IF - Universidade de São Paulo

Small Angle X-Ray Scattering (SAXS) applied to complex fluids

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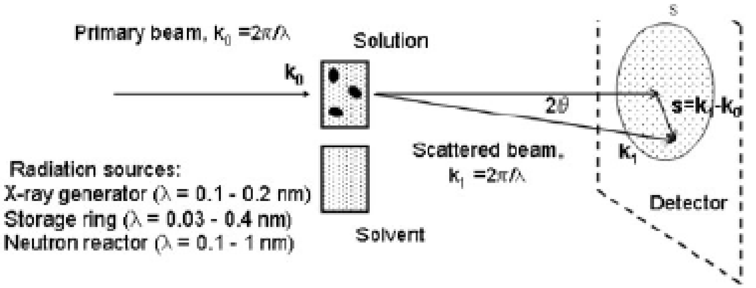
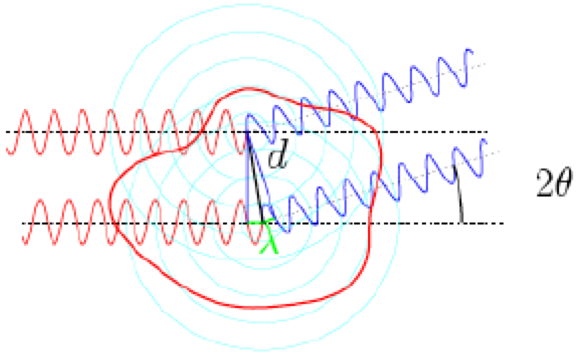
The Bragg Law





$$\boxed{\lambda = 2d\sin\theta}$$

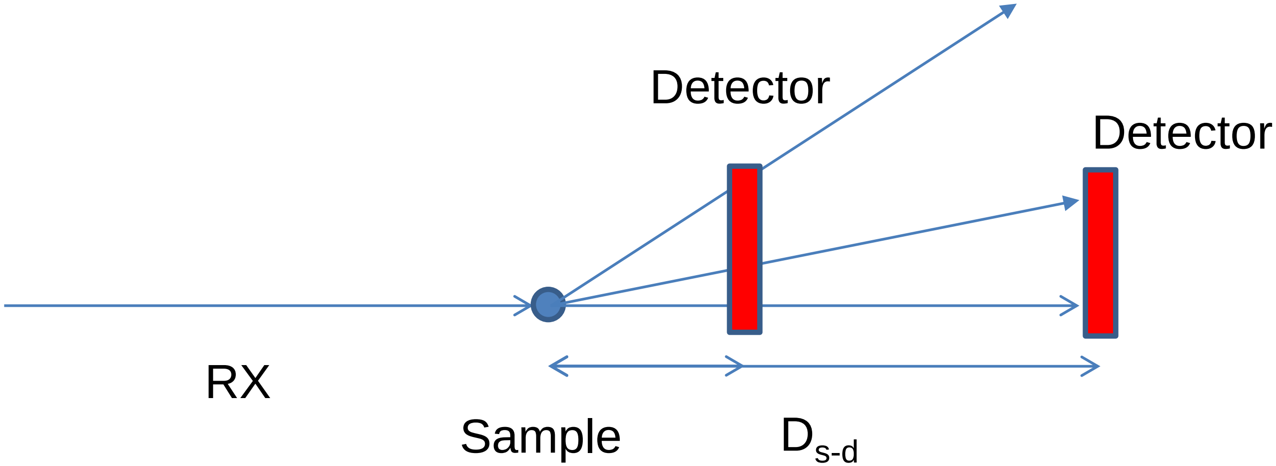
λ = X-ray wavelength

if $d \approx \lambda$, θ is large

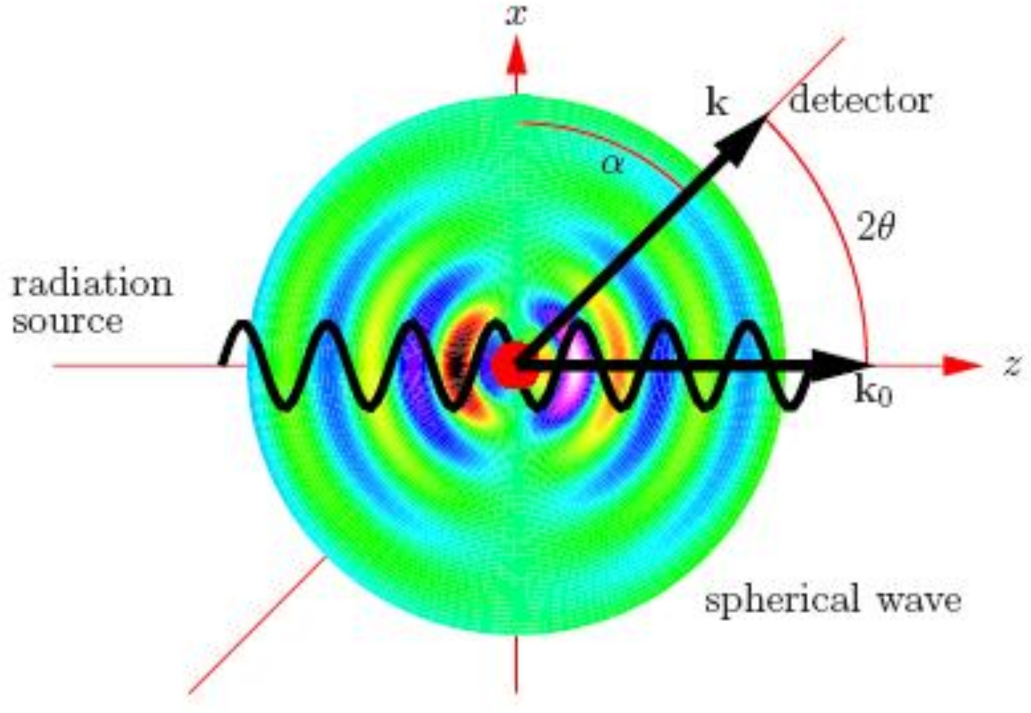
if $d \approx (10 - 100)\lambda$, θ is small

For $d \simeq 100$ Å and $\lambda = 5$ Å, $\theta \simeq 1.4°$

Experimental Set-up

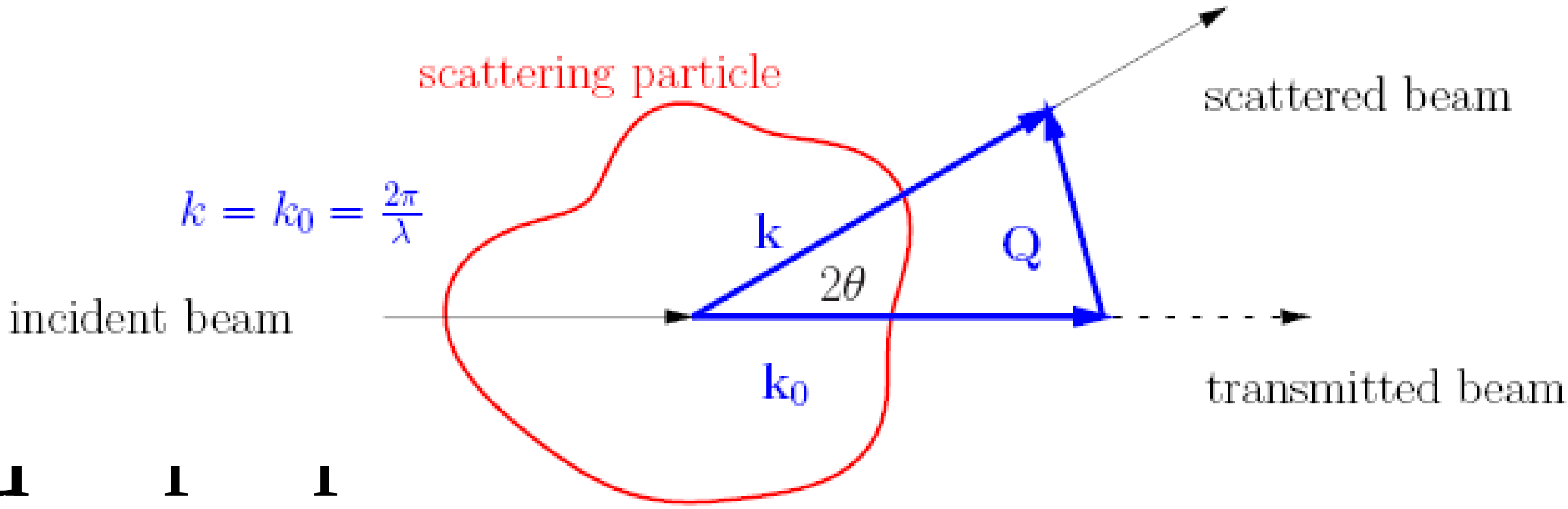


Elastic Scattering



- X-Ray photons interact with electrons, given risen to Thompson scattering: each electron becomes a source of spherical waves, of same frequency (and hence wavelenght) of the incoming beam.

Scattering Vector, Q



$$\vec{Q} = \vec{k} - \vec{k}_0$$

$$Q^2 = k^2 + k_0^2 - 2kk_0\cos(2\theta)$$

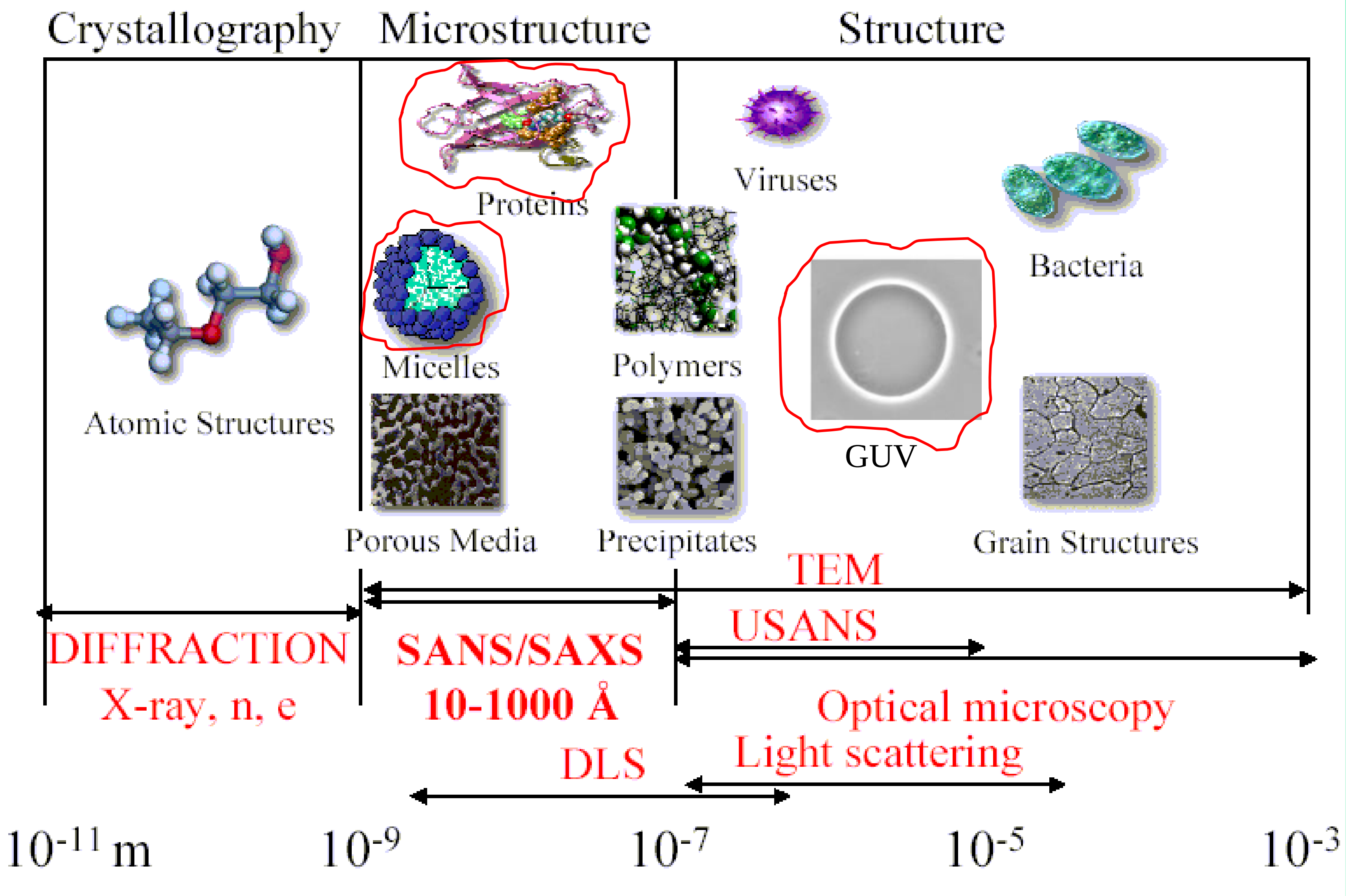
$$= \left(\frac{2\pi}{\lambda}\right)^2 2(1 - \cos(2\theta))$$

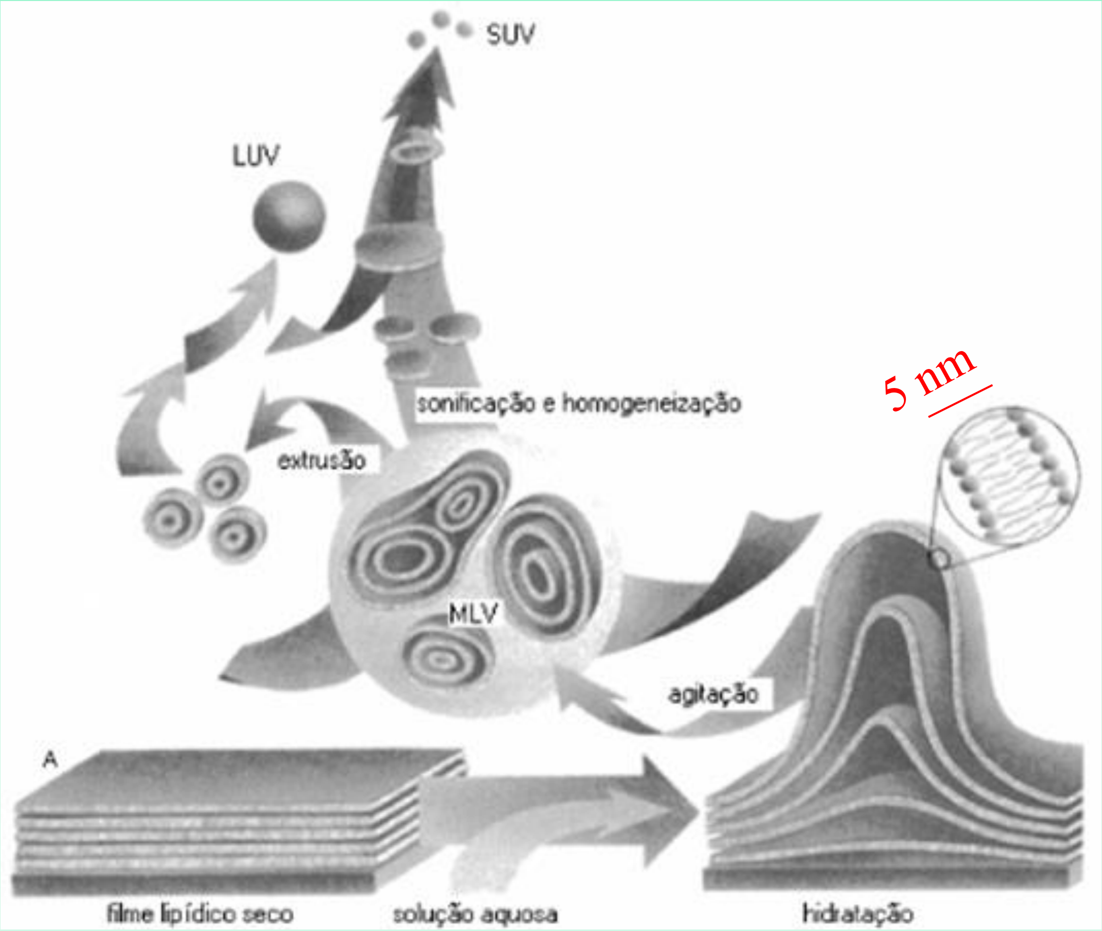
$$= \left(\frac{2\pi}{\lambda}\right)^2 2\left(1 - (\cos^2\theta - \sin^2\theta)\right)$$

$$= \left(\frac{2\pi}{\lambda}\right)^2 2\left(2\sin^2\theta\right)$$

$$Q^2 = \frac{16\pi\sin^2\theta}{\lambda} \therefore Q = \frac{4\pi}{\lambda}\sin\theta$$

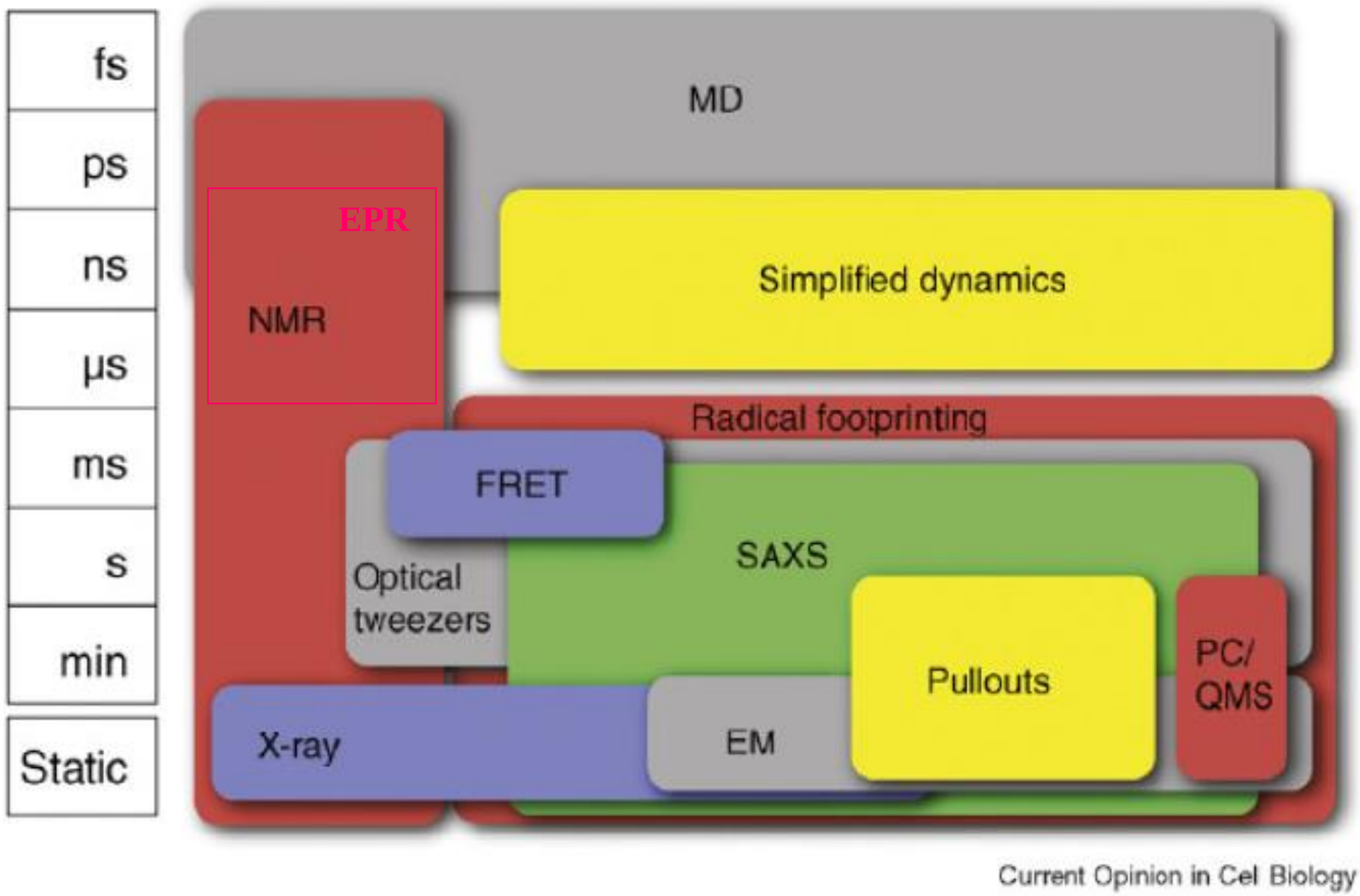
Length scale versus Technique




SUV
LUV
sonificação e homogeneização
extrusão
MLV
agitação
A
filme lipídico seco
solução aquosa
hidratação
5 nm

µm

Time scale versus Technique



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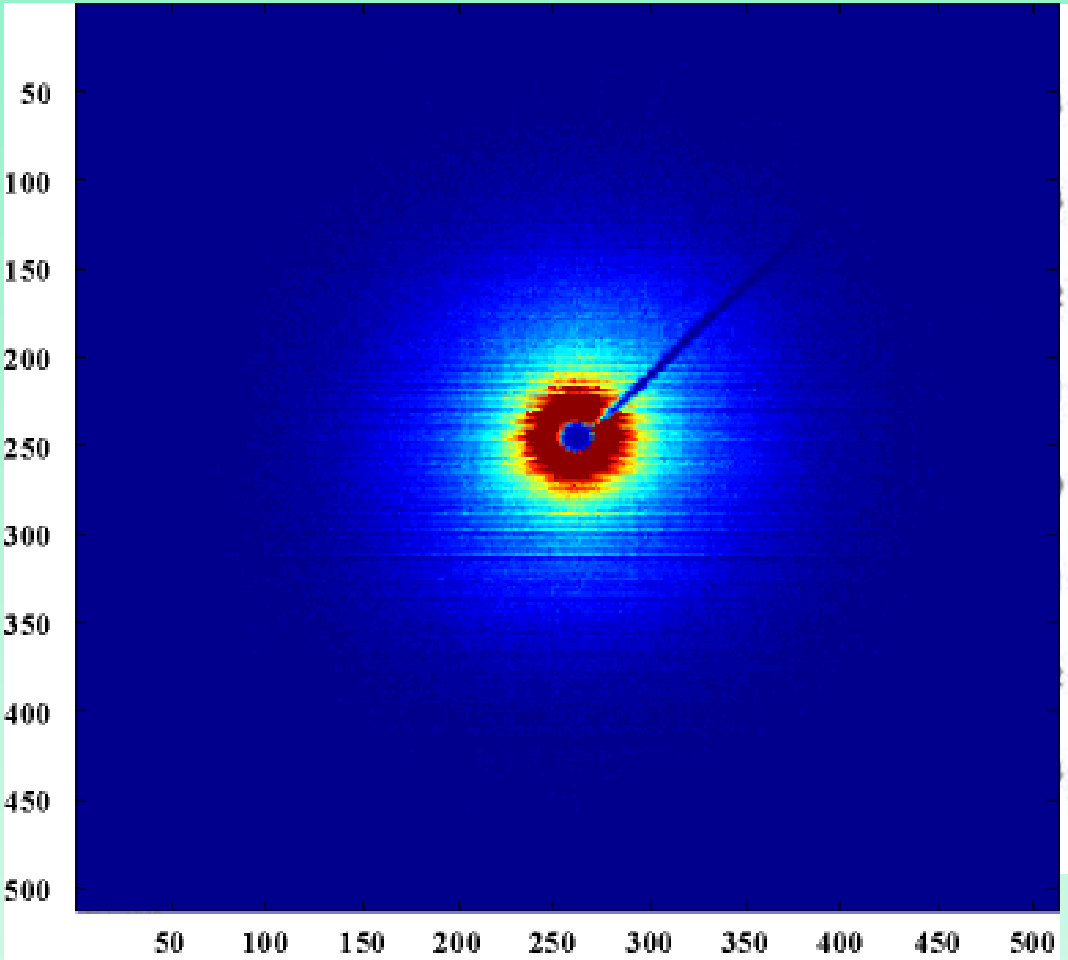
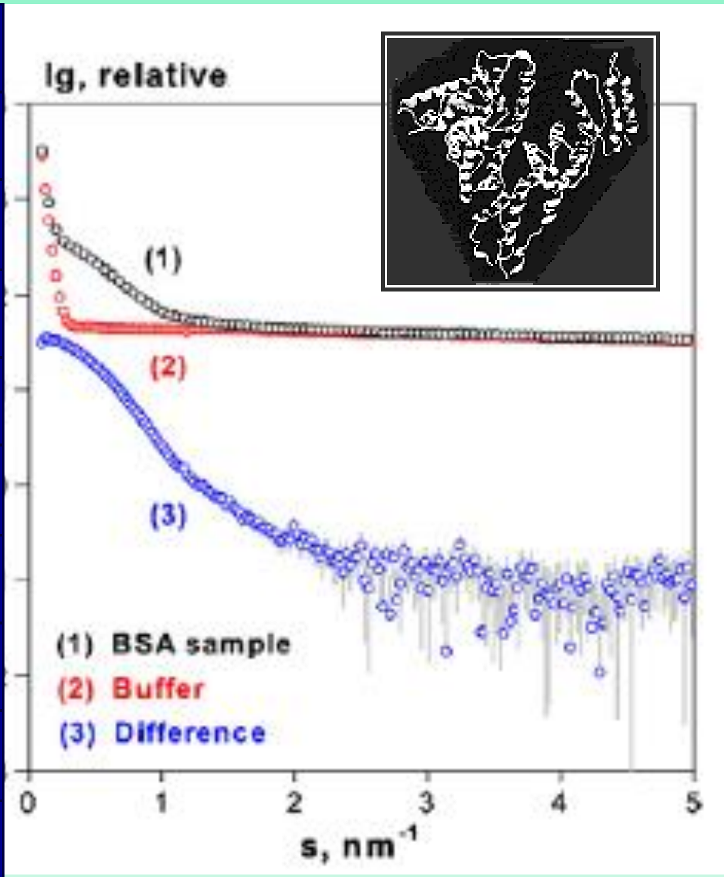
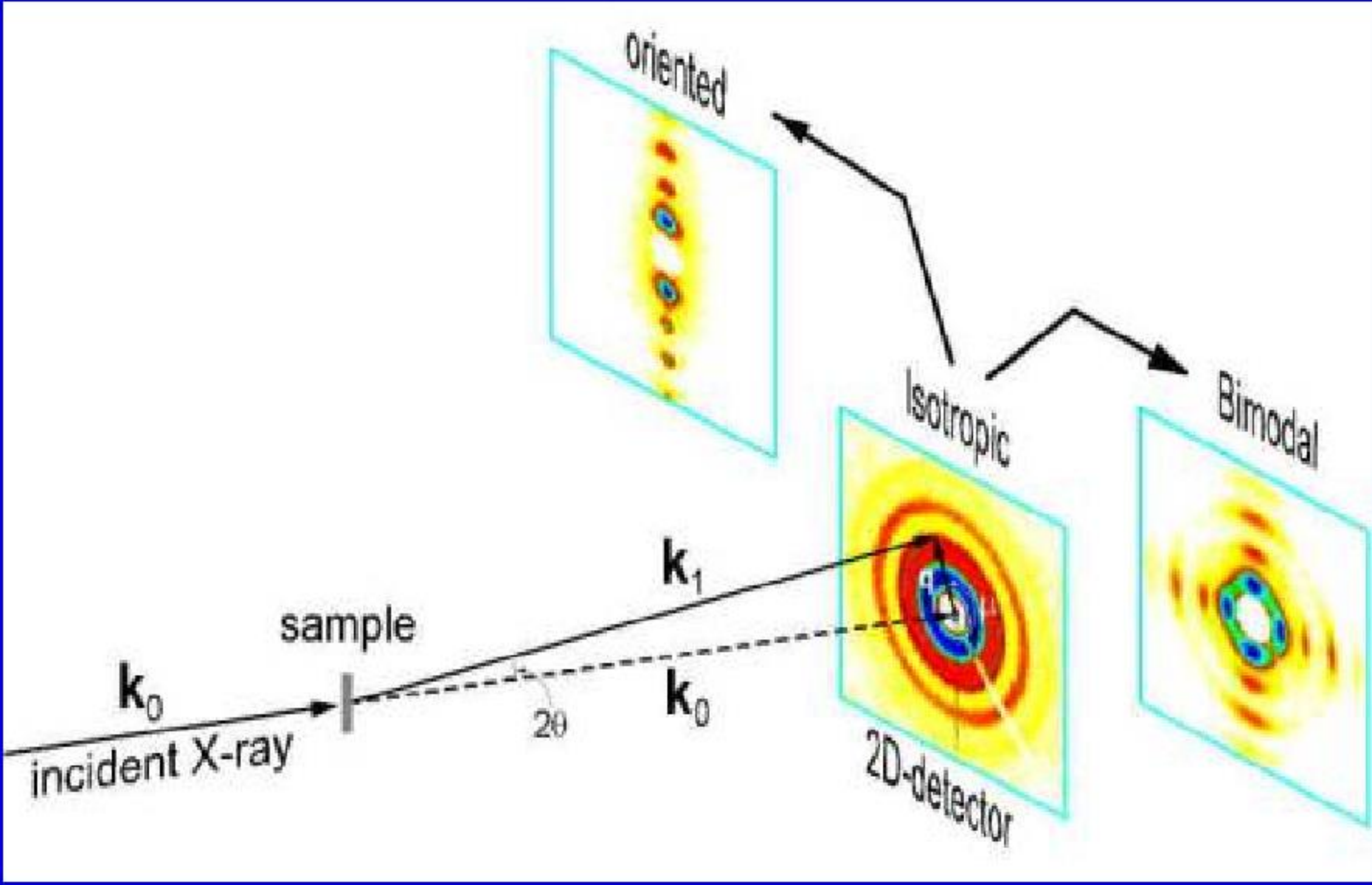
Typical 2D SAXS example





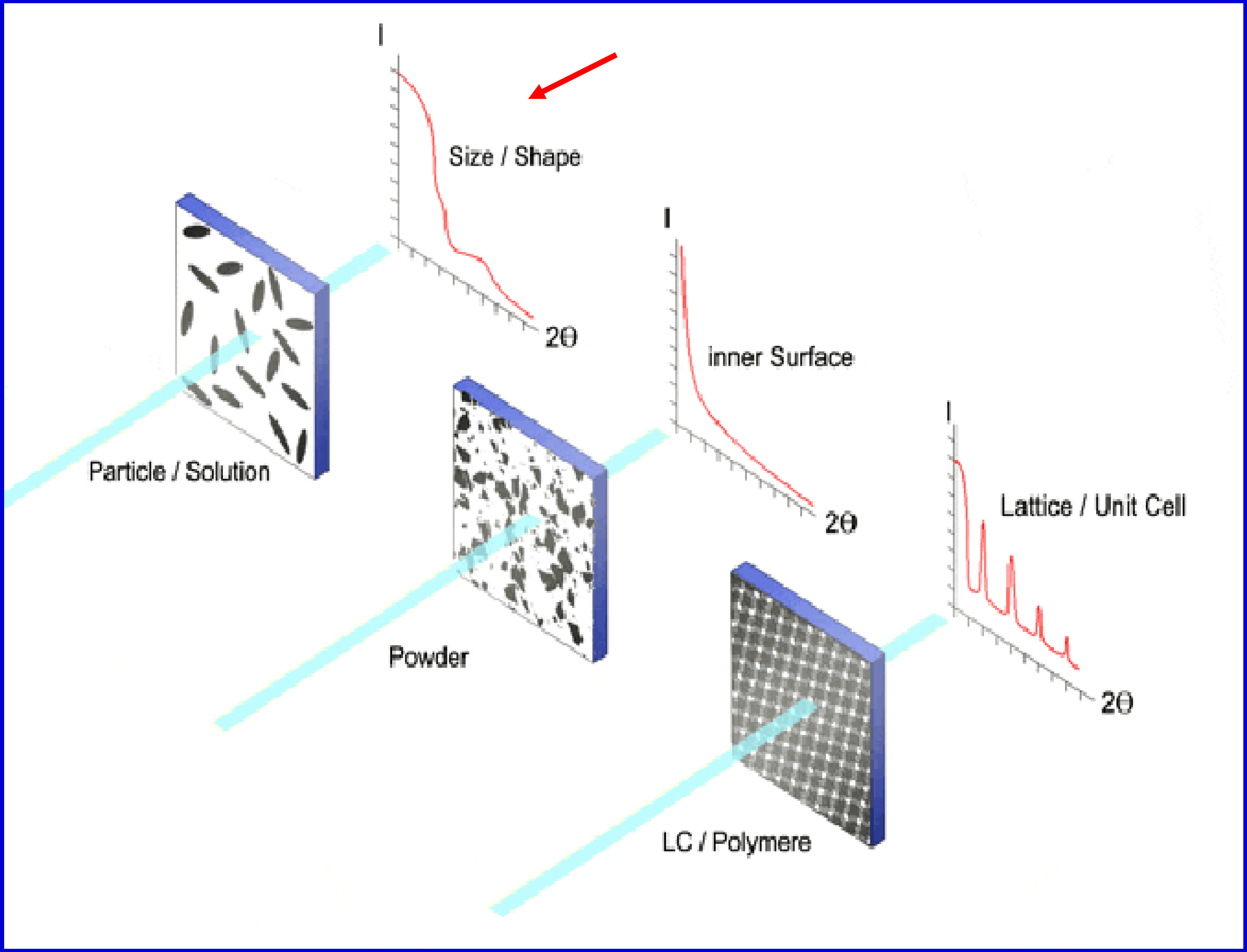
Isotropic Scattering usually show “featureless” decay

A small angle scattering experiment



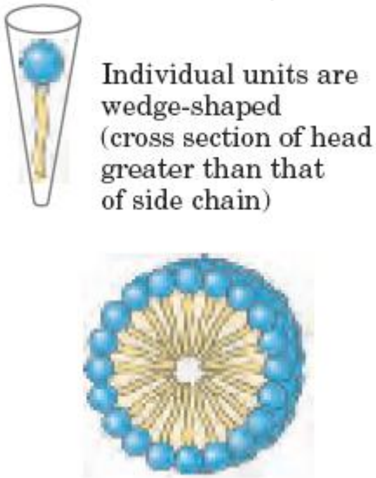
The detected patterns may be isotropic, oriented, bimodal, etc.

SAS processed results

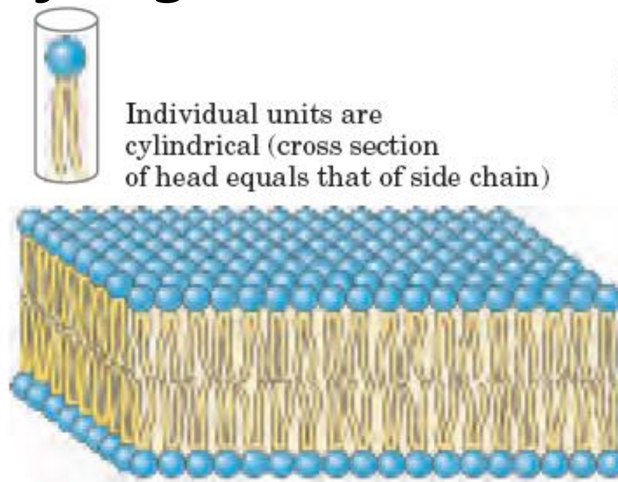


It is helpful to recognize some of the more typical SAXS patterns of isotropic systems, i.e. whether macroscopic orientation exists or not in the scattering volum

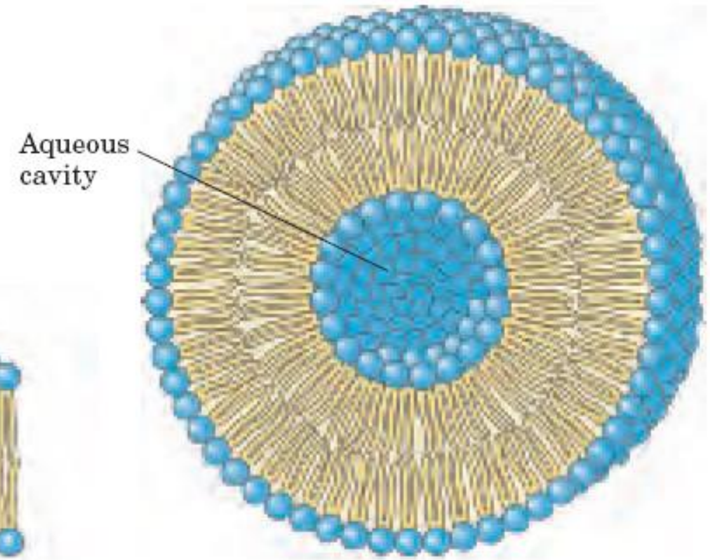
Lipids self-organization!!!



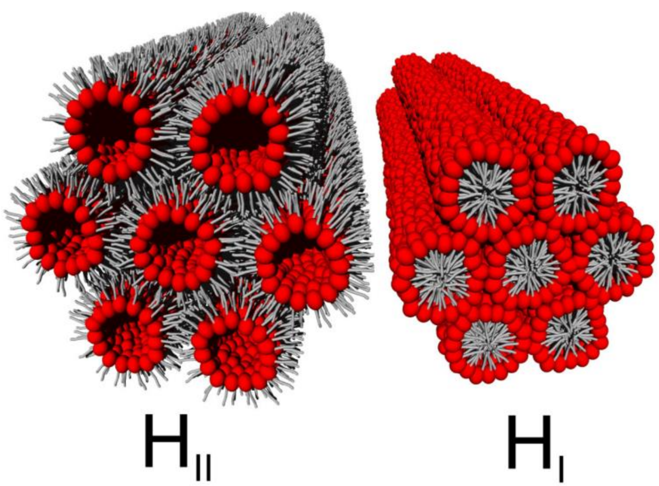
(a) Micelle

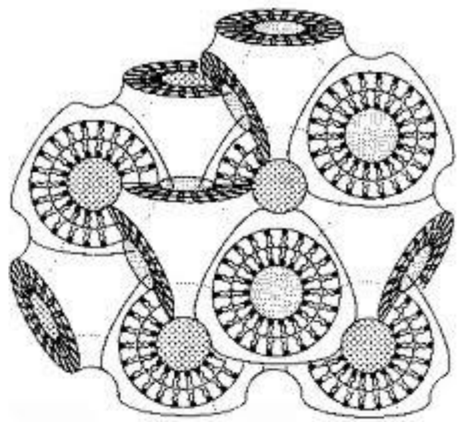


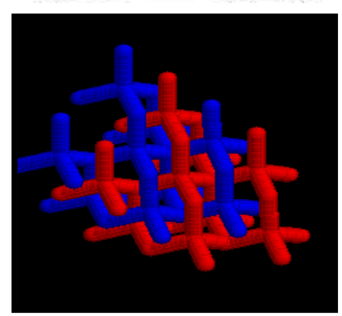
(b) Bilayer

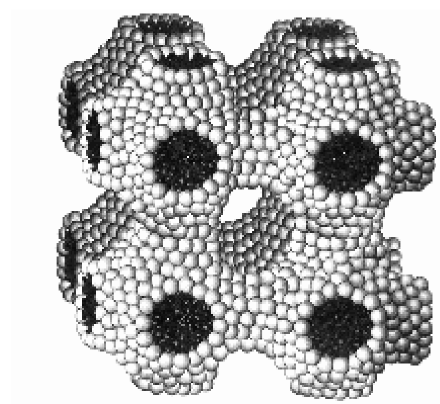


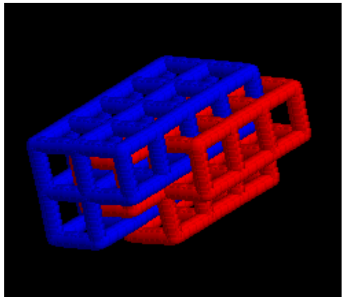
(c) Liposome







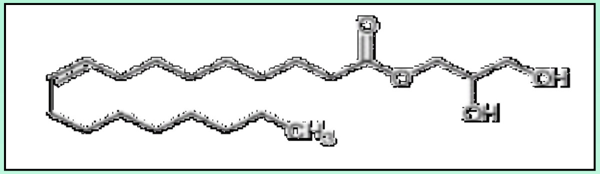




Incorporation of Aqueous-Soluble Proteins in Cubic LC Phases

Collaborators: Prof. Paolo Mariani – Un. Polytech. Marche – Ancona – Italy
PhD student: Serena Mazzoni
Leandro R. S. Barbosa

Monolein



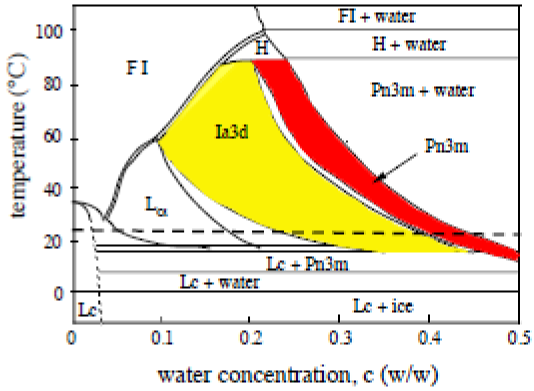
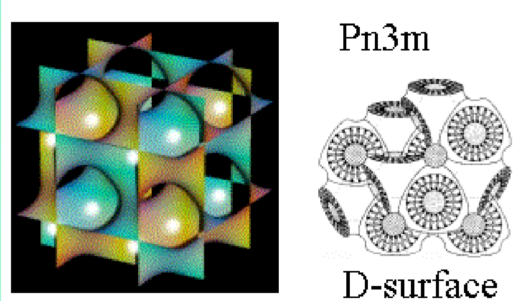
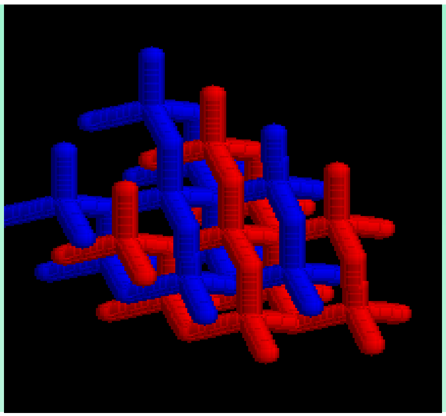


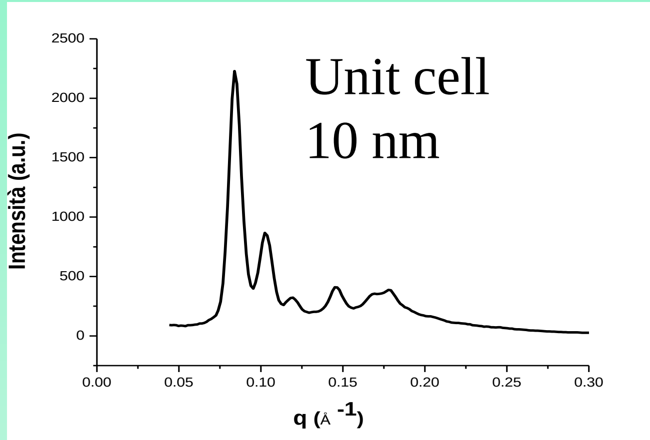
Fig. 1 Monoolein phase diagram in pure water. The different phases are indicated by acronyms: H_{II}, hexagonal phase, type II; FI, fluid isotropic; $Pn3m$, cubic phase of space group Pn3m; $Ia3d$, cubic phase of space group Ia3d; L_α, lamellar phase, with liquid conformation of the hydrocarbon chain; L_c, lamellar crystalline phase. Regions were the assignment is ambiguous are not named. The dotted line shows the phase sequence at 25°C

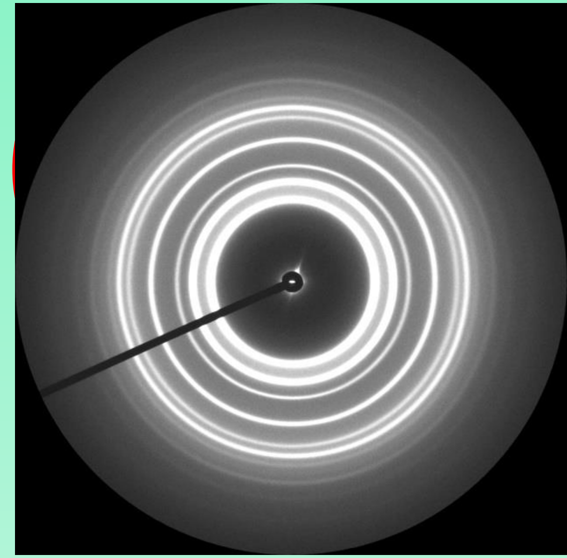


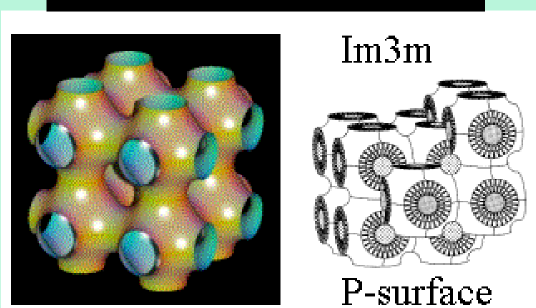


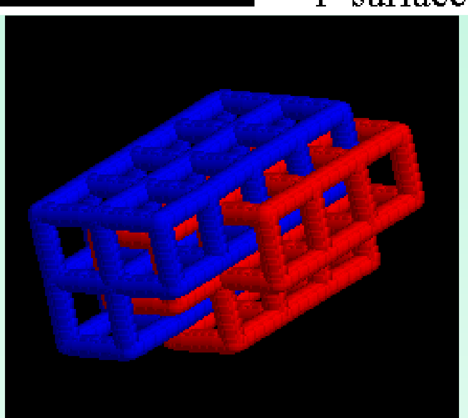
Bragg´s peaks

√2: √4: √6: √8: √10: √12……..



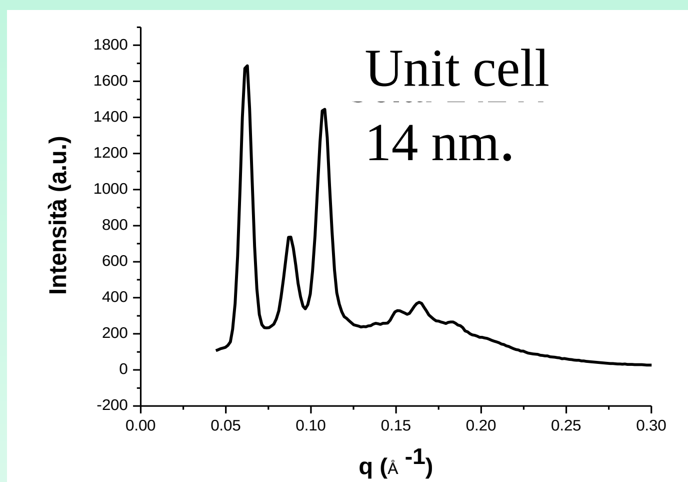


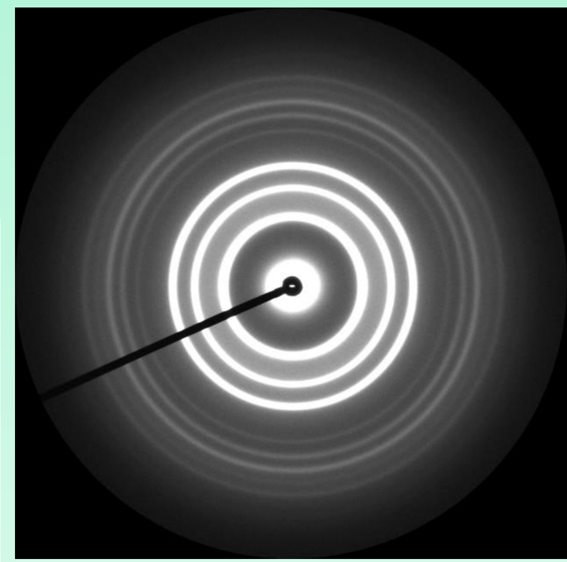




Bragg´s peaks

√2: √3: √4: √6: √8: √9……….





Monolein with cyto-c

Theory: The Master Equation

$$I(q)=n_p\left\{\langle F^2(q)\rangle+4\pi n_p\langle F(q)\rangle^2\int_0^\infty (g(r)-1)\,r^2\frac{sen(qr)}{qr}\,dr\right\}$$
$$=n_p\langle F^2(q)\rangle\bar{S}(q)$$

q = scattering vector **=** $4\pi \sin\theta/\lambda$;

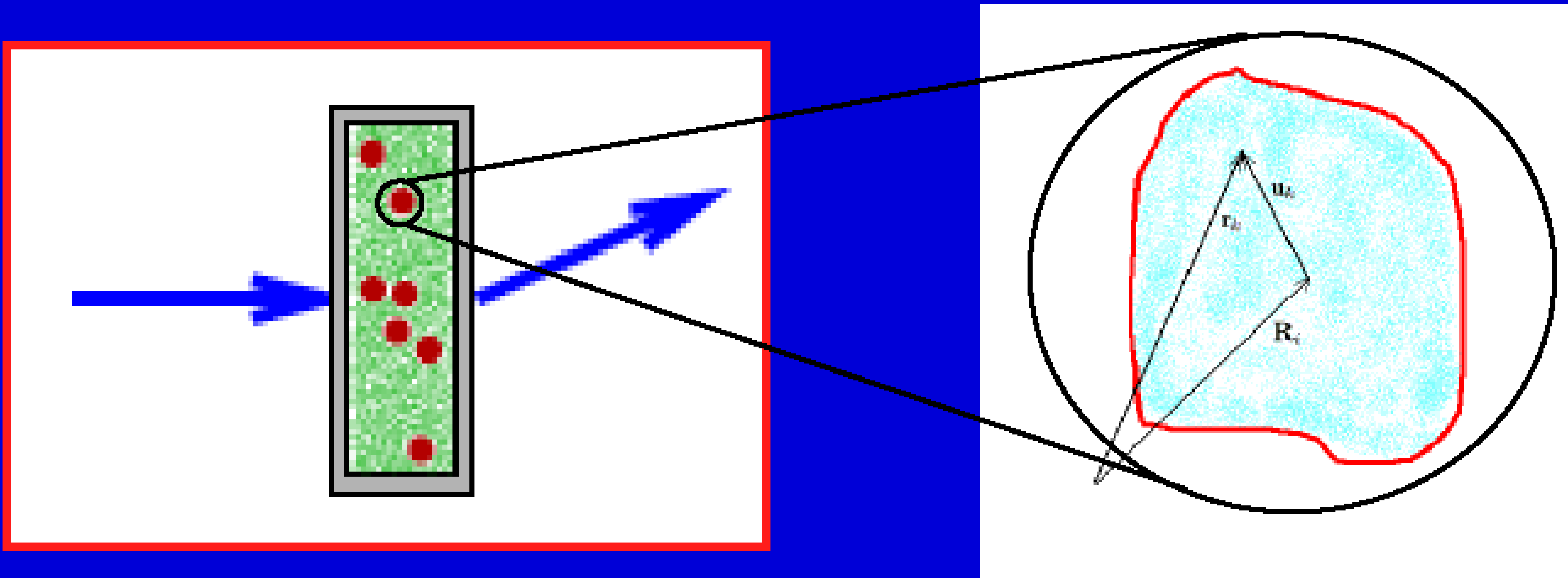
n_p particle number density

F(q) amplitude of form factor - Volume and Electron Density Contrast

P(q) = F²(q) = form factor

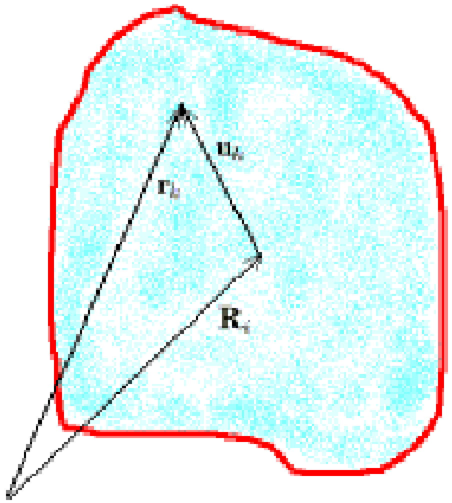
S(q) correlation function (Structure Factor) related to g(r)

g(r) pair correlation function (or radial correlation function) related to a Interaction Potential U(r))



$$\frac{d\Sigma}{d\Omega}(\mathbf{Q}) = \frac{1}{V}\left\langle \left| \int_V d\mathbf{r}\; \delta\rho(\mathbf{r}) e^{i\mathbf{Q}\cdot\mathbf{r}} \right|^2 \right\rangle$$

Form factor



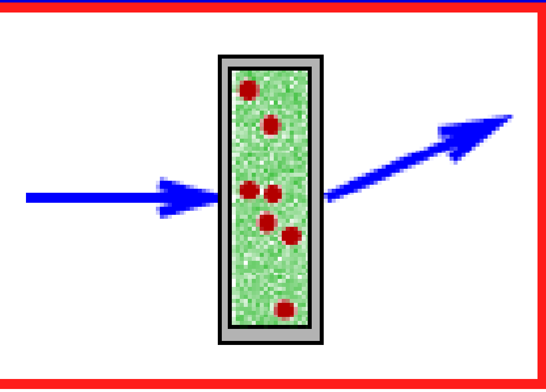
- V_i is the volume of the scattering particle i. It describes the distribution of scattering centres in the i-th particle
- $\mathbf{R}_i$ position vector, $\mathbf{u}_k$ intra-particle vector

$$\mathbf{r}_k = \mathbf{R}_i + \mathbf{u}_k$$

$$F_i(\mathbf{Q}) = \frac{1}{f_i} \int_{V_i} d\mathbf{r}\, \delta\rho_i(\mathbf{r})\, e^{i\mathbf{Q}\cdot\mathbf{r}}$$

$$f_i = \int_{V_i} d\mathbf{r}\, \delta\rho_i(\mathbf{r})$$

Scattering amplitude at zero angle



Form factor of the i-particle

$$
\begin{aligned}
\frac{d\Sigma}{d\Omega}(\mathbf{Q}) &= \frac{1}{V}\left\langle \sum_{i=1}^{N_P}\sum_{j=1}^{N_P}\int_{V_i}\int_{V_j} d\mathbf{u}_i d\mathbf{u}_j \delta\rho_i(\mathbf{u}_i)\delta\rho_j(\mathbf{u}_j) e^{i\mathbf{Q}\cdot(\mathbf{R}_i-\mathbf{R}_j)} e^{i\mathbf{Q}\cdot(\mathbf{u}_i-\mathbf{u}_j)} \right\rangle \\
&= \frac{1}{V}\sum_{i=1}^{N_P} f_i^2 < F_i^2(\mathbf{Q}) >_{\omega_i} \\
&\quad + \frac{1}{V}\sum_{i=1}^{N_P}\sum_{j\neq i}^{N_P} f_i f_j < F_i(\mathbf{Q}) F_j^*(\mathbf{Q}) e^{i\mathbf{Q}\cdot(\mathbf{R}_i-\mathbf{R}_j)} >_{\omega_i,\omega_j,\mathbf{R}_i,\mathbf{R}_j},
\end{aligned}
$$

Particle-particle interaction

Effective form factor and structure factor

- The master equation can be written as

$$\frac{d\Sigma}{d\Omega}(\mathbf{Q}) = n_P < (\Delta\rho V_P)^2 > P(\mathbf{Q}) S(\mathbf{Q})$$

$n_P = N_P/V$ Number density

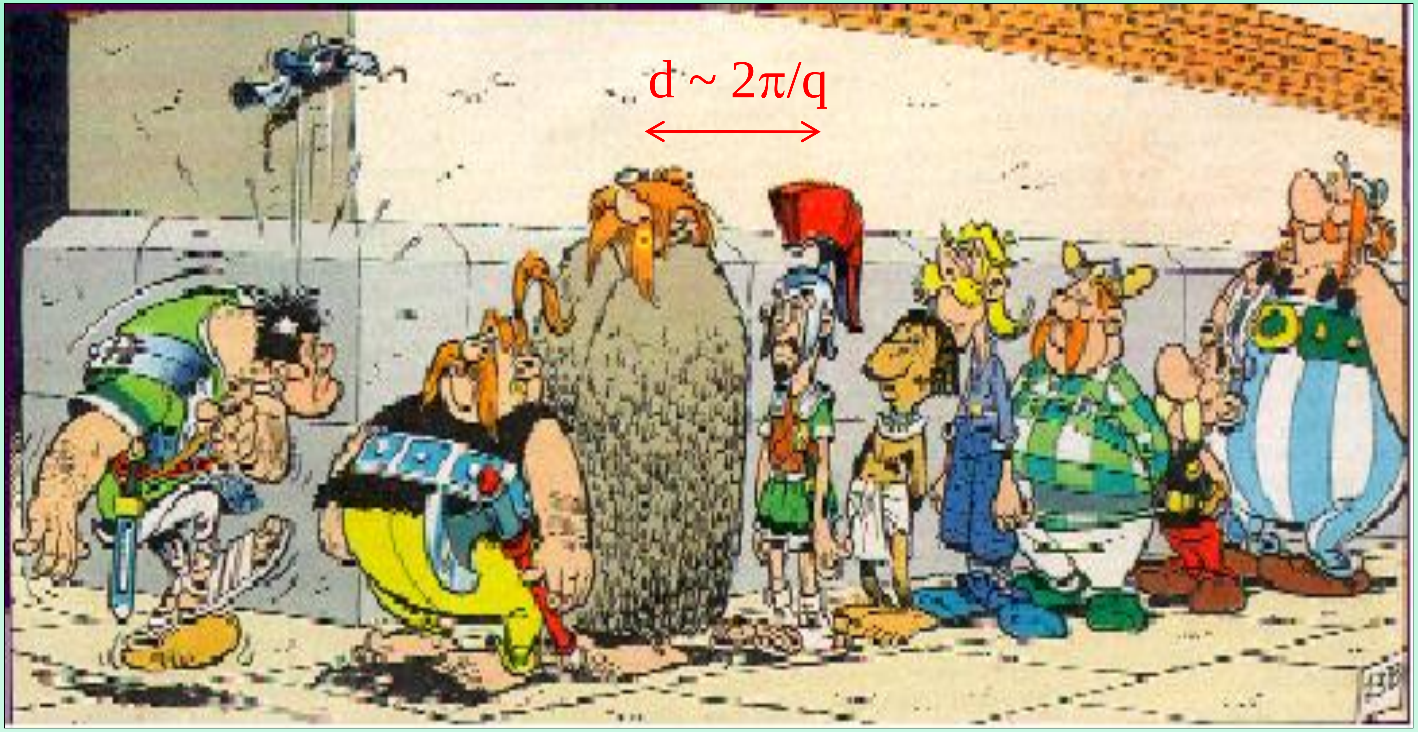
$<(\Delta\rho V_P)^2>$ Average square scattering length per particle

$$<(\Delta\rho V_P)^2> = \frac{1}{N_P}\sum_{i=1}^{N_P} f_i^2$$

$P(\mathbf{Q})$ Effective form factor

$$P(\mathbf{Q}) = \frac{\sum_{i=1}^{N_P} f_i^2 < F_i^2(\mathbf{Q}) >_{\omega_i}}{\sum_{i=1}^{N_P} f_i^2}$$

$S(\mathbf{Q})$ Effective structure factor

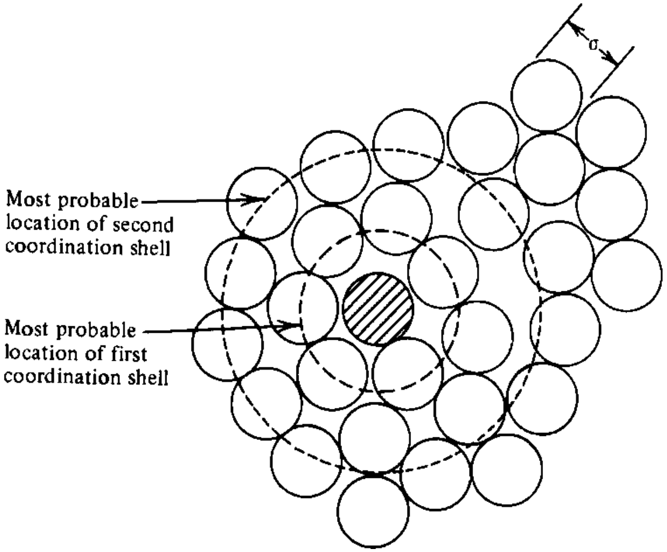

d ~ 2π/q

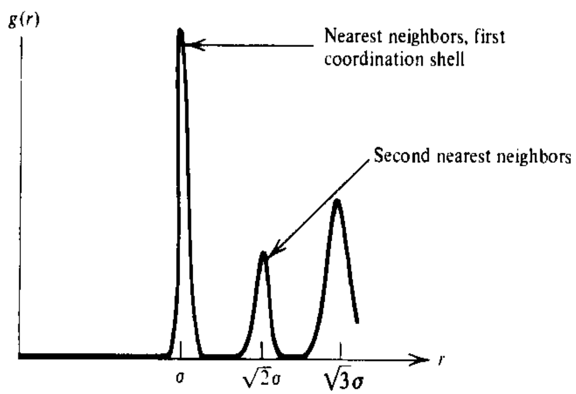

KA-WUMM!!

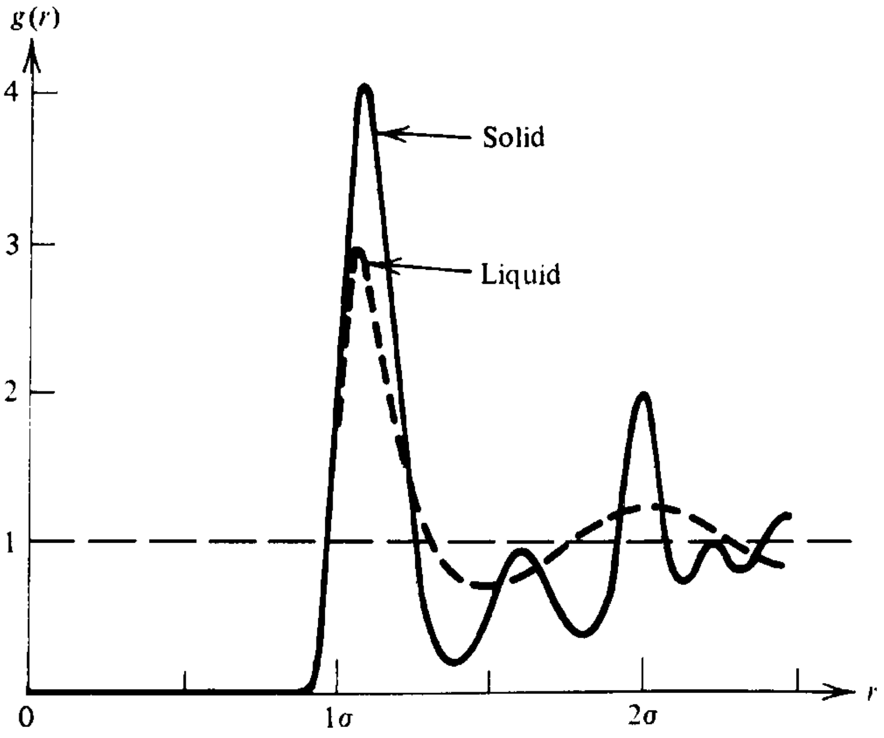
Structure Factor (spherical symmetry)

Describe the interaction between the nearest particles

$$S(q) = \left\{ 1 + 4\pi n_p \frac{\langle F(q) \rangle^2}{\langle F^2(q) \rangle} \int_0^\infty (g(r) - 1) r^2 \frac{sen(qr)}{qr} dr \right\}$$







Interacting, ordered particles

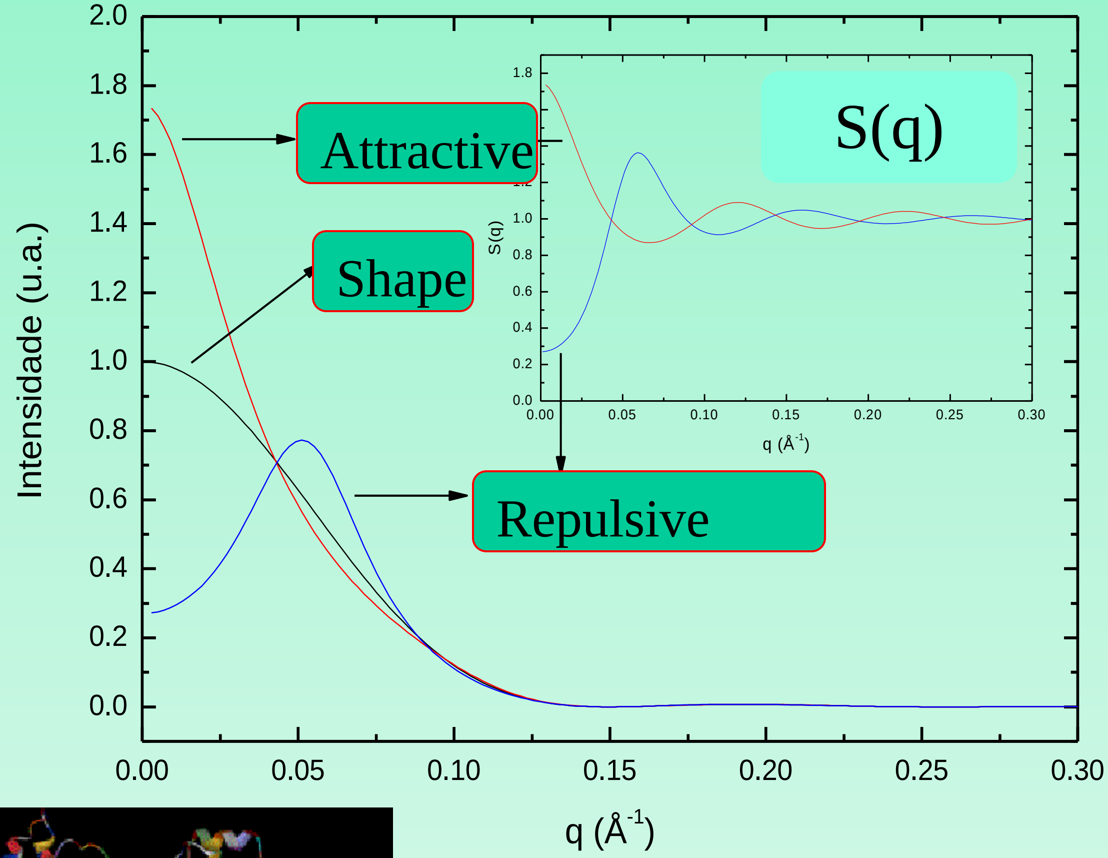
Oriented, homogeneous particles

Homogeneous, not interacting particles

Interacting, not homogeneous particles

Interacting, homogeneous particles

Weakly to Moderately Correlated Systems



$$I(q) = P(q).S(q)$$

BSA with Rg= 30 Å

$$S(q \to 0) = I(0) = k_B T \left(\frac{\partial n_p}{\partial \pi} \right)_T \cong \left(1 + 2Bn_p\right)^{-1}$$

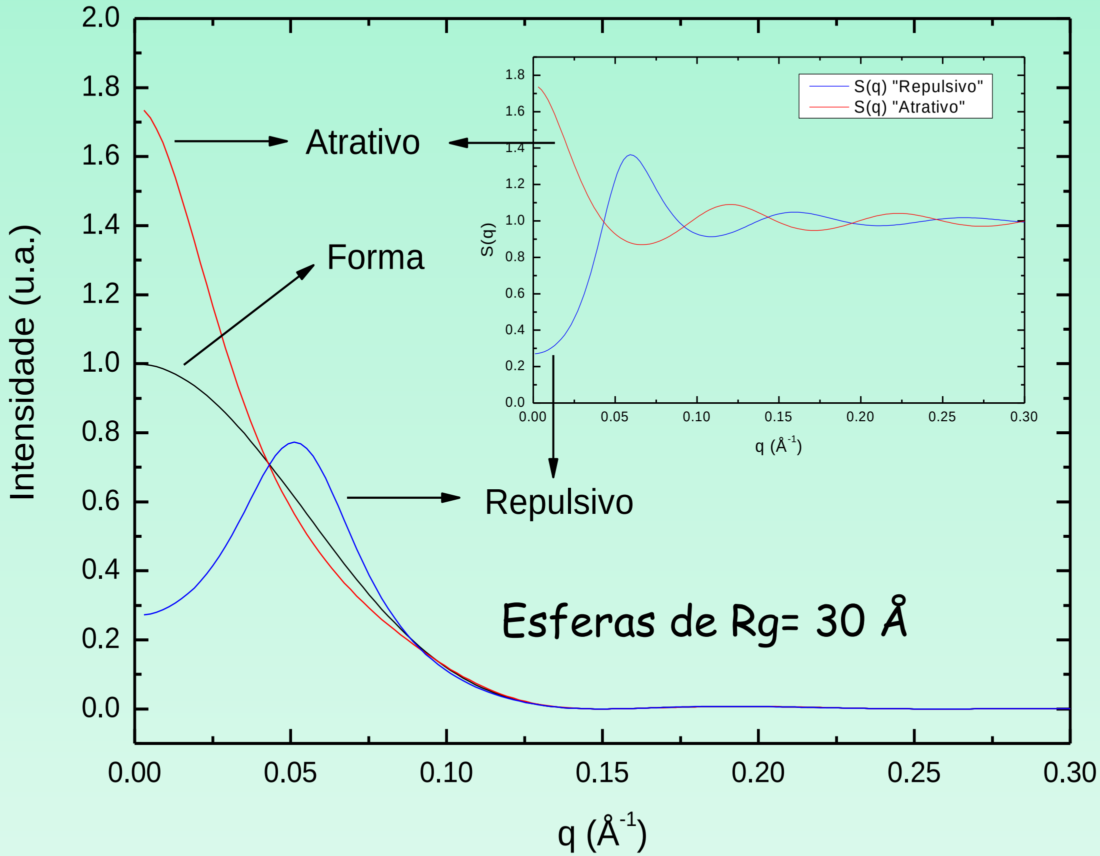
$$B = -2\pi \int_0^\infty (g(r) - 1)\, r^2 dr$$

π = Pressão Osmótica da solução

B = 2nd coeficiente de virial

B> 0 energia interação repulsiva

B<0 energia interação atrativa



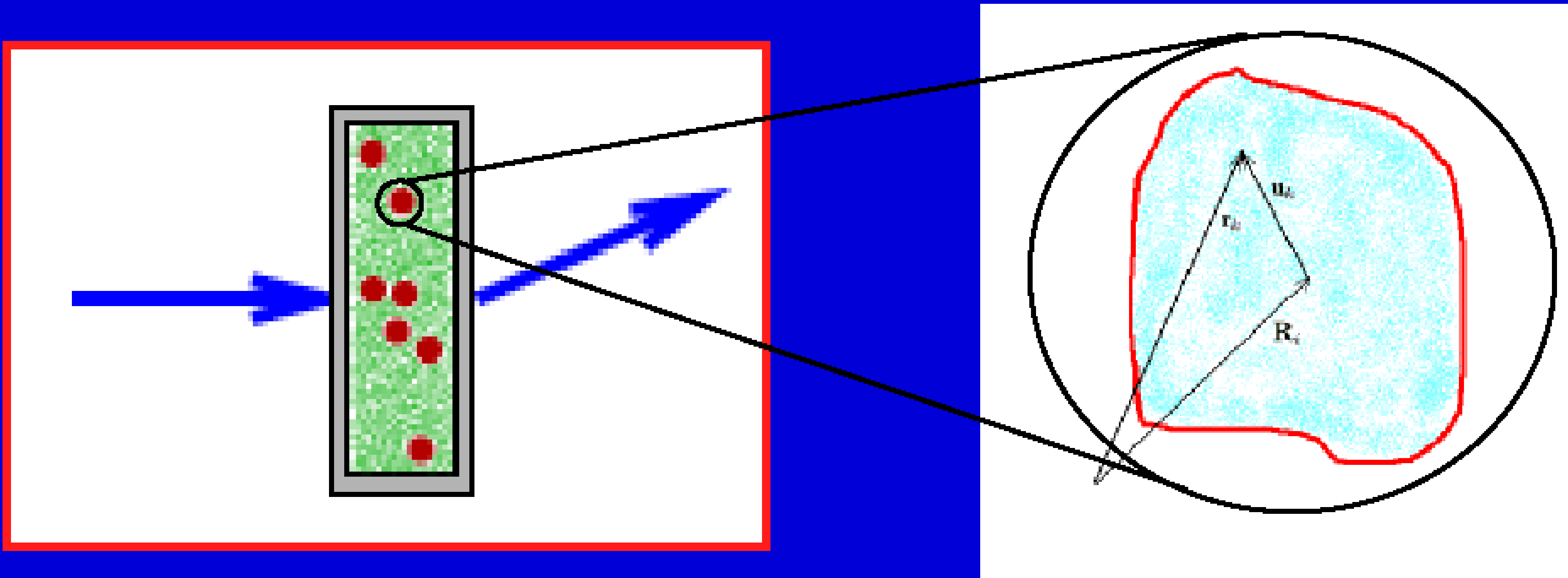
Homogeneous and not interacting particles

Two-phase model

1. N_P identical particles per unit volume with homogeneous scattering length density ρ

2. Particles widely separated, fully isotropic orientation. The structure factor can be neglected (S(q) = 1)

3. Particles imbedded in a matrix of homogeneous scattering length density (electron density) ρ_0

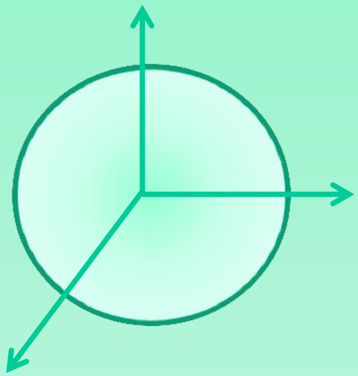


$$\frac{d\Sigma}{d\Omega}(\mathbf{Q}) = \frac{1}{V}\left\langle \left| \int_V d\mathbf{r}\; \delta\rho(\mathbf{r}) e^{i\mathbf{Q}\cdot\mathbf{r}} \right|^2 \right\rangle$$

Média isotrópica

$$\langle e^{iqr} \rangle = \frac{\sin qr}{qr}$$

Example: Sphere of radius ***R*** and electron density $\boldsymbol{\rho_S}$ dispersed in a medium of ellectron density $\boldsymbol{\rho_0}$.



$$\rho(\vec{r}) = \begin{cases} \rho_S & r \le R \\ \rho_0 & r > R \end{cases}$$

$$I(q) \propto \left\{ \int_V \left(\rho(\vec{r}) - \rho_0 \right) e^{i\vec{q}\cdot\vec{r}} d\vec{r} \right\}^2$$

$$= \left\{ 4\pi \left(\rho_E - \rho_0 \right) \int_0^R r^2 \frac{sen(qr)}{qr} dr \right\}^2$$

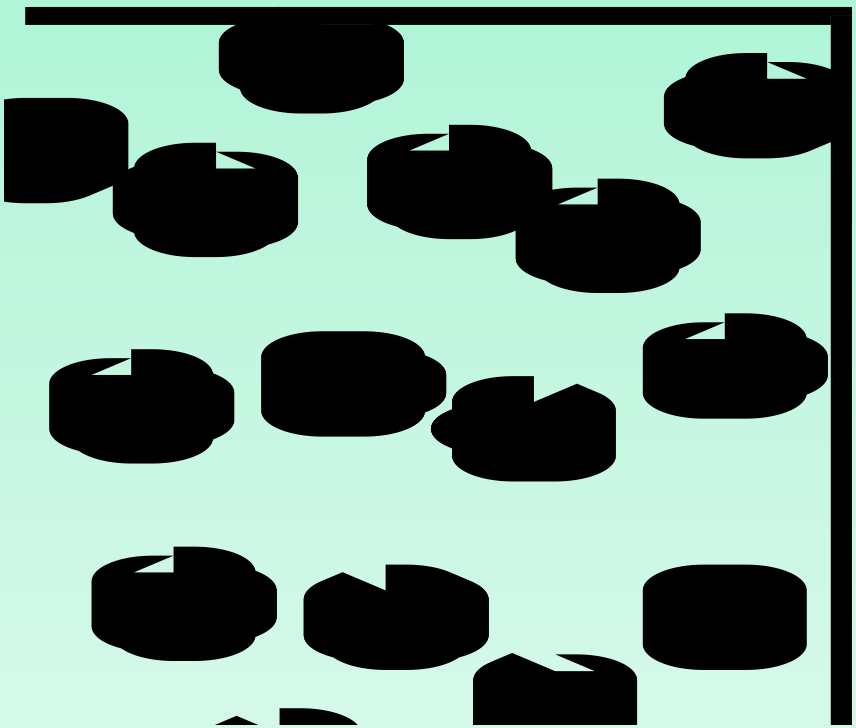
$$P(q) = \left\{ 3V \left(\rho_E - \rho_0 \right) \left\{ \frac{sen(qR) - qR\cos(qR)}{(qR)^3} \right\} \right\}^2$$

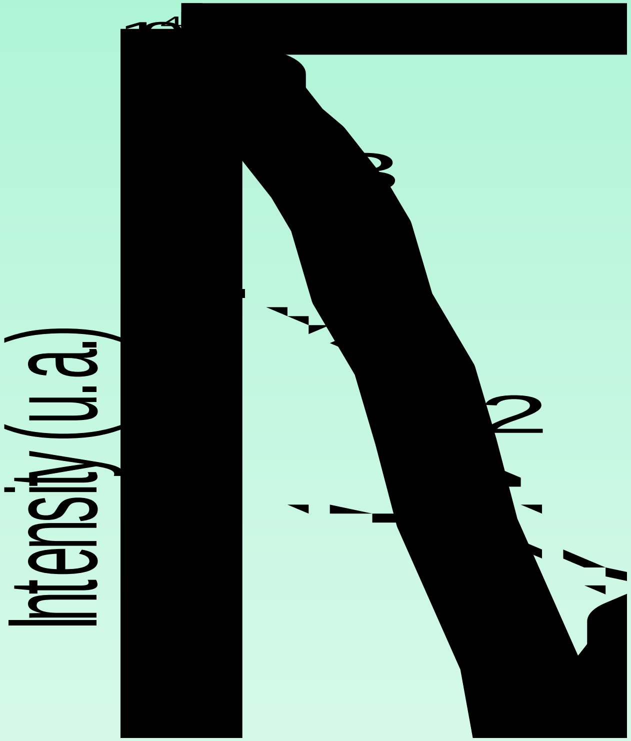
Same procedure for spheres, cylinders... Describe the electron density of the scattering particle

Form Factor for Homogeneous Spheres

$$F(q) = 3V(\rho_1 - \rho_2)\frac{\sin(qR) - qR\cos(qR)}{(qR)^3}$$

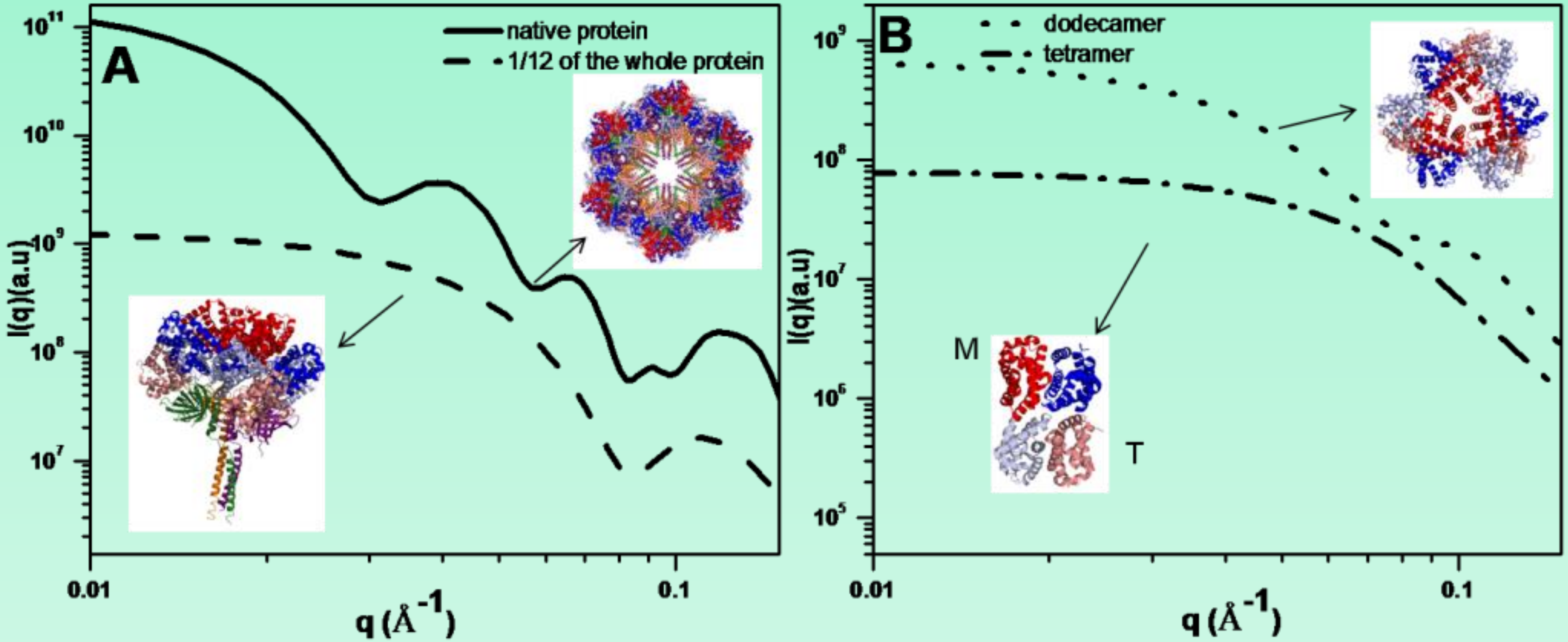
$$I(q) = n_p\left[< F(q) >\right]^2$$





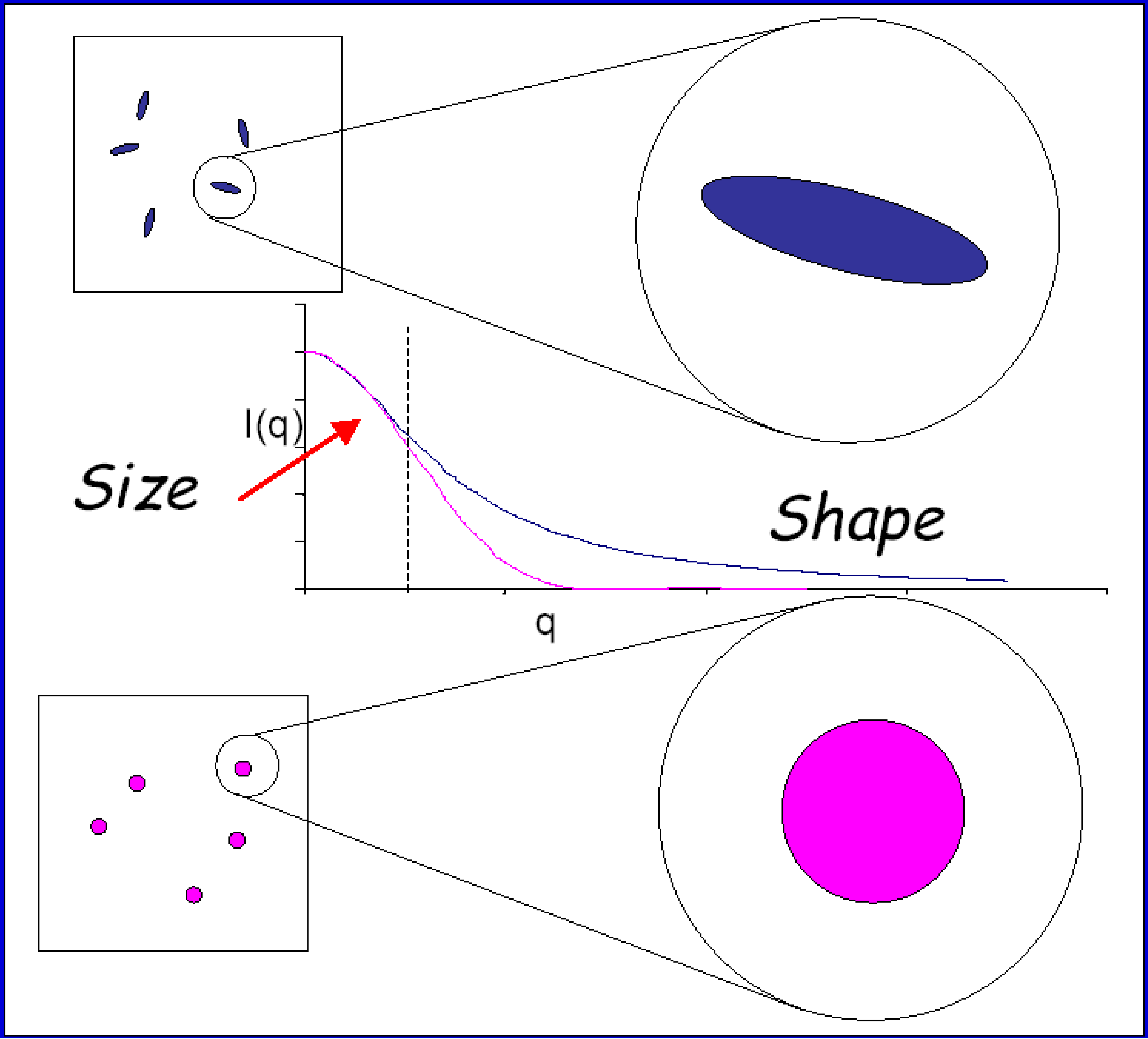
Example: *On the temperature stability of extracellular hemoglobin of Glossoscolex paulistus, at different oxidation states: SAXS and DLS studies*

Tabak, Itri et al Biophys Chem (2012)



A) Native HbGp is a hexagonal bilayer consisting of twelve protomers (***abcd***) $_3$***L***$_3$. Half of the native molecule corresponds to one hexagonal layer of six protomers. **B**) Dodecamer (***abcd***)$_3$is an assembly of four hetero-tetramers (***abcd***), each composed of a disulfide-bonded trimer (***abc***=T) and a monomer (***d***=M). ***L*** corresponds to the linker chains.

Form factor: particle size and shape



$\Rightarrow$ Not Interacting Systems:

Guinier's region

$$I(q)_{q\to 0} = n_p \Delta\rho^2 V^2 \exp(-q^2 R_g^2 / 3)$$

$$I(q) = n_p < F^2(q) >= n_p P(q)$$

$S(q) \to 1$

$$I(q)_{q\to\infty} = n_p (\Delta\rho)^2 2\pi S_{up} / q^4$$

Porod's region

Homogeneous Particles with sharp electronic density contrast between the particle and the medium.

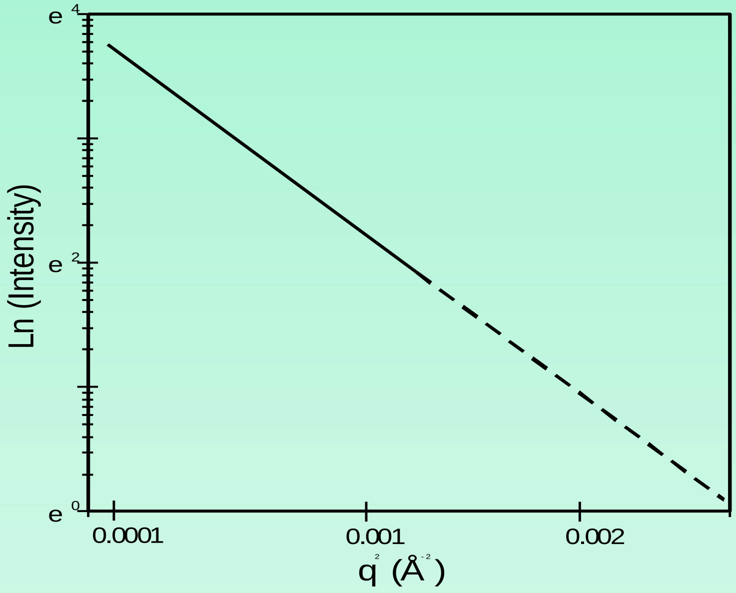
Rg = radius of gyration!!!

qRg < 1.3!!!

Guinier Law

$$I(q) = N(\rho_1 - \rho_2)^2 v^2 e^{-\frac{R_g^2 q^2}{3}} \quad , q \to 0$$

$(qRg \le 1)$



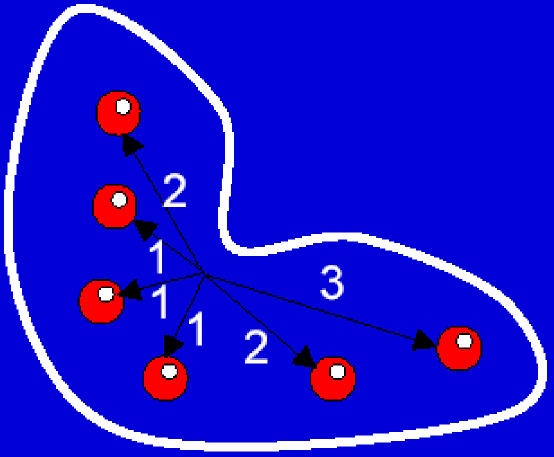
$$\text{slope } \alpha = \frac{1}{3} R_g^2$$

$$R_g = \sqrt{3\alpha}$$

Then,what do we mean by “size”?

$$R_g^2 = \frac{1}{V_P}\int_{V_P} d\mathrm{r}\, r^2$$

$R_g{}^2$ is the average squared distance of the scatterers from the centre of the object



$R_g{}^2 = (1^2 + 1^2 + 1^2 + 2^2 + 2^2 + 3^2)/6 = 20/6$

$R_g = \sqrt{3.333} = 1.82$

How about polydispersity???

Langmuir
Article
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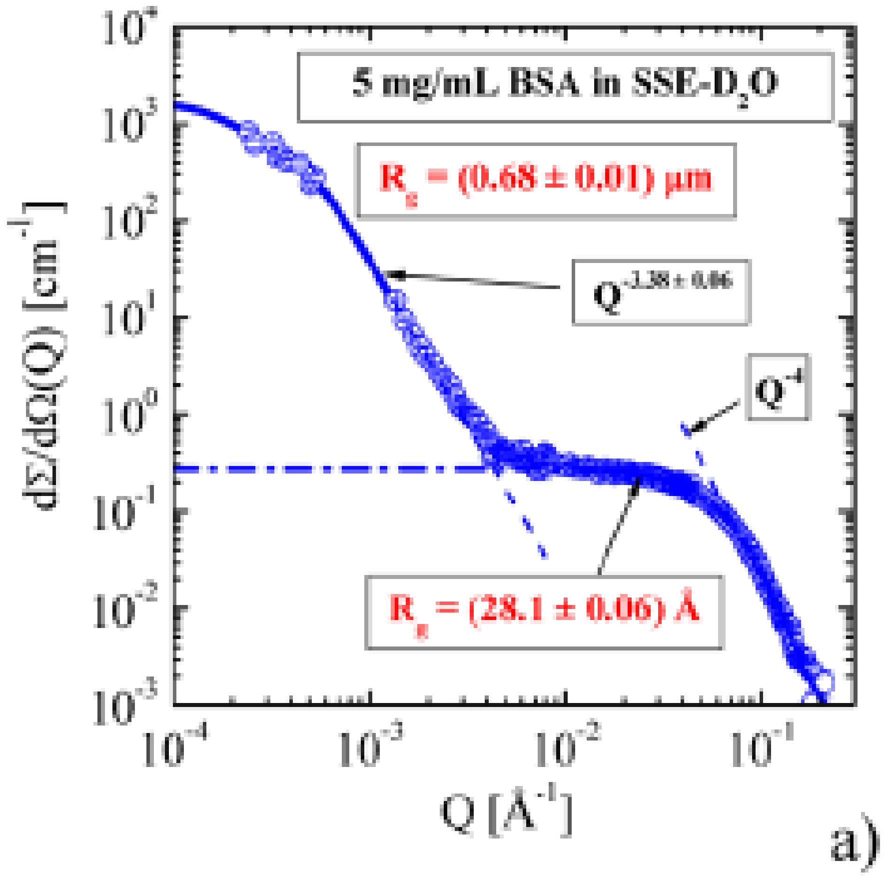
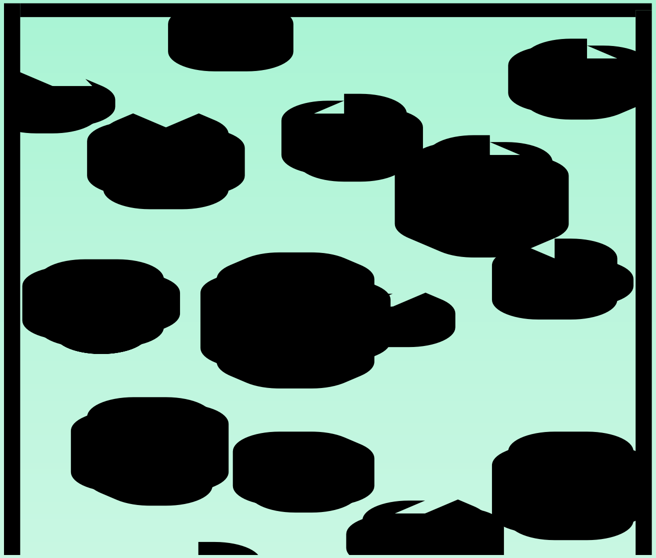
Effects of Biological Molecules on Calcium Mineral Formation Associated with Wastewater Desalination as Assessed using Small-Angle Neutron Scattering

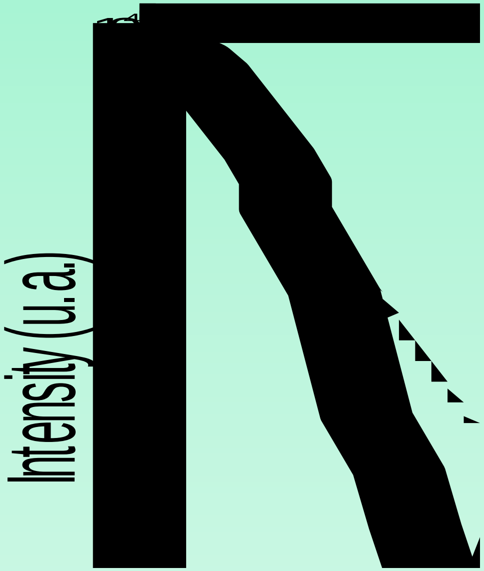
Vitaliy Pipich,[†] Yara Dahdal,[‡,§] Hanna Rapaport,[∥] Roni Kasher,[‡] Yoram Oren,[‡] and Dietmar Schwahn*[,§,⊥]



Case 2

$$I(q) = np\int N(R)[F(q)]^2\, dR$$

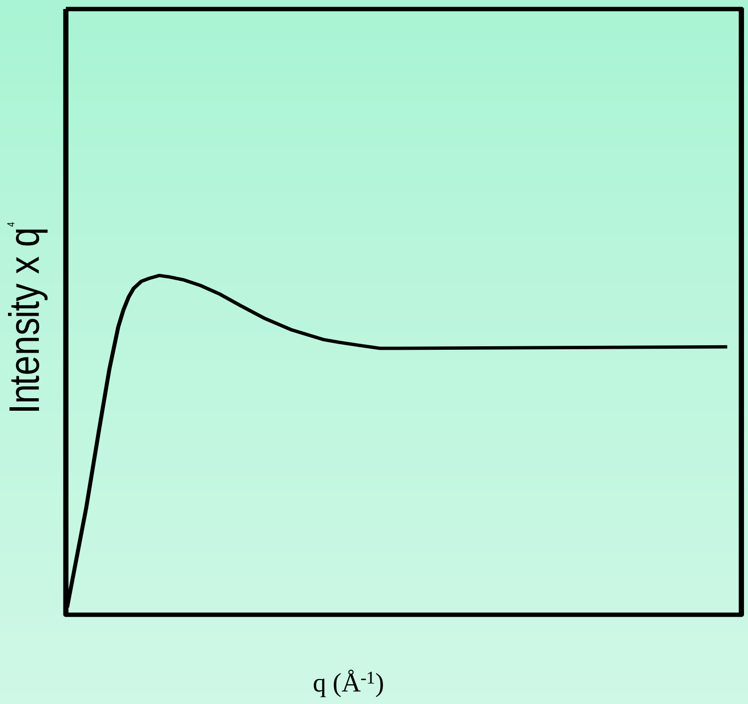




Porod Law

$$\lim_{q\to\infty} I(q) = \frac{2\pi(\rho_1 - \rho_2)^2 S}{q^4}$$

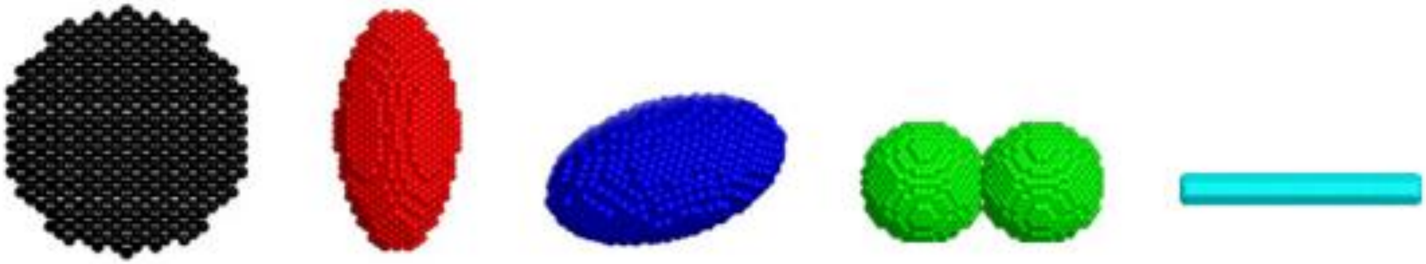
i.e. lim I(q)q^4 = constant

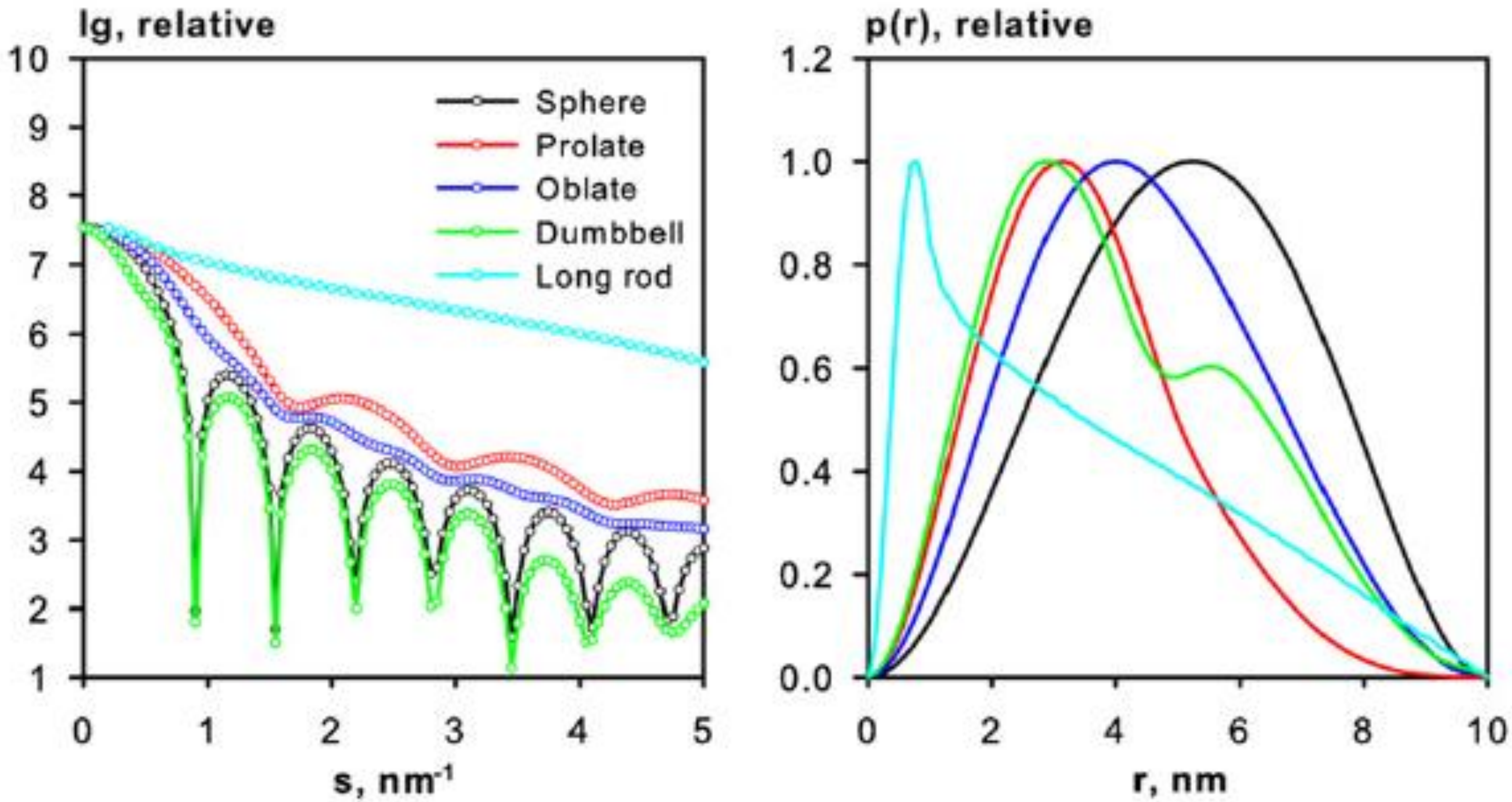


Distance distribution function, p(r)

$$p(r)=\frac{1}{2\pi^2}\int_0^{\infty} I(q).qr.sen(qr)dq$$

$$Rg^2=\frac{\int_0^{D_{MÁX}} p(r)r^2dr}{2\int_0^{D_{MÁX}} p(r)dr}$$

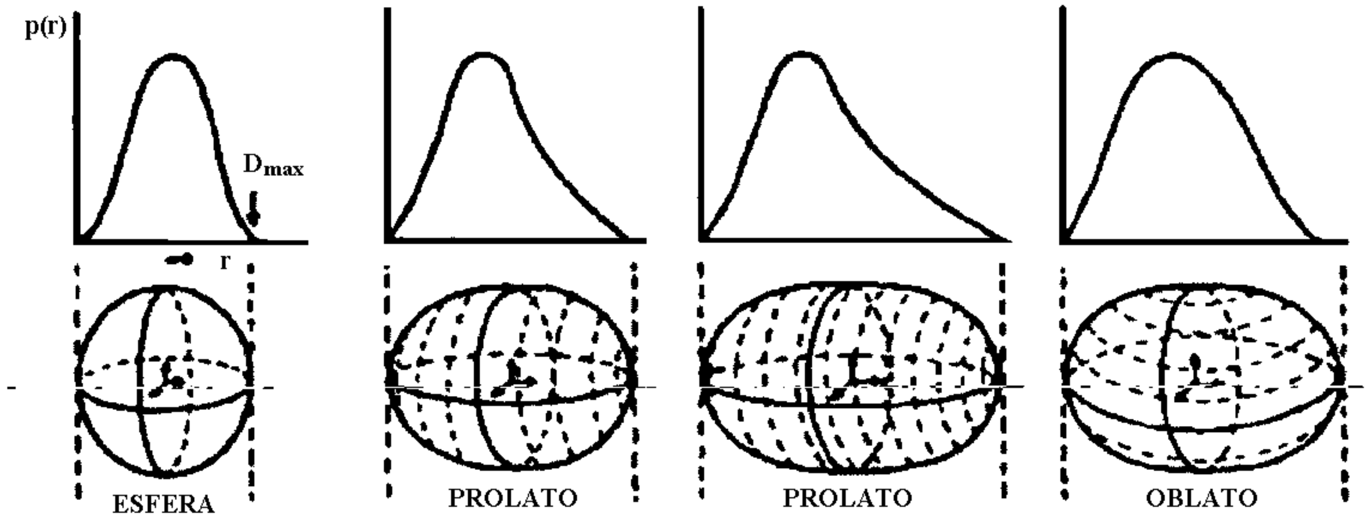


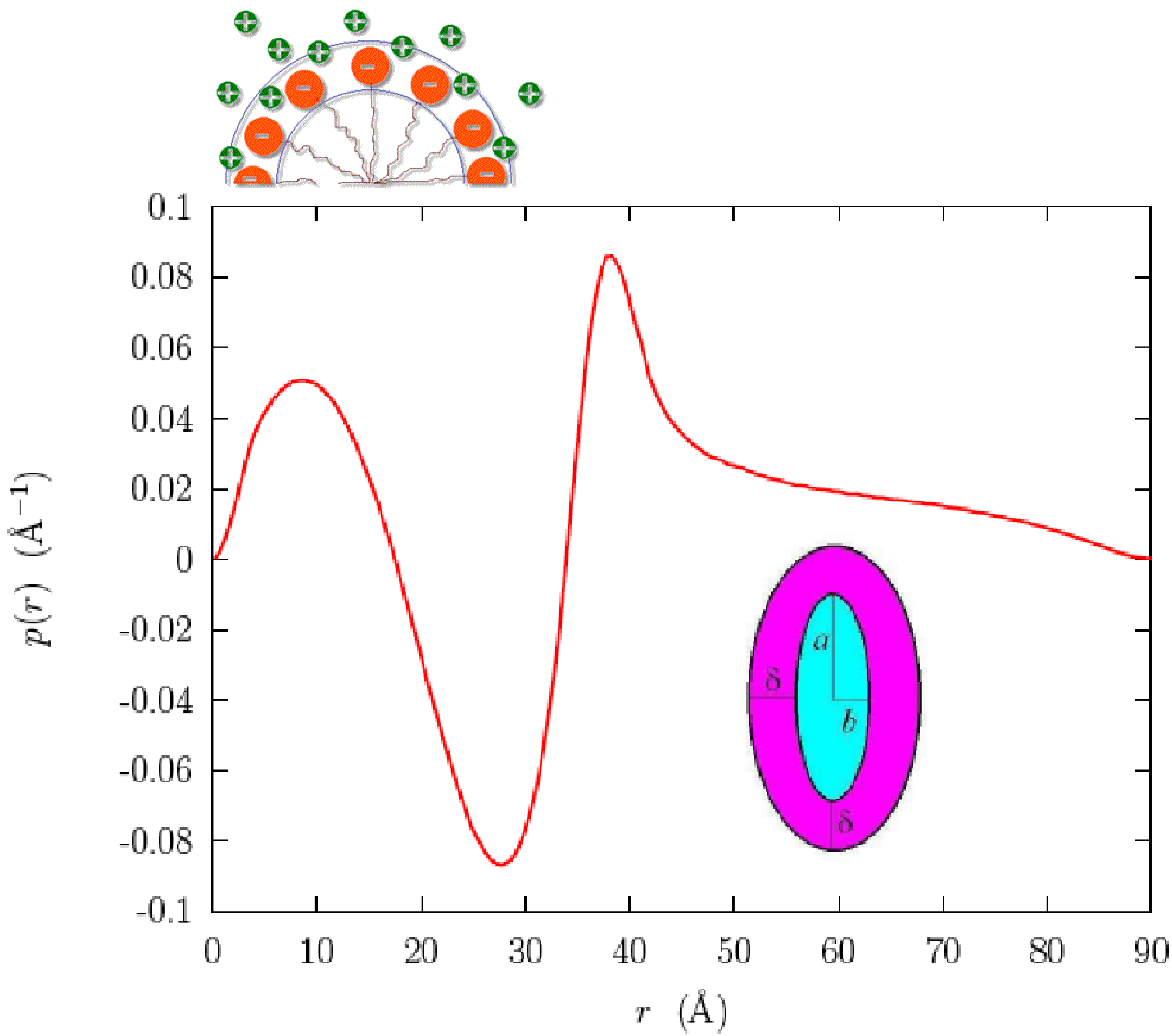


$$p(r)=\frac{1}{2\pi^2}\int_0^{\infty} I(q)(qr)\,sen(qr)\,dq$$

$$R_g^2=\frac{\int_0^{D_{\max}} p(r)r^2dr}{2\int_0^{D_{\max}} p(r)\;dr}$$

$$Dmax$$





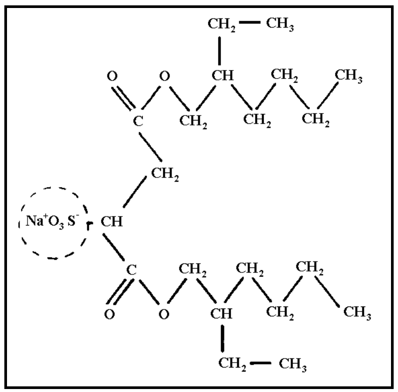
0.1
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
-0.08
-0.1
0
10
20
30
40
50
60
70
80
90
$p(r)$ (Å^{-1})
r (Å)
a
b
δ
δ

Most known available softwares

- GNOM –D. Svergum
- GIFT package – Otto Glatter
- GENFIT package – Francesco Spinozzi

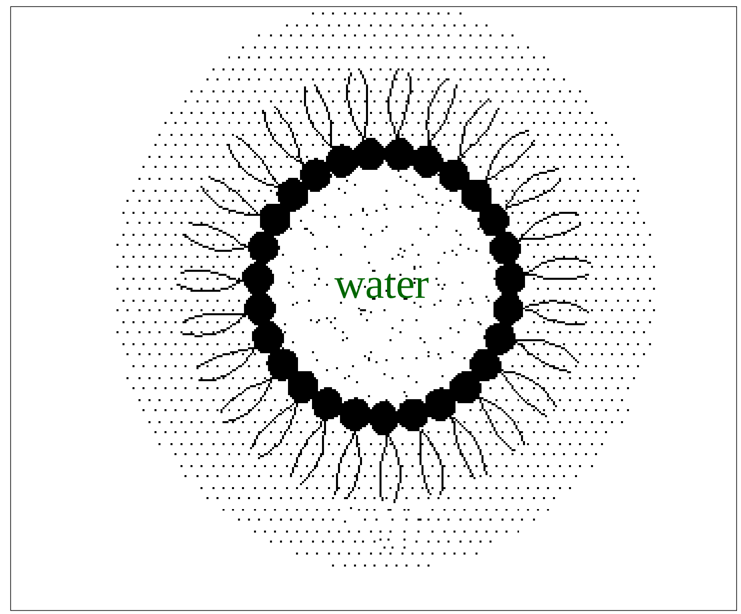
Reversed Micelles. A Study by SAXS

AOT

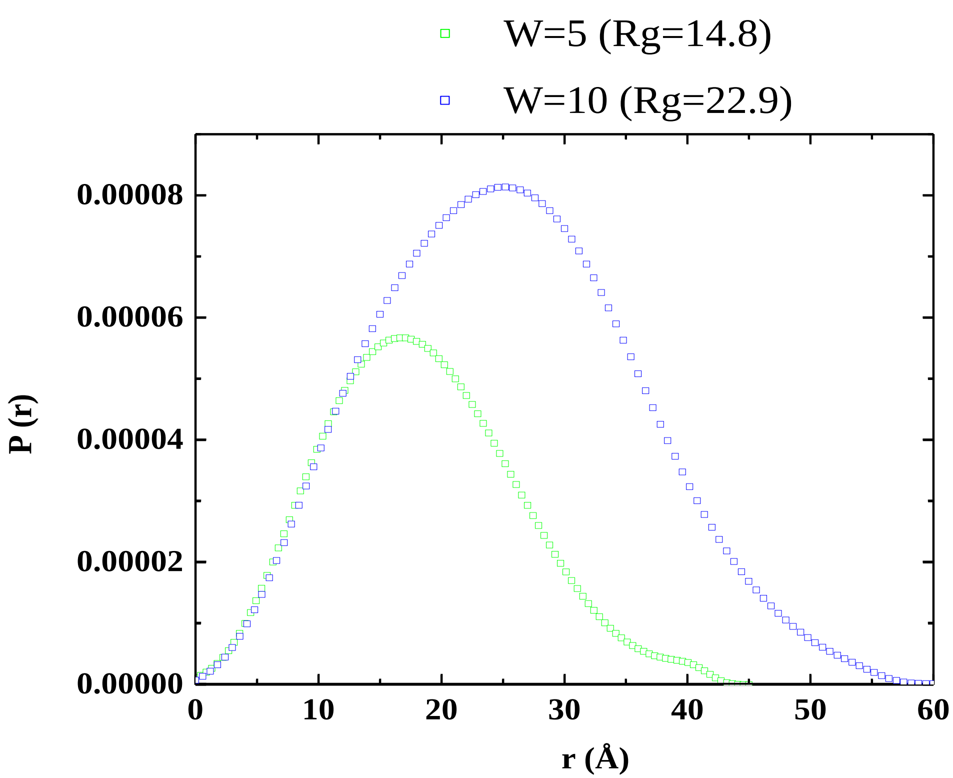


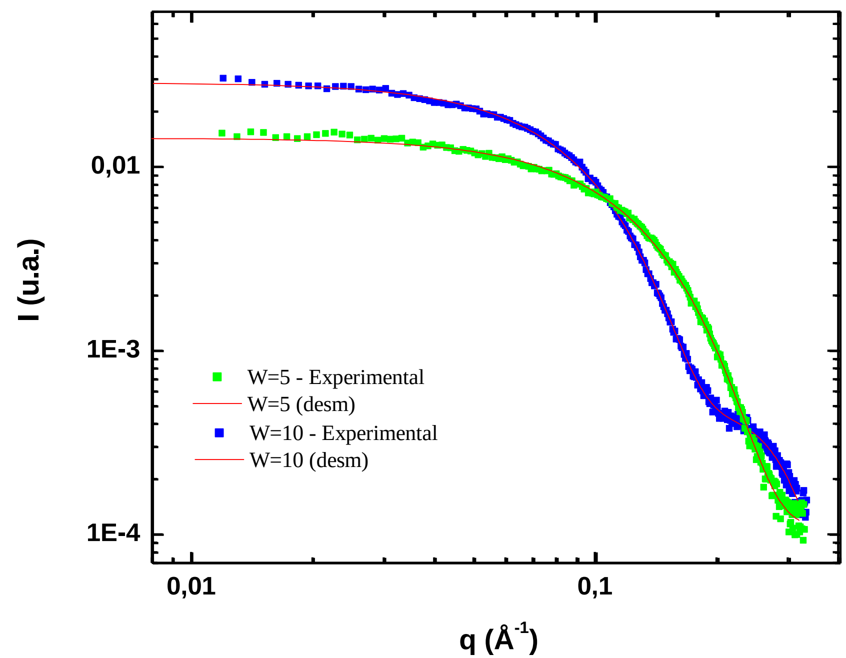
water

n-hexano



W = [water]:[AOT]


W=5 (Rg=14.8)
W=10 (Rg=22.9)
P (r)
0.00008
0.00006
0.00004
0.00002
0.00000
0
10
20
30
40
50
60
r (Å)


I (u.a.)
0,01
1E-3
1E-4
W=5 - Experimental
W=5 (desm)
W=10 - Experimental
W=10 (desm)
0,01
0,1
q (Å-1)

Configuração de Nanopartículas Magnéticas em Solução Aquosa

Jerome Depeyrot, Francisco A. Tourinho (UnB), Evandro L. Duarte (IFUSP) e R. Itri (IFUSP)

MFe_2O_4 M = Mn, Co, Zn, Ni e Cu

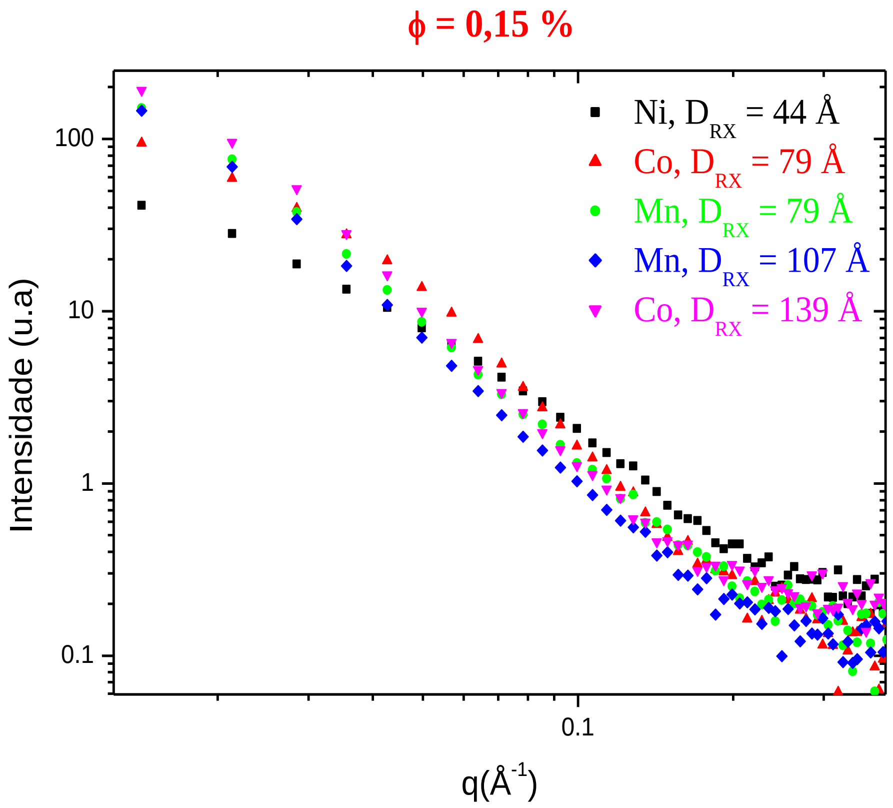
Partícula coloidal suspensa num líquido

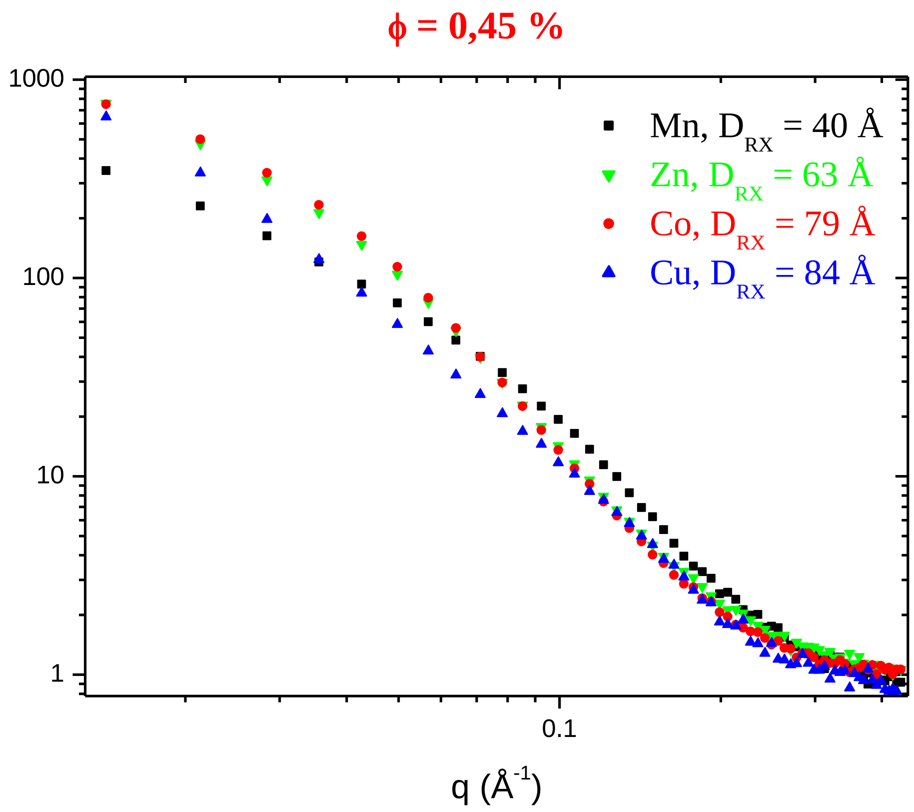
$$\boxed{D \leq \left(\frac{\Omega^2 k_B T}{\rho_s (\Delta\rho)^2 g^2} \right)^{1/3}}$$

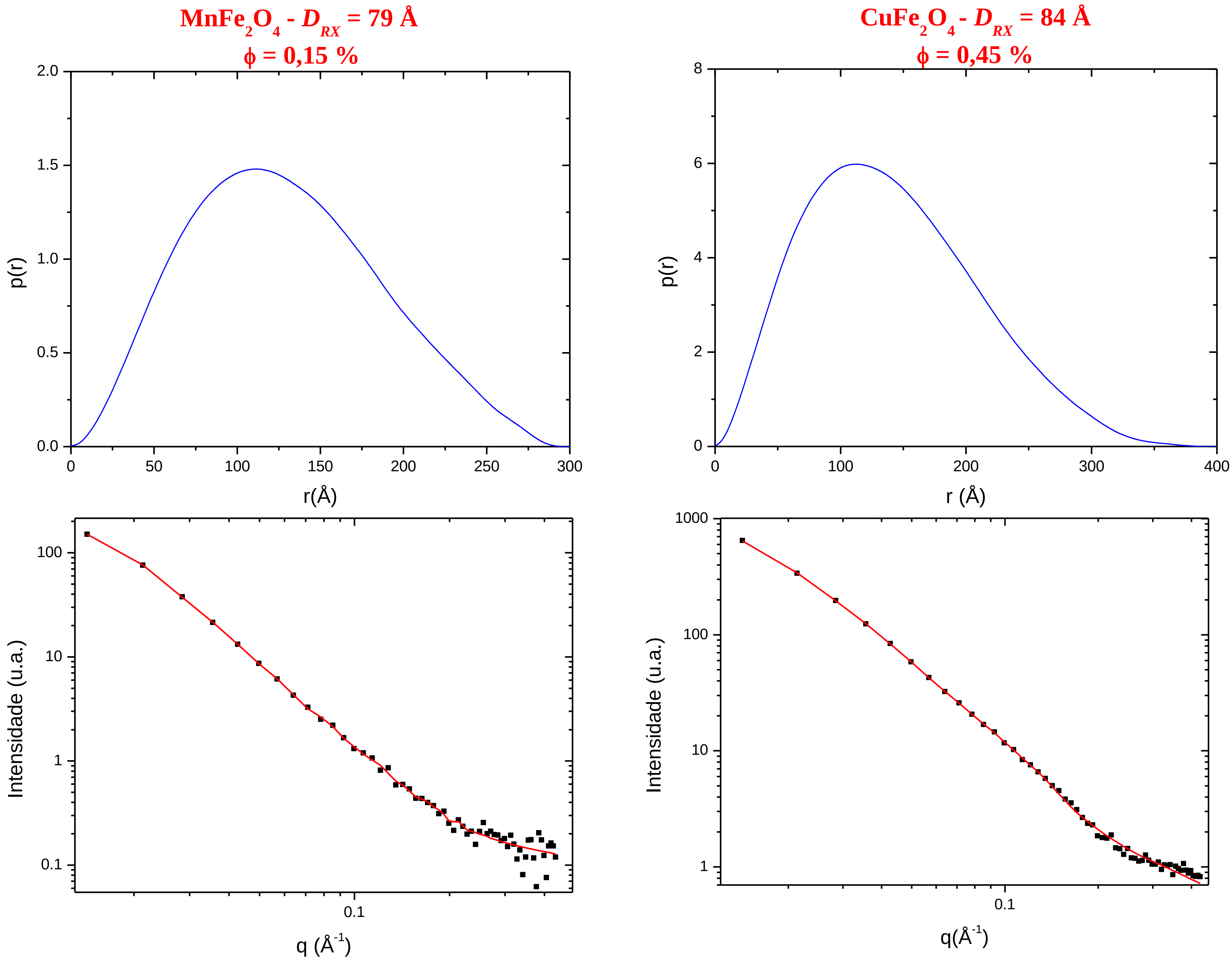
Nanopartícula magnética ⇒ interação dipolar magnética
concentração - forças de van der Walls ⟶ **precipitação**

Criar forças de repulsão ⇒ fluídos magnéticos surfactados em meio apolar
⇒ de dupla camada elétrica em meio aquoso

Resultados de *SAXS*: gerador de RX de anodo rotatório (LCr)

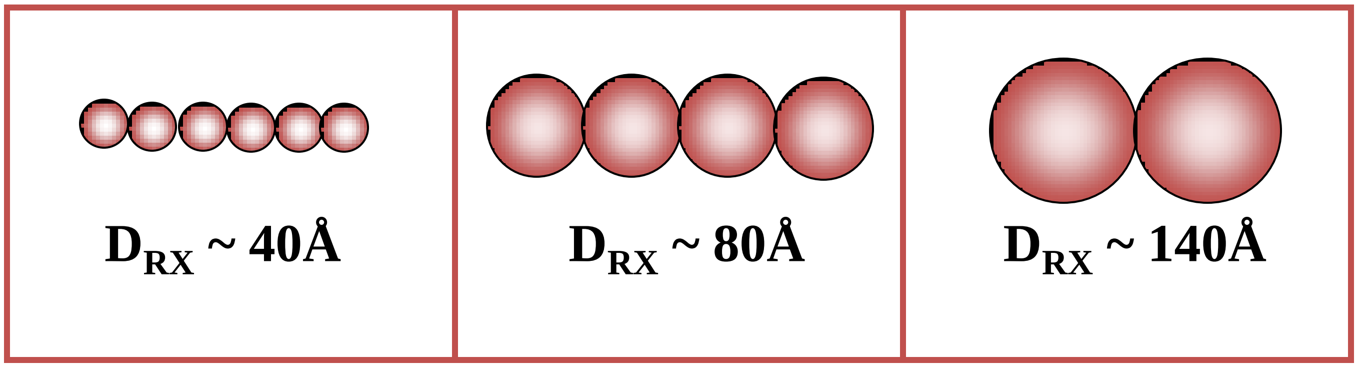





MnFe2O4 - DRX = 79 Å
ϕ = 0,15 %
CuFe2O4 - DRX = 84 Å
ϕ = 0,45 %
p(r)
r(Å)
r (Å)
Intensidade (u.a.)
q (Å-1)
q(Å-1)

MFe_2O_4	*par.rede*(Å)	D_{RX} (Å)	r_I (Å)	D_{max} (Å)	N_{na}
Mn	8,499	40[b]	30	250±10	6
		79[a]	78	280±10	3-4
		107[a]	90	330±10	3
Co	8,380	79[a]	50	280±10	3-4
		79[b]	50	260±20	3-4
		139[a]	80	330±10	2-3
Ni	8,199	44[a]	34	240±10	6
Cu	8,349	84[b]	77	330±10	4
Zn	8,441	63[b]	50	240±10	4

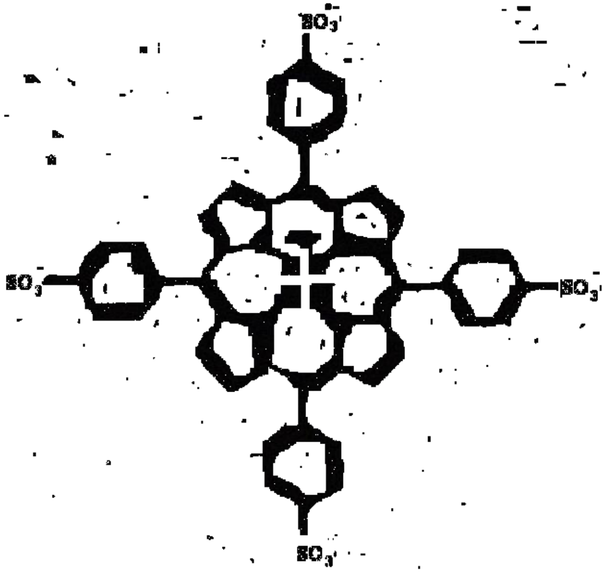
[a] fração volumétrica da nanopartícula magnética de 0,15 %; [b] 0,45 %.


$D_{RX} \sim 40Å$
$D_{RX} \sim 80Å$
$D_{RX} \sim 140Å$

Meso-tetrakis(4-sulfonatophenyl) porphyrin ($TPPS_4$) in aqueous solution.

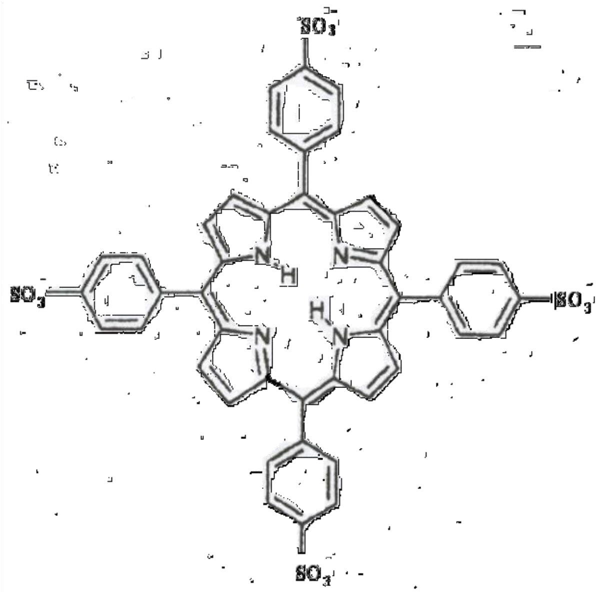
Shirley Gandini, Marcel Tabak (IQSC-USP) and R. Itri

Biophys. J. (2003)



pH 4.0

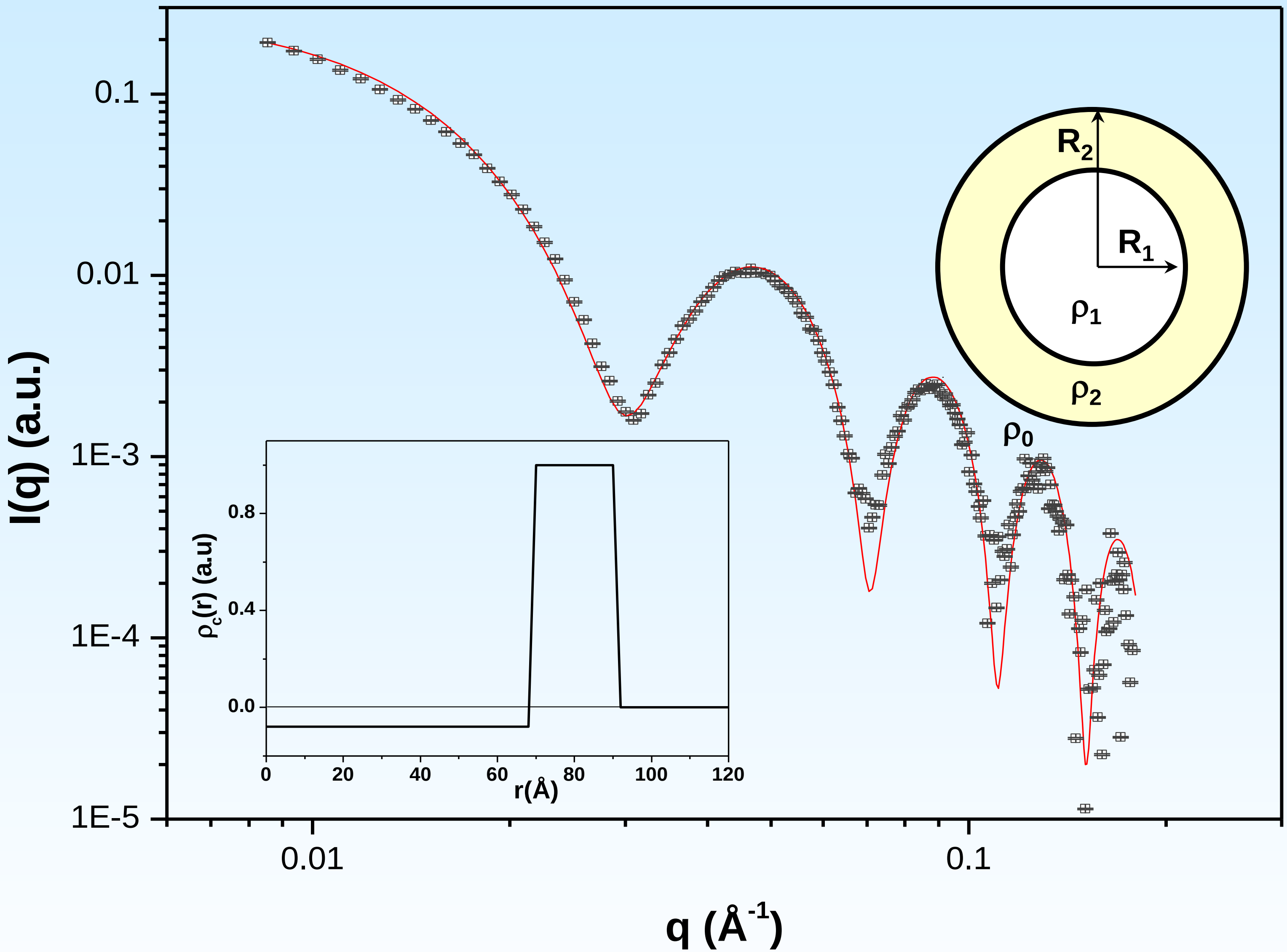
H_4 $TPPS_4$ $^{2-}$

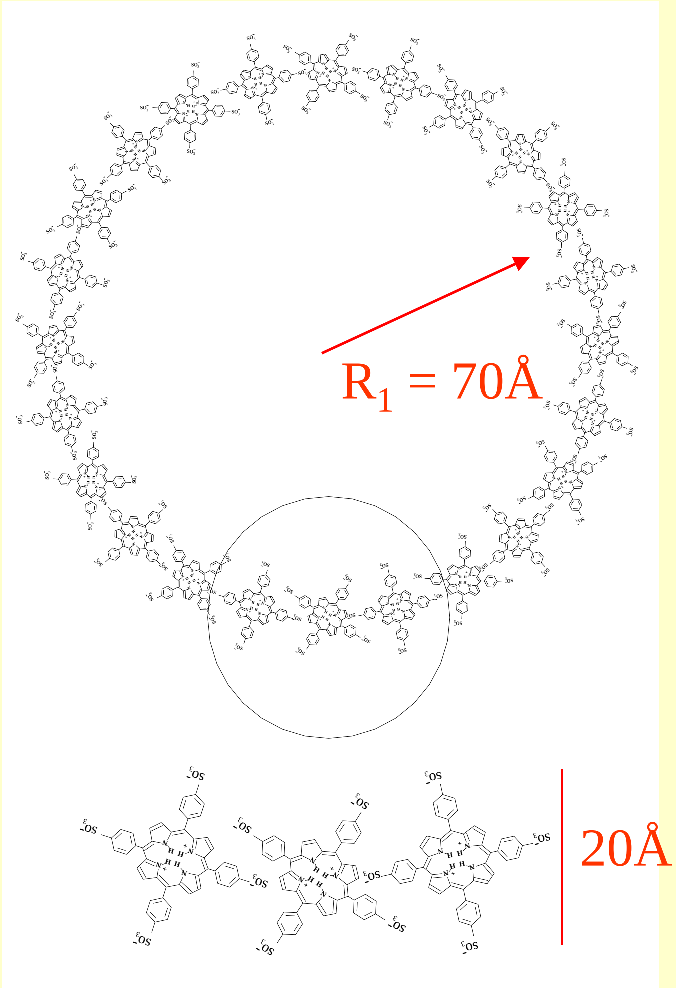


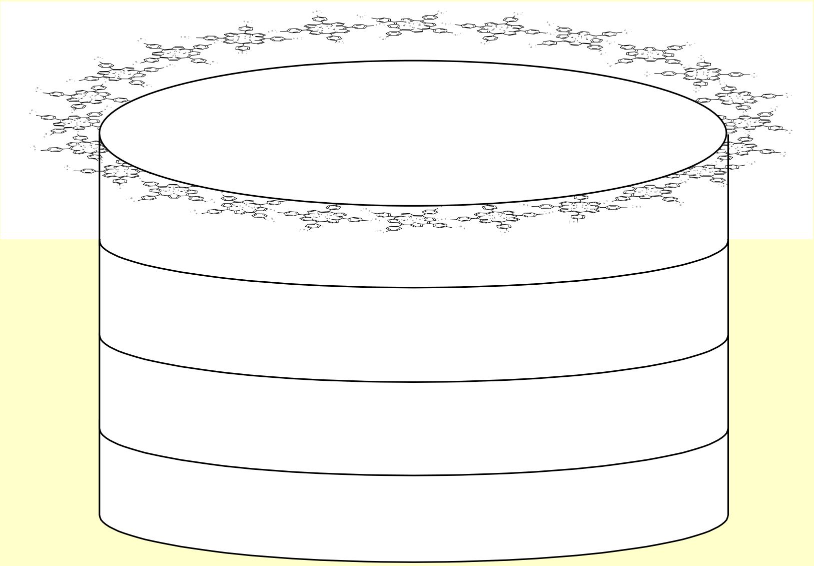
pH 9.0

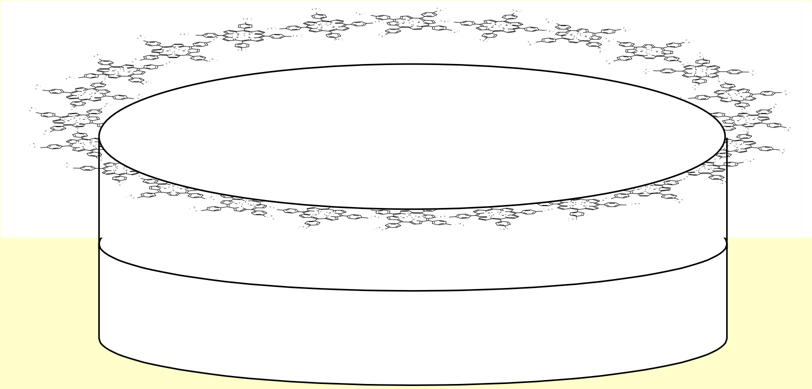
H_2 $TPPS_4$ $^{4-}$

TPPS$_4$ 10 mM at pH 4.0









S.C. Gandini, R. Itri e M. Tabak, *Biophys. J., 85, 1 (2003)*

Auto-associação de peptídeos: o Caso do GHRP-6

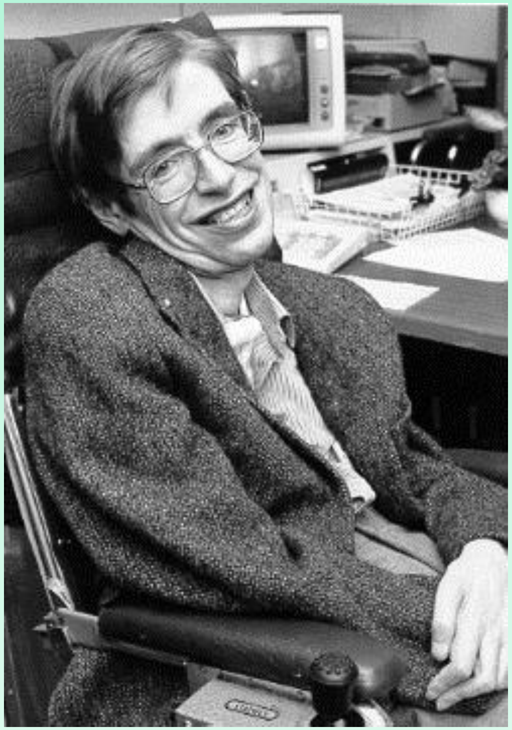
Leandro R. S. Barbosa, César Avila(Un. Tucuman), Hector Santana Millan, Rolando Paez (Centro de Biotecnologia Cuba), Rosangela Itri

Epidermal growth factor (EGF) and **Growth Hormone Releasing Peptide-6** (GHRP-6) have exhibited a variety of physiological and pharmacological properties in some diseases.

GHRP-6 induces the insulin growth factor-1 (IGF-1) hormone expression in the central nervous system [1,2]. The IGF-1 is a well known important neurotrophic factor in both **ALS (Amyotrophic lateral sclerosis)** and **ALS's** animal models. (induce the survival, development, and function of neurons).

EGF and **GHRP-6** have been considered as good candidates for the treatment of **ALS**, due to their well documented effects in **brain cell** survival mechanisms [3].

Amyotrophic lateral sclerosis: (ALS) is a disease of the central nervous system characterized by irreversible loss of spinal motor neurones, for which **no effective treatment exists**, and where patient evolves quickly to death in a few years [3,4].

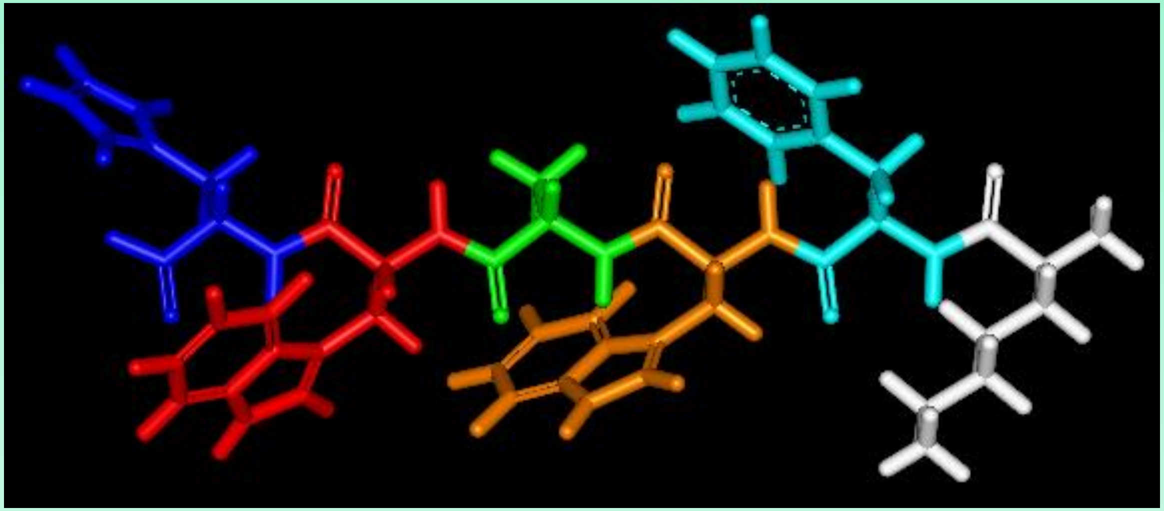


1. L. M. Frago et al. **Endocrinology (2002) 143(10):4113–4122**
2. L. M. Frago et al**. J Neuroendocrinol (2005) 17(11):701–710**
3. Diana Garcıa del Barco, et al. **Neurotox Res (2011) 19:195–209**.
4. T. Niidome et al. **Eur J Pharmacol (2006) 548(13):1–8.**
5. J.P. Crow et al. **Ann Neurol (2005) 58(2):258–265.**
6. E. Beghi et al. **Curr Med Chem (2007) 14(30): 3185–3200.**

So, what is GHRP-6 ?

GHRP-6 is a synthetic hexapeptide:

(His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2)



Extended Chain Length ~ 20 Å

***GHRP-6 has* Self-Assemble Properties;**

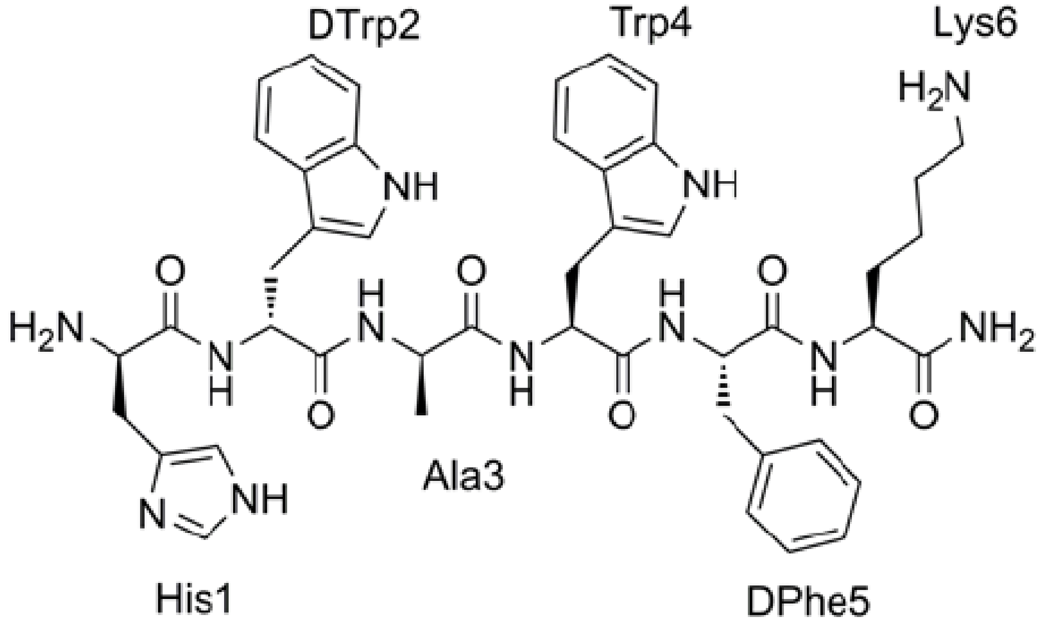
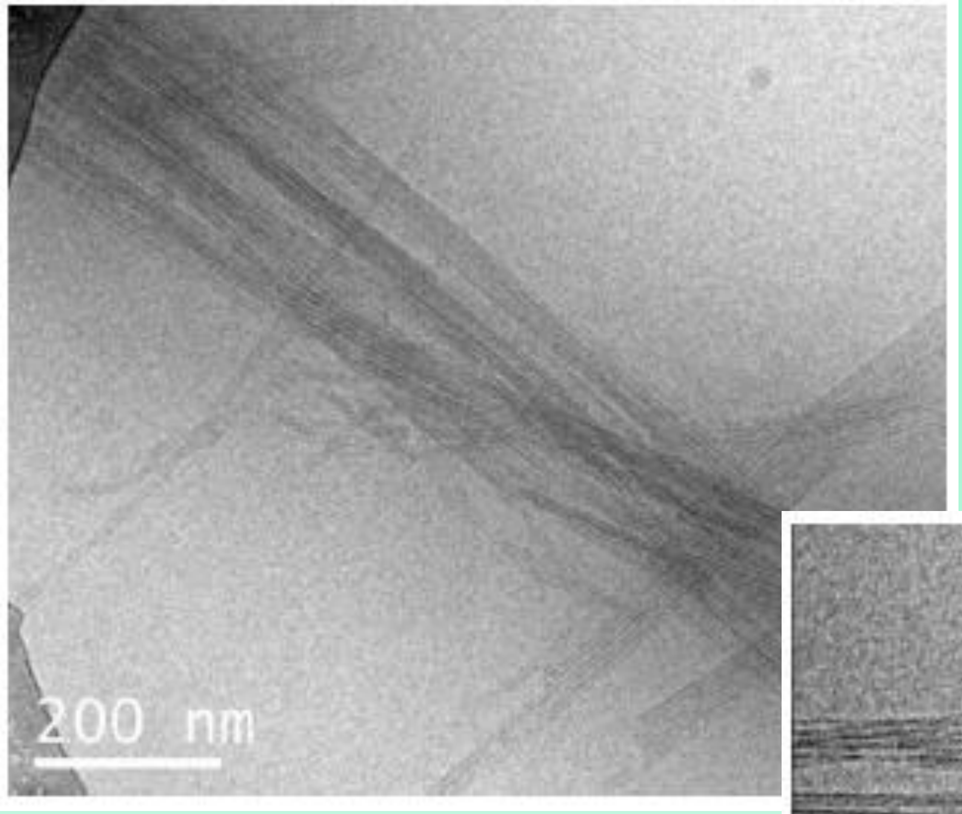
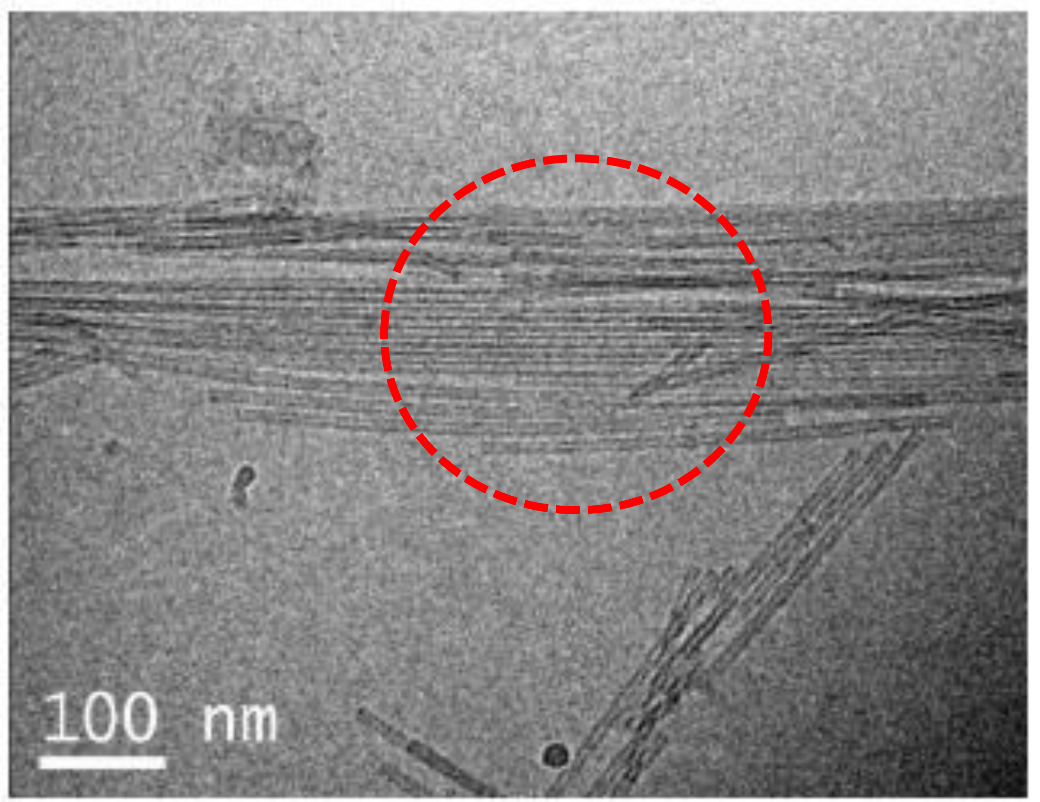


Figure 1



Electronic Microscopy shows the formation of organized Fibers.

The importance of self-assembling of small peptides as : drug-delivery, nanowires,...

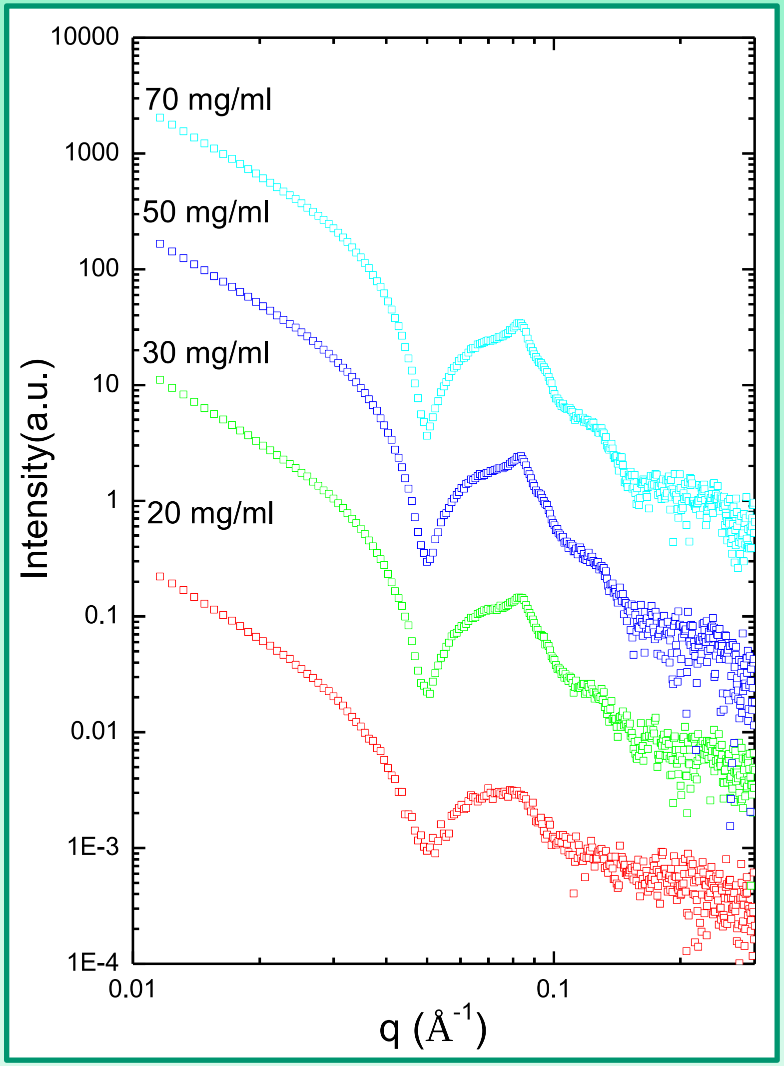


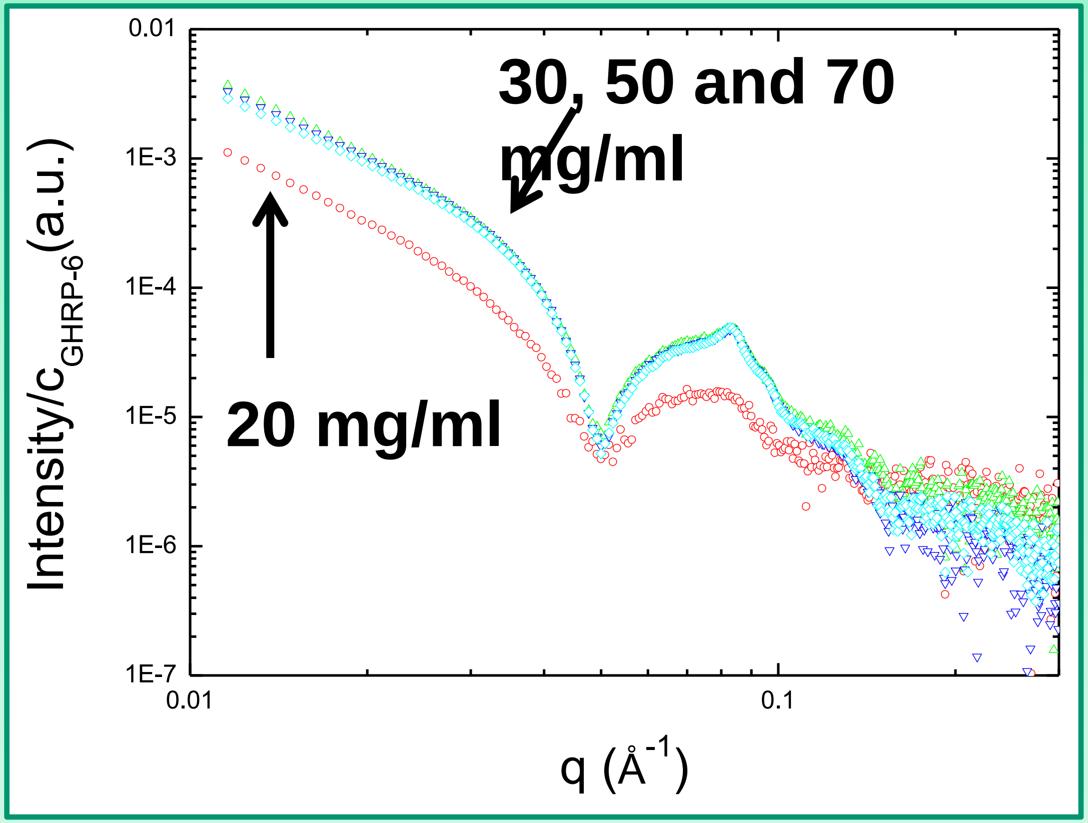
Time and buffer-dependent

And how about SAXS measurements?

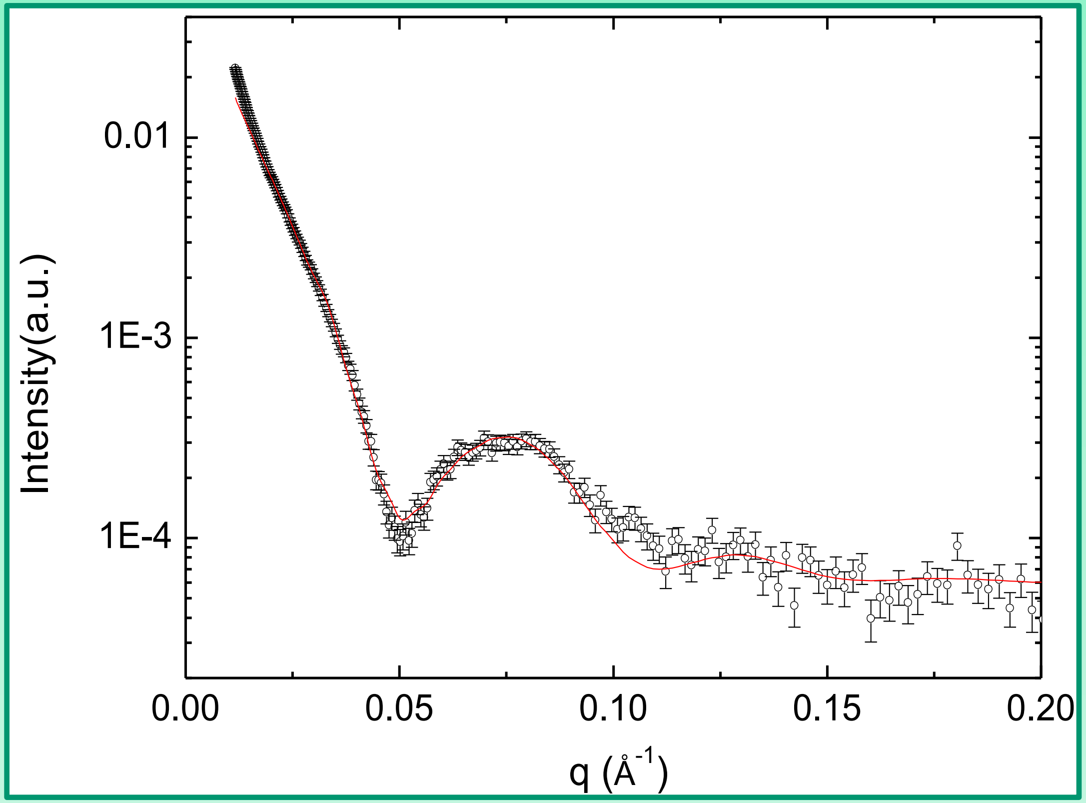
GHRP-6 at 20, 30, 50 and 70 mg/ml

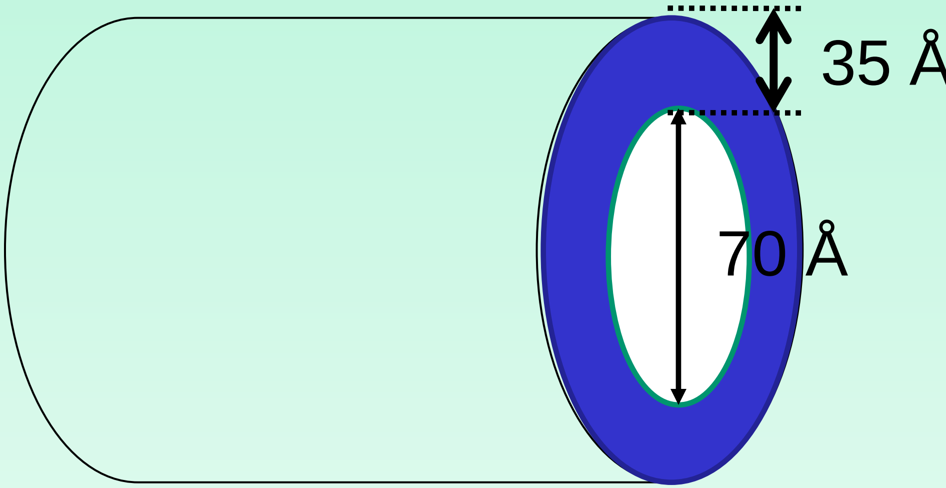
GHRP-6


10000
1000
100
10
1
0.1
0.01
1E-3
1E-4
70 mg/ml
50 mg/ml
30 mg/ml
20 mg/ml
Intensity(a.u.)
0.01
0.1
q (Å-1)


0.01
1E-3
1E-4
1E-5
1E-6
1E-7
30, 50 and 70 mg/ml
20 mg/ml
Intensity/cGHRP-6(a.u.)
0.01
0.1
q (Å-1)

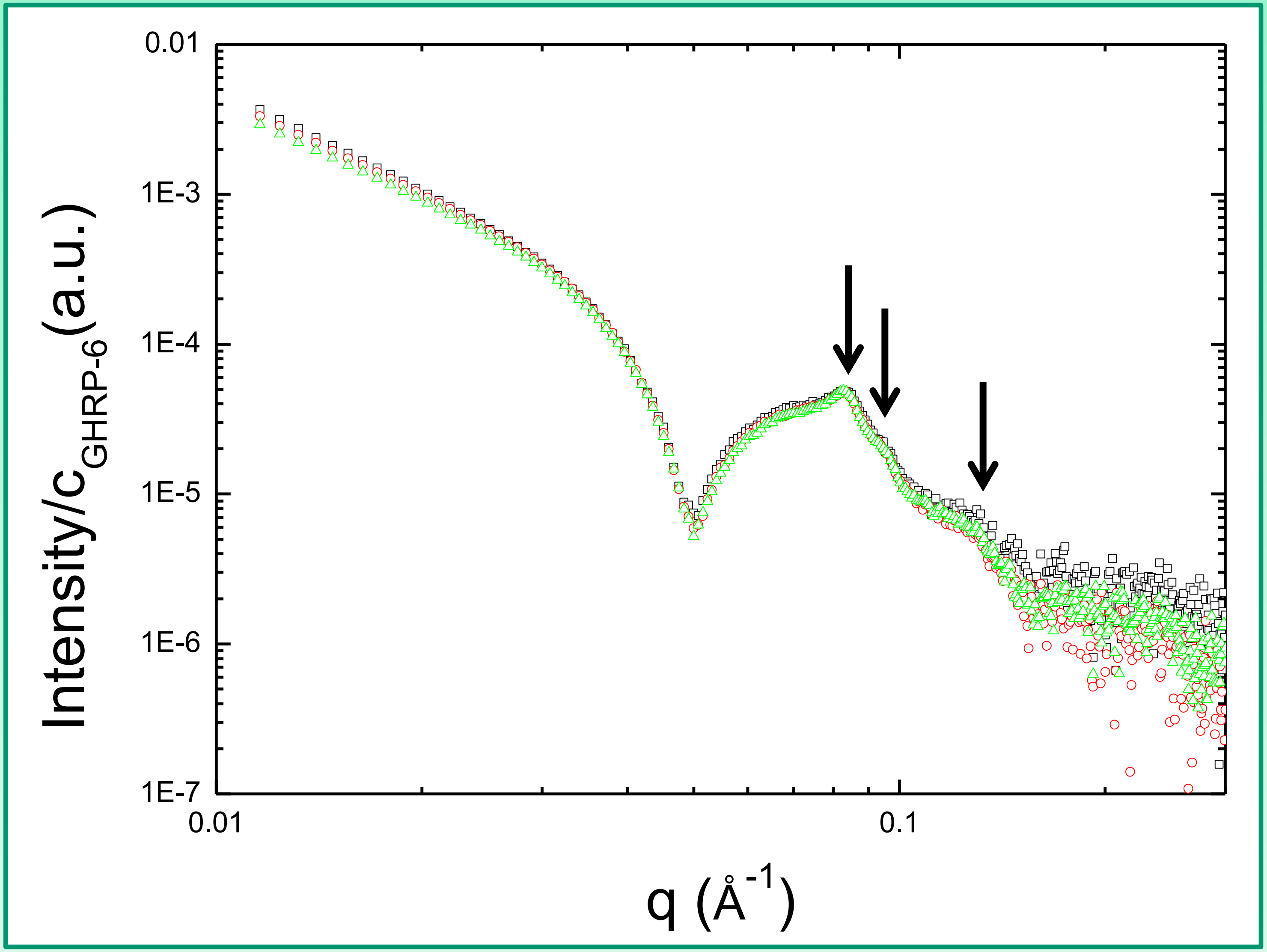
GHRP-6 - 20 mg/ml





Hollow Cylinder;
"Infinite length" (> 600 Å)
And at higher concentrations?

GHRP-6 - 30, 50 and 70 mg/ml

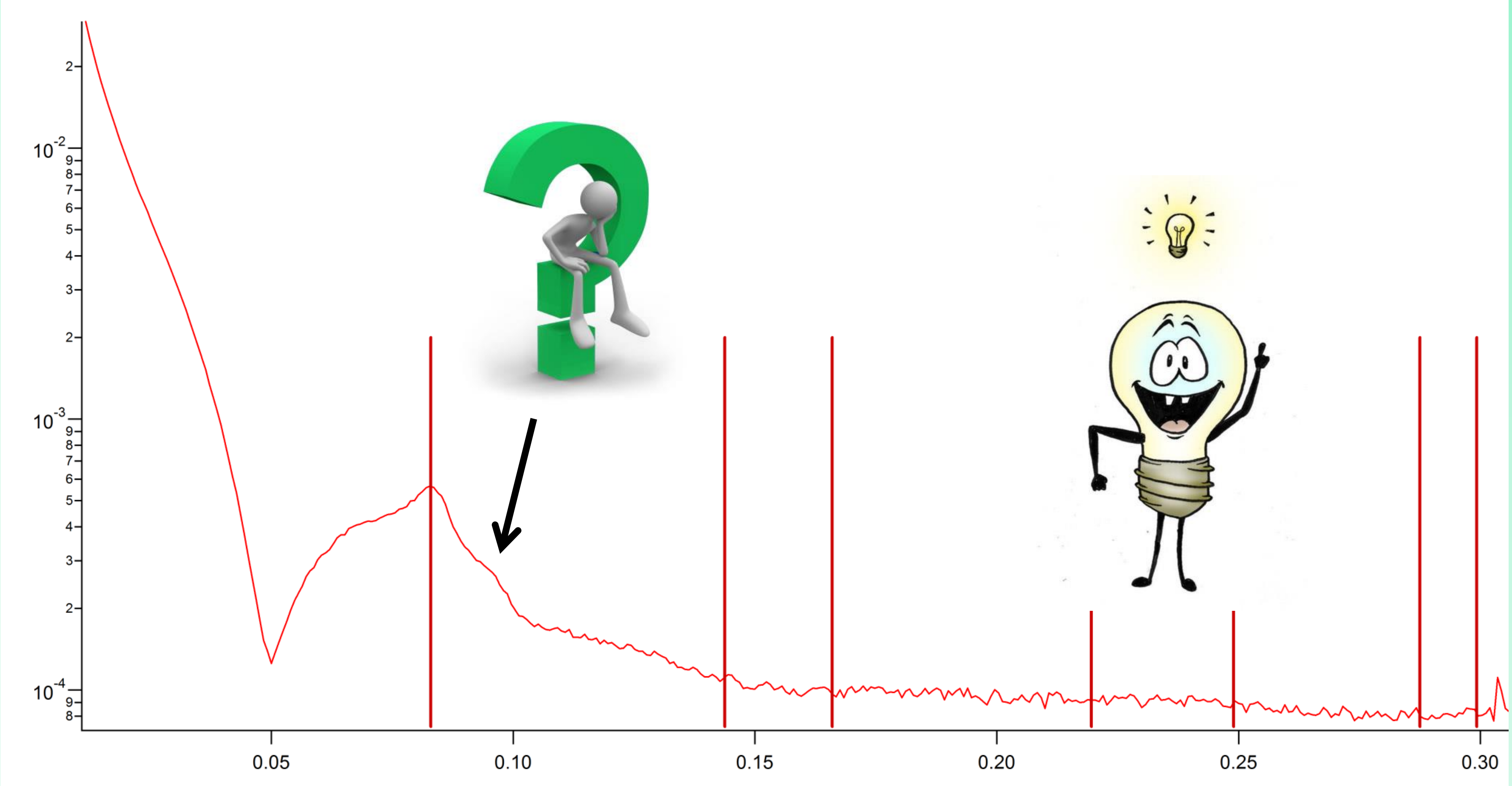


GHRP-6 - 30, 50 and 70 mg/ml

Lammelar Staking?

GHRP-6 - 30, 50 and 70 mg/ml

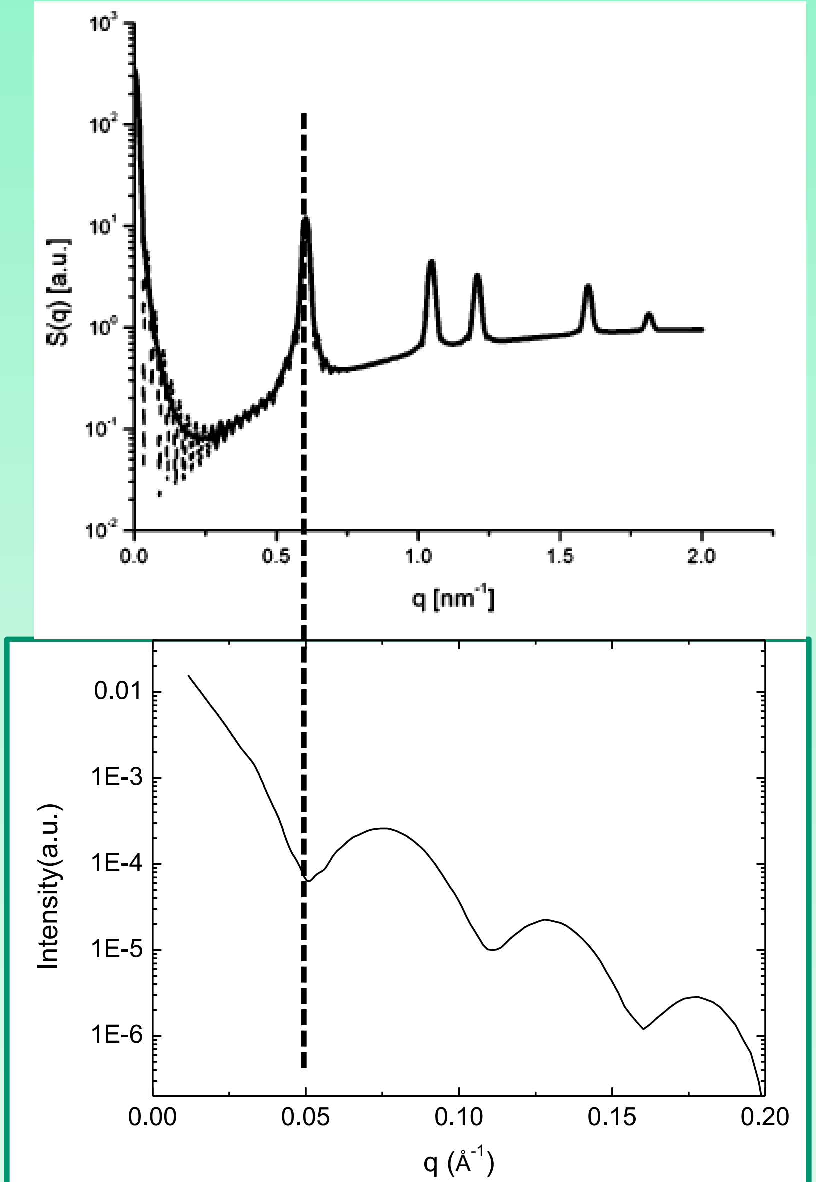
Hexagonal Staking?



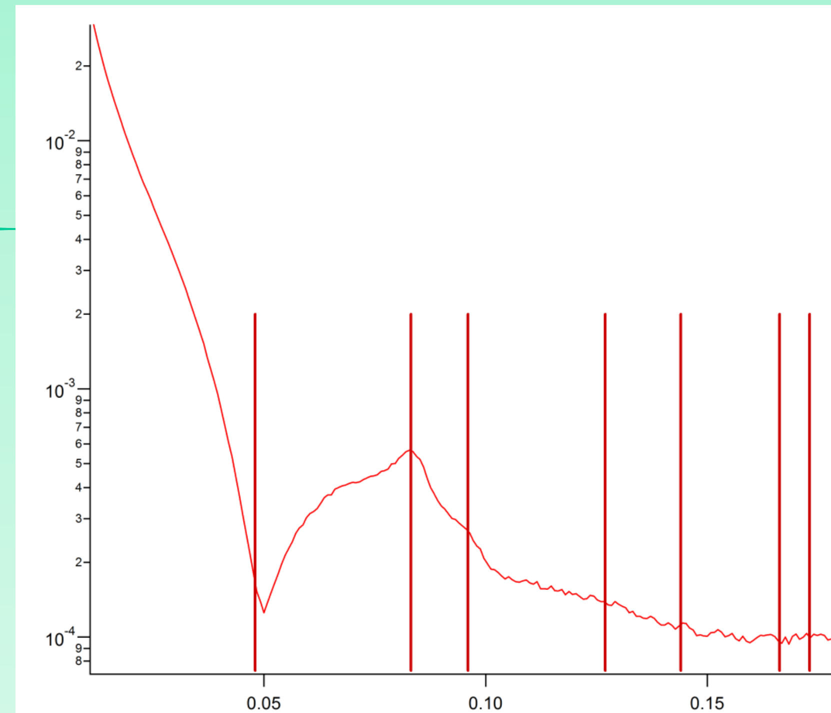
a = 87 Å

Smaller then the Cylinder diameter

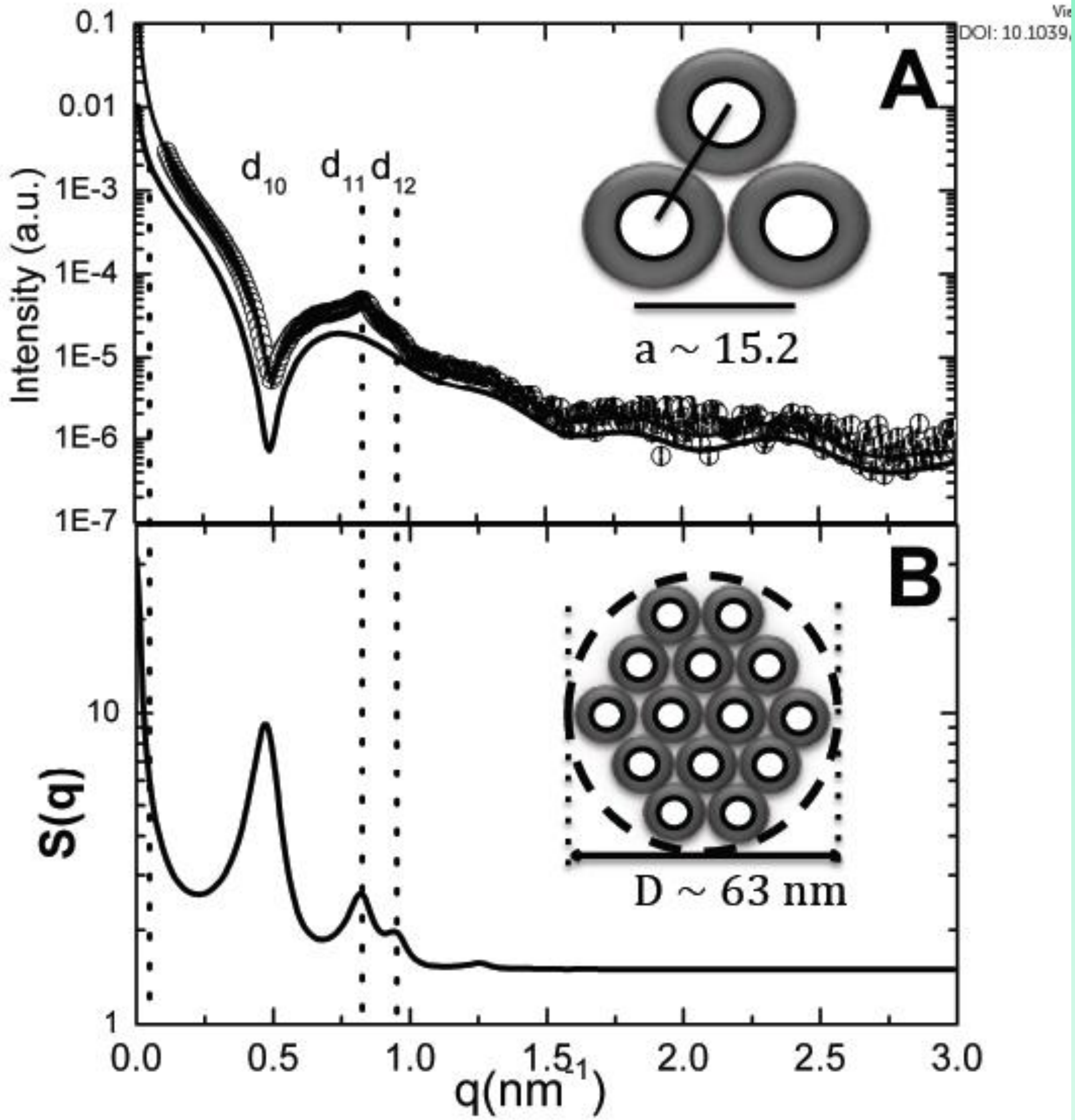
From the SAXS point of view...



The maximum of the S(q) is at the same position as the Minimum of the P(q)! No “evident” first Peak!!



N. Freiberger and O. Glatter* - *J. Phys. Chem. B* **2006,** *110,* 14719-14727



Vie
DOI: 10.1039/
A
B
d10
d11
d12
a ~ 15.2
D ~ 63 nm
Intensity (a.u.)
S(q)
q(nm-1)

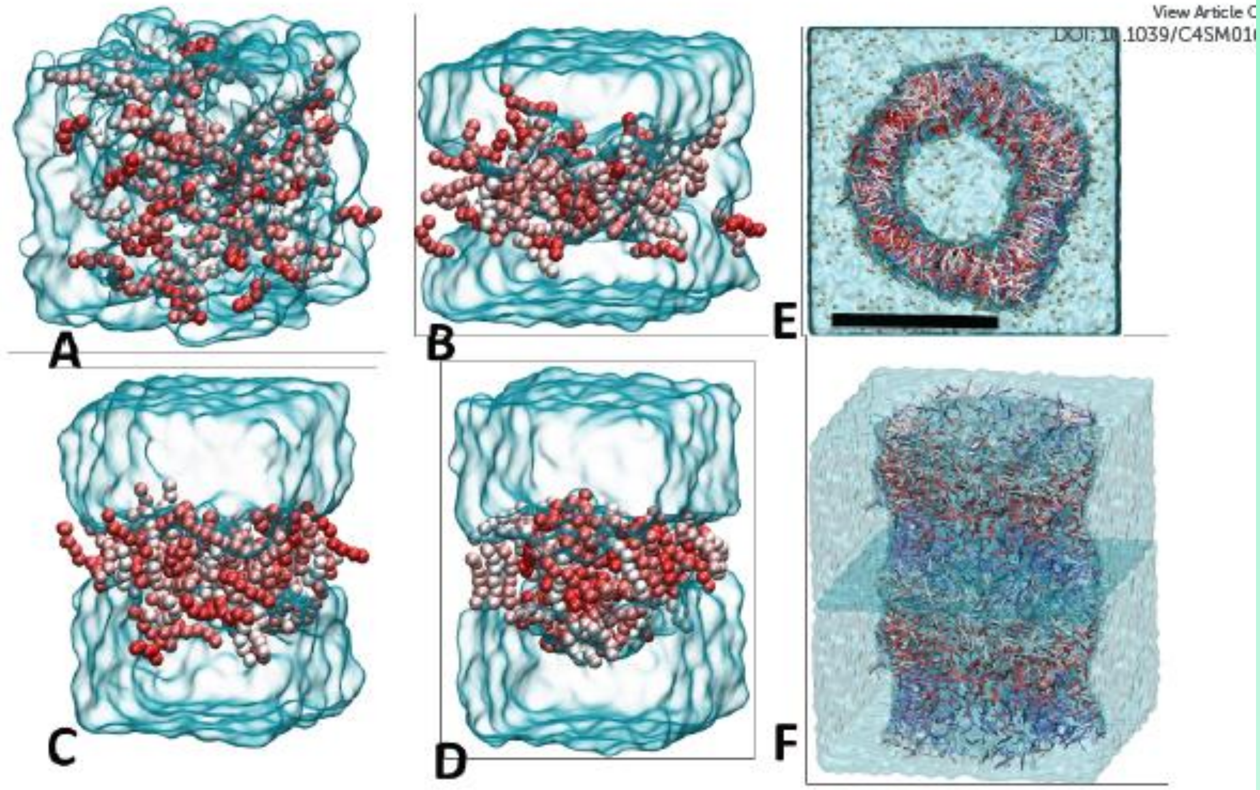


Figure 5 – Snapshots of the spontaneous aggregation of GHRP-6 peptide in a simulation box at (A) 0 ns, (B) 150ns, (C) 300ns and (D) 500ns. (E) Backbone representation of the peptide in a cylindrical aggregate after 20 μs of simulation. (F) Cross-section of the cylinder after 20 μs of simulation. The bar on 5E has 10 nm.

Coarse-grained – Martini force field

Table I. Comparison of external and inner cross-sections of GHRP-6 self-assembled aggregate, as well as its thickness, obtained using Cryo-TEM, TEM, SAXS and MDS (molecular dynamic simulation) techniques with the associated standard deviation.

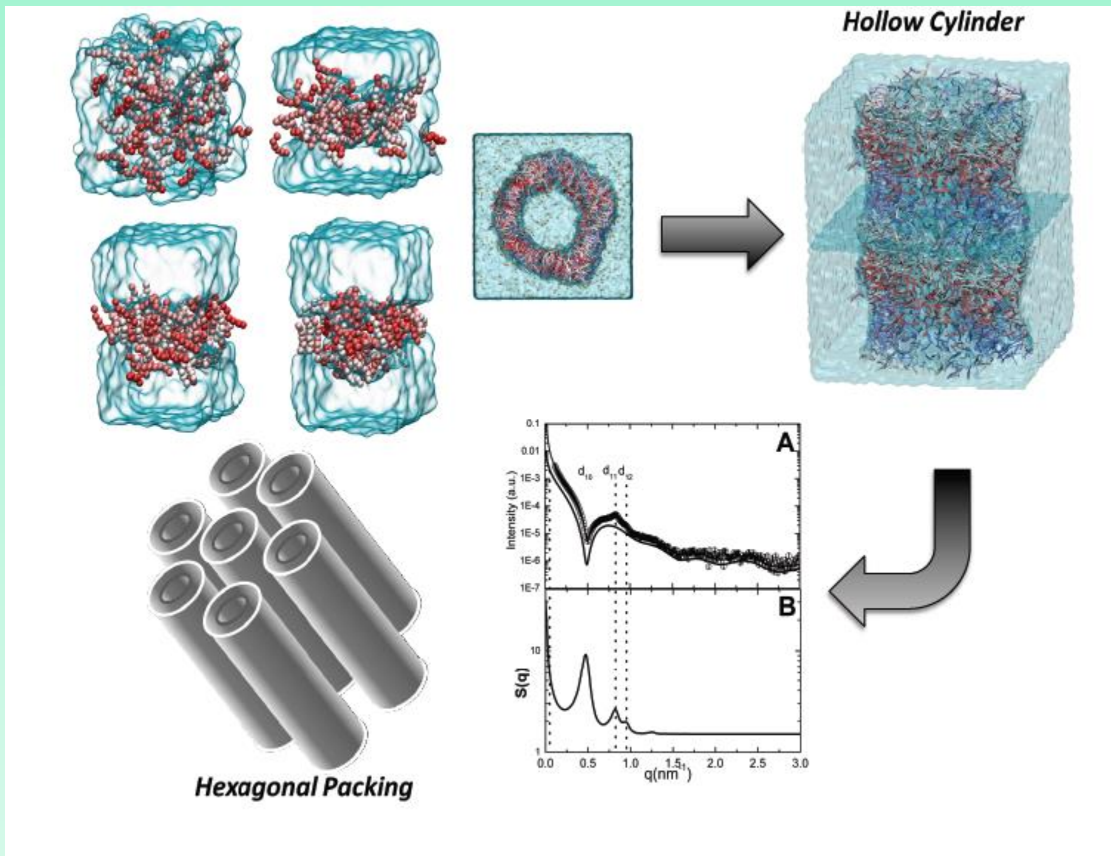
	Cryo-TEM	TEM	SAXS	MDS
External cross-section (nm)	11.9 (8)	14.2 (6)	13.4 (5)	15.0
Inner cross-section (nm)	5.8 (7)	7.0 (7)	7.4 (2)	9.0
Thickness (nm)	3.1 (4)	3.6 (3)	3.0 (2)	3.0

Legend: The values in parenthesis represent the standard deviation

Conclusions

- GHRP-6 self-assembles into long hollow cylinders - ***Nanorods***
- From 30 up to 70 mg/ml such cylinders are closed packed into a hexagonal arrangement.

Soft Matter 2014



Interacting, ordered particles

Interacting, not homogeneous particles

Oriented, homogeneous particles

Homogeneous, not interacting particles

Interacting, homogeneous particles

Theory: The Master Equation

$$I(q)=n_p\left\{\langle F^2(q)\rangle+4\pi n_p\langle F(q)\rangle^2\int_0^\infty(g(r)-1)r^2\frac{sen(qr)}{qr}dr\right\}$$

$$=n_p\langle F^2(q)\rangle\bar{S}(q)$$

q = scattering vector **=** $4\pi \sin\theta/\lambda$;

n_p particle number density

F(q) amplitude of form factor - Volume and Electron Density Contrast

P(q) = *F²(q)* = form factor

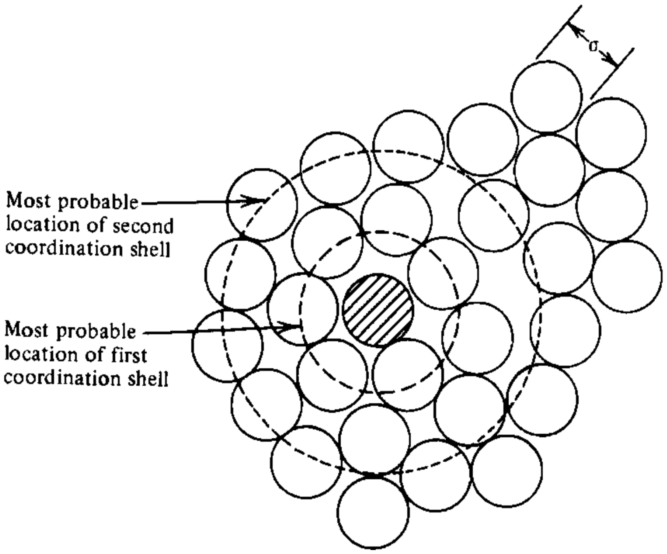
S(q) correlation function (Structure Factor) related to g(r)

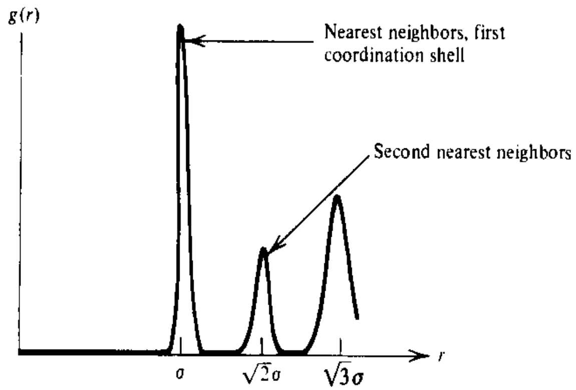
g(r) pair correlation function (or radial correlation function) related to a Interaction Potential U(r))

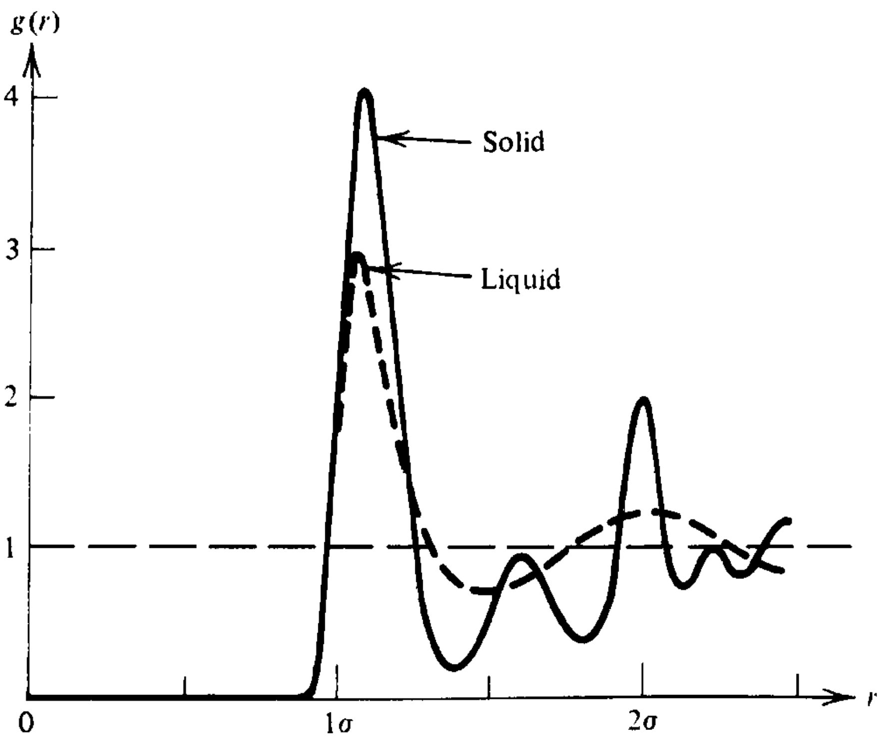
Structure Factor (spherical symmetry)

Describe the interaction between the nearest particles

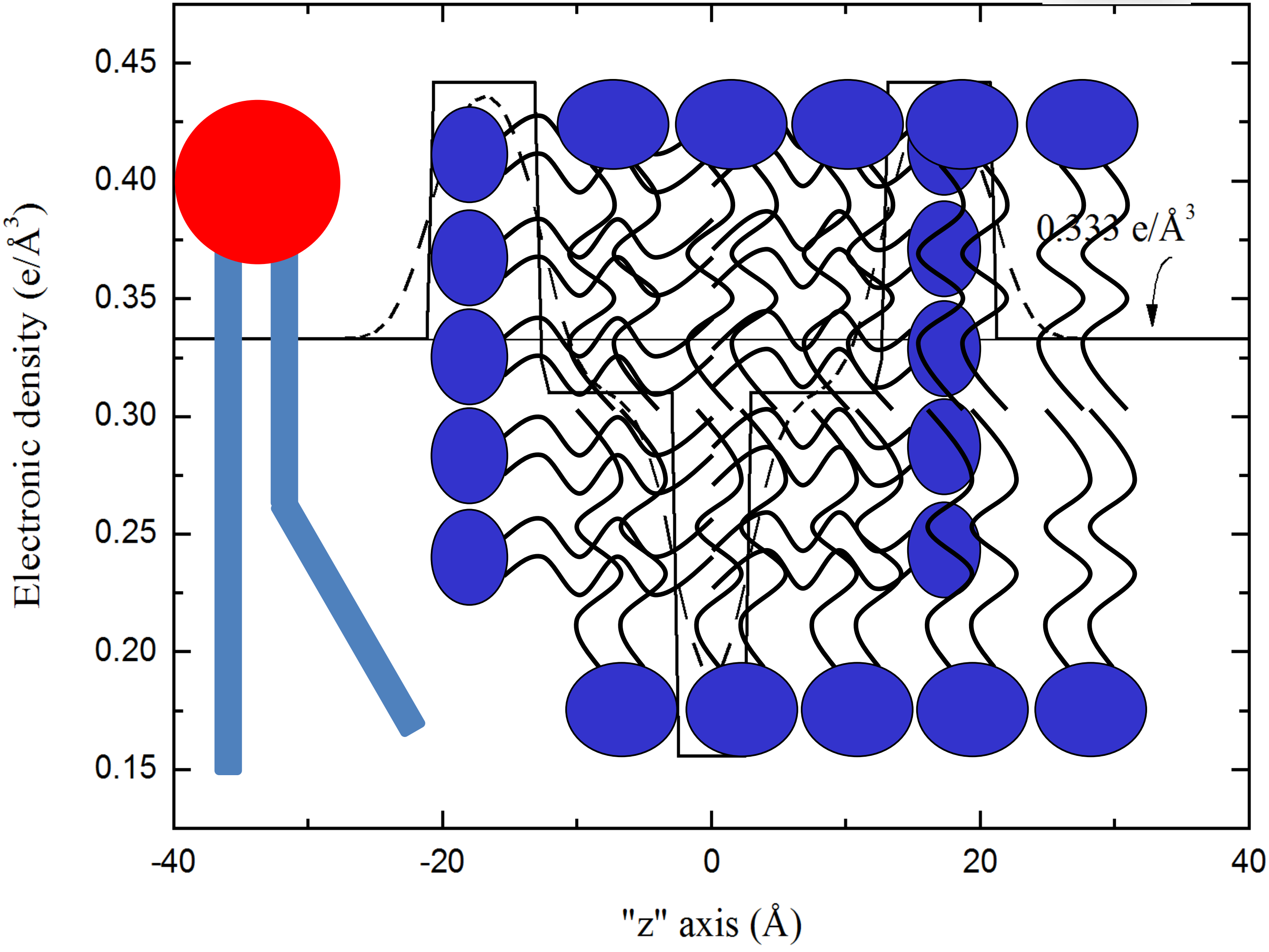
$$S(q)=\left\{1+4\pi n_p\frac{\langle F(q)\rangle^2}{\langle F^2(q)\rangle}\int_0^\infty (g(r)-1)\,r^2\frac{sen(qr)}{qr}\,dr\right\}$$



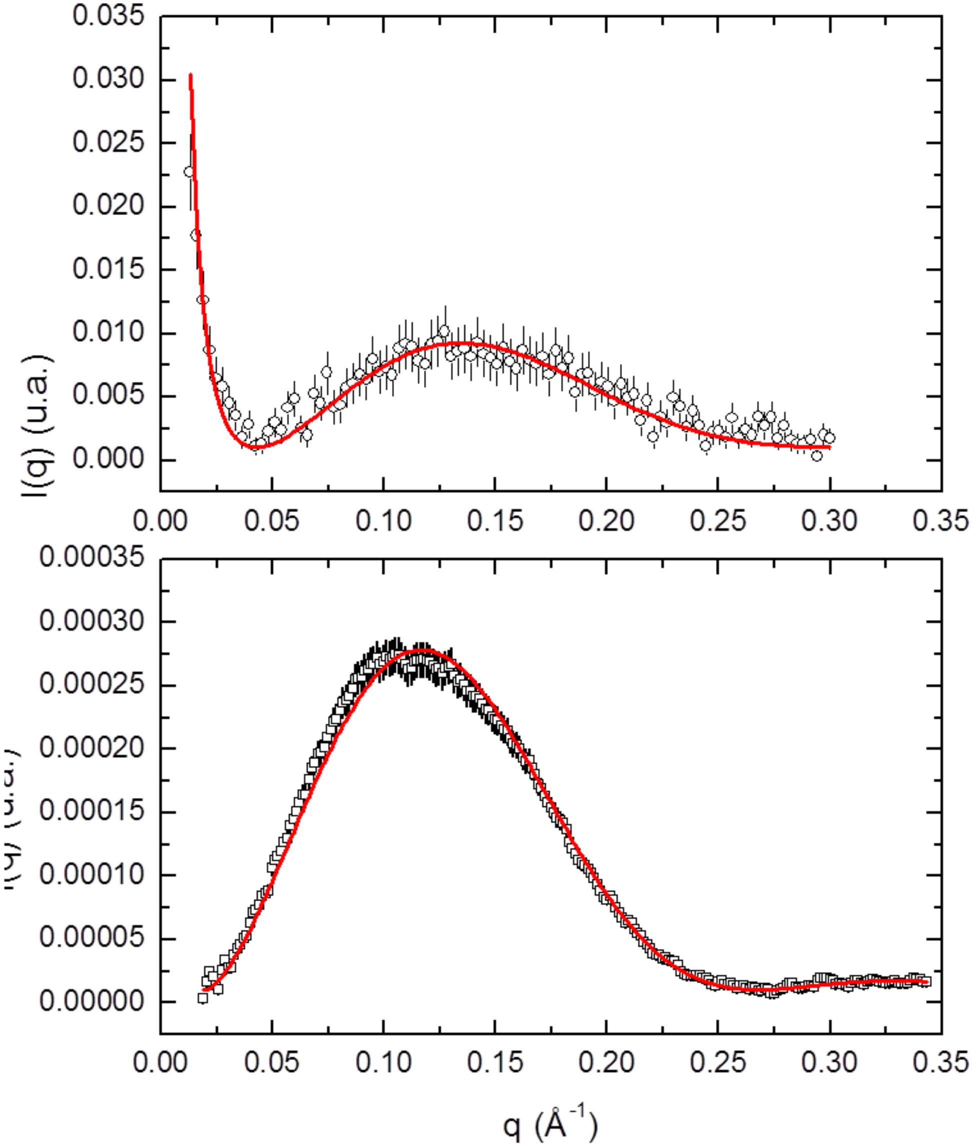




In the case of lipid bilayer...

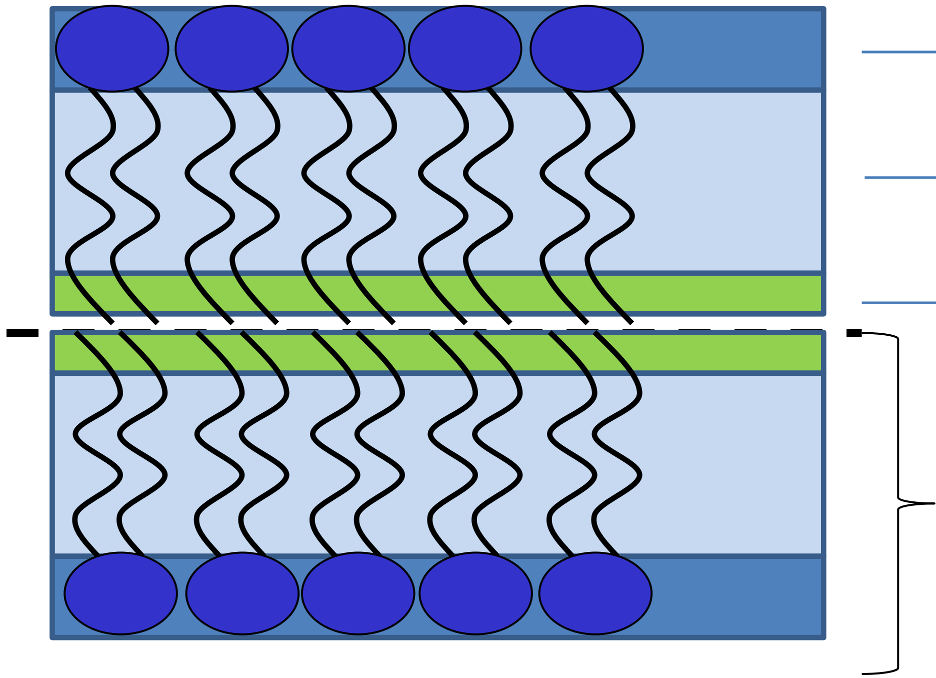


How about SAXS???



LUVs

SAXS adjustments parameters.....



R_{pol} (Å), $\boldsymbol{\sigma}_{pol}$ (e/Å^3)

R_{CH2} (Å), $\boldsymbol{\sigma}_{CH2}$ (e/Å^3)

R_{CH3} (Å), $\boldsymbol{\sigma}_{CH3}$ (e/Å^3)

Thickness $= 2(R_{pol} + R_{CH2} + R_{CH3})$

$$P(q) = \frac{2\pi A}{q^2} P_t(q)$$

Infinity Plane

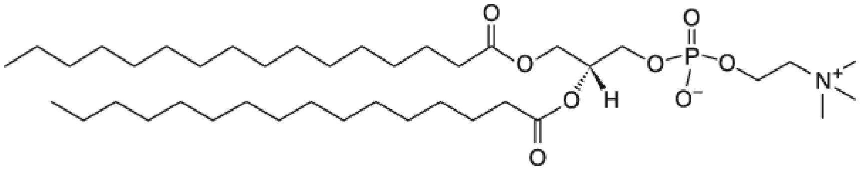
$$F(q)=\int_{-d/2}^{d/2}\rho(z)\exp(-iqz)dz\ .$$

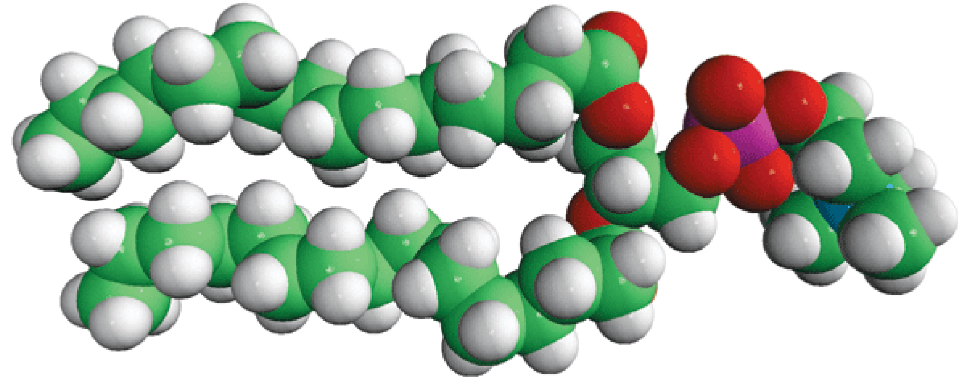
$$P(q)=\frac{2\pi A}{q^2}P_t(q)$$

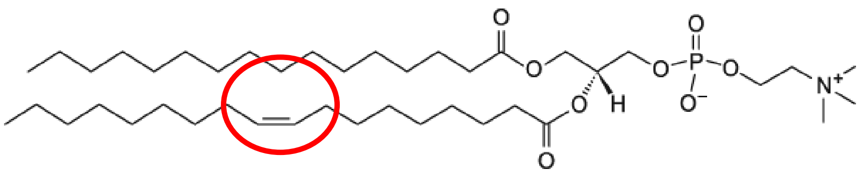
$$P_t(q)=\left\{\frac{2}{q}\left\{\Delta\rho_{CH_3}\sin qR_{CH_3}+\Delta\rho_{par}\left[\sin q\left(R_{par}+R_{CH_3}\right)-\sin qR_{CH_3}\right]+\right.\right.$$

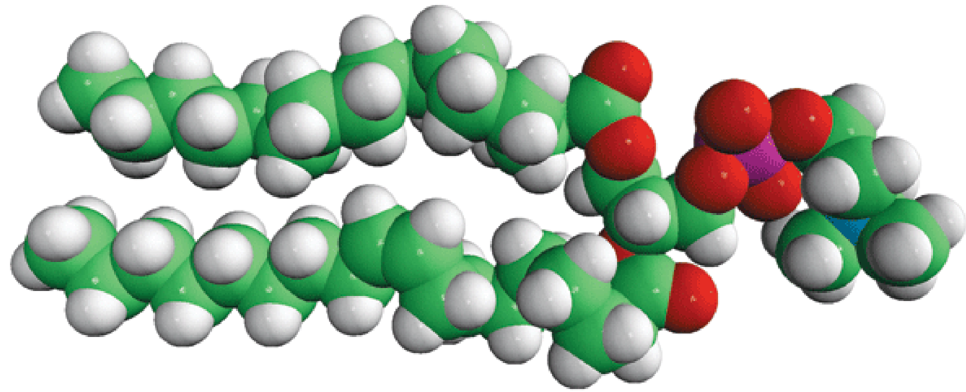
$$\left.\left.\Delta\rho_{pol}\left[\sin q\left(R_{pol}+R_{par}+R_{CH_3}\right)-\sin q\left(R_{par}+R_{CH_3}\right)\right]\right\}\right\}^2$$

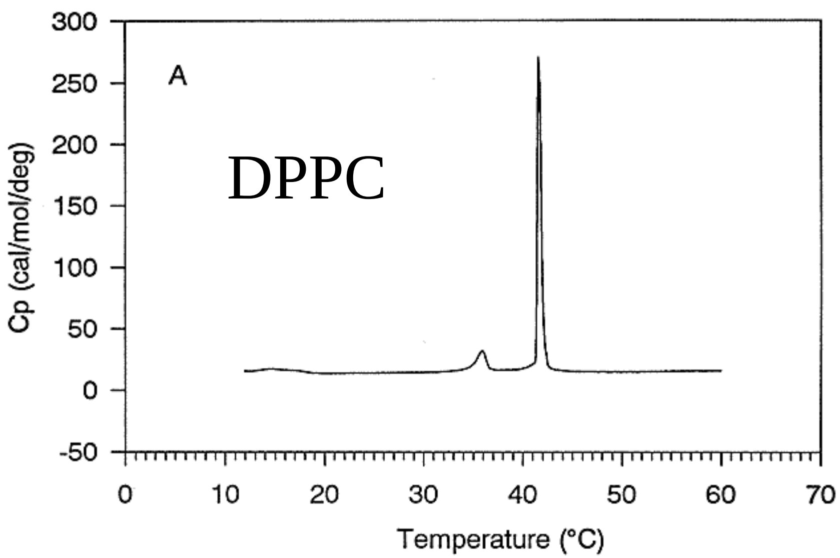
DPPC and POPC Structures

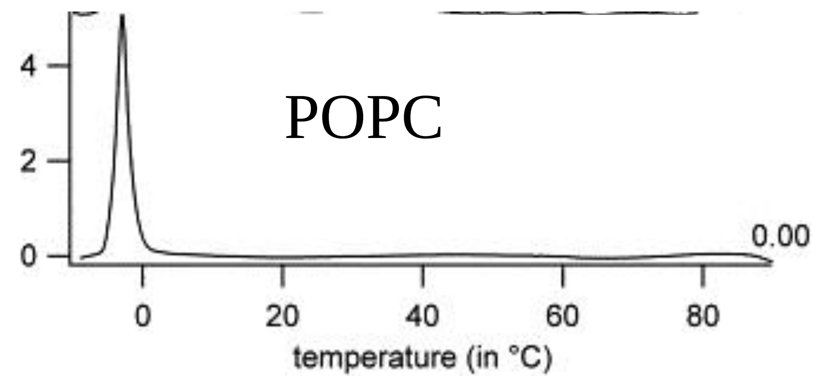


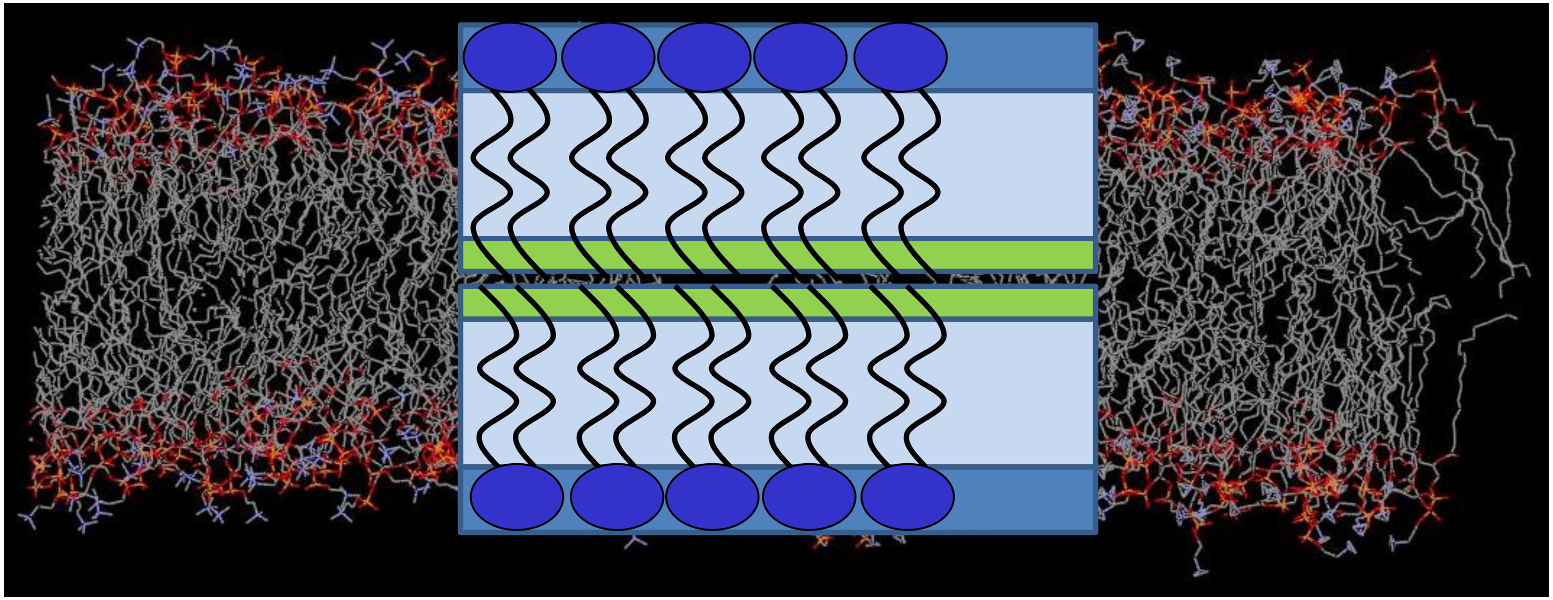






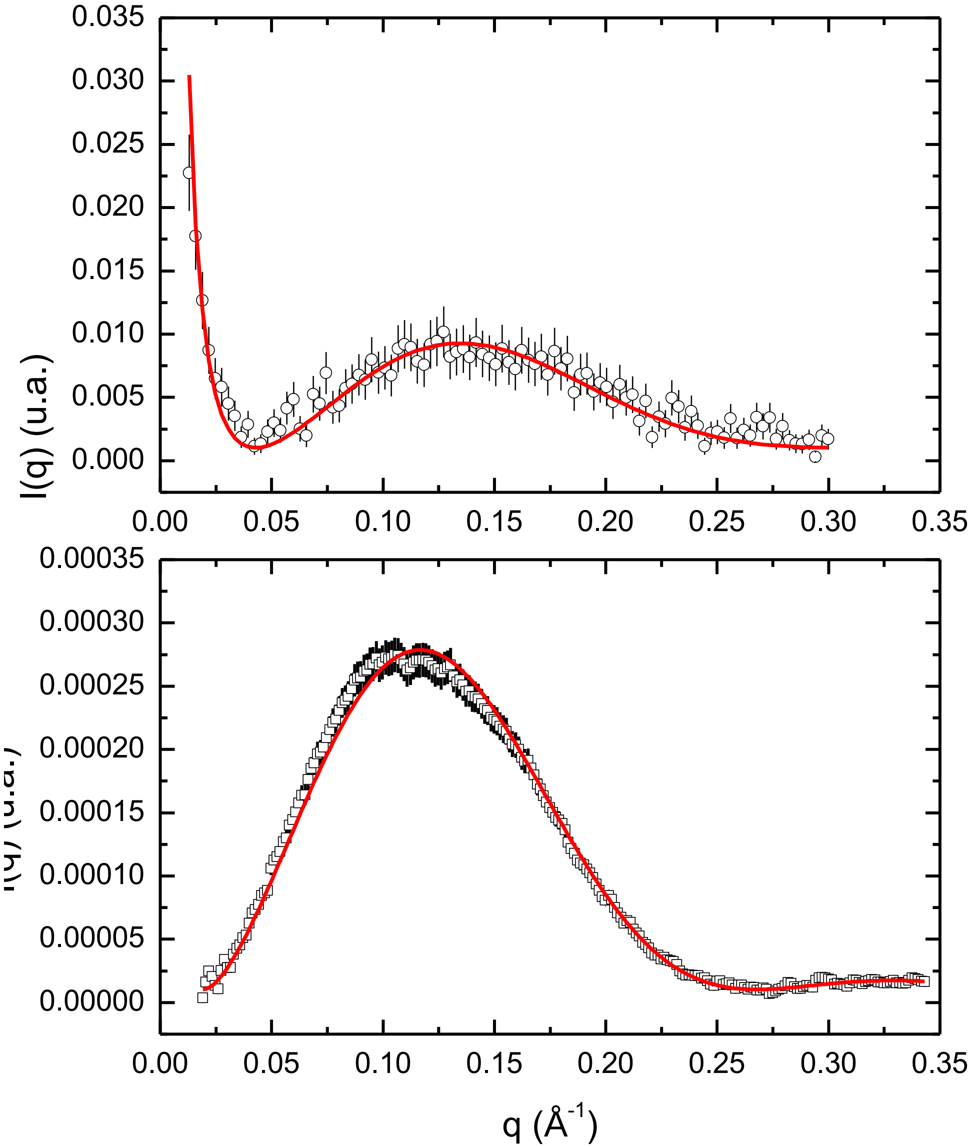






1) CHAPTER 1 - LIPID STRUCTURE, C: Dynamics of Membrane Lipids, BIOCHEMISTRY - DR. JAKUBOWSKI,
2) Fidorra, Heimburg,‡ and Bagatolli† Biophys J. 2009 July 8; 97(1): 142–154.
3) MEMBRANE PDBs. Dr. Peter Tieleman: http://moose.bio.ucalgary.ca/index.php?page=Structures_and_Topologies

And From the SAXS point of view?



R_{pol} (Å) = 8.4(6)

ρ_{pol} (e/Å^3) = 0.442(7)

R_{CH2} (Å) = 10(1)

ρ_{CH2} (e/Å^3) = 0.310(5)

R_{CH3} (Å) = 2.3(3)

ρ_{CH3} (e/Å^3) = 0.16(2)

DPPC

R_{pol} (Å) = 6.7(6)

ρ_{pol} (e/Å^3) = 0.444(7)

R_{CH2} (Å) = 12(1)

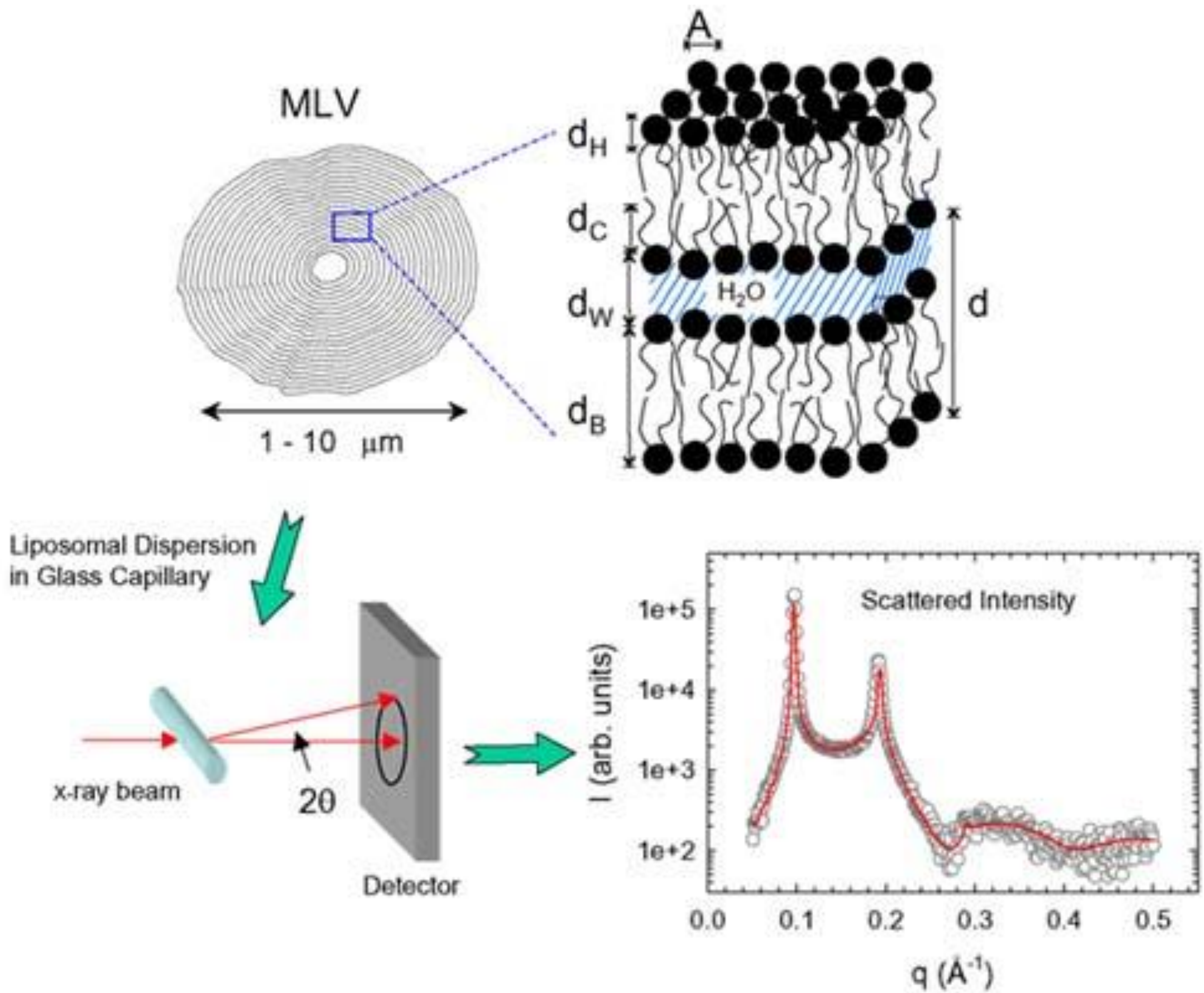
ρ_{CH2} (e/Å^3) = 0.300(5)

R_{CH3} (Å) = 3.5(3)

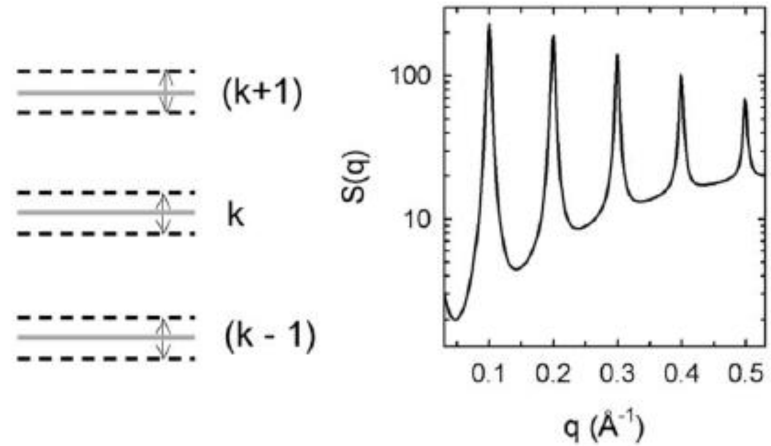
ρ_{CH3} (e/Å^3) = 0.22(2)

POPC

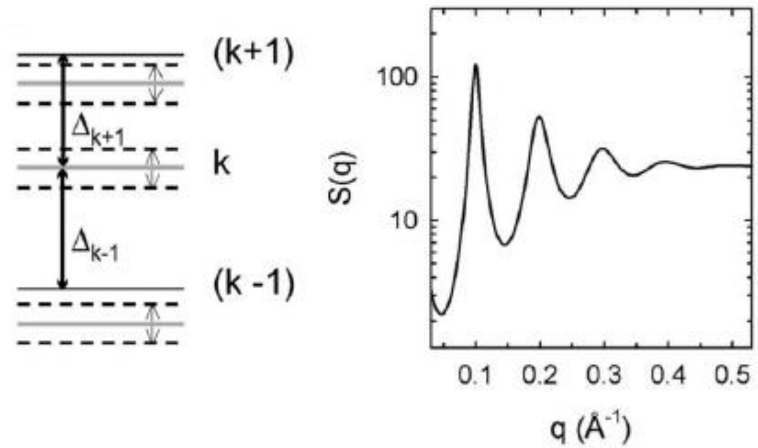
Multilayer Stacking



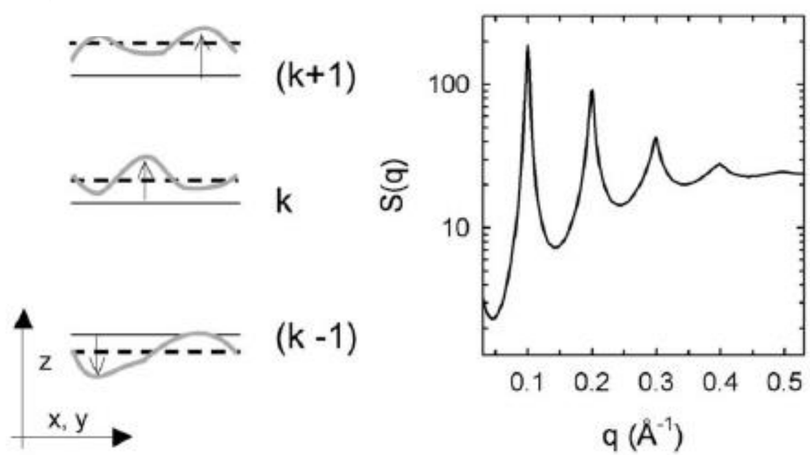
Thermal Disorder



Paracrystaline Theory



Modified Caillè Theory



- Pabst, G., Koschuch, R., Pozo-Navas, B., Rappolt,M., Lohner, K. and Laggner, P.. Structural analysis of weakly ordered membrane Stacks. J. Appl. Cryst. (2003). 36, 1378±1388.

Protein-Membrane Interaction

Characteristics of Fibers Formed by Cytochrome *c* and Induced by Anionic Phospholipids†

Juha-Matti Alakoskela,‡ Arimatti Jutila,‡ Adam C. Simonsen,§ Jussi Pirneskoski,‡ Sinikka Pyhäjoki,‖ Raija Turunen,‖ Sani Marttila,‖ Ole G. Mouritsen,§ Erik Goormaghtigh,⊥ and Paavo K. J. Kinnunen*,‡,§

Biochemistry **2006,** *45,* 13447−13453

Mechanism of formation???

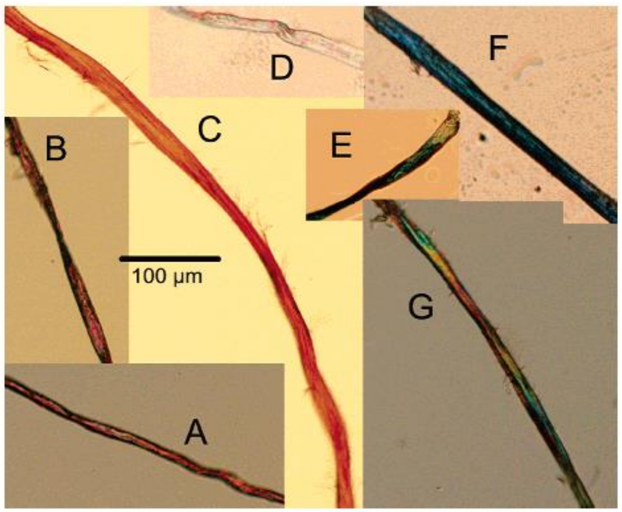
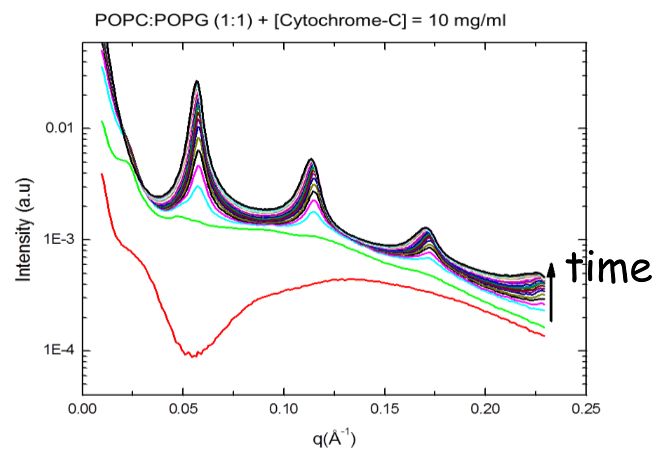
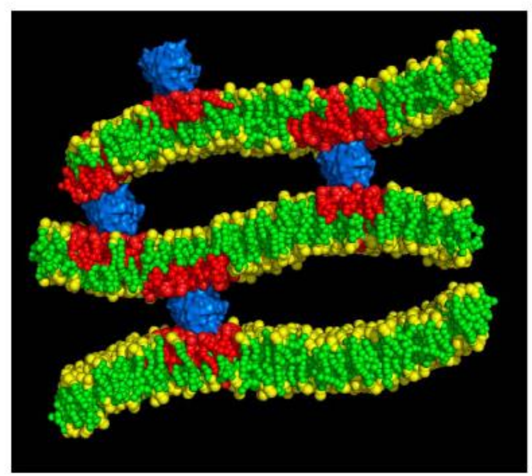


FIGURE 1: Microscopy images for sections of some cyt *c* fibers induced by liposomes. The nominal sample lipid:protein ratios for fibers A−G are 5:1, 100:1, 10:1, 25:1, 10:1, 10:1, and 10:1, respectively. In fibers A, B, D, F, and G, liposomes were composed of PC and PS (80:20), and in fibers C and E, liposomes were composed of PC and PG (80:20). The scale bar applies for all panels.



SAXS

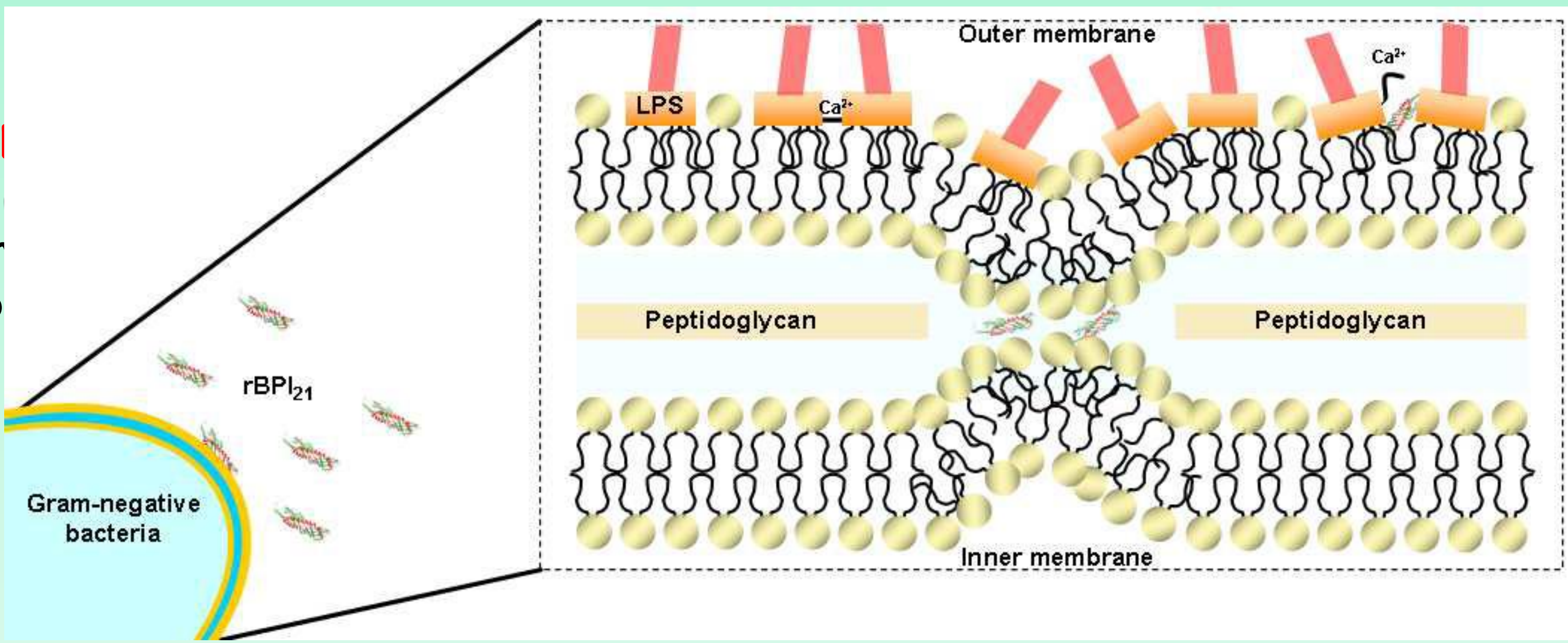


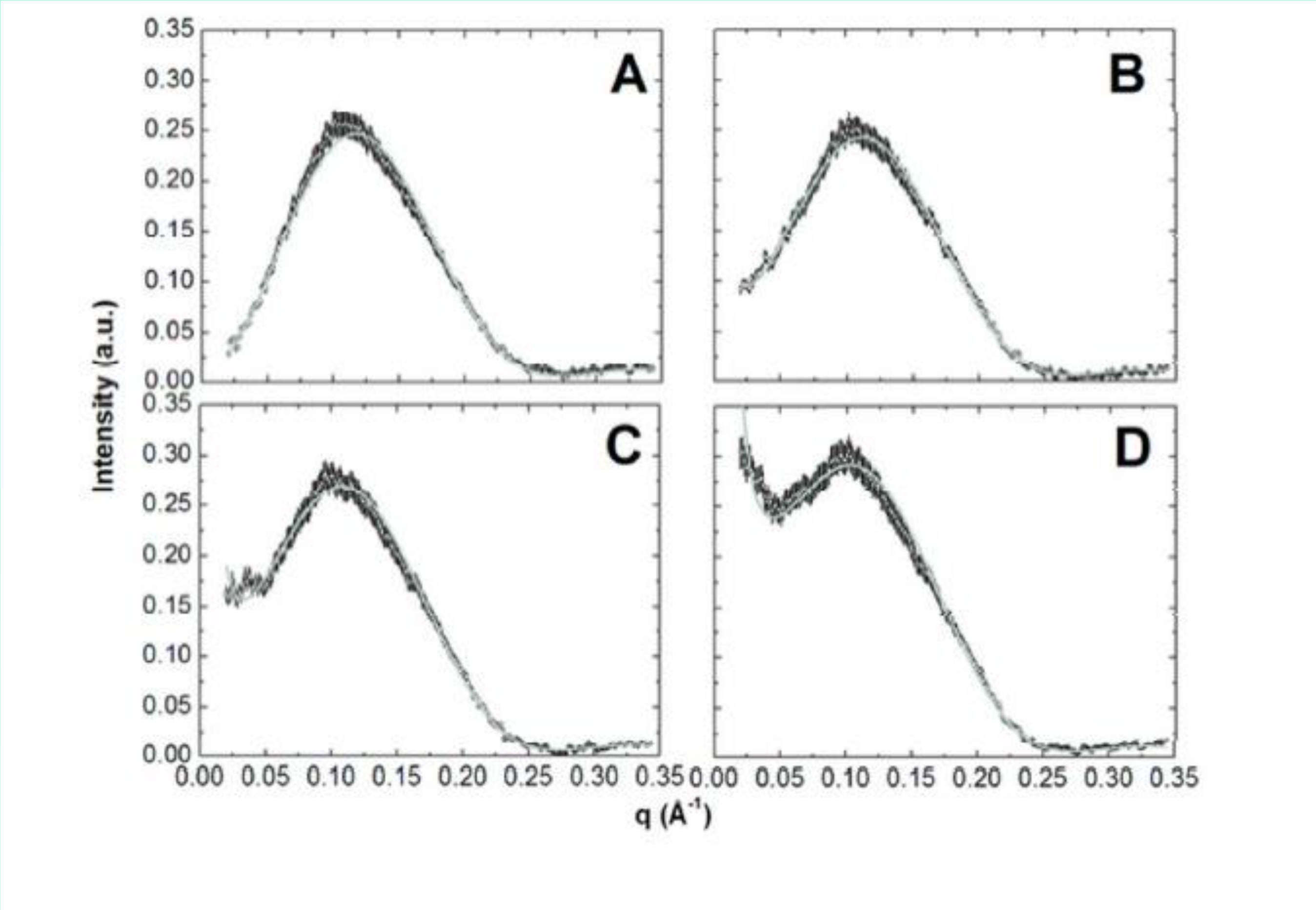
FRET
M. Prieto et al, BJ (2008) 4726

Antimicrobial peptide rBPI21 interacts with negative membranes promoting the formation of rigid multilamellar structures

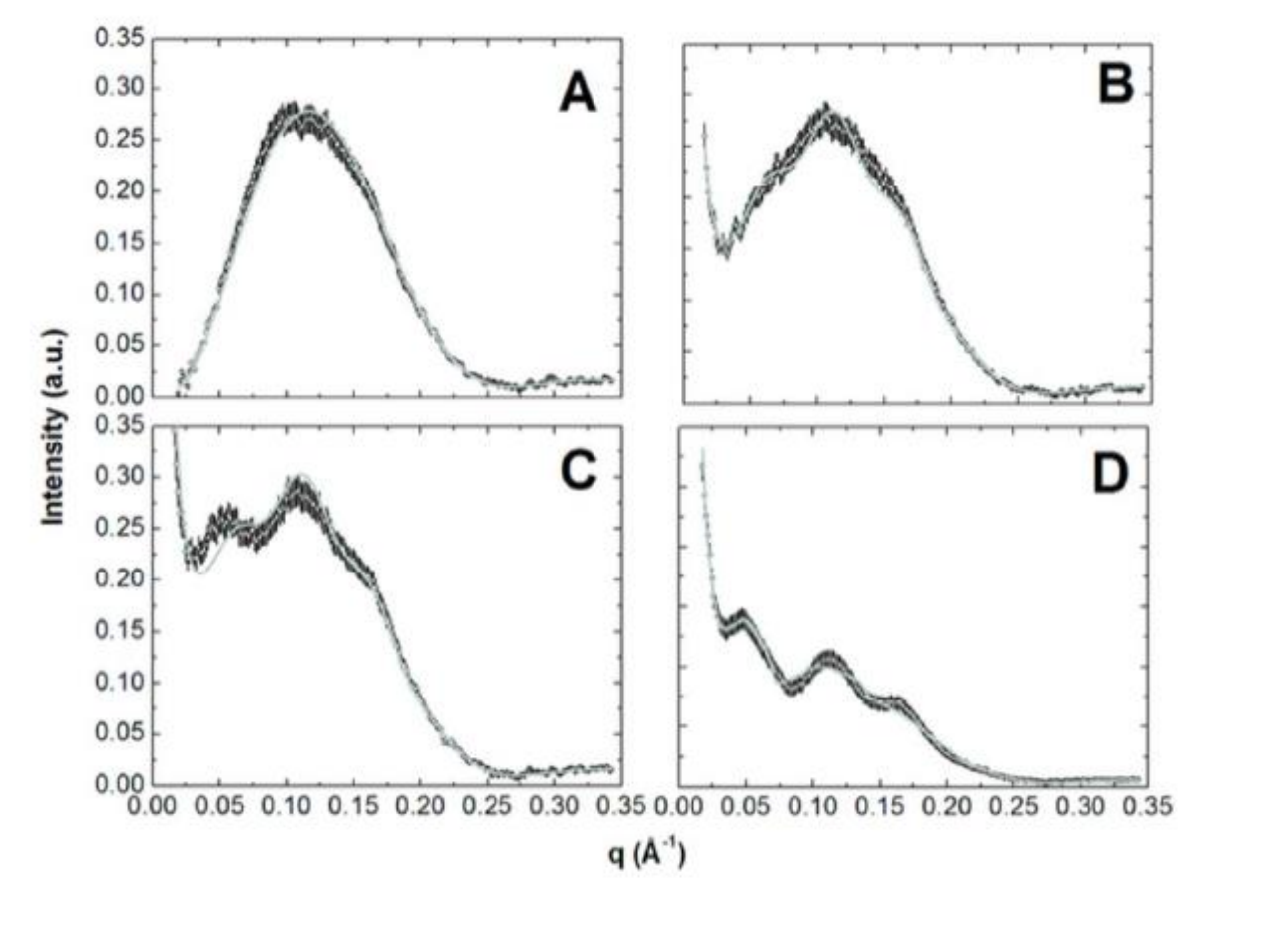
Marco Domingues, Manoel Castanho and Nuno Santos (Fac.Med- Un. Lisboa)
Leandro R. S. Barbosa and R. Itri (IFUSP) – BBACta 2014

r... al
f... is
m... e
b

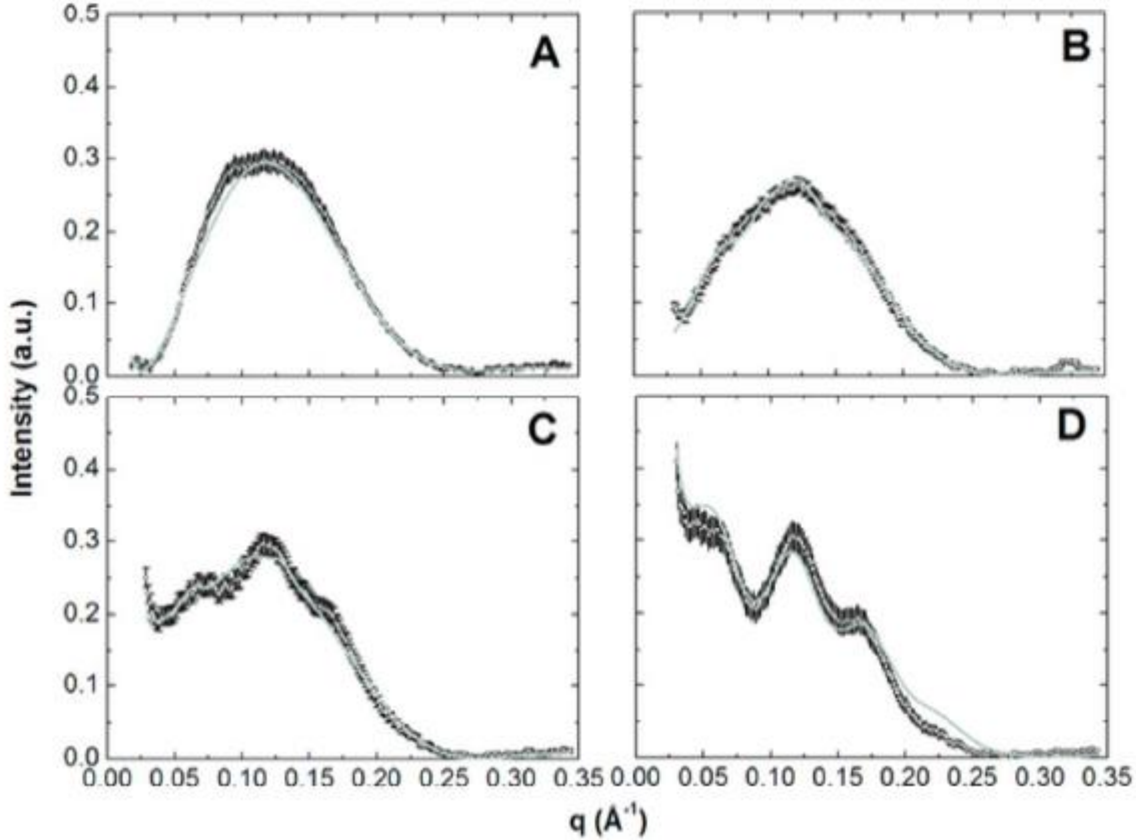




Experimental SAXS curves of POPC in the absence (A) and presence of 5 μM (B), 10 μM (C) and 20 μM (D) of rBPI21 in 20 mM sodium phosphate buffer pH 7.4, containing 150 mM NaCl. The solid lines represent the best fittings of Eqs. (1-2) to the experimental data. The fitting parameters are reported on Table 2.



SAXS curves of POPC:POPG 80:20 in the absence (A) and presence of 5 µM (B), 10 µM (C) and 20 µM (D) of rBPI21 in 20 mM sodium phosphate buffer pH 7.4, containing 150 mM NaCl. The solid lines represent the best fittings of Eqs. (1-4) to the experimental data. The fitting parameters are reported on Table 2.



SAXS curves of POPC:POPG 55:45 in the absence (A) and presence of 5 µM (B), 10 µM (C) and 20 µM (D) of rBPI21 in 20 mM sodium phosphate buffer pH 7.4, containing 150 mM NaCl. The solid lines represent the best fittings of Eqs. (1-4) to the experimental data. The fitting parameters are reported on Table 2.

POPC:POPG 80:20								
[rBPI$_{21}$] (μM)	0	5	10	20	0	5	10	20
w_i (%)						2.0±0.2	3.5±0.4	6.3±0.7
R_{pol} (Å)	9.7±0.8	9.6±0.7	9.5±0.6	8.5±0.7	-	9.6±0.8	9.5±0.6	8.5±0.6
R_{par} (Å)	9.8±0.8	9.8±0.8	9.8±0.8	9.8±0.8	-	9.8±0.8	9.8±0.8	9.8±0.8
R_{CH3} (Å)	3.4±0.2	3.4±0.2	3.4±0.2	3.4±0.2	-	3.4±0.2	3.4±0.2	3.4±0.2
ρ_{pol} (e/Å^3)	0.41±0.01	0.40±0.01	0.40±0.01	0.40±0.01	-	0.40±0.01	0.40±0.01	0.40±0.01
ρ_{par} (e/Å^3)	0.29±0.01	0.29±0.01	0.29±0.01	0.29±0.01	-	0.29±0.01	0.29±0.01	0.29±0.01
ρ_{CH3} (e/Å^3)	0.24±0.01	0.24±0.01	0.24±0.01	0.24±0.01	-	0.24±0.01	0.24±0.01	0.23±0.01
N	-	-	-	-	-	2±1	5±1	7±1
$\eta_{Caillé}$	-	-	-	-	-	0.18±0.02	0.18±0.02	0.18±0.02
$D_{Lamella}$ (Å)	-	-	-	-	-	112±5	112±5	110±5
$\sigma_{Caillé}$ (Å)	-	-	-	-	-	14.0±0.8	14.0±0.8	13.8±0.8

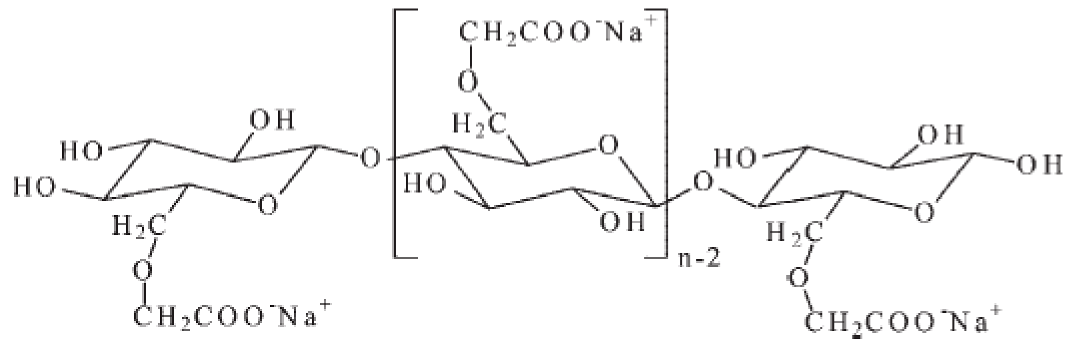
POPC:POPG 55:45								
[rBPI$_{21}$] (μM)	0	5	10	20	0	5	10	20
w_i (%)						6±1	8±1	12±2
R_{pol} (Å)	9.4±0.6	8.2±0.6	6.5±0.5	6.5±0.5	-	8.2±0.7	6.5±0.6	6.5±0.5
R_{par} (Å)	11.2±0.7	11.2±0.7	12.1±0.7	10.7±0.7	-	11.2±0.5	12.1±0.7	10.7±0.7
R_{CH3} (Å)	3.0	2.8±0.3	2.3±0.2	2.3±0.2	-	2.8±0.3	2.3±0.3	2.3±0.2
ρ_{pol} (e/Å^3)	0.43±0.01	0.39±0.01	0.40±0.01	0.40±0.01	-	0.39±0.01	0.40±0.01	0.40±0.01
ρ_{par} (e/Å^3)	0.31±0.01	0.31±0.01	0.31±0.01	0.31±0.01	-	0.31±0.01	0.31±0.01	0.30±0.01
ρ_{CH3} (e/Å^3)	0.17±0.01	0.18±0.01	0.18±0.01	0.25±0.01	-	0.18±0.01	0.18±0.01	0.25±0.01
N	-	-	-	-	-	3±1	5±1	8±1
$\eta_{Caillé}$	-	-	-	-	-	0.085±0.003	0.085±0.003	0.085±0.003
$D_{Lamella}$ (Å)	-	-	-	-	-	114±5	111±5	107±5
$\sigma_{Caillé}$ (Å)	-	-	-	-	-	9.8±0.6	9.6±0.6	9.2±0.6

Small-Angle X-Ray Scattering on Solutions of Carboxymethylcellulose and Bovine Serum Albumin

Sabrina M. Pancera, Denise F. S. Petri and **R. Itri**

Macromol. Bioscience, 5, 331 (**2005**)

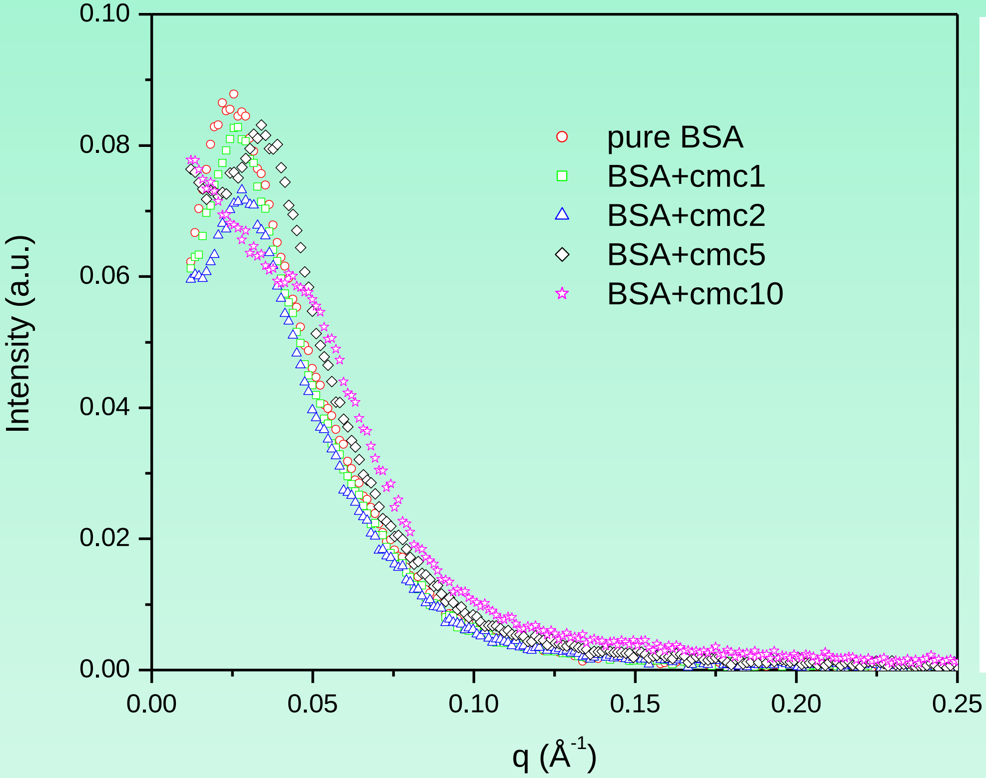
(pH 7.0, no buffer)



Scheme 1. Chemical structure of CMC.

É possível a complexação????

10 mg/ml BSA, pH 7.0 (>pI=5.4)



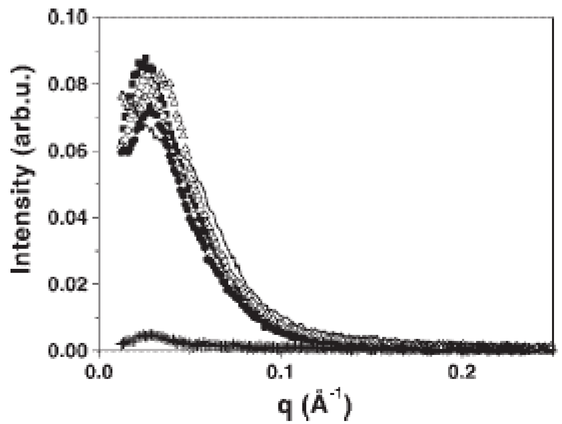
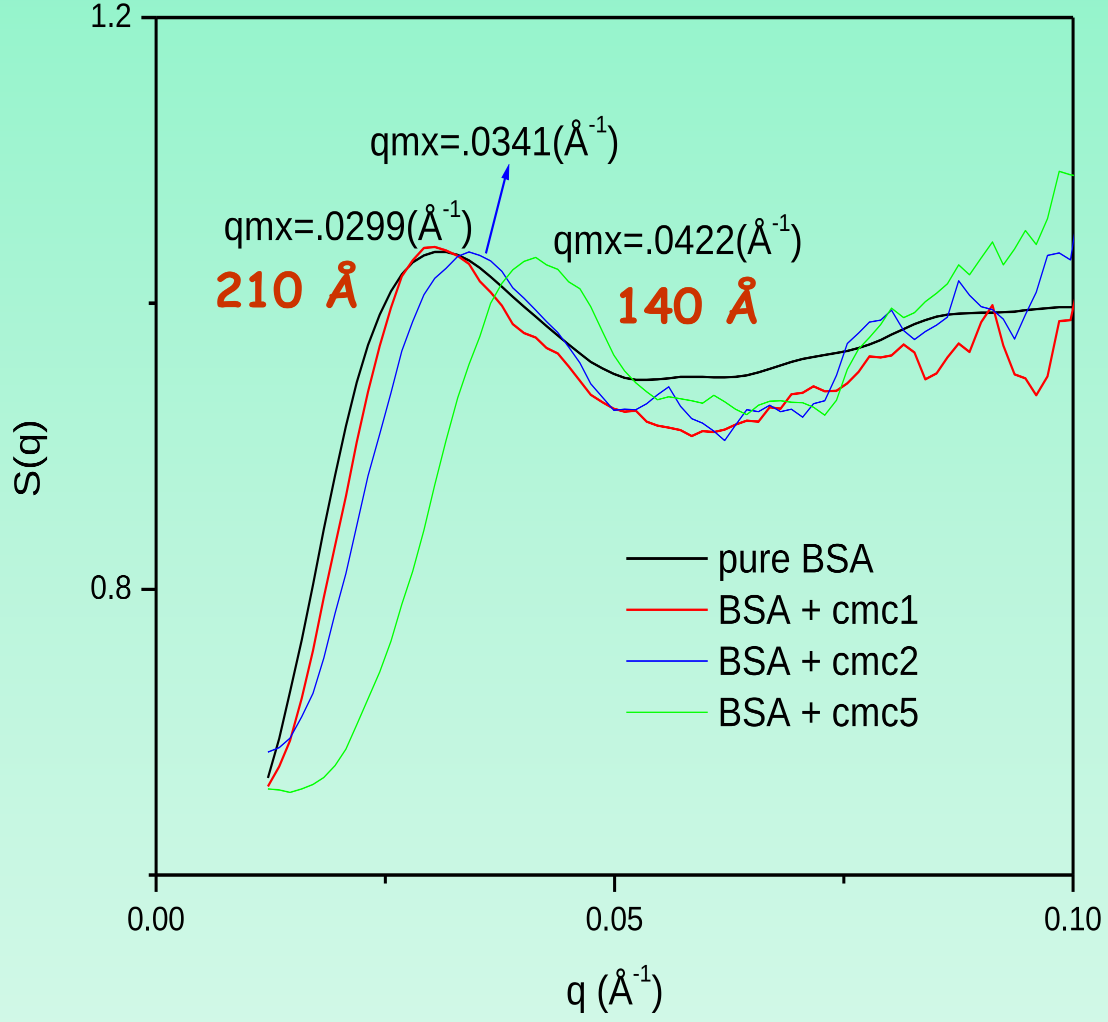
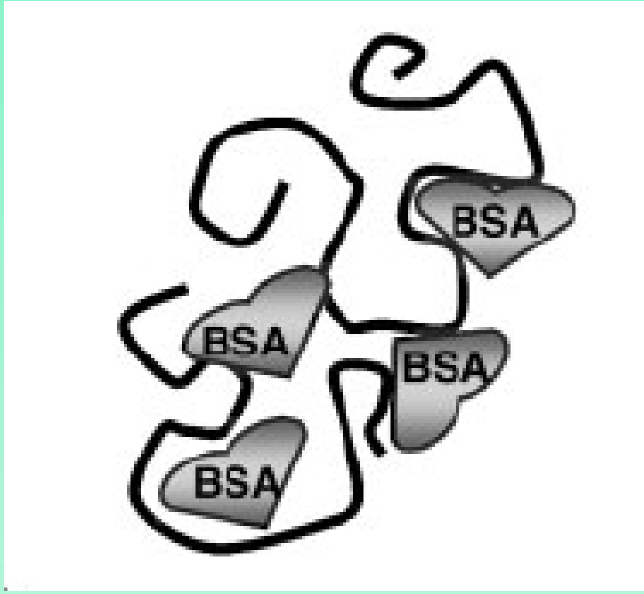


Figure 2. SAXS curves obtained for pure BSA solution at a concentration of 10×10^{-3} g · mL^{-1} (■) and for mixtures of BSA (10×10^{-3} g · mL^{-1}) and CMC at $C_p = 1 \times 10^{-3}$ g · mL^{-1} (○), $C_p = 2 \times 10^{-3}$ g · mL^{-1} (●), $C_p = 5 \times 10^{-3}$ g · mL^{-1} (△) and $C_p = 10 \times 10^{-3}$ g · mL^{-1} (solid line). The SAXS curve of a protein-free CMC solution at 5 mg · mL^{-1} (+) is also included.

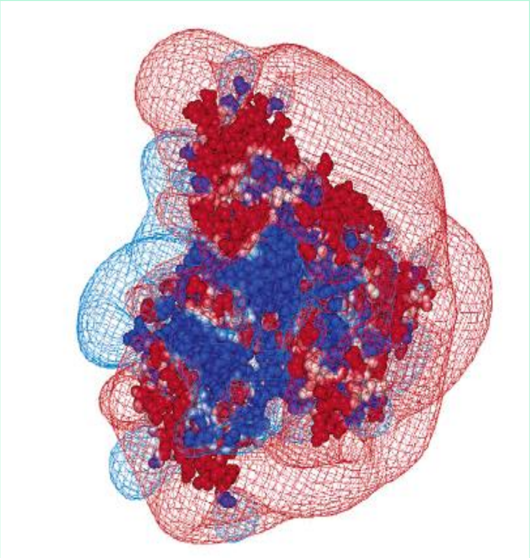


qmx=.0341(Å-1)
qmx=.0299(Å-1)
210 Å
qmx=.0422(Å-1)
140 Å
1.2
0.8
S(q)
pure BSA
BSA + cmc1
BSA + cmc2
BSA + cmc5
0.00
0.05
0.10
q (Å-1)

Qual a nossa proposta ???



Dublin et al, **Biomacromolecules**, 4, 273-282 (**2003**)



BSA a pH 7.0

Polyelectrolyte–protein complexation driven by charge regulation

Fernando Luís Barroso da Silva[*ab] and Bo Jönsson[b] Soft Matter, 2009, 5, 2862–2868

$$w(r) \simeq l_B Z_\alpha \left(\frac{\langle Z\rangle_0}{R}\right) - l_B^2 Z_\alpha^2 \left(\frac{C}{2R^2} + \frac{\langle \mu\rangle_0^2}{6R^4}\right)$$

$l_B = e^2/4\pi\varepsilon_0\varepsilon_s k_B T$ is the Bjerrum length.

$$C \equiv \langle Z^2\rangle_0 - \langle Z\rangle_0^2 \propto -\frac{\partial Z}{\partial \mathrm{pH}}$$

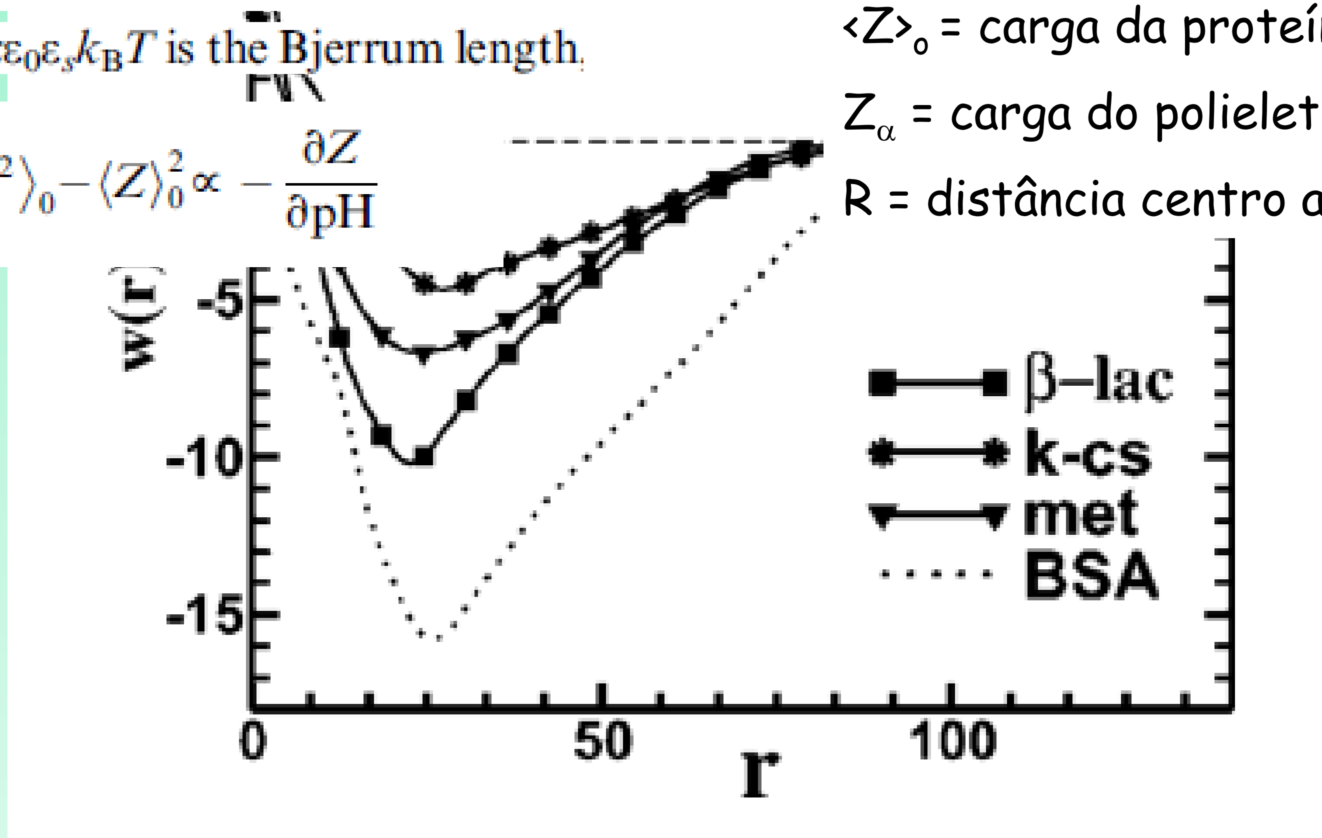
C = capacitância da proteína

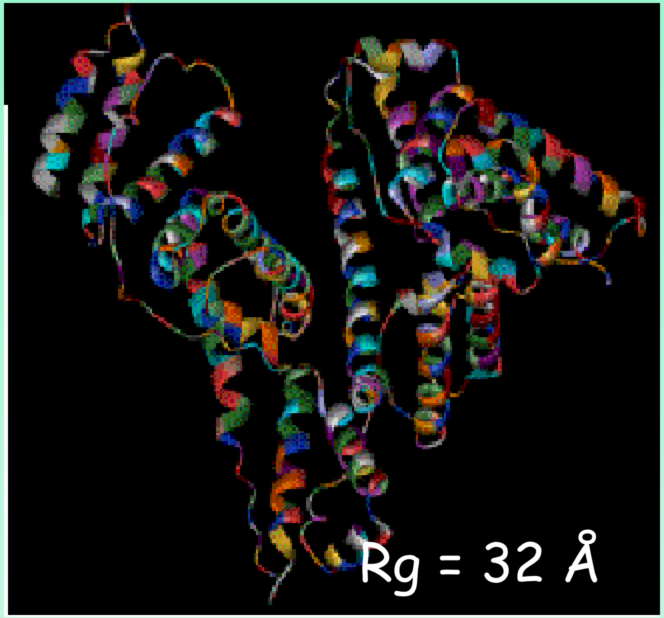
μ= mom. Dipolo elétrico

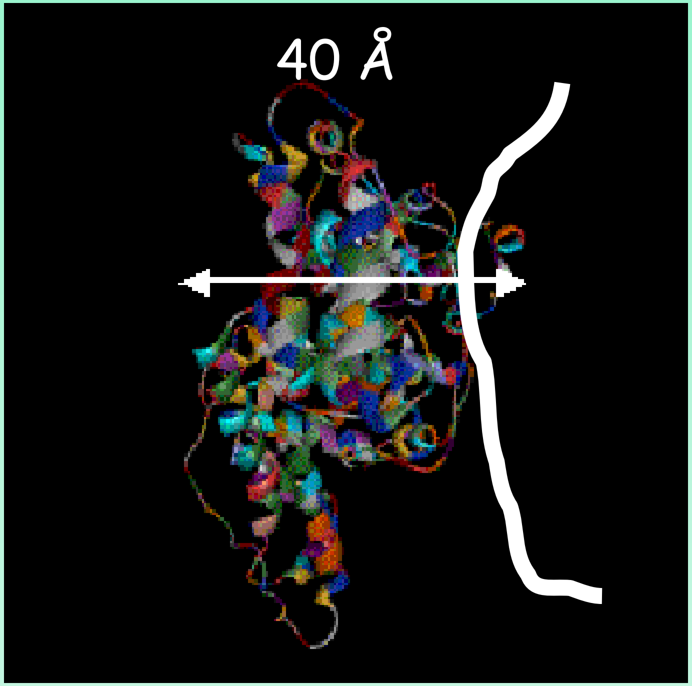
$\langle Z\rangle_o$ = carga da proteína

Z_α = carga do polieletrólito

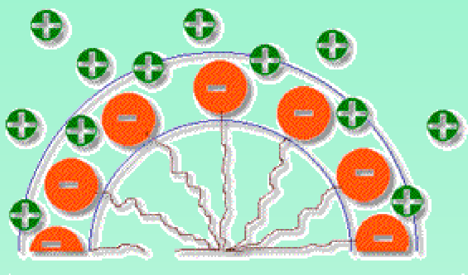
R = distância centro a centro

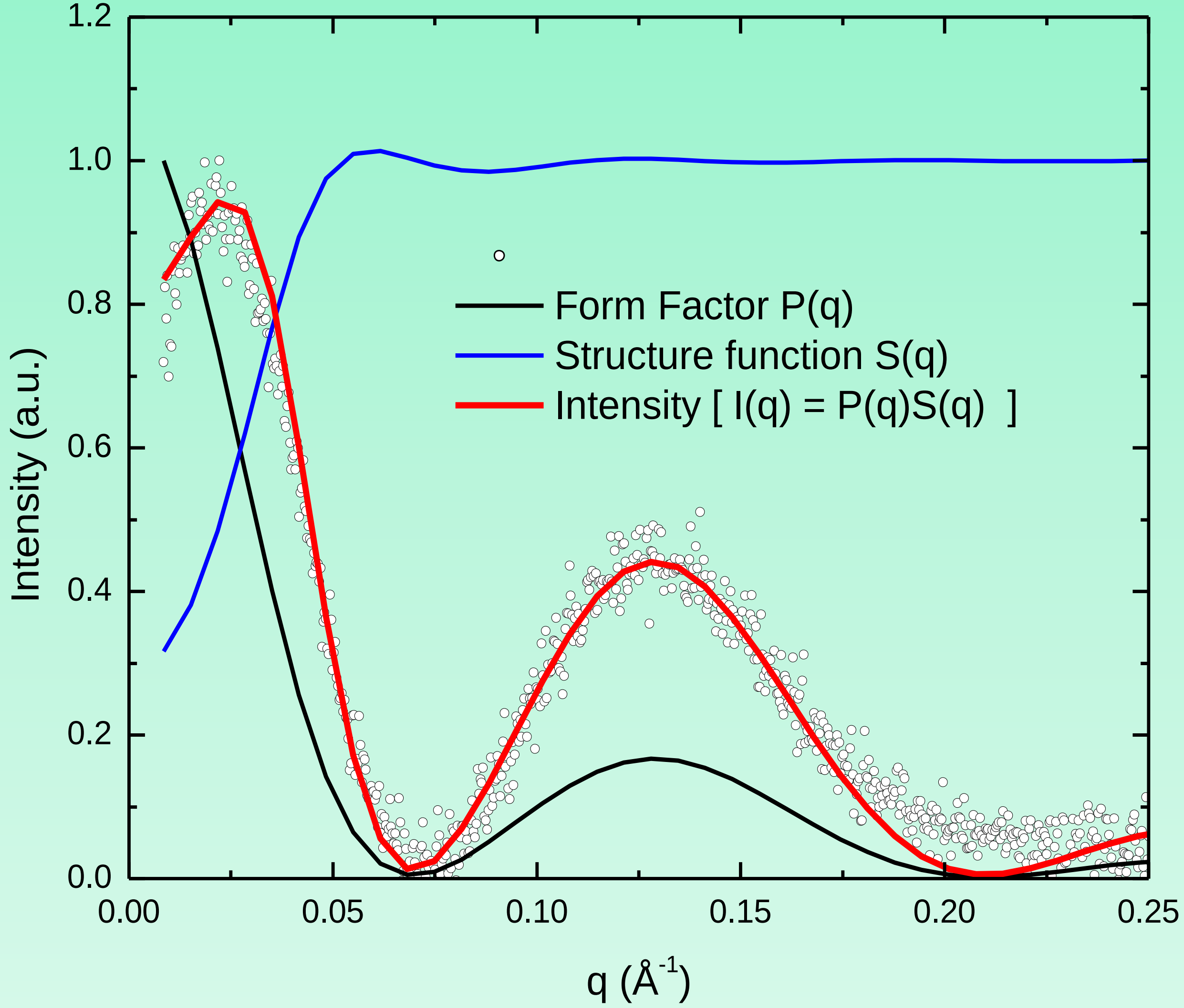



Rg = 32 Å


40 Å

Surface Charged Micelles interacting through a Screeend Coulomb Potential





Itri&Amaral, PRE 1993

Master Equation

$$I(q) = n_p \left\{ \langle F^2(q) \rangle + 4\pi n_p \langle F(q) \rangle^2 \int_0^\infty (g(r)-1)\, r^2 \frac{sen(qr)}{qr}\, dr \right\}$$

$$= n_p \langle F^2(q) \rangle \bar{S}(q)$$

S(q)   correlation function (structure factor) related to g(r)

g(r) pair correlation function

$g(r) = \exp(-W(r)/k_B T)$

W(r) = Mean Field potential

$h(r) = g(r) - 1$ **h(r) = função de correlação total**

Equação de Ornstein-Zernicke: $$h(r_{12}) = c(r_{12}) + n_p \int c(r_{13}) h(r_{23}) dr_3$$

relacionar c(r) com h(r) → relações de fechamento para um determinado *U*:

Percus-Yevick $$c(r) = \{1 - \exp[k_B TU(r)]\}(h(r) + 1)$$

Hipperneted Chain Approximation $$c(r) = -k_B TU(r) + h(r) - \ln(h(r) + 1)$$

Mean Spherical Approximation

$$c(r) = -k_B TU(r) \qquad r > 2R$$

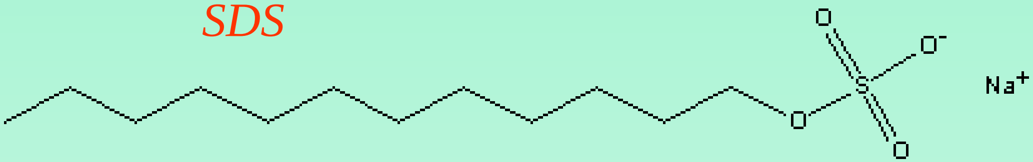
$$h(r) = -1 \qquad r \leq 2R$$

Complexes of Chloropromazine (CPZ) and Sodium Dodecyl Sulfate (SDS)Micelles

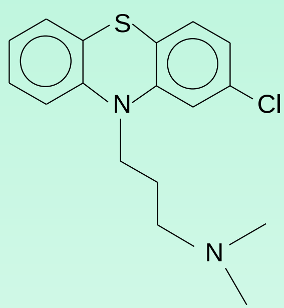
Wilker Caetano, Marcel Tabak (IQUSP-SC) e R. Itri (IFUSP)

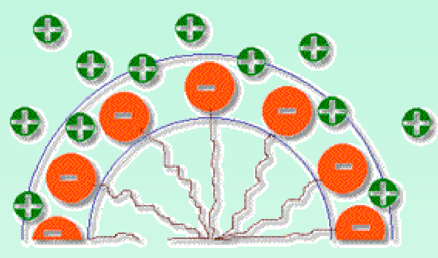
J. Coll. Interf. Science, 248, 149 (2002)

SDS



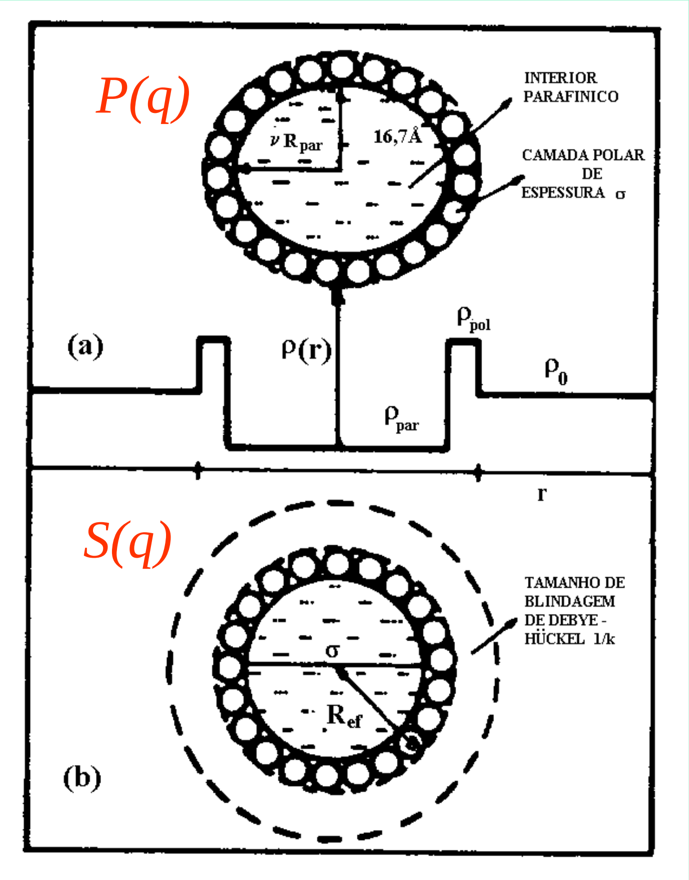
\+ água





CPZ - pKa = 10 in SDS → pH = 4,0, 7,0 e 9,0

Analysis Method



DLVO

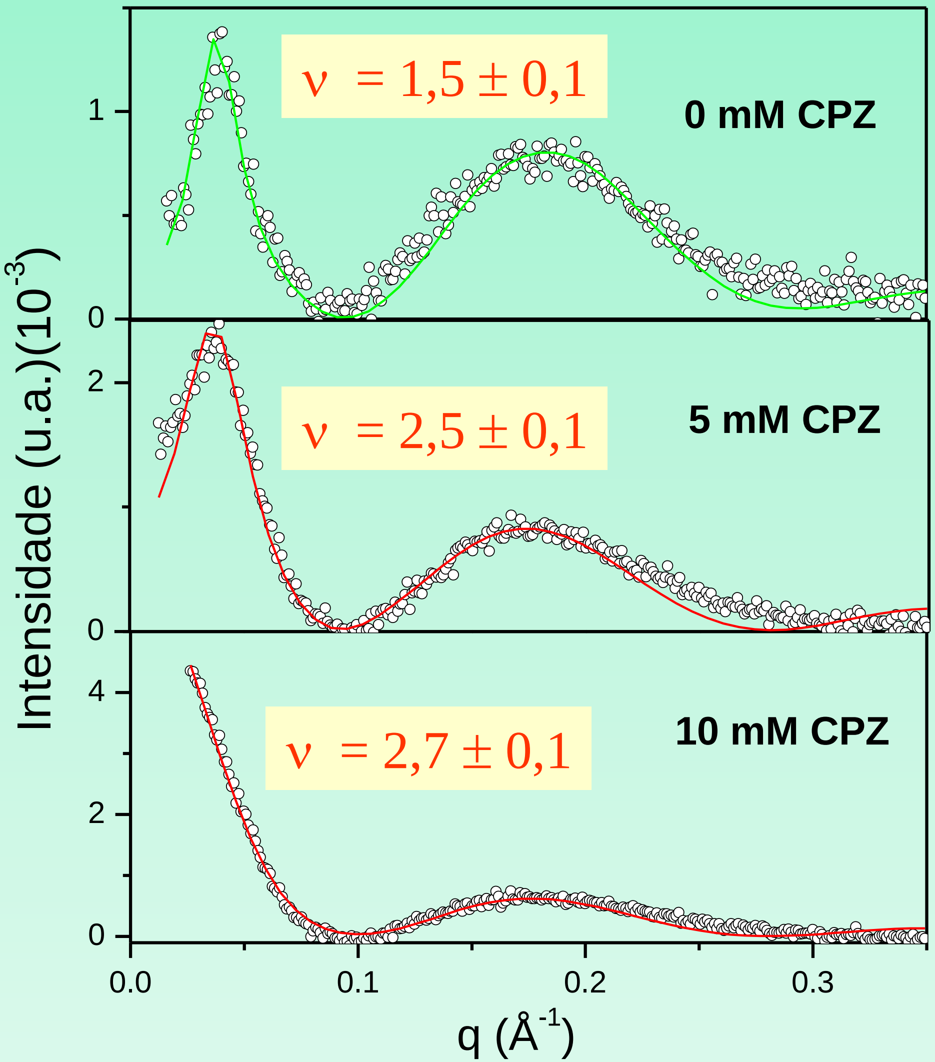
$$U(r) = \pi\varepsilon_0 \varepsilon D^2 \Phi_0^2 \frac{\exp[-k_{DB}(r-D)]}{r} \qquad r > 2R_{ef}$$

$$U(r) = \infty \qquad r \leq 2R_{ef}$$

S(q) (MSA) - Hayter & Penfold,81

Results - *SAXS beam line LNLS*

40 mM SLS *pH 9,0*
(20 mM borato de sódio)



$R_{par} = 16{,}7 \pm 0{,}3$ Å
$\sigma = 5{,}5 \pm 0{,}5$ Å

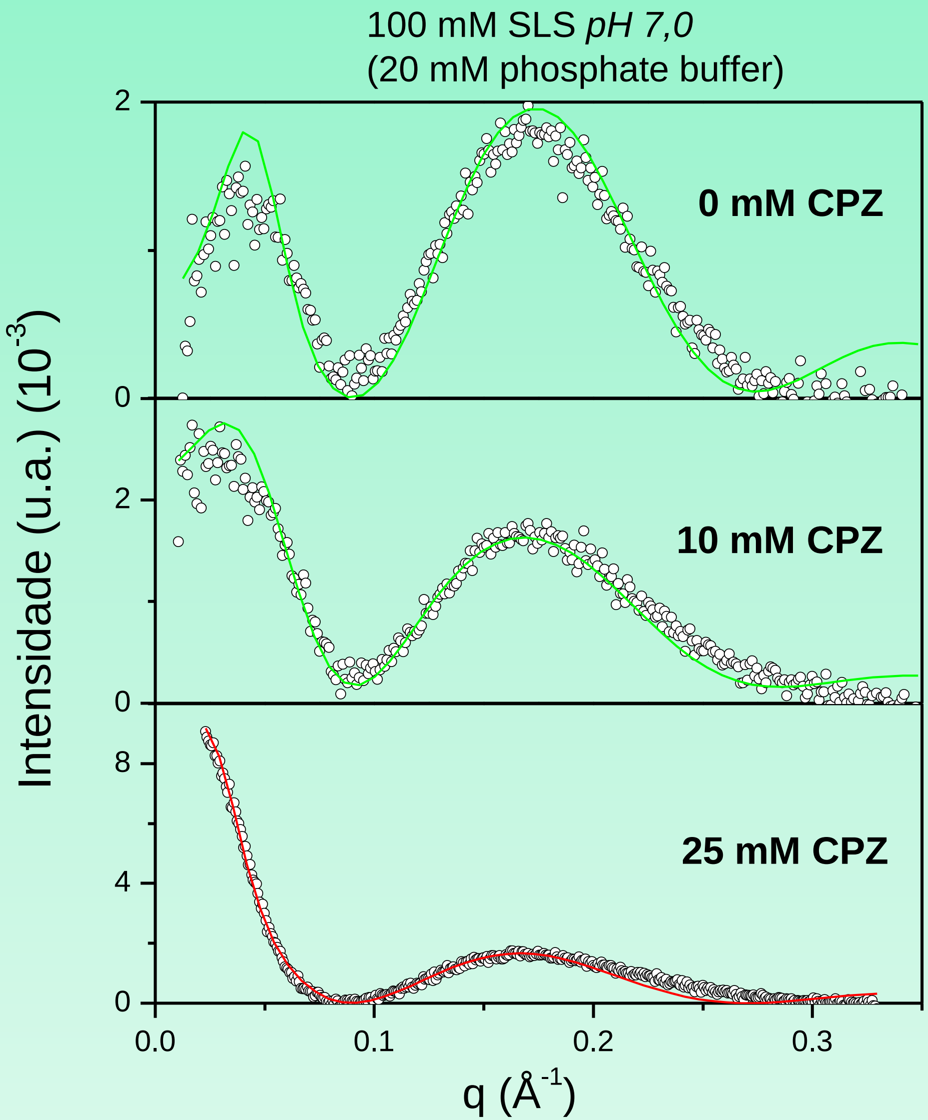
Prolate ellipsoid

$\rho_{pol} \approx 0{,}40$ e/Å^3

cylinder

$\rho_{pol} \approx 0{,}50$ e/Å^3

Results - *SAXS beam line LNLS*



Prolate ellipsoid

$\nu = 1{,}6 \pm 0{,}1$

$\nu = 2{,}0 \pm 0{,}1$

cylinder

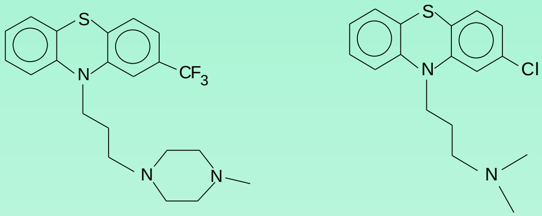
$\nu = 4{,}0 \pm 0{,}2$


40 mM SLS
ν = 1,5
Ausência de droga
ν = 2,5
1 CPZ : 8 SLS
ν = 2,7
1 CPZ : 4 SLS
100 mM SLS
ν = 1,6
Ausência de droga
ν = 2,0
1 CPZ : 10 SLS
ν = 4,0
1 CPZ : 4 SLS

Interaction of Chlorpromazine (CPZ) and Trifluoperazine (TFP) with Zwitterionic Micelles

Leandro Barbosa, Wilker Caetano e R. Itri (IFUSP), Marcel Tabak (IQUSP-SC)

TFP CPZ



CPZ - pKa = 10 in SDS

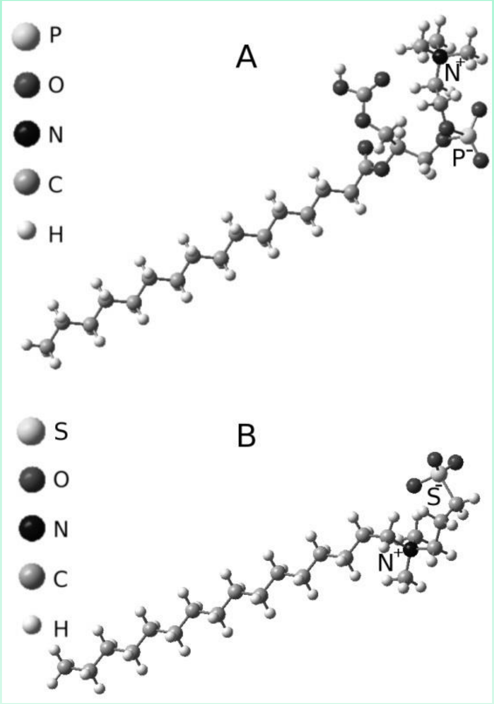
→ pH = 4.0, 7.0 and 9,0

Co-Micelle

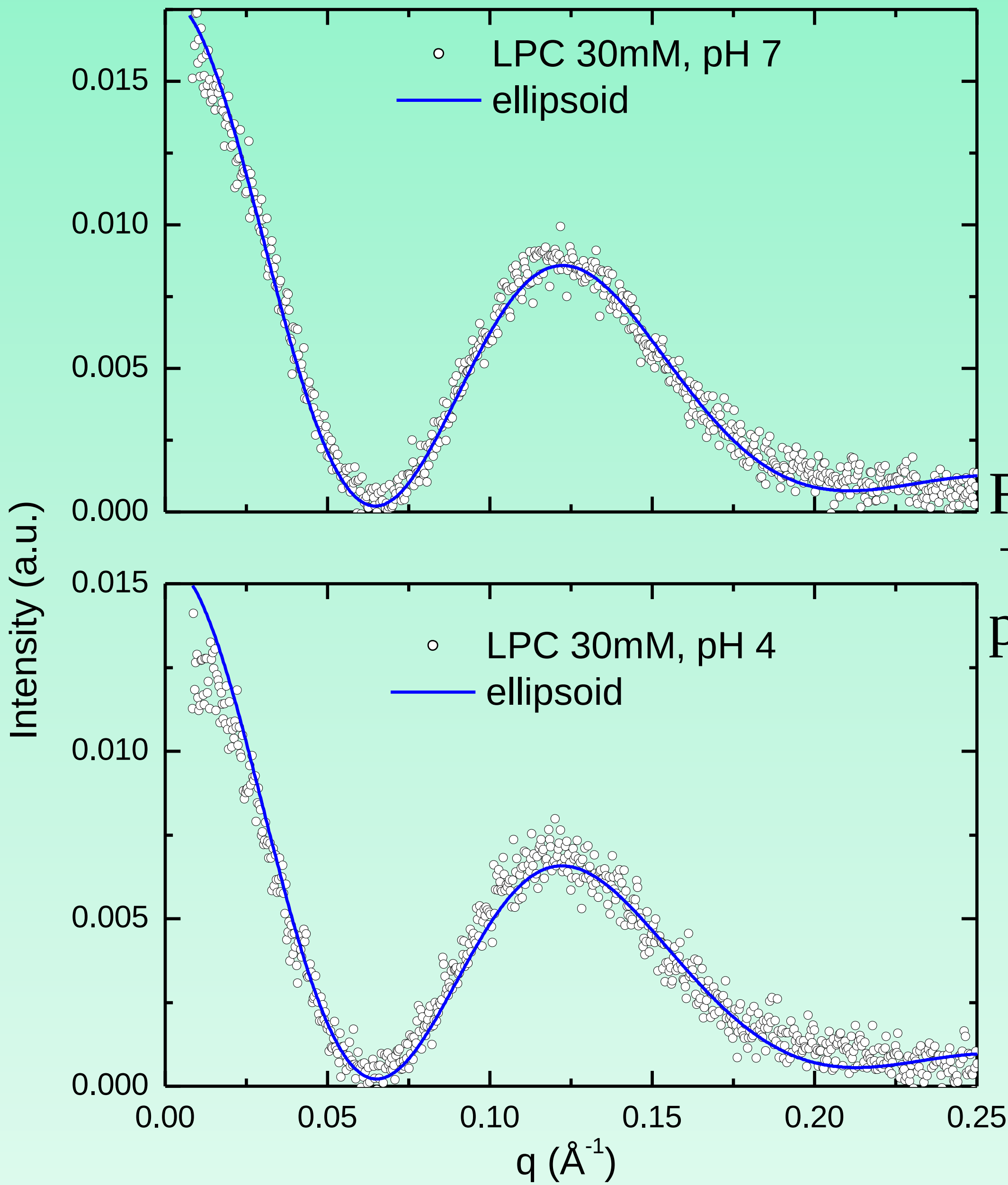
Obs:smaller binding to HPS

LPC

HPS



SAXS curves



Fitting parameters

$\alpha = 0$

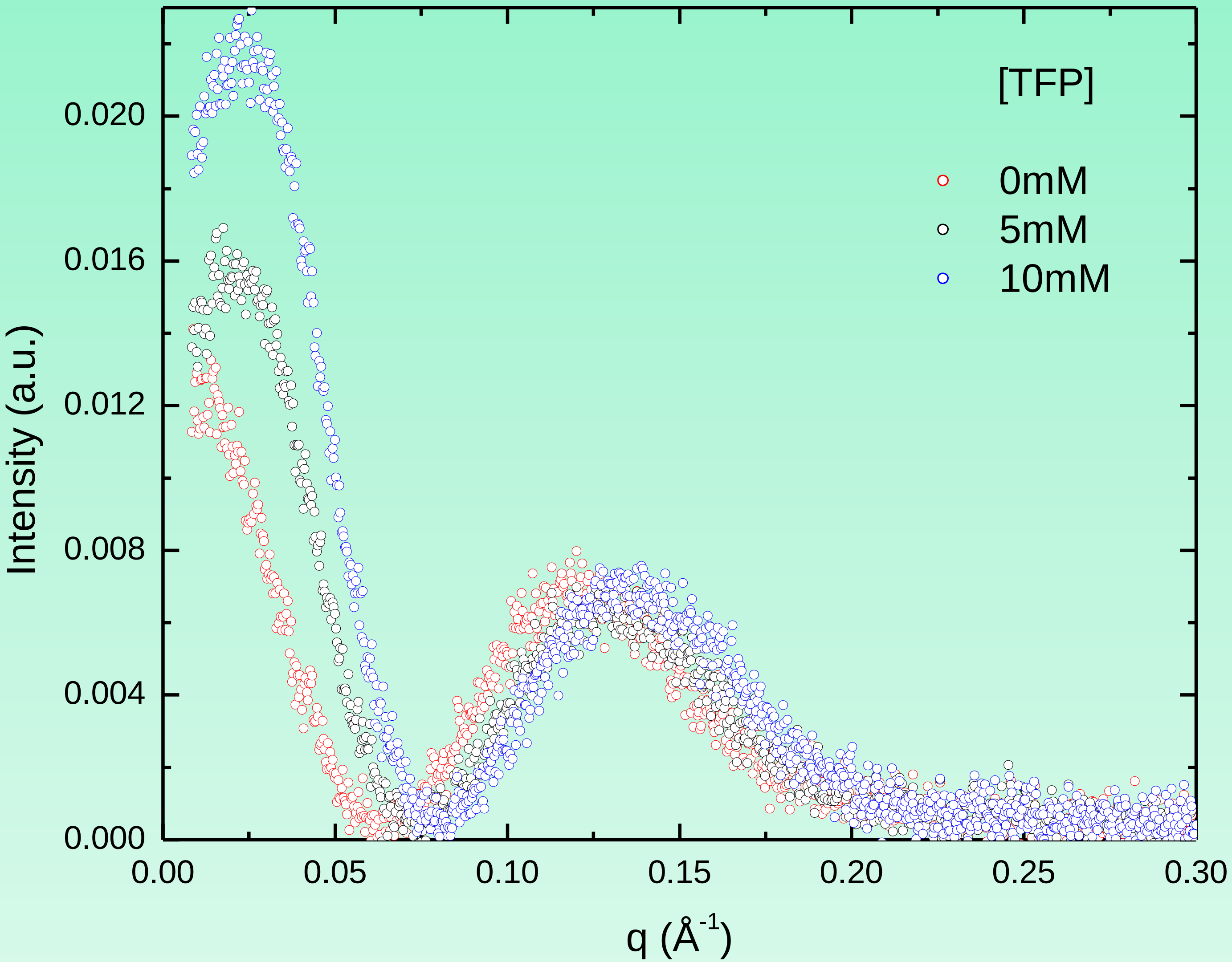
$R_{par} = 23.0(2)$ Å

$\rho_{pol} = 0.39(1)$ e/Å^3

$\sigma_{pol} = 9.1(2)$ Å

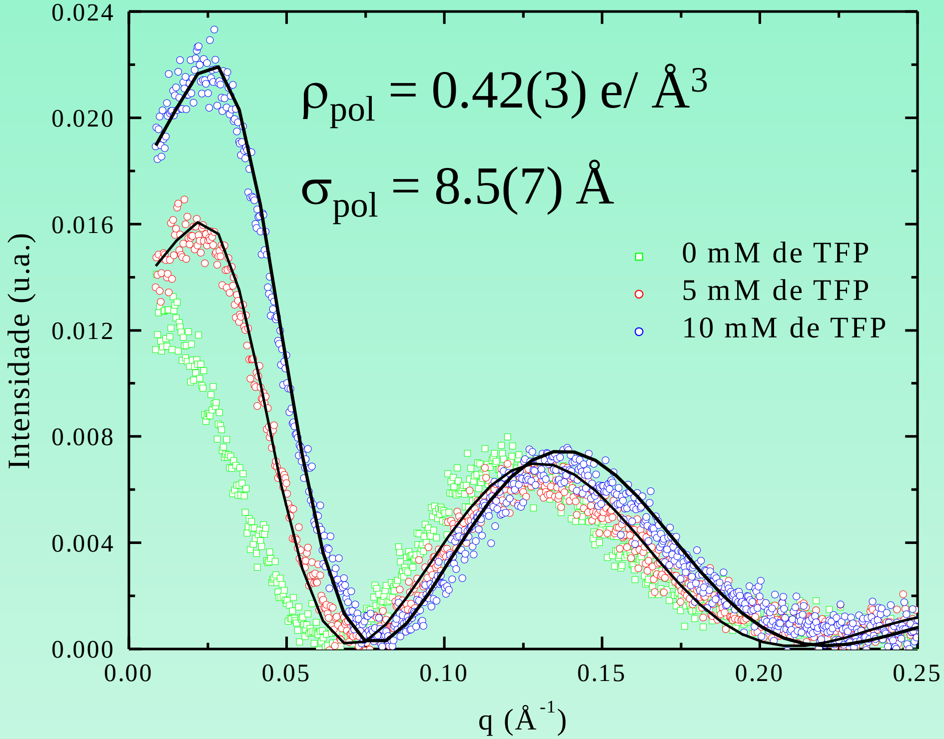
$\nu = 1.70(5)$

30mM LPC at pH 4 + TFP

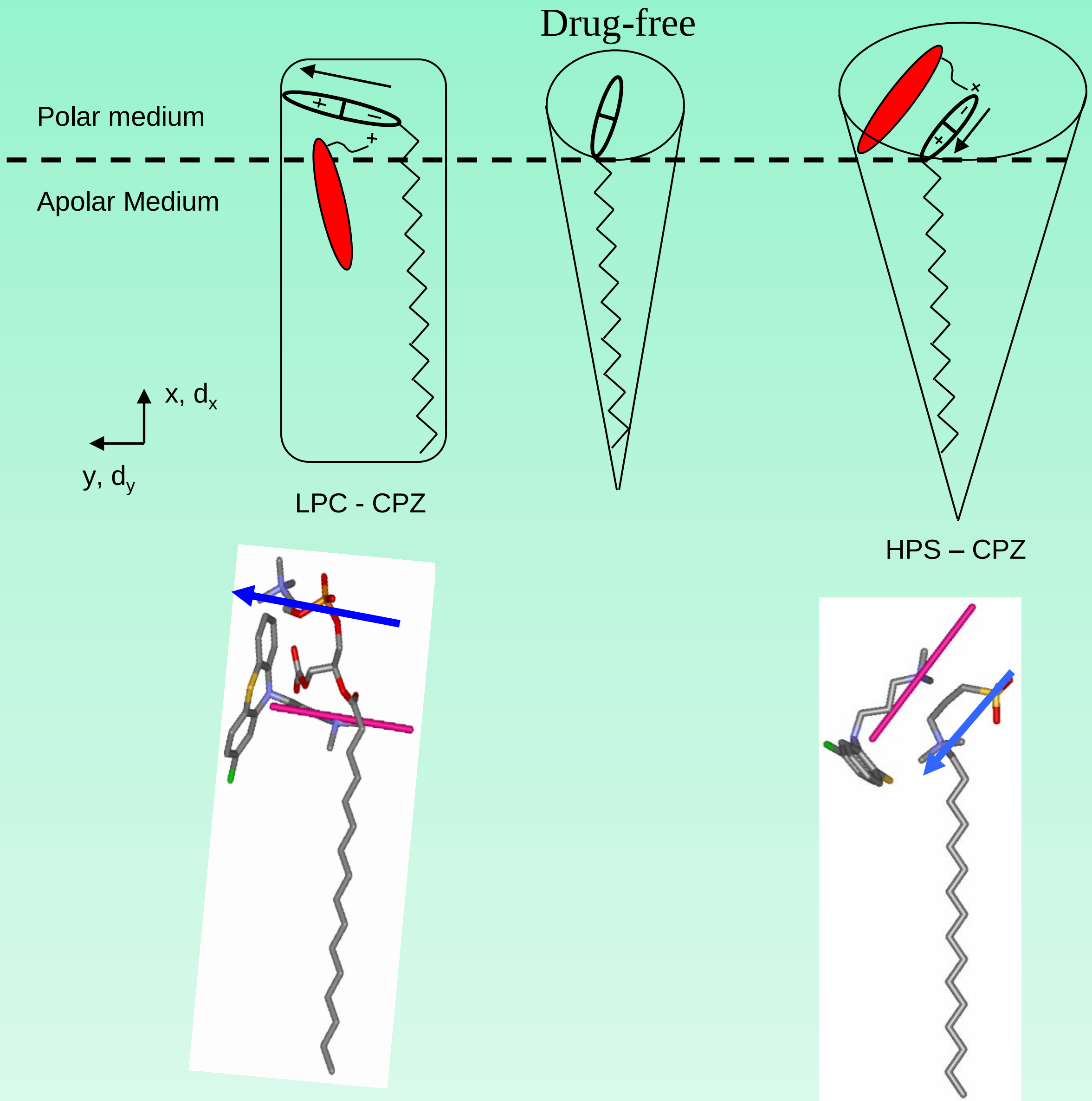


pH 4

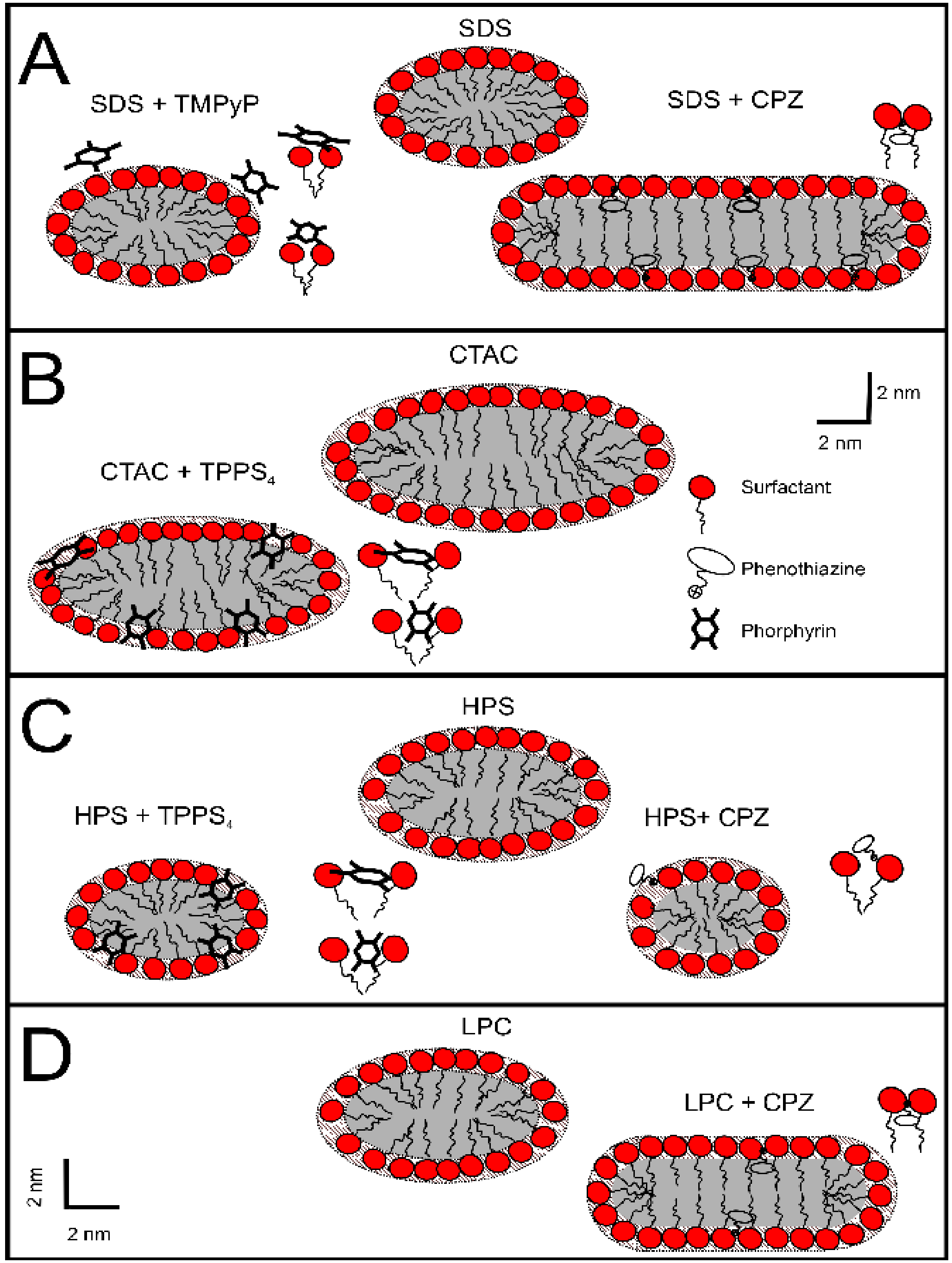
LPC



	R_{par} (Å)	ν	
0mM	22.4(2)	1.74(2)	Elipsoid
5mM	21.0(4)	2.5(2)	Cylinder
10mM	20.0(4)	2.4(2)	



Drug-free
Polar medium
Apolar Medium
x, d_x
y, d_y
LPC - CPZ
HPS – CPZ



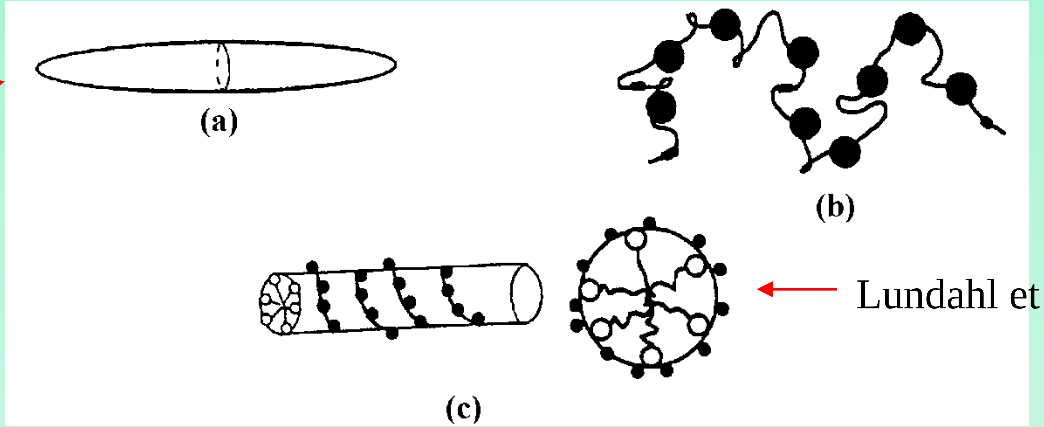
A
SDS
SDS + TMPyP
SDS + CPZ
B
CTAC
CTAC + TPPS$_4$
2 nm
2 nm
Surfactant
Phenothiazine
Phorphyrin
C
HPS
HPS + TPPS$_4$
HPS+ CPZ
D
LPC
LPC + CPZ
2 nm
2 nm

Interaction between Bovine Serum Albumin and SDS

Dino Zanette (Depto. Química - UF Santa Catarina) and R. Itri (IFUSP)

Structural Models of protein/surfactant complexes

Reynolds & Tanford, JBC, 1970 (viscosity)

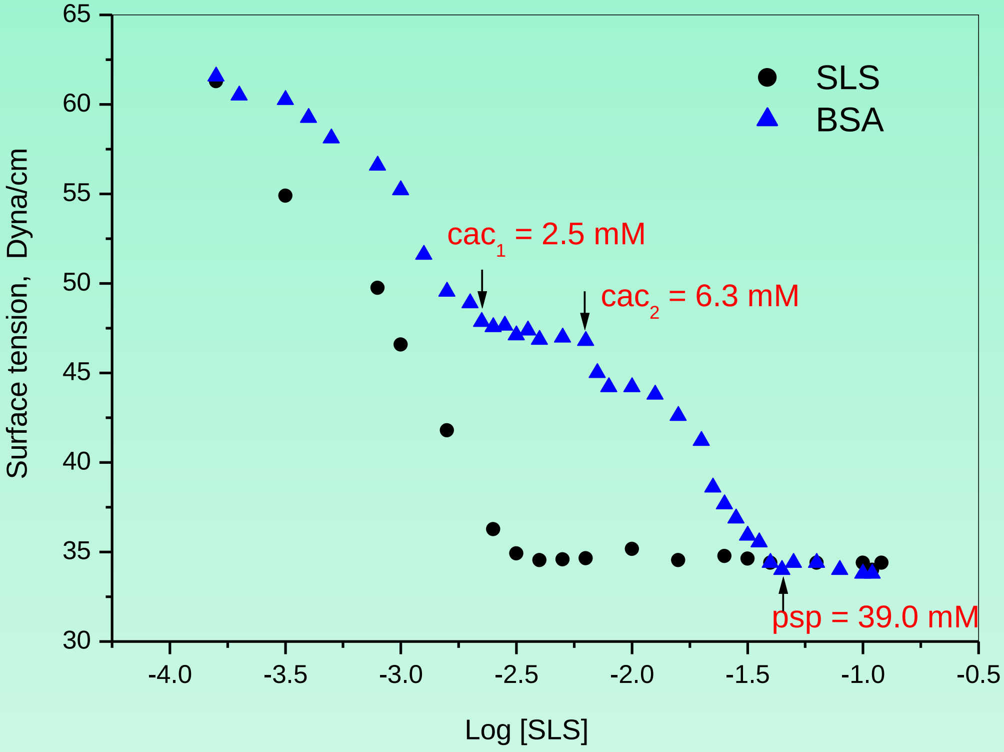


Lundahl et al, BBA,1986

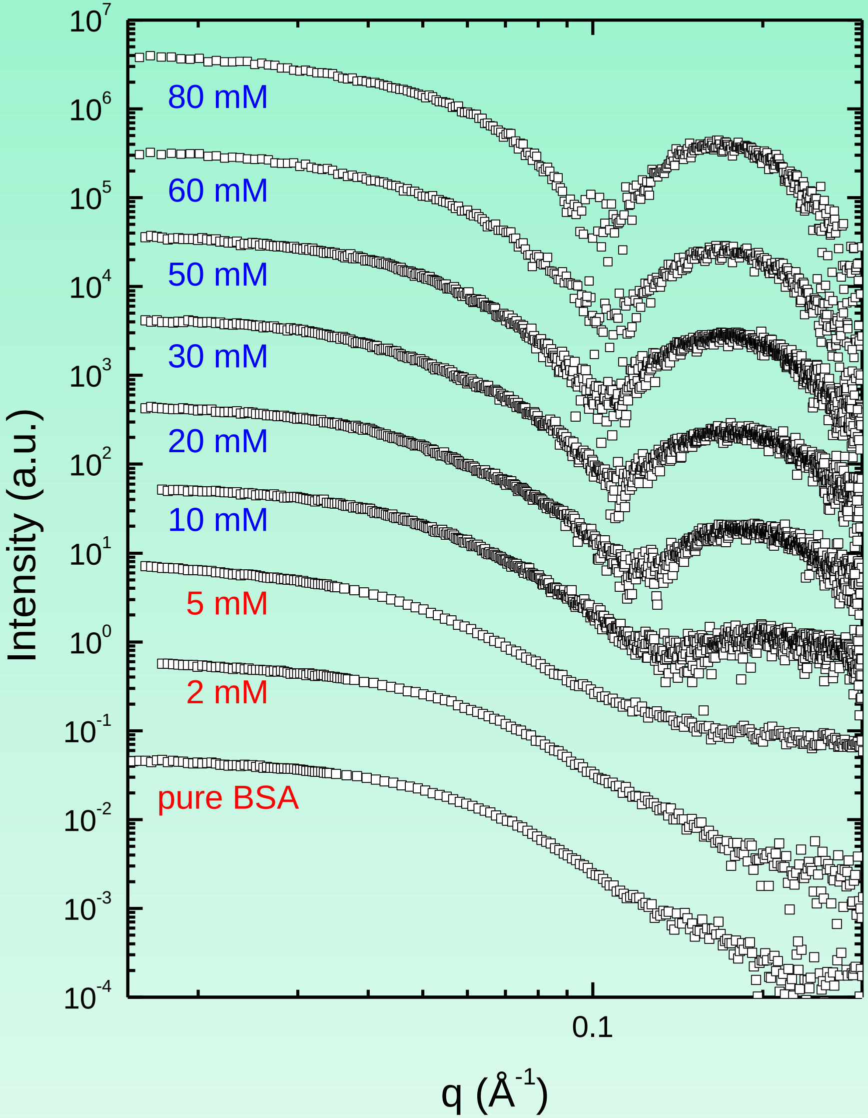
(b) Pearl-Necklace model

- Shirahama et al (J. Bioch, 1974)
- Chen, Tanner e col (JCP, 1982)- DLS
- Chen, Teixeira e col (PRL, 1986, Biopolymers, 1990) - *SANS* of BSA/LiLS and OVA/SLS
- Turro et al (Langmuir, 1995) - NMR, fluorescence and EPR

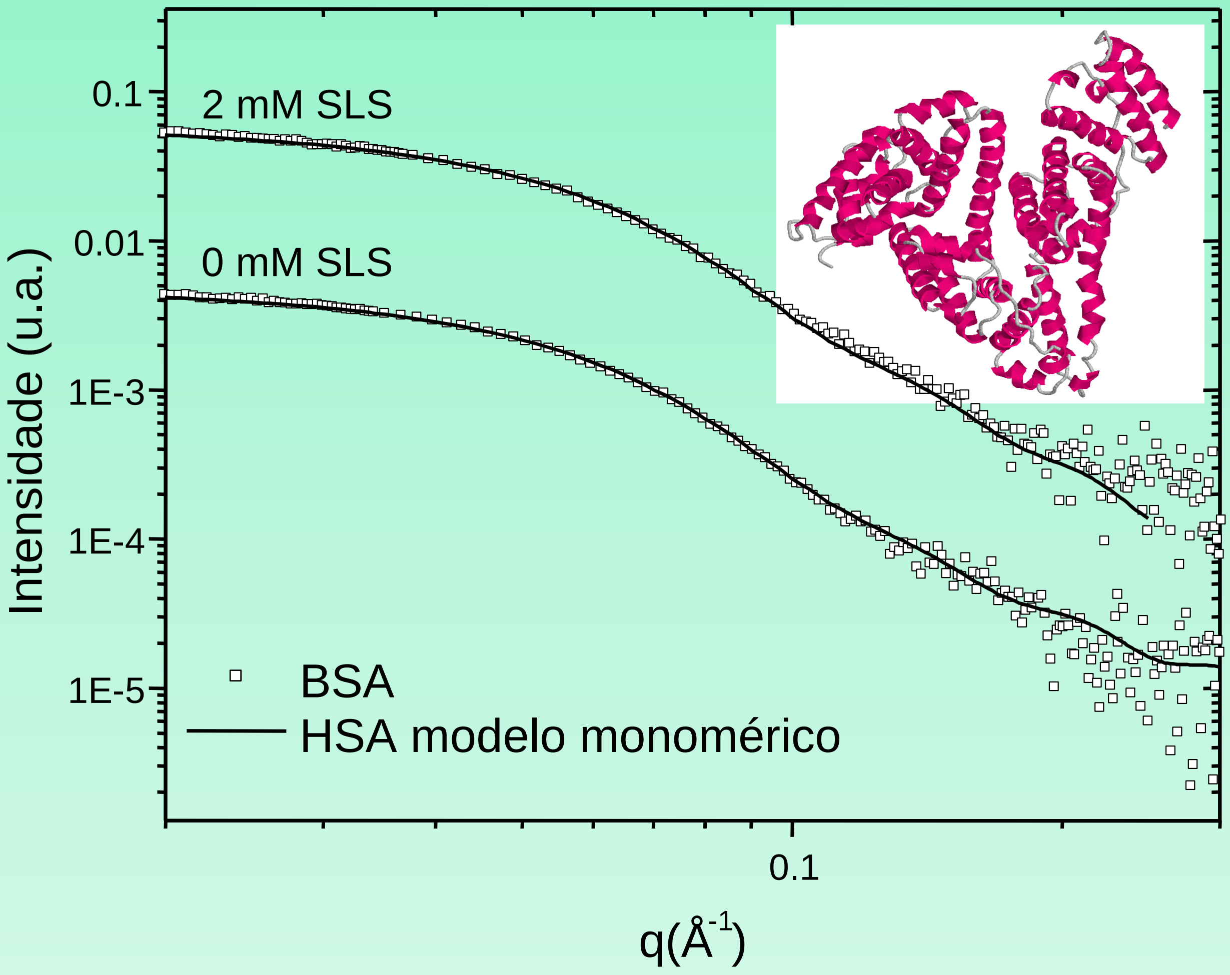
1 wt % BSA, 20 mM sodium succinate, pH 5.6



Results - *SAXS beam line LNLS*

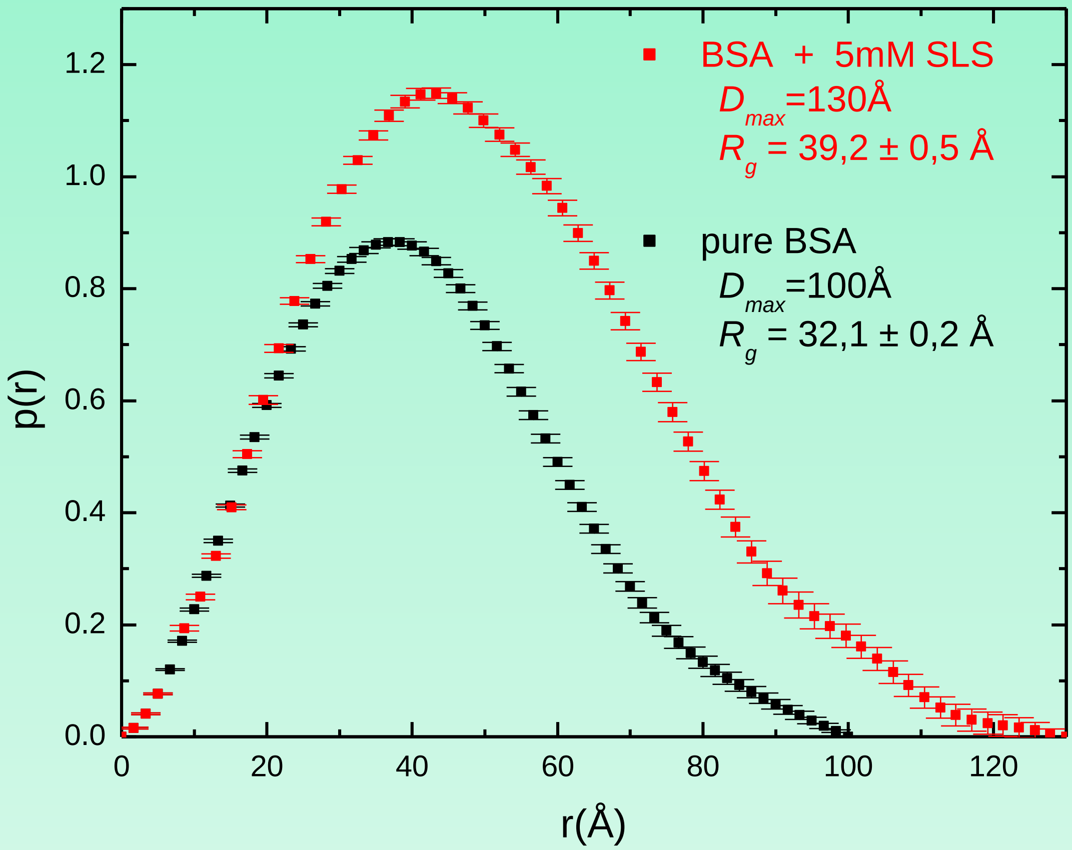


1 wt % BSA, 20 mM sodium succinate, pH 5.4



Crysol software- Svergun et al, J.App.Cryst.1995

1 wt % BSA, 20 mM sodium succinate, pH 5.4



Analysis Method for [SLS] >10 mM

Pearl-Necklace Model

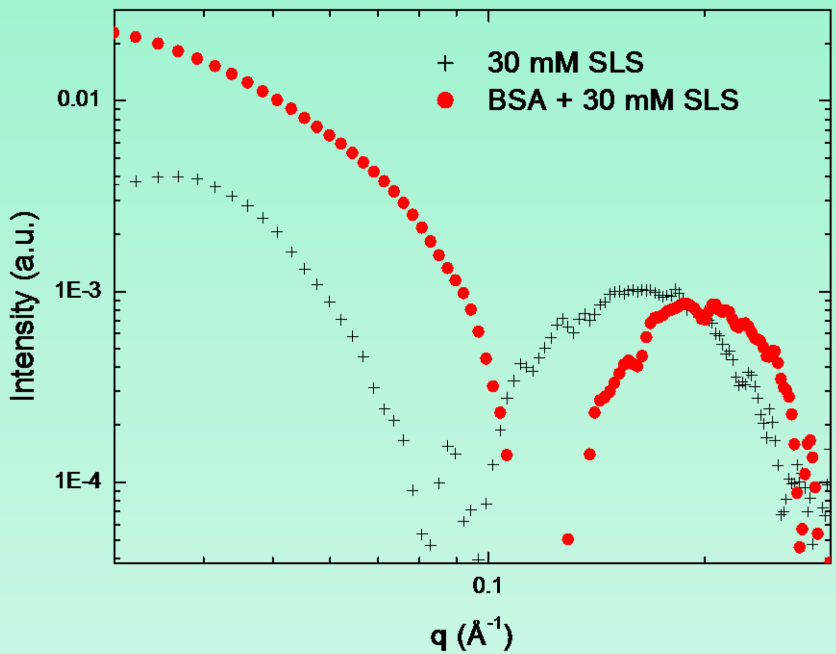
Form Factor P(q)→ prolate ellipsoid micelles

Interference Function S(q) → fractal model (SANS, Teixeira, Chen , PRL, 1986)

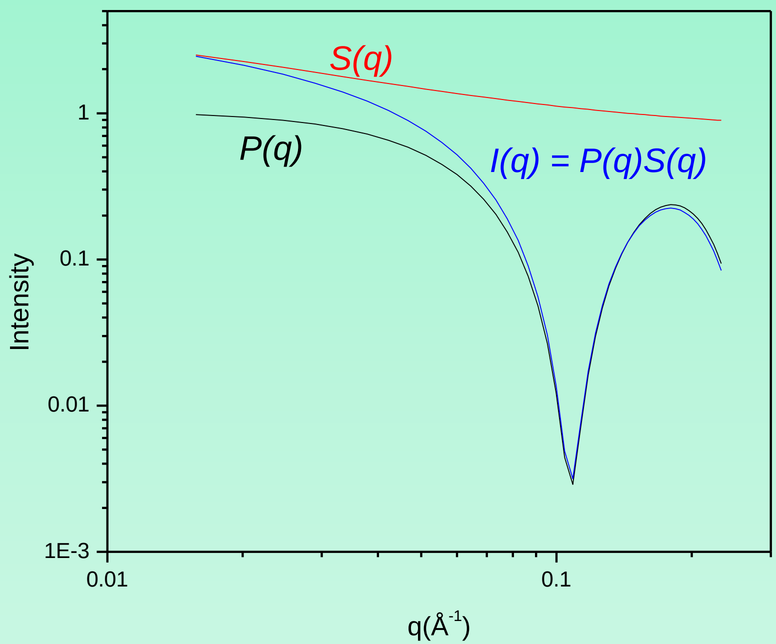
$$S(q) = 1 + \frac{1}{(qR)^{d_f}} \frac{d_f \Gamma(d_f - 1)}{\left[1 + (q\xi)^{-2}\right]^{(d_f - 1)/2}} \, sen\left[(d_f - 1)\tan^{-1}(q\xi)\right]$$

d_f =fractal dimension → 3 compact arrangement; → < 3 open structures packing

ξ = correlation length (persistent length for polymers)

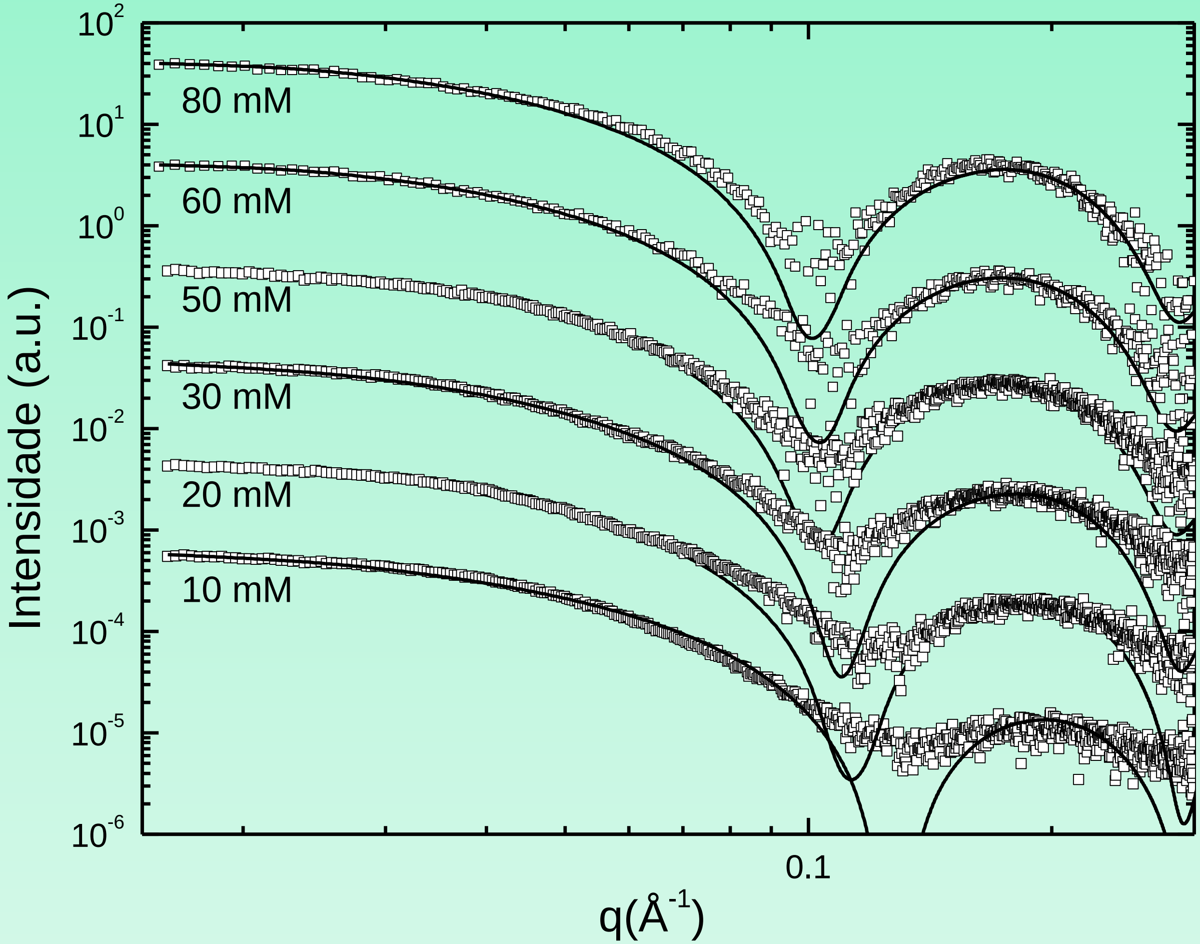


30 mM SLS
BSA + 30 mM SLS
0.01
1E-3
1E-4
0.1
Intensity (a.u.)
q (Å^{-1})



S(q)
P(q)
I(q) = P(q)S(q)
1
0.1
0.01
1E-3
0.01
0.1
Intensity
q(Å^{-1})

1 wt % BSA, 20 mM sodium succinate, pH 5.4



1 wt % BSA, 20 mM buffered, pH 5.4

Rpar = 16.7 Å σ = 7.5 ± 0.5 Å ρ_{pol} = 0.4 e/Å^3

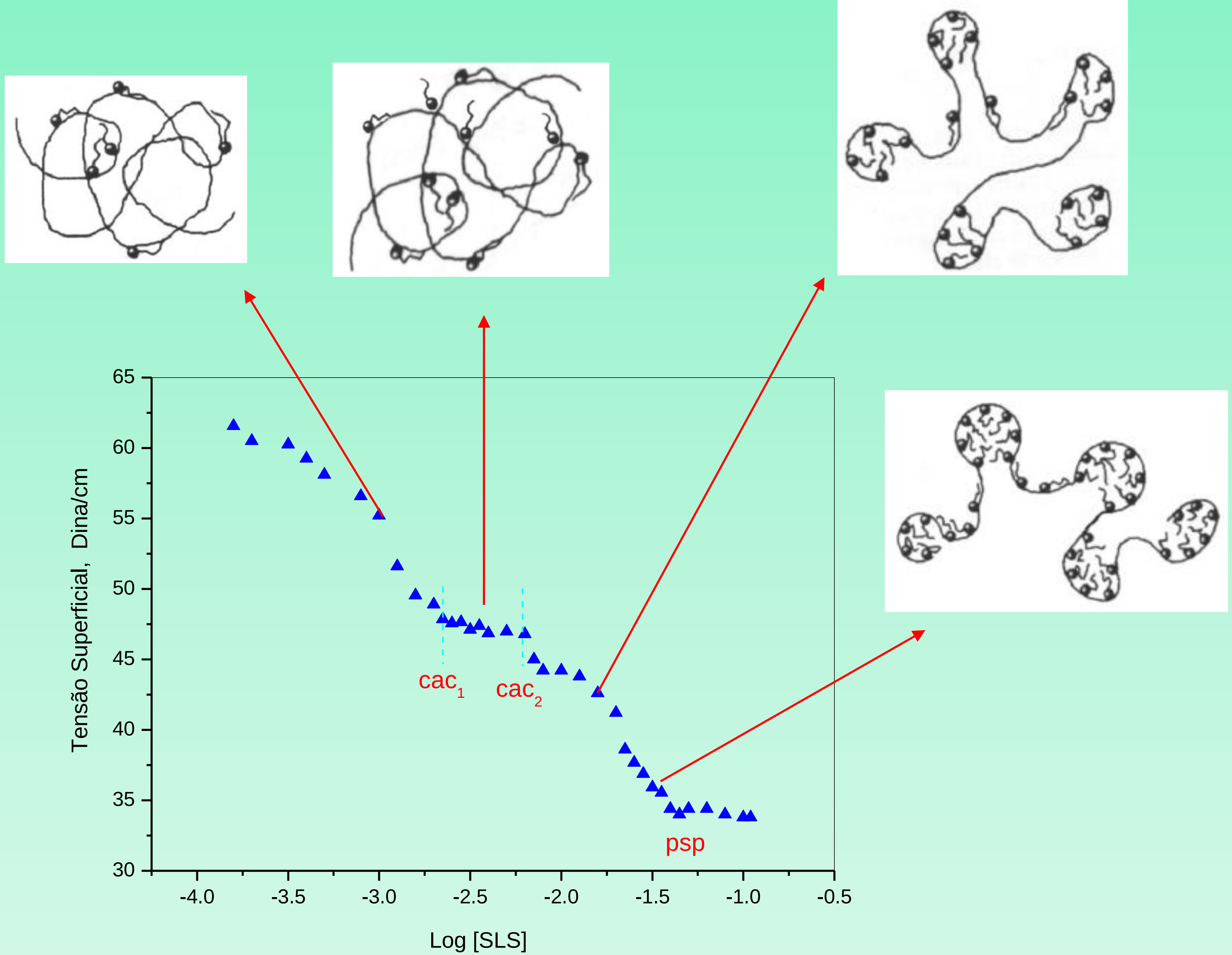
SLS	10 mM	20 mM	30 mM	50 mM	60 mM	80 mM	
d_f	1.01	1.01	1.01	1.01	1.01	1.01	
ξ (Å)	100	100	100	100	100	100	
ν	1.0	1.1	1.2	1.3	1.3	1.3	
n	56	61	67	72	72	72	
n	27±2	36	40	59±6	----	66	fluorescence

psp

⇒ 5 micelles/protein at 50 mM SLS

⇒ Interaction with polar head

⇒ after psp →micelles in solution may coexist with those complexed (same size and shape)



J. Coll. Interf. Science, 262, 400 (2003)

J. Colloid and Interf. 277, 285-291 Science (2004); 471-182 (2004)

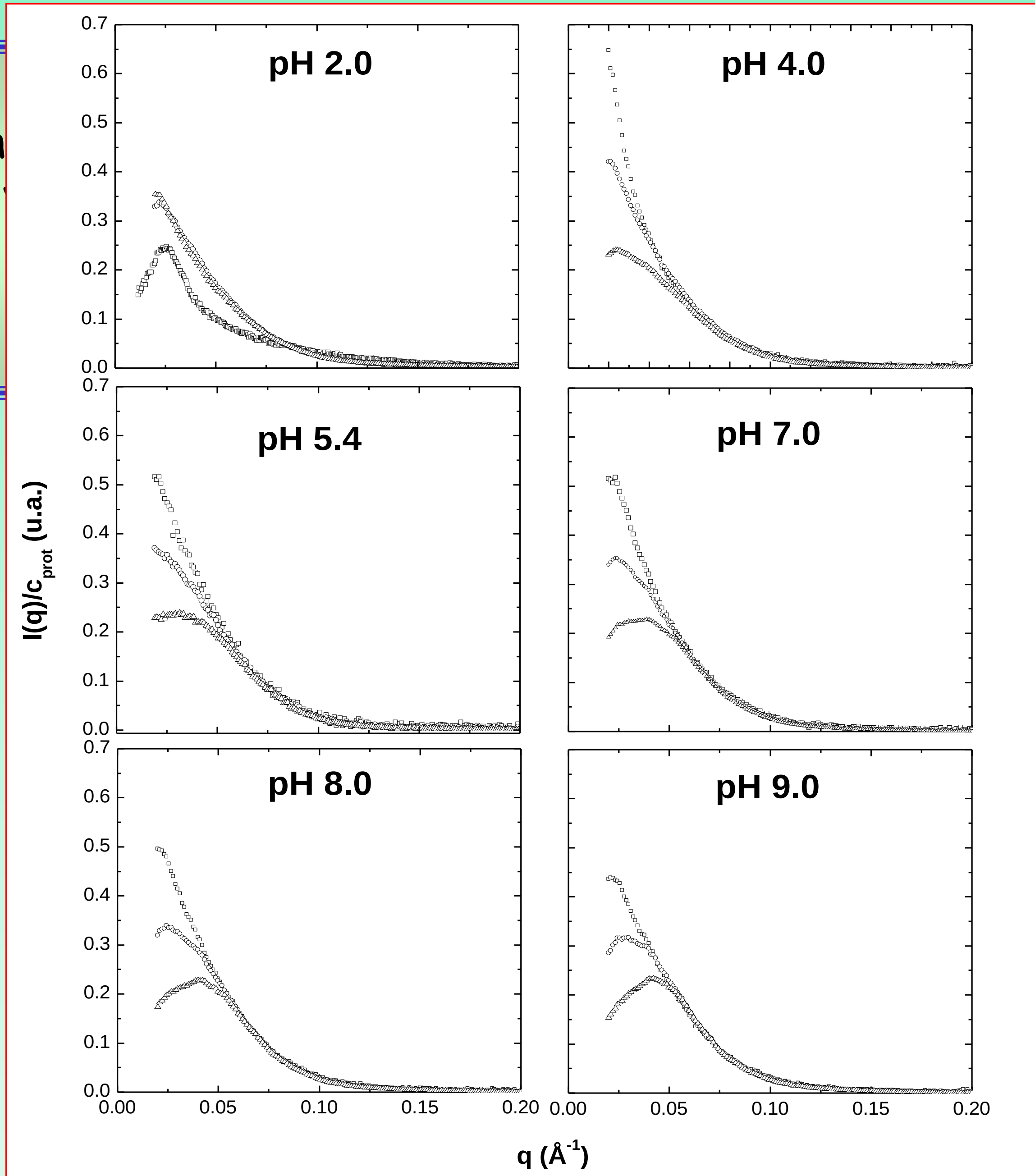
And the protein concentration effect???

BSA is found at 50 mg/ml in the serum

Influen ns induced by pH of

Bo (SAXS) study

tri



10 mg/ml,

25 mg/ml

50 mg/ml

$$U_{pp}(r) = U_{HS}(r) + U_{Coulomb}(r) + U_{Atr}(r)$$

$$U_{Atr}(r) = -J\left(\frac{\sigma_{eff}}{r}\right)e^{-\frac{\left(r-\sigma_{eff}\right)}{d}}$$

J profundidade do potencial

d alcance do potencial

J. Narayanan & X.Y. Liu, Protein interactions in under saturated and supersaturated solutions: A study using light and X-ray scattering; **Biophys. J**. 84, 523 - 532 (**2003**).

Relação de Fechamento : Random Phase Approximation (RPA)

$$c(r) = c_0(r) - \beta w(r)$$

$w(r) = U_{Coulomb}(r) + U_{atr}(r)$

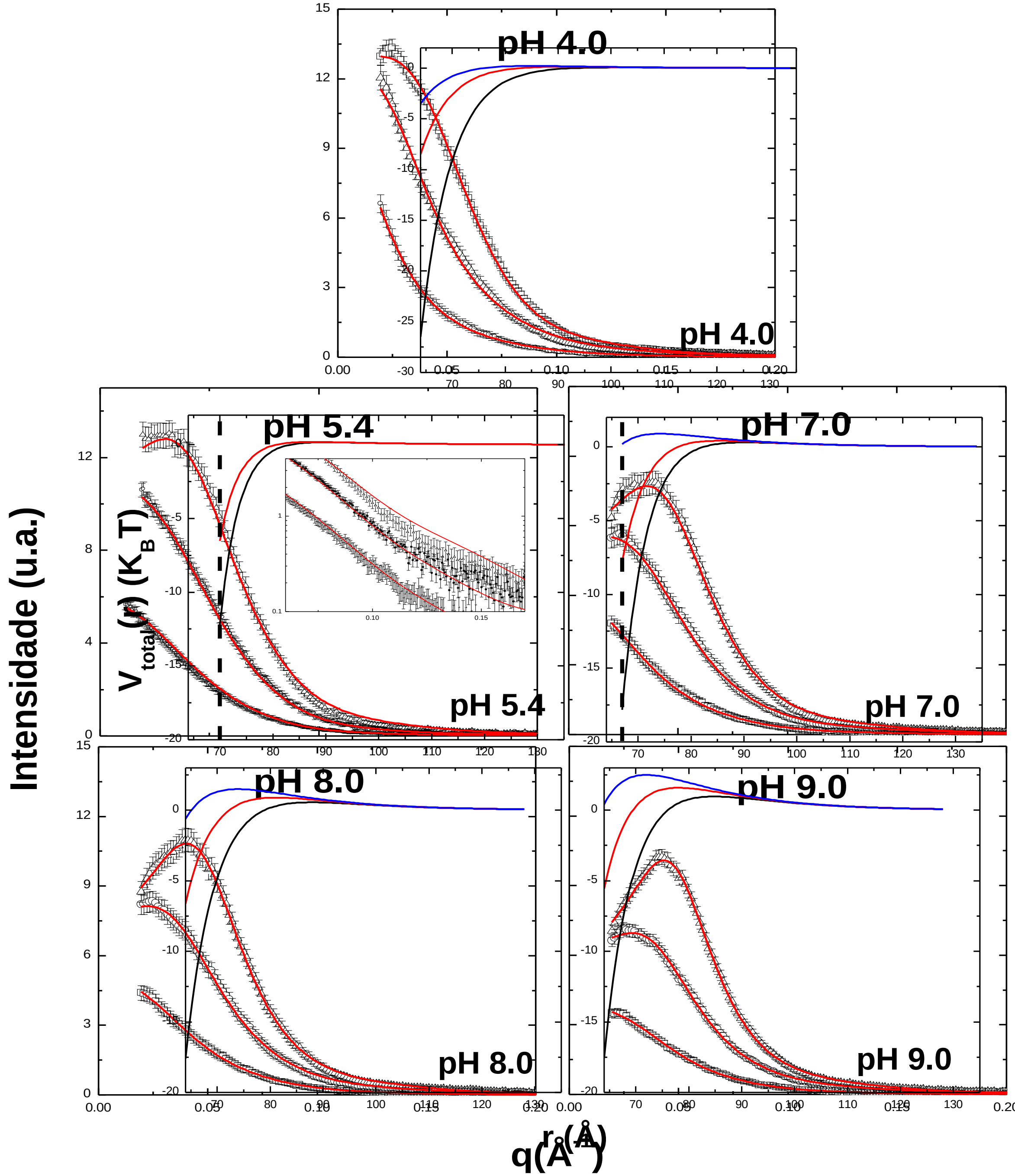
termo perturbativo

$$S(q) = \frac{S_0(q)}{1 + \beta n_p S_0(q)\phi(q)}$$

$\phi(q)$ é a transformada de Fourier de *$w(r)$*

pH	4.0	5.4	7.0	8.0	9.0
[BSA] (mg/ml)	*10 25 50*	*10 25 50*	*10 25 50*	*10 25 50*	*10 25 50*
$J\ (K_BT)^*$	28 10 5	13 7 --	20 10 2	23 12 6	26 14 8
d (Å)**	6.4	3.5	5.0	6.4	6.4
σ_{ef} (Å)*	64	70	67	64	64
Força Iônica*** (mM)	27	27	29	32	36
z_{ef} (e)	10(2)	8(1)	13(2)	20(2)	26(2)

Genfit - Spinozzi, F. http://www.isf.univpm.it/biophysics/software.htm

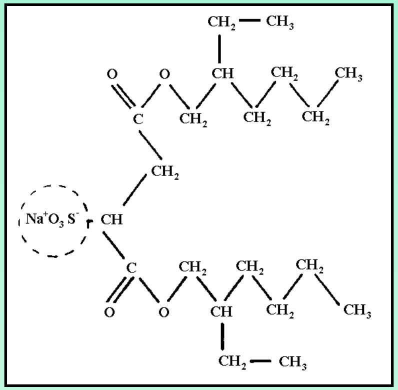


pH 4.0
pH 5.4
pH 7.0
pH 8.0
pH 9.0
Intensidade (u.a.)
V_total(r) (K_B T)
q(Å^-1)
r (Å)

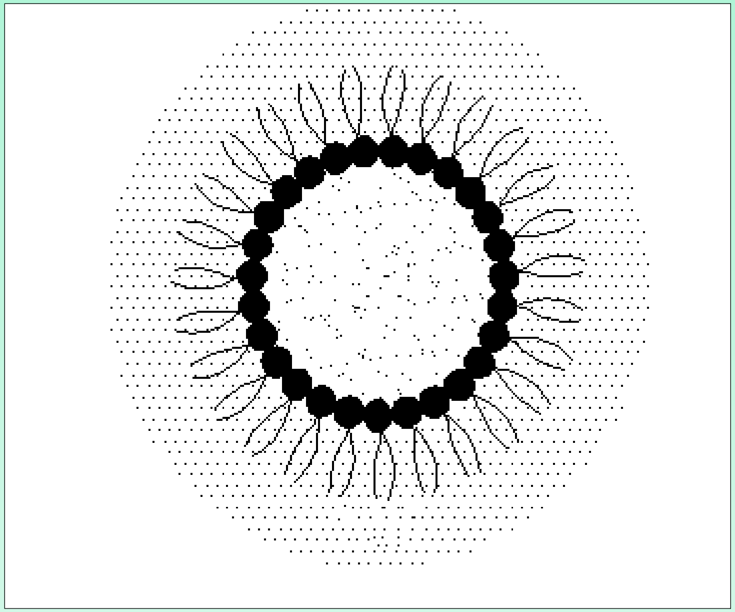
Urea Effect on AOT/*n*-Hexano/Water Reversed Micelles

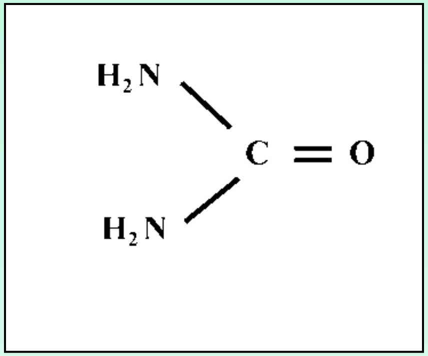
Carmem L. C. Amaral, M. J. Politi (IQUSP) e R. Itri (IFUSP)

AOT

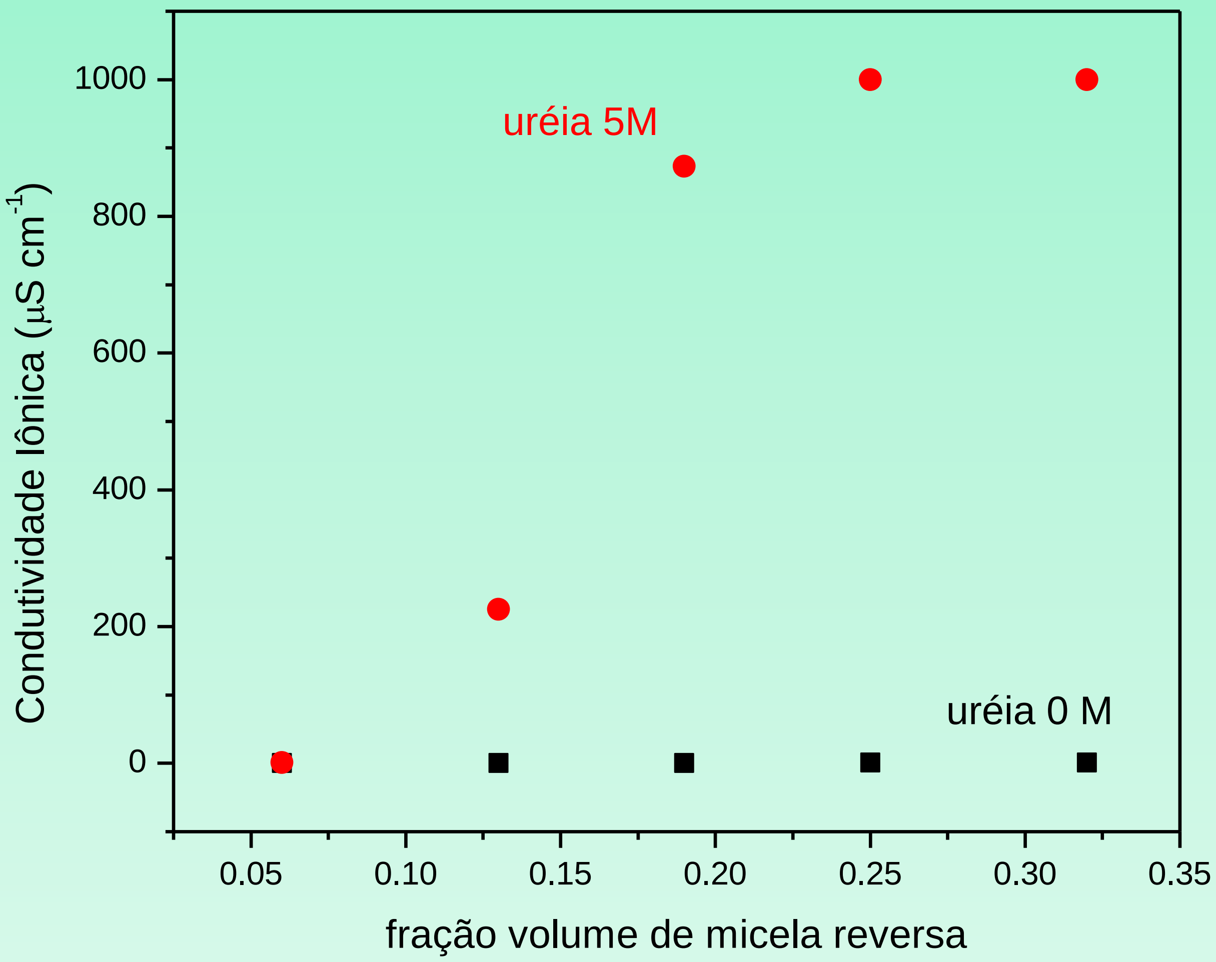


$$W = [water]/[AOT]$$



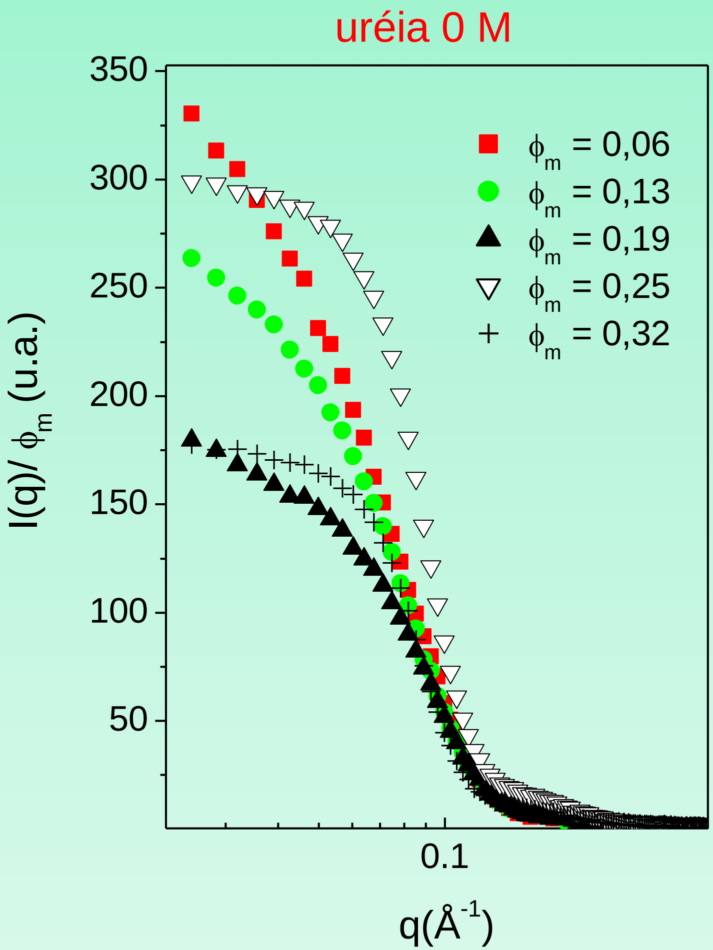


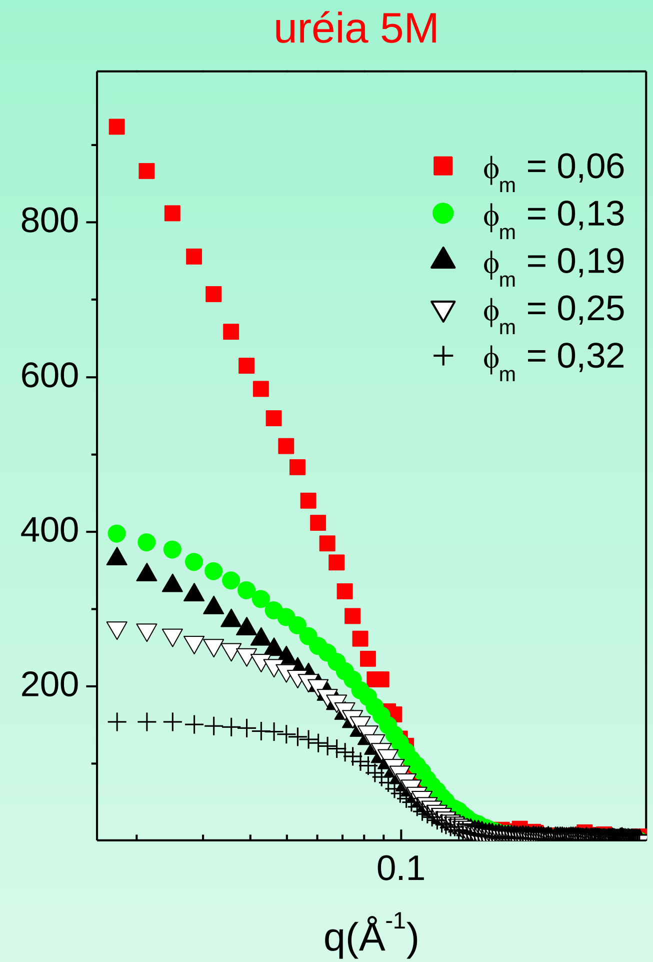
Conductivity W= [water]/[AOT] = 10



SAXS :conventional generator (LCr)

W= [water]/[AOT] = 10





	Static Light Scattering			SAXS		
[uréia] M	R_H (Å)	$Coef_V$	n	ϕ_m	R_g (Å)	R_m (Å)
0	35,0±0,9	6,0±0,1	202±15			
				0,06	22,8±0,1	29,4±0,2
				0,13	22,1±2,0	28,5±2,4
				0,19	22,0±2,0	28,4±2,4
3	43,0±1,5	-2,4±1,1	200±38			
5	55,0±2,9	-6,5±0,8	197±48			
				0,06	23,3±0,3	30,1±0,3
				0,13	21,9±2,0	28,3±2,4
				0,19	25,2±2,0	32,5±2,4

Langmuir, 12, 4638-4643 (1996)

Analysis Method

Form Factor → polydisperse spheres

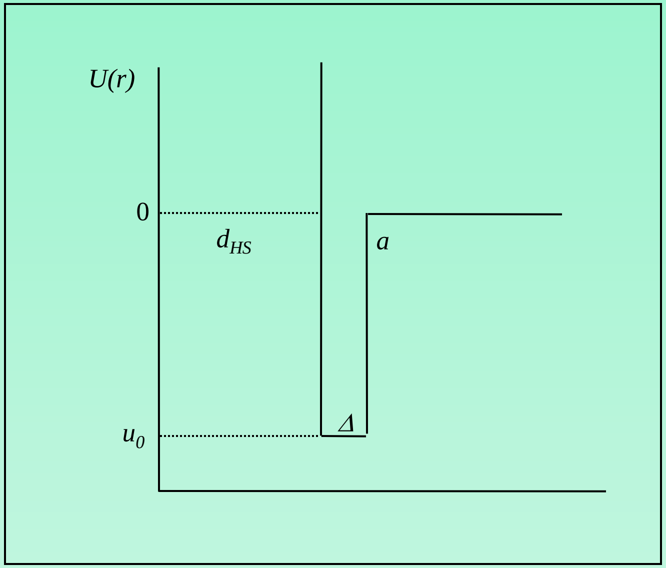
$$P(q) = \langle F^2(q) \rangle = \int_0^\infty F^2(q)\ f(R)\ dR$$

$$\langle F(q) \rangle^2 = \left[\int_0^\infty F(q)\ f(R)\, d\,R \right]^2$$

$$f(R) = \left(2\pi\varpi^2\right)^{-\frac{1}{2}} \exp\left[-\left(R - \overline{R}\right)^2 / 2\varpi^2 \right]$$

with ϖ = Gaussian distribution width

Interference Function



U(r) = + infinito $\quad 0 < \mathrm{r} < d_{HS}$

$= k_B T \ln[12\tau\Delta/a] \quad d_{HS} < \mathrm{r} < a$

$= 0 \quad \mathrm{r} > a$

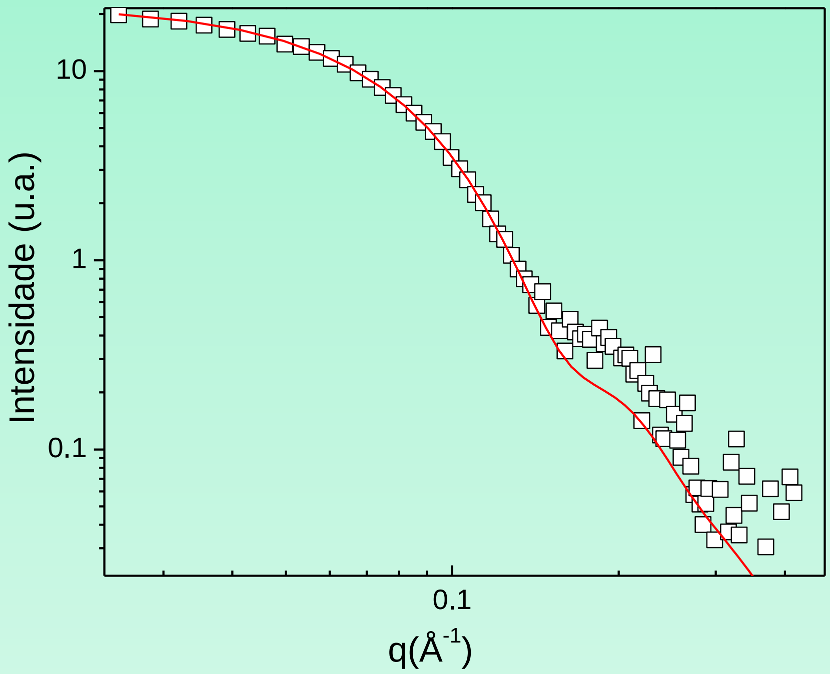
Adhesion parameter: $\tau = \left(\frac{12\Delta}{a}\right)^{-1} \exp\left(\frac{u_0}{k_B T}\right)$

$$S(qa) = [1 - n_p c(qa)]^{-1}$$

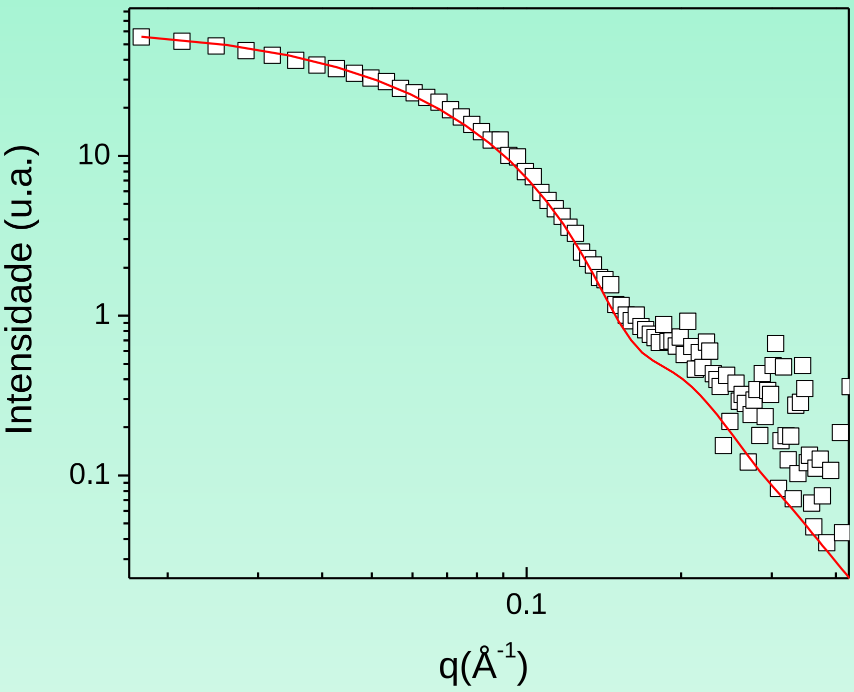
PY

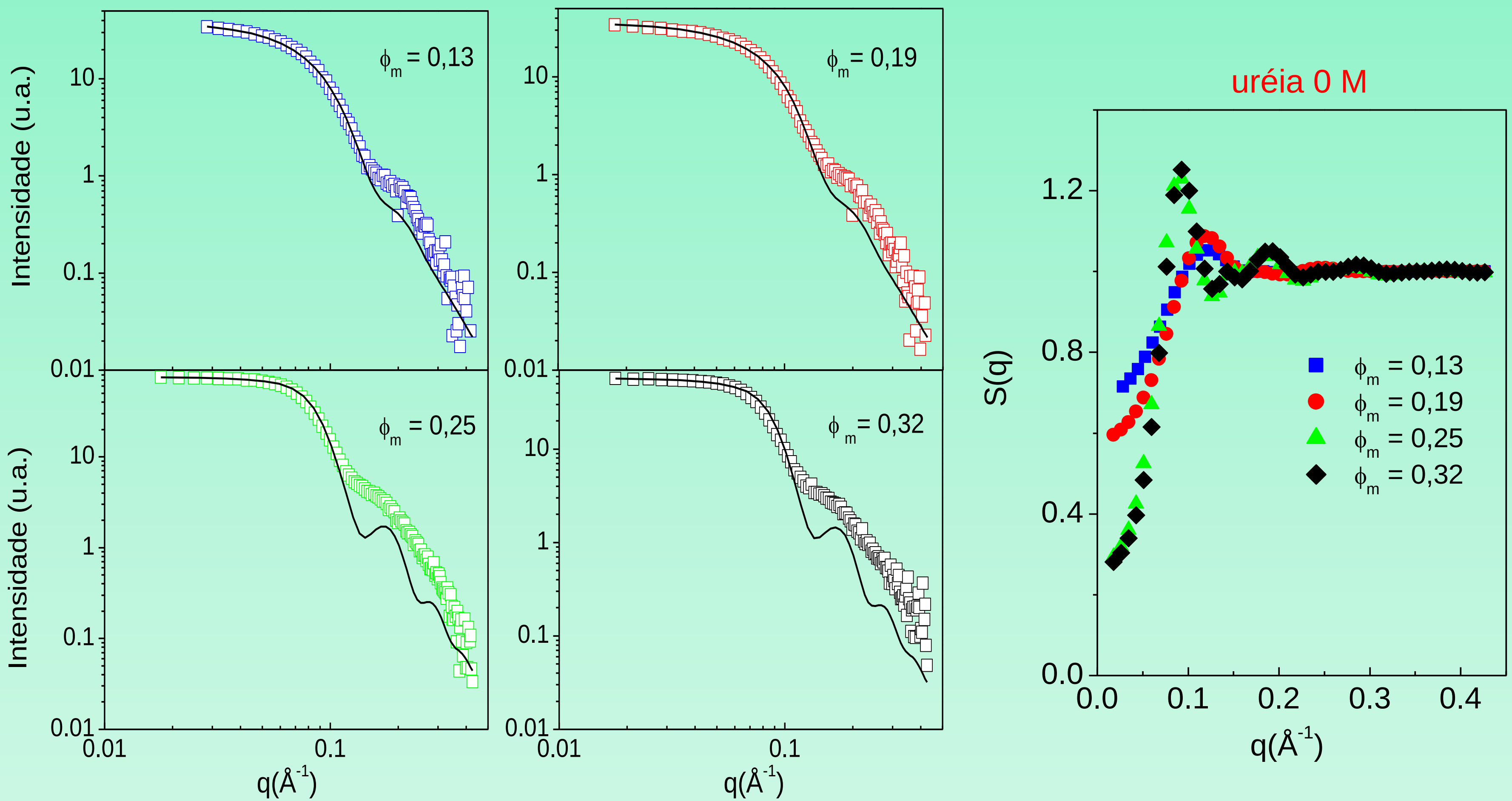
Results: $\phi_m = 0{,}06$

0 M Uréia

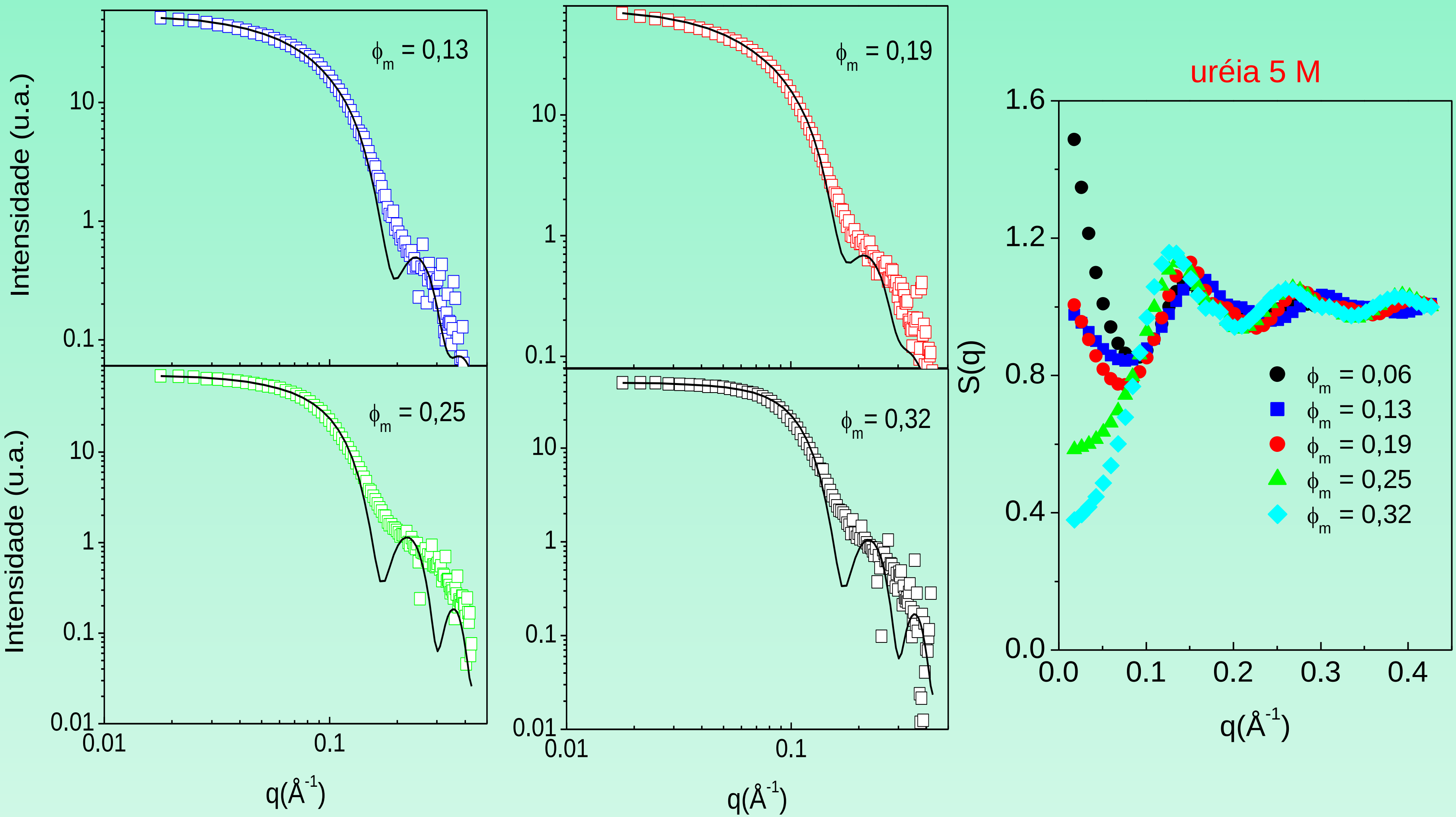


5 M Uréia





Intensidade (u.a.)
10
1
0.1
0.01
ϕ_m = 0,13
ϕ_m = 0,19
ϕ_m = 0,25
ϕ_m = 0,32
0.01
0.1
q(Å^{-1})
uréia 0 M
S(q)
1.2
0.8
0.4
0.0
0.1
0.2
0.3
0.4
ϕ_m = 0,13
ϕ_m = 0,19
ϕ_m = 0,25
ϕ_m = 0,32



J. Chemical Physics, 1999

ϕ_m	$\bar{R}_c$ (Å)	R_{HS} (Å)	$\varpi/\bar{R}_c$	u_0 ($unid.\ k_BT$)	Δ (Å)	[Uréia M
0,06	24,0 ± 1,0	-----------	0,20 ± 0,05	*sem*	*interferência*	0
	24,5 ± 0,5	24,5 ± 0,5	0,18 ± 0,03	4,0 ± 0,1	0,8 ± 0,1	5
0,13	24,5 ± 0,5	24,5 ± 0,5	0,18 ± 0,03	0	0	0
	22,5 ± 0,5	22,5 ± 0,5	0,08 ± 0,03	3,0 ± 0,1	0,8 ± 0,1	5
0,19	24,5 ± 0,5	24,5 ± 0,5	0,18 ± 0,03	0	0	0
	24,0 ± 1,0	24,0 ± 1,0	0,12 ± 0,03	3,2 ± 0,2	0,8 ± 0,1	5
0,25	29,5 ± 0,5	34,0 ± 0,5	0,12 ± 0,02	0	0	0
	24,5 ± 0,5	24,5 ± 0,5	0,07 ± 0,02	2,5 ± 0,1	0,8 ± 0,1	5
0,32	31,0 ± 0,5	33,5 ± 0,5	0,12 ± 0,02	0	0	0
	25,0 ± 0,5	25,0 ± 0,5	0,07 ± 0,02	1,8 ± 0,1	0,8 ± 0,1	5

Obrigada!

itri@if.usp.br