

Espalhamento de raios-X em baixos ângulos (SAXS)

Requer amostras com 1-2 mg de proteína purificada em solução

Pode avaliar efeitos de condições da solução (pH, temperatura, ligantes, etc...) sobre a forma, flexibilidade e oligomerização da proteína

Gera informações sobre tamanho e forma da proteína

Referências

Principles of Physical Biochemistry

van Holde, Johnson e Ho

Structural characterization of proteins and complexes using small-angle X-ray solution scattering

Merten e Svergun

Journal of Structural Biology 172, 128-141 (2010)

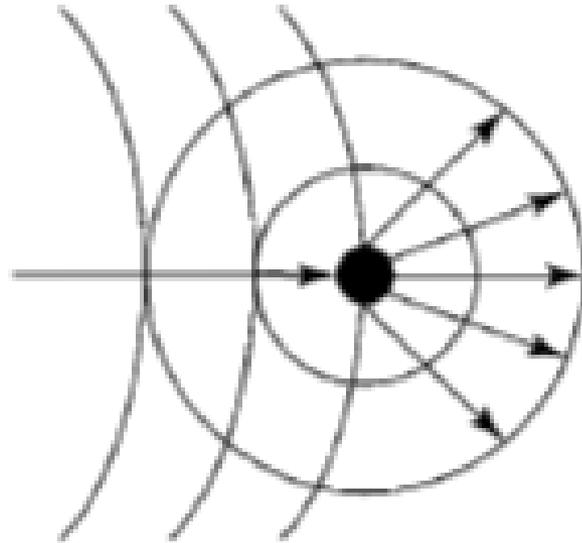
Papel das redes estruturais proteicas nas propriedades de uma beta-glicosidase

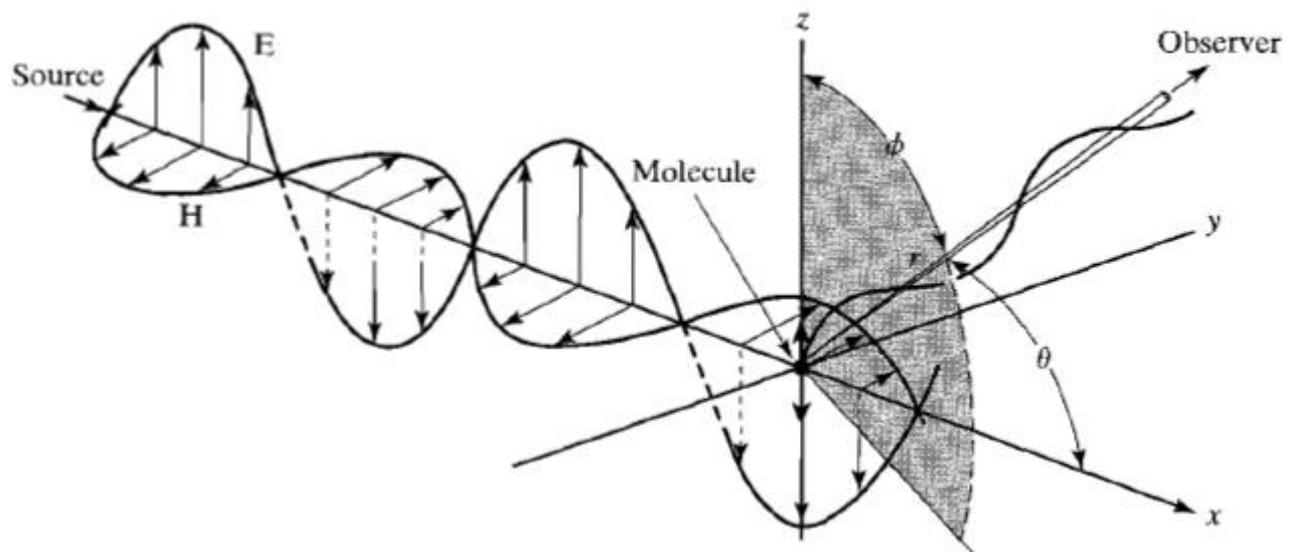
Valquiria Pianheri Souza – Setembro/2017

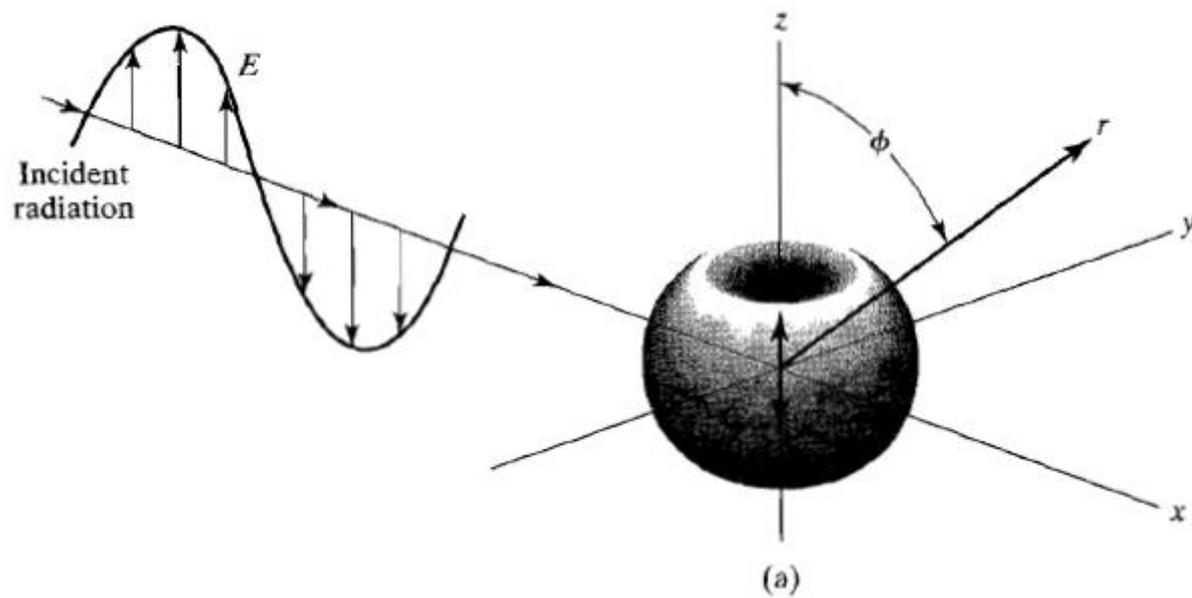
Tese de Doutorado disponível no Banco de Teses da USP

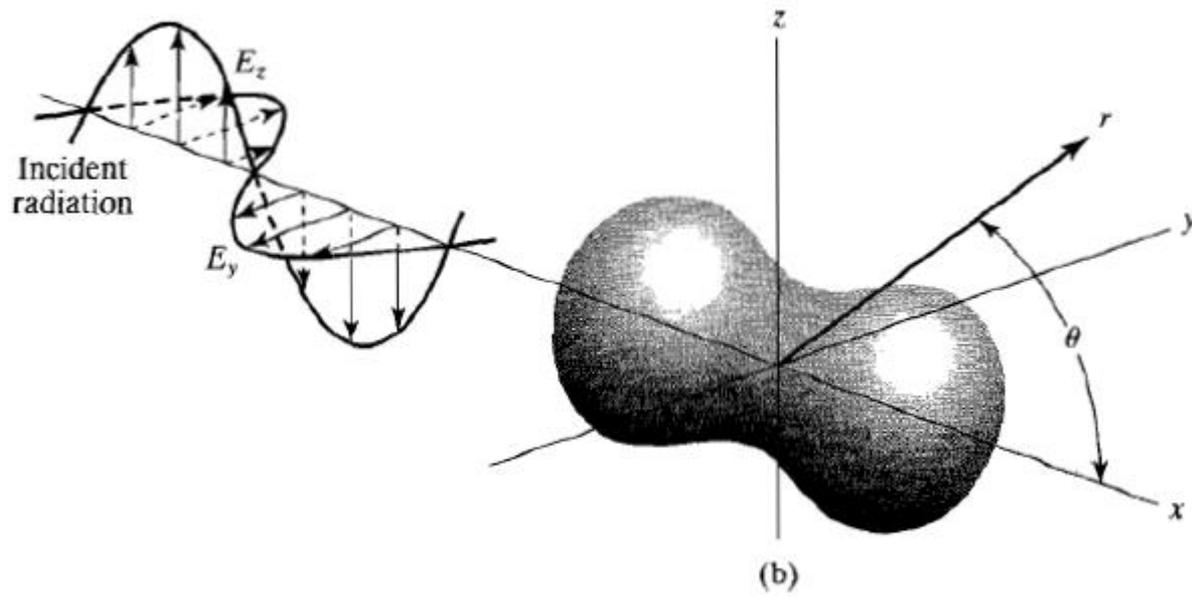
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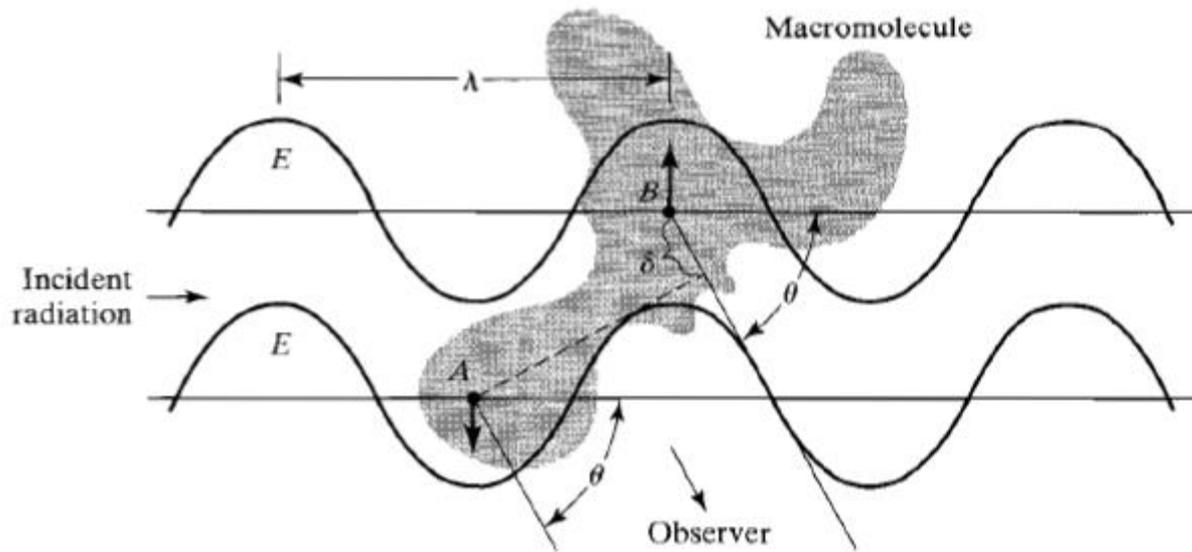
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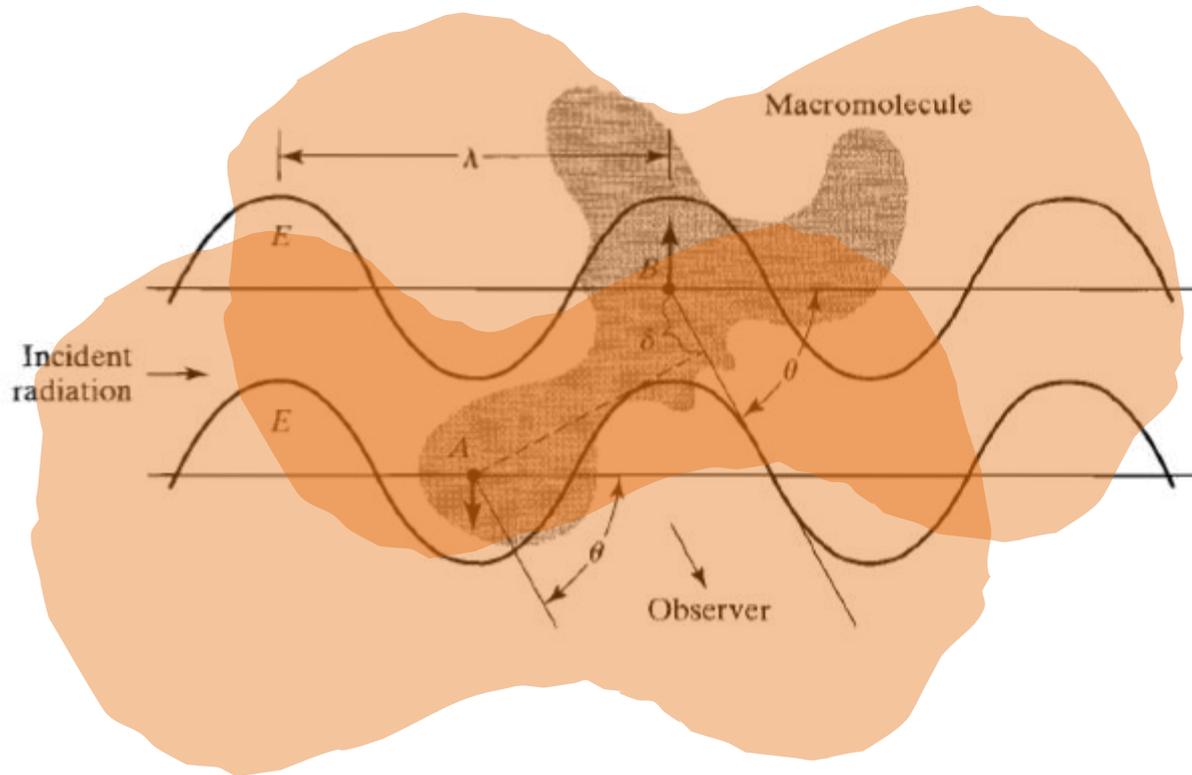


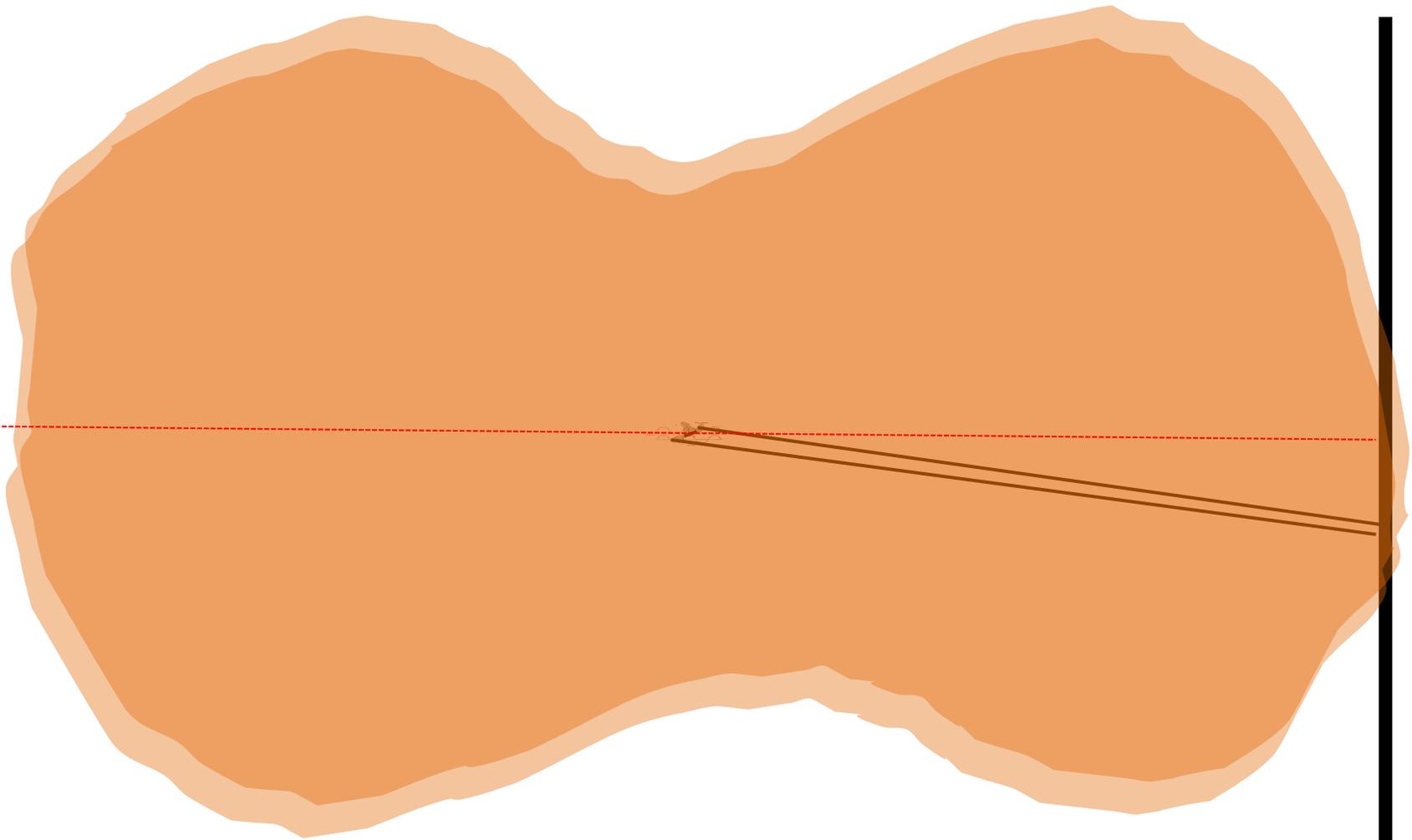


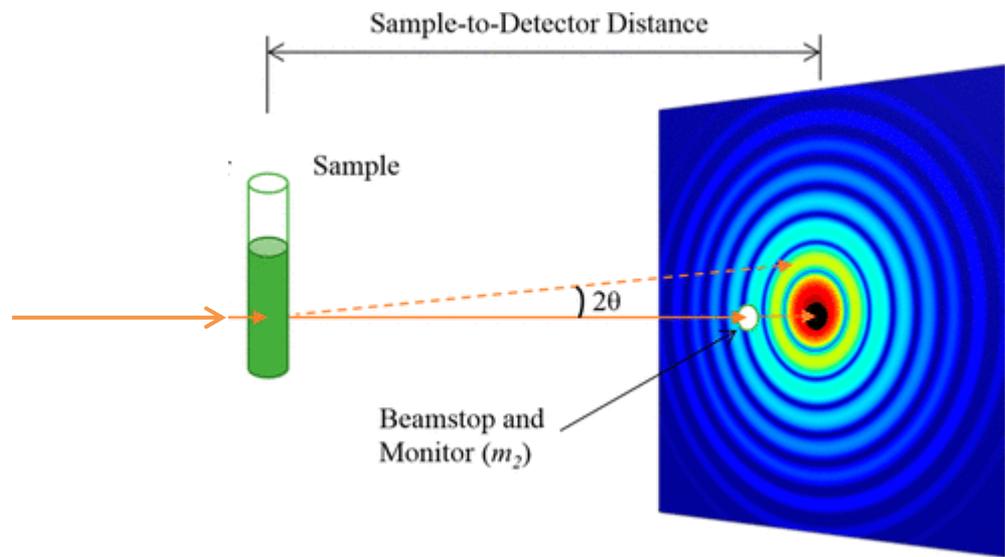


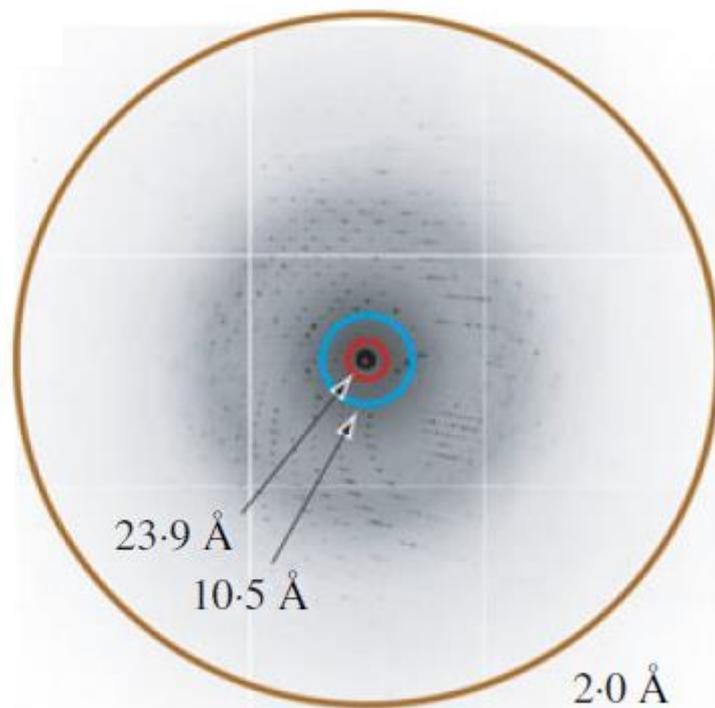
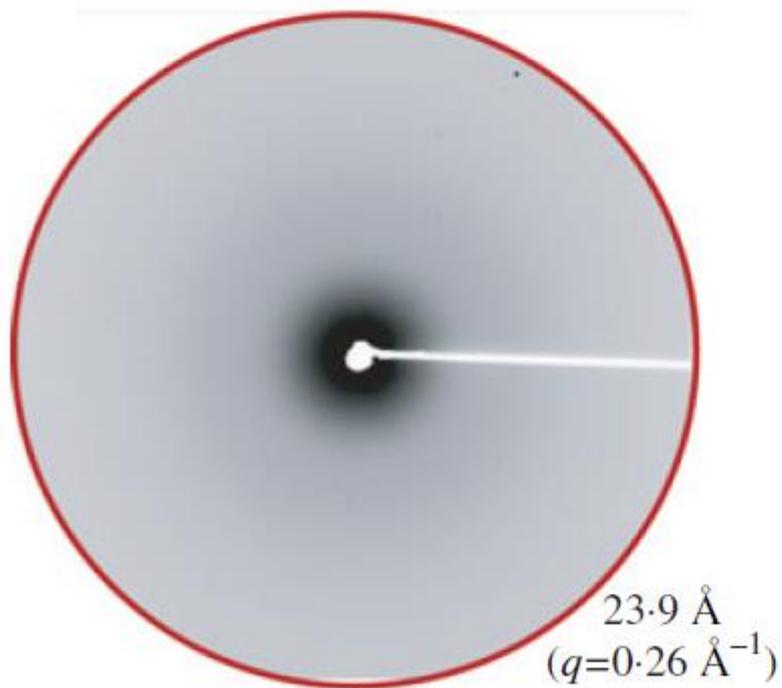
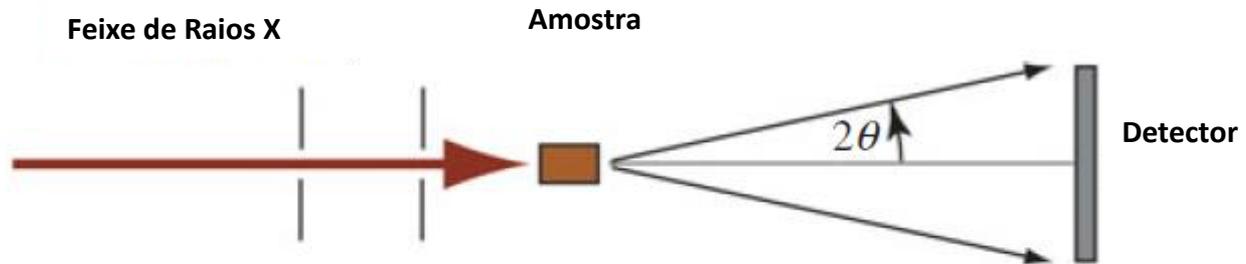


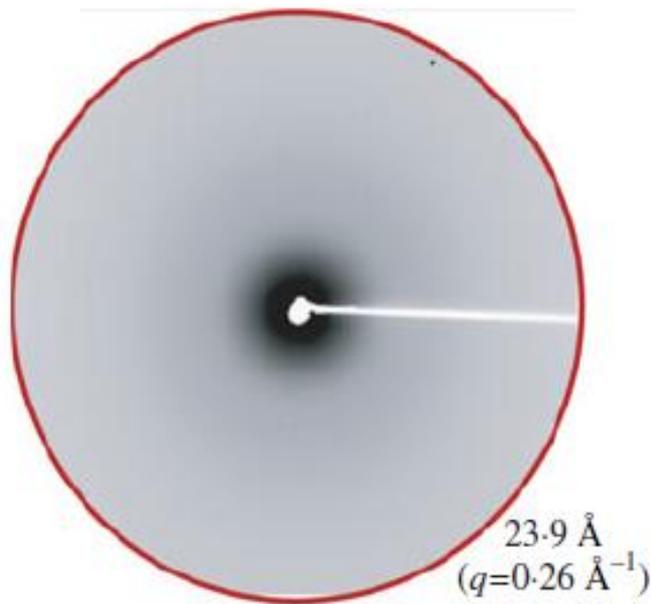
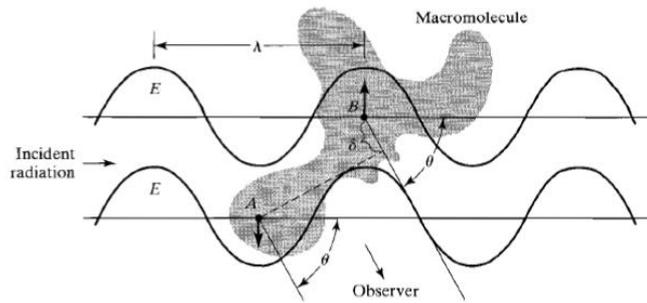






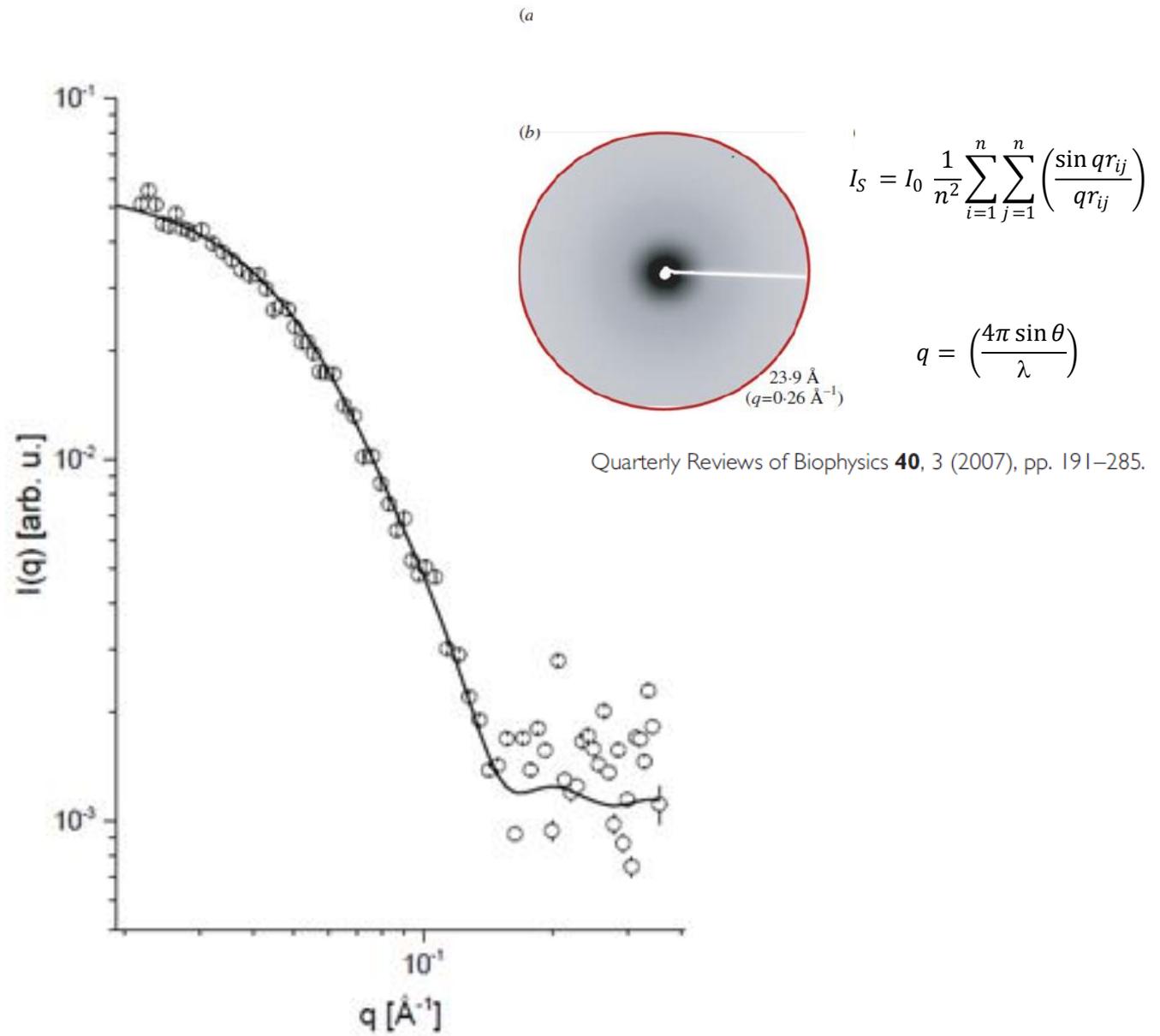


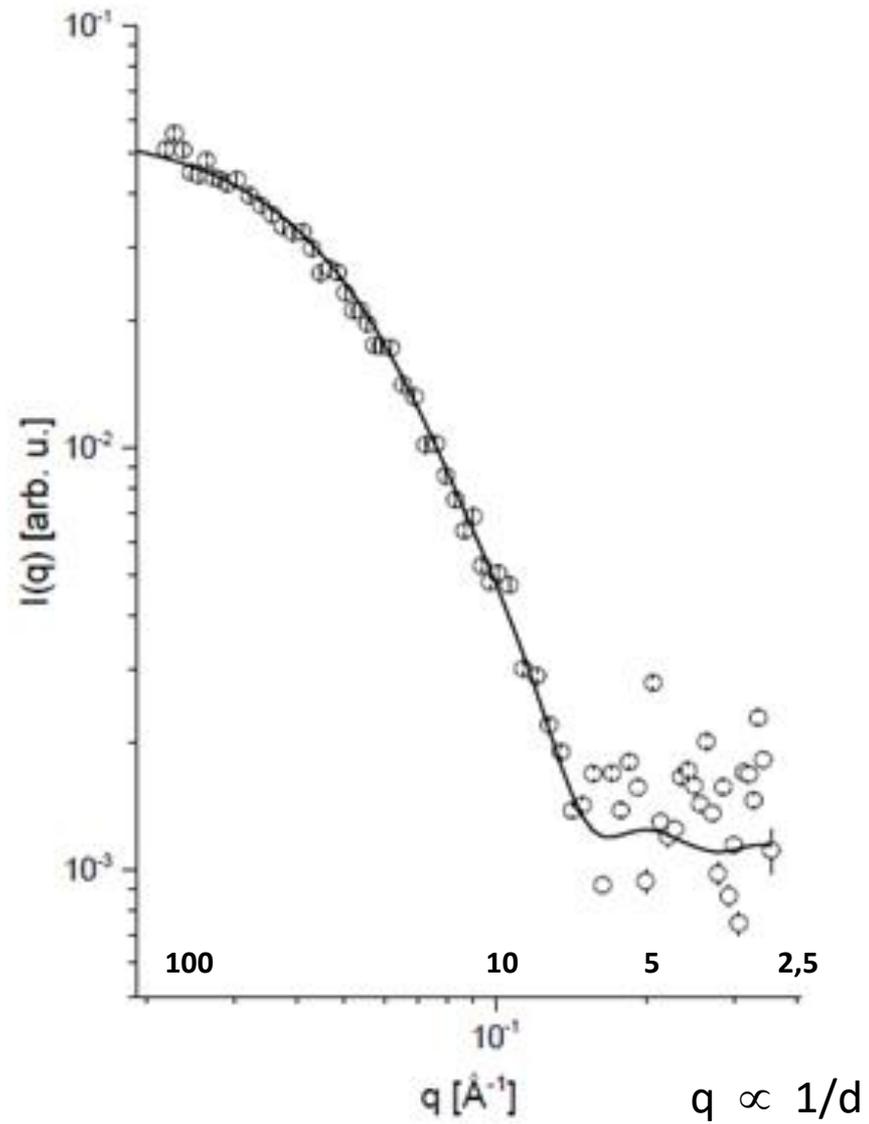




$$I_s = I_0 \frac{1}{n^2} \sum_{i=1}^n \sum_{j=1}^n \left(\frac{\sin qr_{ij}}{qr_{ij}} \right)$$

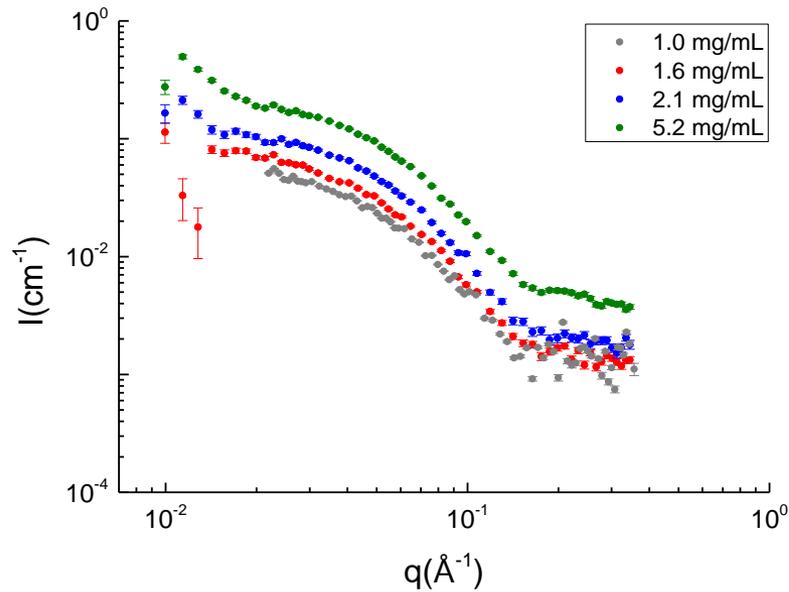
$$q = \left(\frac{4\pi \sin \theta}{\lambda} \right)$$





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Análise de Guinier



$$I_S = I_0 \frac{1}{n^2} \sum_{i=1}^n \sum_{j=1}^n \left(\frac{\sin qr_{ij}}{qr_{ij}} \right)$$

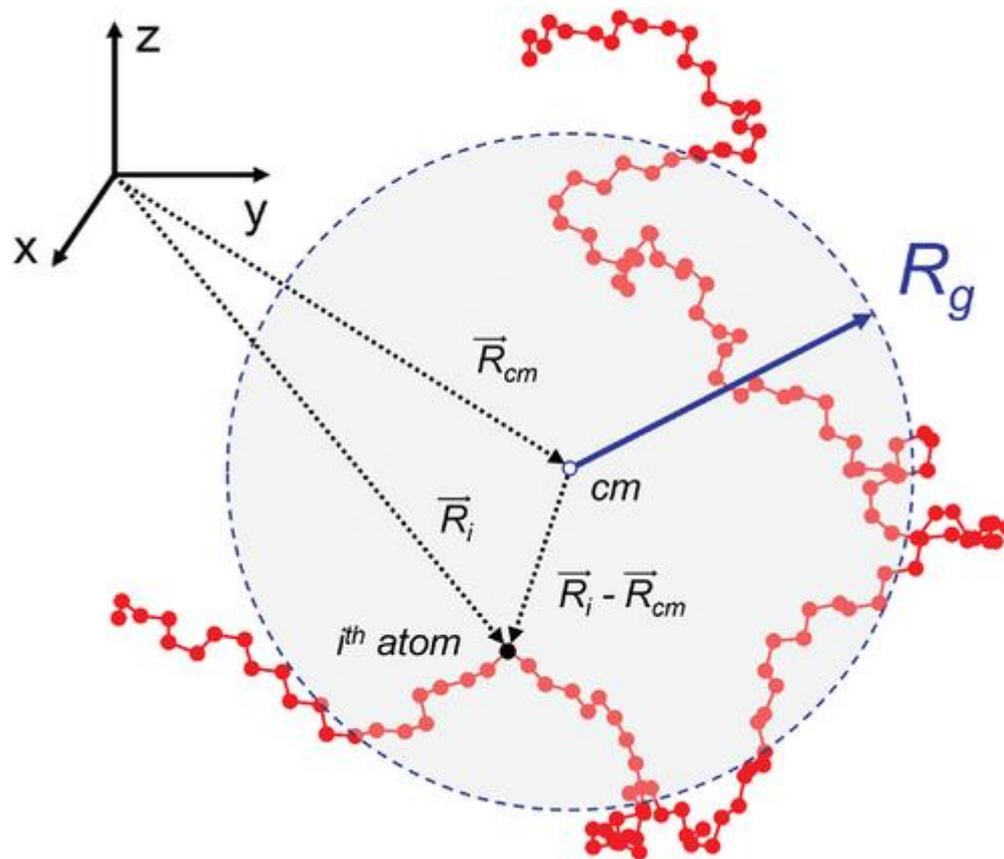
$$I_S = I_0 \left(\frac{1}{n^2} \sum_{i=1}^n \sum_{j=1}^n (1) - \frac{q^2}{6n^2} \sum_{i=1}^n \sum_{j=1}^n (r_{ij}^2) \right)$$

$$R_g^2 = \frac{1}{2n^2} \sum_{i=1}^n \sum_{j=1}^n r_{ij}^2$$

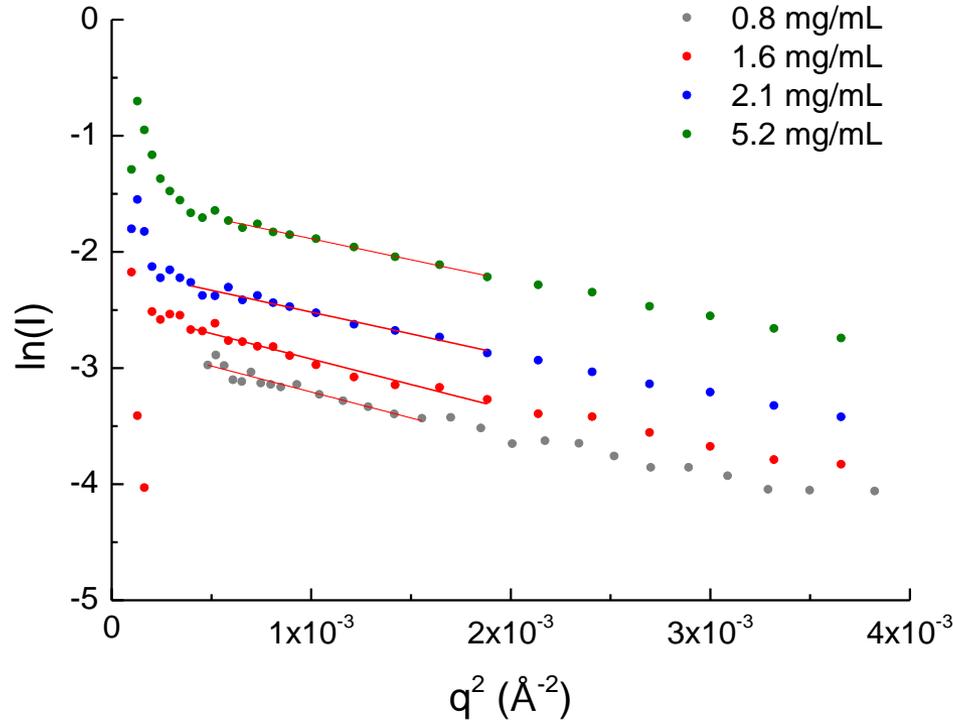
$$I_S = I_0 \left(1 - \frac{q^2}{3} R_g^2 \right)$$

$$I_S \cong I_0 \exp \left(-\frac{q^2}{3} R_g^2 \right)$$

$$\ln I_S = \ln I_0 - \frac{1}{3} R_g^2 q^2$$



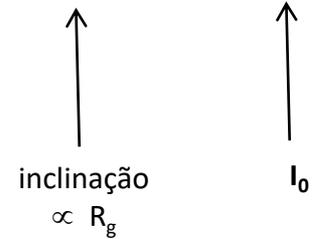
Análise de Guinier



$$\ln I_s = \ln I_0 - \frac{1}{3} R_g^2 q^2$$

Ajuste linear – plote de Guinier

0,8 mg/mL	$\ln(I) = (-450 \pm 40) \times q^2 + (-2,76 \pm 0,03)$
1,6 mg/mL	$\ln(I) = (-440 \pm 30) \times q^2 + (-2,48 \pm 0,03)$
2,1 mg/mL	$\ln(I) = (-380 \pm 20) \times q^2 + (-2,14 \pm 0,02)$
5,2 mg/mL	$\ln(I) = (-363 \pm 13) \times q^2 + (-1,52 \pm 0,01)$

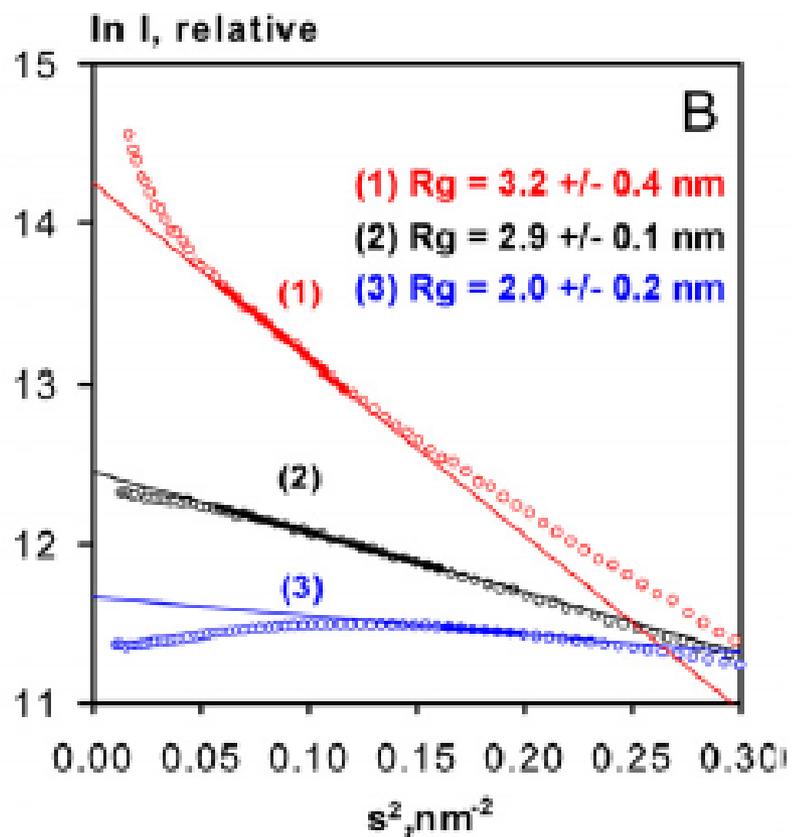


$$R_g^2 = \frac{1}{2n^2} \sum_{i=1}^n \sum_{j=1}^n r_{ij}^2$$

$I_0 \propto$ densidade eletrônica \propto massa molecular

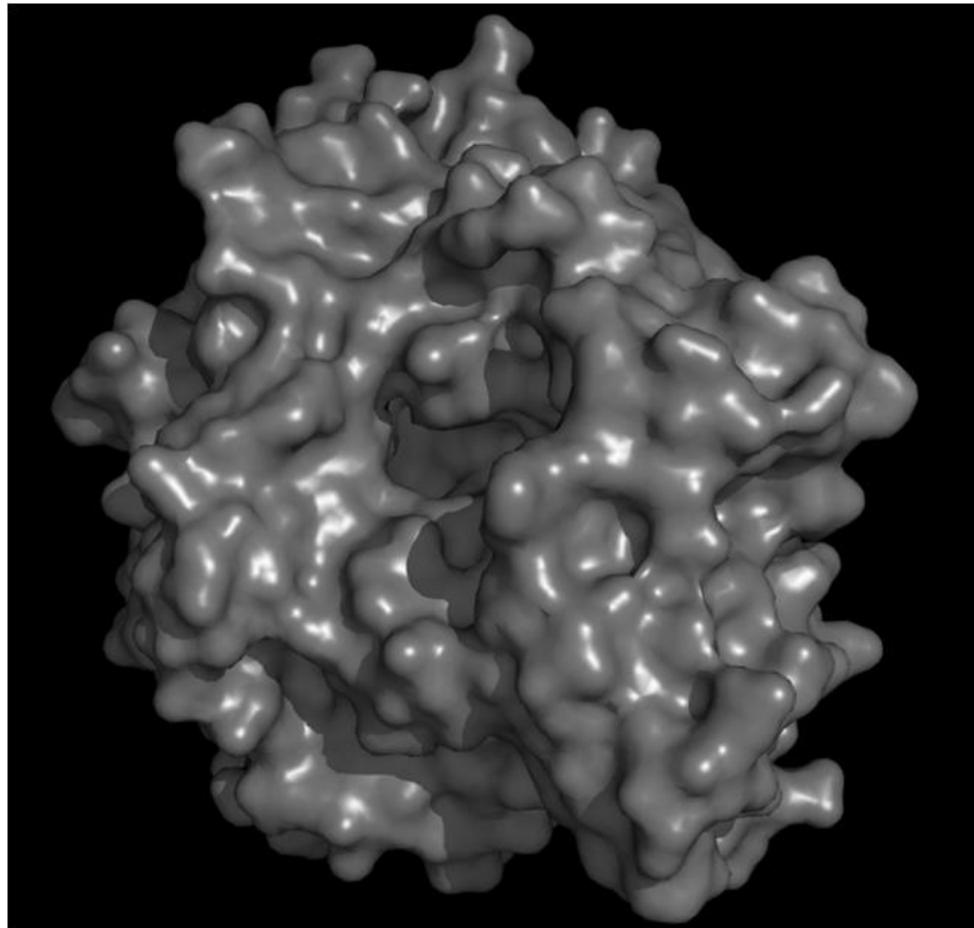
Experimental
 $R_g \approx 34 \text{ \AA}$
 MW ≈ 135.000

Calculado (software Crystol)
 21Å 32Å
 58.000 116.000



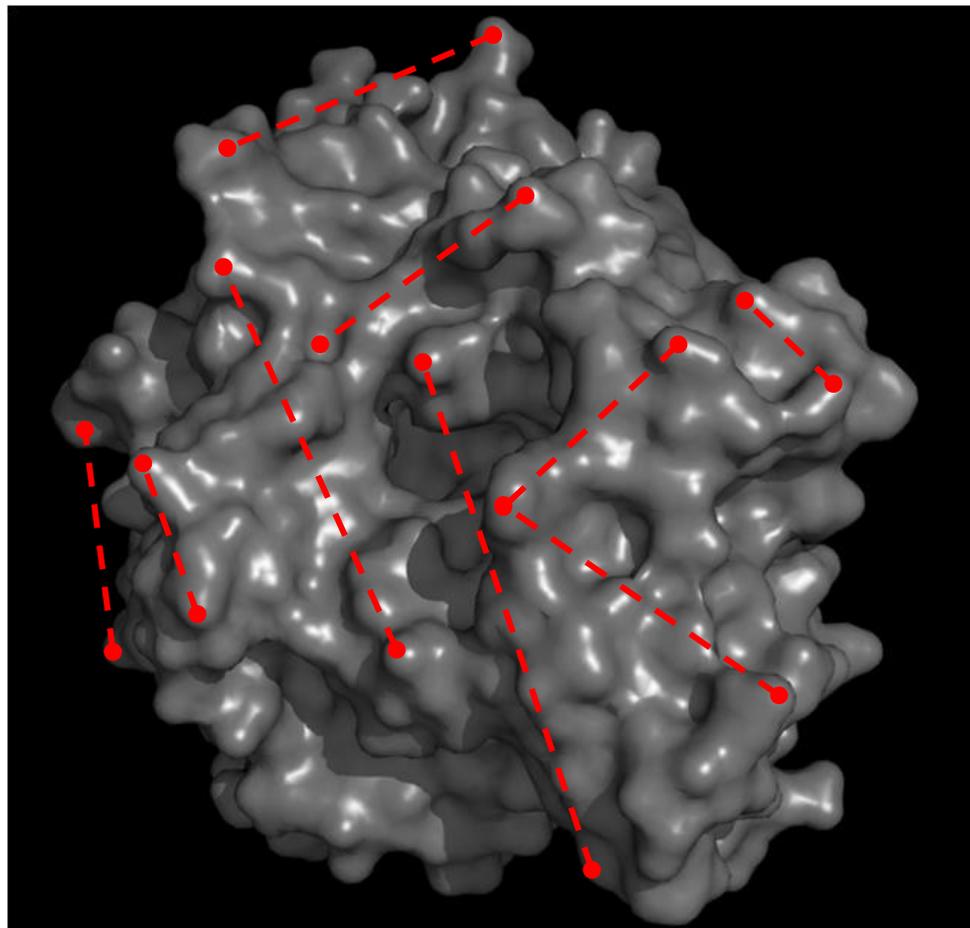
Agregação
 Proteínas isoladas
Repulsão

Distribuição de Pares



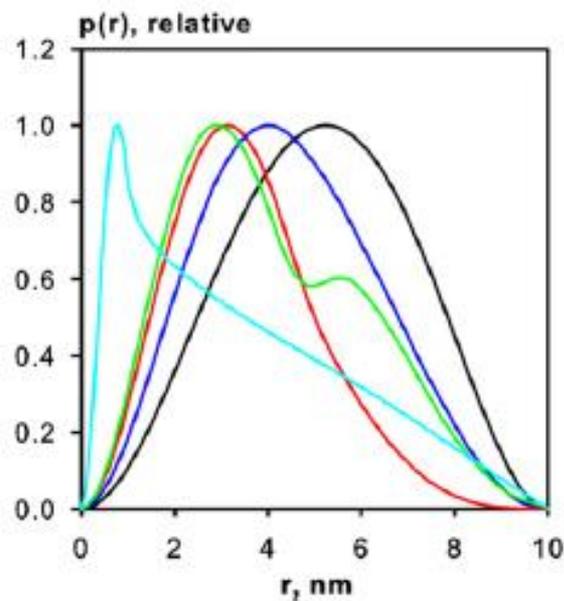
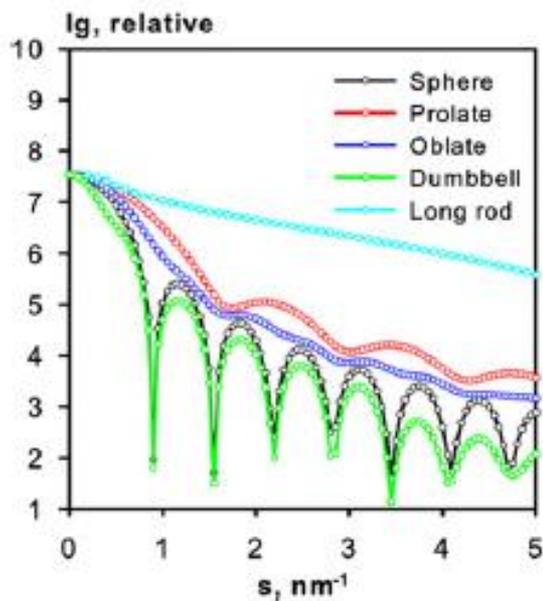
PDB 5CG0

Distribuição de Pares



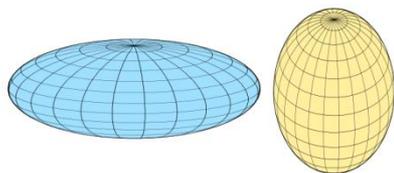
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Distribuição de Pares

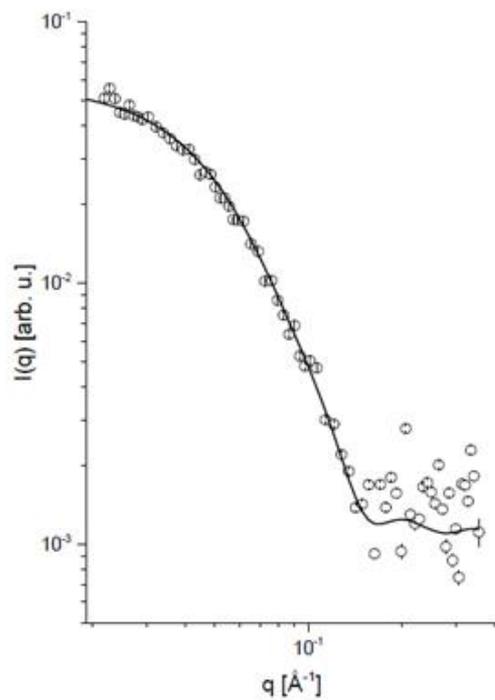


Oblato

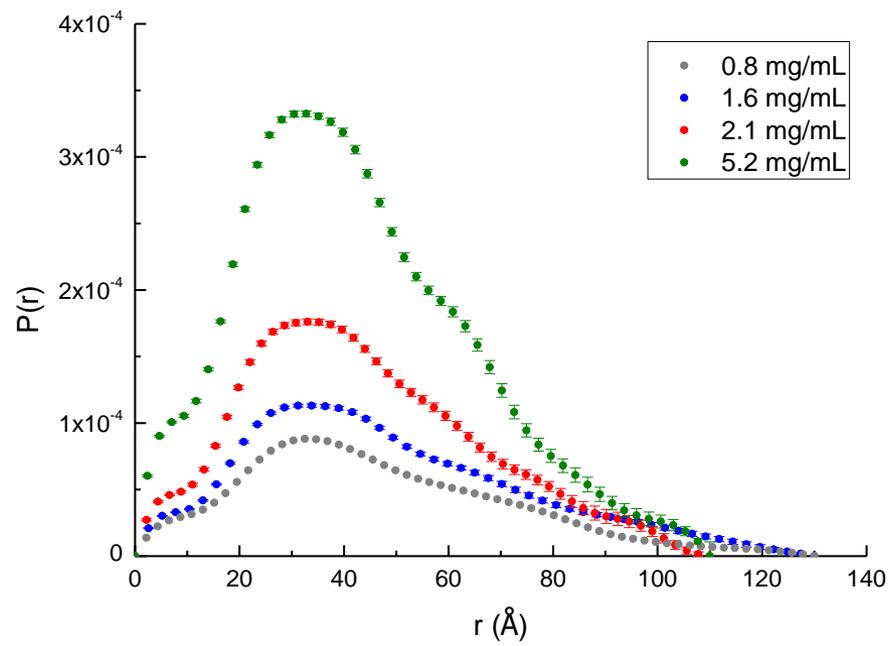
Prolato

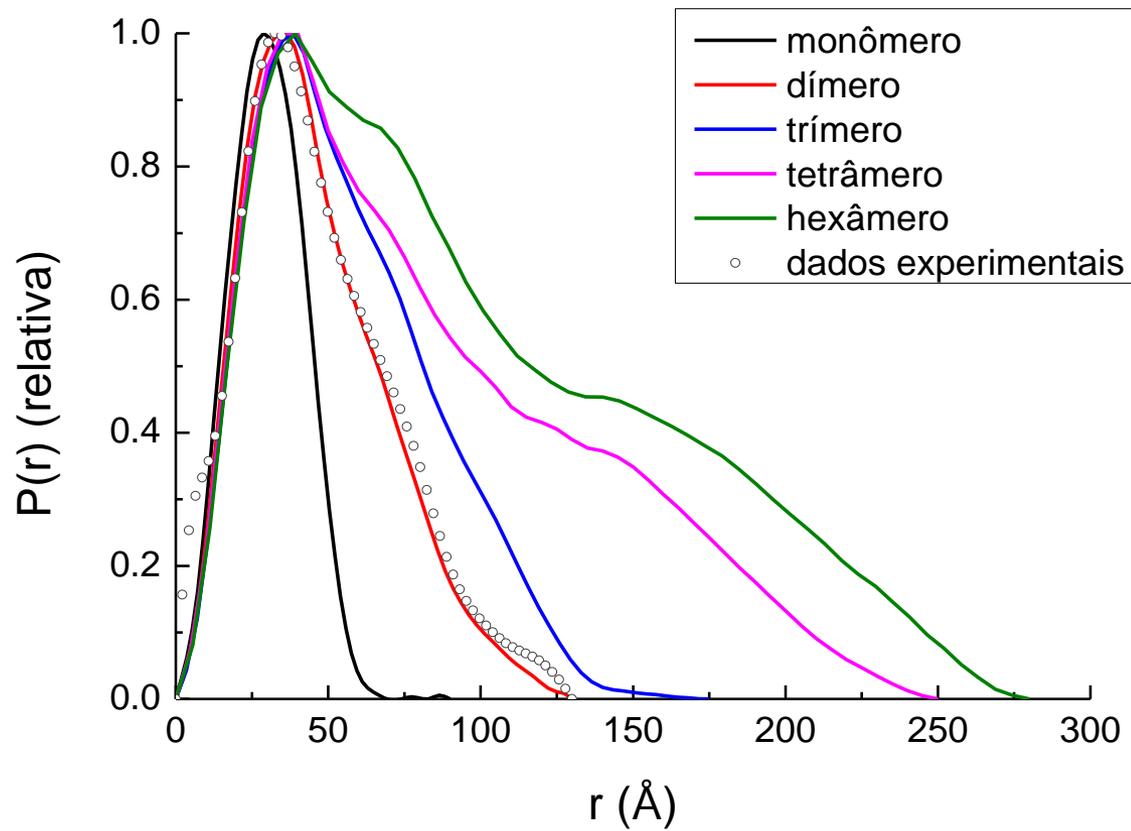


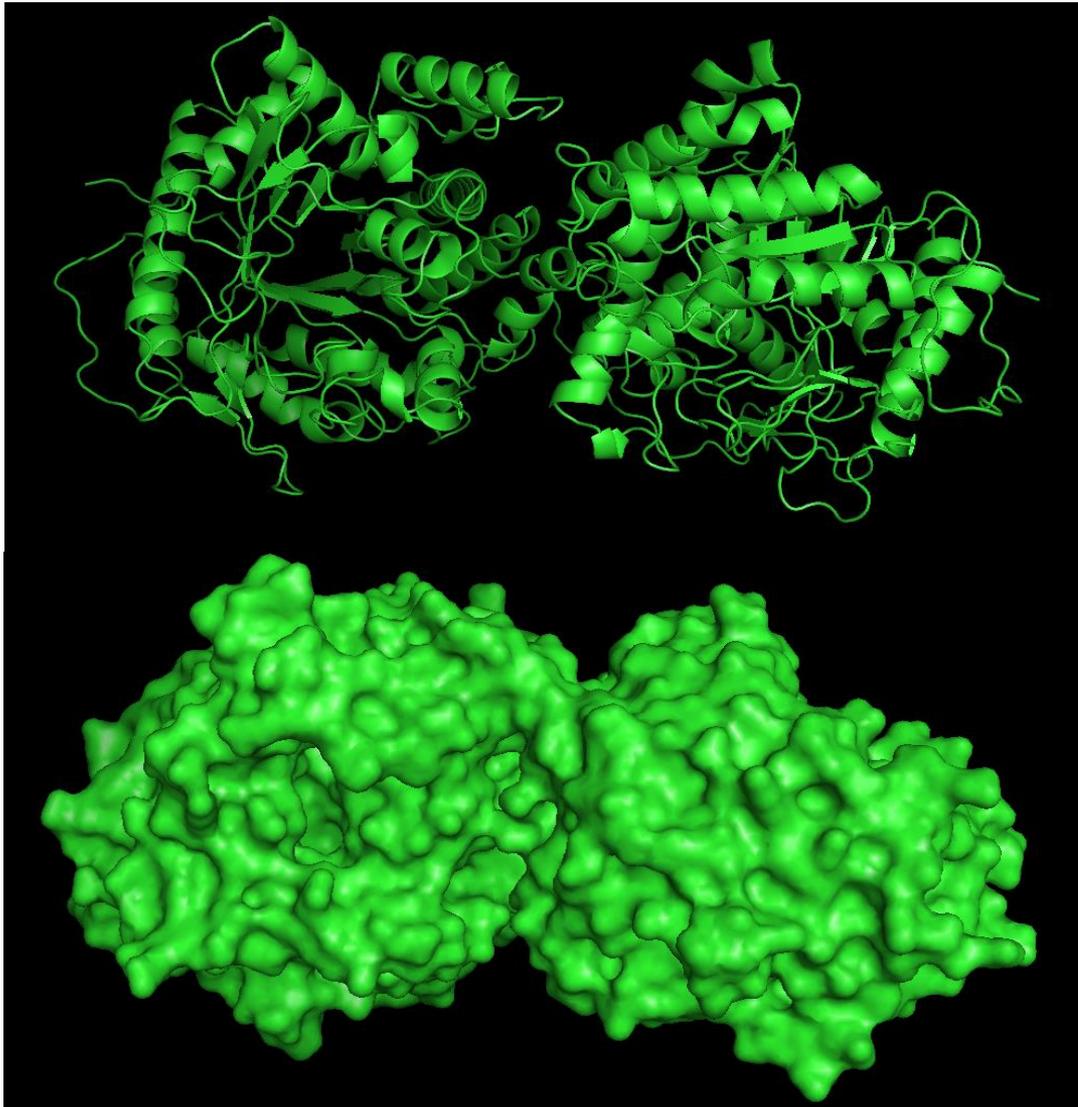
Distribuição de Pares



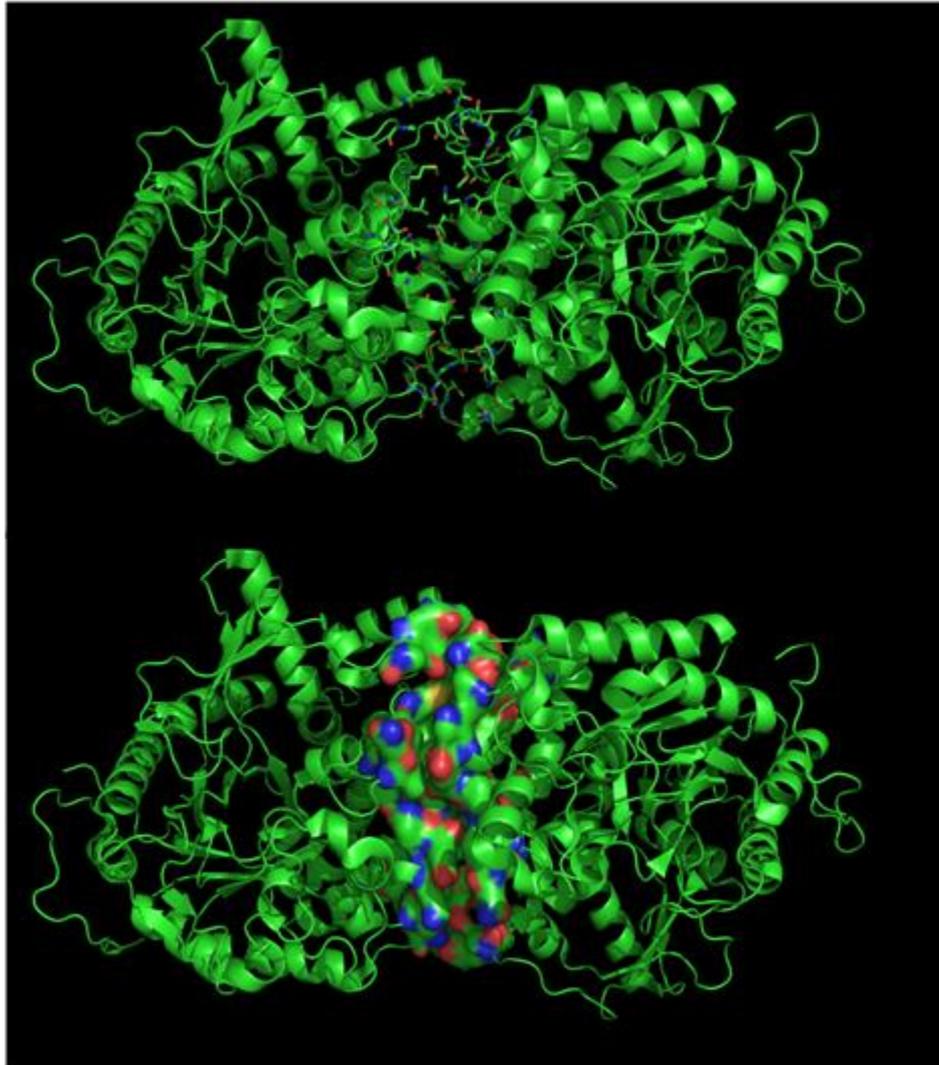
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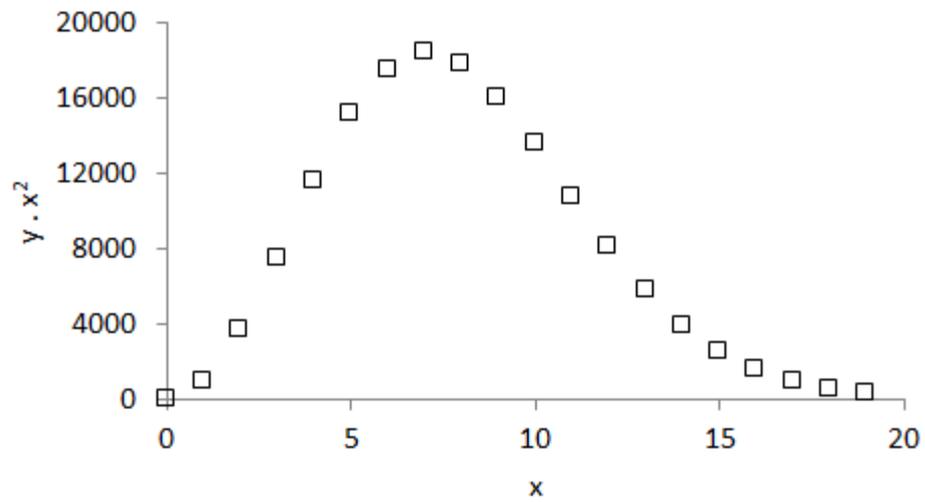
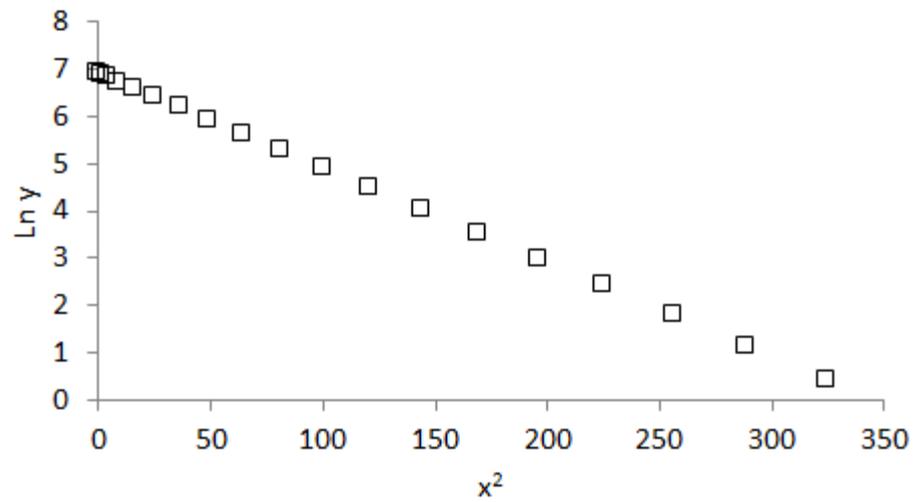


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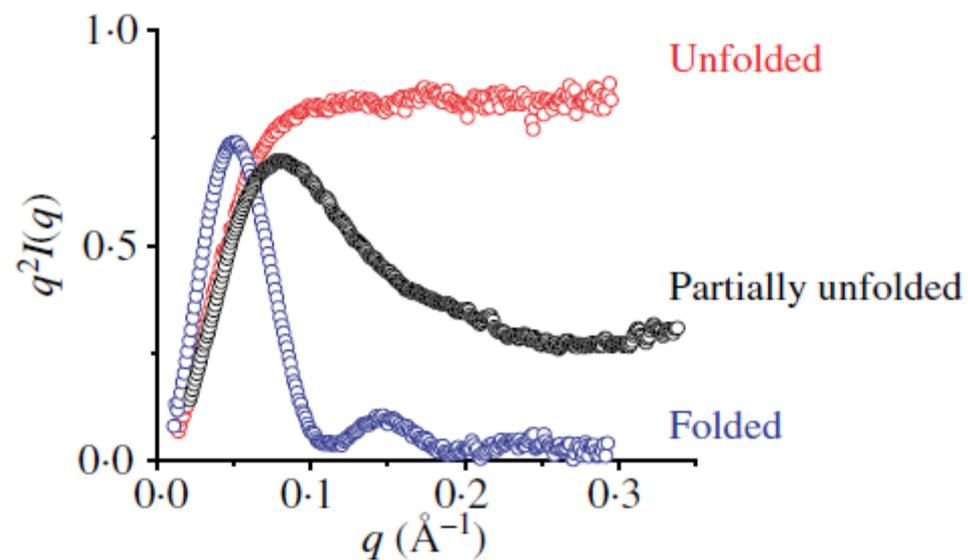


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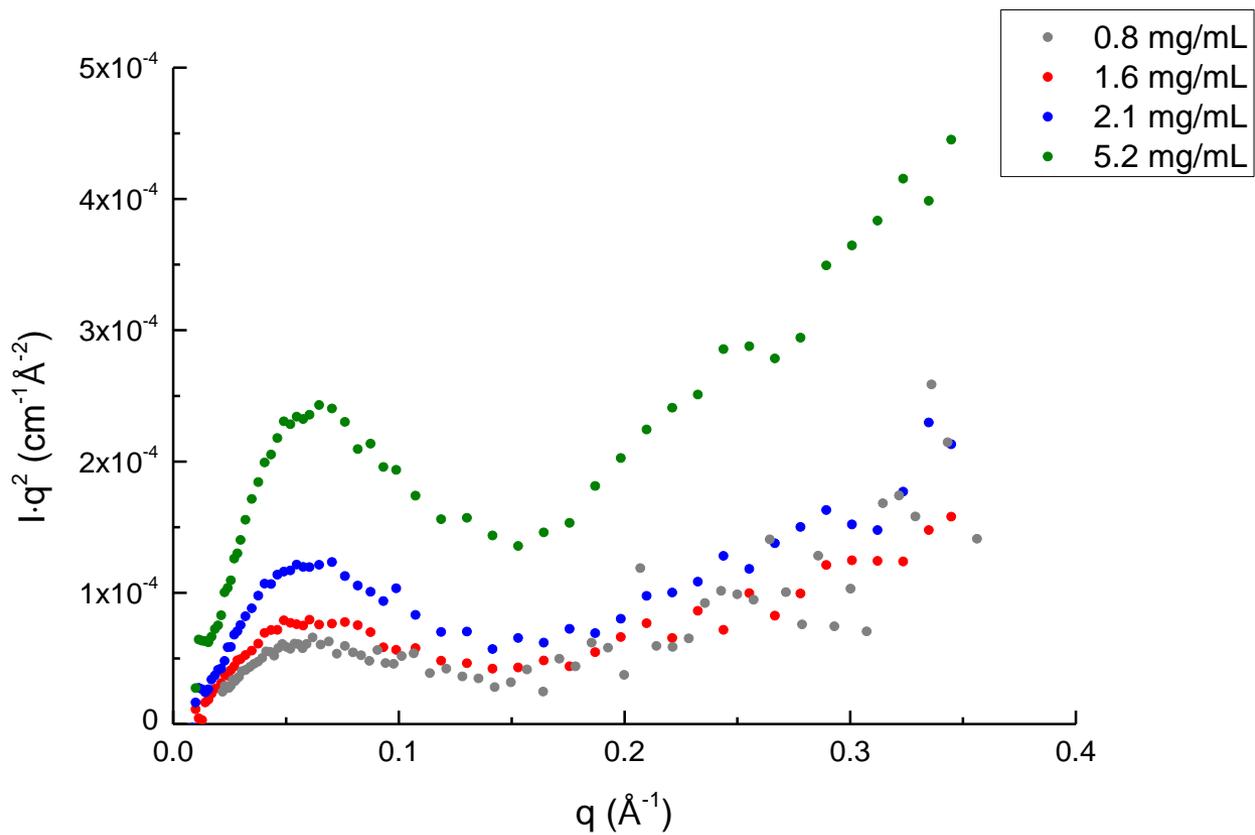
Análise de Kratky



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