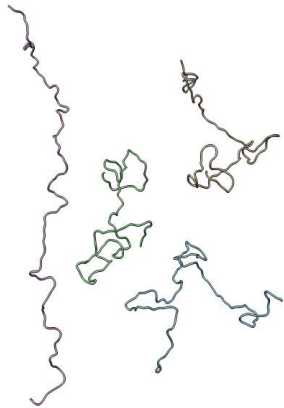
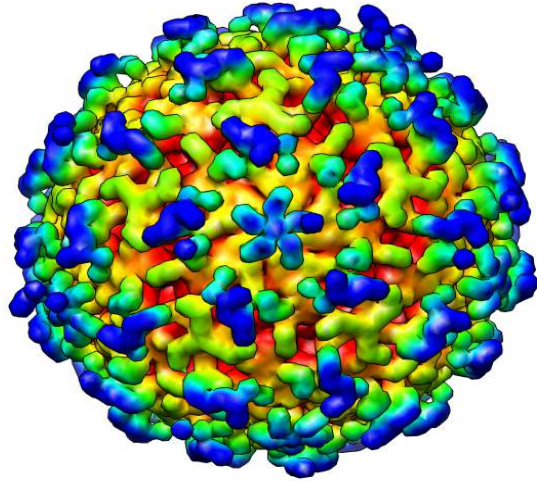


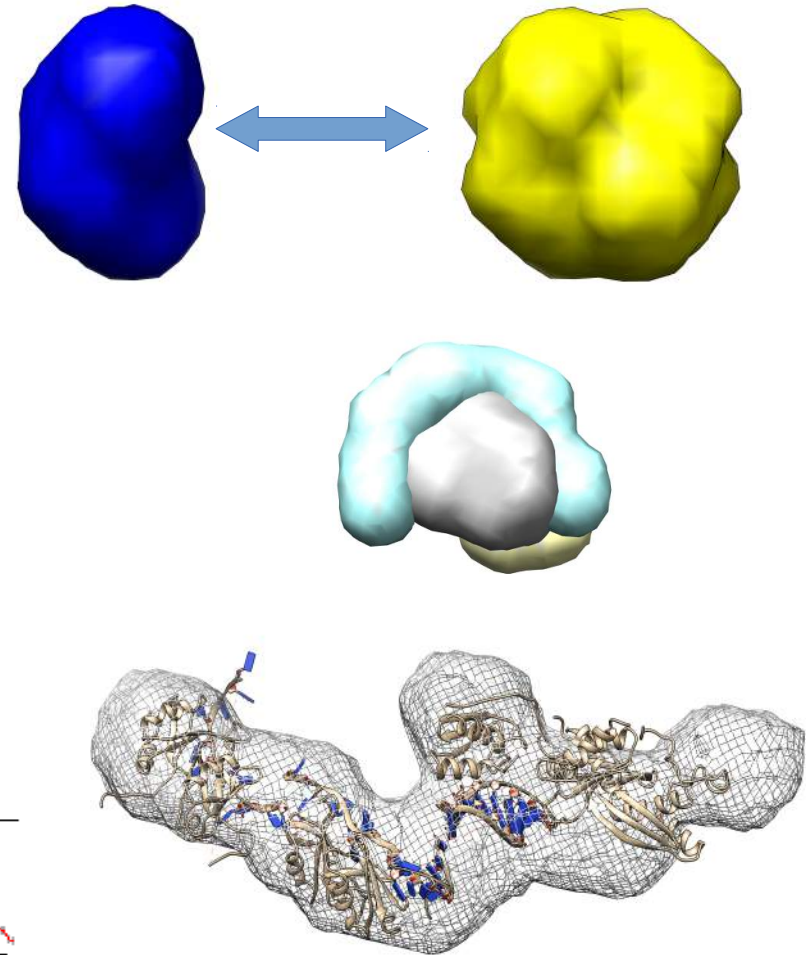
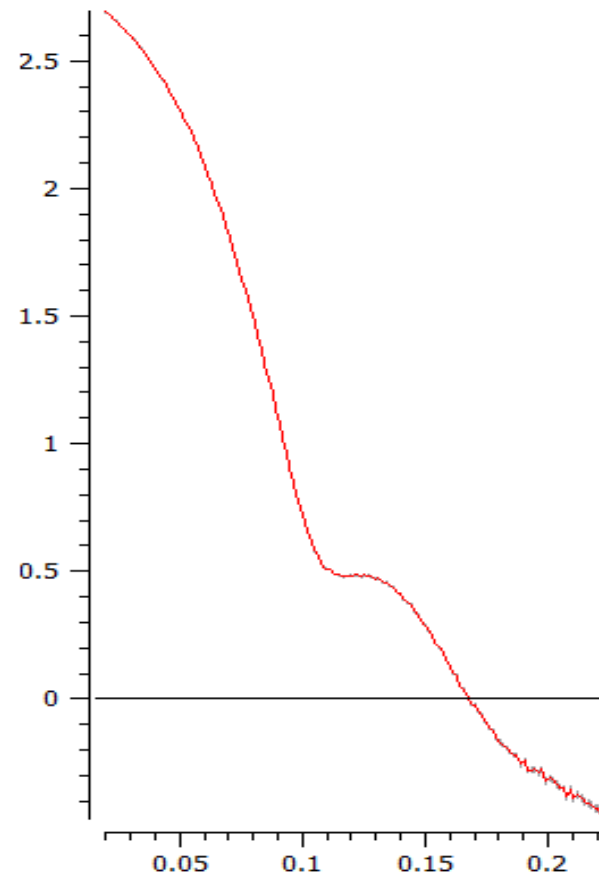
Biologický Small-Angle X-ray Scattering

v

CF RTG difrakce a bioSAXS,
CEITEC-MU, Brno



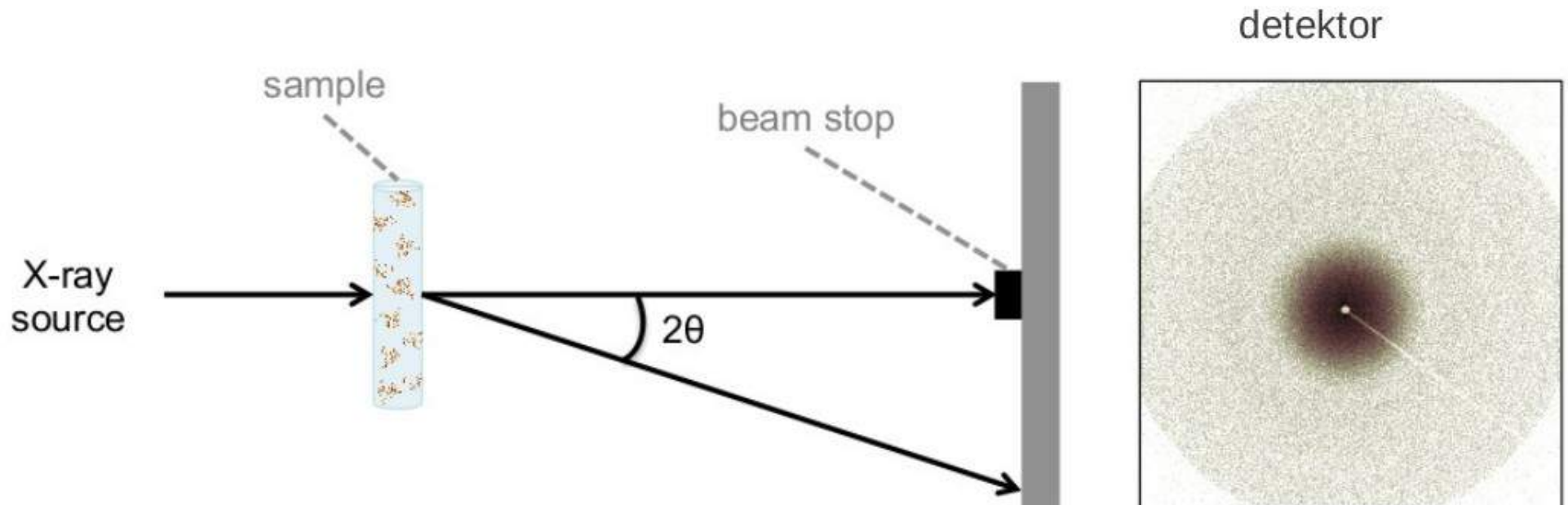
Tomáš Klumpler

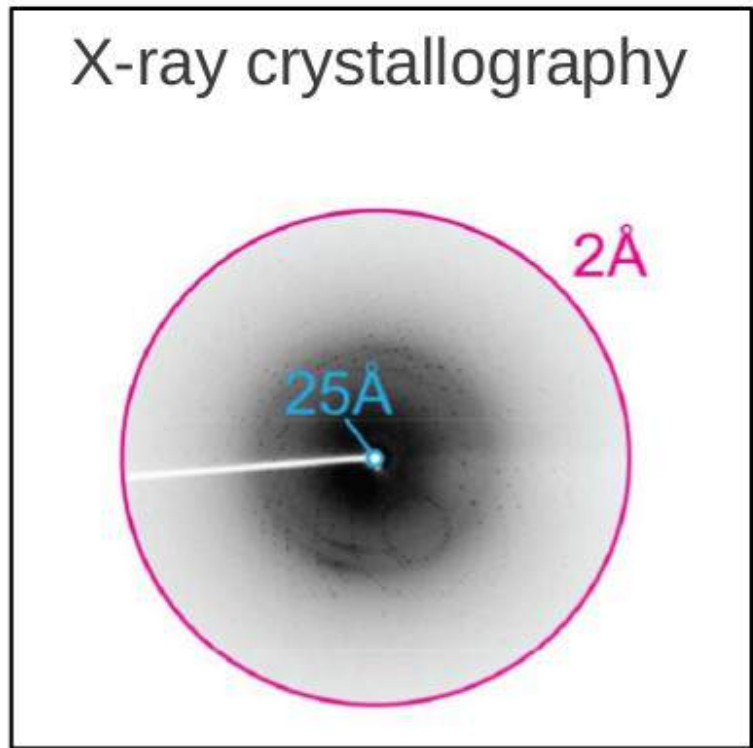
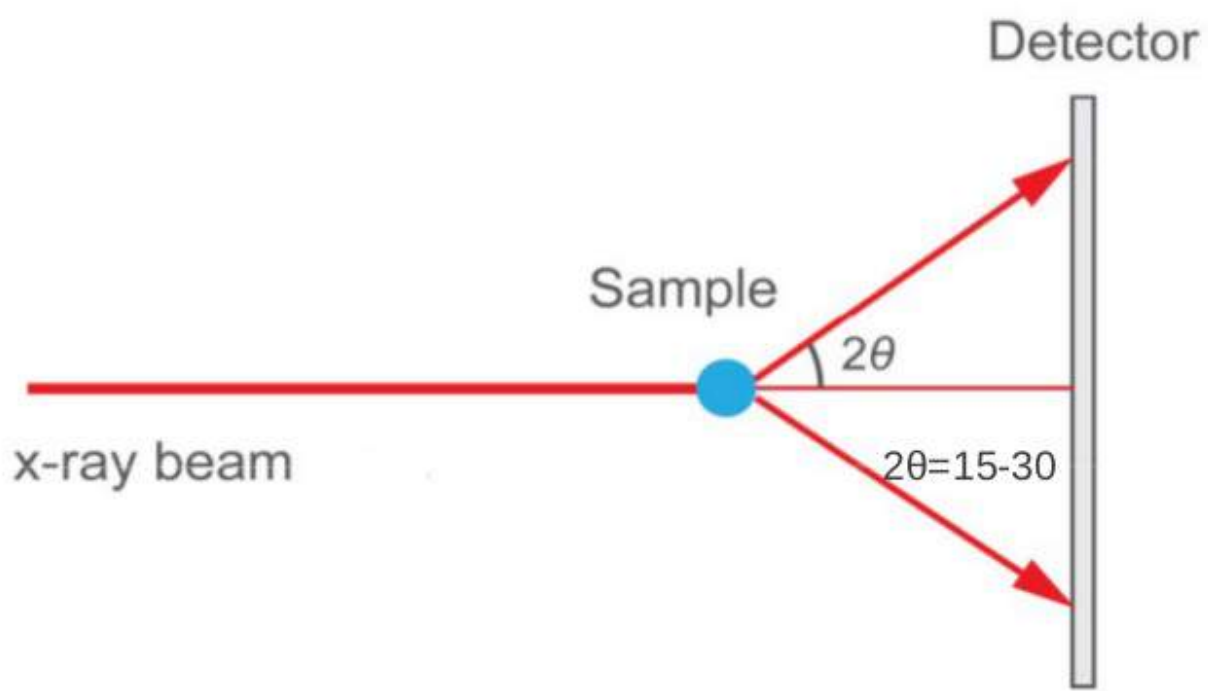


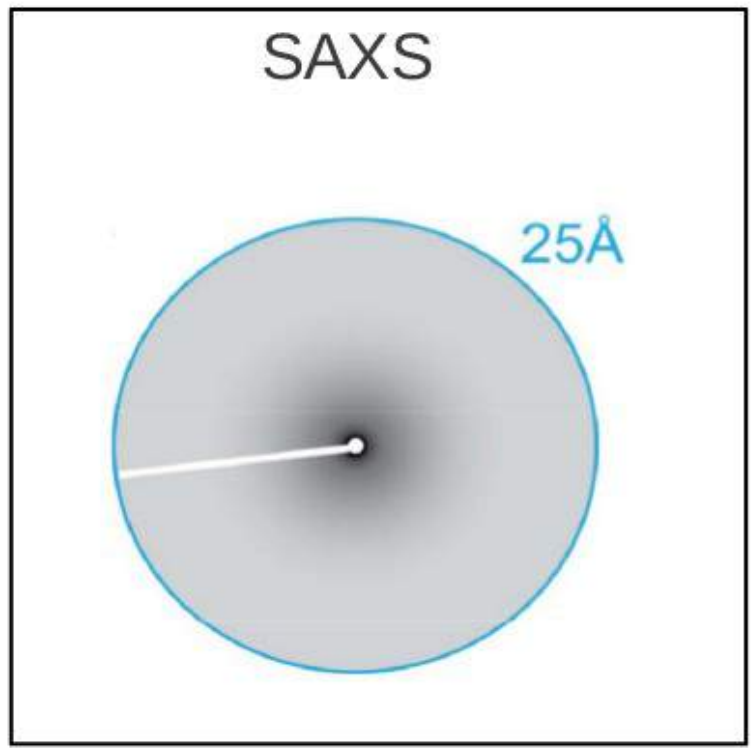
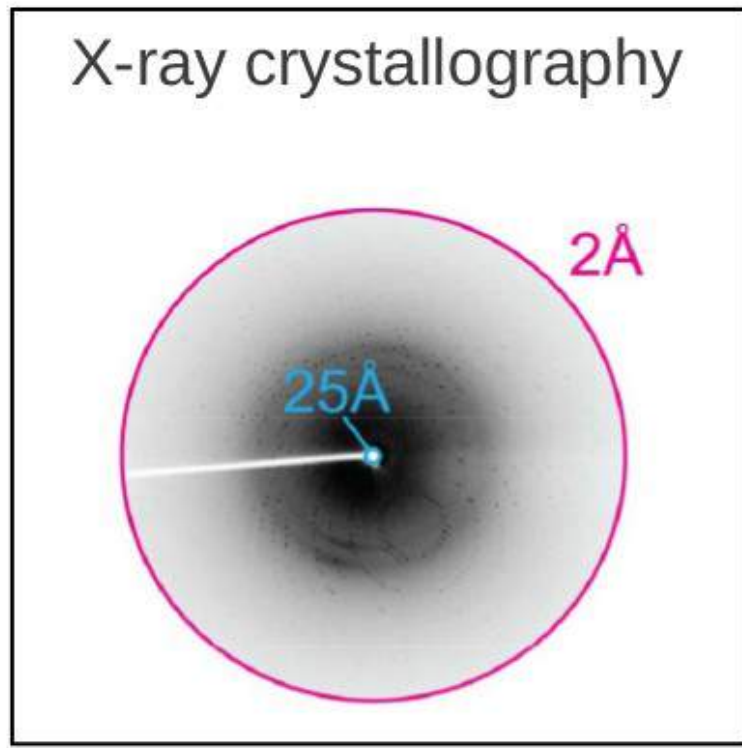
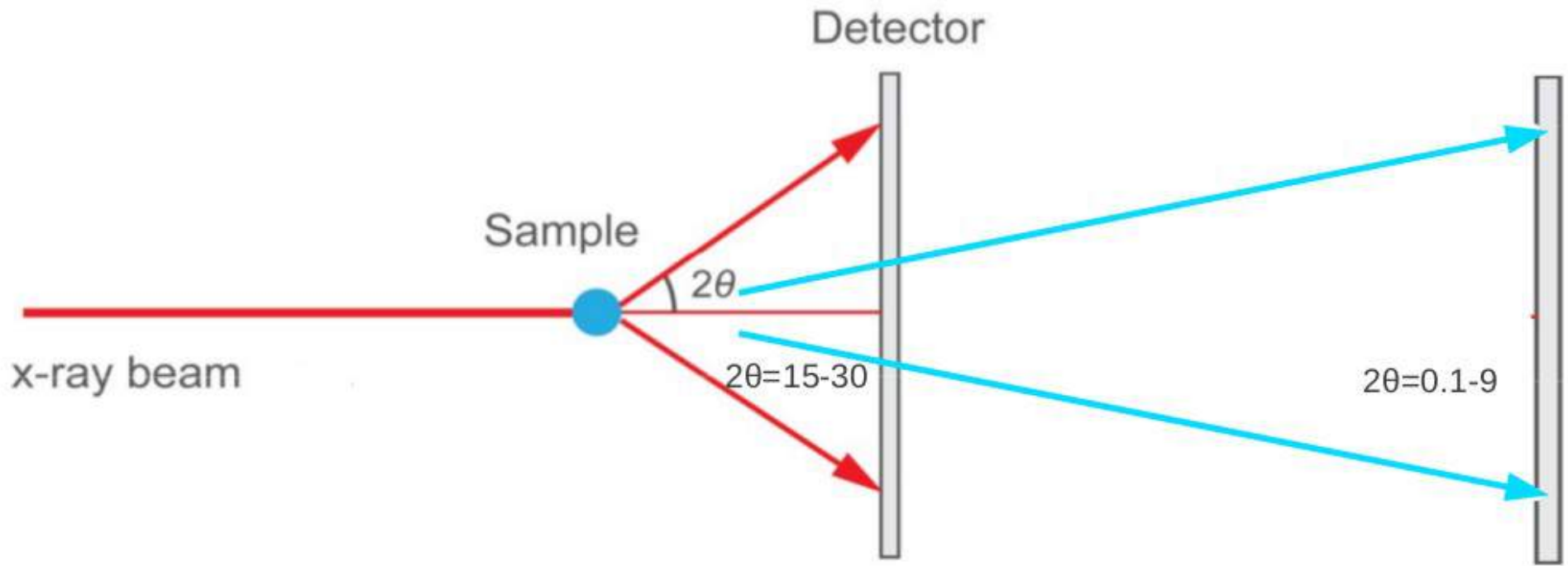
SAXS

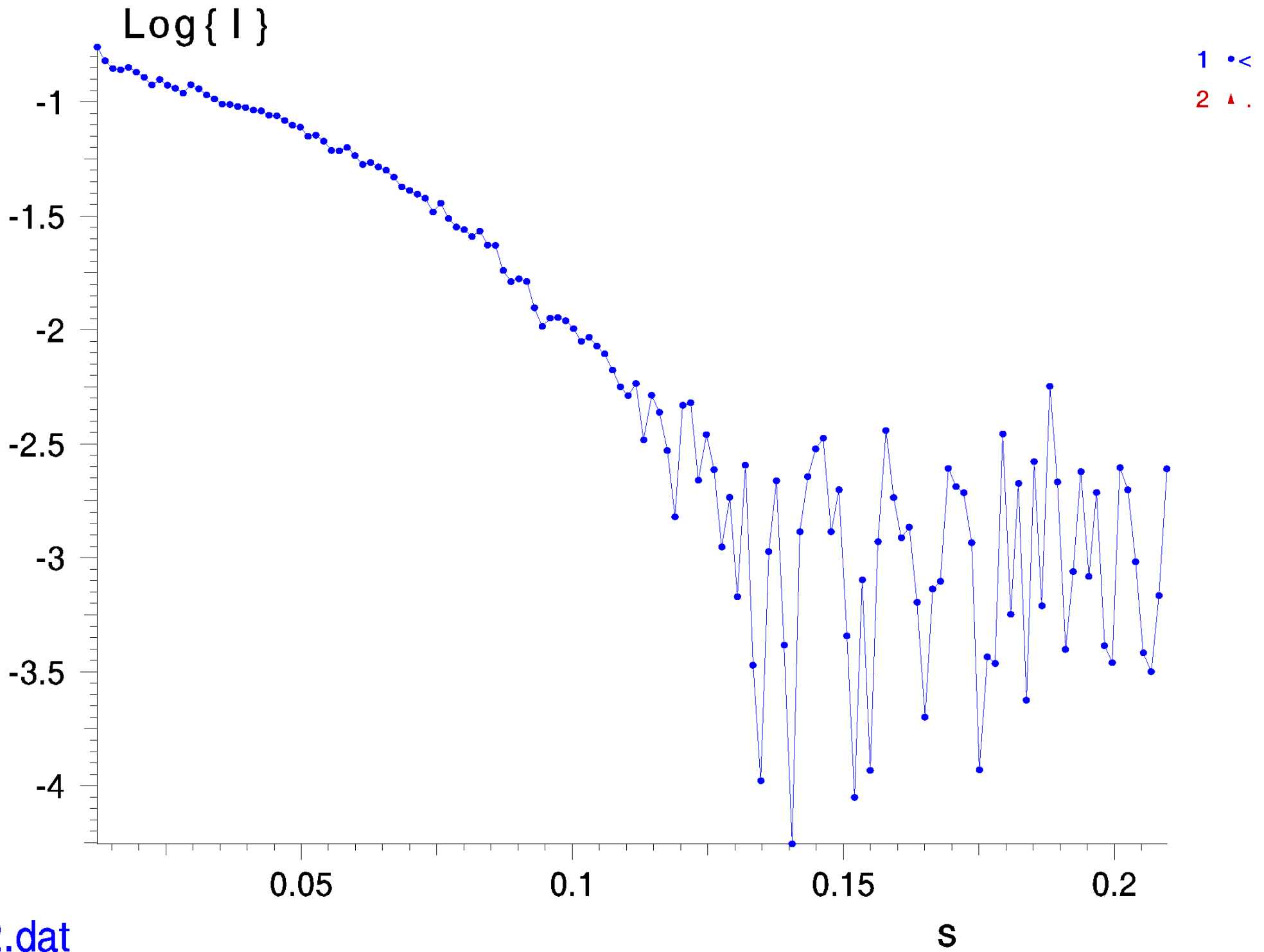
Small angle X-ray scattering

- rozptyl rentgenového záření v malých úhlech

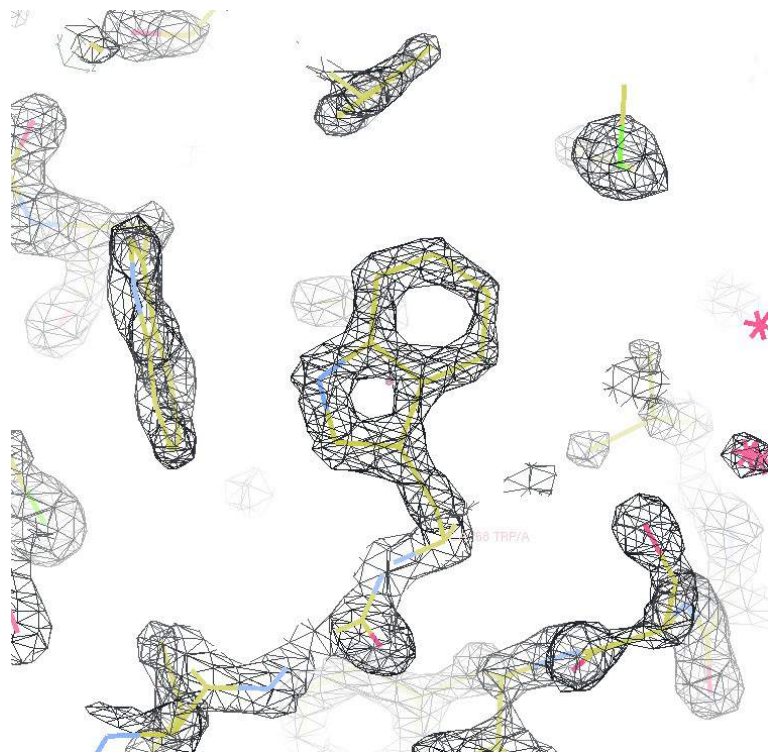






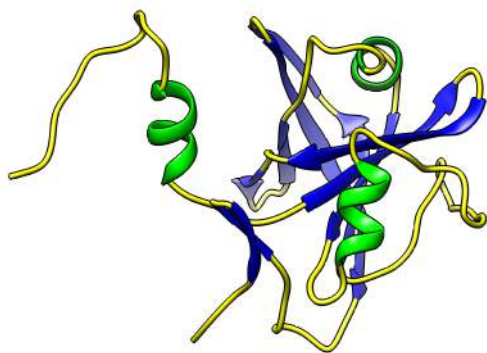
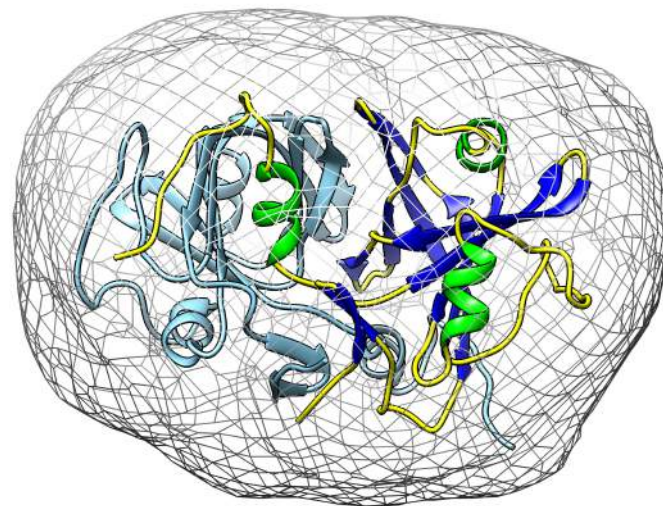


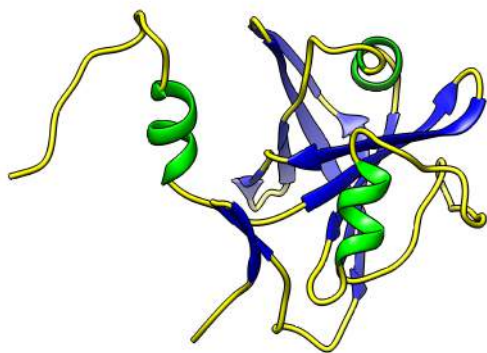
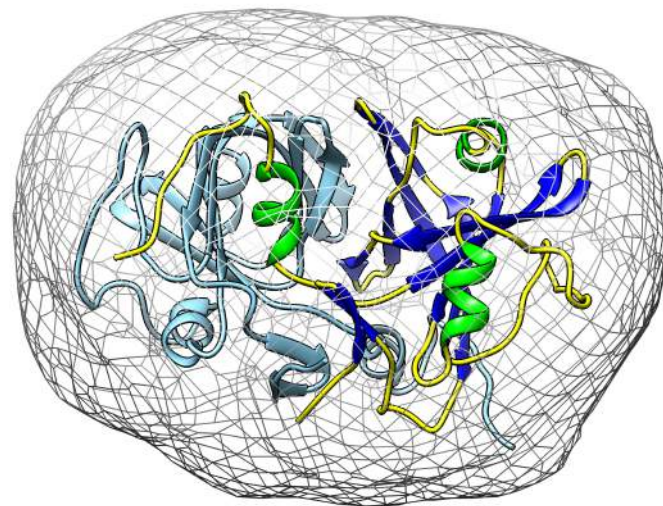
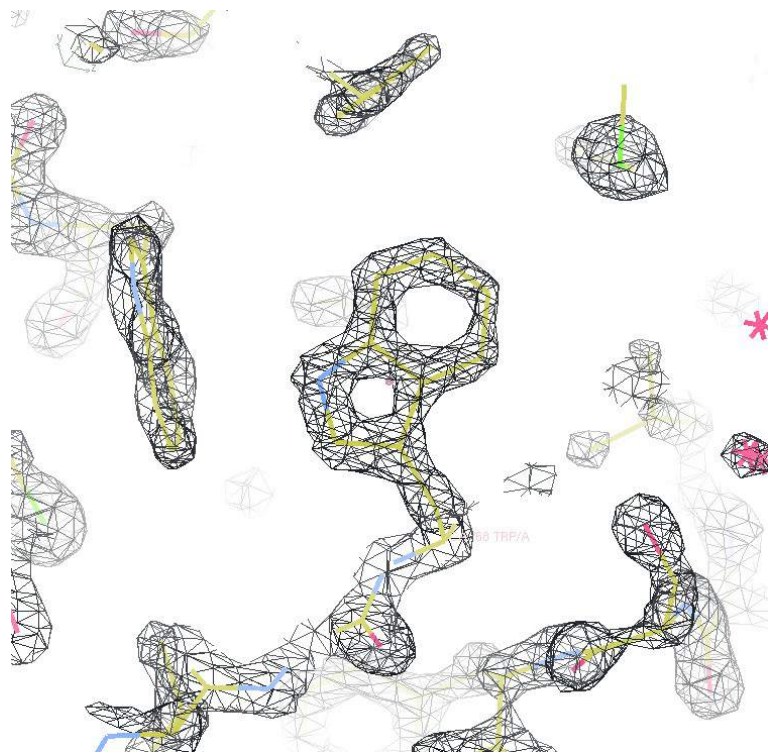
c2.dat



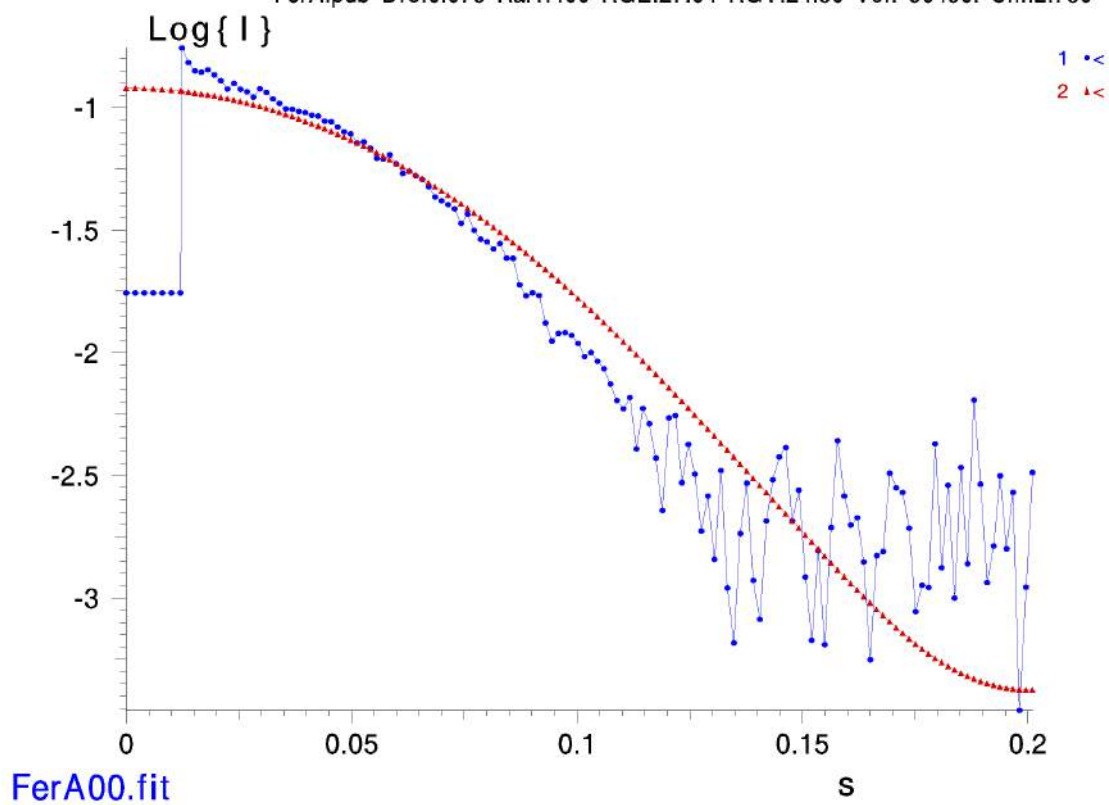
5A

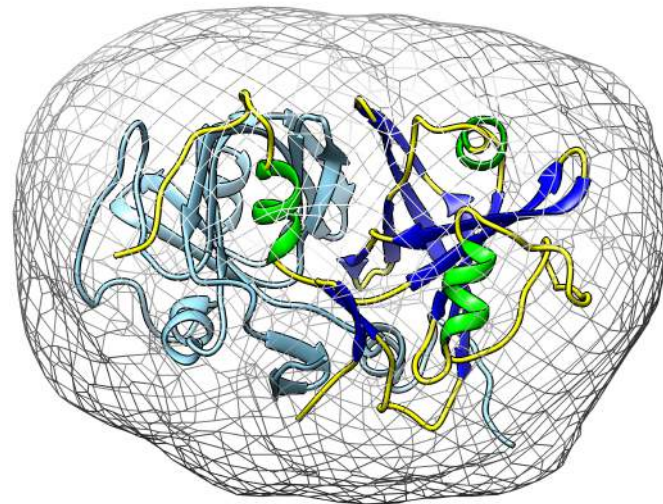
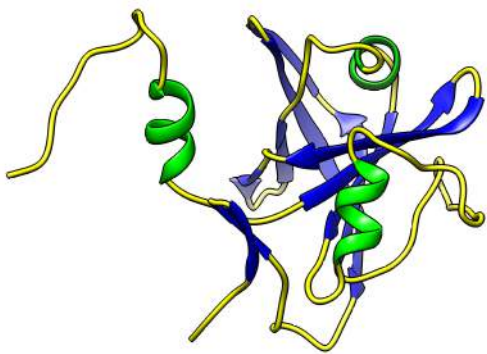
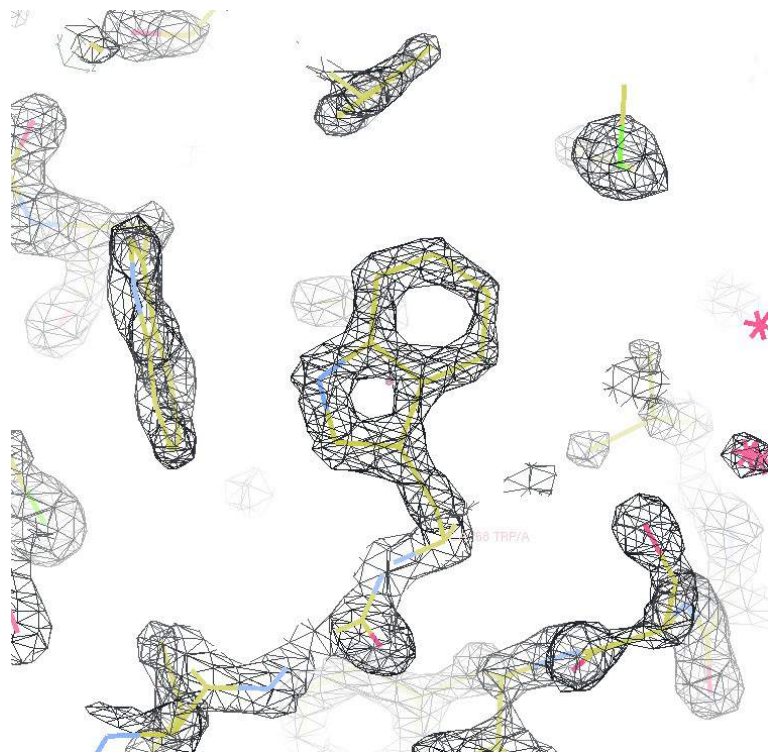
40A



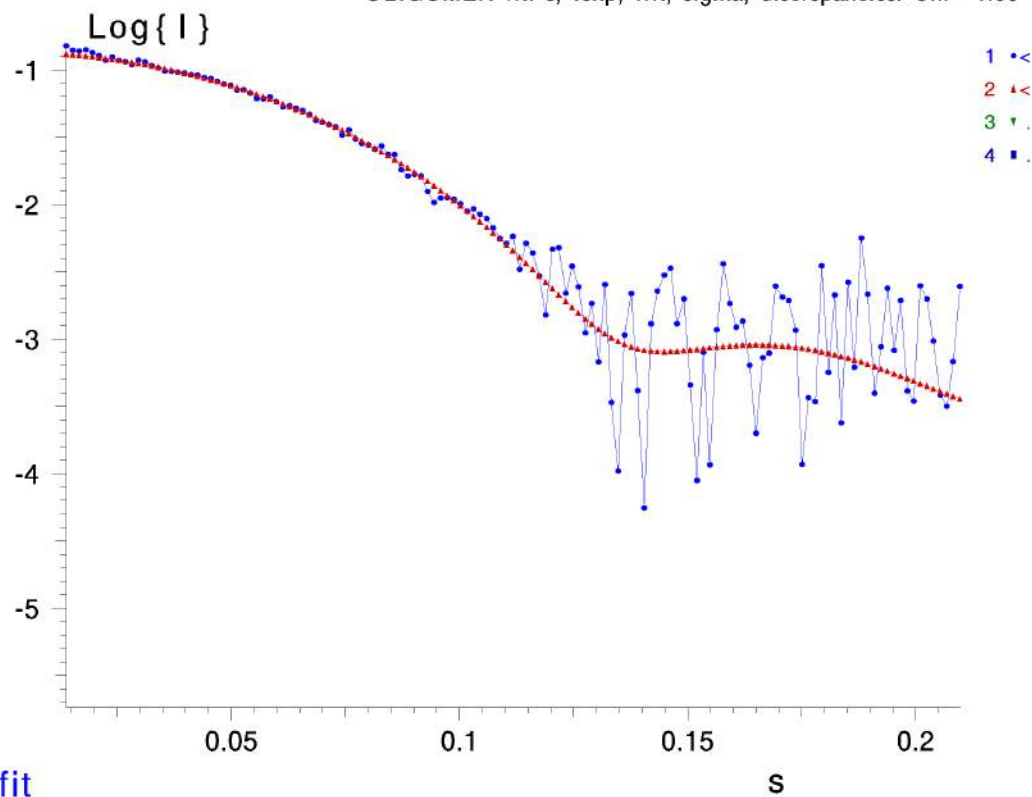


FerA.pdb Dro:0.075 Ra:1.400 RGE:27.64 RGT:24.30 Vol: 56456. Chi:2.730

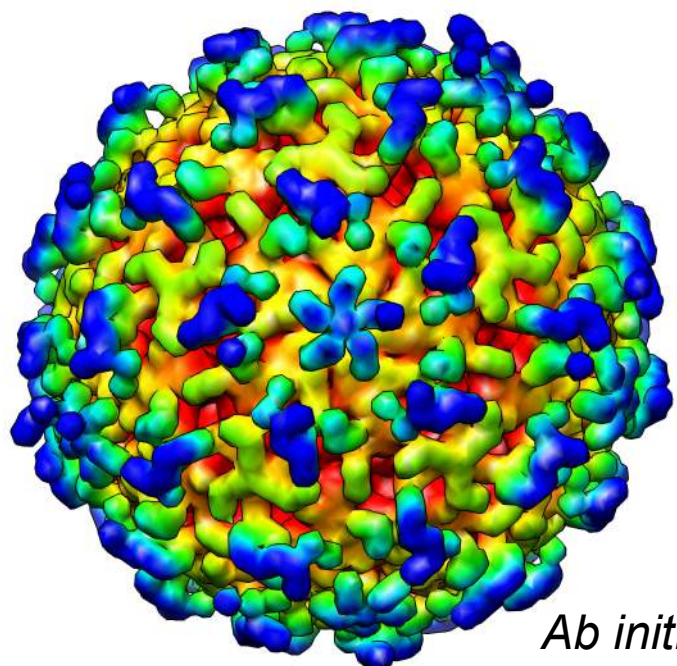




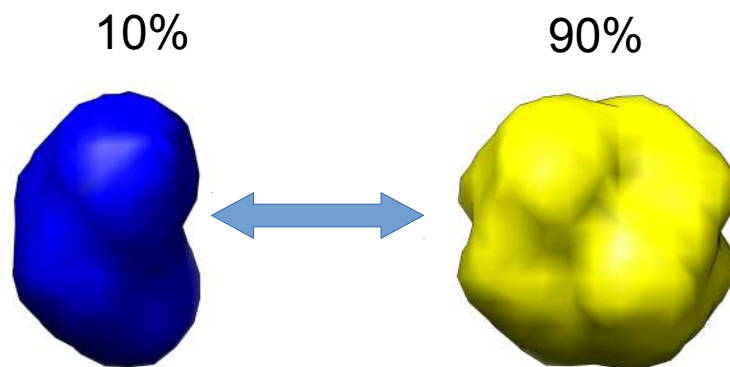
OLIGOMER fit: s, l_{exp}, l_{fit}, sigma, discrepancies. Chi= 1.06



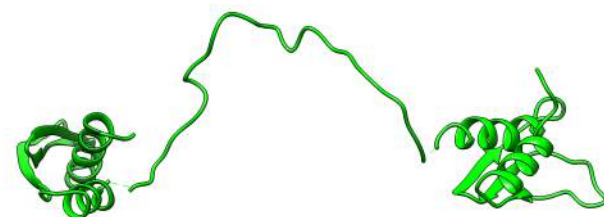
c2.fit



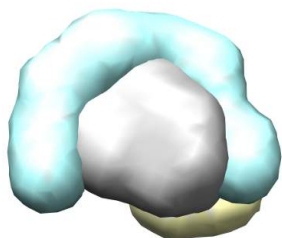
Ab initio



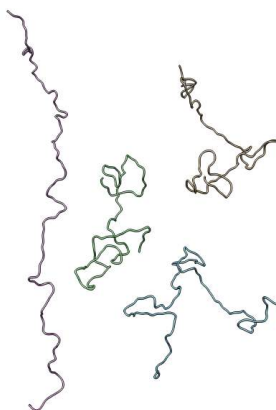
oligomeric state in solution/hierarchical structures



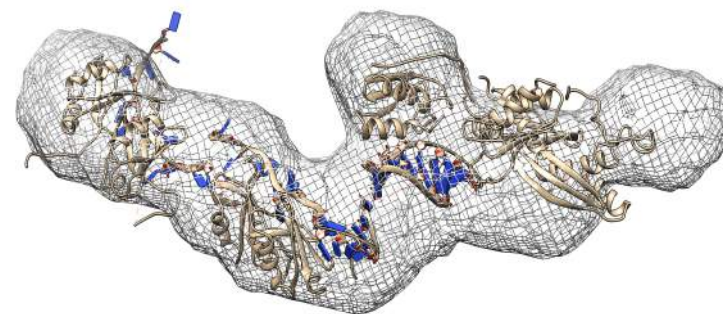
missing fragments



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_{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

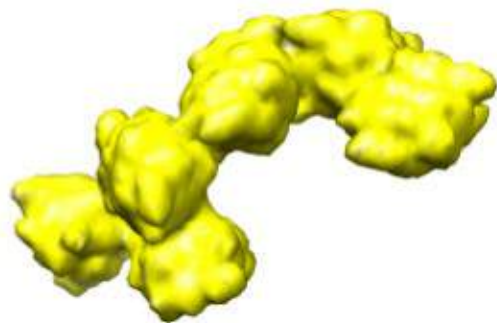
“low resolution structure” ~ bez číselné hodnoty
odpovídá mapě el. hustoty s rozlišením cca 20Å

Rozlišení

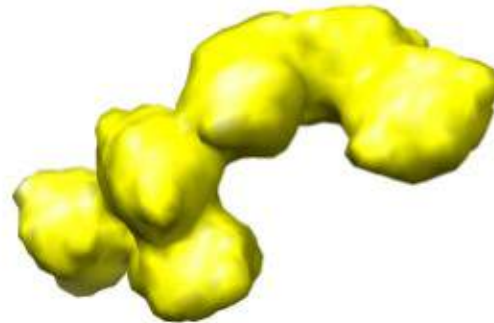
SAXS 10-20Å



5Å



10Å



15Å

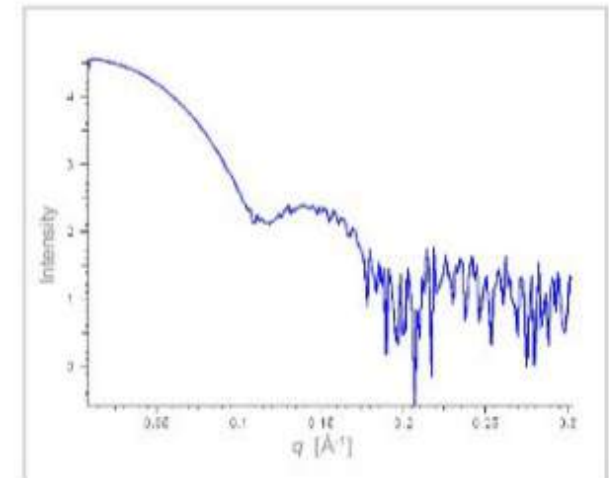
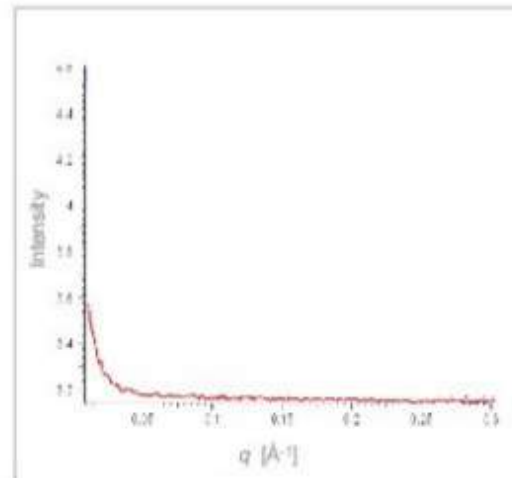
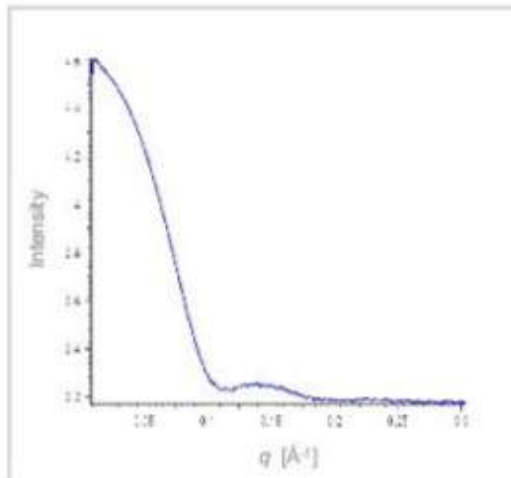
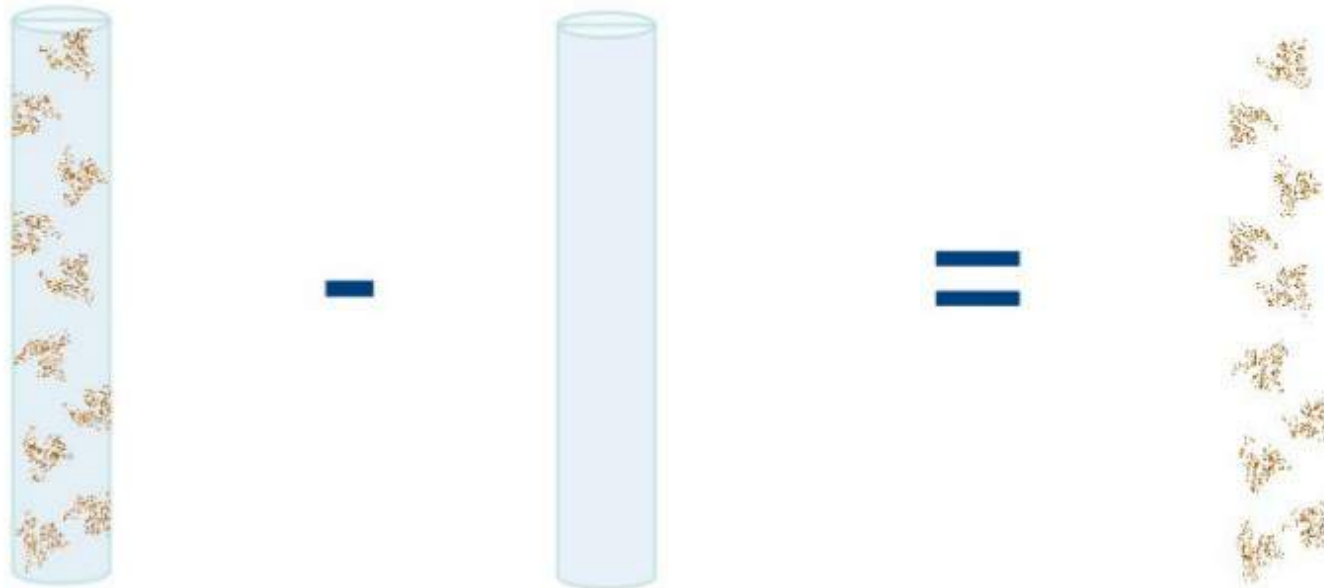


20Å

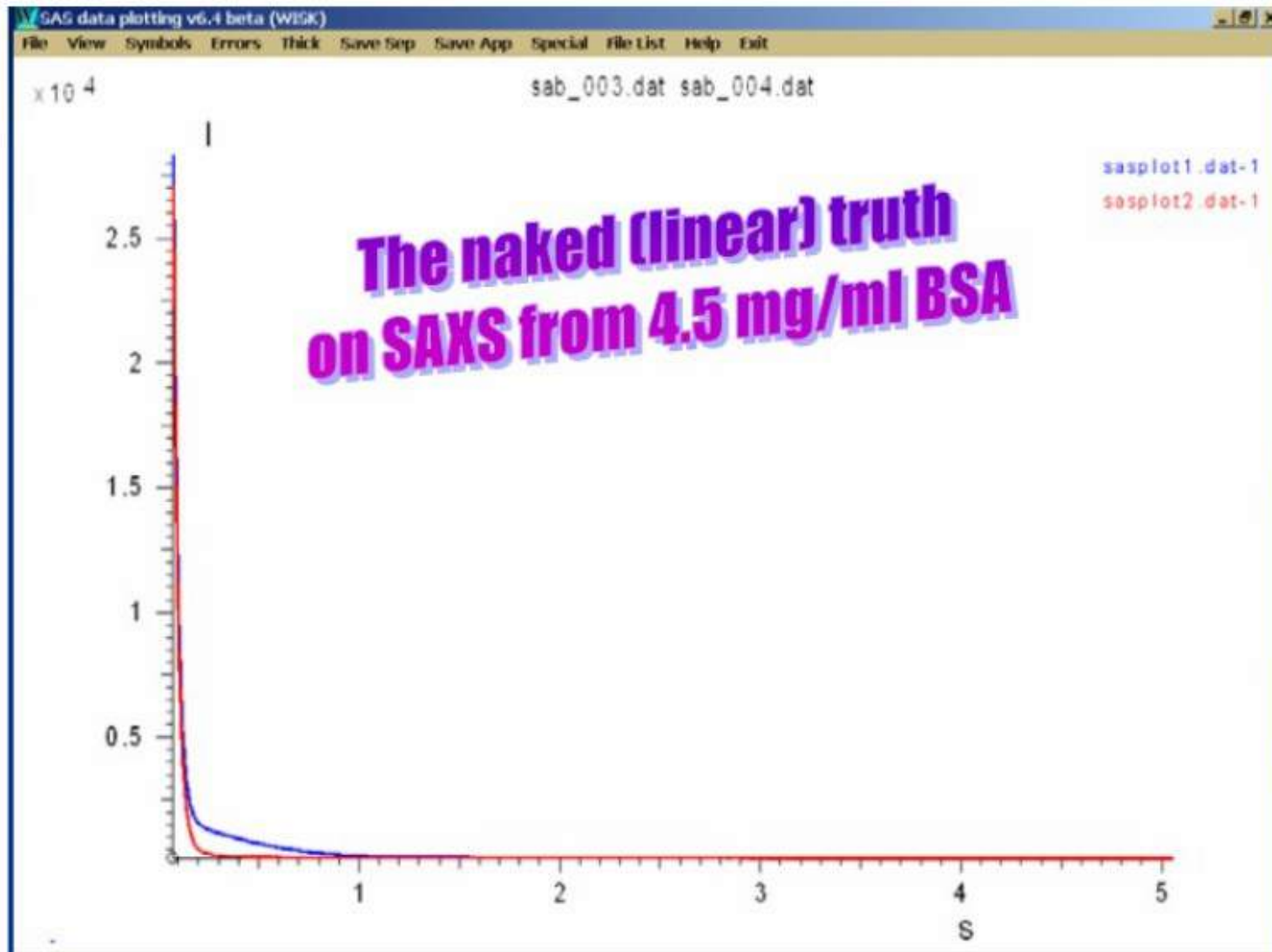
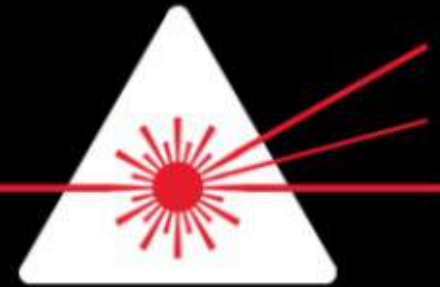
50Å

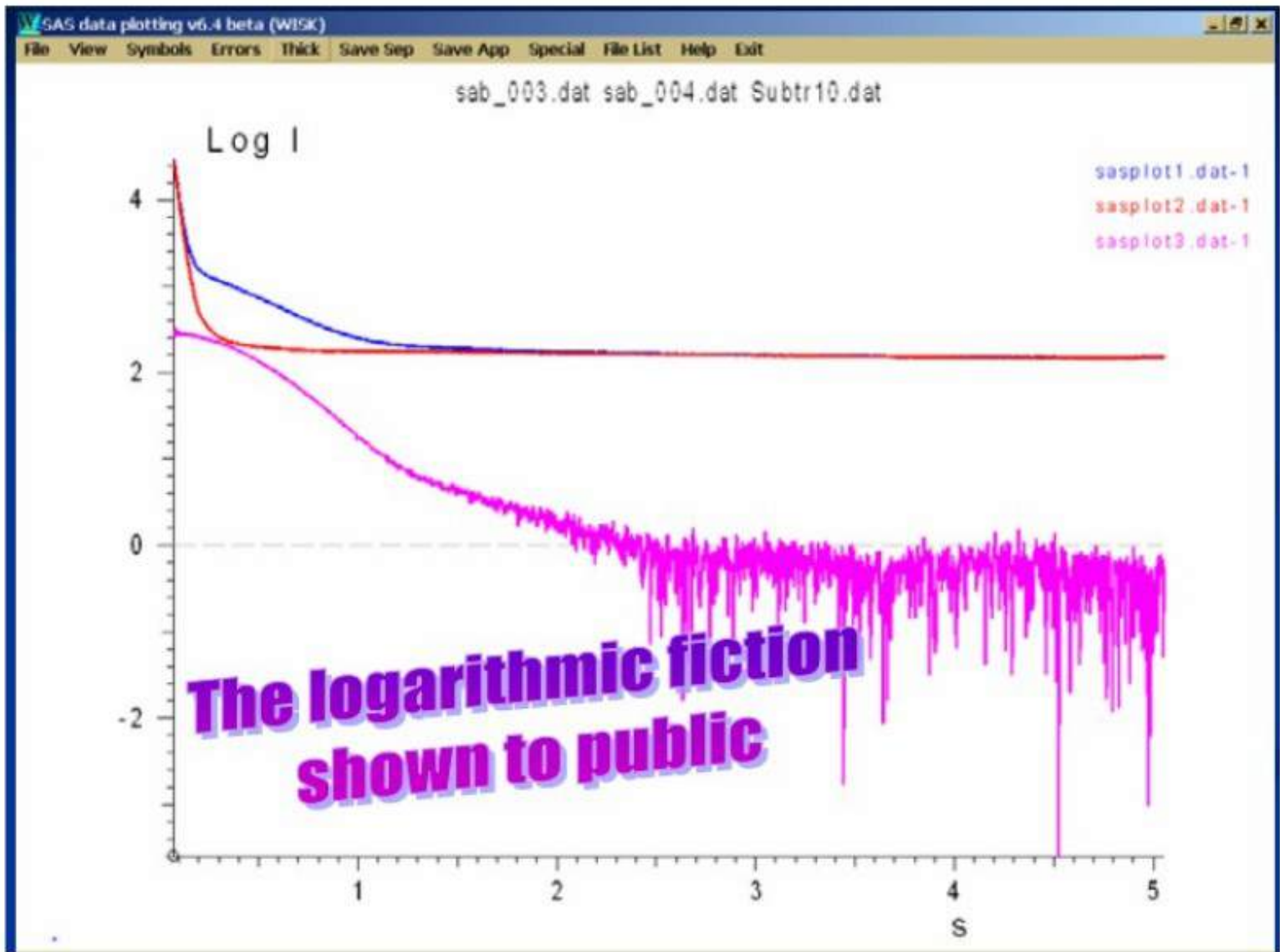
RTT complex ~7000atoms

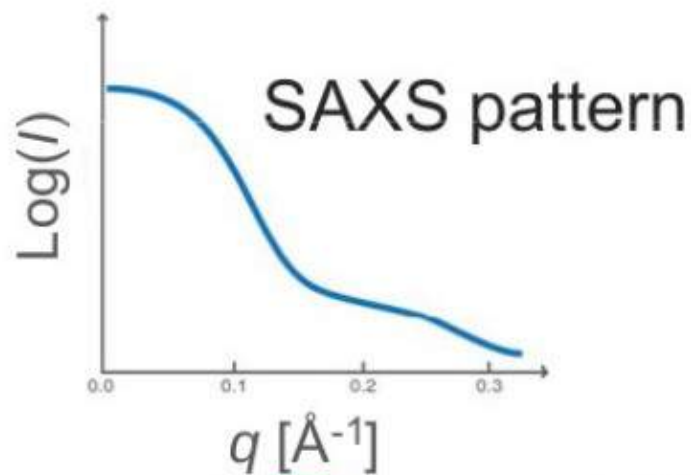
Odečtení příspěvku solventu



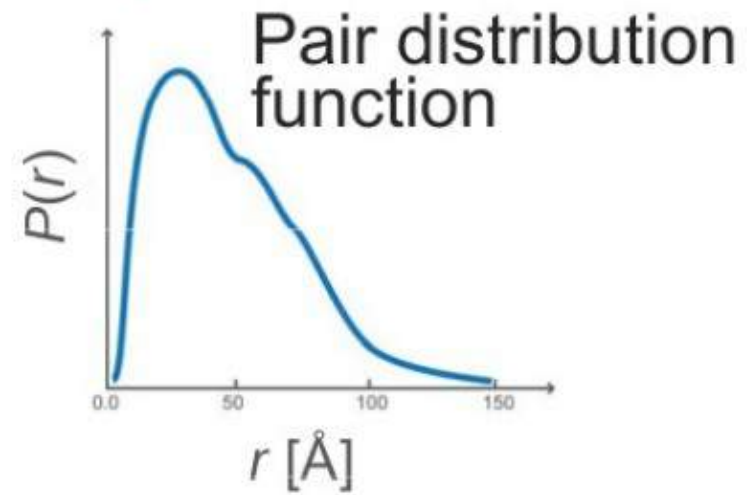
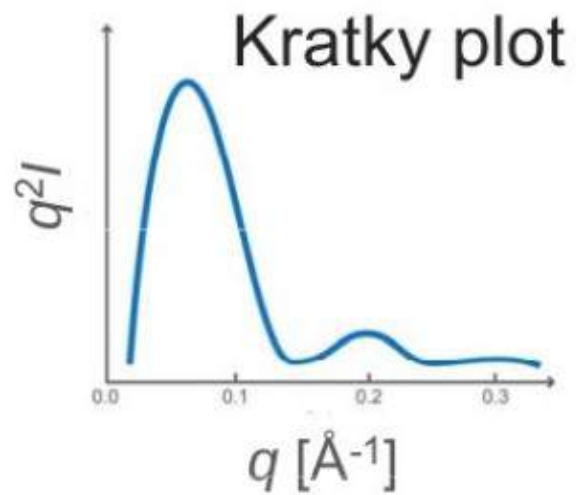
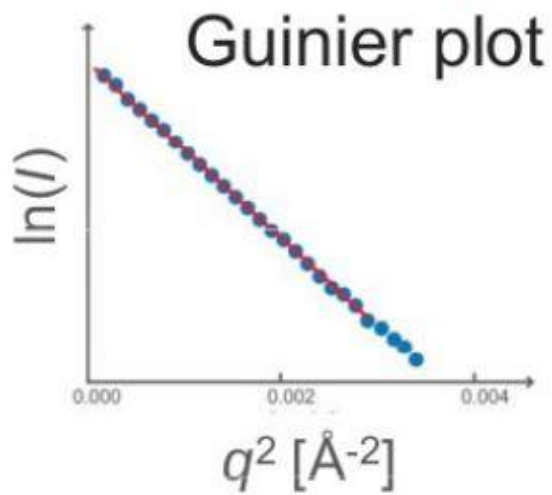
Looking At Nothing





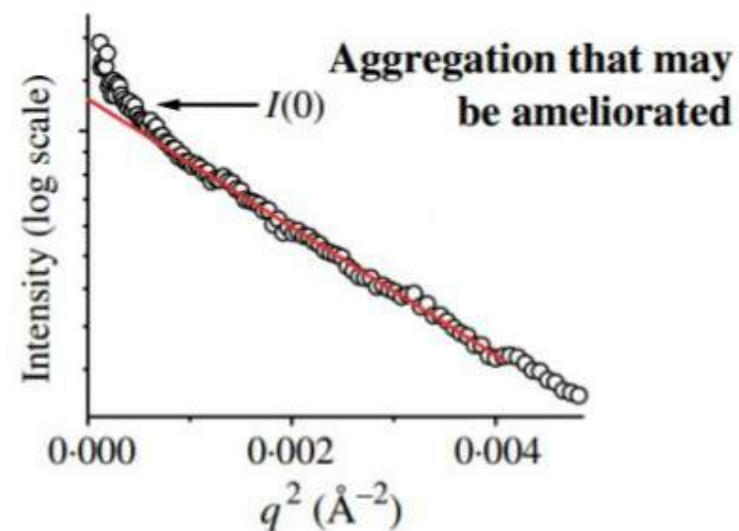
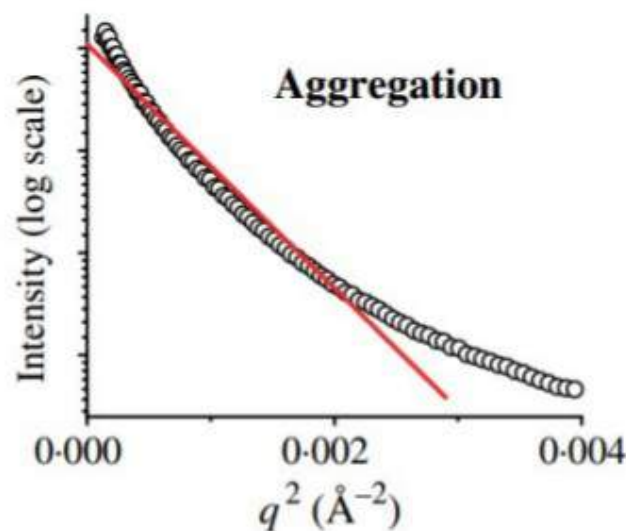
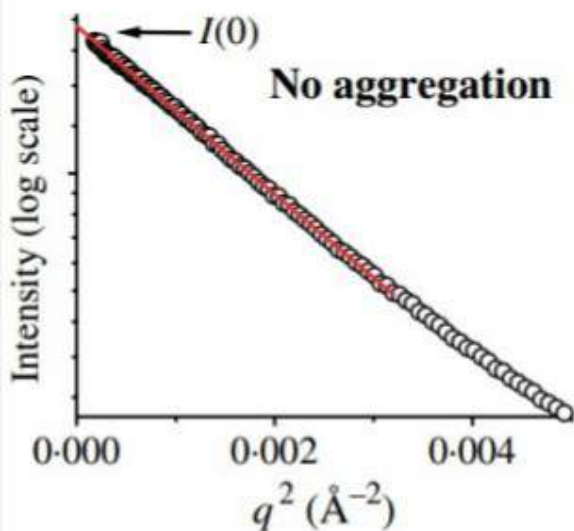


Fourier Transform



Guinier plot

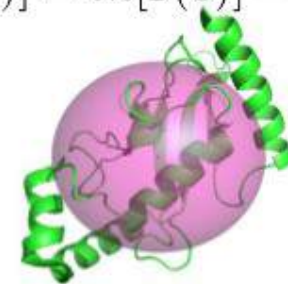
Analýza rozptylové křivky ve velmi malých úhlech



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

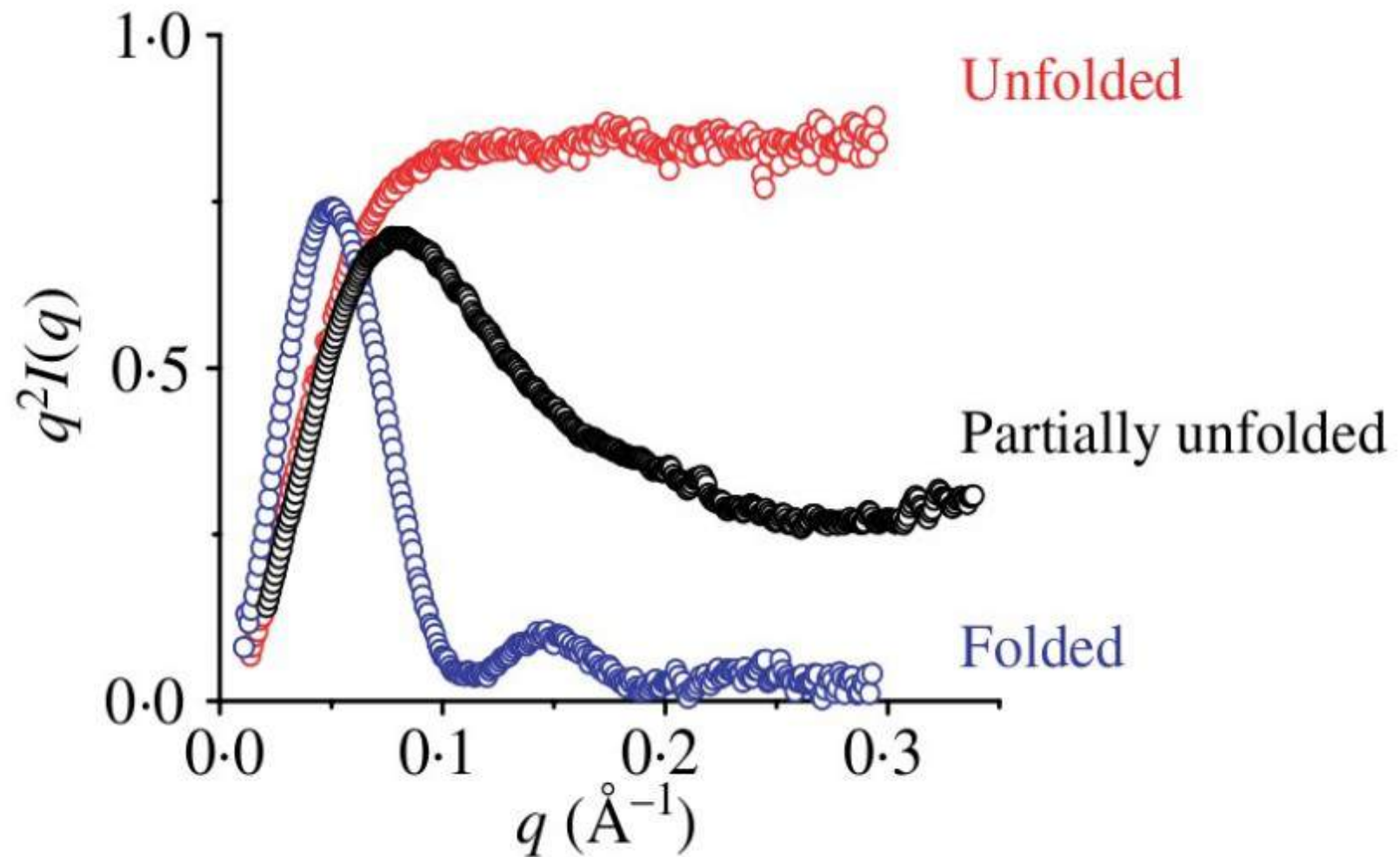
2. **Poloměr gyrace**

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



Kratky plot

Kompaktnost molekuly

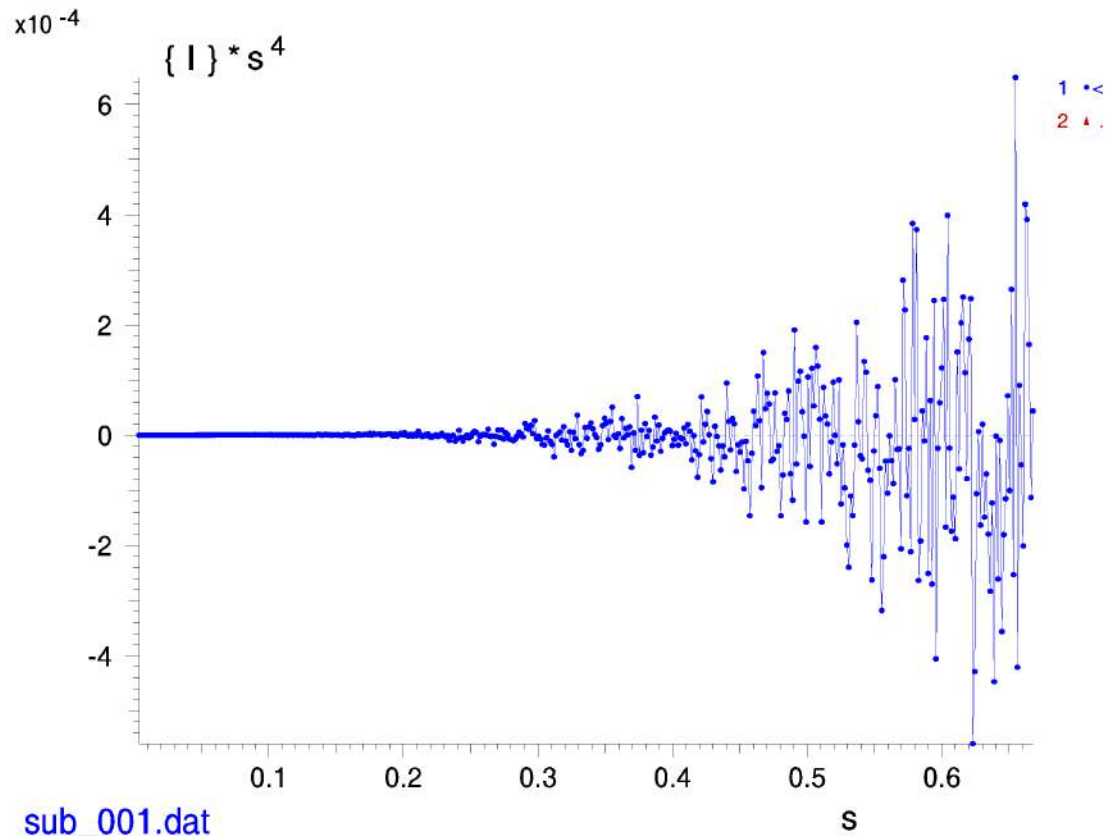


Porod volume

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

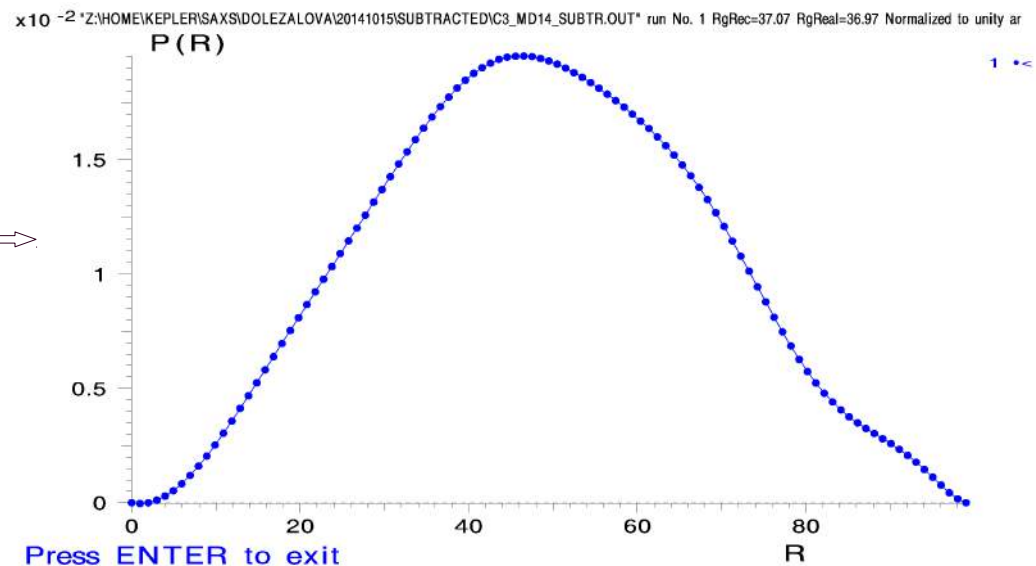
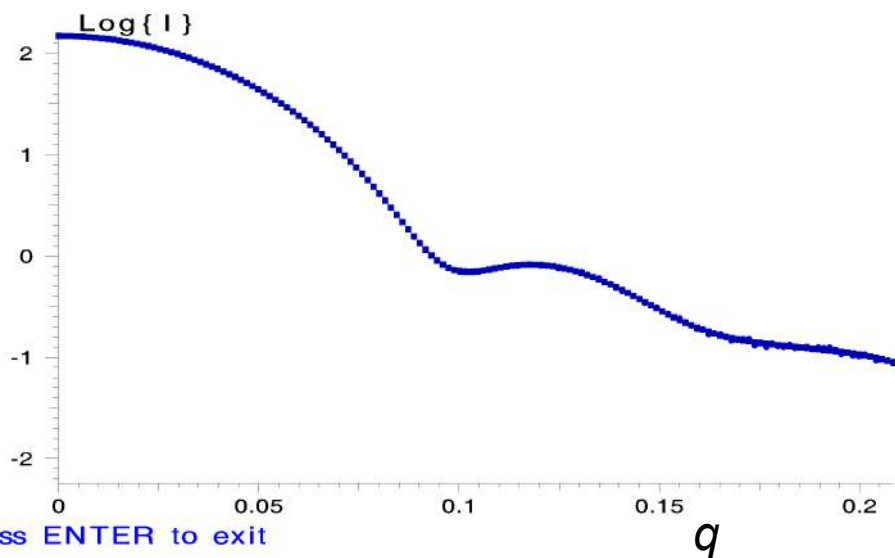
$$I(0) = (\Delta\rho V)^2$$

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

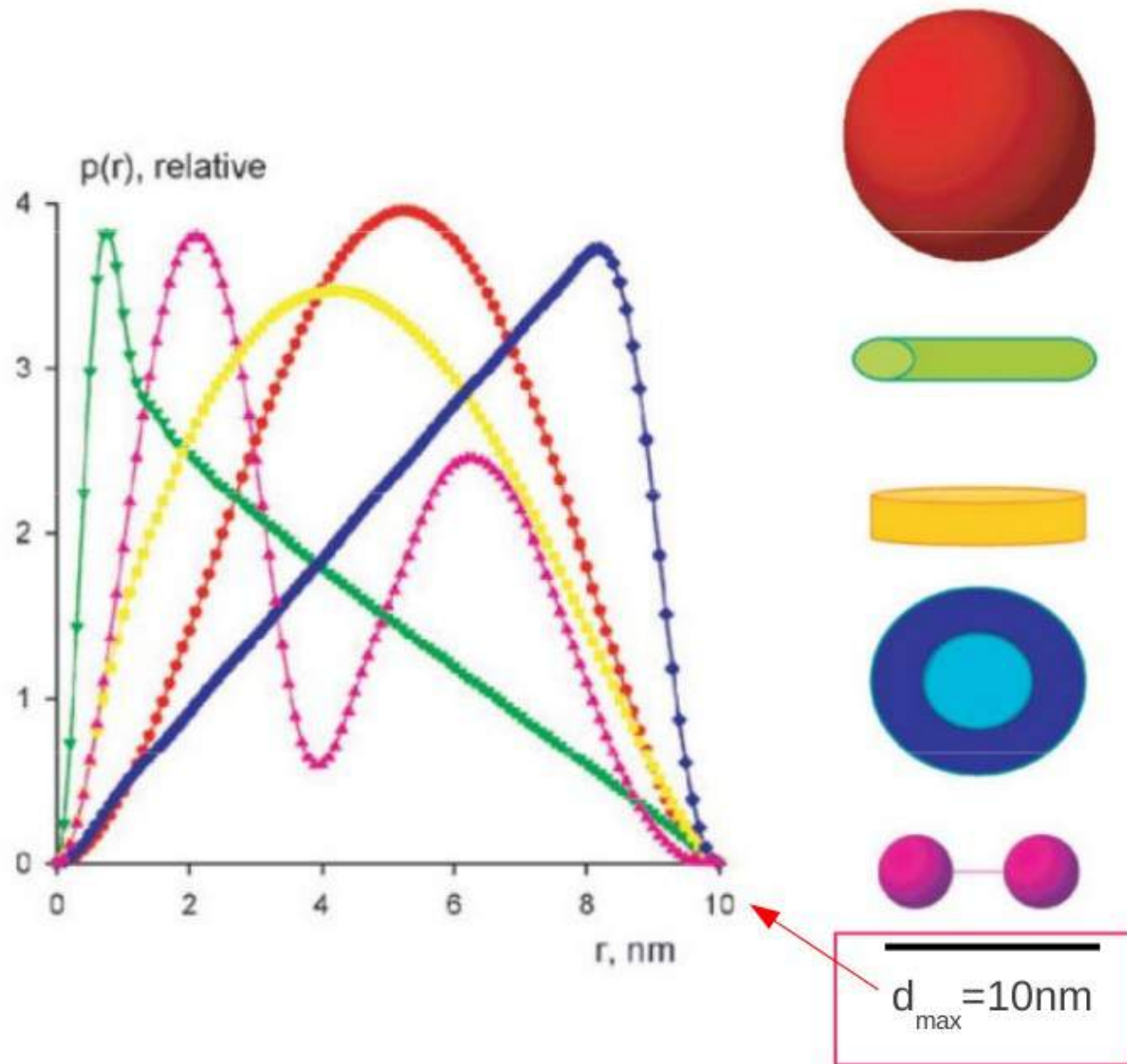


Pair distribution function

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



Pair distribution function

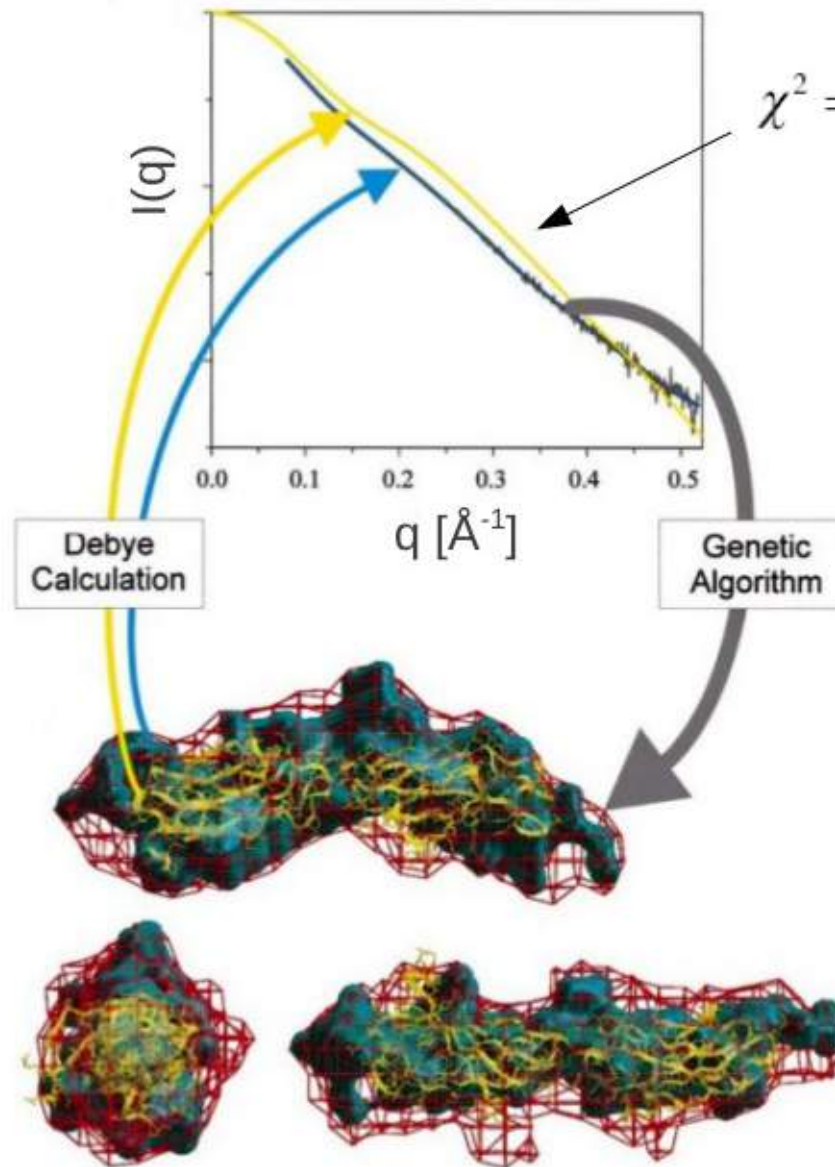


SAXS modelování tvaru *ab initio*

test shody:

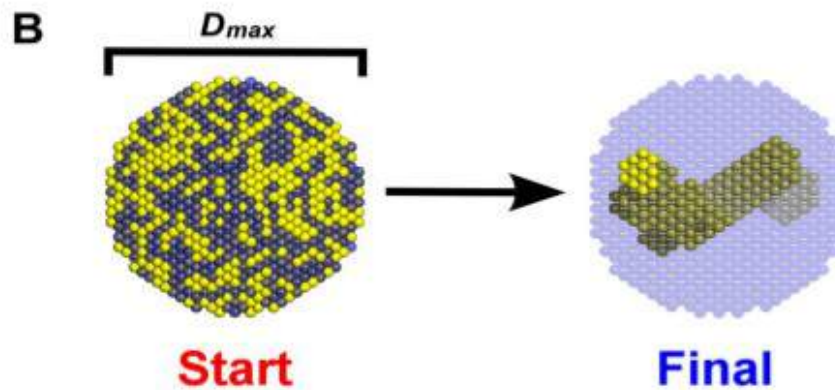
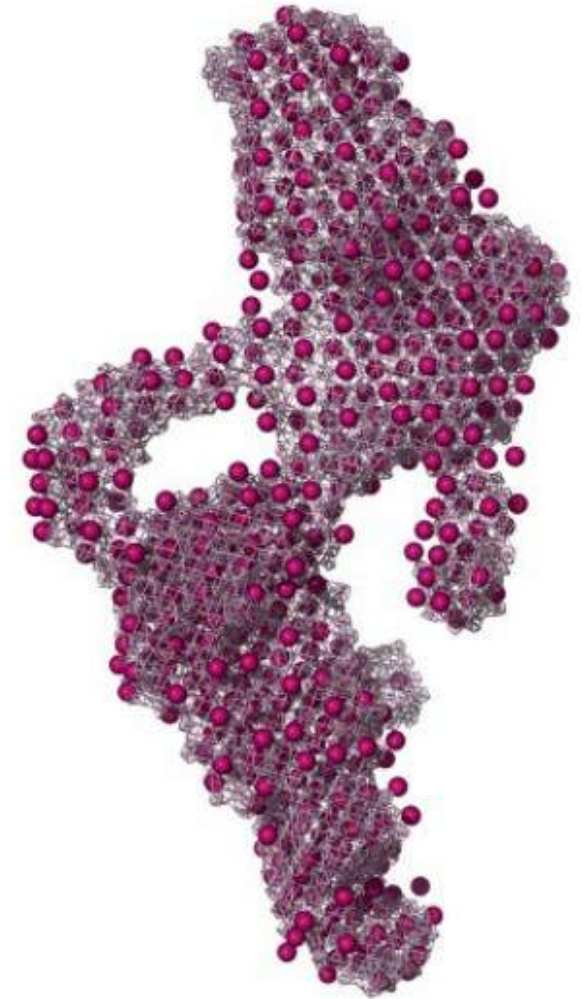
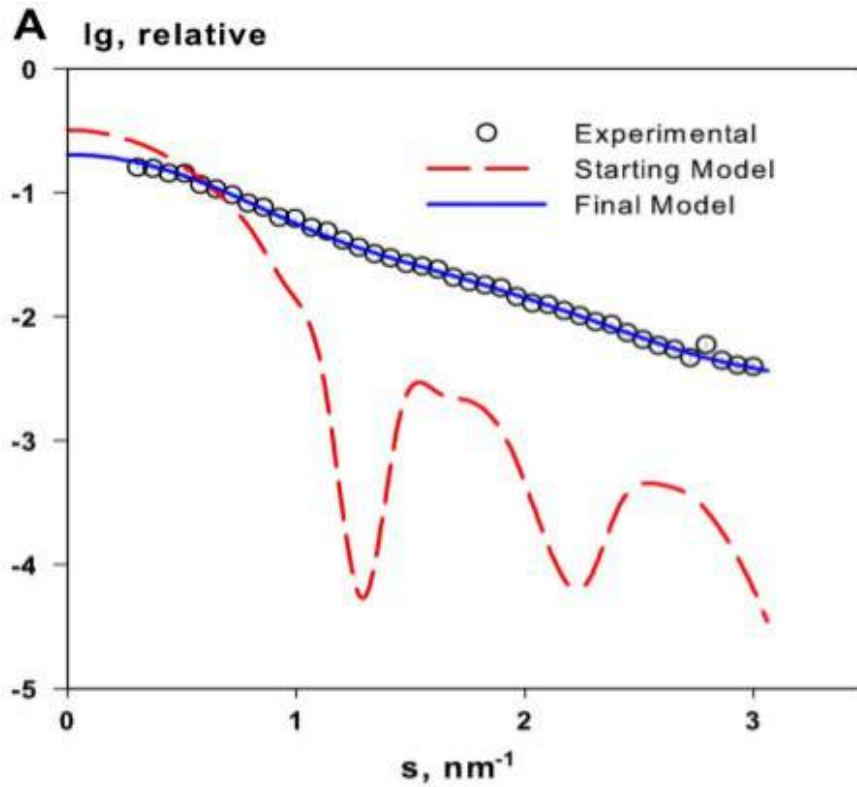
$$\chi^2 = \sum_{k=1}^Q \left(\frac{I_{\text{exp}}(q_k) - c \cdot I_{\text{m}}(q_k)}{\sigma_{\text{exp}}(q_k)} \right)^2$$

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



SAXS modelování tvaru

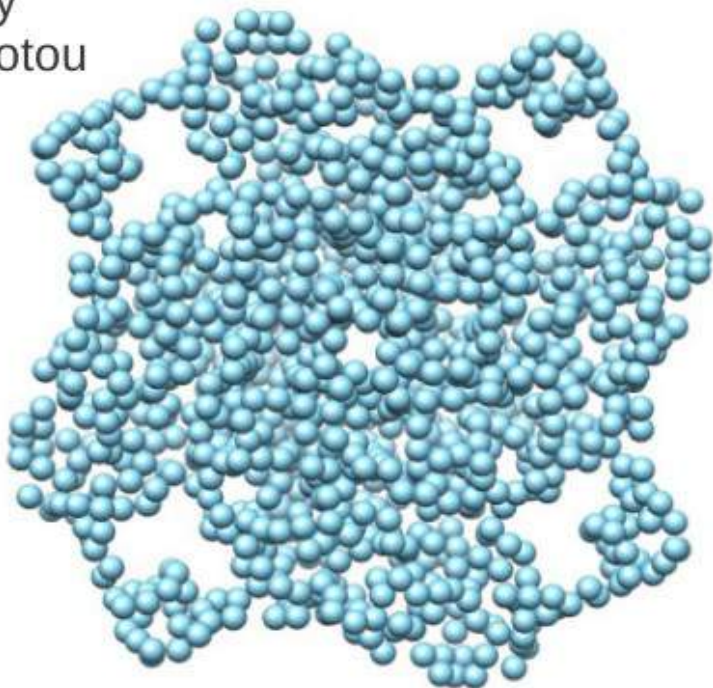
Program DAMMIN



SAXS modelování tvaru

Program GASBOR

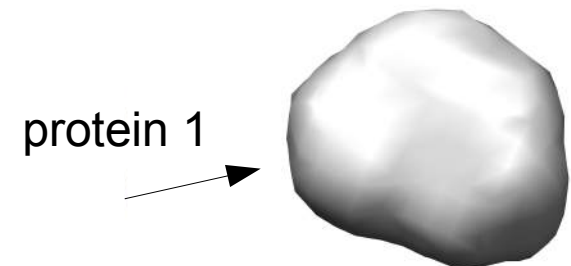
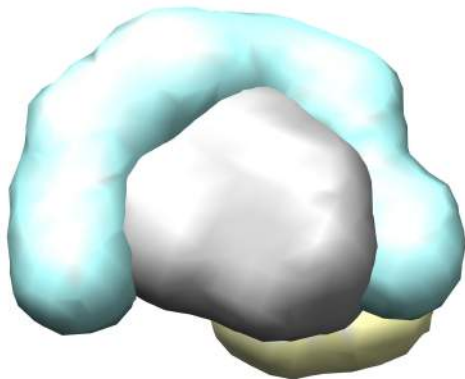
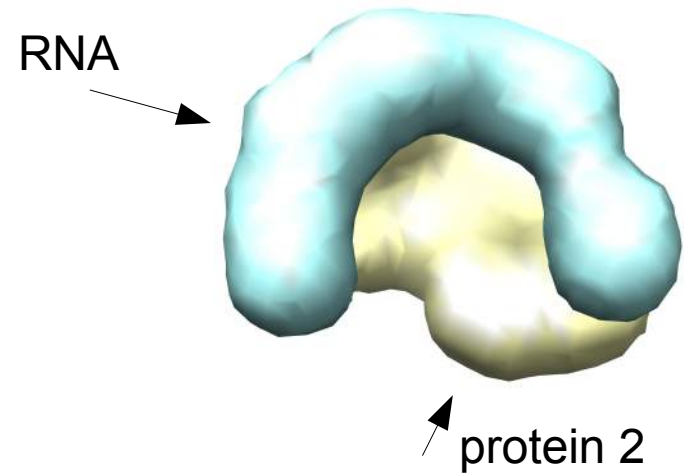
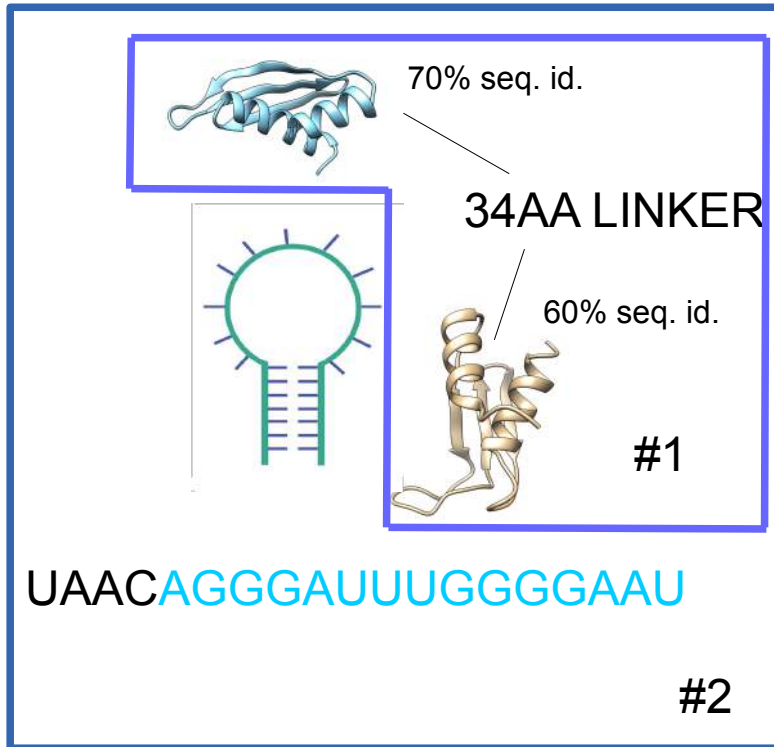
Chain-like GASBOR model složen z univerzálních residuí (Dummy residues) s průměrnou el. hustotou a rozestupy ~ 0.38 nm



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

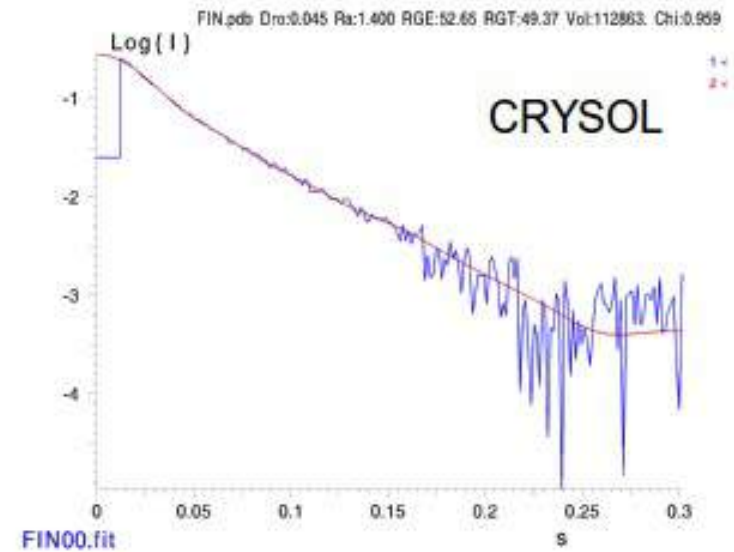
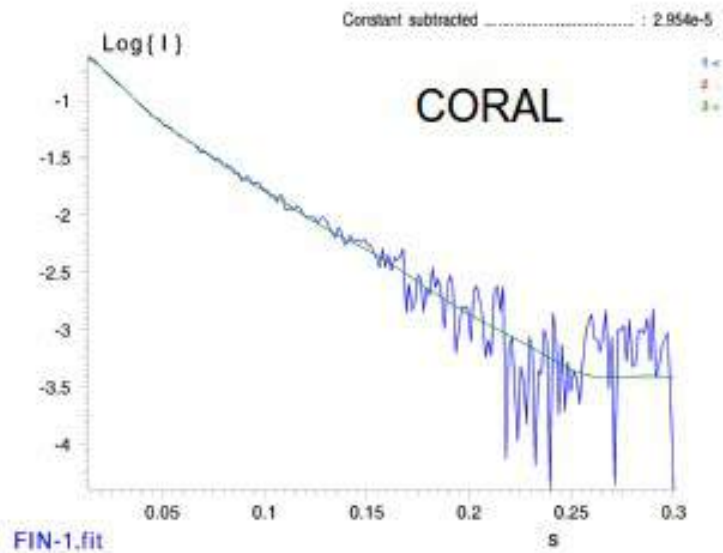
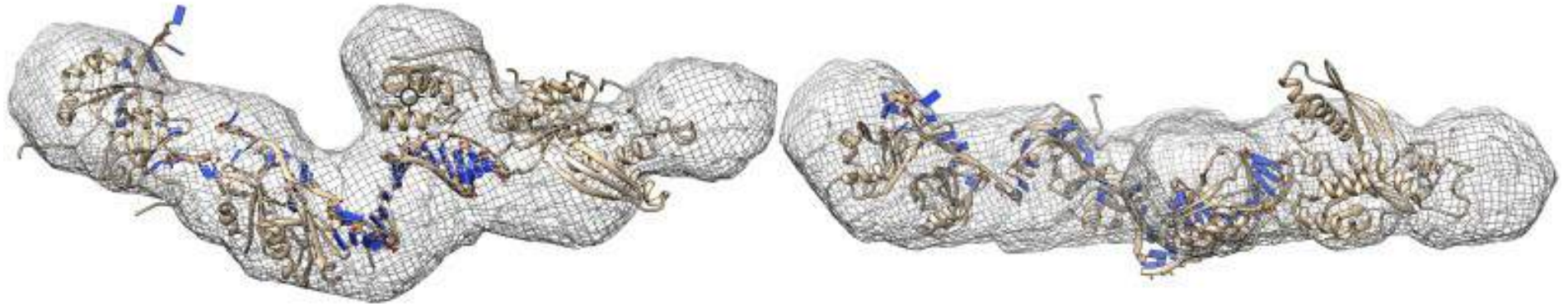
SAXS modelování tvaru

multi-phase program MONSA



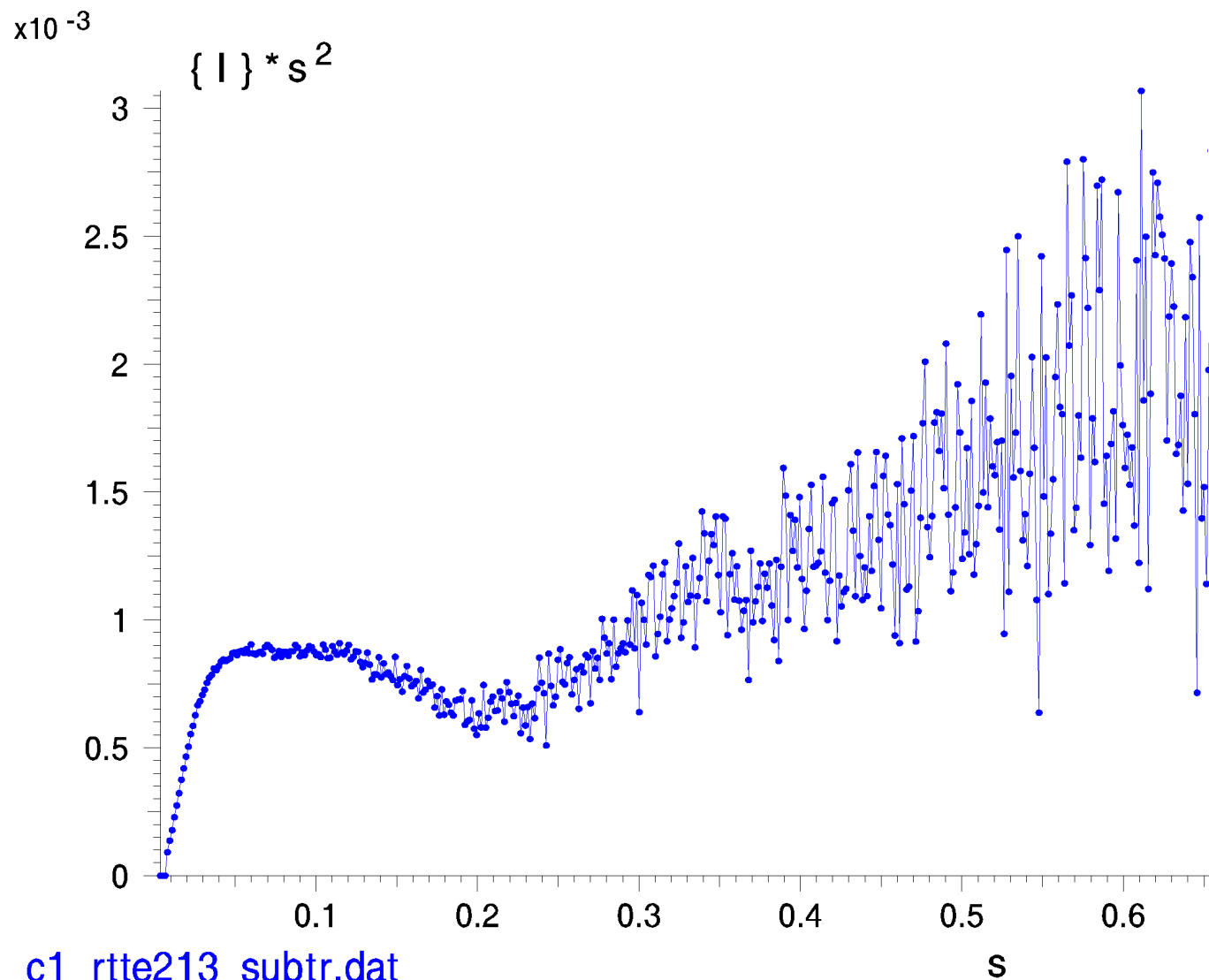
RIGID BODY modeling

programy SASREF, BUNCH, CORAL,...

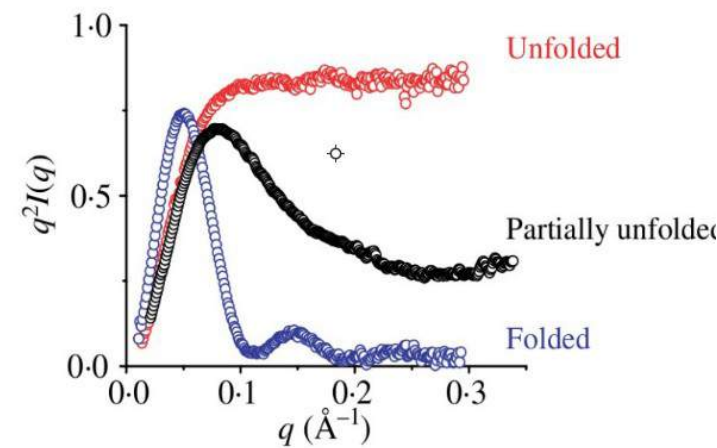


Flexibilní systémy

program EOM

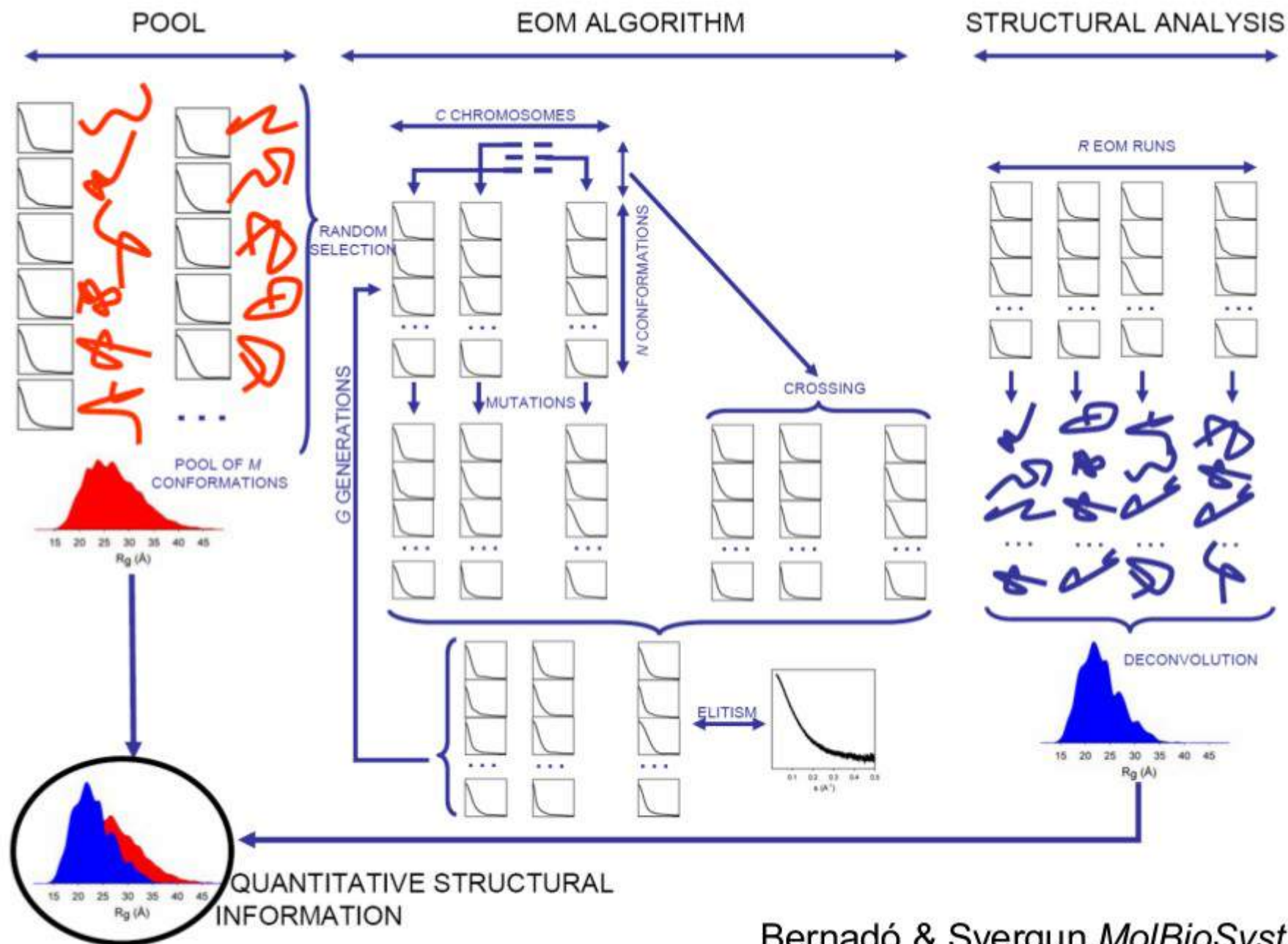


c1_rtte213_subtr.dat



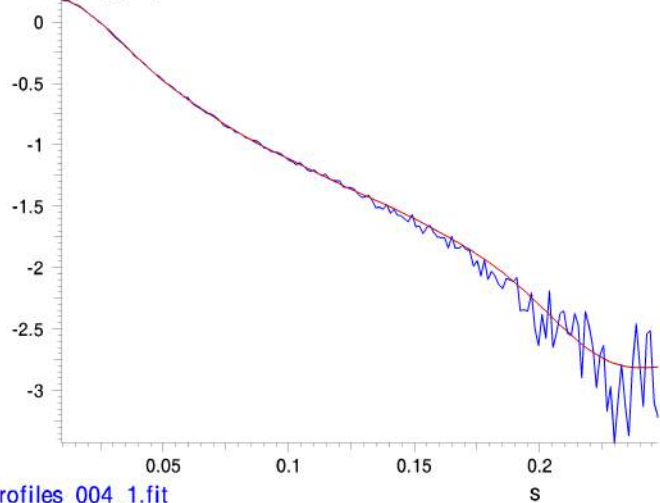
Flexibilní systémy

program EOM



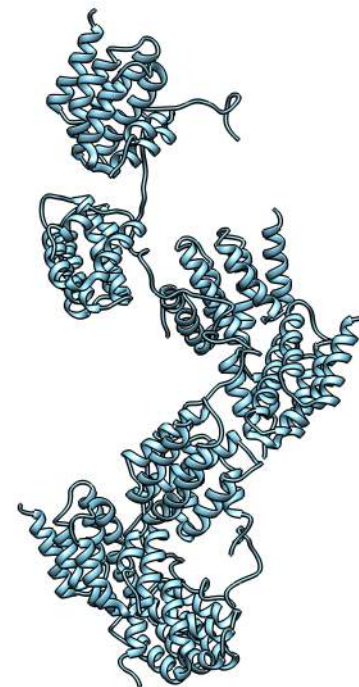
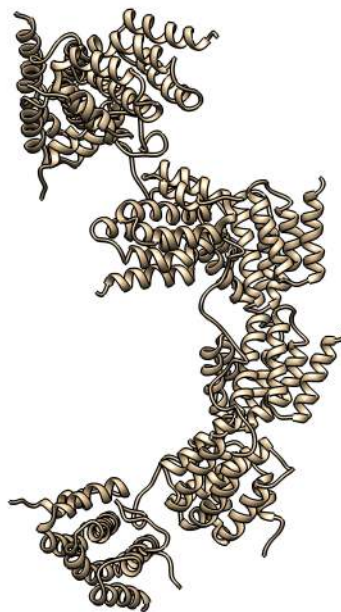
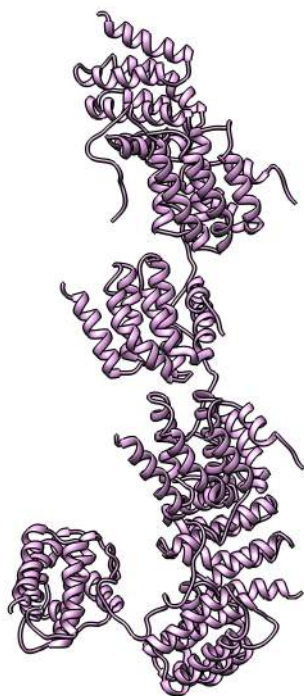
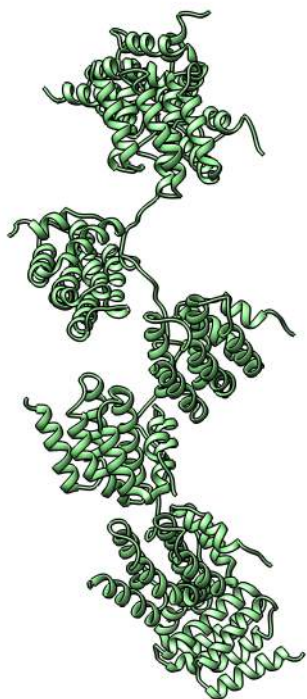
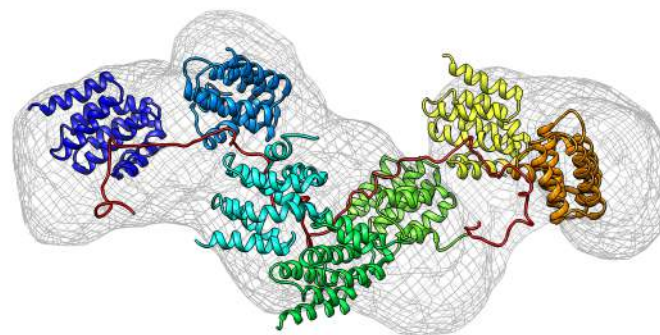
CYCLE: 87 Chi: 1.160 GENER.:1000 ENSEMBLES: 50 CURVES: 24

Log{I}



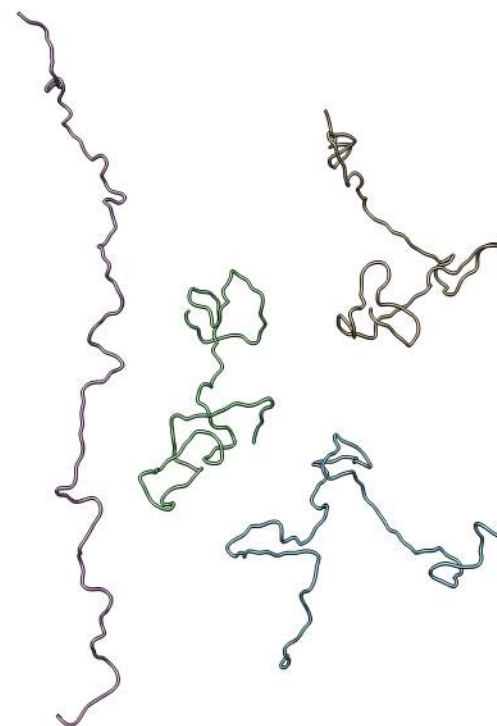
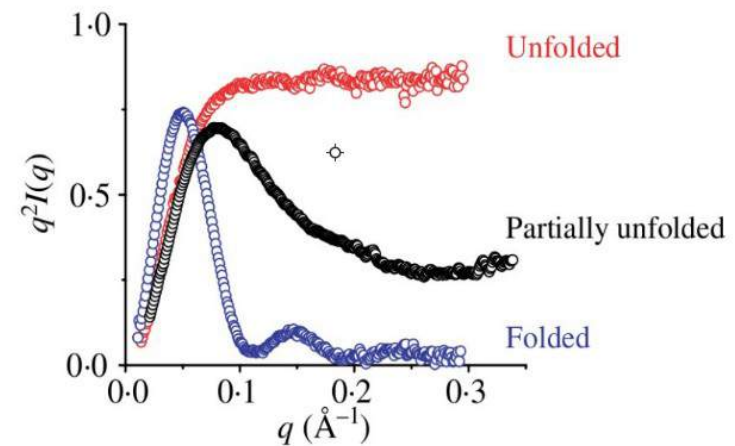
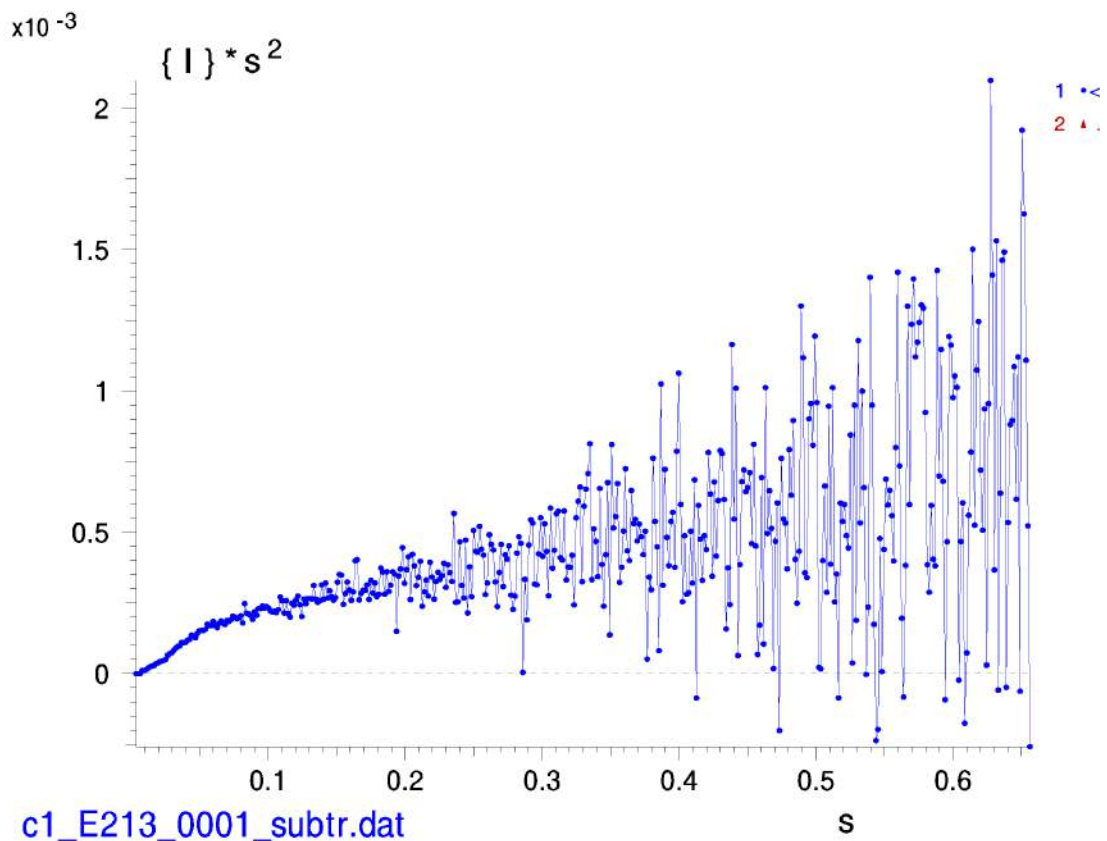
1 <
2 <

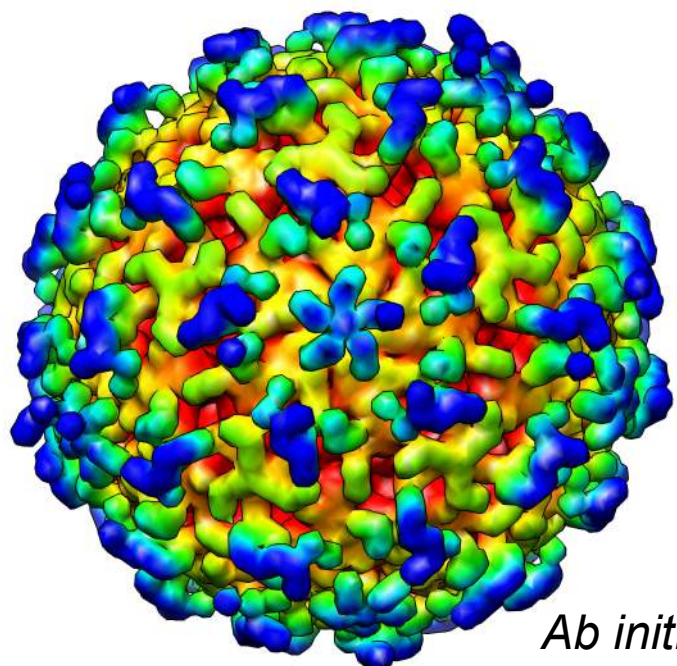
profiles_004_1.fit



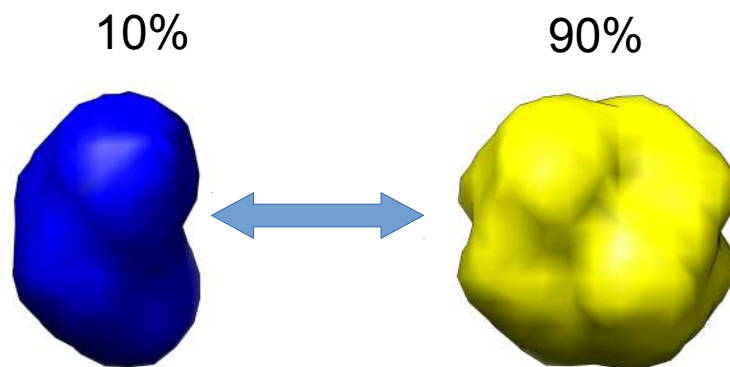
Flexibilní systémy

program EOM

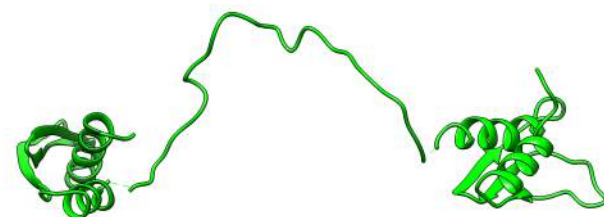




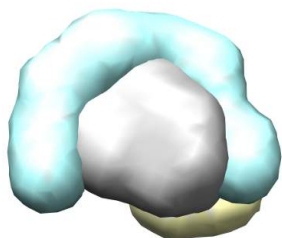
Ab initio



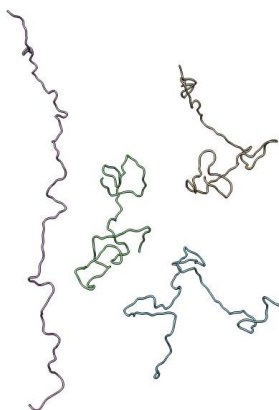
oligomeric state in solution/hierarchical structures



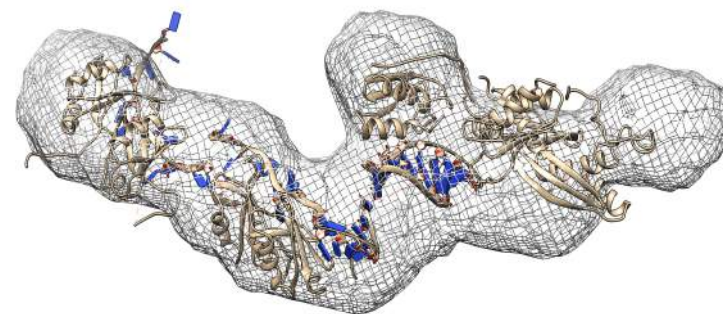
missing fragments



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 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