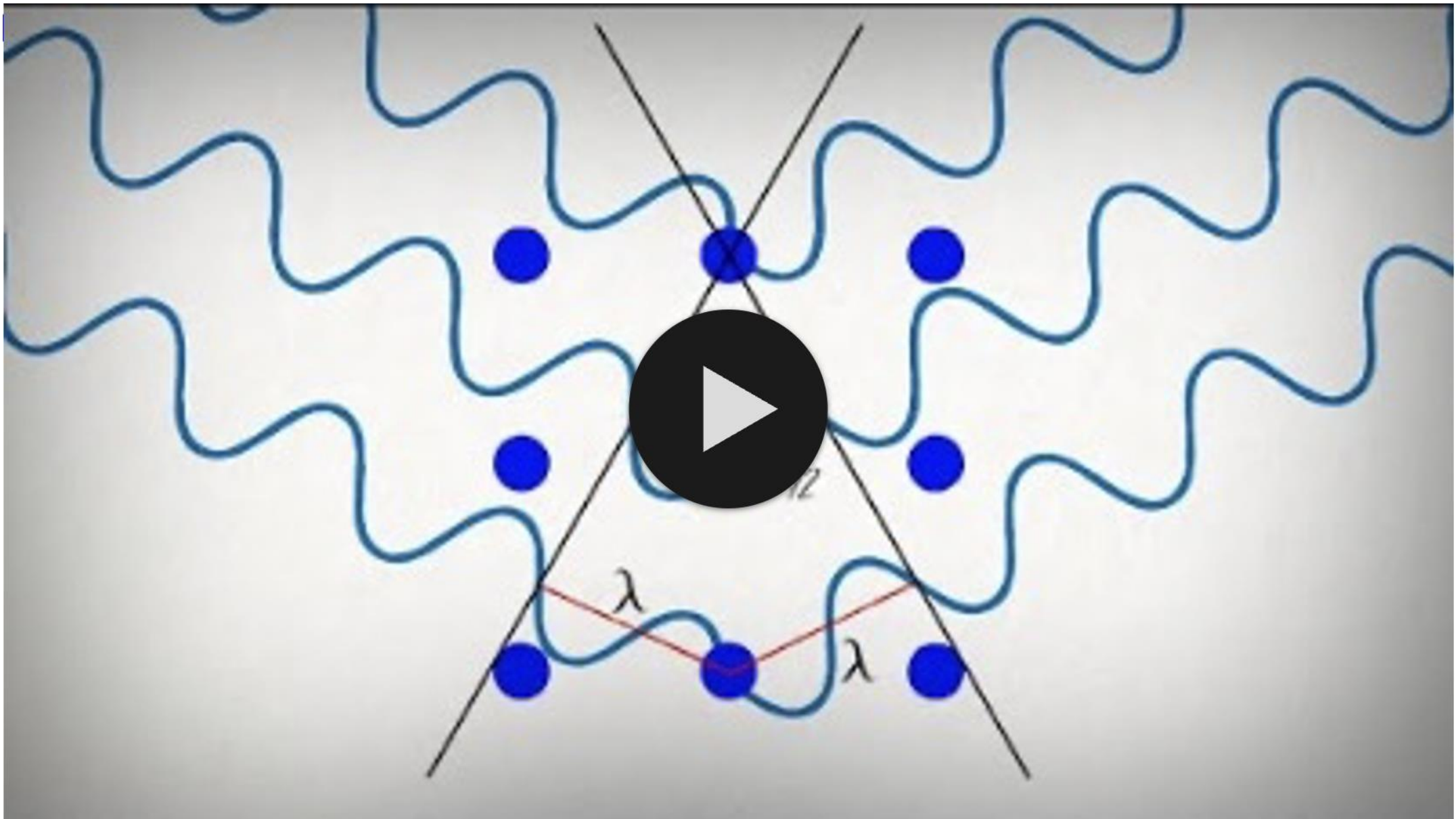


Rentgenová strukturní analýza

Experimentální metody biofyziky

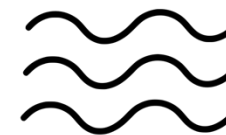


<https://youtu.be/xBA09PXPPR4?si=VlyOm4sjZj6JqGIA>

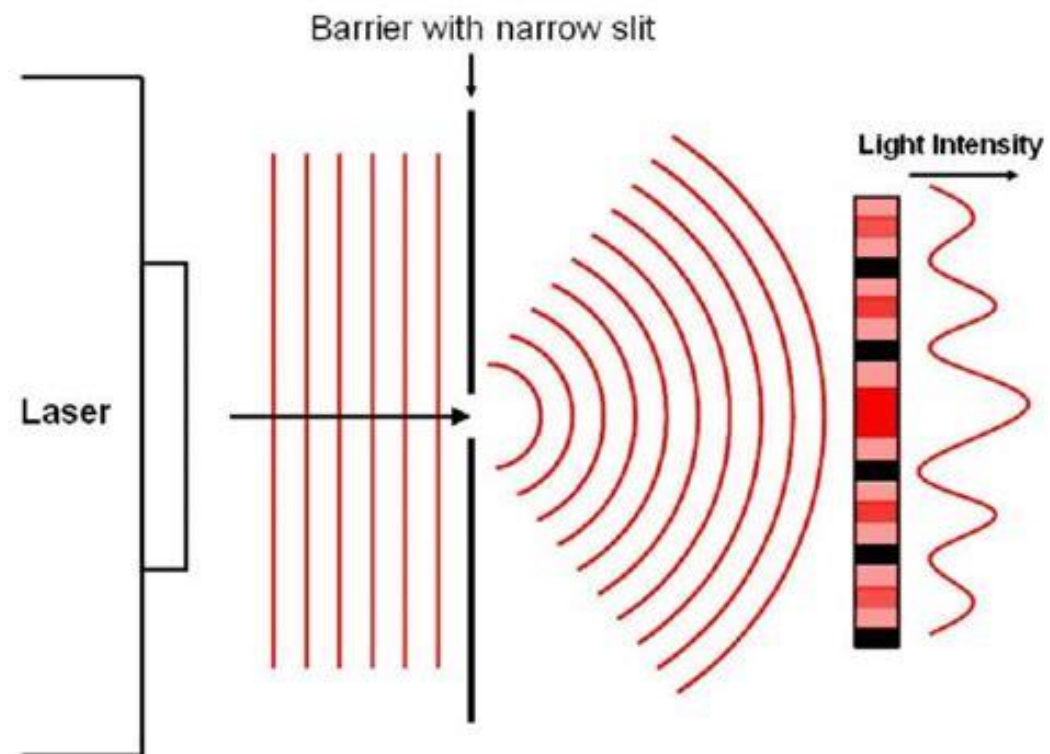
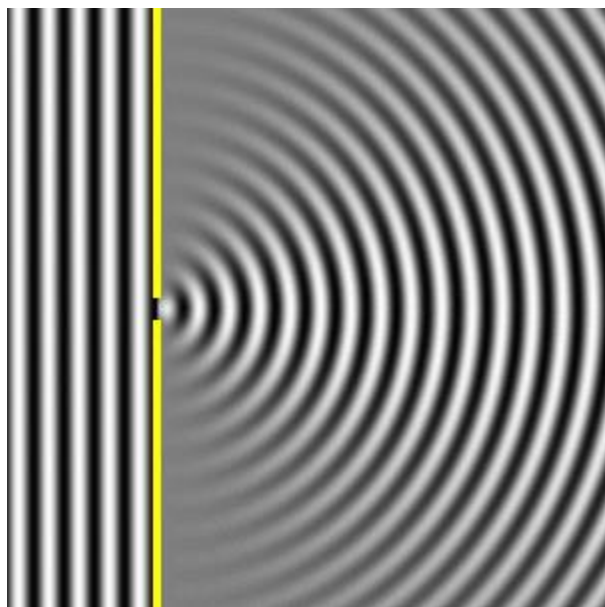
Rozptyl světla na elektronech

- Fotoabsorbce elektronu
 - Foton absorbován
- Neelastický rozptyl
 - Modifikovaná frekvence a fáze
- Elastický rozptyl
 - NENÍ modifikovaná fáze ani frekvence
 - "Rayleigh scattering" nebo "Thomson scattering"

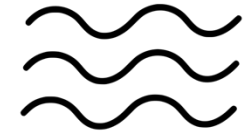
Difrakce světla



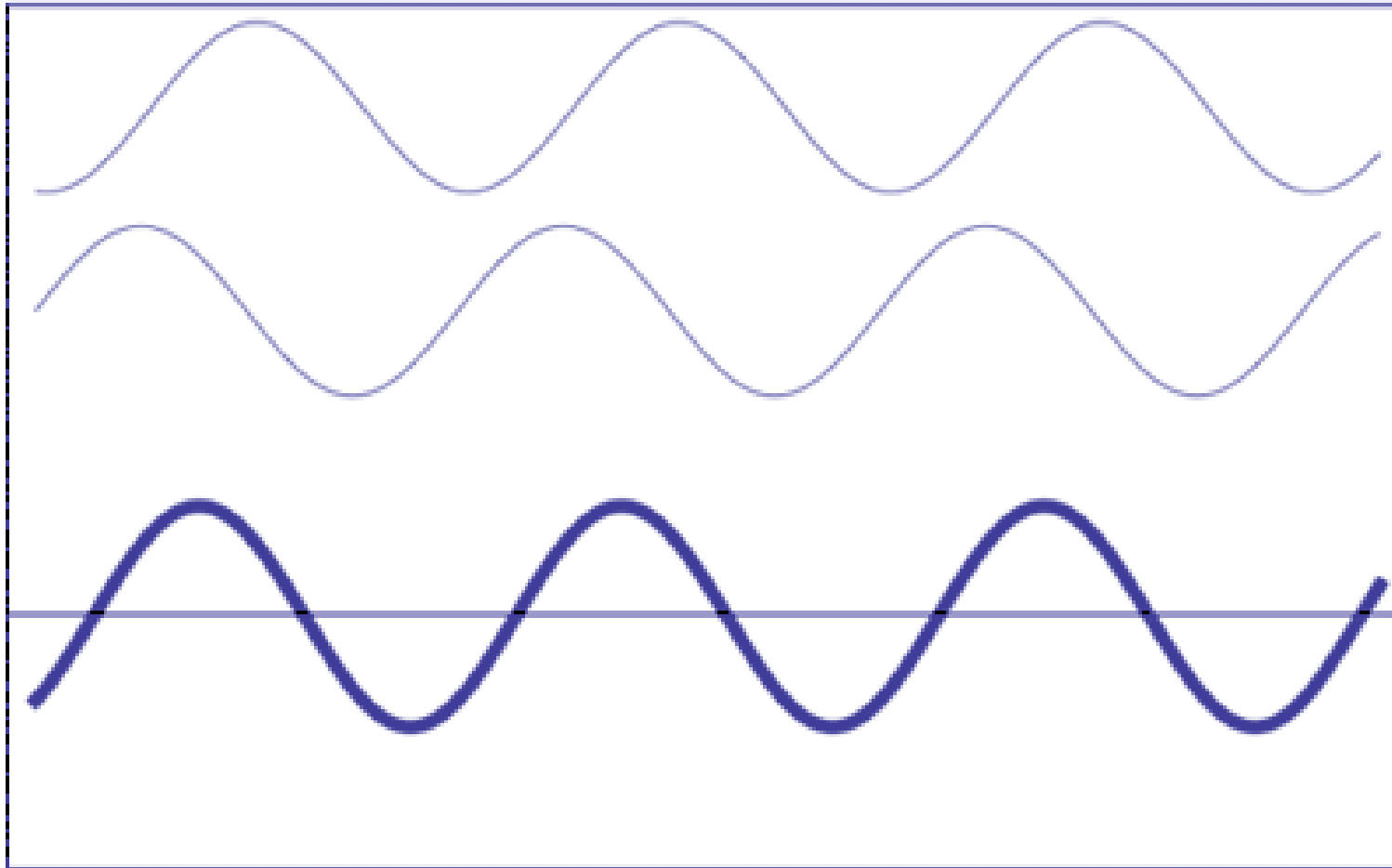
Created by wony hi
from Noun Project

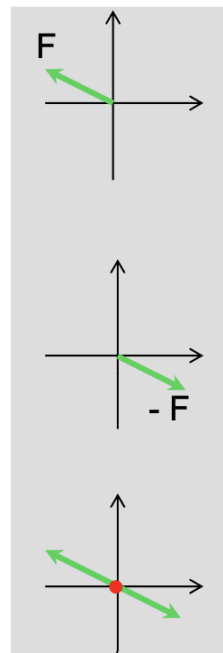
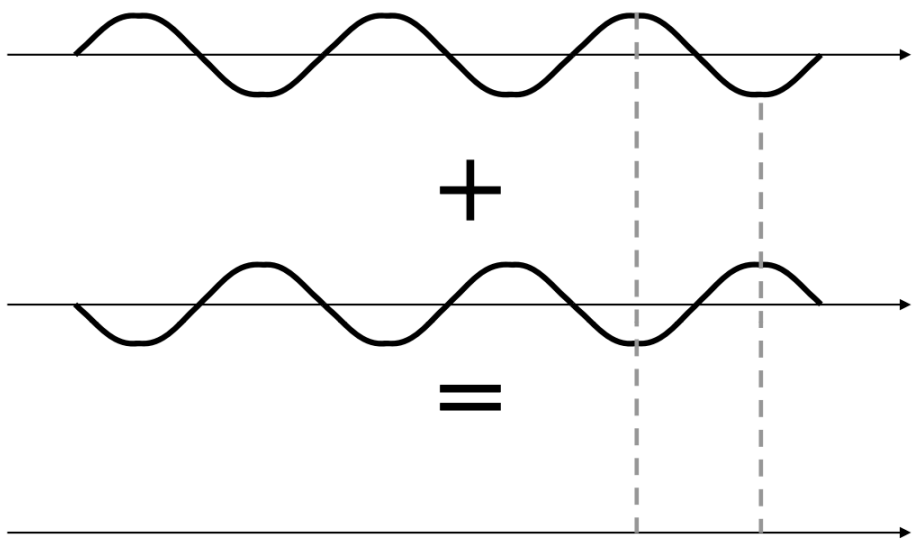
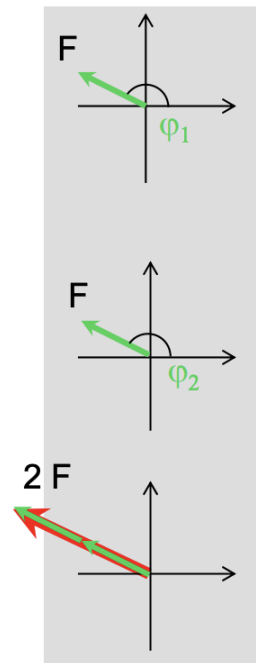
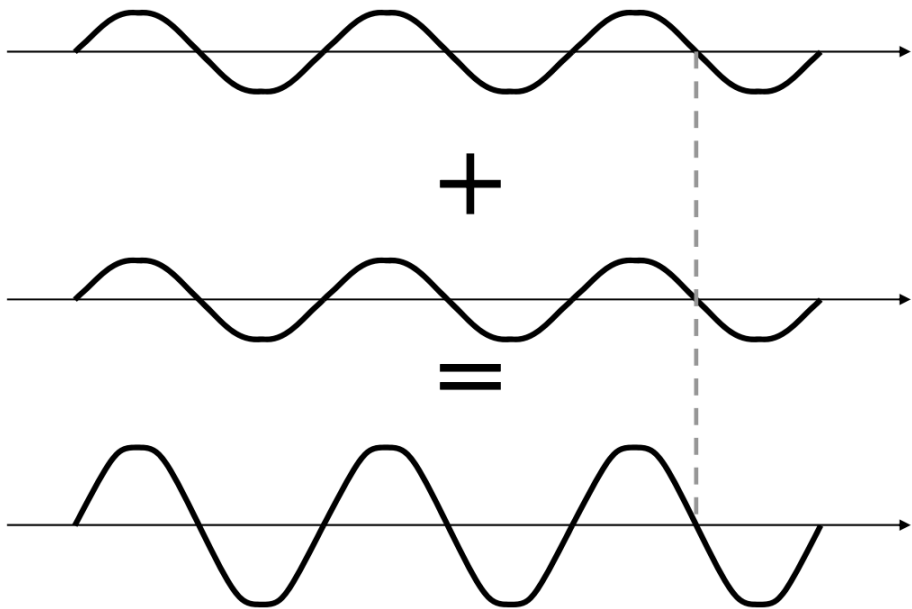


Skládání vln

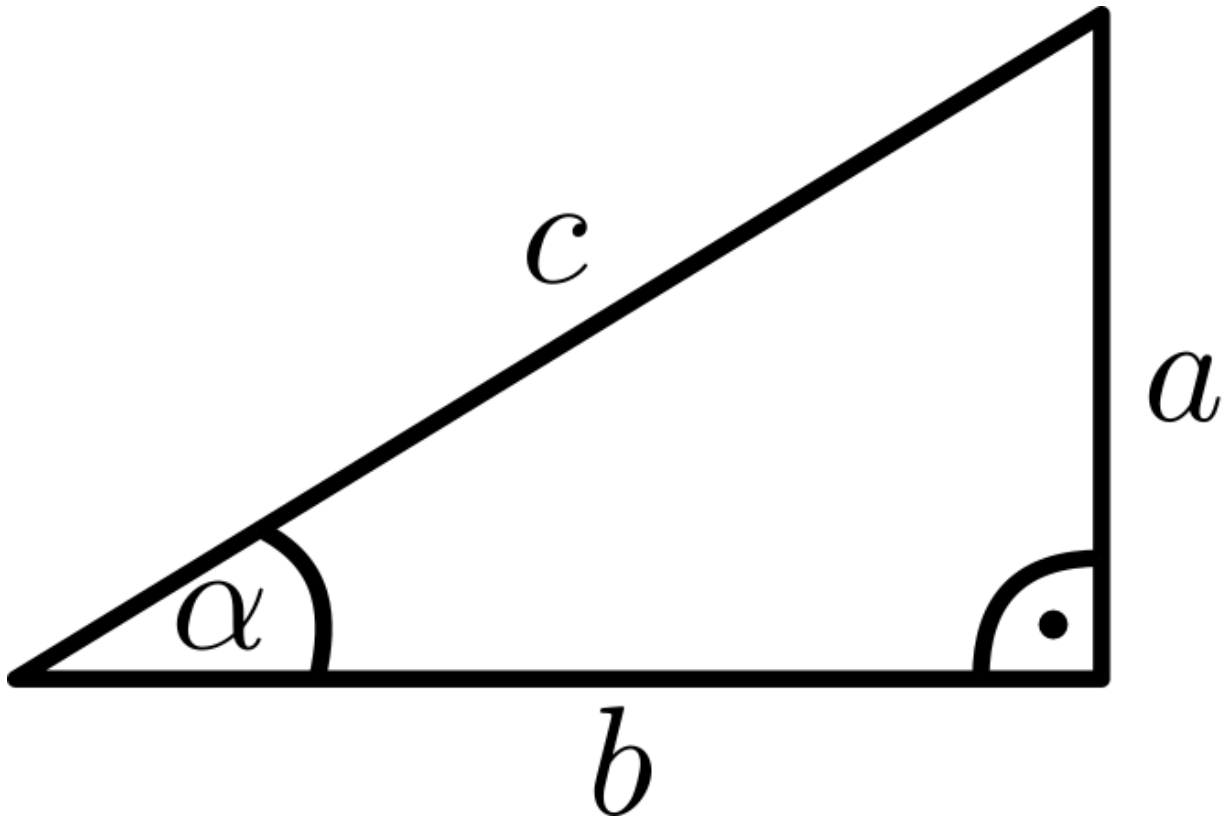


Created by wonny hi
from Noun Project





Goniometrické funkce

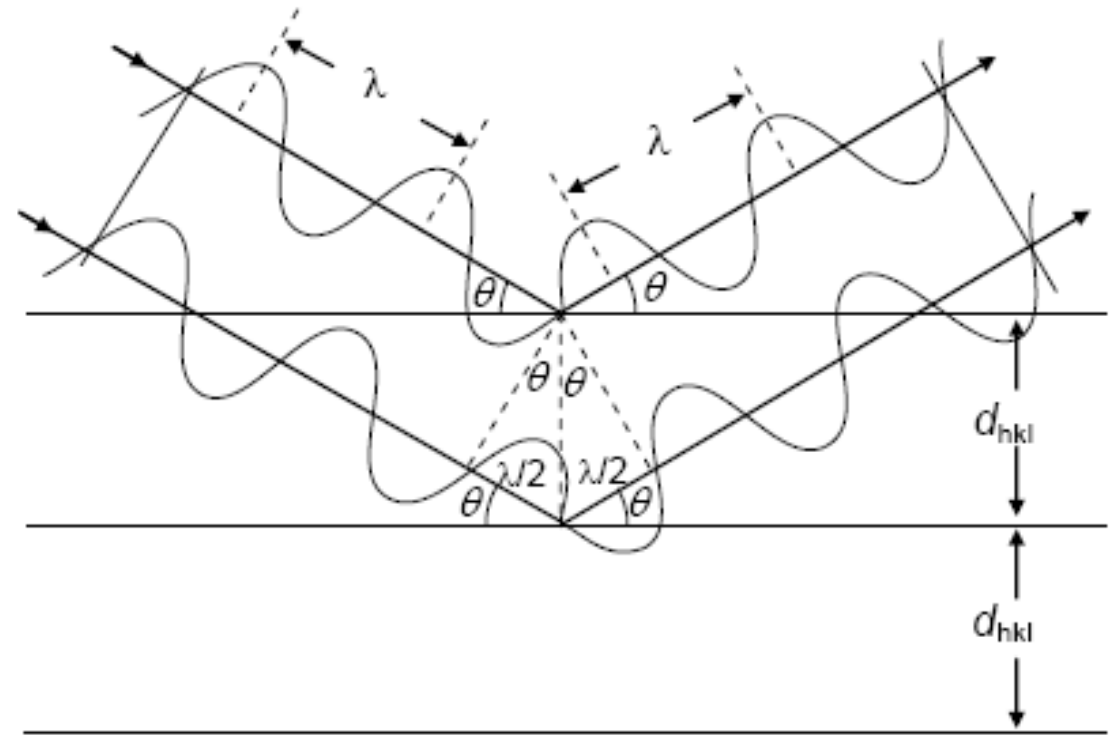
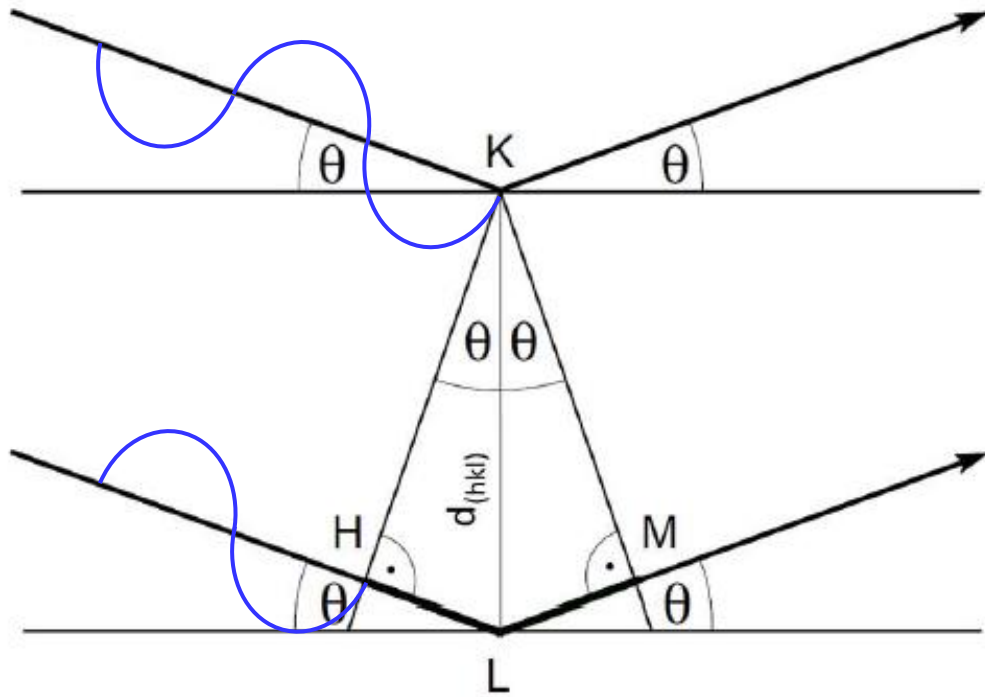


$$\sin(\alpha) = \frac{a}{c}$$

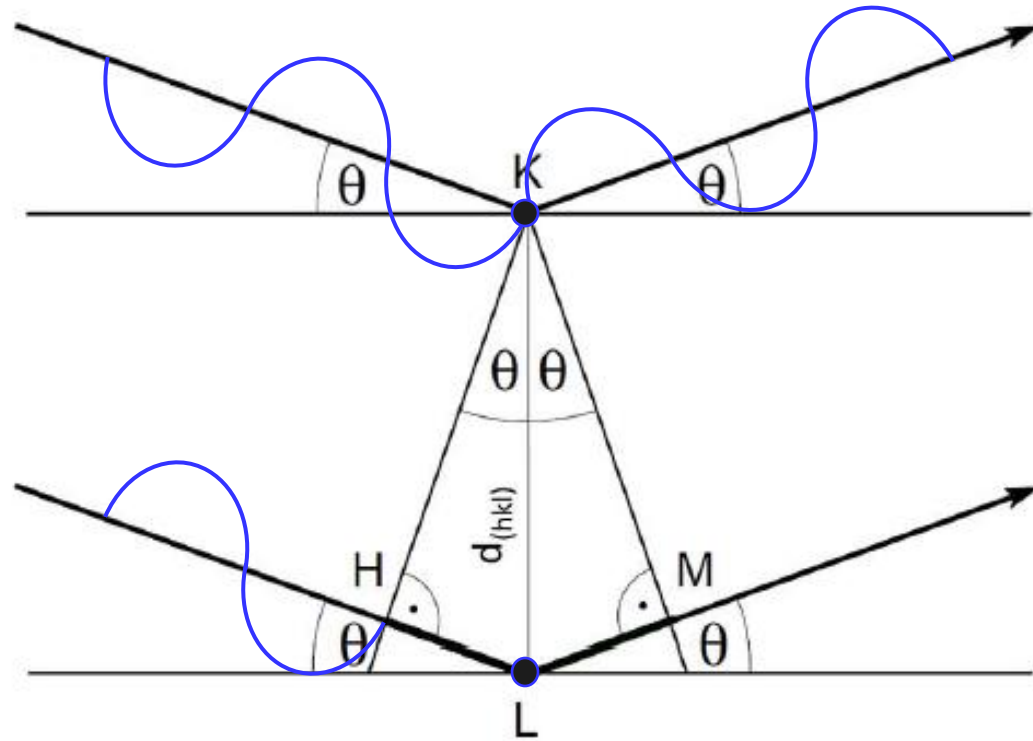
$$\cos(\alpha) = \frac{b}{c}$$

$$\tan(\alpha) = \frac{a}{b}$$

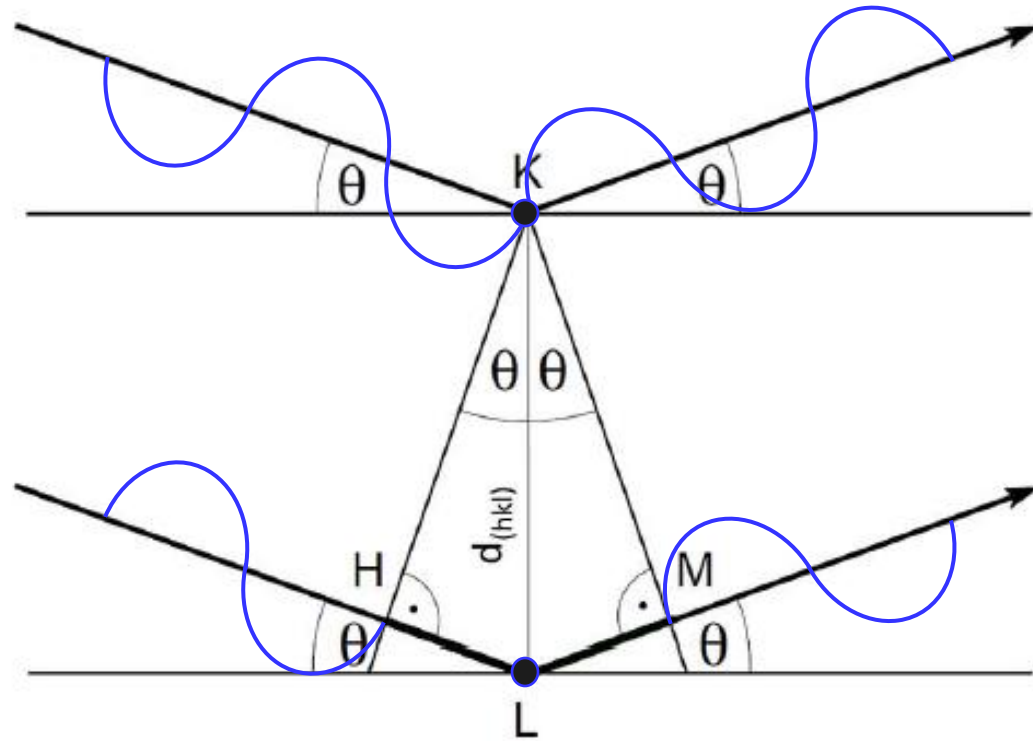
Braggův zákon



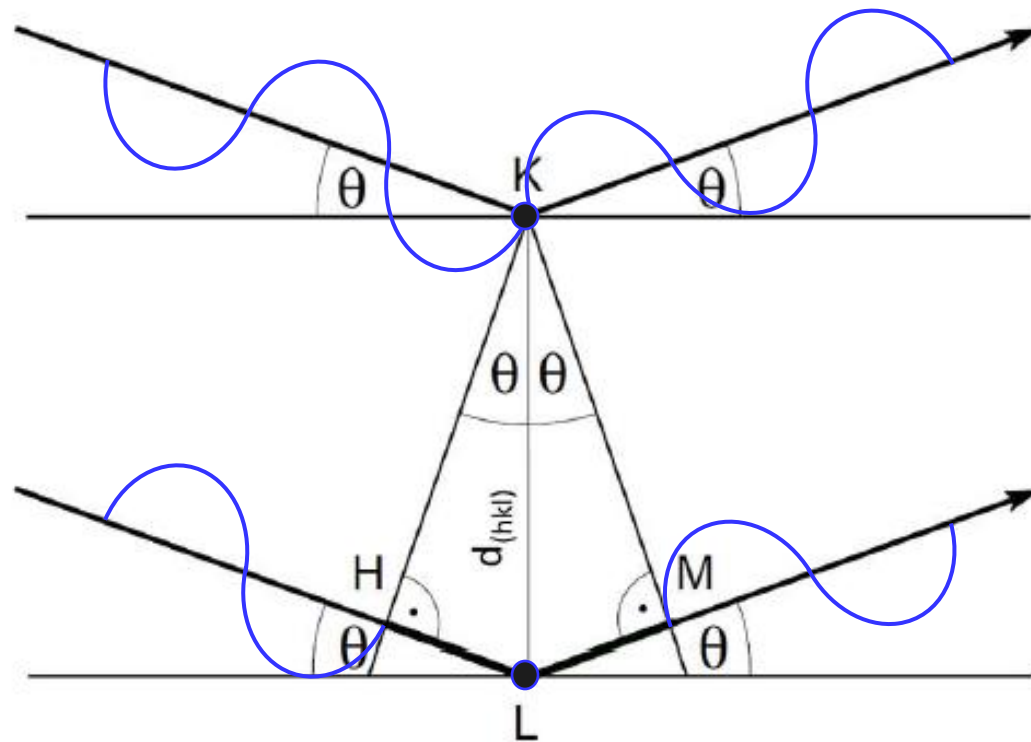
Braggův zákon



Braggův zákon



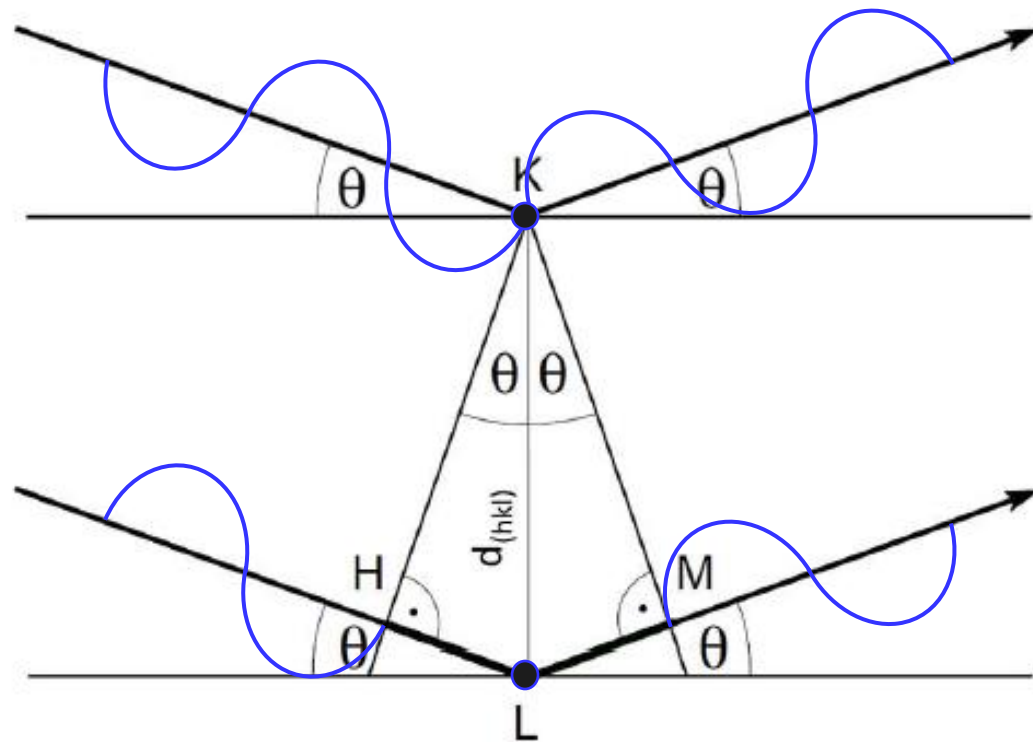
Braggův zákon



Extra vzdálenost

$$HL + LM = n\lambda$$

Braggův zákon

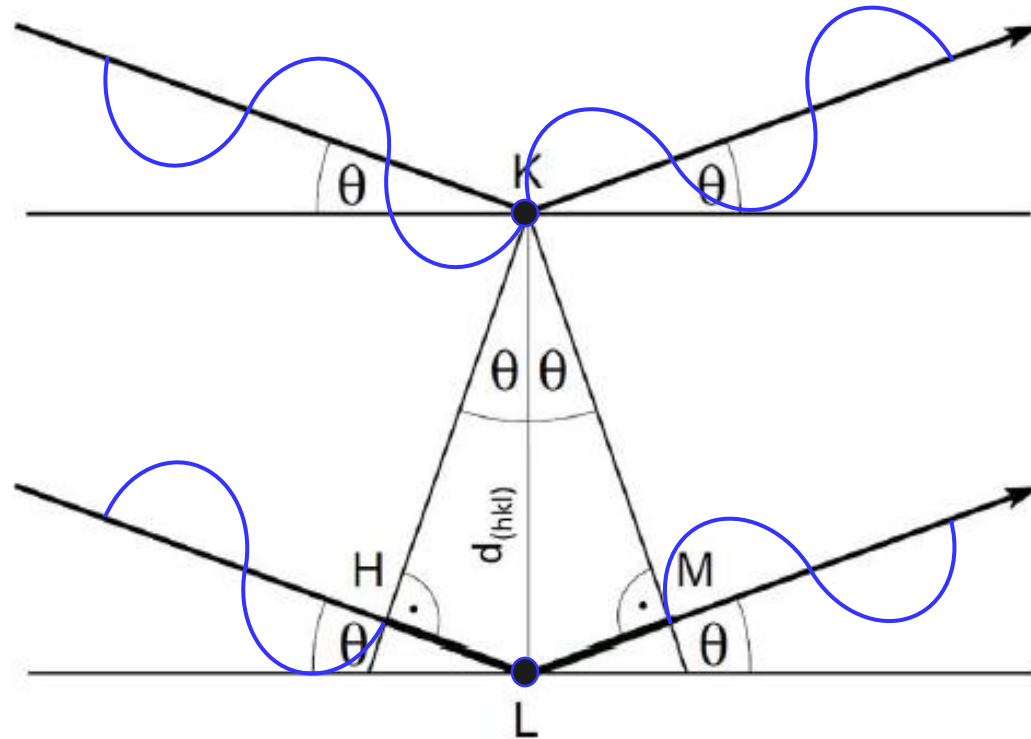


$$HL + LM = n\lambda$$

$$\sin \theta = HL/d$$

$$\sin \theta = LM/d$$

Braggův zákon

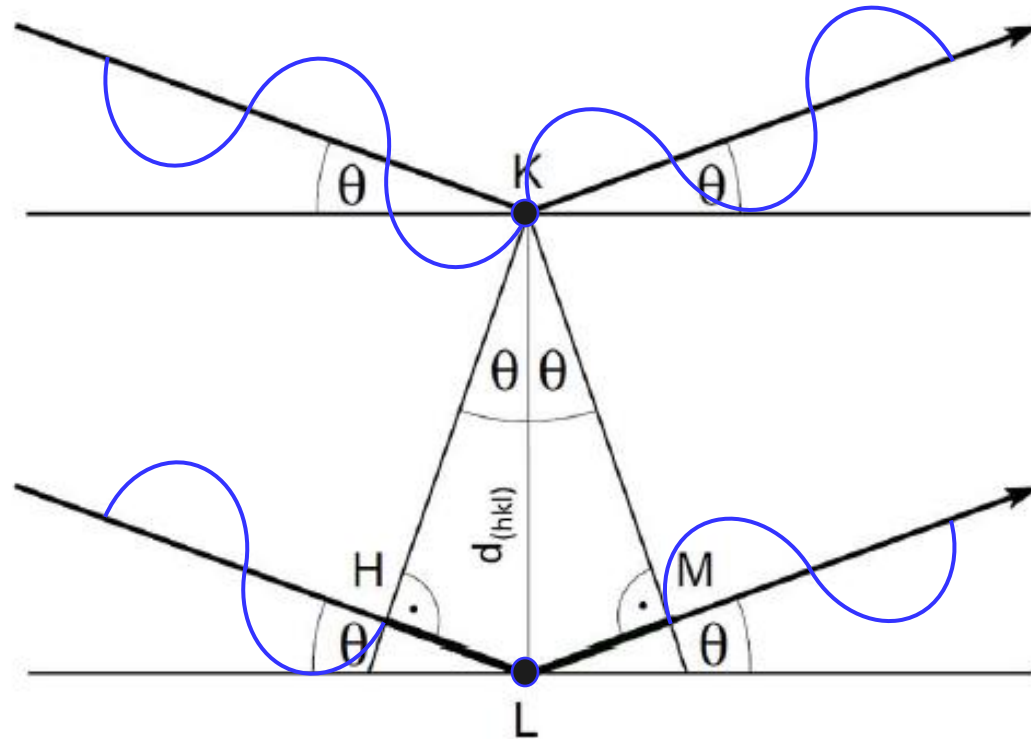


$$HL + LM = n\lambda$$

$$\sin \theta = HL/d$$
$$d \cdot \sin \theta = HL$$

$$\sin \theta = LM/d$$
$$d \cdot \sin \theta = LM$$

Braggův zákon



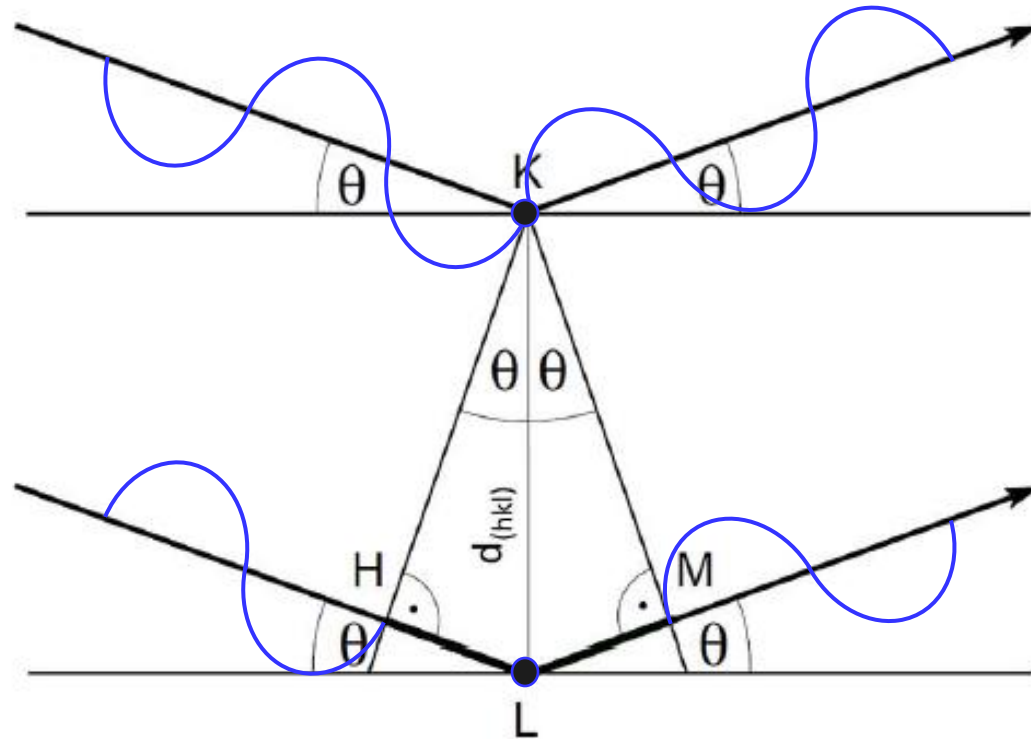
$$HL + LM = n\lambda$$

$$\sin \theta = HL/d$$
$$d^* \sin \theta = HL$$

$$\sin \theta = LM/d$$
$$d^* \sin \theta = LM$$

$$HL + LM = n\lambda$$
$$d^* \sin \theta + d^* \sin \theta = n\lambda$$

Braggův zákon



$$HL + LM = n\lambda$$

$$\sin \theta = HL/d$$
$$d^* \sin \theta = HL$$

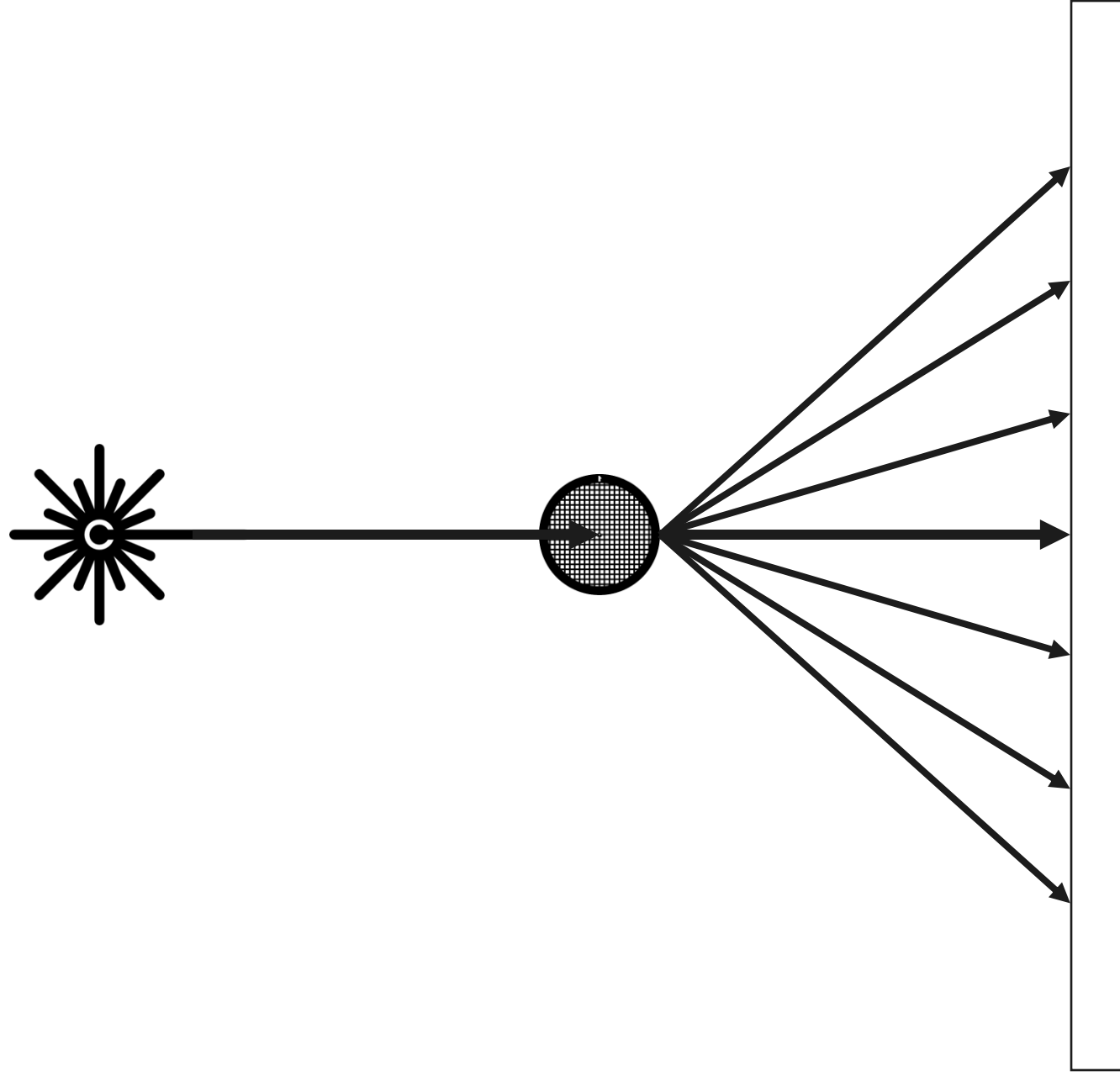
$$\sin \theta = LM/d$$
$$d^* \sin \theta = LM$$

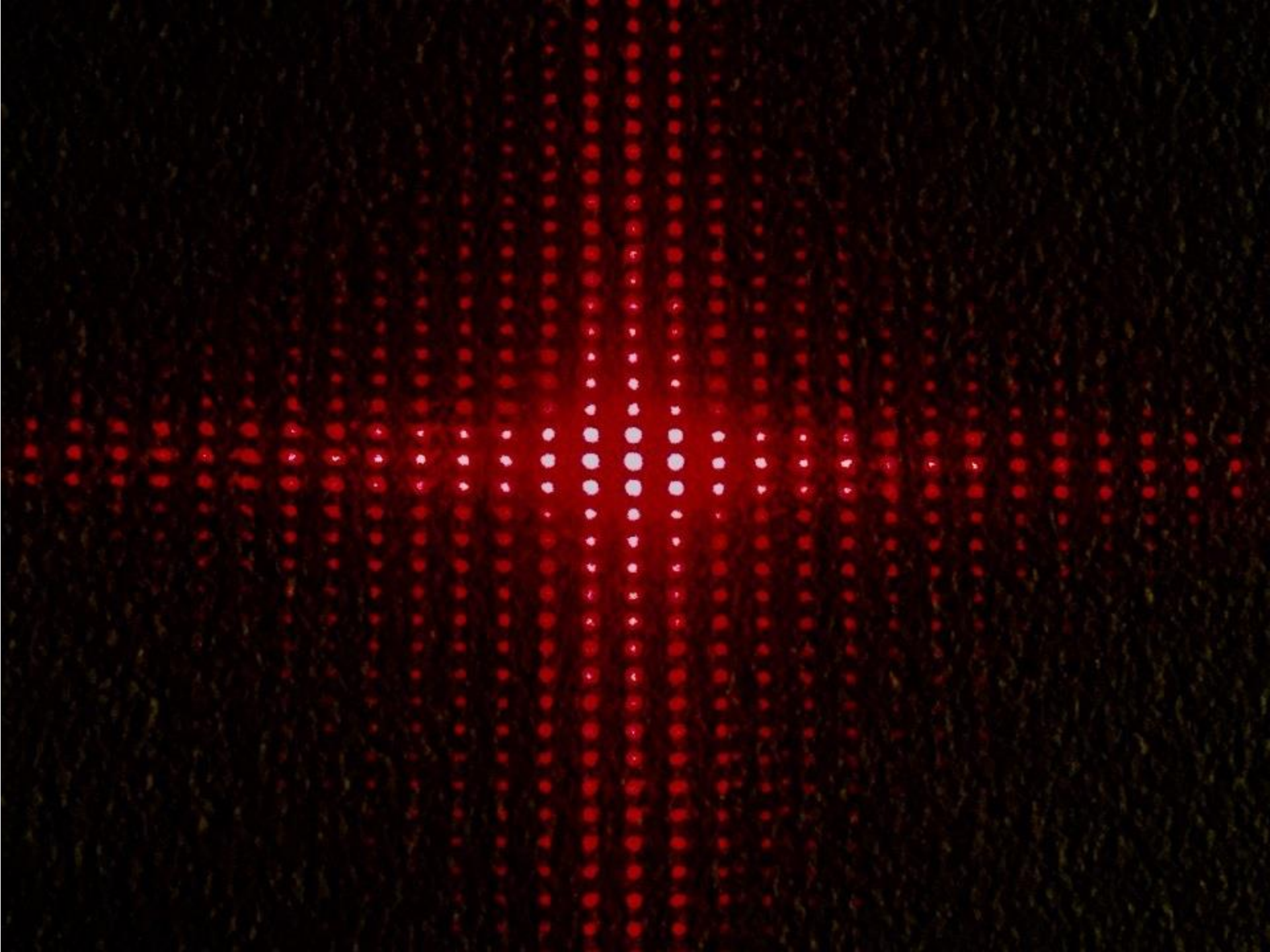
$$HL + LM = n\lambda$$
$$d^* \sin \theta + d^* \sin \theta = n\lambda$$

$$2d \sin \theta = n\lambda$$



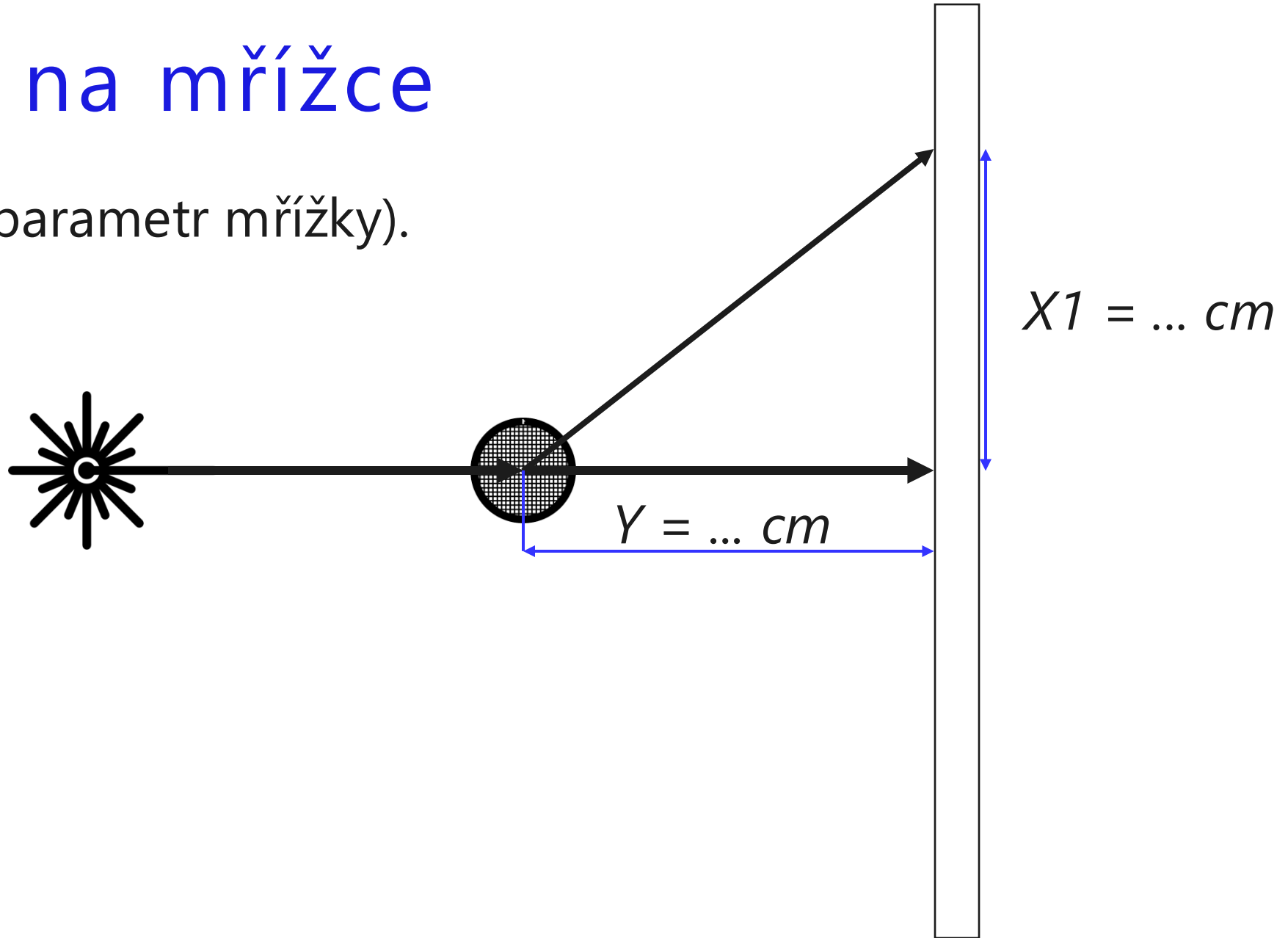
Rozptyl světla na mřížce





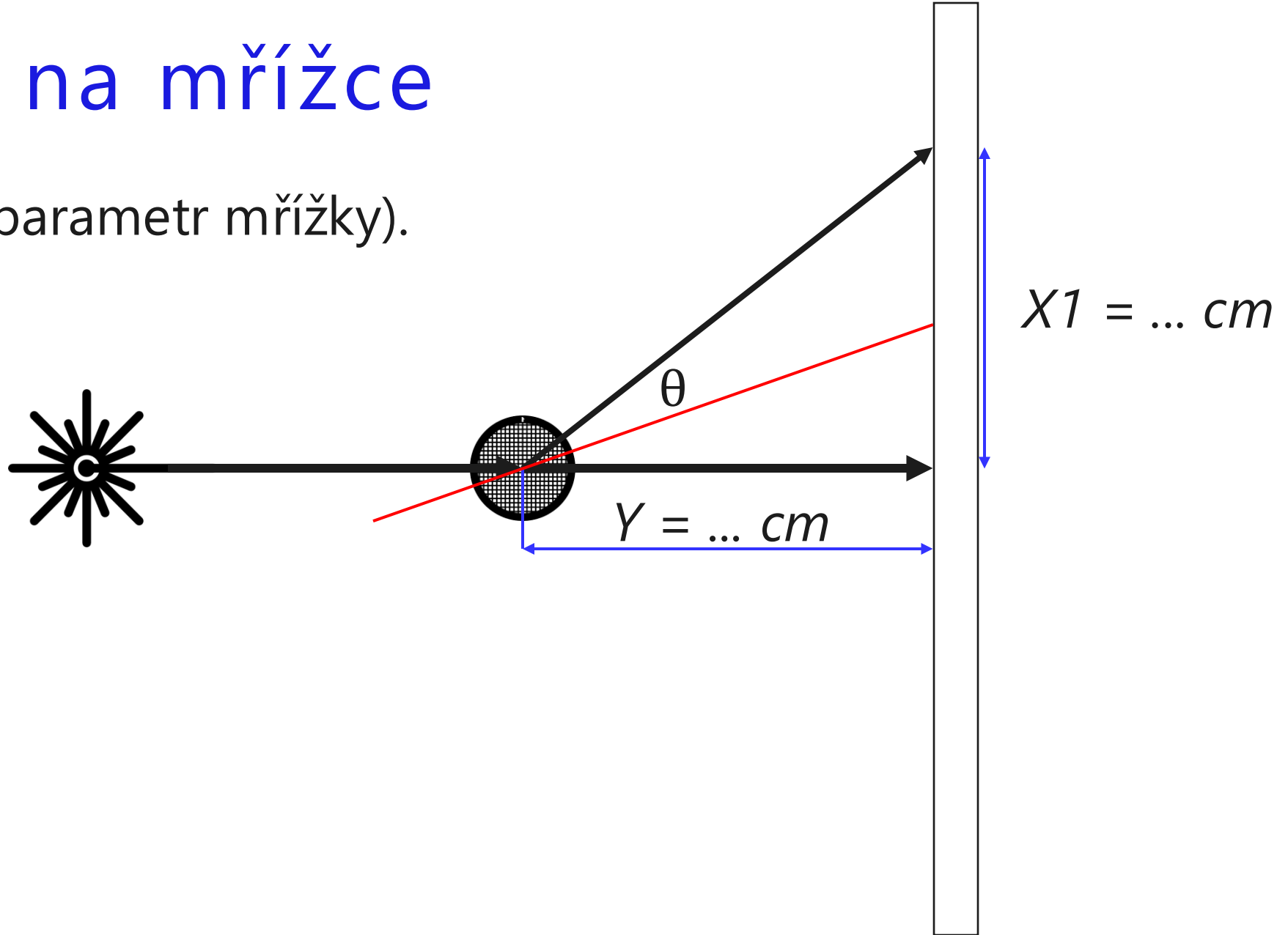
Difrakce na mřížce

Vypočítejte **d** (parametr mřížky).



Difrakce na mřížce

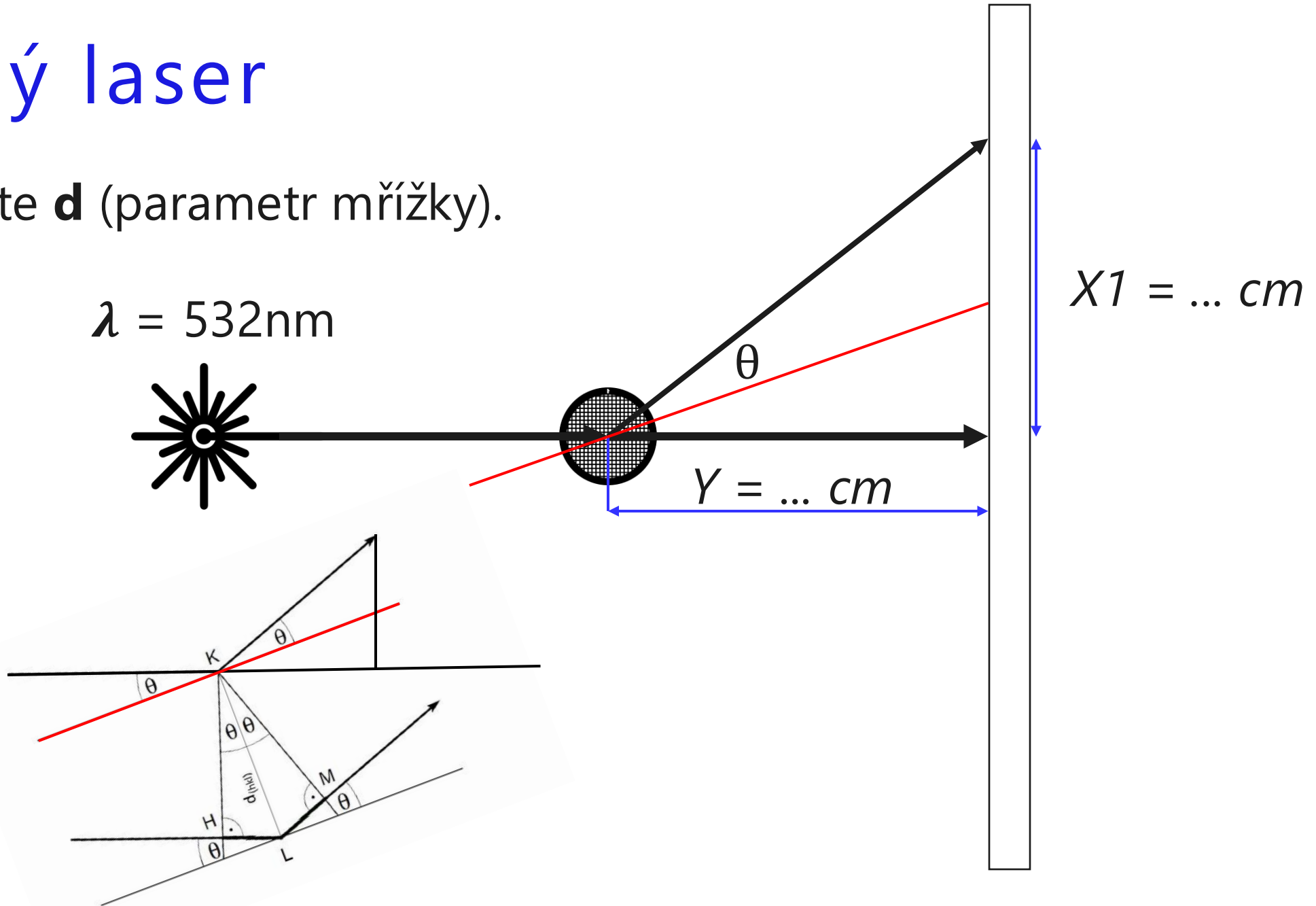
Vypočítejte **d** (parametr mřížky).



Zelený laser

Vypočítejte **d** (parametr mřížky).

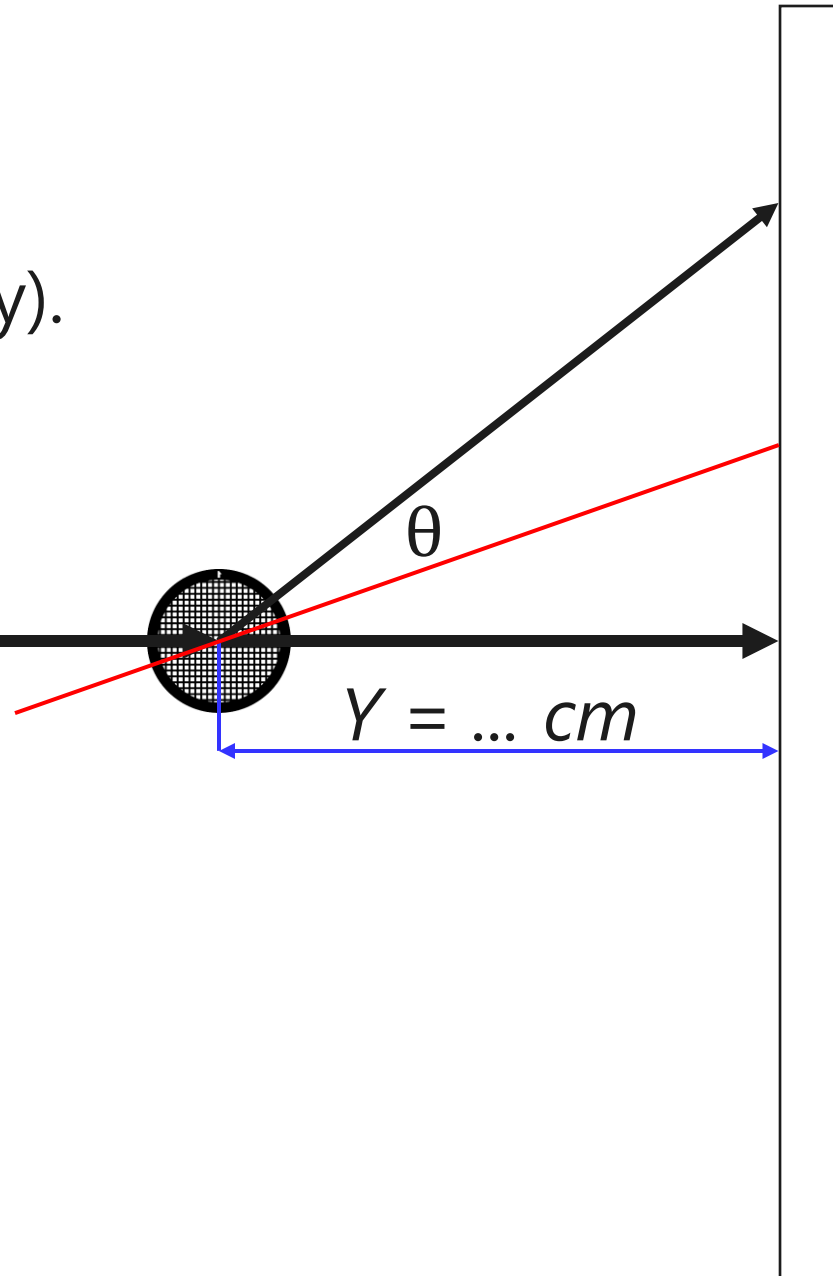
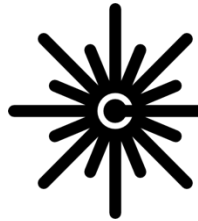
$$\lambda = 532\text{nm}$$



Zelený laser

Vypočítejte **d** (parametr mřížky).

$$\lambda = 532\text{nm}$$

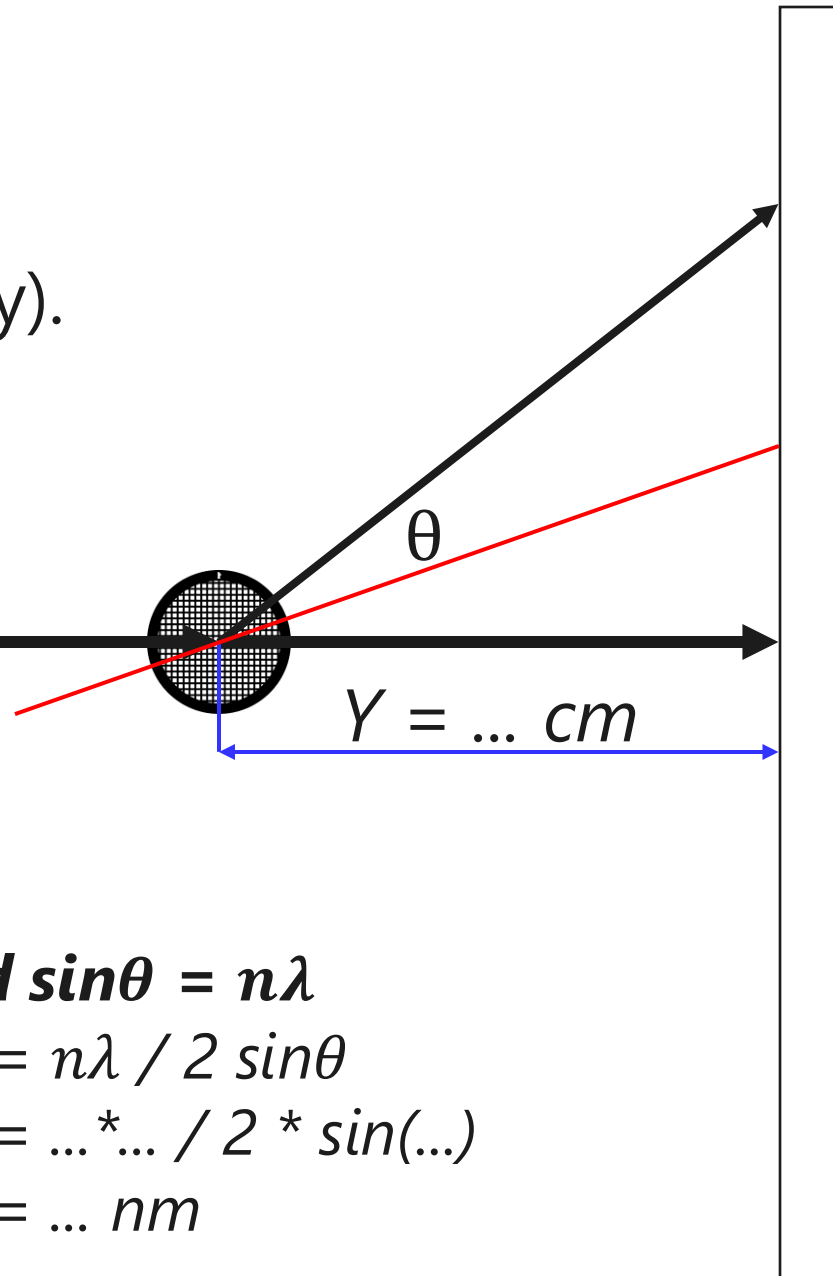
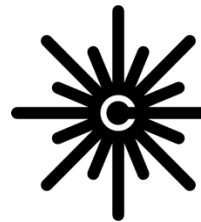


$$\begin{aligned} \text{tg}(2\theta) &= X1/Y \\ 2\theta &= \text{tg}^{-1}(X1/Y) \\ 2\theta &= \dots^\circ \\ \theta &= \dots^\circ \end{aligned}$$

Zelený laser

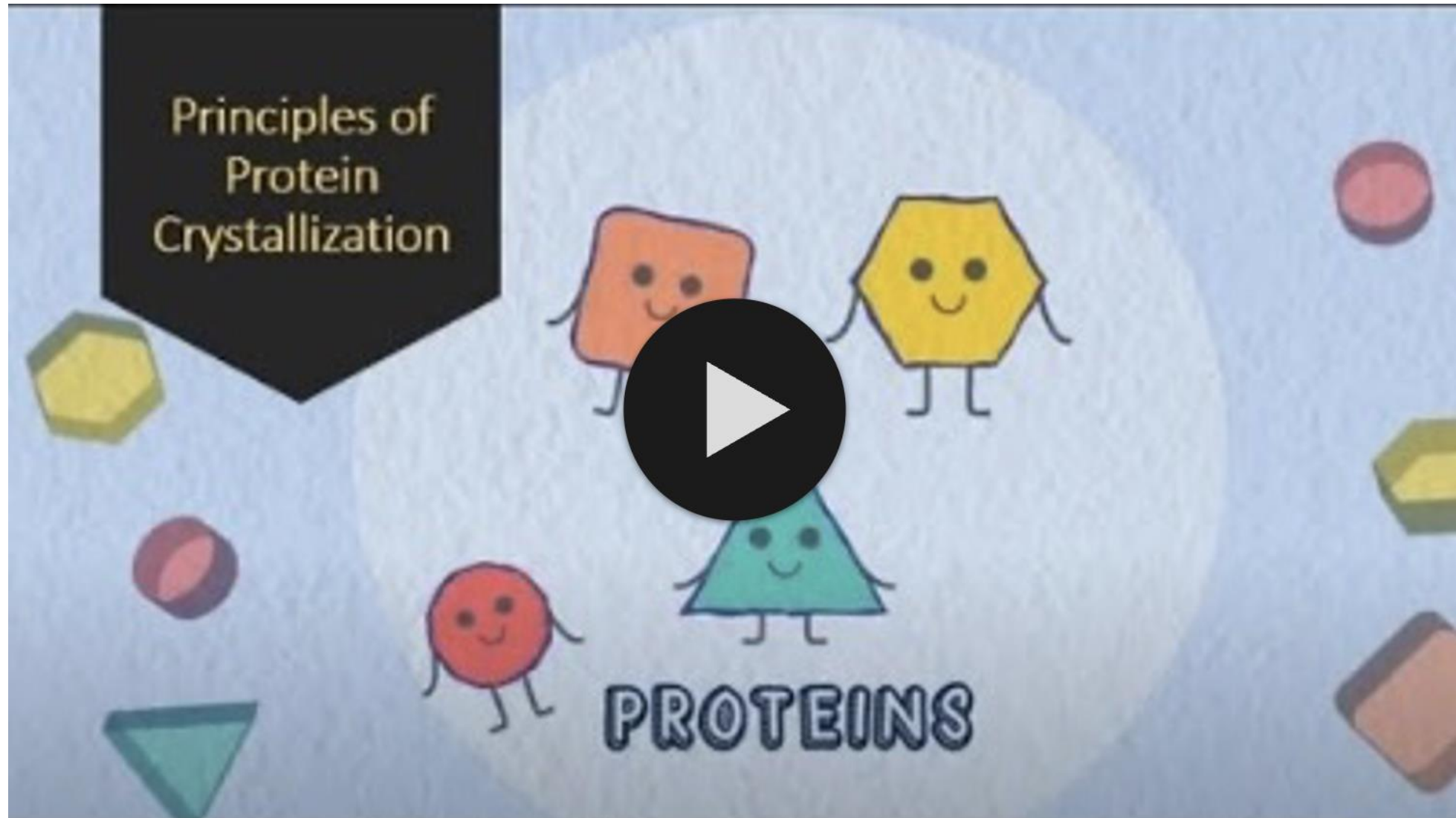
Vypočítejte **d** (parametr mřížky).

$$\lambda = 532\text{nm}$$

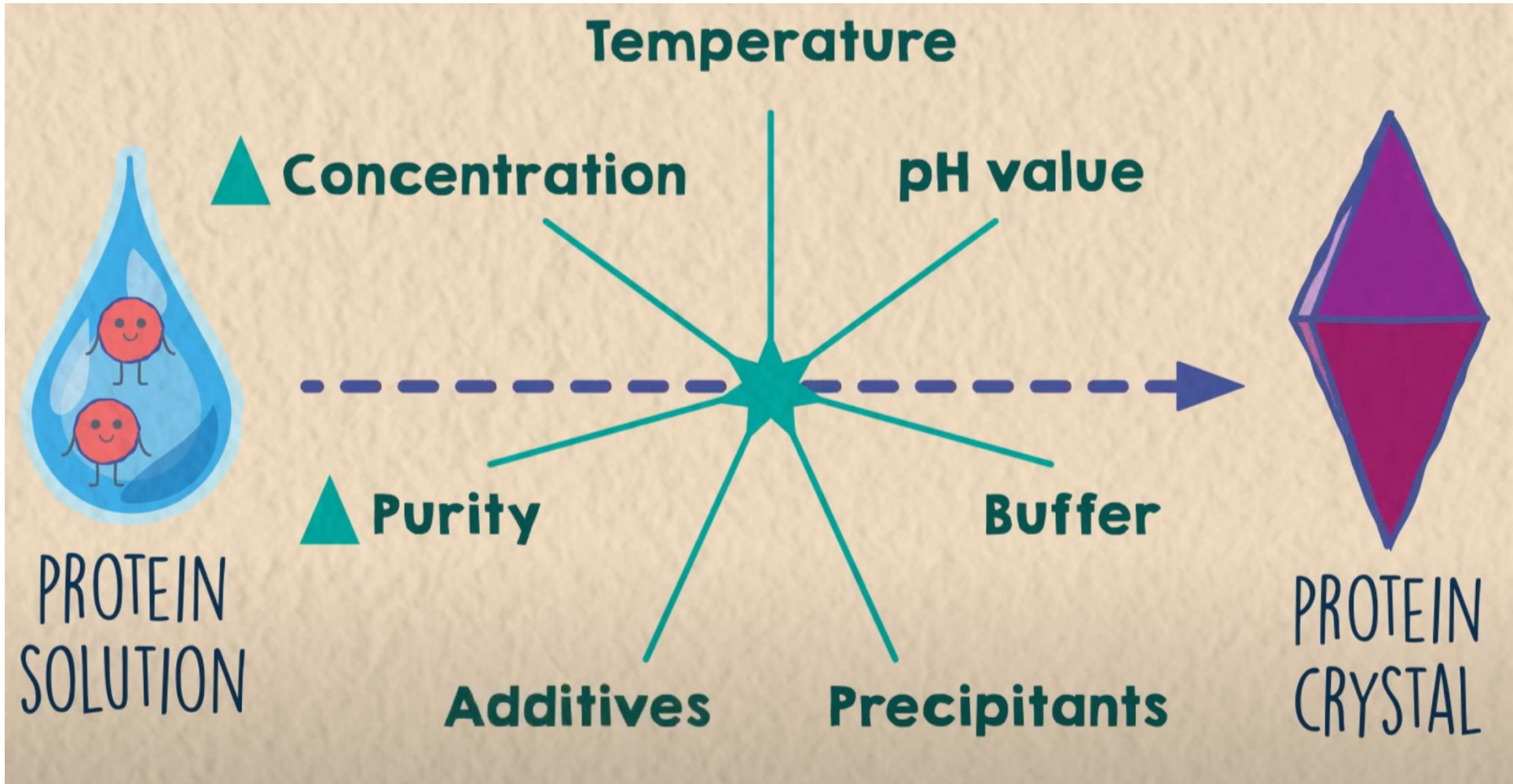


$$\begin{aligned} \text{tg}(2\theta) &= X1/Y \\ 2\theta &= \text{tg}^{-1}(X1/Y) \\ 2\theta &= \dots^\circ \\ \theta &= \dots^\circ \end{aligned}$$

$$\begin{aligned} 2d \sin\theta &= n\lambda \\ d &= n\lambda / 2 \sin\theta \\ d &= \dots * \dots / 2 * \sin(\dots) \\ d &= \dots \text{ nm} \\ d &= \dots \mu\text{m} \end{aligned}$$



Real video: <https://youtu.be/kPX6-Ab1pYw?si=dFBcu3qhfE-17EdU>

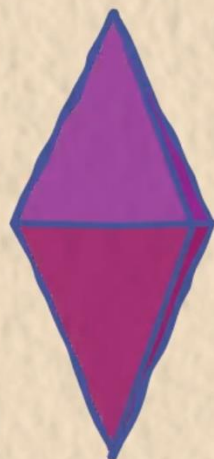


Protein Solution



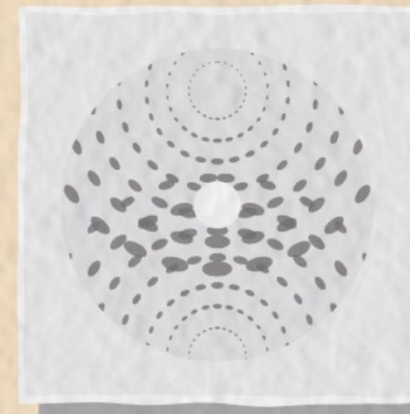
- PURIFIED
- CONCENTRATED

Protein Crystal



- PURE
- STABLE

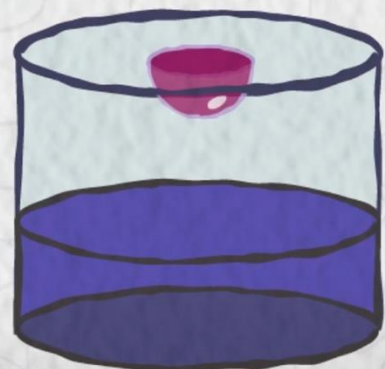
Diffraction Data



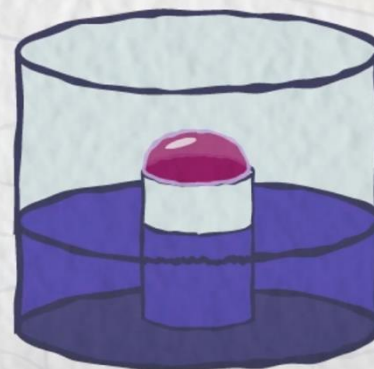
- CLEAR
- PRECISE

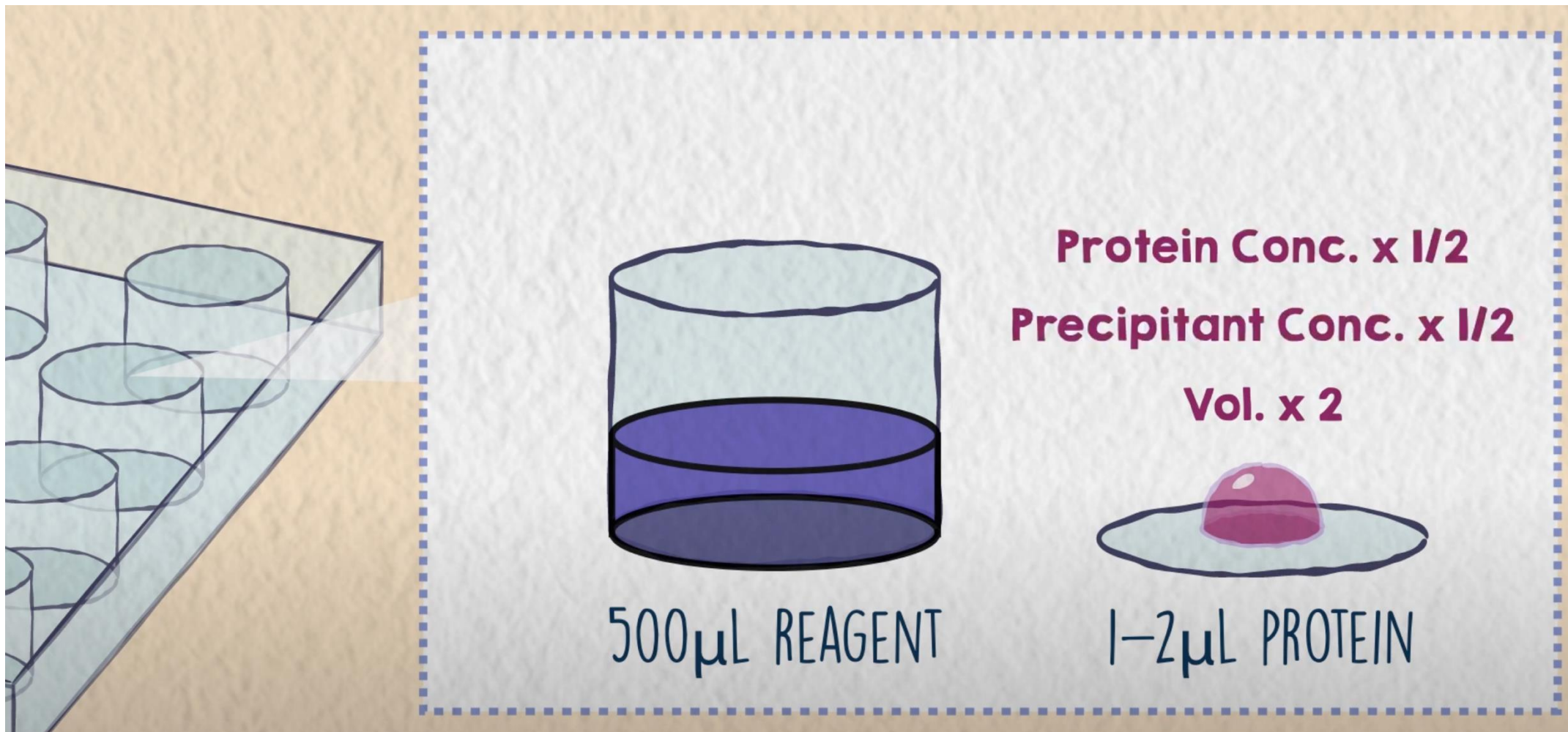
VAPOR DIFFUSION

HANGING DROP



SITTING DROP

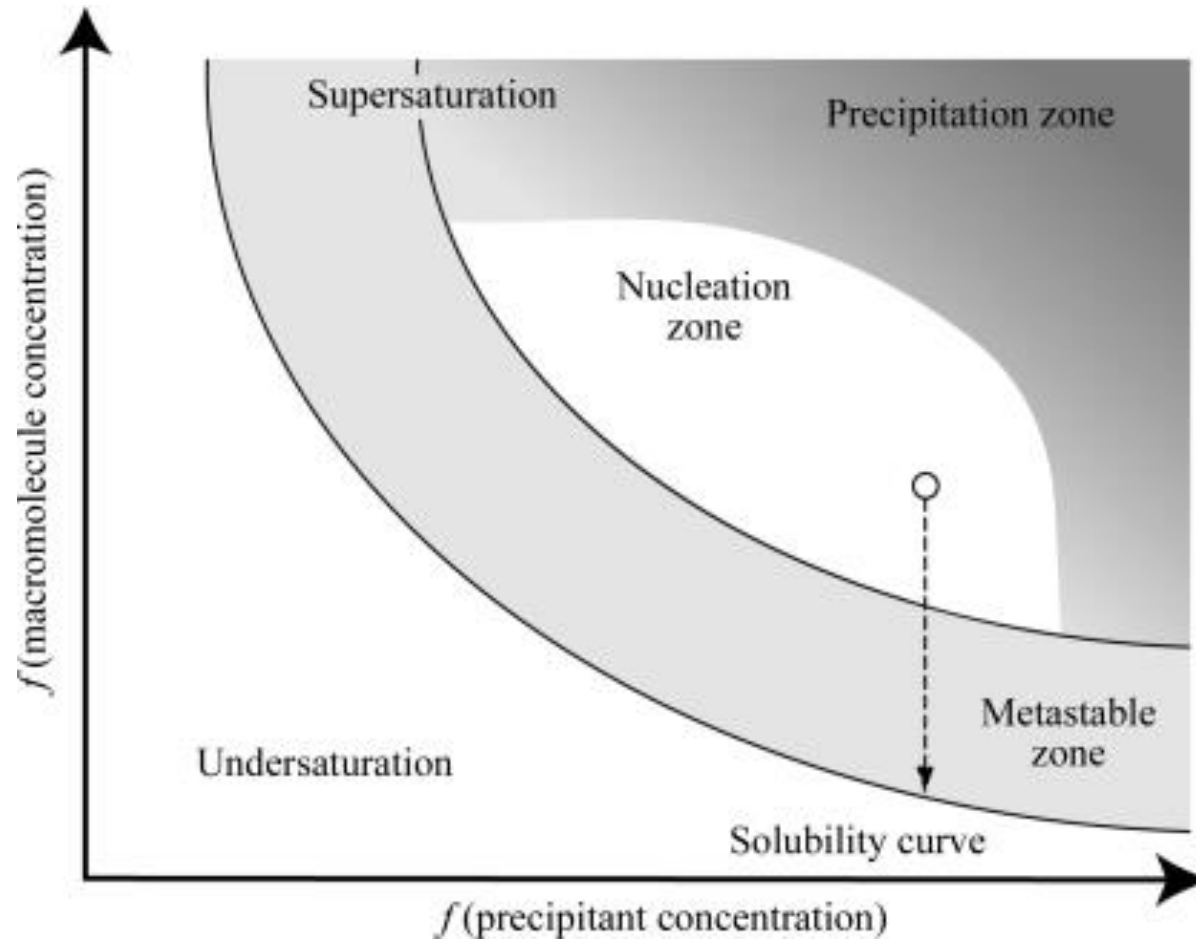




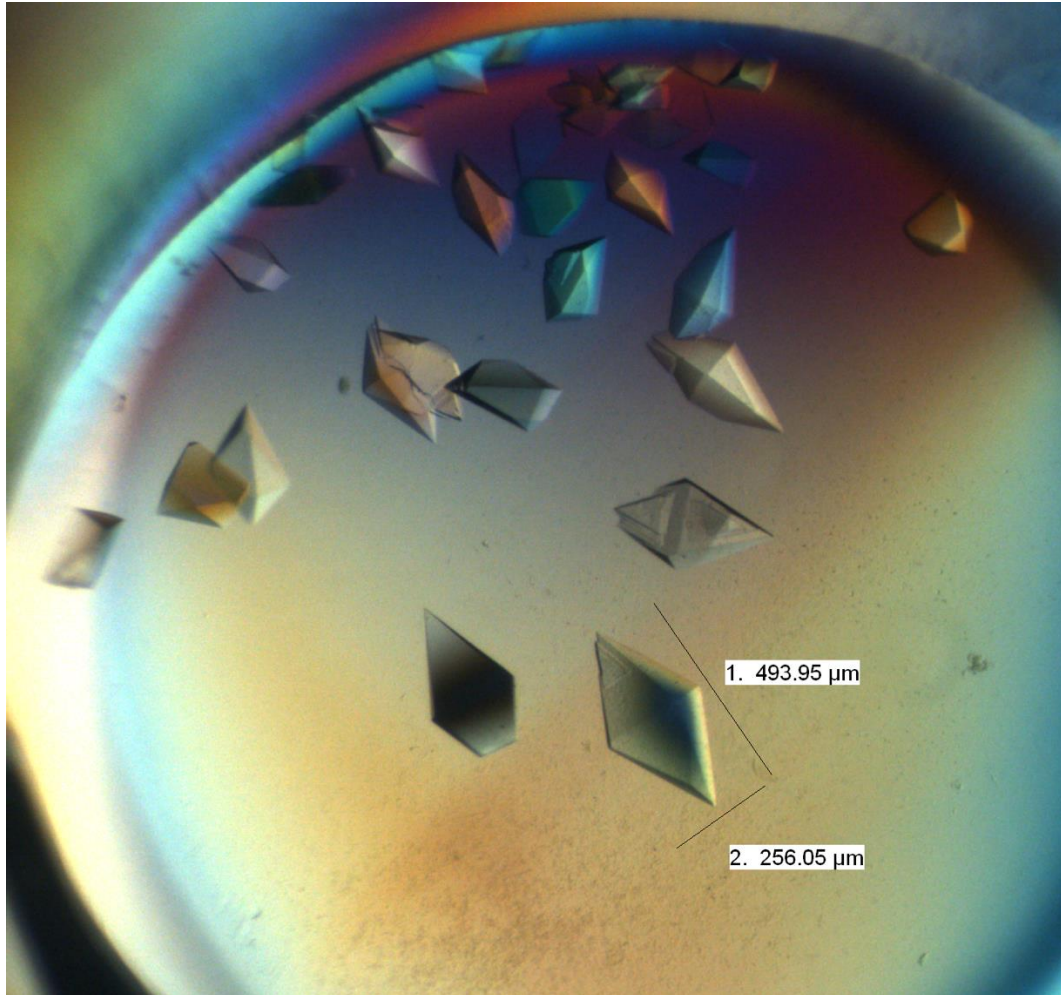
4 - 25 °C

SUPERSATURATION

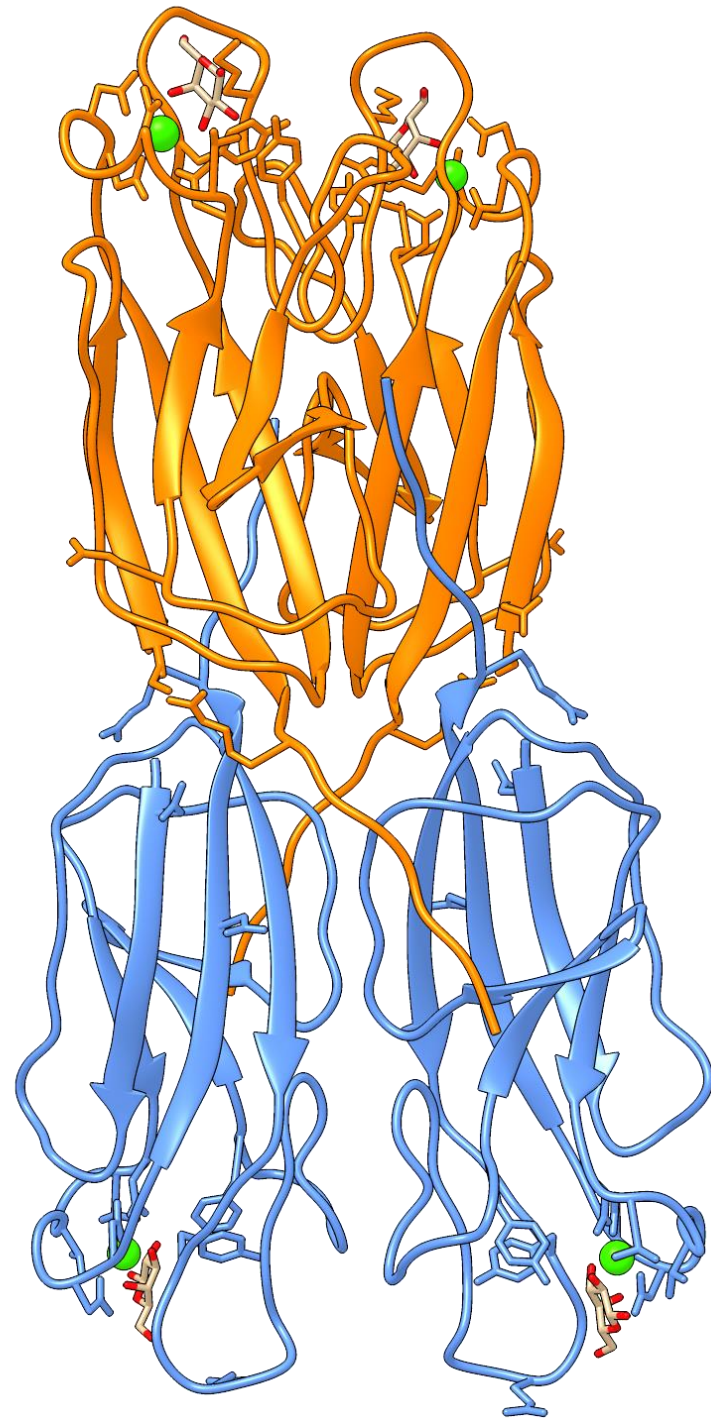
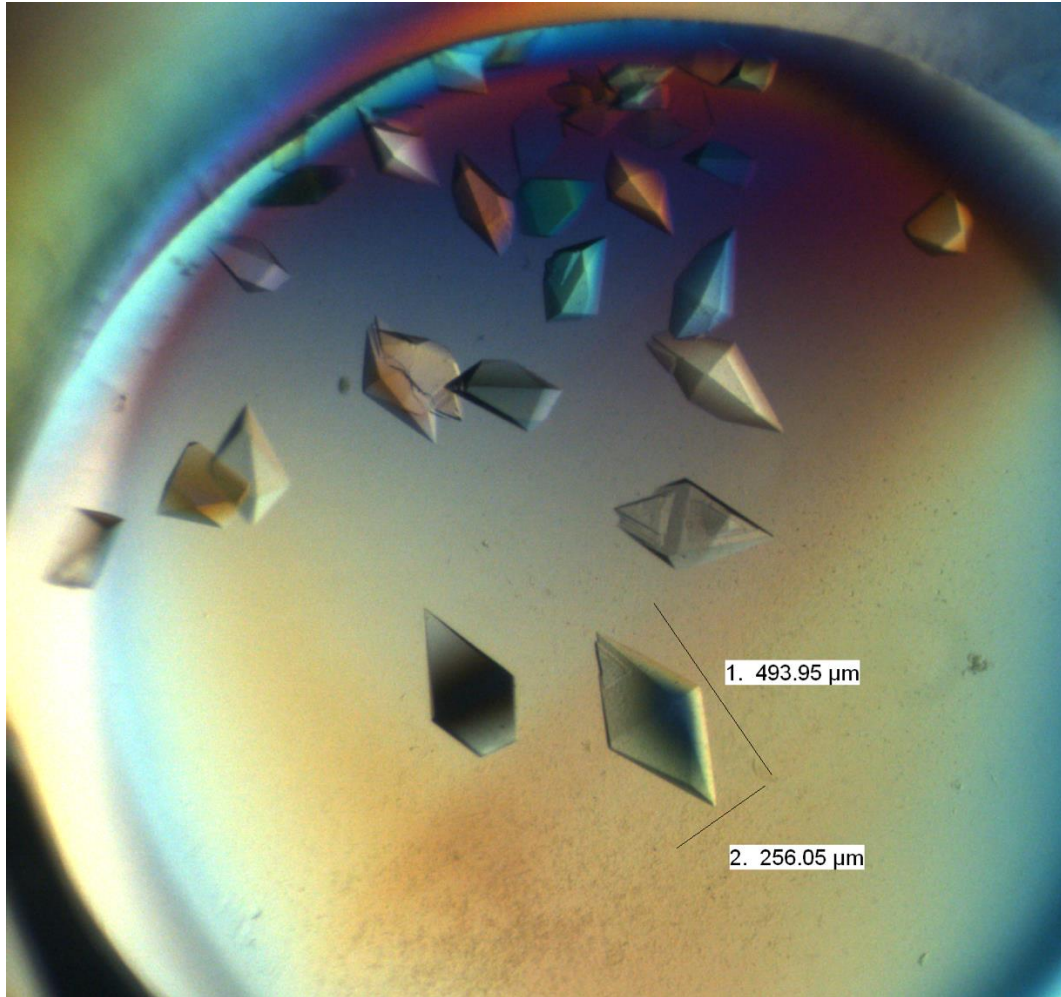
Fázový diagram krystalizace

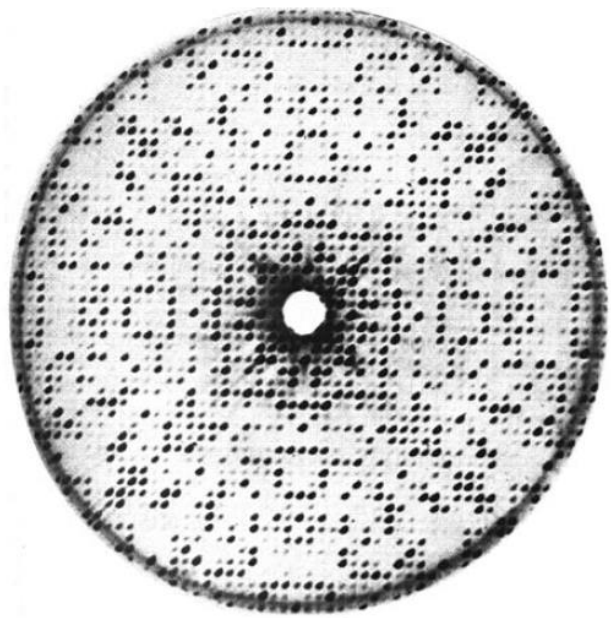


Proteinové krystaly - PluLec

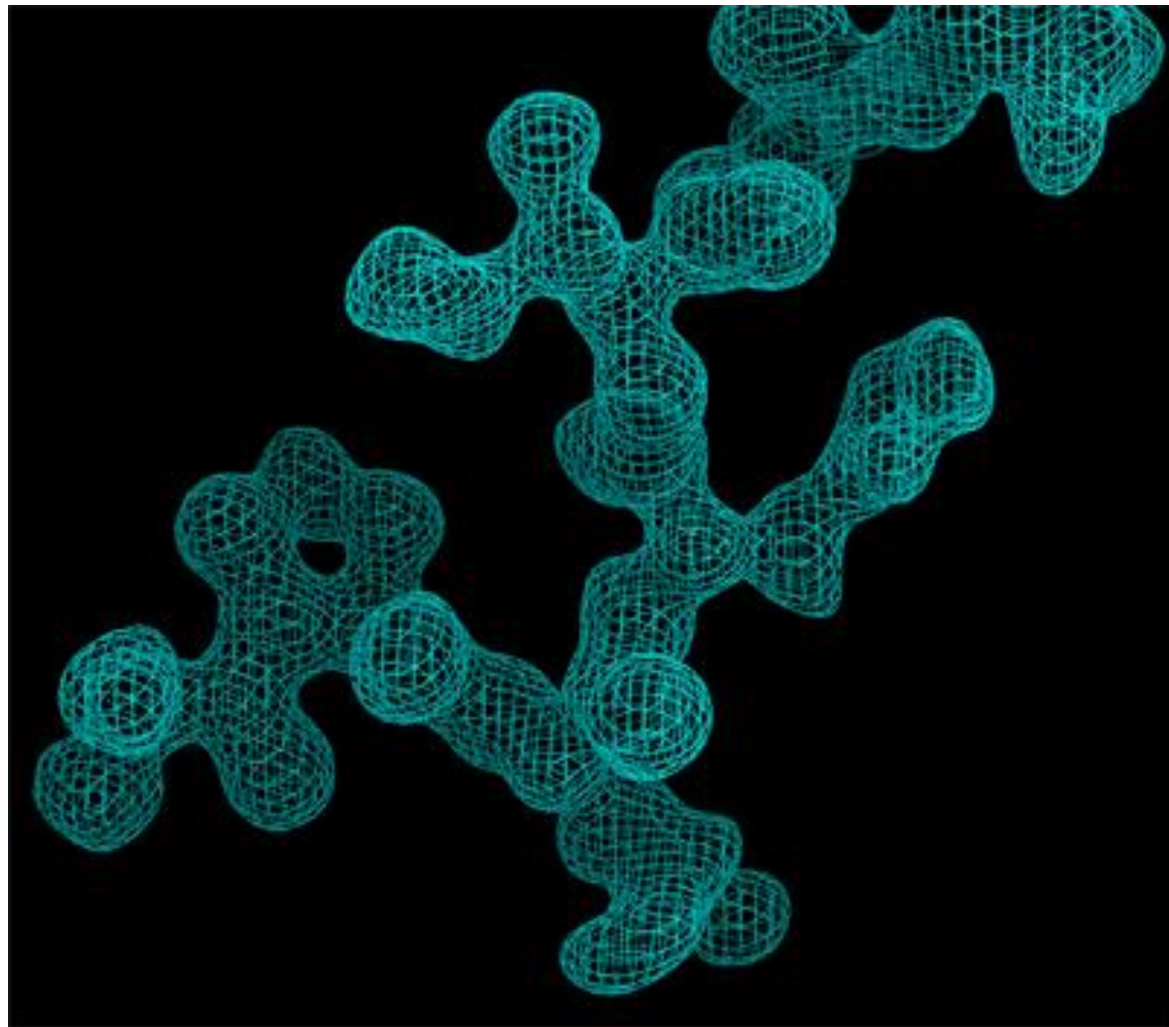


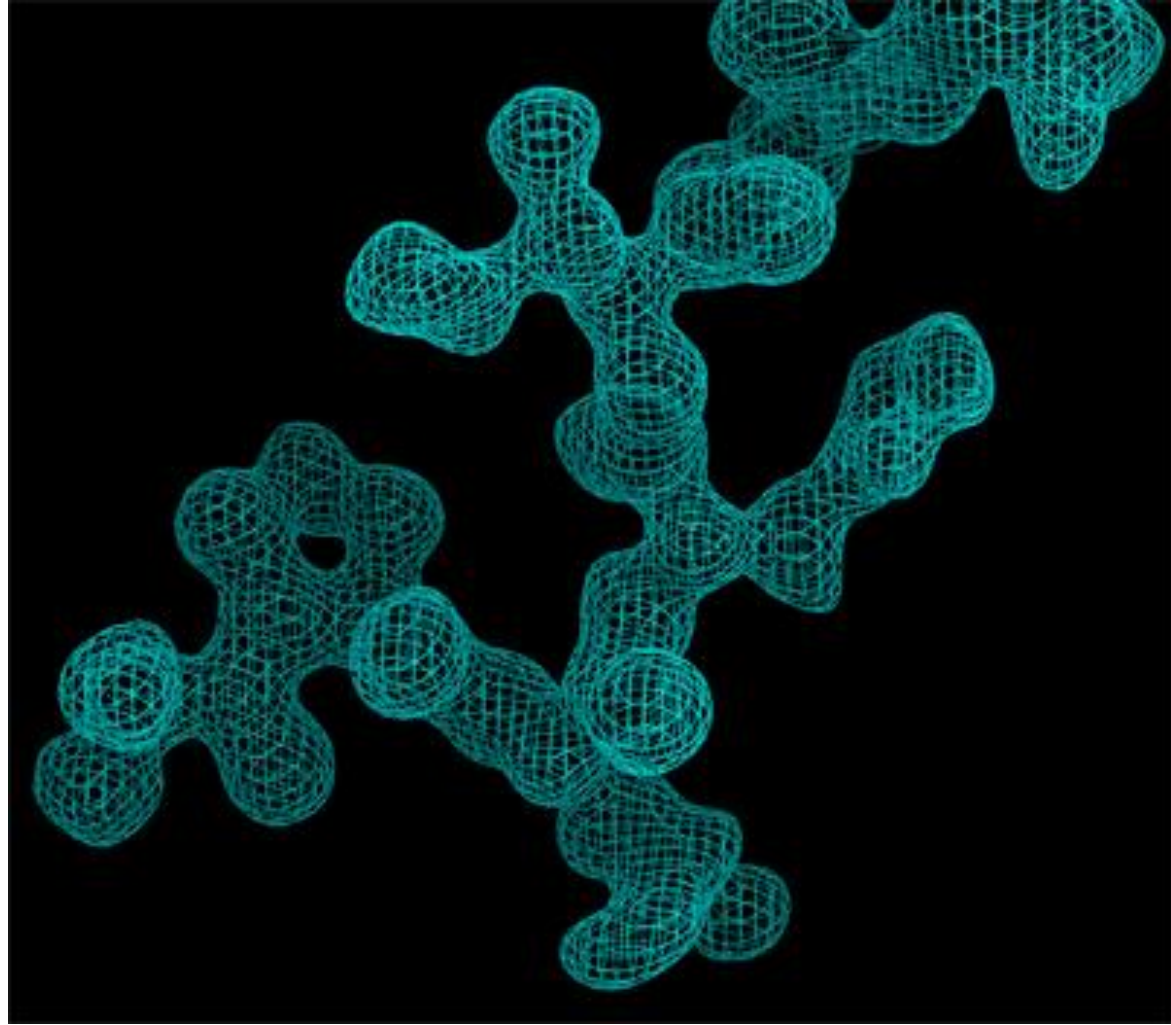
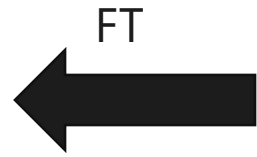
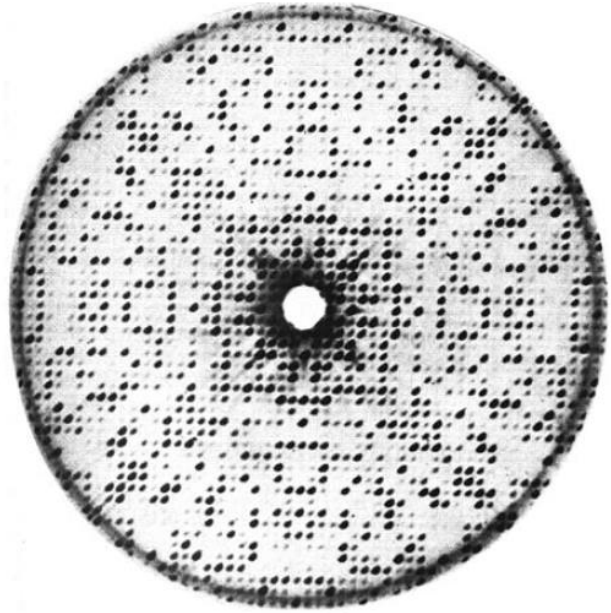
Proteinové krystaly

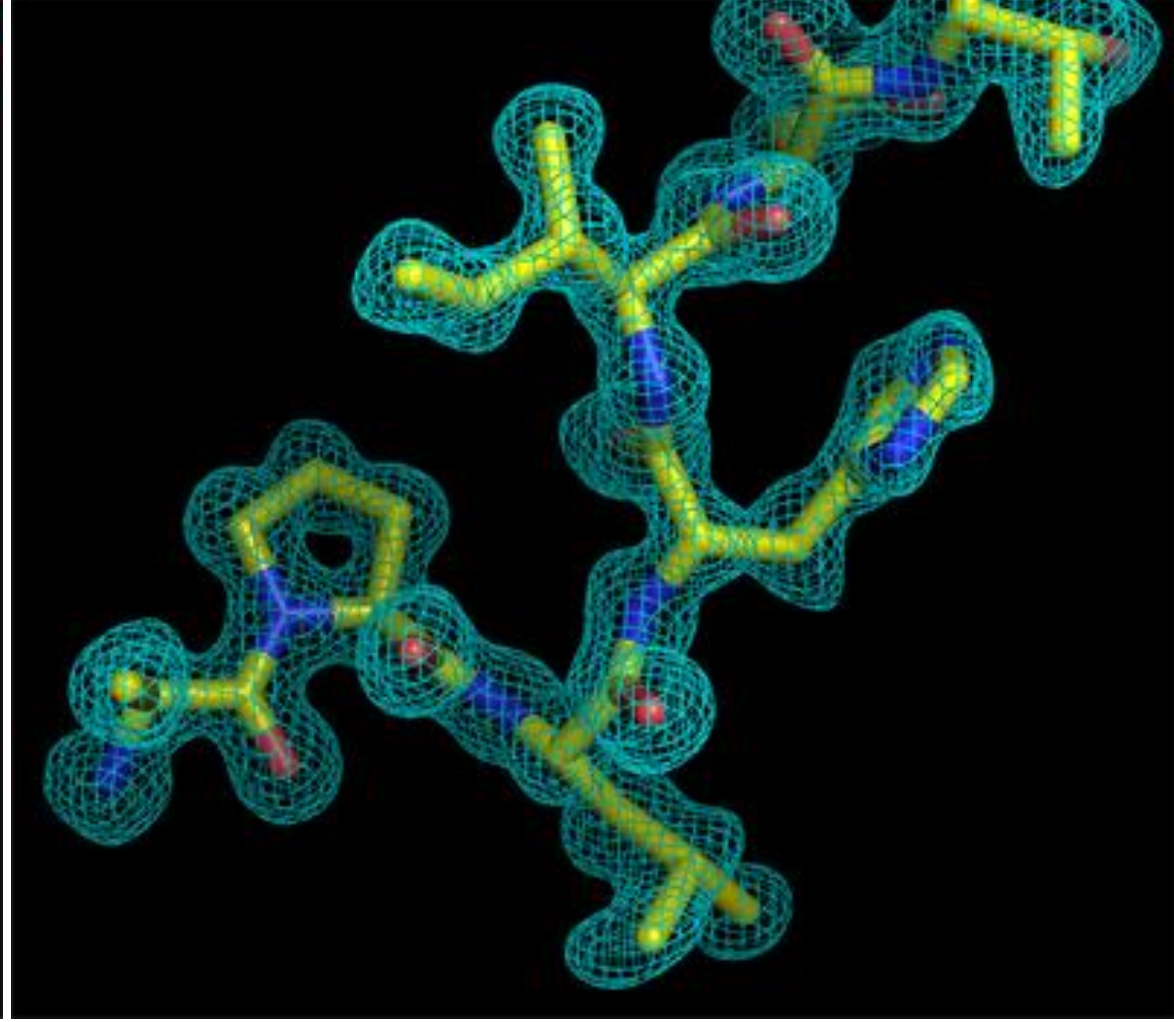
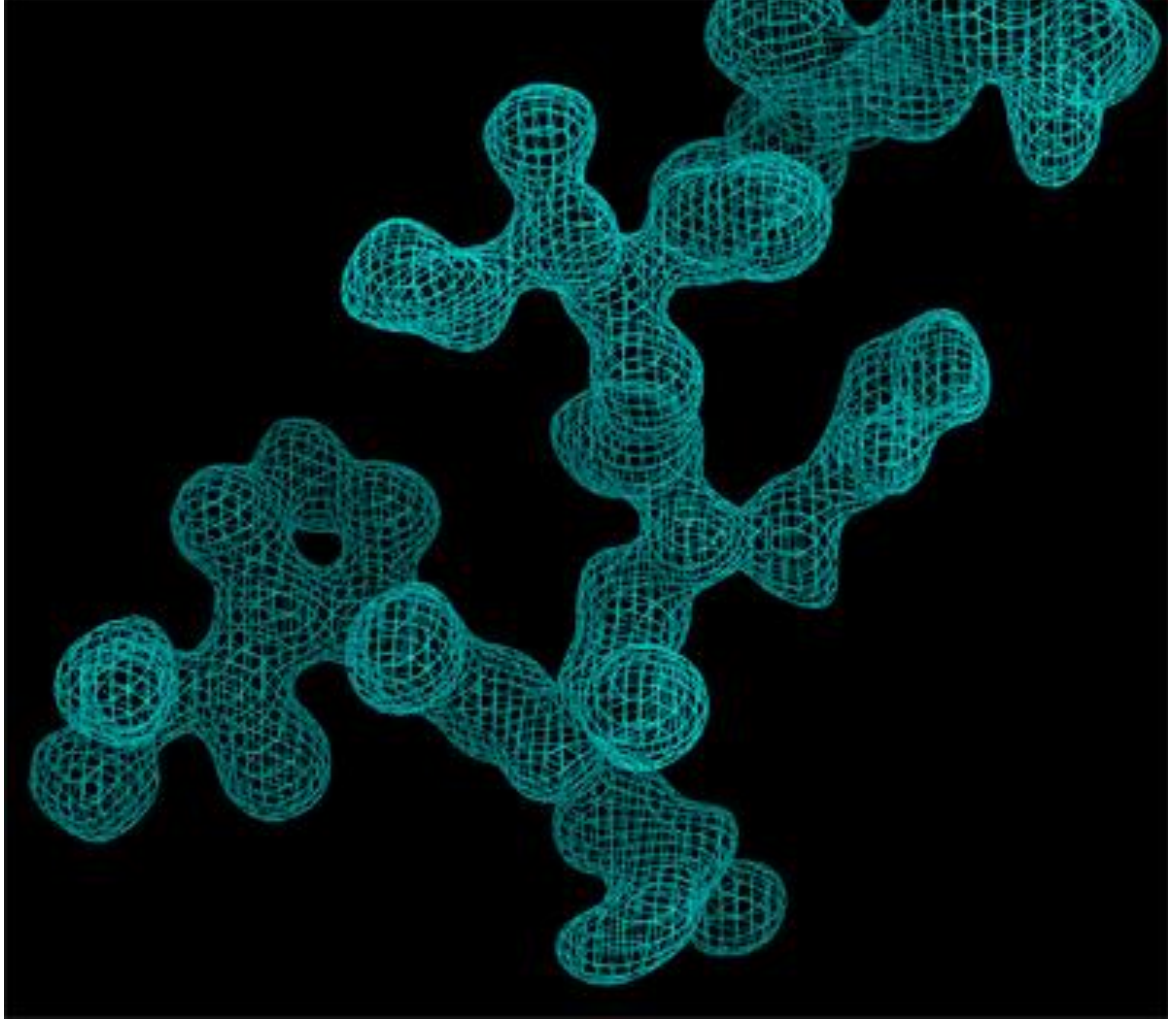




FT
→



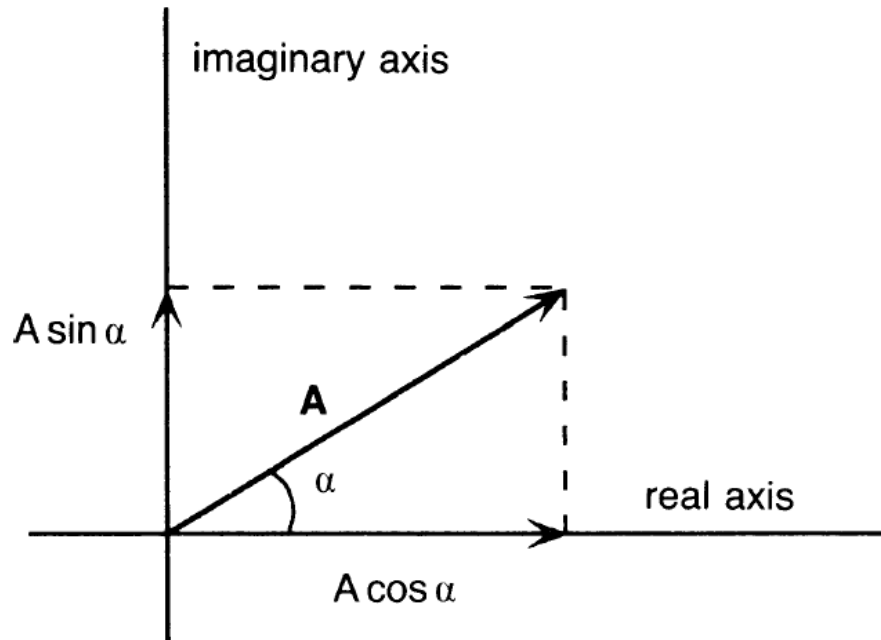




Argandův diagram

$$A = |A| (\cos \alpha + i \sin \alpha)$$

$$A = |A| \exp i \alpha$$



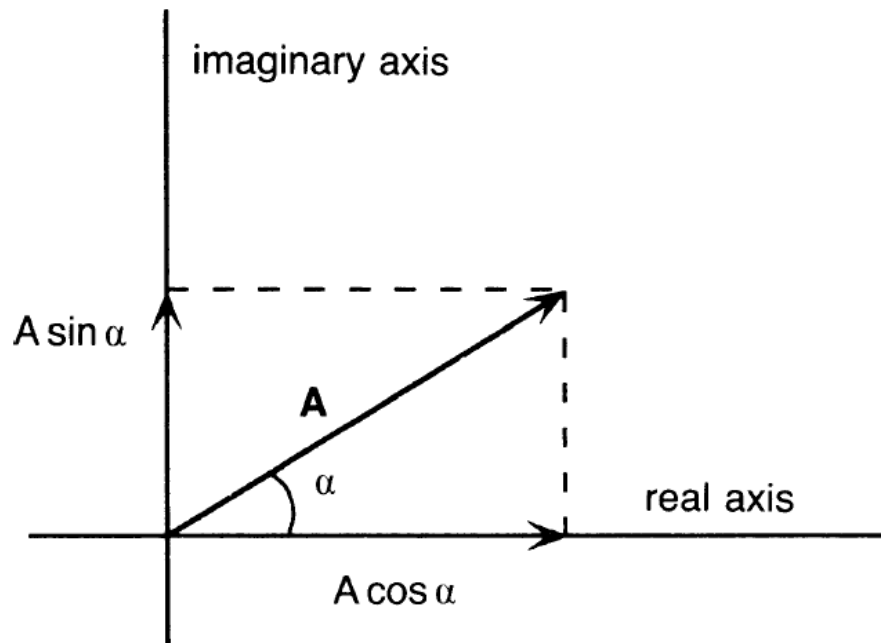
Argandův diagram

$$A = |A| (\cos \alpha + i \sin \alpha)$$

$$A = |A| \exp i \alpha$$

$$F_{(hkl)} = |F_{(hkl)}| (\cos \alpha_{(hkl)} + i \sin \alpha_{(hkl)})$$

$$F_{(hkl)} = |F_{(hkl)}| \exp [i \alpha_{(hkl)}]$$



Argandův diagram

$$A = |A| (\cos \alpha + i \sin \alpha)$$

$$A = |A| \exp i\alpha$$

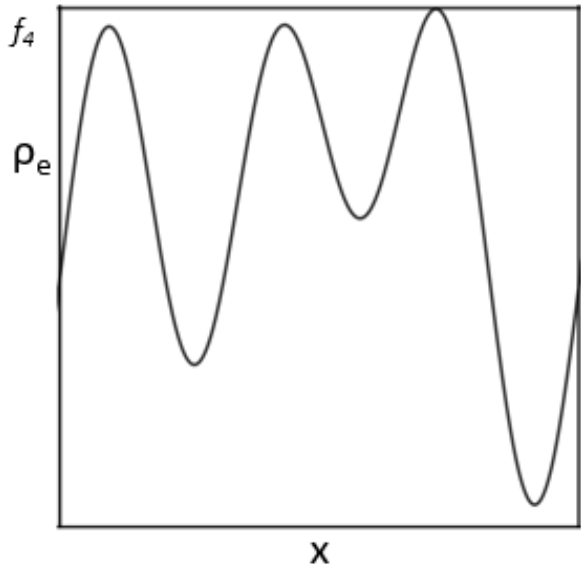
$$F_{(hkl)} = |F_{(hkl)}| (\cos \alpha_{(hkl)} + i \sin \alpha_{(hkl)})$$

$$F_{(hkl)} = |F_{(hkl)}| \exp [i\alpha_{(hkl)}]$$

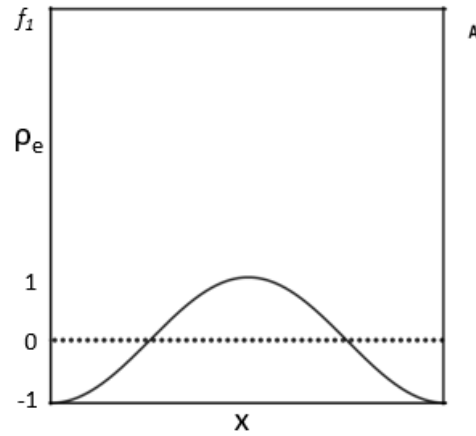
$$\rho_{(xyz)} = \frac{1}{V_C} \sum_h \sum_k \sum_l |F_{(hkl)}| \exp [-2\pi i(hx + ky + lz) + i\alpha_{(hkl)}]$$

FT složené funkce f_4

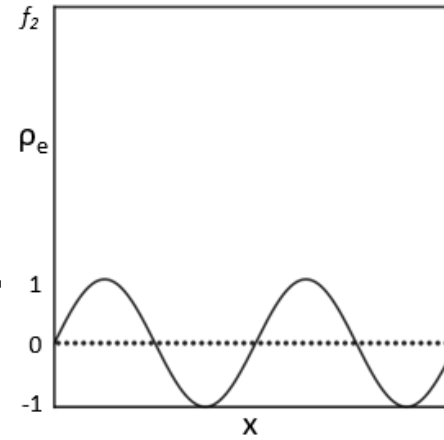
$$f_4 = f_1 + f_2 + f_3$$



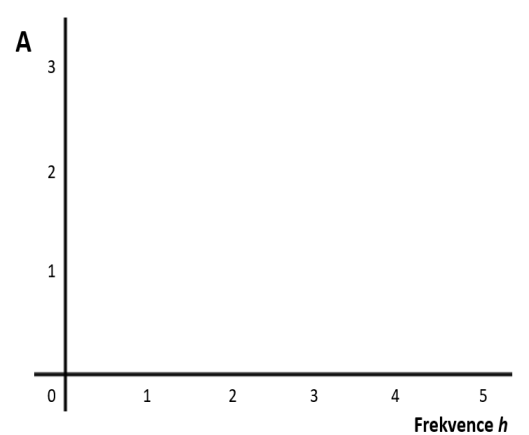
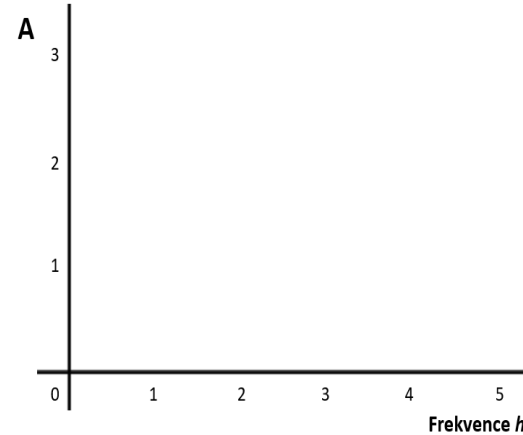
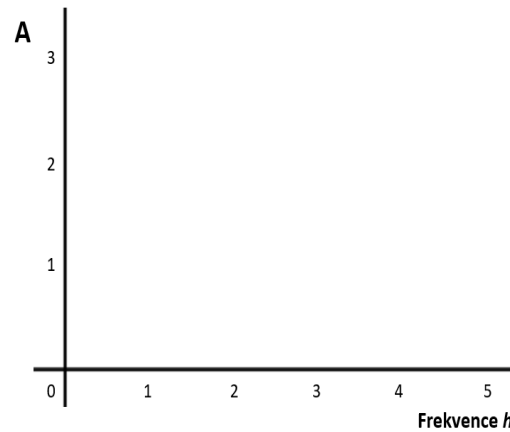
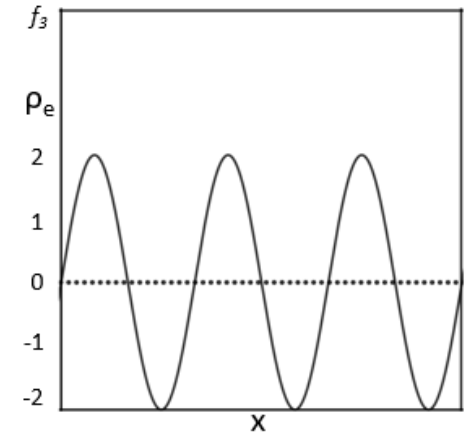
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+

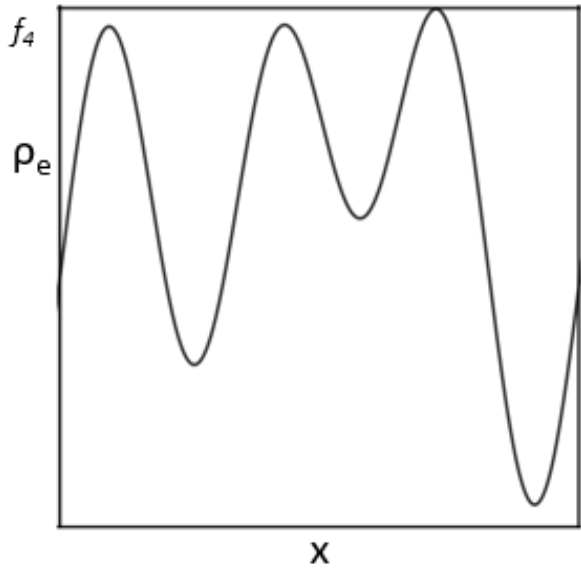


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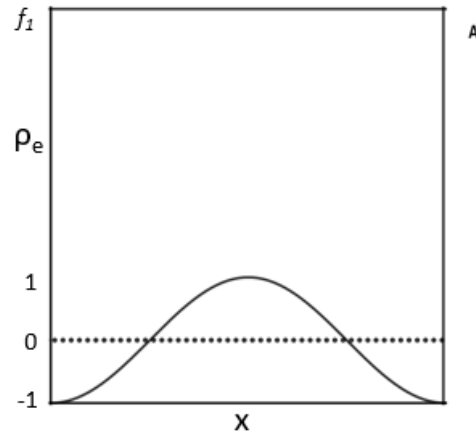


FT složené funkce f_4

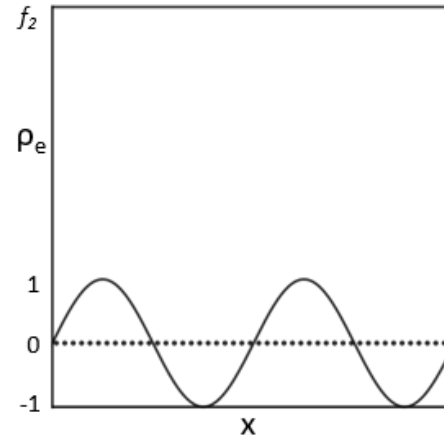
$$f_4 = f_1 + f_2 + f_3$$



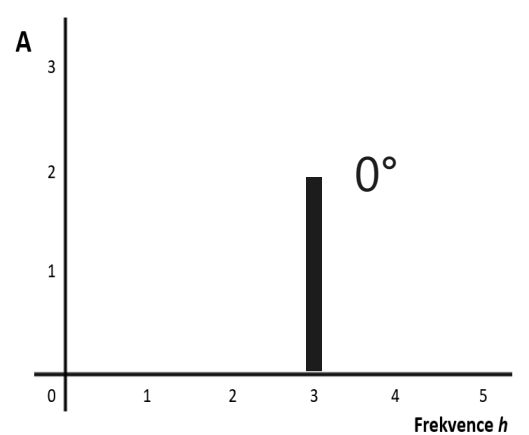
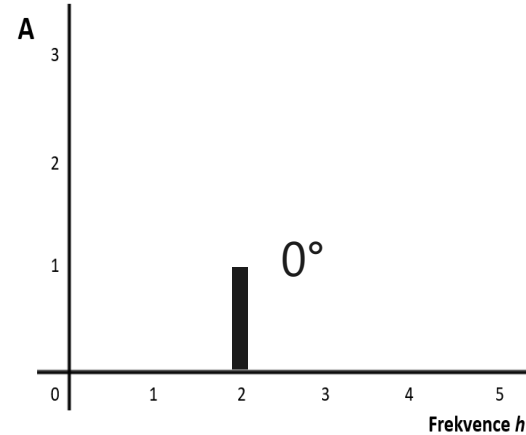
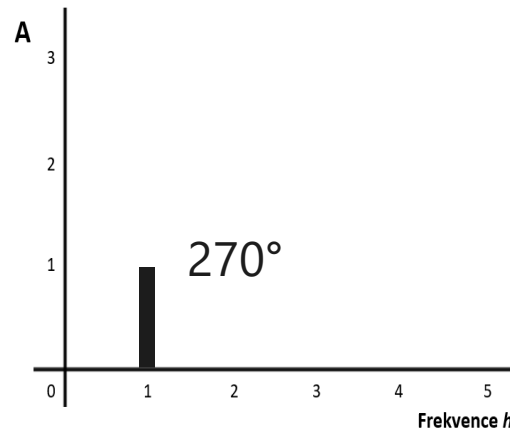
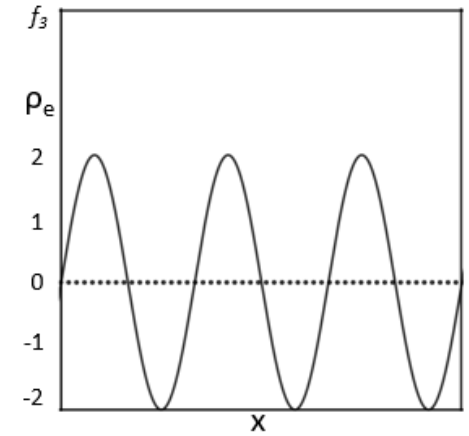
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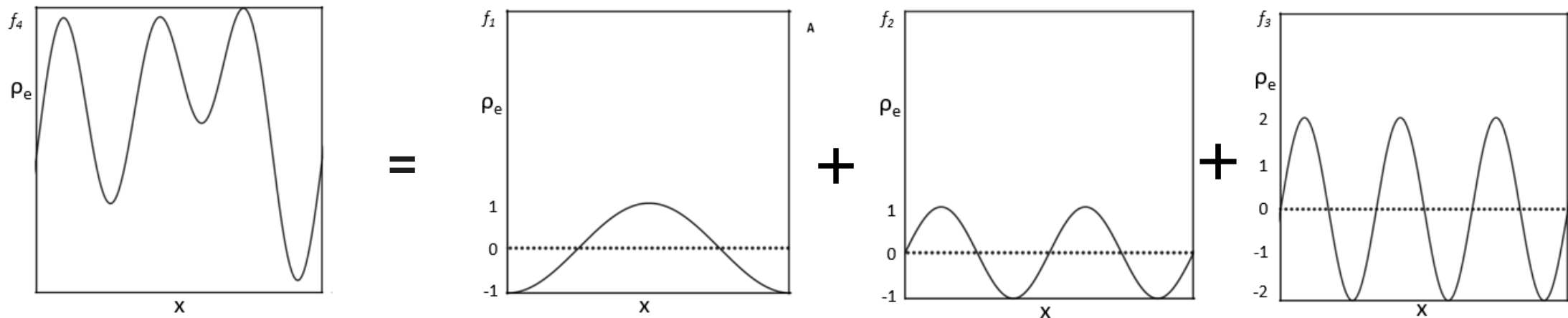


+



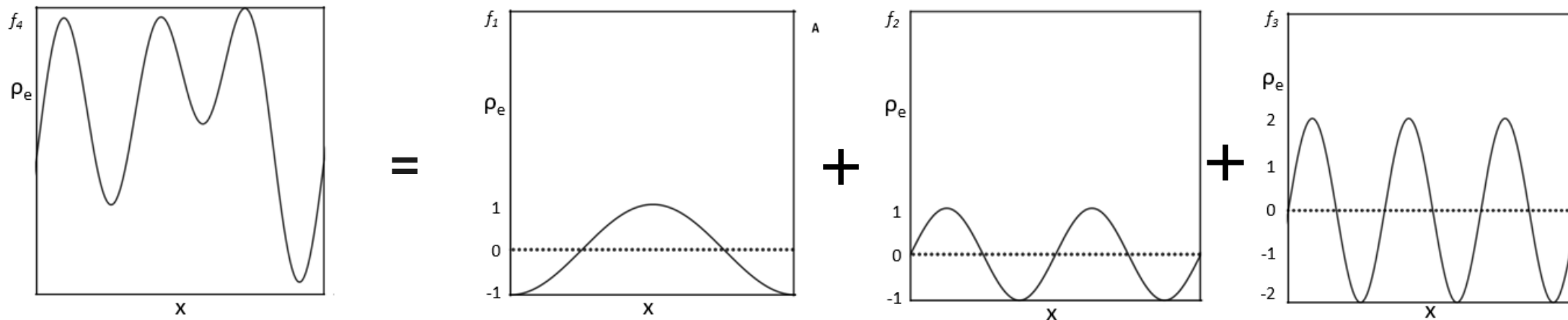
+





$$\rho_{(xyz)} = \frac{1}{V_C} \sum_h \sum_k \sum_l |F_{(hkl)}| \exp [-2\pi i(hx + ky + lz) + i\alpha_{(hkl)}]$$

Elektronová hustota ← $\rho(x) = \frac{1}{l} \sum_{n=1}^N |F_n| \exp [-2\pi i hx + i\alpha_{(n)}]$



Elektronová hustota



$$\rho(x) = \frac{1}{l} \sum_{n=1}^N |F_h| \exp \left[-2\pi i h x + \cancel{\psi} \right]$$

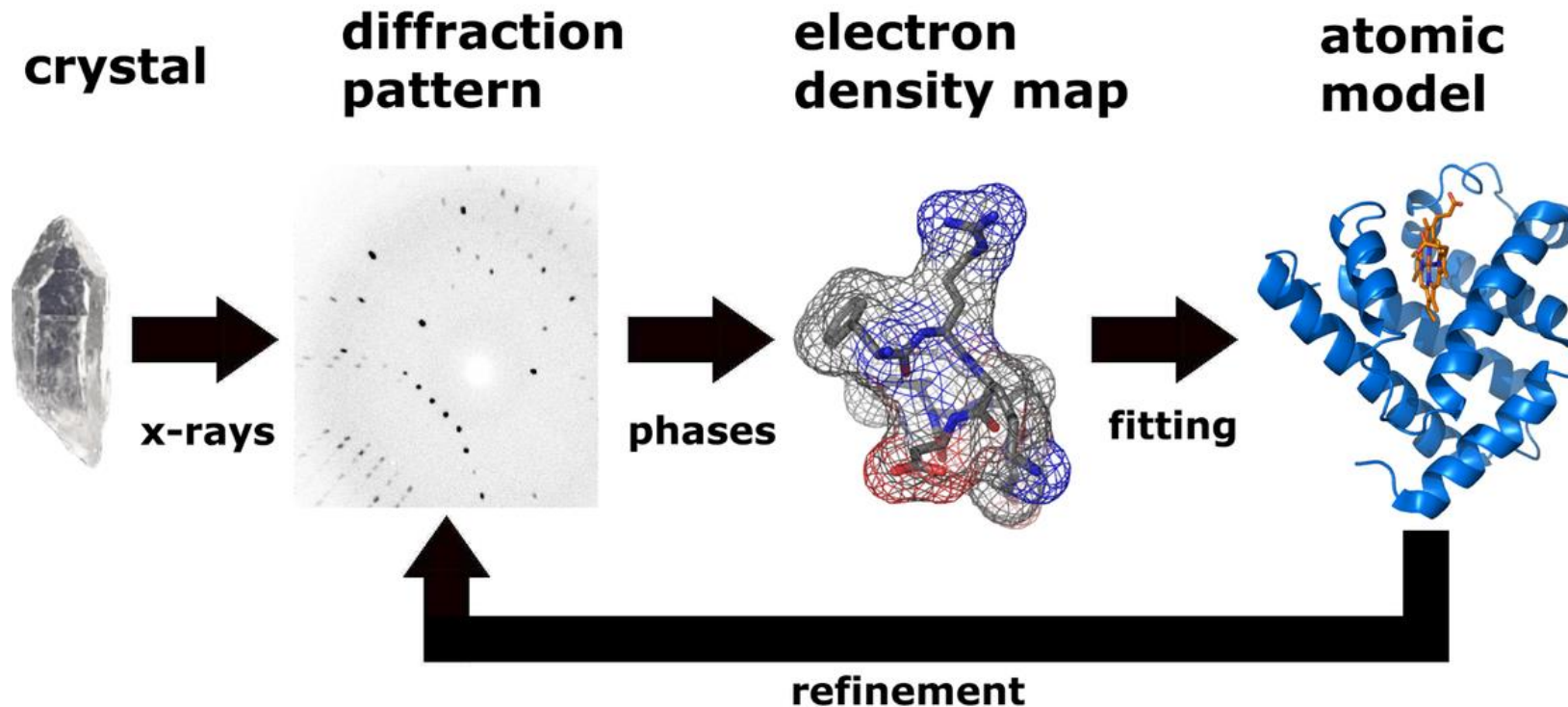
Amplituda

Frekvence

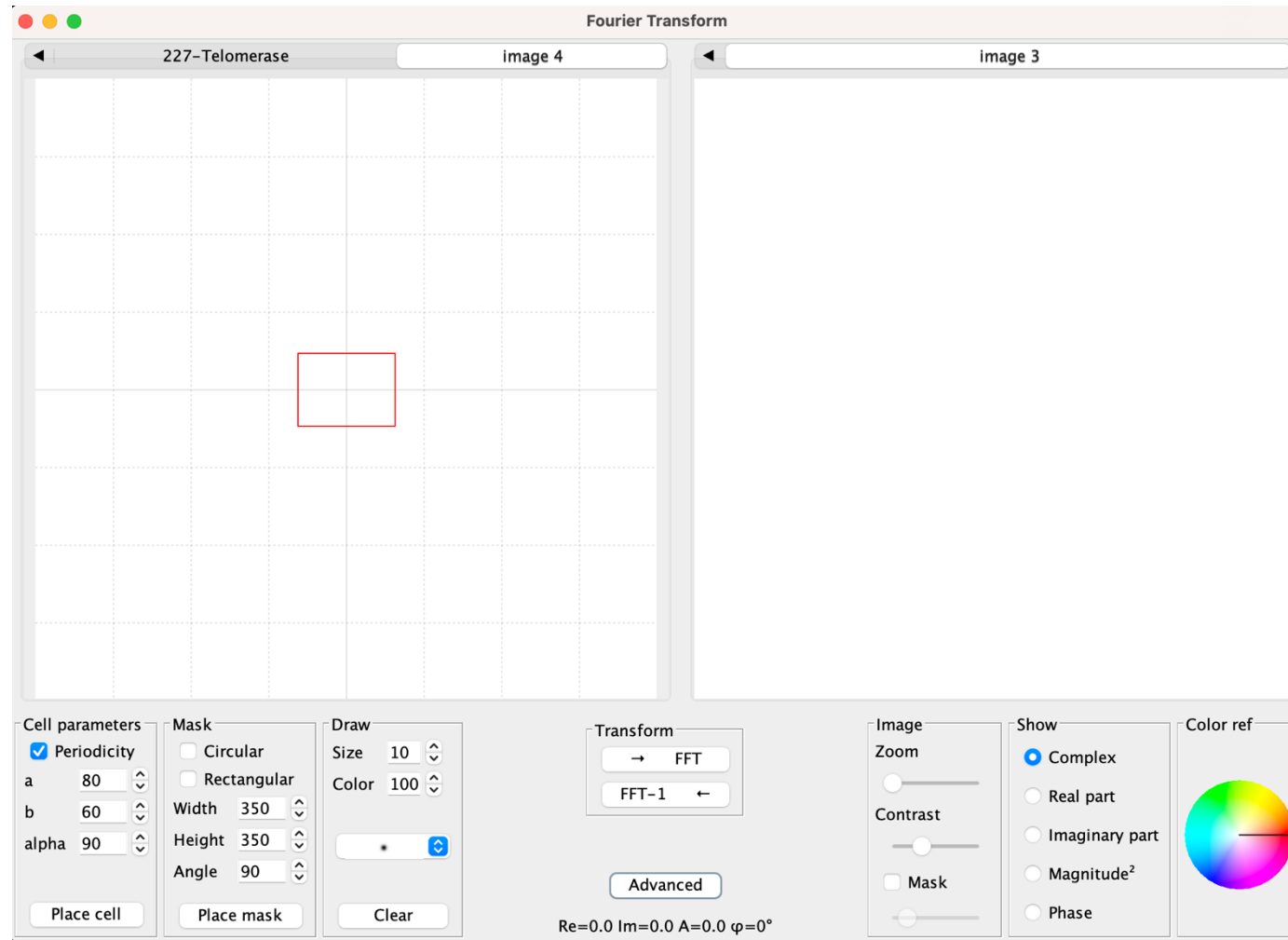
Fáze

Metody řešení fázového problému

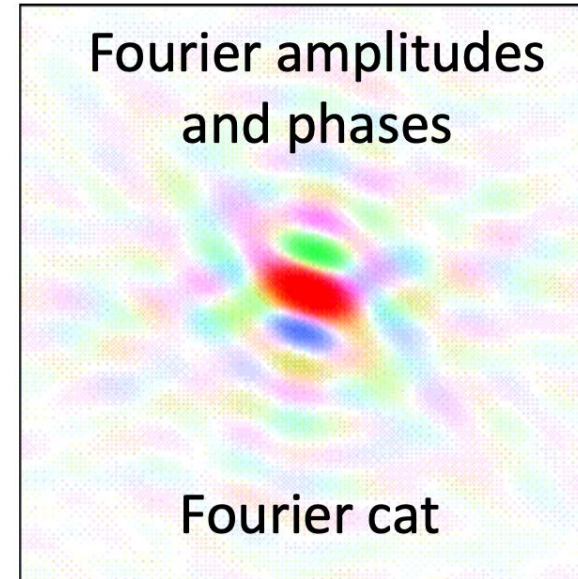
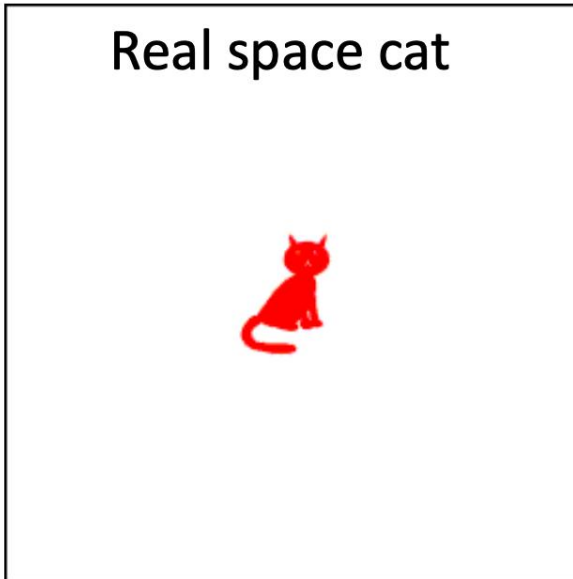
- Metoda molekulárního nahrazení
- Metoda izomorfního nahrazení
- Metoda anomálního rozptylu



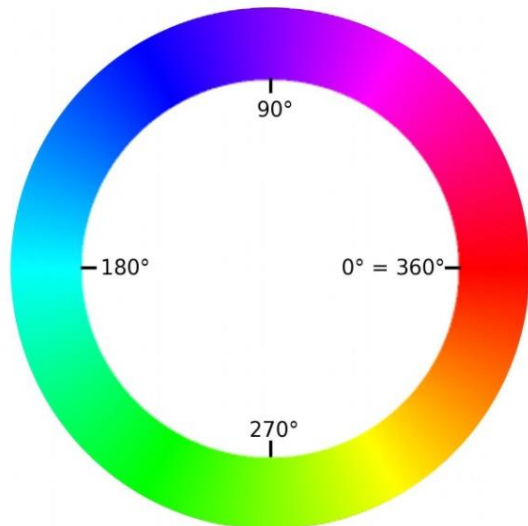
Fourierova transformace obrázku



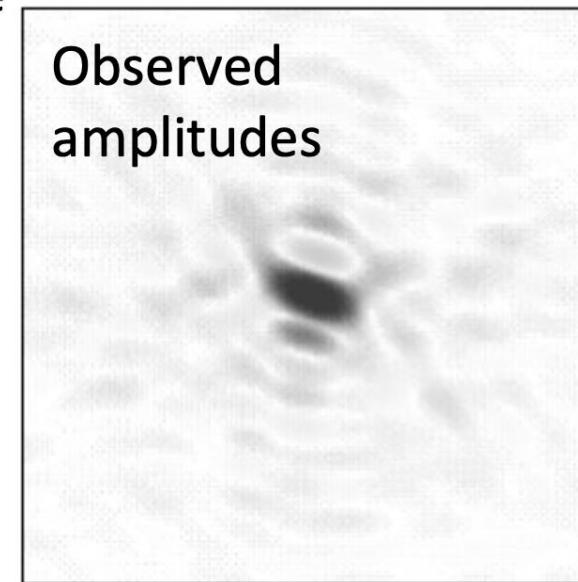
[Odkaz program ZDE](#) je potřeba mít java na PC




Circular rainbow scale of phases



Linear intensity scale of amplitude size

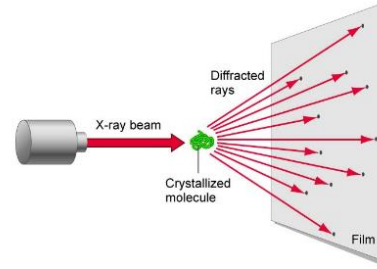


**Unknown structure,
unknown orientation**

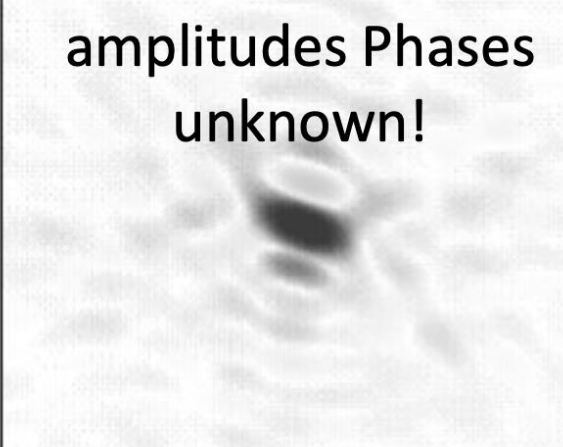


Cat

Diffraction
experiment




Observed
amplitudes Phases
unknown!

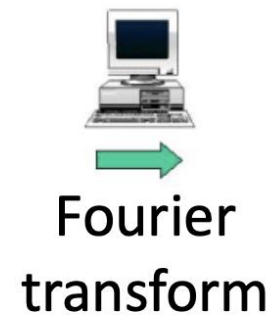


Fourier cat

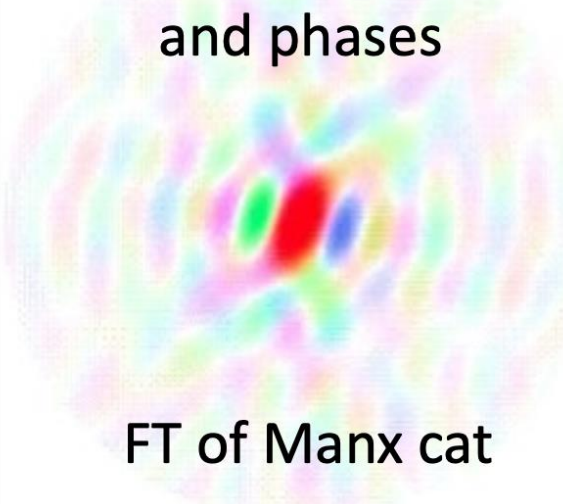
Known structure



Manx cat



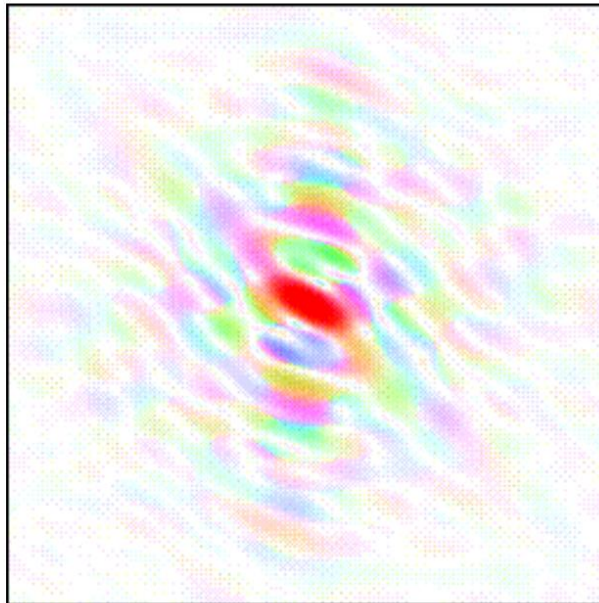
Calculated amplitudes
and phases




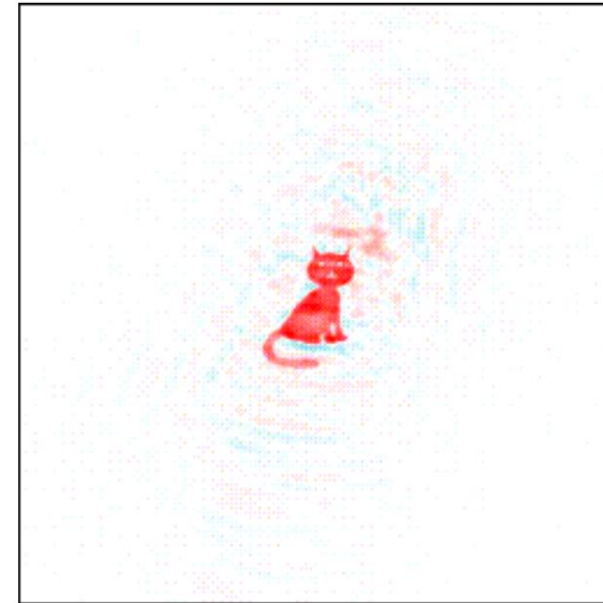
FT of Manx cat

Wrong orientation!

Observed **amplitudes** (tailed cat), calculated **phases** (Manx cat)

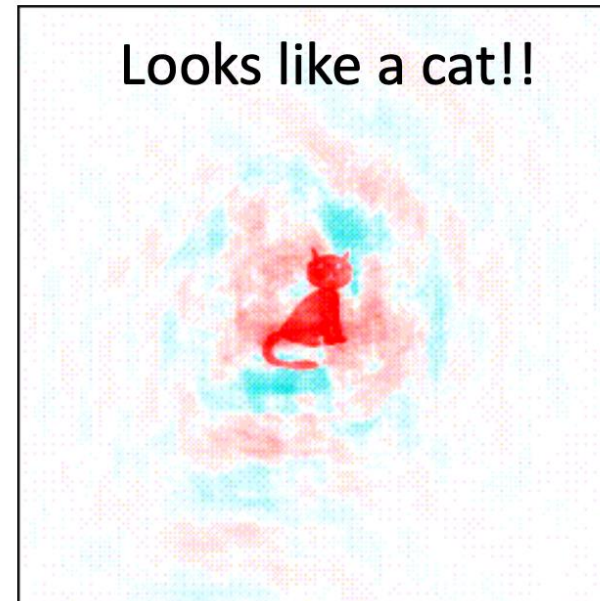
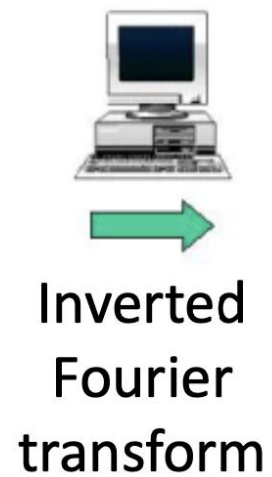
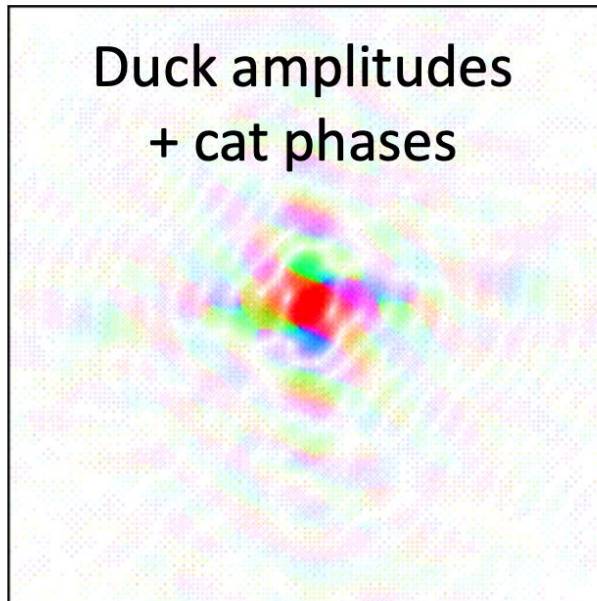
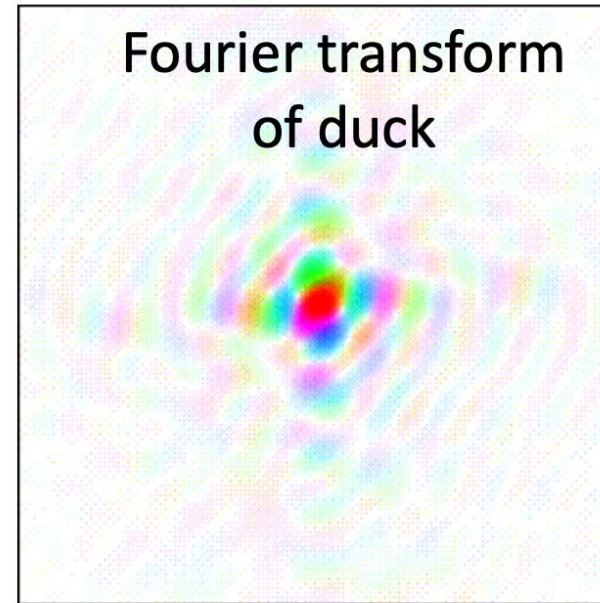
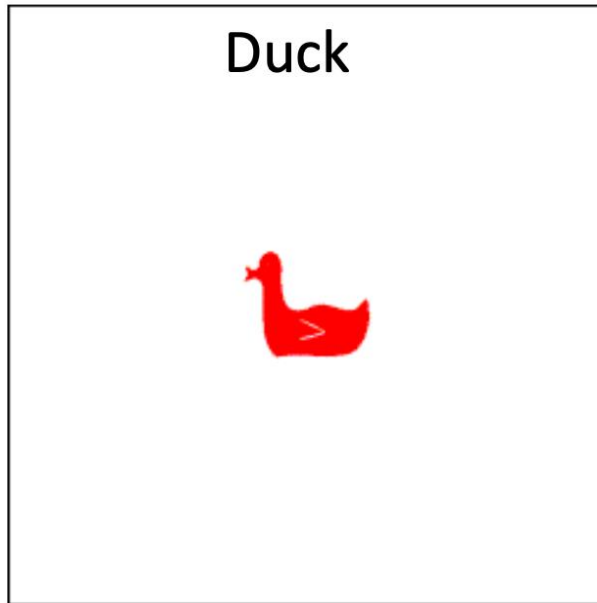



→
Inverted
Fourier
transform



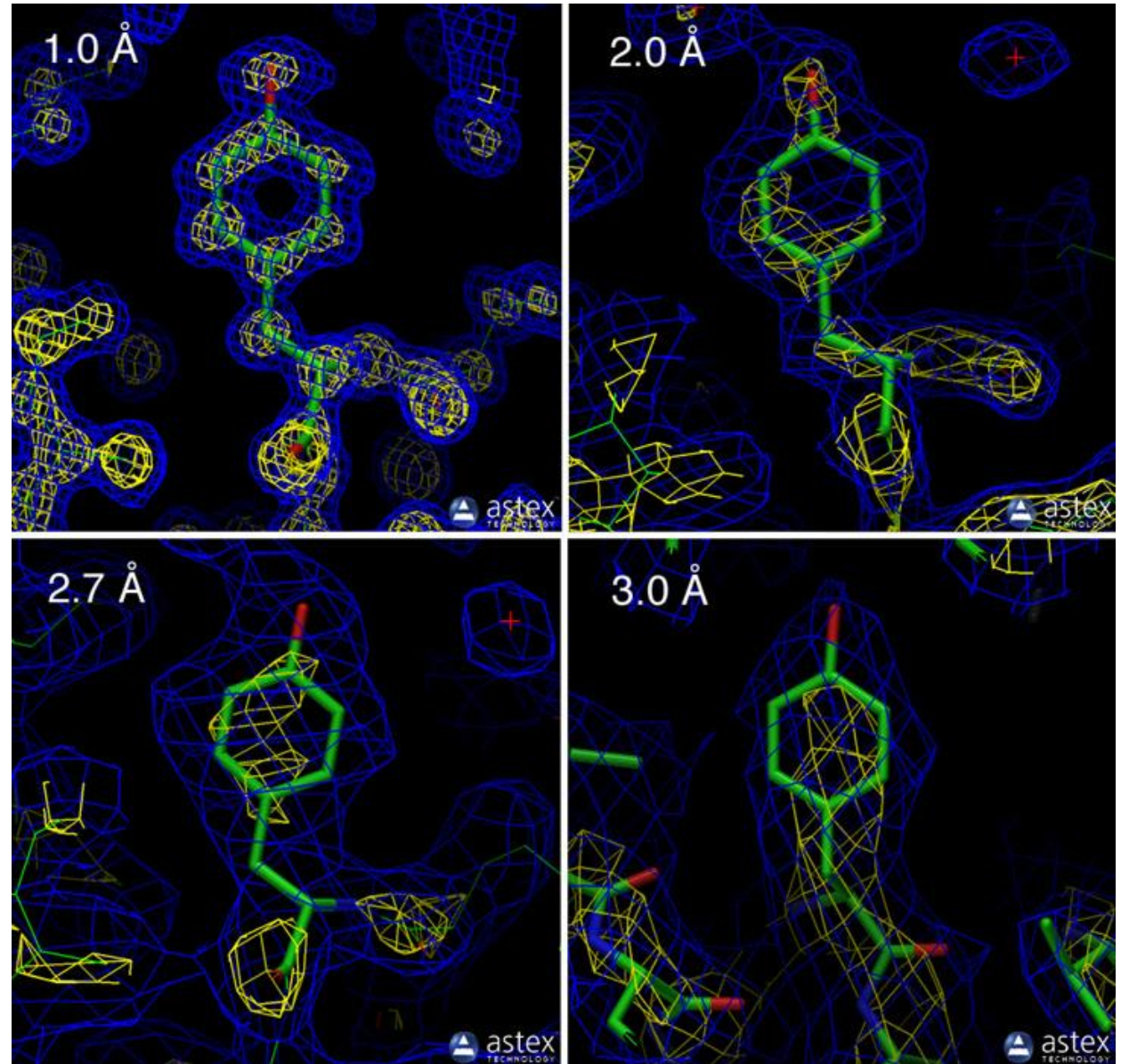
Even the tail becomes visible!

Model Bias

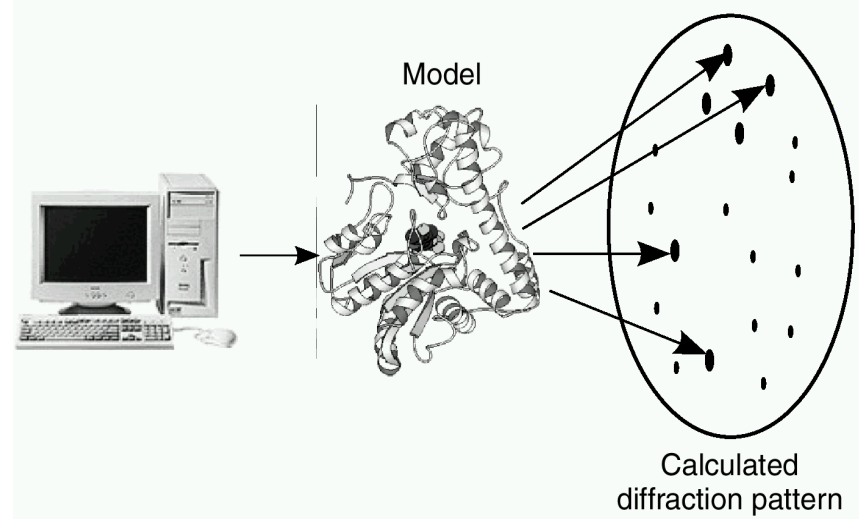
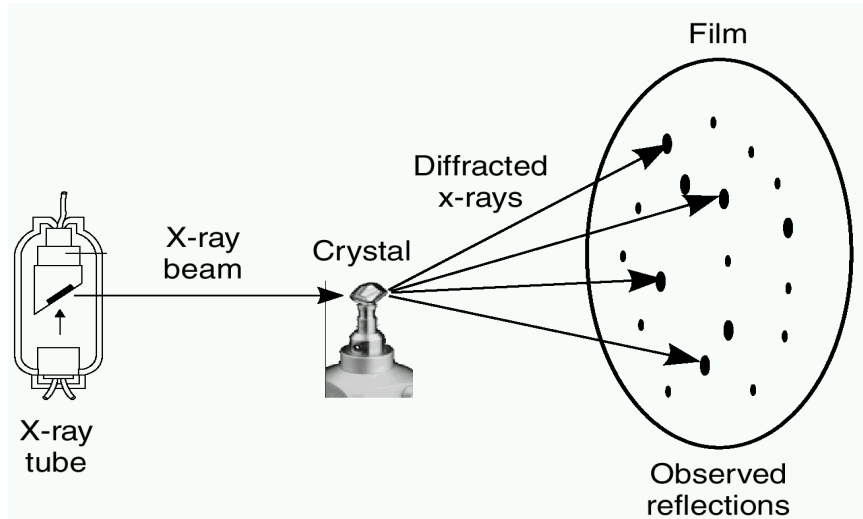


Rozlišení modelu

- Proteinové struktury mívají mezi 1-2 Å
- Flexibilní části často nejsou pozorovatelné



R-factor a R_{free} factor – ověření



R-factor

$$R = \frac{\sum_{hkl} ||F_{\text{obs}}| - k|F_{\text{calc}}||}{\sum_{hkl} |F_{\text{obs}}|}$$

R_{free} factor

$$R_{\text{free}} = \frac{\sum_{hkl \subset T} ||F_{\text{obs}}| - k|F_{\text{calc}}||}{\sum_{hkl \subset T} |F_{\text{obs}}|}$$

Výpočet struktury z náhodných fází

The interface is divided into several sections:

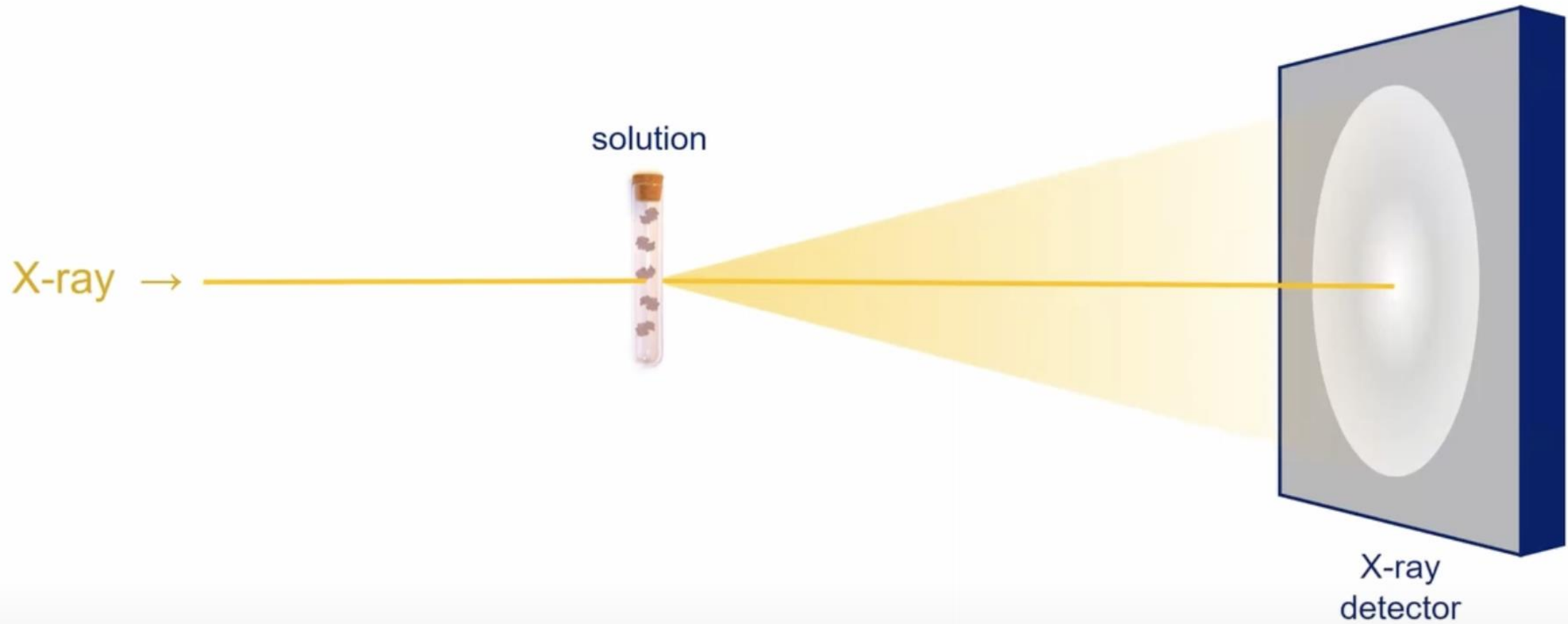
- Original structure:** A grid for drawing the initial structure.
- Reconstructed structure:** A grid for displaying the reconstructed structure.
- Flowchart:** A central diagram showing the iterative process: **Draw a Structure** (orange box) leads to **FFT**, which produces **F**. **F** is processed by **Random Phases** to produce **ρ**. **ρ** is processed by **Flip charges ($\rho < \delta$)** to produce **g**. **g** is processed by **FFT** to produce **G**. **G** is processed by **Restore amplitudes** to produce **F**. **F** is processed by **FFT-1** to produce **ρ**.
- Graph:** A plot showing the progress of the reconstruction. The y-axis ranges from 0% to 100%. The legend indicates: **Total Charge G(0)** (red line) and **R factor** (blue line).
- Control Panel:**
 - Drawing:** Pen size (10), Darkness (100), Position (-109 95), Clear button.
 - Run:** FFT, Do one cycle, Loop, Reset buttons.
 - Parameters:** Mask Radius (checked), Delta (0.4279), Dynamic (checked, 10%), Weak reflections (0%), Re=0.0 Im=0.0 A=0.0 $\varphi=0^\circ$, Default button.
 - Image:** Zoom, Contrast, Rotate 180° buttons.
 - Show:** Complex (checked), Real part, Imaginary part, Magnitude², Phase radio buttons.
 - Color ref:** A color wheel for reference.

[Odkaz program ZDE](#) je potřeba mít java na PC

No crystal – No problem

Small-angle X-ray scattering
SAXS

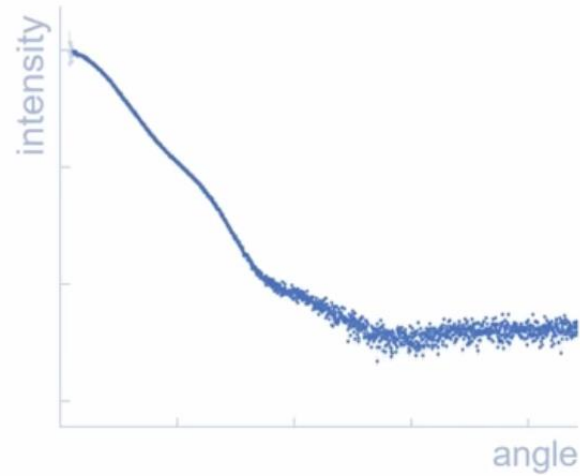
Maloúhlý rozptyl rentgenového světla



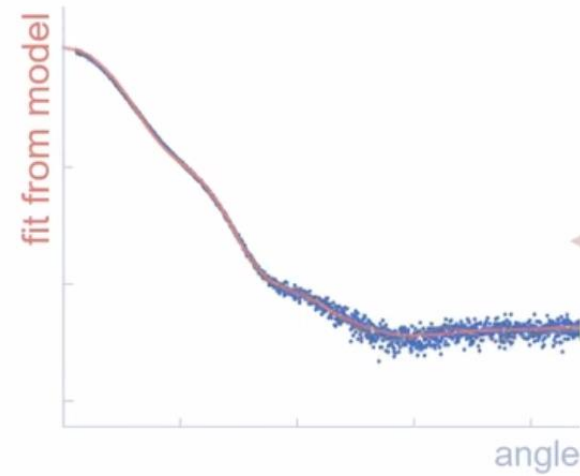
Maloúhľý rozptyl rentgenového svetla



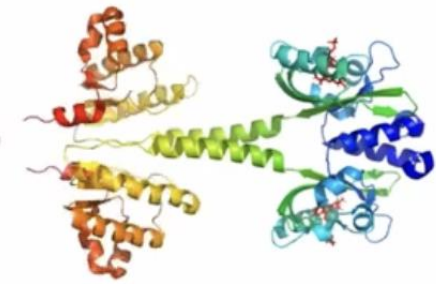
X-ray detector

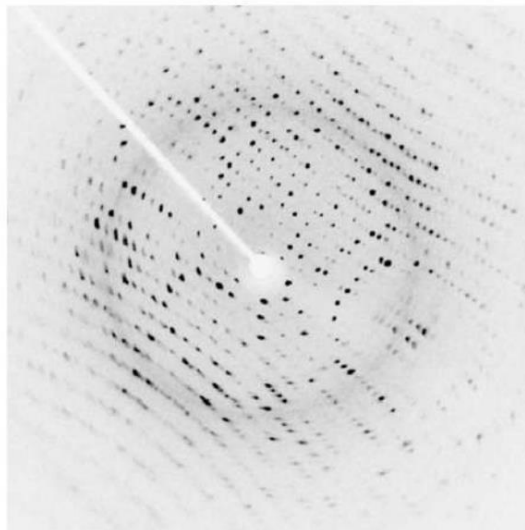
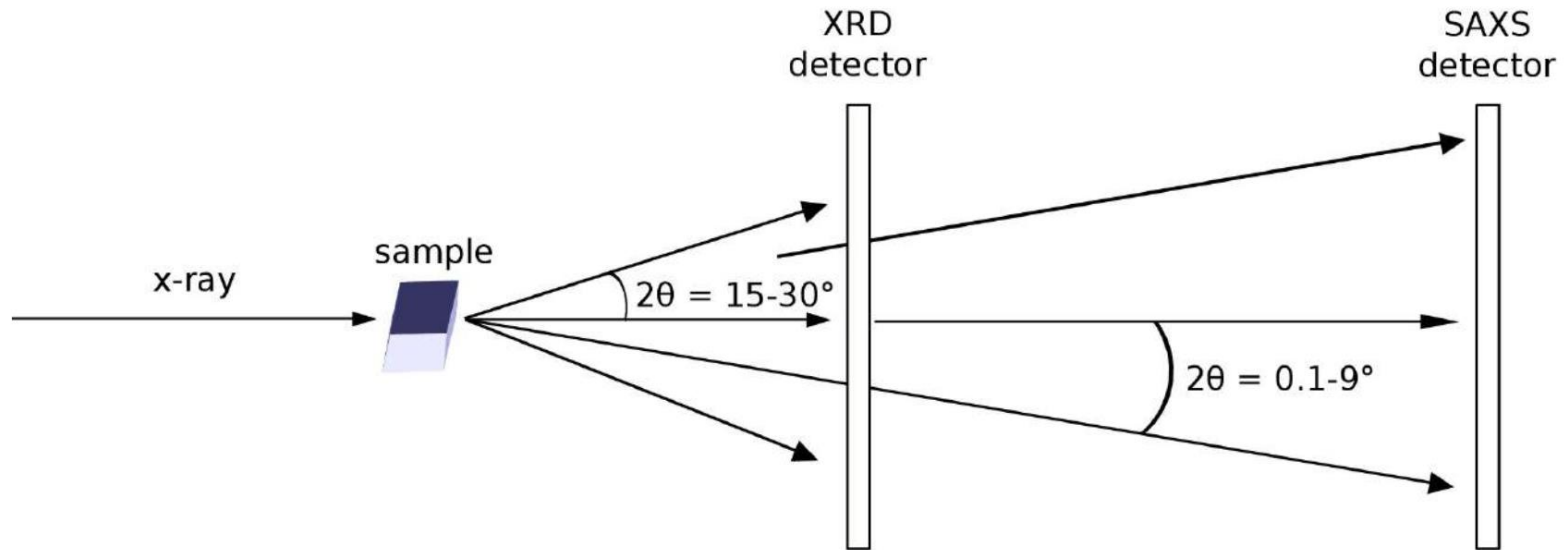


SAXS data

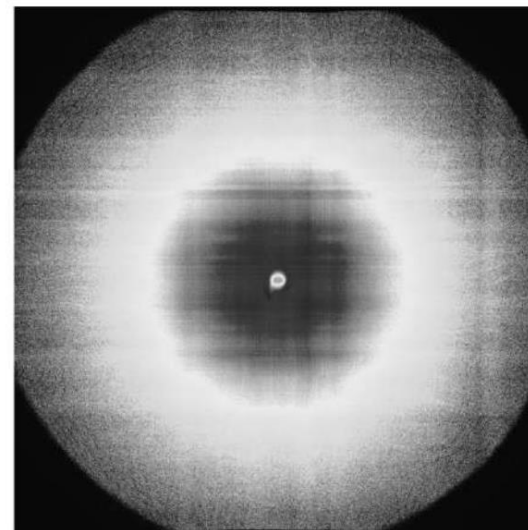


Model

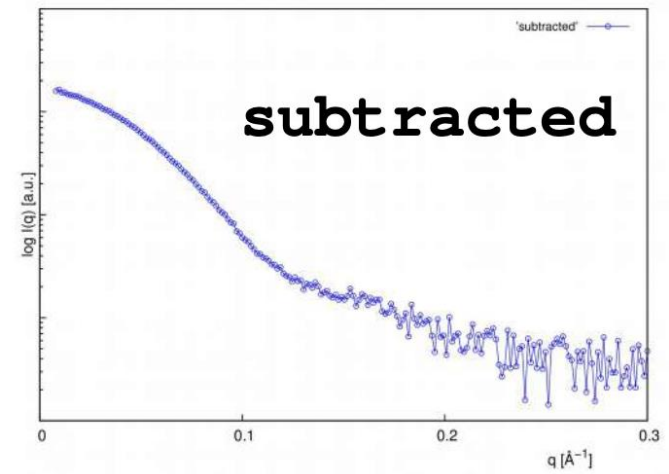
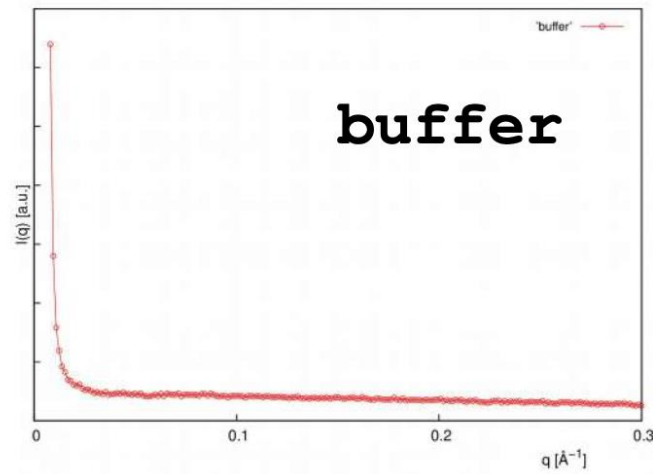
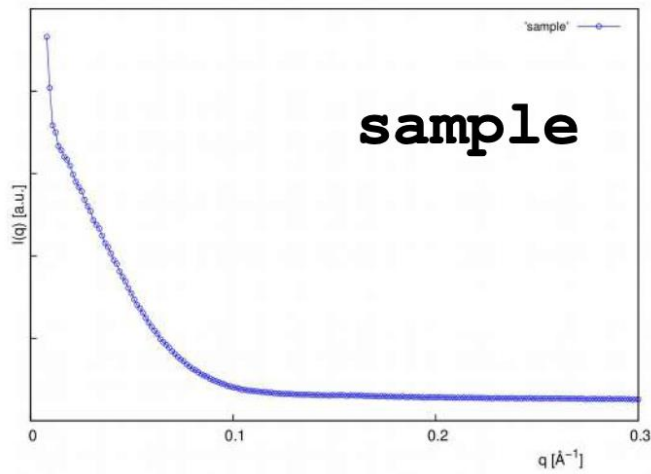
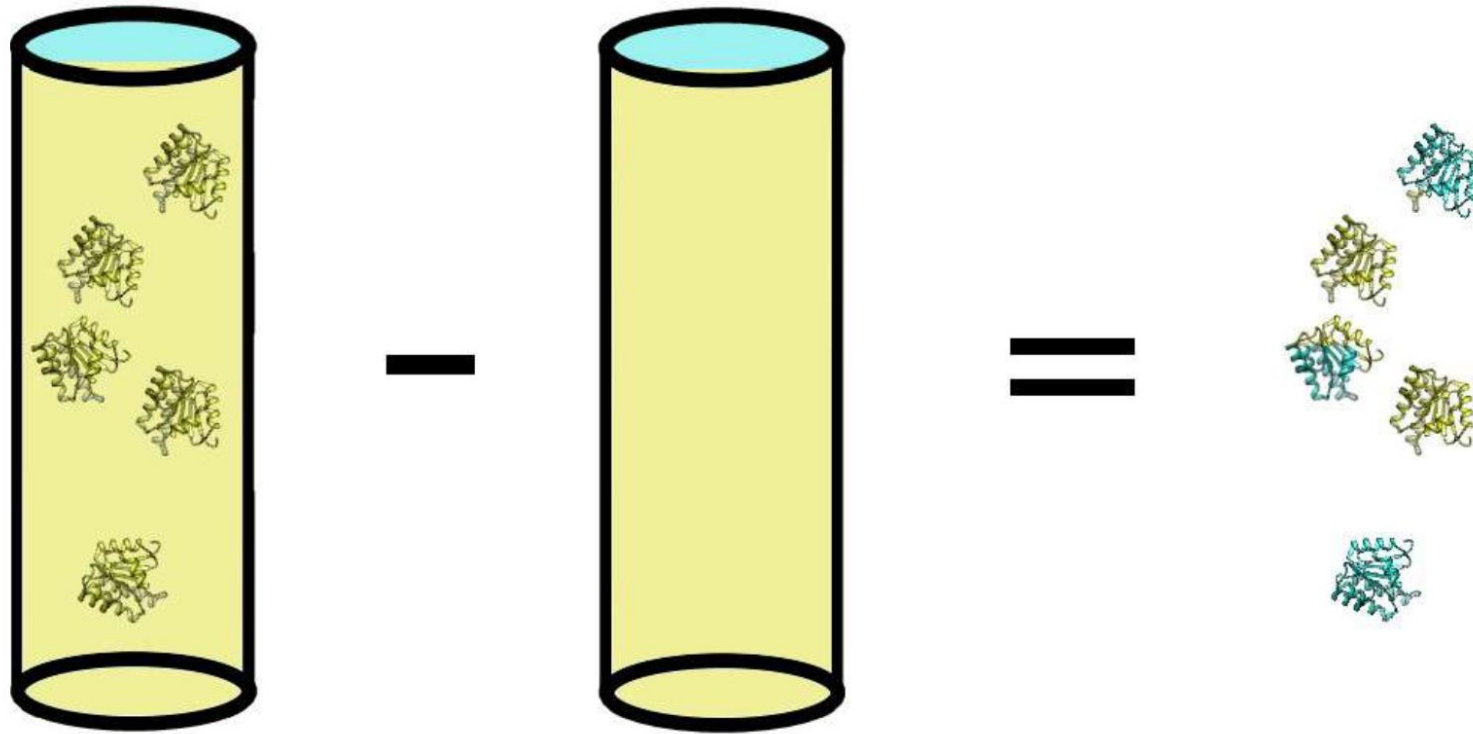




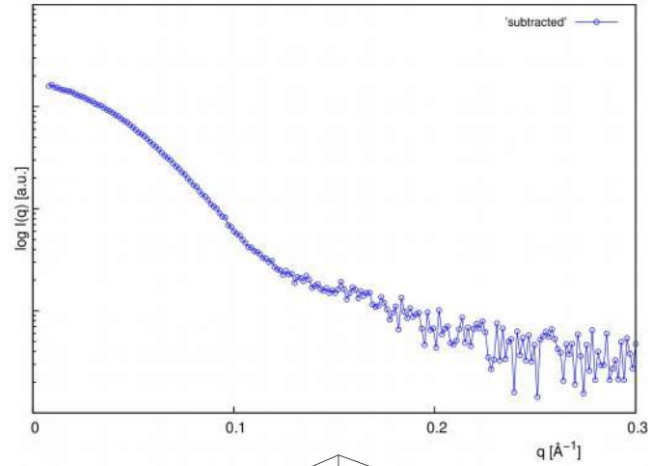
XRD detector image
diffraction spots



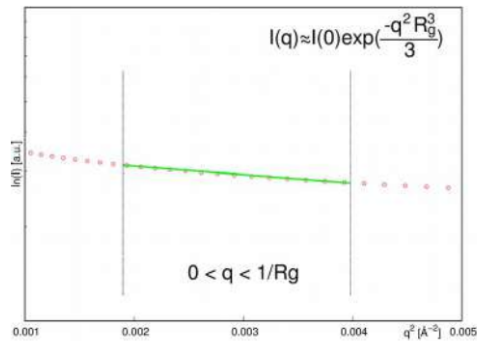
SAXS detector image
x-ray scattering



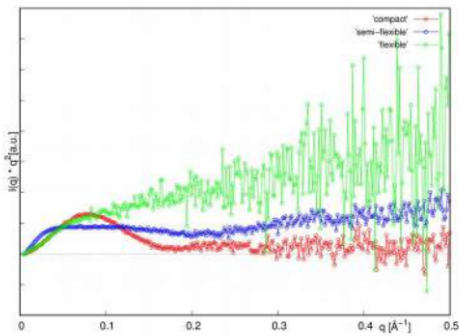
Subtracted SAXS data



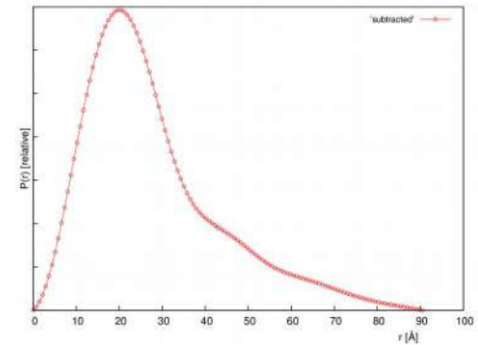
Guinier plot

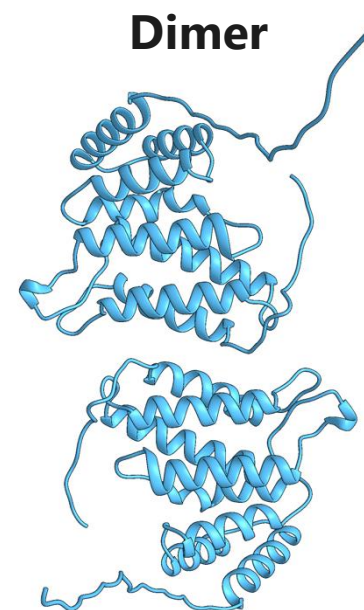
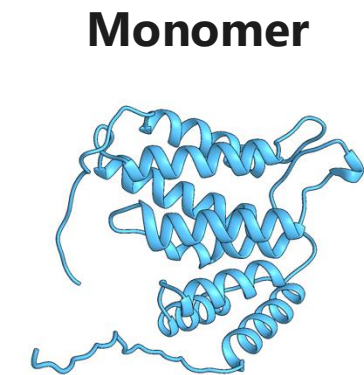
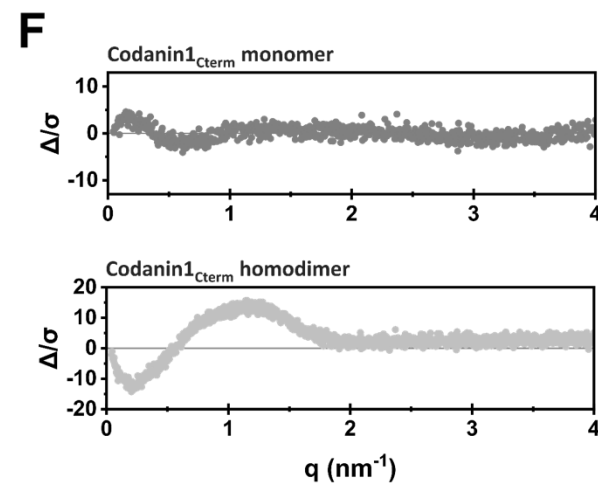
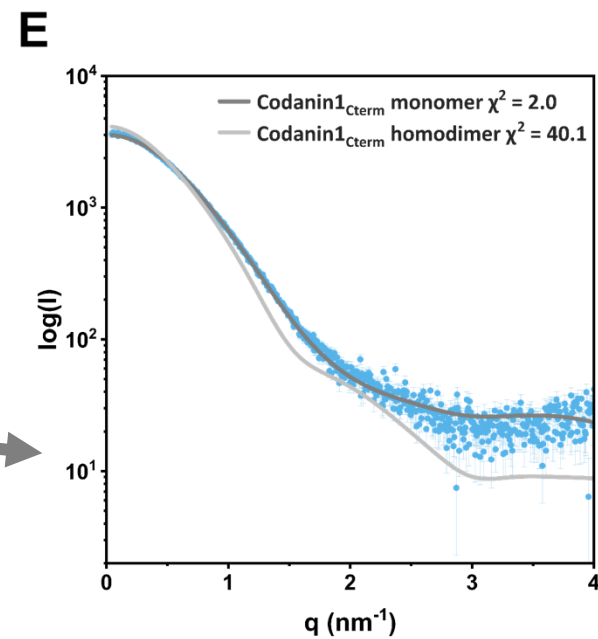
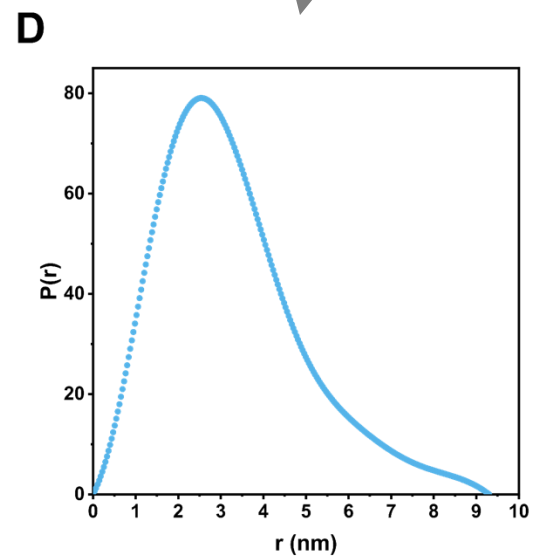
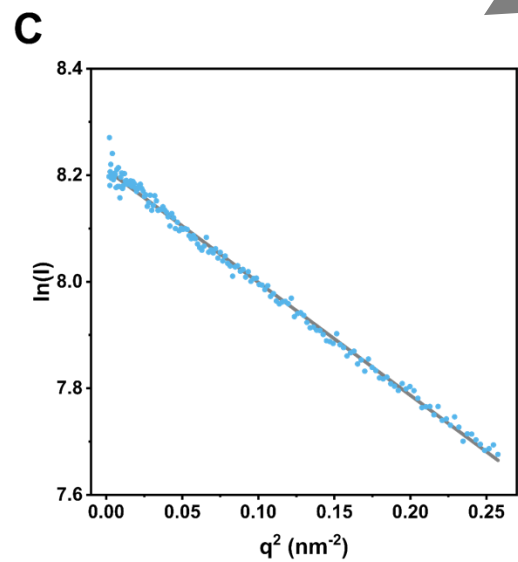
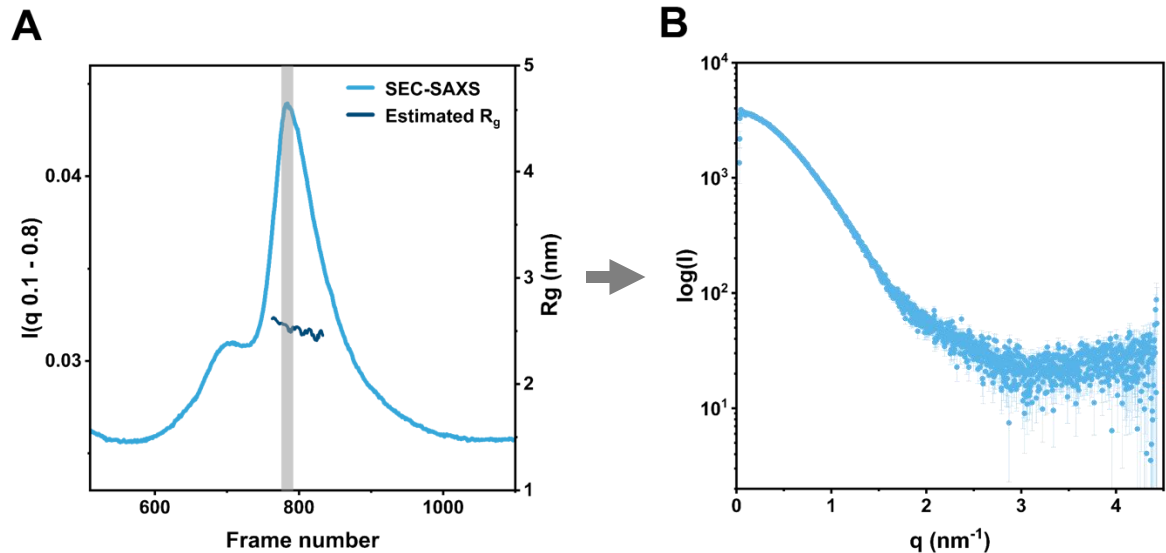


Kratky plot



P(r) Pair distance distribution function





Shrnutí krystalografie