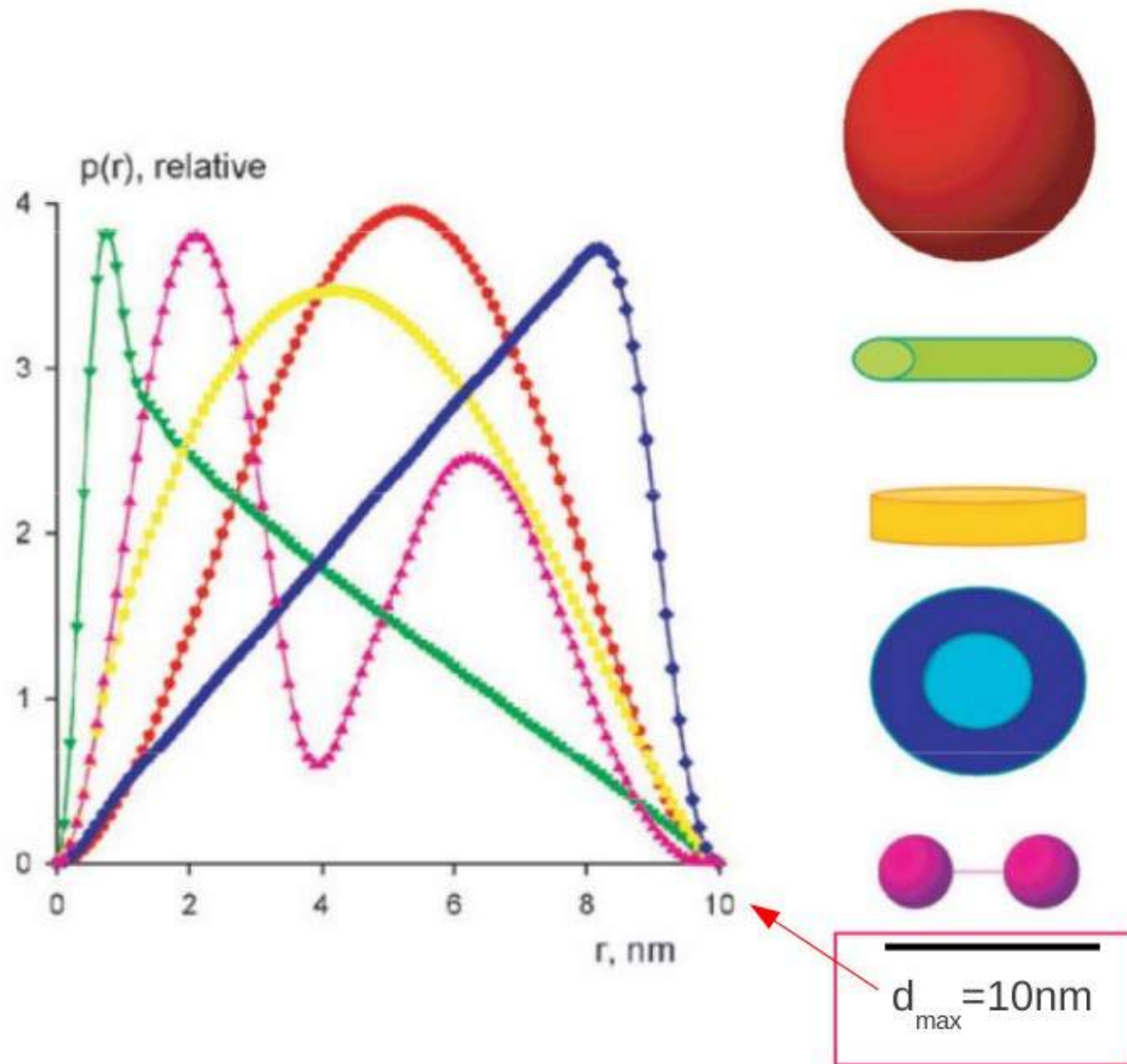


# Pair distribution function

$$I(q) = 4\pi \int_0^{D_{\max}} p(r) \frac{\sin(qr)}{qr} dr \iff p(r) = \frac{r^2}{2\pi^2} \int_0^\infty q^2 I(q) \frac{\sin(qr)}{qr} dq$$



# Pair distribution function

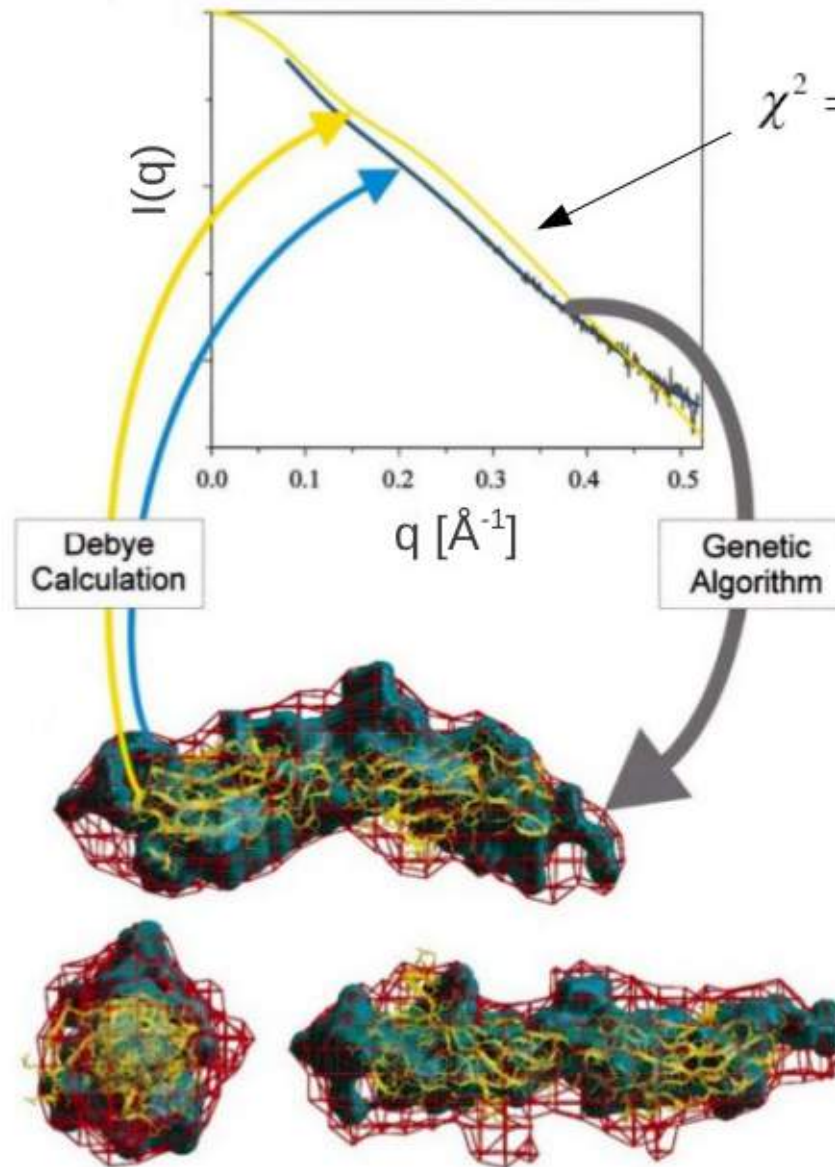


# SAXS modelování tvaru *ab initio*

test shody:

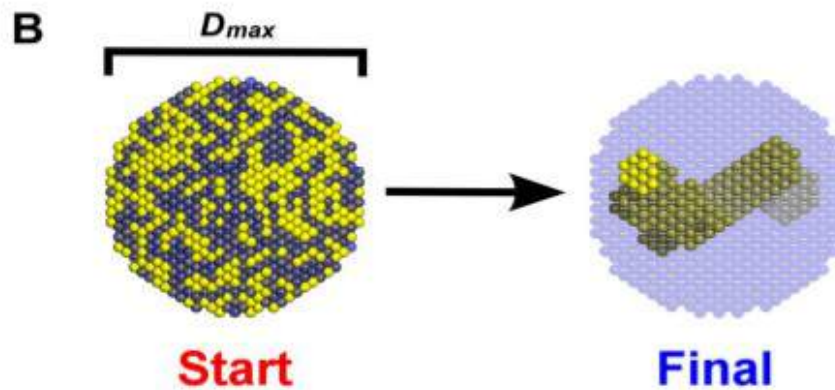
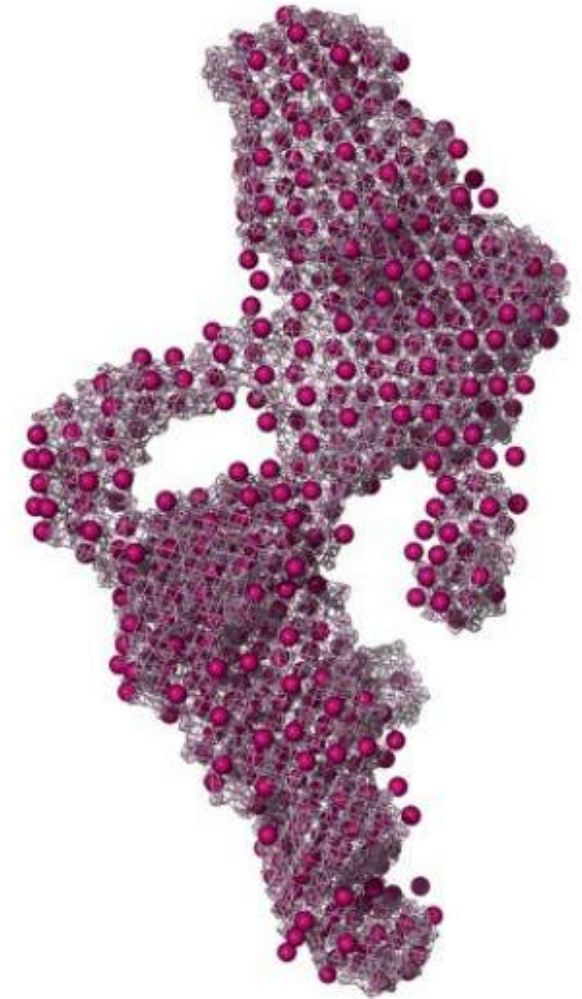
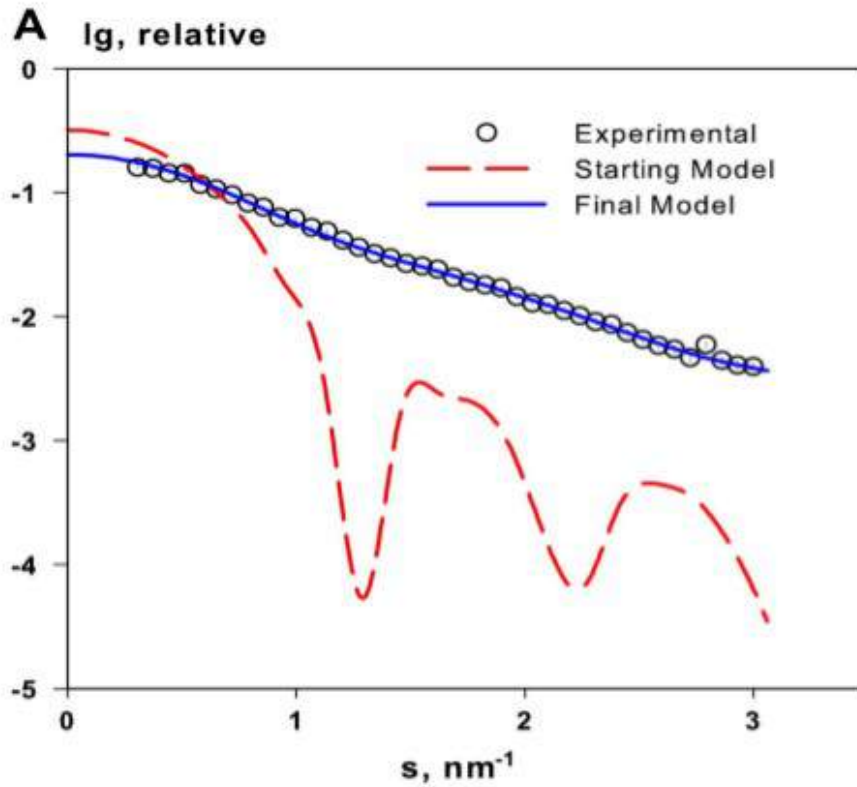
$$\chi^2 = \sum_{k=1}^Q \left( \frac{I_{\text{exp}}(q_k) - c \cdot I_{\text{m}}(q_k)}{\sigma_{\text{exp}}(q_k)} \right)^2$$

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



# SAXS modelování tvaru

Program DAMMIN

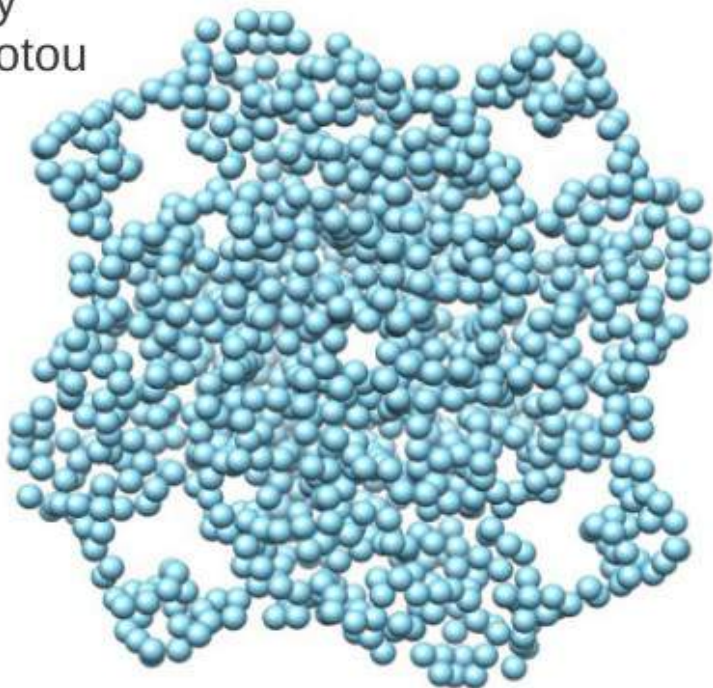




# SAXS modelování tvaru

Program GASBOR

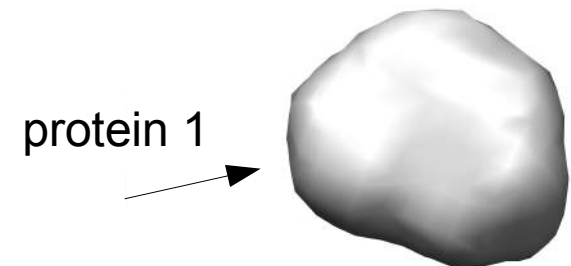
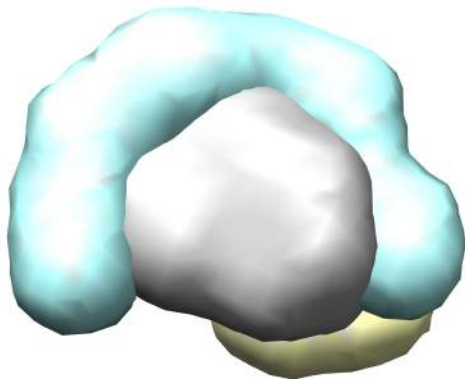
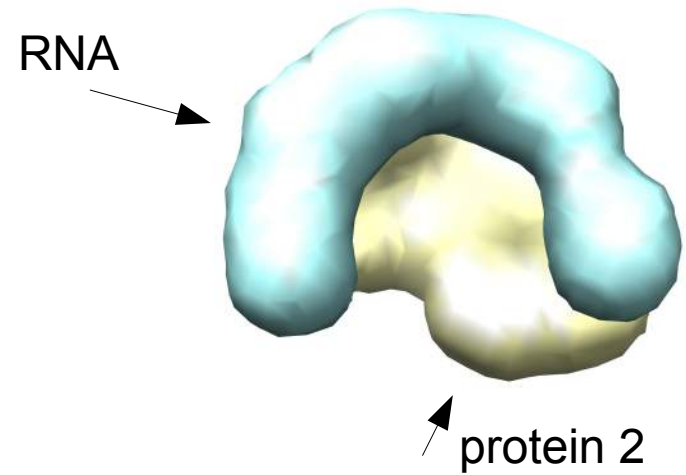
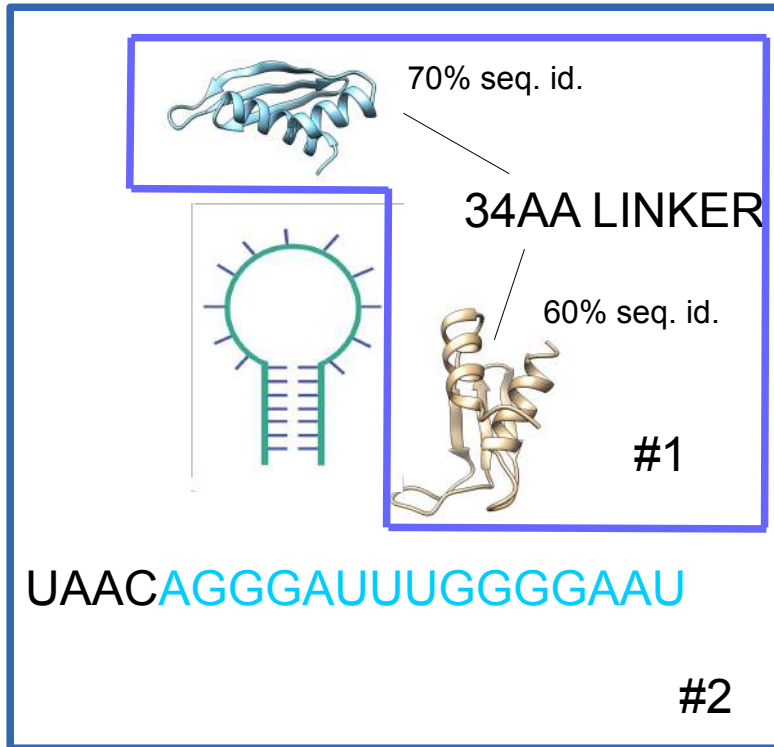
Chain-like GASBOR model složen z univerzálních residuí (Dummy residues) s průměrnou el. hustotou a rozestupy  $\sim 0.38$  nm



Pozice středu kuliček odpovídá  $C^\alpha$   
Počet kuliček odpovídá počtu aminokyselin  
v proteinu

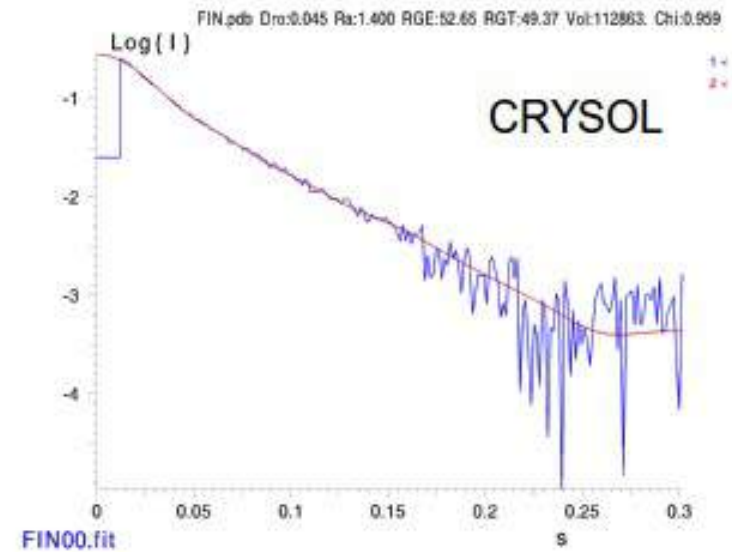
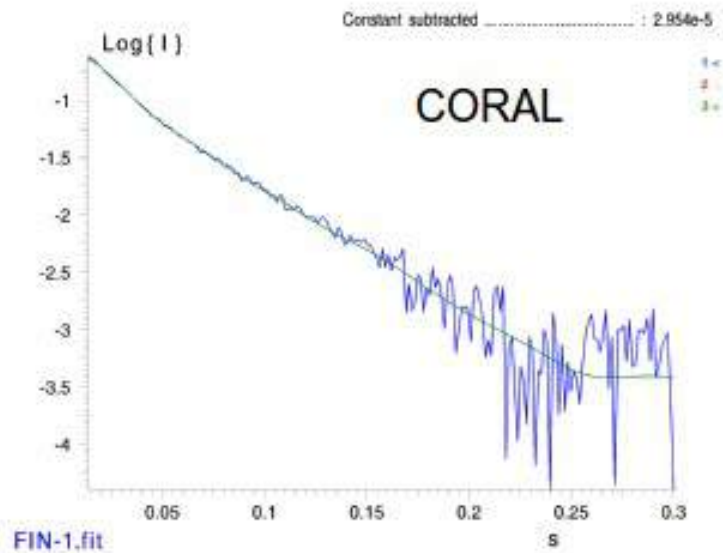
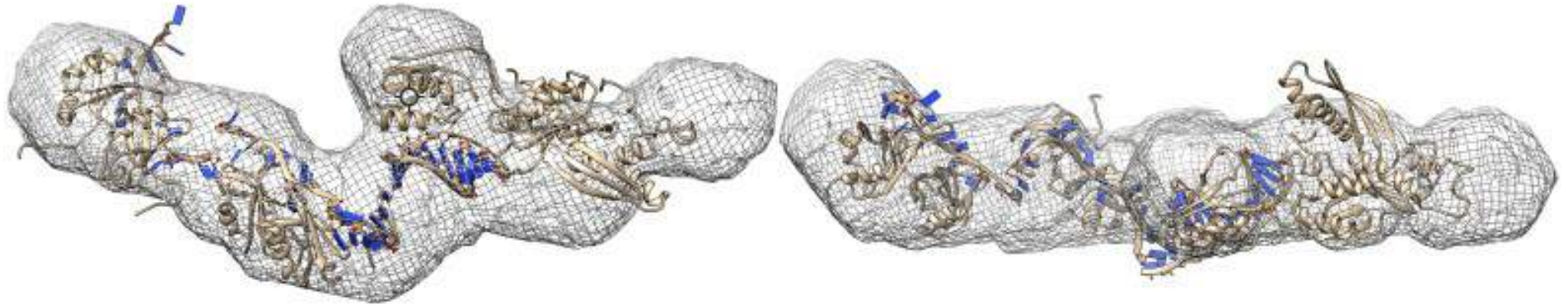
# SAXS modelování tvaru

multi-phase program MONSA



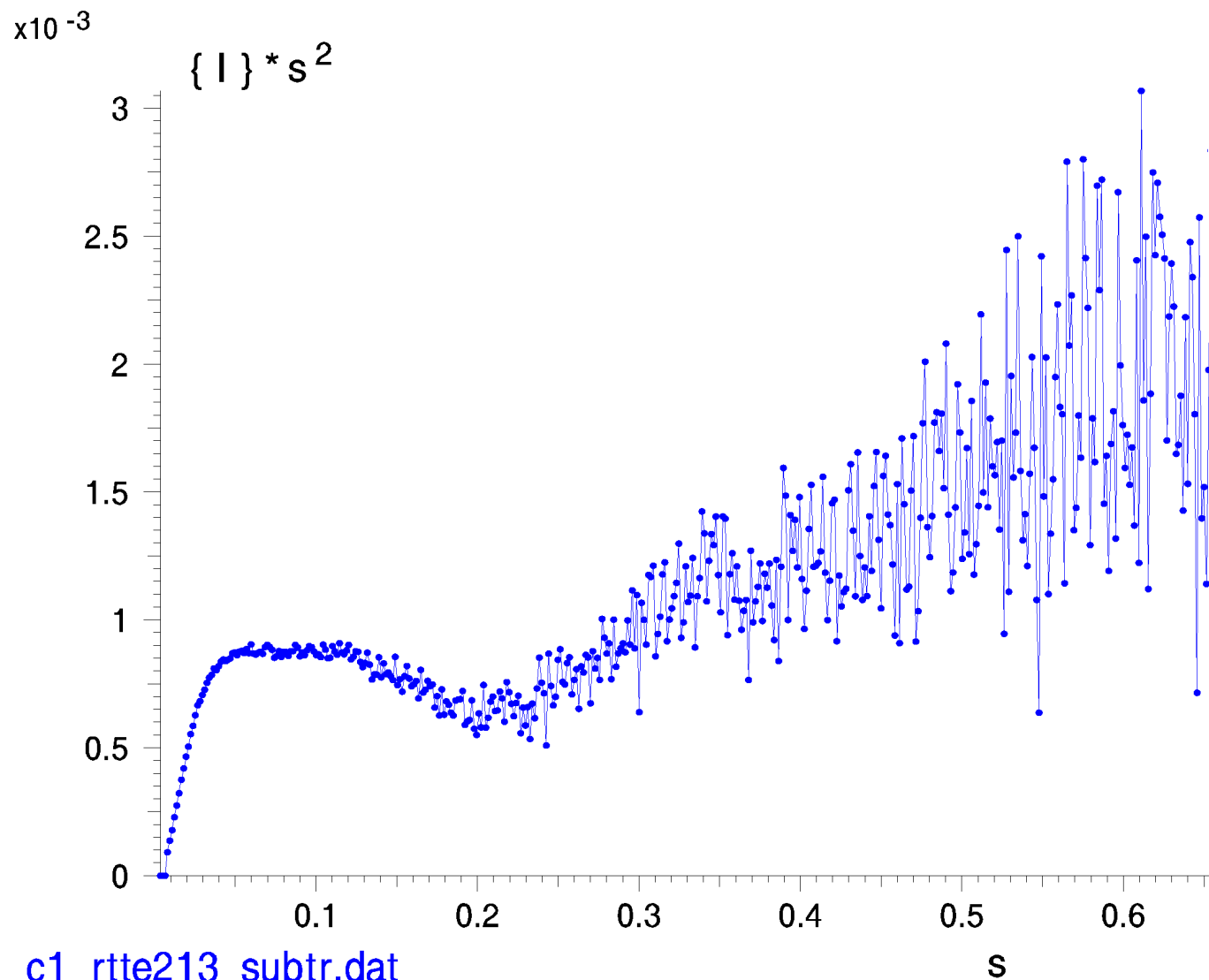
# RIGID BODY modeling

programy SASREF, BUNCH, CORAL,...

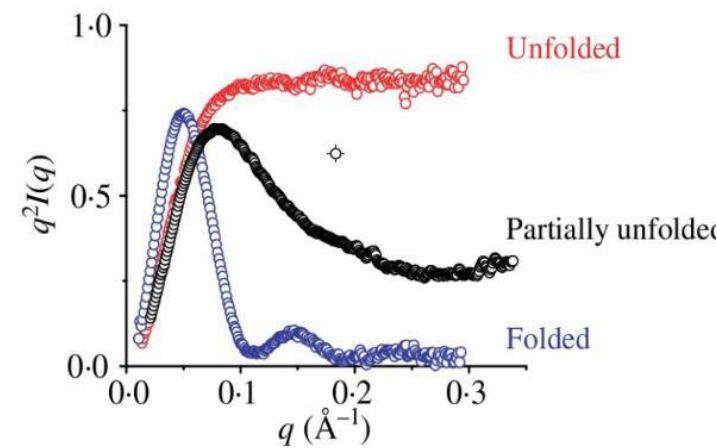


# Flexibilní systémy

program EOM

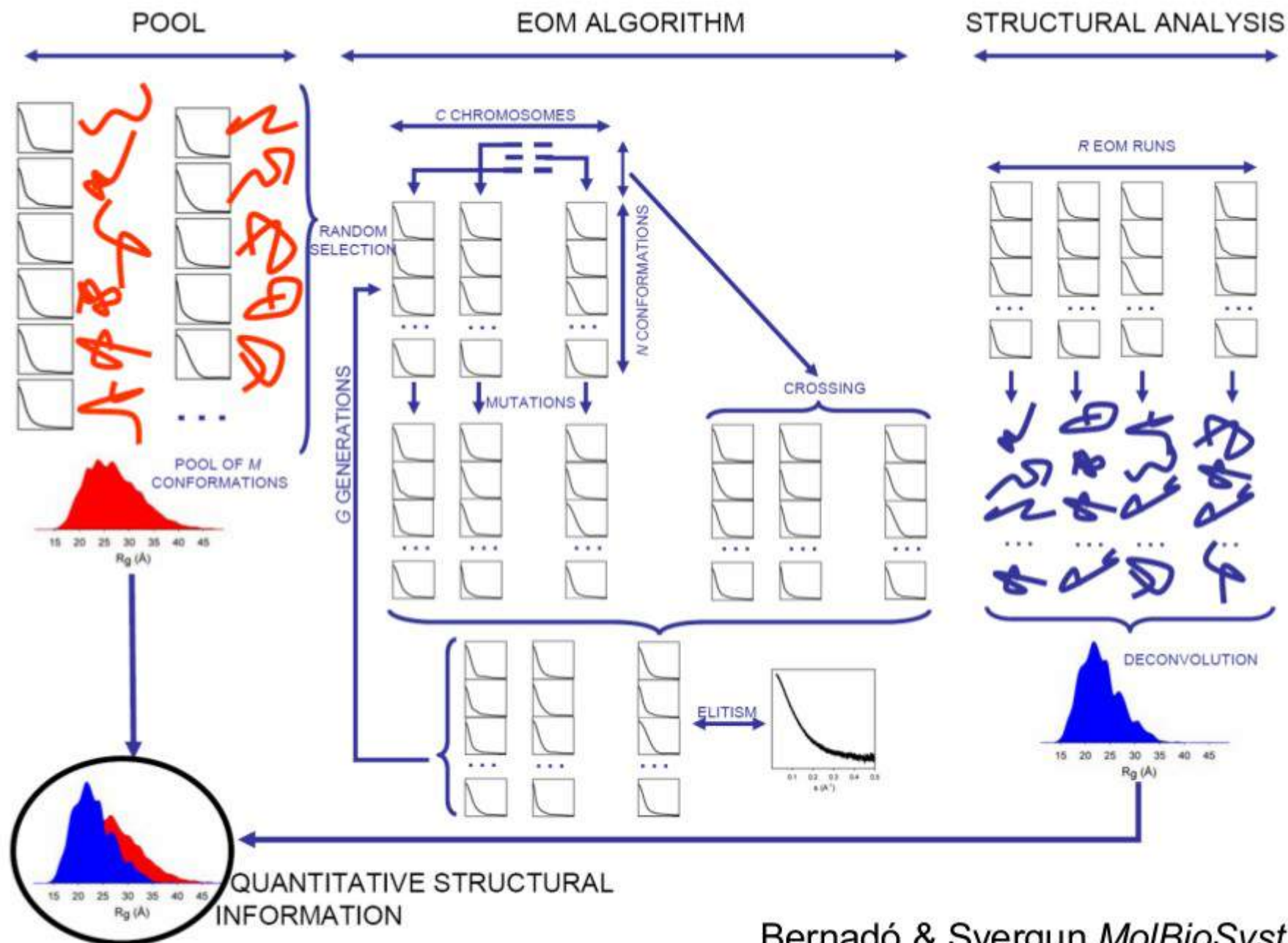


c1\_rtte213\_subtr.dat



# Flexibilní systémy

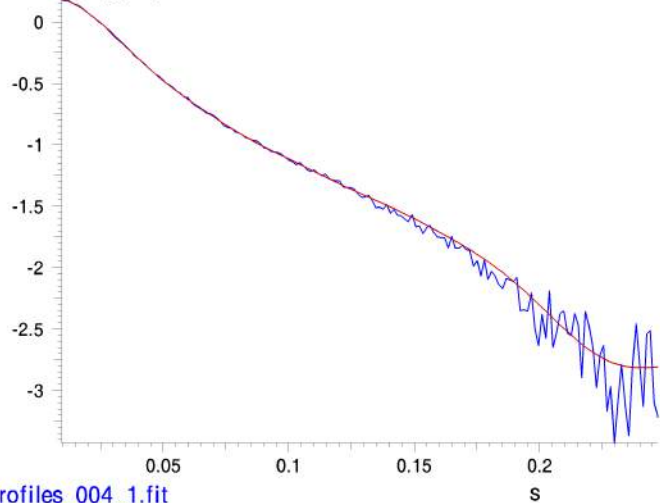
program EOM



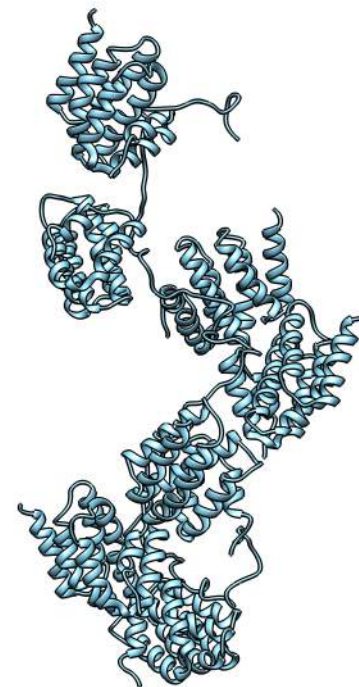
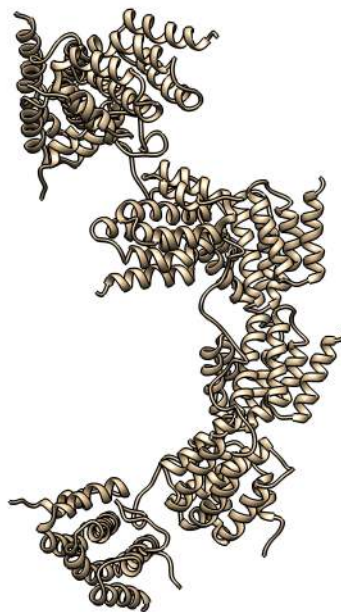
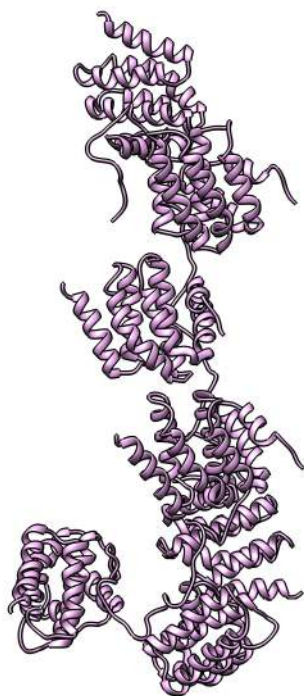
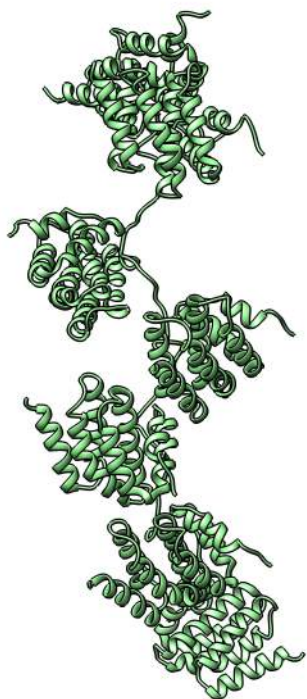
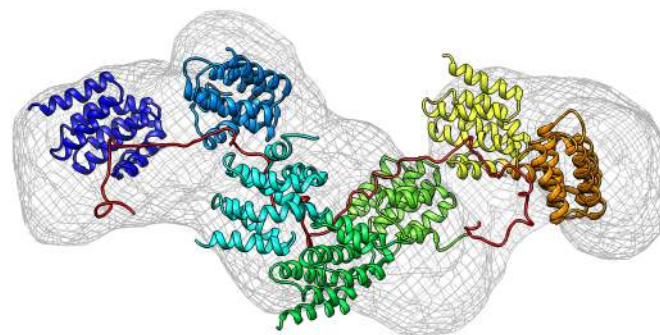


CYCLE: 87 Chi: 1.160 GENER.:1000 ENSEMBLES: 50 CURVES: 24

Log{I}

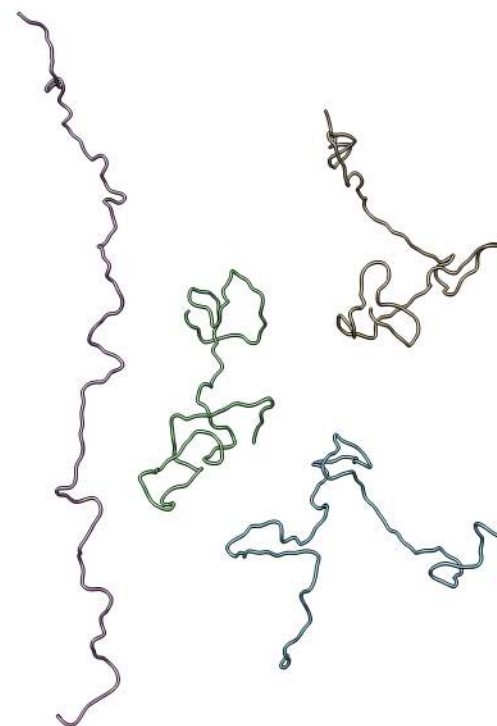
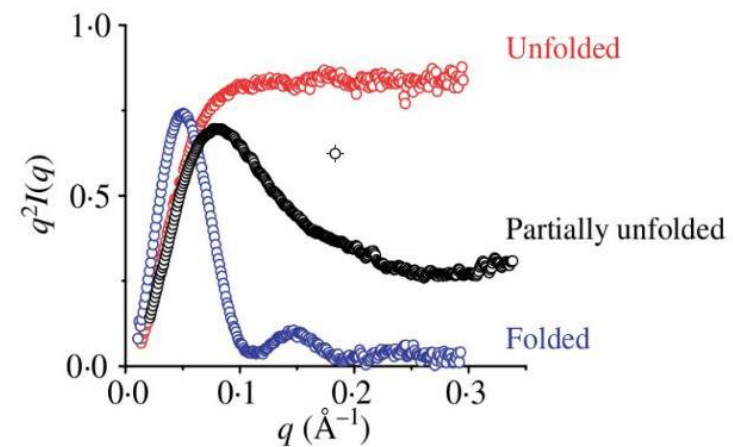
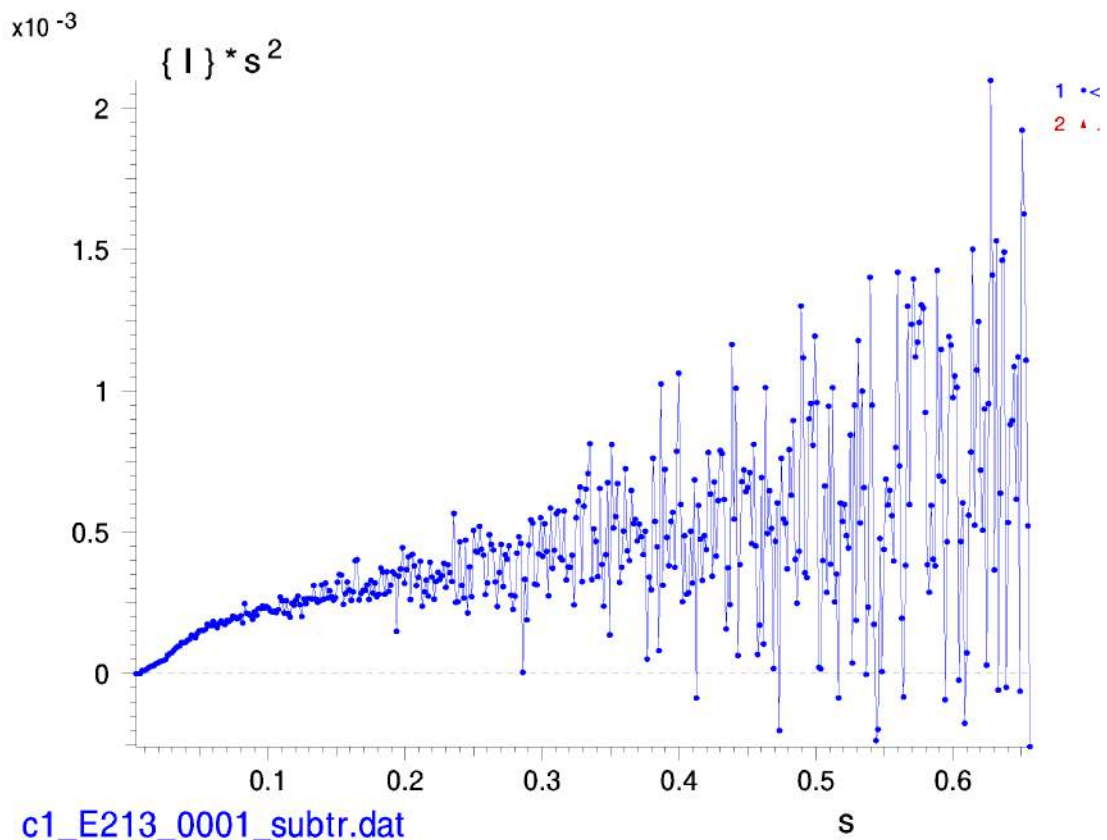


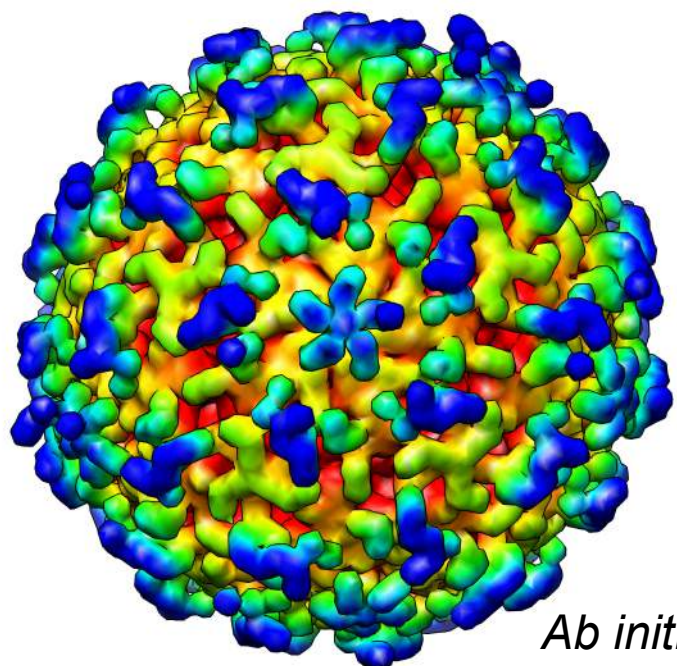
profiles\_004\_1.fit



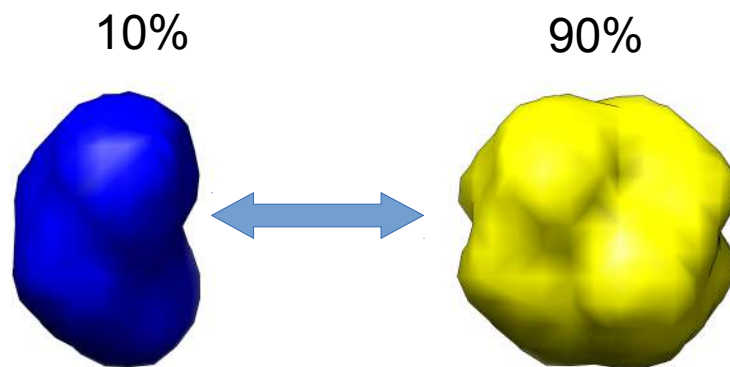
# Flexibilní systémy

program EOM

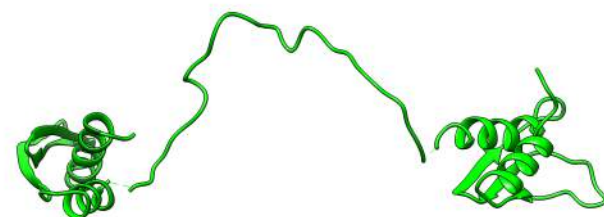




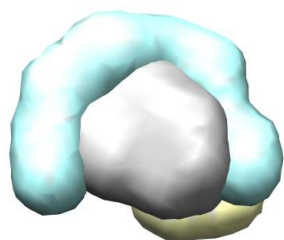
*Ab initio*



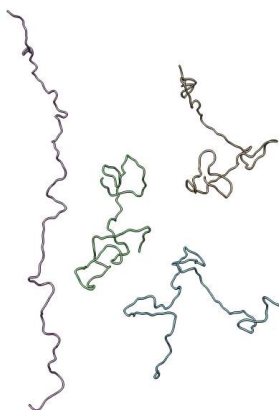
oligomeric state in solution/hierarchical structures



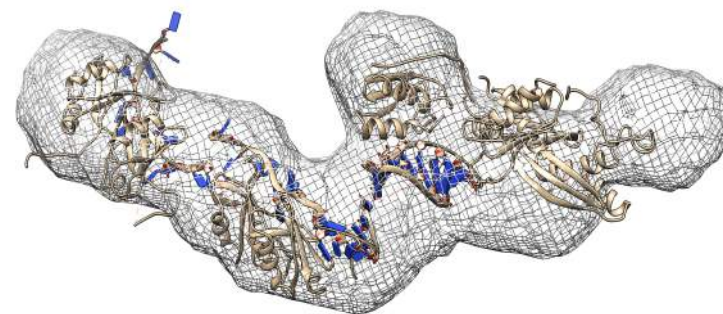
missing fragments



*Ab initio*: multi-phase



flexible systems



rigid body modeling

# bioSAXS - shrnutí

pokud máme kvalitní data:

1. model independent:
  - obecné strukturní parametry:  $R_g$ ,  $D_{max}$ ,  $V_{porod}$
  - stav proteinu: agregace, folding/flexibilita
  - *ab initio* rekonstrukce tvaru
  
2. model dependent:
  - validace RTG / NMR struktur
  - oligomerní stavy / hierarchické struktury
  - kvartérní struktura komplexů
  - doplnění chybějících částí v RTG strukturách
  - studium flexibilních systémů

# Praktické informace pro uživatele

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

- Reservation system:

[http://www.sci.muni.cz/~necas/new/index\\_saxs.html](http://www.sci.muni.cz/~necas/new/index_saxs.html)

- Local contact:

[klumpler@sci.muni.cz](mailto:klumpler@sci.muni.cz)

[marek@chemi.muni.cz](mailto:marek@chemi.muni.cz)