Gruppo di ricerca

Fluidi complessi ed interfasi

Lab. 20-21-22 – Dipartimento di Fisica

- Sviluppo di microscopia a scansione di sonda su film di idratazione
- Proteine e stati aggregati in soluzione

Ricercatori strutturati:

Marcello Carla'
Cecilia Gambi
Leonardo Lanzi (in congedo)

Ricercatori precari:

Stefania Marchetti
David Dolci
Federico Bacci

Fonti di finanziamento (2007-2009)

Miur 60% (10.42 k€)
Ente Cassa di Risparmio di Firenze 2007 (39 k€)
Ente Cassa di Risparmio di Firenze 2008 (20 k€)

Prevista richiesta di un assegno cofinanziato per il 2010.

http://fluidi.fisica.unifi.it/ http://spm.polosci.unifi.it

Gruppo di ricerca

Fluidi complessi ed interfasi

Collaborazioni:

- Dip. Di Chimica – Universita' di Firenze
- Istituto Sistemi Complessi CNR – Firenze
- Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali
- Centro per lo Studio delle Dinamiche Complesse (CSDC)
- Dip. Anatomia, Istologia e Medicina Legale – Universita' di Firenze
- Dip. Di Fisica, Universita' di Roma - La Sapienza
- Dip. Di Fisisca – Universita' di Messina
- Dip. Di Fisica – Universita' di Trento
- Laboratorio Lèon Brillouin, Saclay

Proteine in soluzione: folding-unfolding e processo di aggregazione

collaborazioni: ISC-CNR Firenze, Dip. di Anatomia, Istologia e Medicina legale, Universita` di Firenze.

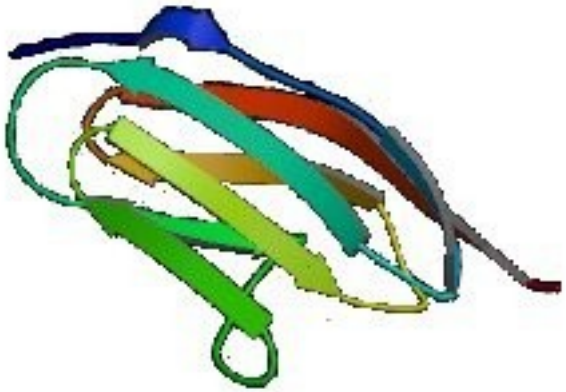
Beta-connettina del muscolo cardiaco umano

Ottamero di Ig della banda elastica: I27-I34

Singolo dominio ellissoidale: asse lungo 4.3 nm
e asse corto 2.1 nm

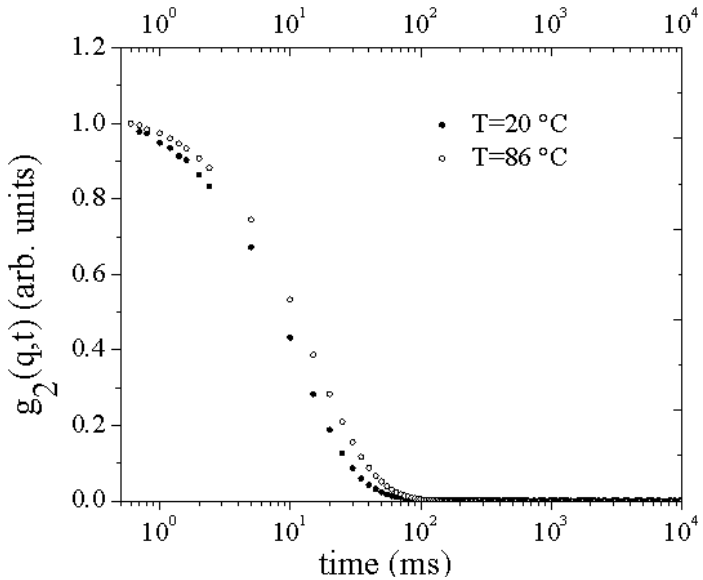
Beta sheet formato da 80 amminoacidi

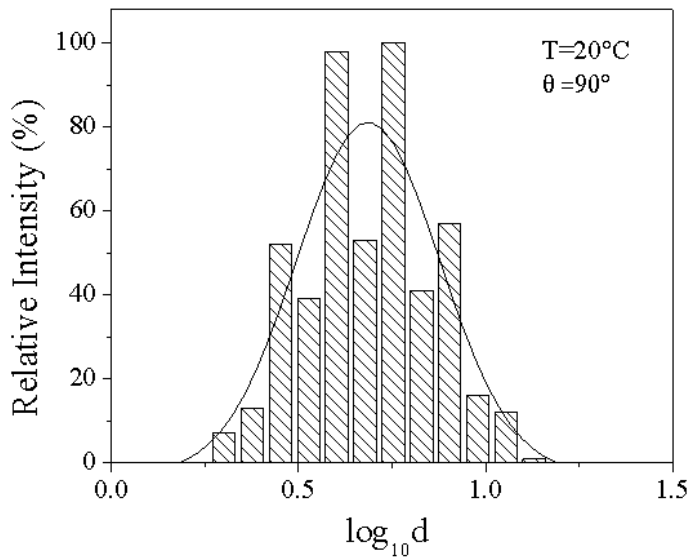
Peso molecolare: 87 kDa, concentrazione C= 0.1 mg/ml

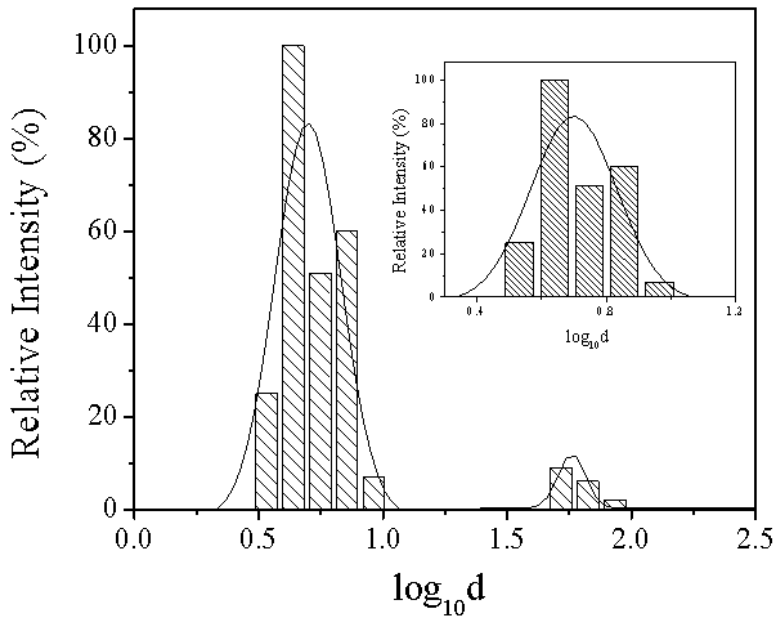


S. Marchetti, F. Sbrana, R. Raccis, L. Lanzi, C. M. C. Gambi, M. Vassalli, B. Tiribilli, A. Pacini, A. Toscano "Dynamic light scattering and atomic force microscopy imaging on fragments of beta-connectin from human cardiac muscle", Phys. Rev. E, 77, 021910 (2008).
V. Pini, B. Tiribilli, C.M.C. Gambi, M. Vassalli “Dynamical characterization of vibrating AFM cantilevers forced by photothermal excitation” Phys. Rev. B, 81, 054302 (2010).

Scattering di luce laser dinamico (DLS)







Risultati di DLS

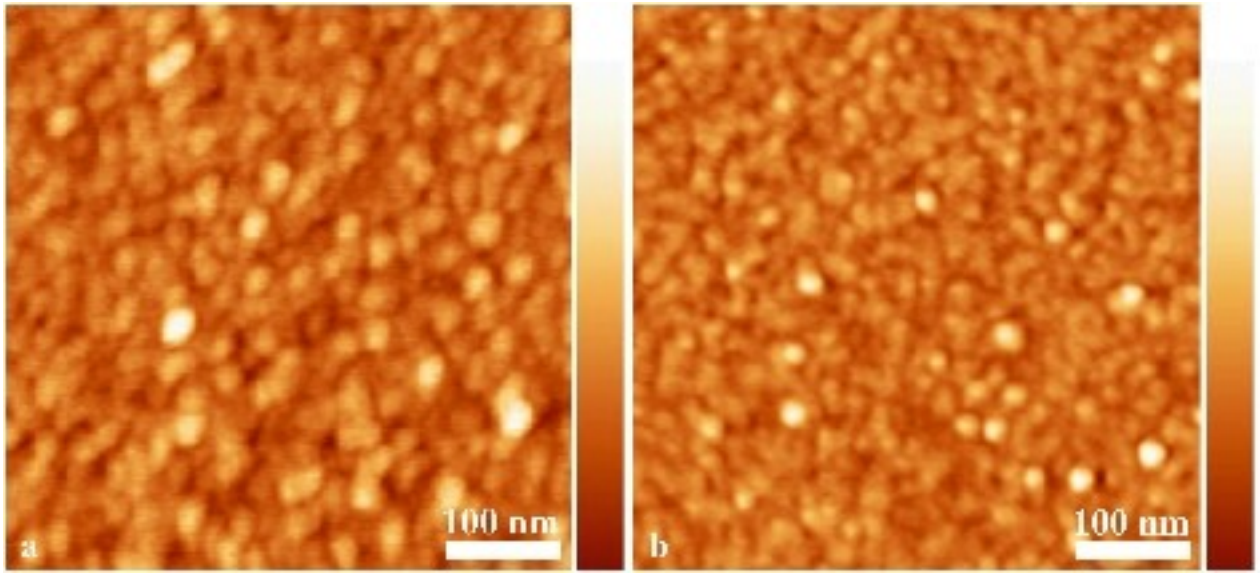
Ottamero:
Forma nativa
Dimensione della proteina (diametro idrodinamico):

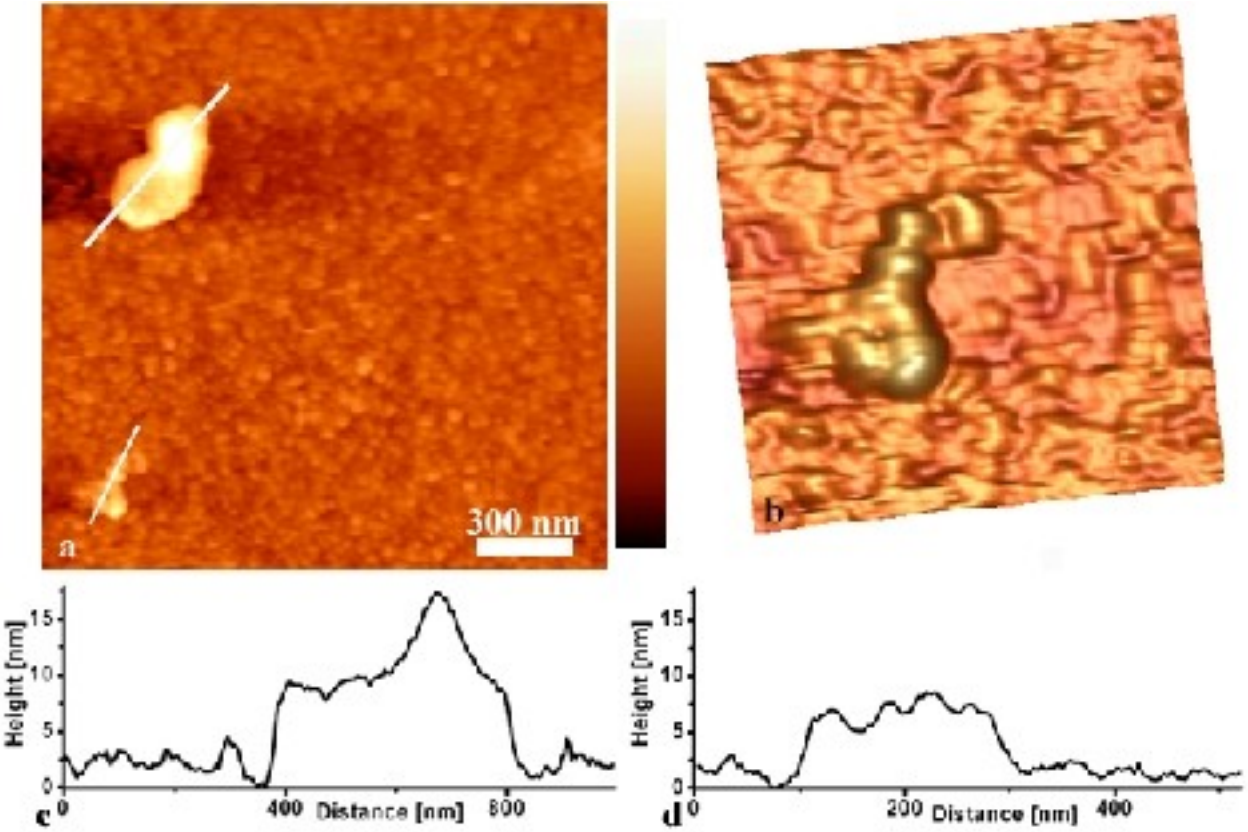
5.18 +/- 0.07 nm temperatura 20 C
5.0 +/- 0.8 nm temperatura 50 C
6.3 +/- 0.8 nm temperatura 60 C
14 +/- 1 nm temperatura 70 C denaturazione
22 +/- 1 nm temperatura 80 C denaturazione -- aggregati 14-33 nm
24 +/- 4 nm temperatura 86 C denaturazione -- aggregati 13-46 nm

25.2 +/- 0.9 nm temperatura 20 C denaturata

una volta innescato il processo di denaturazione, continua il processo di aggregazione

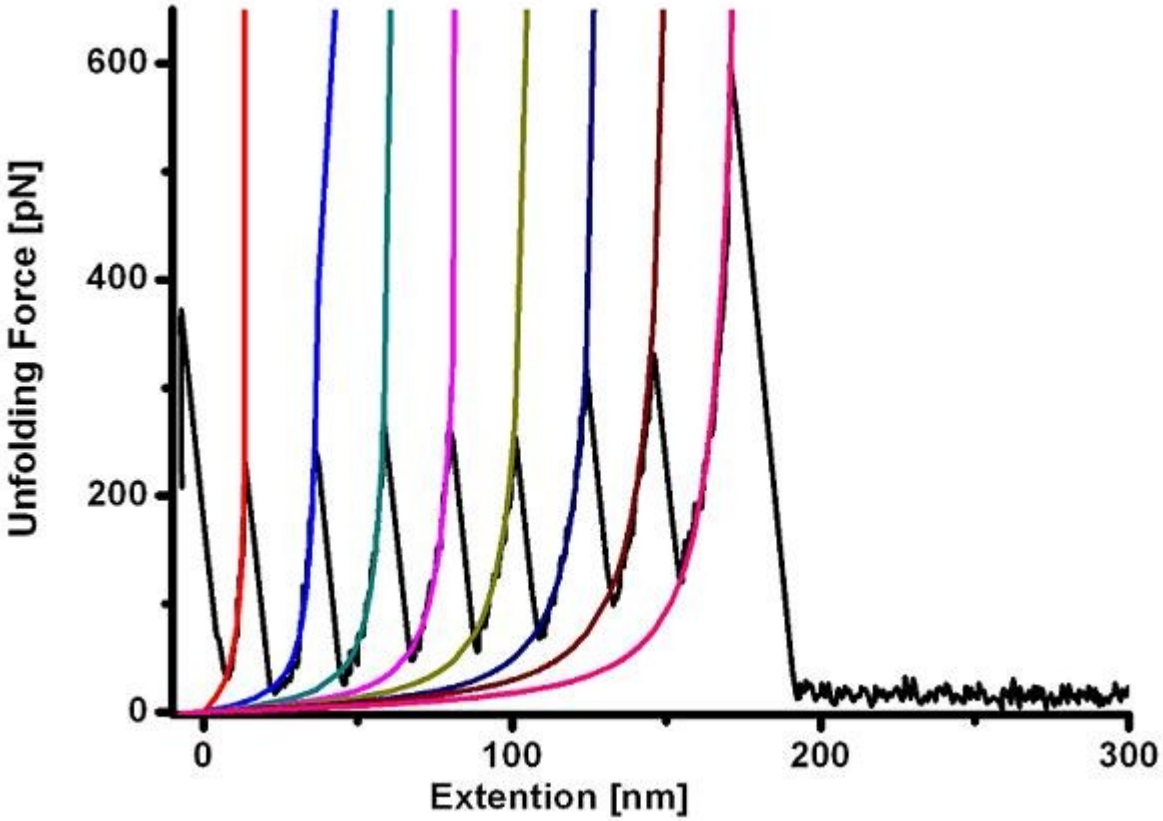
Microscopia a forza atomica




300 nm
a
b
Height [nm]
15
10
5
0
0
400
800
Distance [nm]
c
Height [nm]
15
10
5
0
0
200
400
Distance [nm]
d

Spettroscopia a Forza Atomica

Spettroscopia a Forza Atomica AFM



SAXS ANALYSIS OF PROTEINS IN SOLUTION

CHARGE	-13
ASSE MAGGIORE (A)	33
ASSE MINORE (A)	13
DIAMETRO EFFETTIVO (A)	49

Peptide in soluzione

collaborazioni: CSDC presso il Dip. di Fisica, Dip. di Chimica Universita` di Firenze.

Peptide 101-108 MOG-derivato; MW 1064 Dalton

fornito da Ditta Epsichem del Polo Scientifico e Tecnologico, Universita` di Firenze

purezza > 95 %

misure di Dicroismo Circolare confermano i dati della Simulazione di Dinamica Molecolare

C. Guardiani, S. Marsili, S. Marchetti, C. M. C. Gambi, P. Procacci, R. Livi
“Conformational structure of the MOG derived peptide 101-108 in solution”, (submitted).

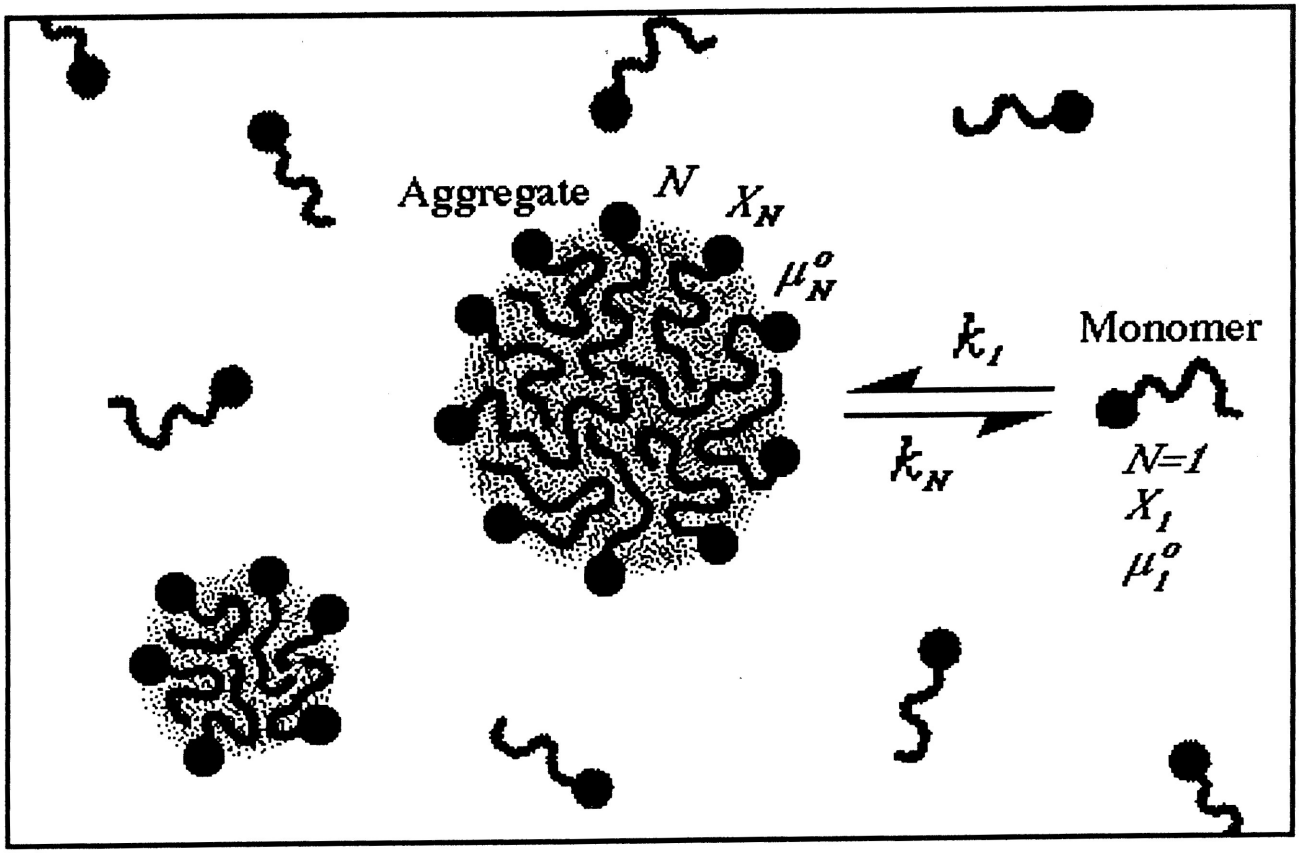
“Cluster Phases” di micelle con leganti macrociclici

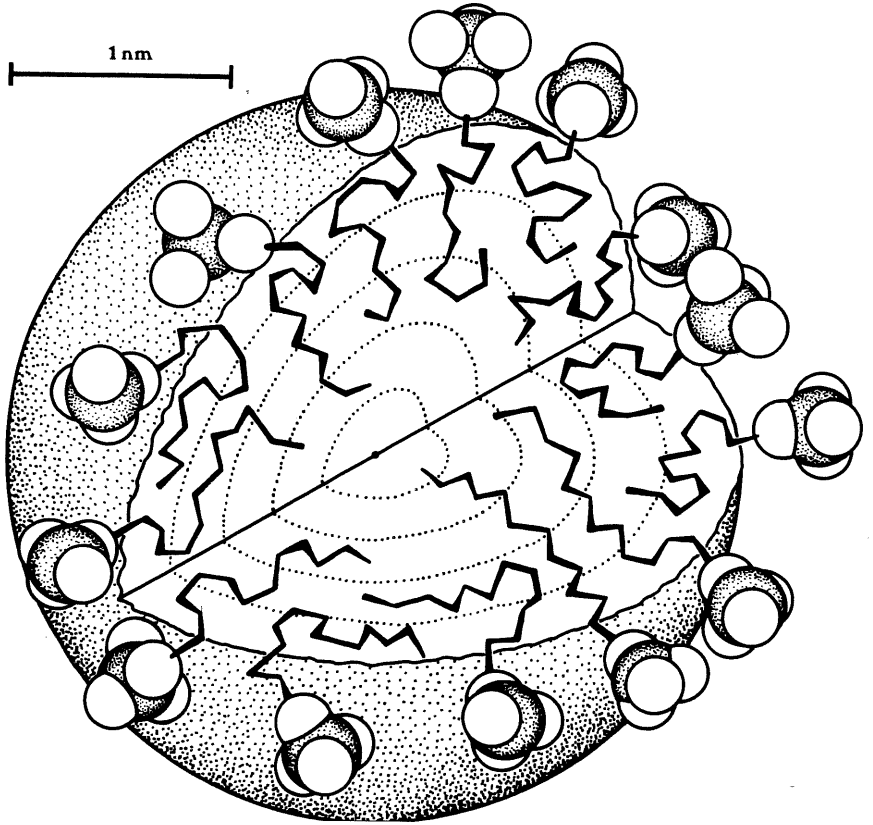
collaborazioni:

Dip. di Chimica Universita` di Firenze,

Dip. di Fisica Universita` di Roma “La Sapienza”,

Dip. di Fisica Universita` di Trento





Surfactant polar head: SO4- and Na+

Counterion: Na+

micelle composition

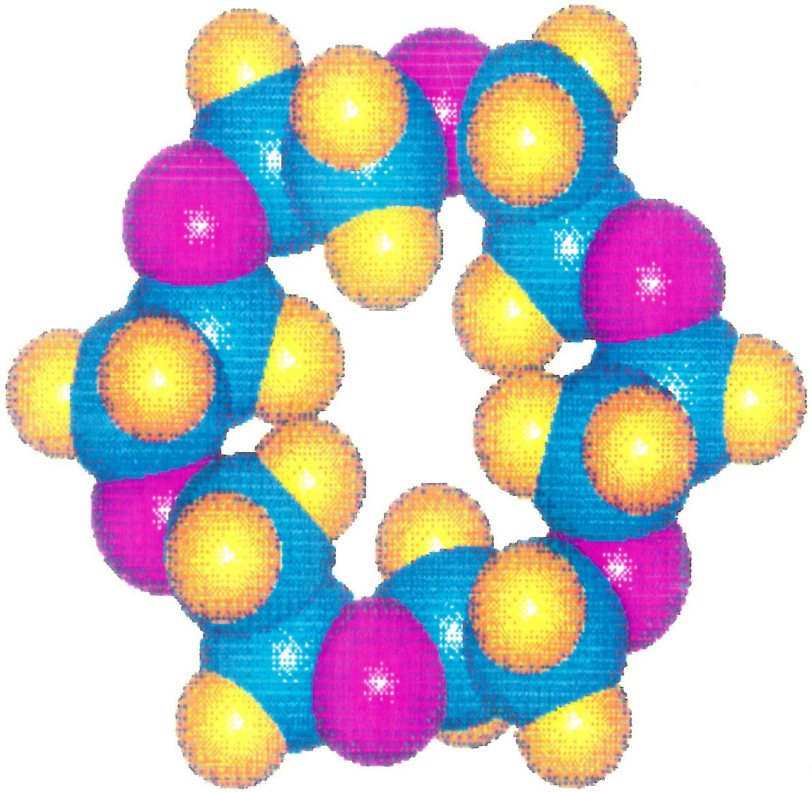
1) hydrophobic core

2) interfacial layer (polar head + some counterions + bound water)

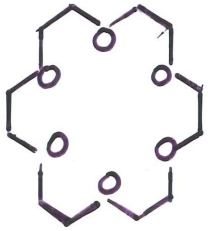
3) diffuse layer with the remaining unbound counterions in bulk water.

SDS concentration in water=260 mM CMC=8 mM at 20 C

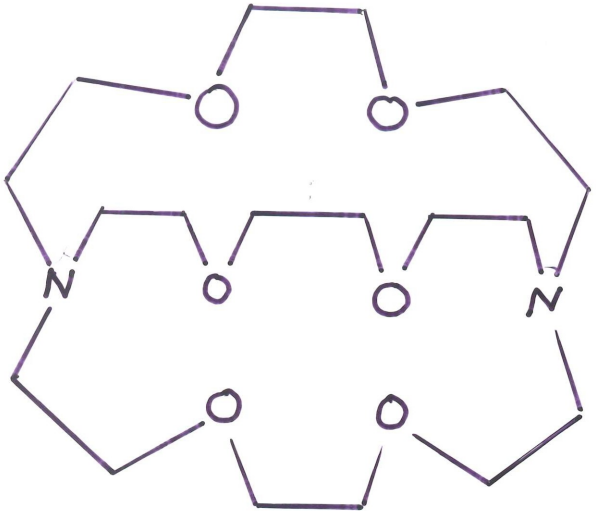
18- CROWN- 6



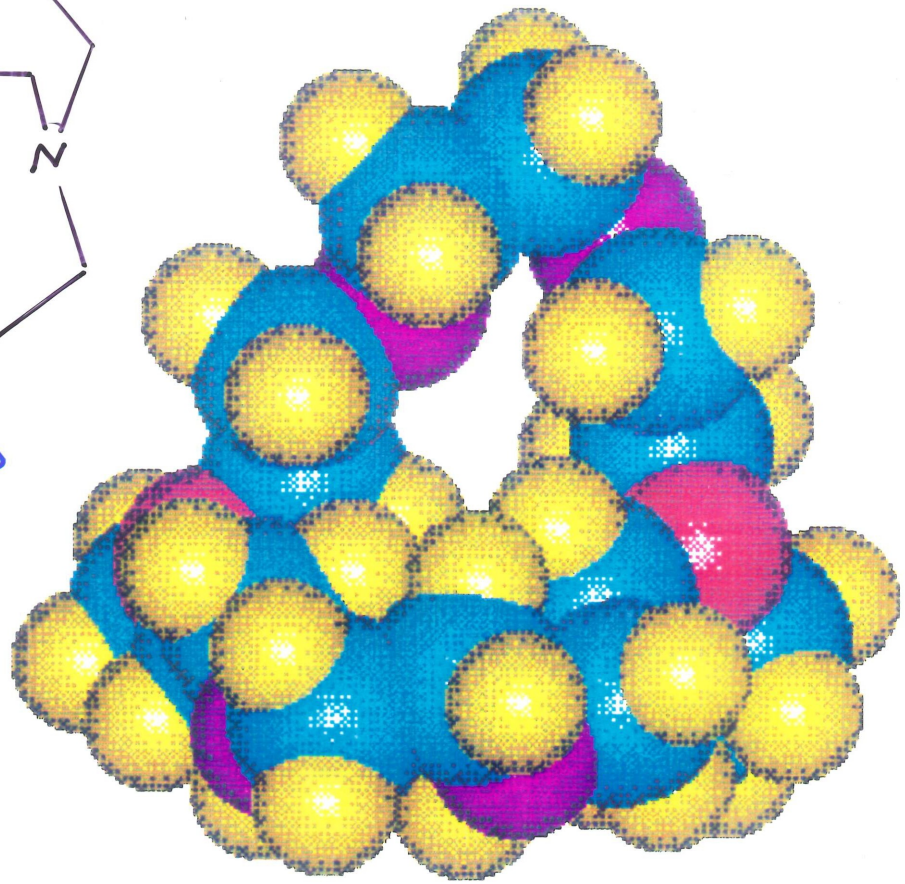
$C_{12}H_{24}O_6$



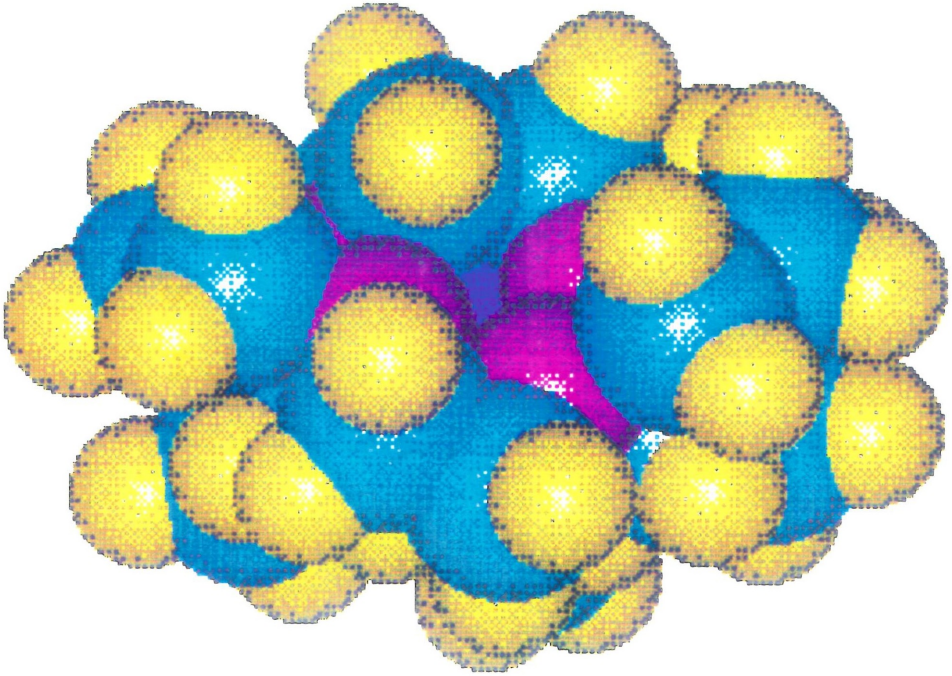
RAGGIO 5.7 Å



[2.2.2] - CRYPTAND

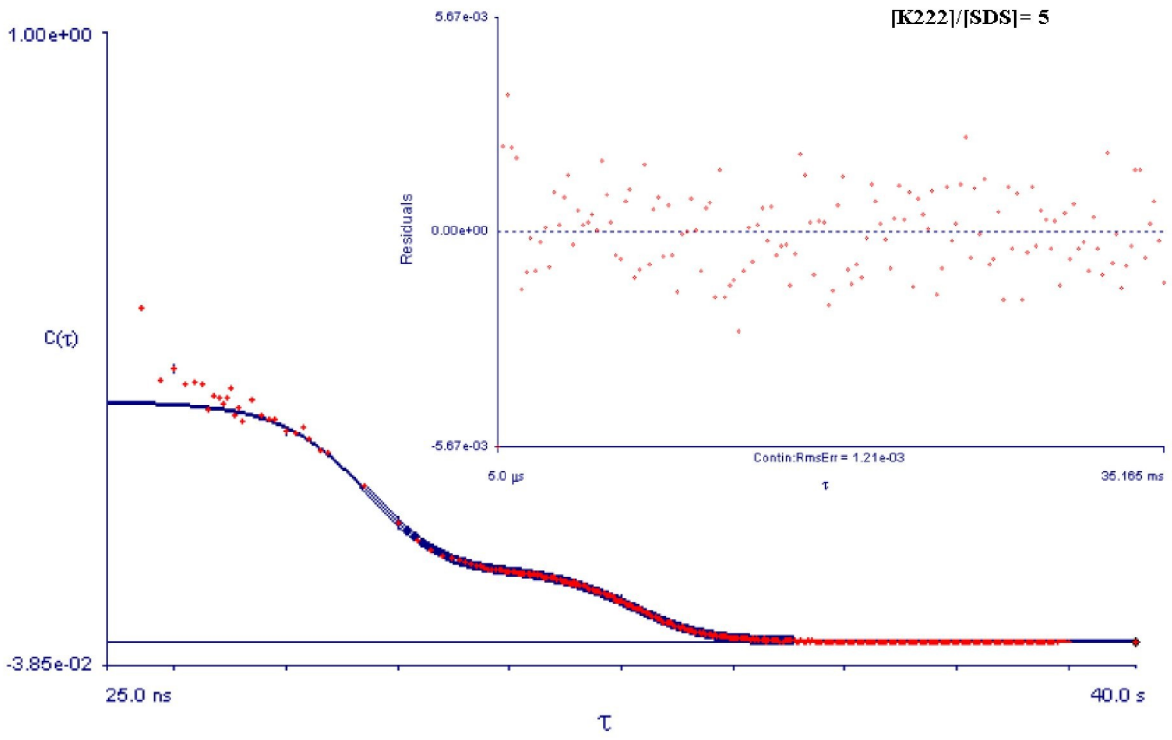


10 Å



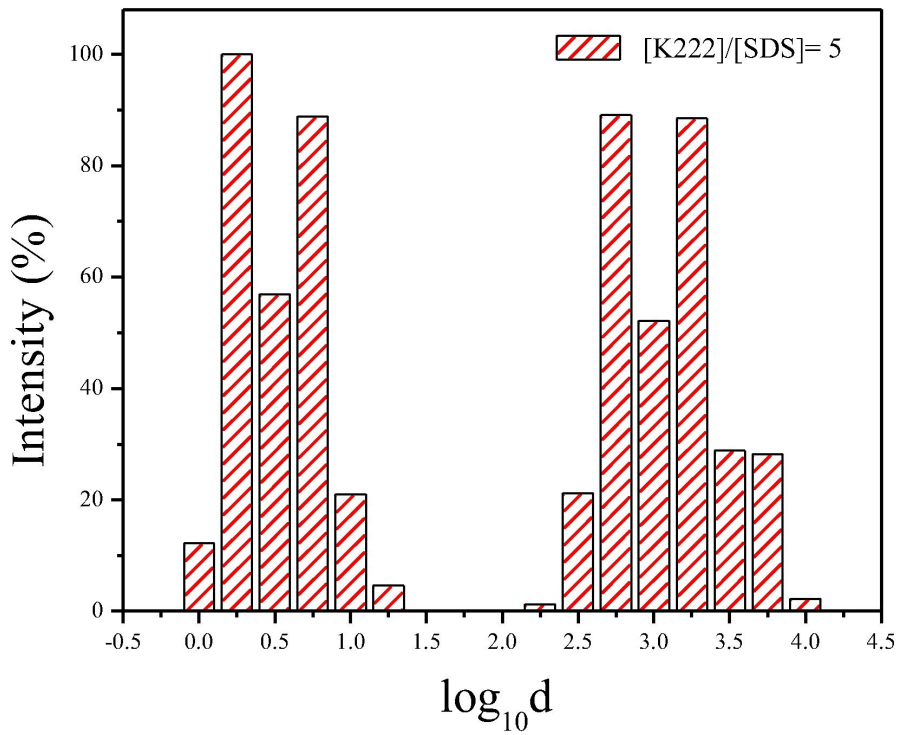
Dynamic Light Scattering or Photon correlation spectroscopy

Autocorrelation function of the light intensity scattered by the sample



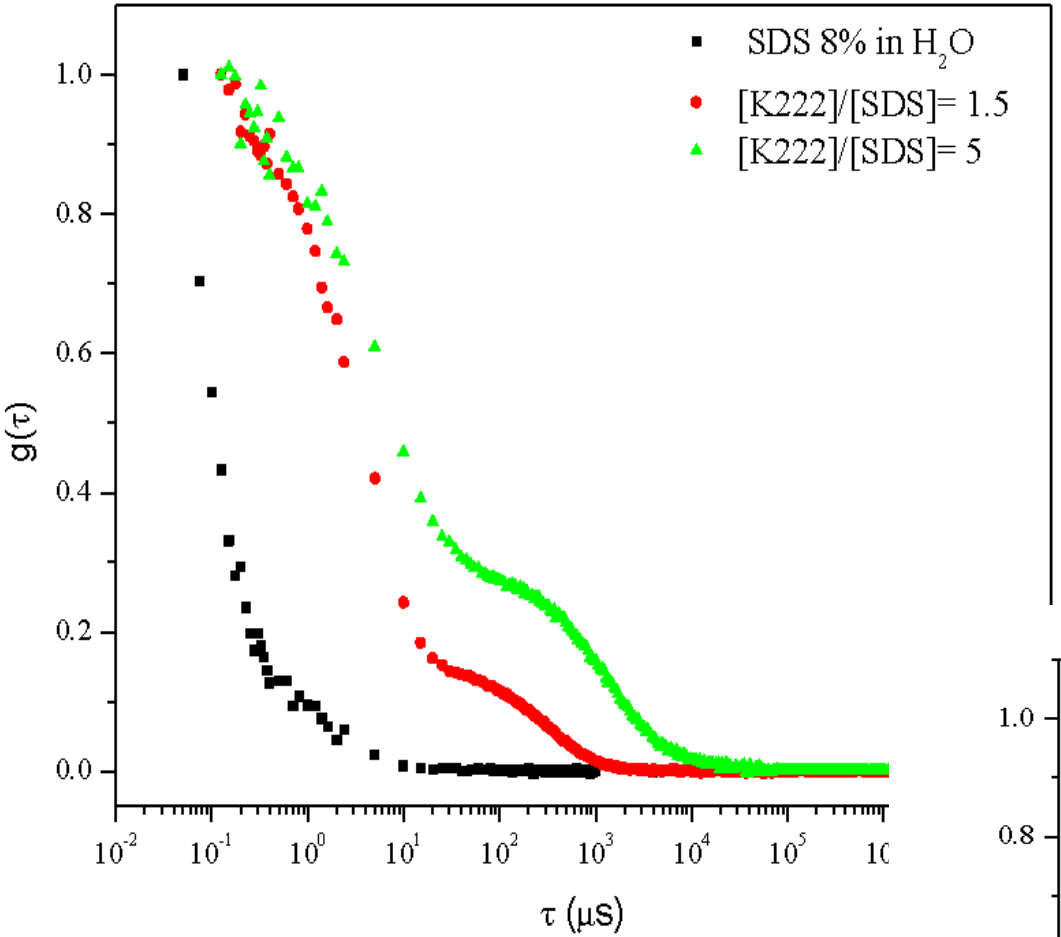
K222/SDS=5

Two populations are shown



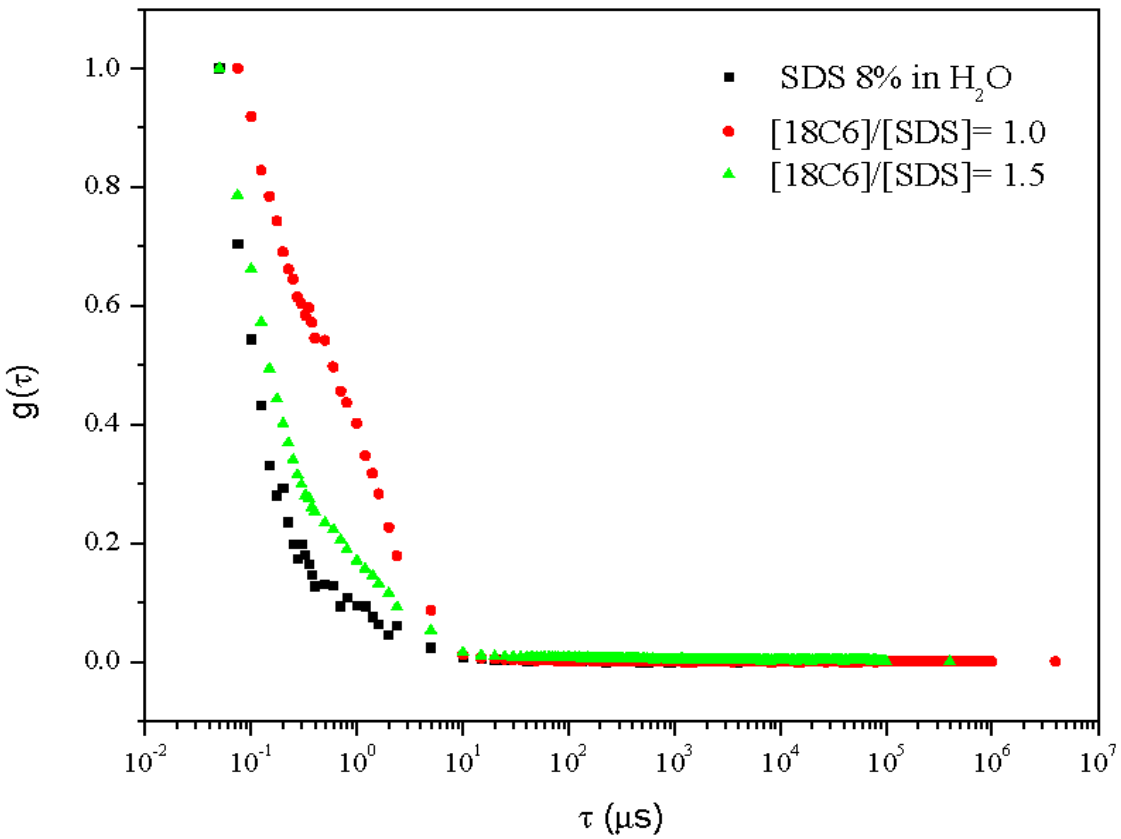
d	%	d	%
1.00	12	562.34	89
1.78	100	1000.00	52
3.16	57	1778.28	89
5.62	89	3162.28	29
10.00	21	5623.41	28
17.78	5	10000.00	2
31.62	0		
56.23	0		
100.00	0		
177.83	1		
316.23	21		

TWO POPULATIONS: MICELLES AND AGGREGATES OF MICELLES



SDS in WATER C=8% wt/wt

for all the samples



Γ is the inverse relaxation time of the given process.

The average diffusion coefficient D is related to Γ by the relationship:

$$D = \Gamma / q^2$$

where $q = (4\pi n/\lambda) sin(\theta/2)$ is the scattering wave vector, n is the index of refraction of the sample, and θ is the scattering angle.

When D is the translational diffusion coefficient, it is related to the hydrodynamic diameter $d = 2R_H$ (where R_H is the hydrodynamic radius of the fragment) through the Stokes-Einstein relationship:

$$D = K_B T / (6\pi\eta R_H)$$

where $K_B T$ is the thermal energy and η is the viscosity of the solvent.

Results are obtained by CONTIN and CUMULANT expansion.

DINAMIC LIGHT SCATTERING ON "Cluster Phases"

θ angular degrees	micelles diameter nm	%	aggreg. medium nm	%	aggreg. large nm	%
K222/SDS=5.0						
150	1-6	84	-	-	300-500	16
135	1-8	44	-	-	450-2200	56
90	2-7	13	-	-	400-2000	87
50	5	3	-	-	1000-2300	97
15	5	3	-	-	1700-3500	97
K222/SDS=1.5						
150	1-5	67	-	-	200-300	33
90	r 1-5	89	-	-	200-300	11
50	2-3	12	-	-	260-560	88
15	4-8	27	50-90	11	4000-8000	62

DYNAMIC LIGHT SCATTERING RESULTS AT 20 C

MICELLES: diameter 5 nm

AGGREGATES OF MICELLES (at different scattering angles 150-50 a.d.):

K222/SDS=5.0

diameters 300-2000 nm

K222/SDS=1.5

diameters 200-560 nm

18C6/SDS=1.5

diameters 300-700 nm

18C6/SDS=1.0

diameters 100-500 nm

18C6/SDS=0.5

diameters 100-400 nm

DYNAMIC LIGHT SCATTERING RESULTS
TEMPERATURE dependance

K222/SDS=1.5 MICELLES: diameter 5 nm

AGGREGATES OF MICELLES (at different scattering angles 150-50 a.d.):

FOR EACH TEMPERATURE THE SAMPLE IS STIRRED AND MEASURED

AFTER ONE NIGHT OF STABILIZATION

20 C 200-1000 nm

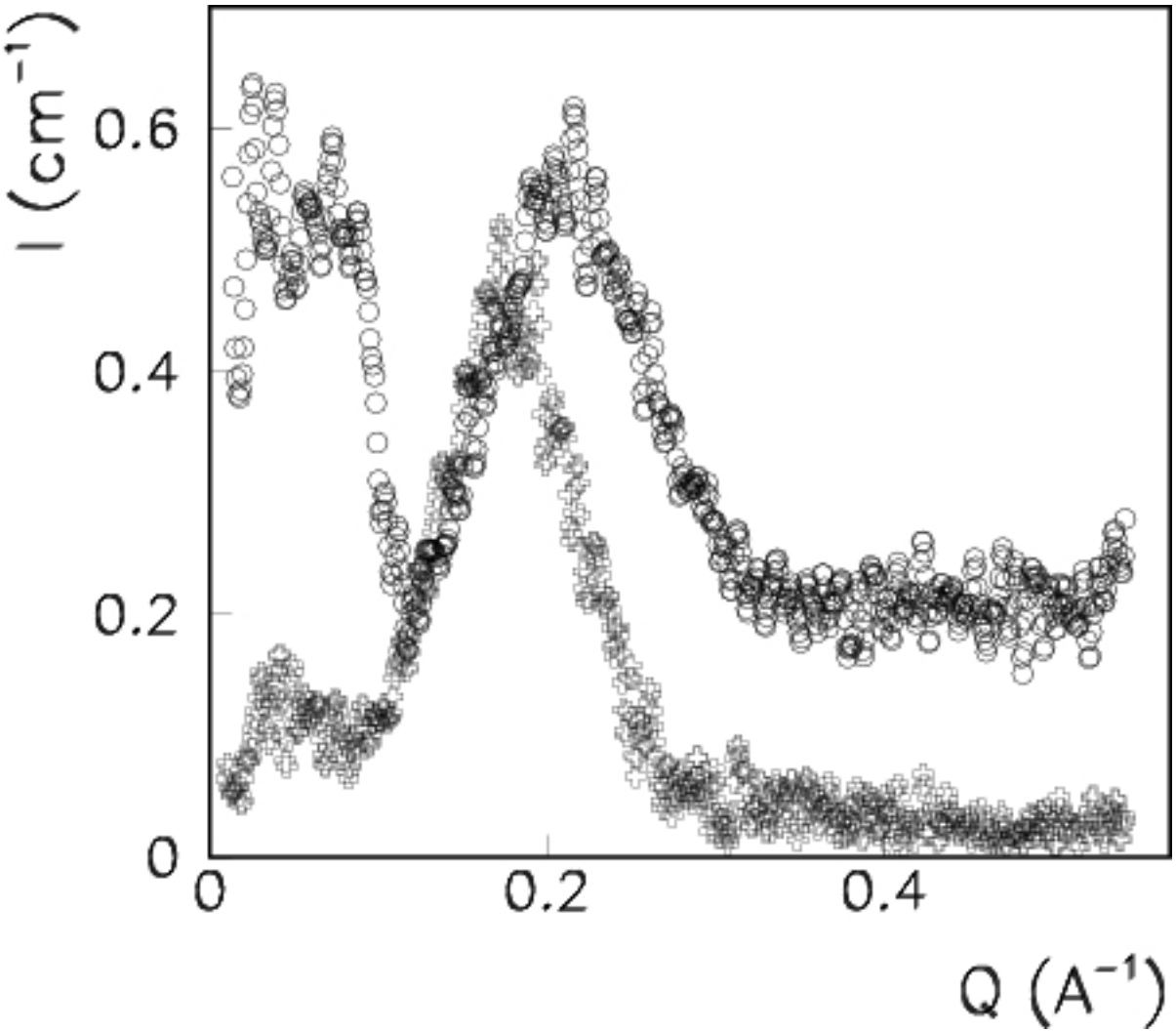
30 C 200-800 nm

40 C 400-30000 nm

without stirring		with stirring	
20 C	no aggregates	20 C	200-1000 nm

PRECIPITATION OF AGGREGATES ? THE AGGREGATES APPEAR AGAIN

SAXS EXPERIMENTS OF SDS MICELLES AND SDS+K222



SDS crosses

SDS+K222(1.5) circles

RESULTS OF THE SAXS EXPERIMENTS ON MICELLES

Z	N	t(A)	Nc	N lig(%)	a/b	Diam(A)	D.length	V(KB T)	vol.fract.
K222/SDS SANS* analysis									
1.5	12	54	12	9.5	65	1.5	56	16	2.3	0.242
1.0	15	59	11	8.9	68	1.8	54	17	4.1	0.211
0.5	21	74	10	12	40	1.3	57	16	6.7	0.206
18C6/SDS SAXS analysis									
1.5	20	84	5.5	12	15	1.5	50	17	9.7	0.115
1.0	27	74	5.5	12	12	1.3	48	14	14	0.121
0.5	18	103	5.5	12	10	1.85	52	19	8.2	0.116

* L. Scaffei, L. Lanzi, C. M. C. Gambi, R. Giordano, P. Baglioni and J. Teixeira, J. Phys. Chem. B, 106, 10771 (2002)

L. Scaffei, L. Lanzi, C. M. C. Gambi, R. Giordano, P. Baglioni and J. Teixeira, J. Phys. Chem. B, 106, 10771 (2002)

P. Baglioni, C. M. C. Gambi, R. Giordano e J. Teixeira, Colloids and Surfaces A, 121, 47-52 (1997)

M. Carla`, C. M. C. Gambi e P. Baglioni "Adsorption properties of cryptand 222 at the charged mercury - solution interface" J. Phys. Chem. 100, 11067 (1996)

P. Baglioni, C.M.C. Gambi, R. Giordano and J. Teixeira, Physica B, 213&214, 597 (1995)

Dielectric Spectroscopy
In collaboration with C. CAMETTI and S. SENNATO:
Dept. of Physics "La Sapienza", Universita` di Roma

MICELLES IN WATER WITHOUT LIGANDS

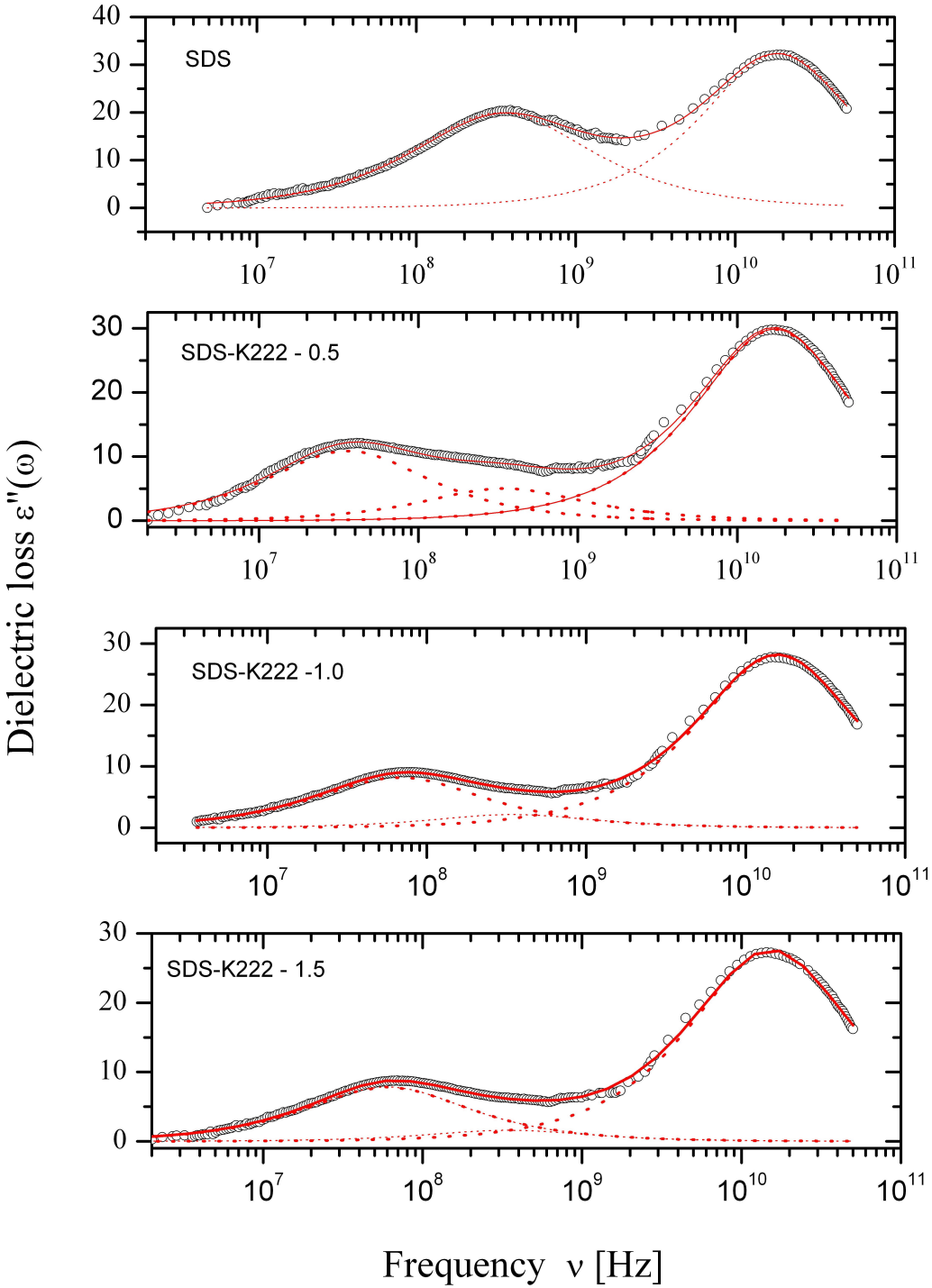
IN * *, TWO DEBYE' S RELAXATION TIMES WERE IDENTIFIED :

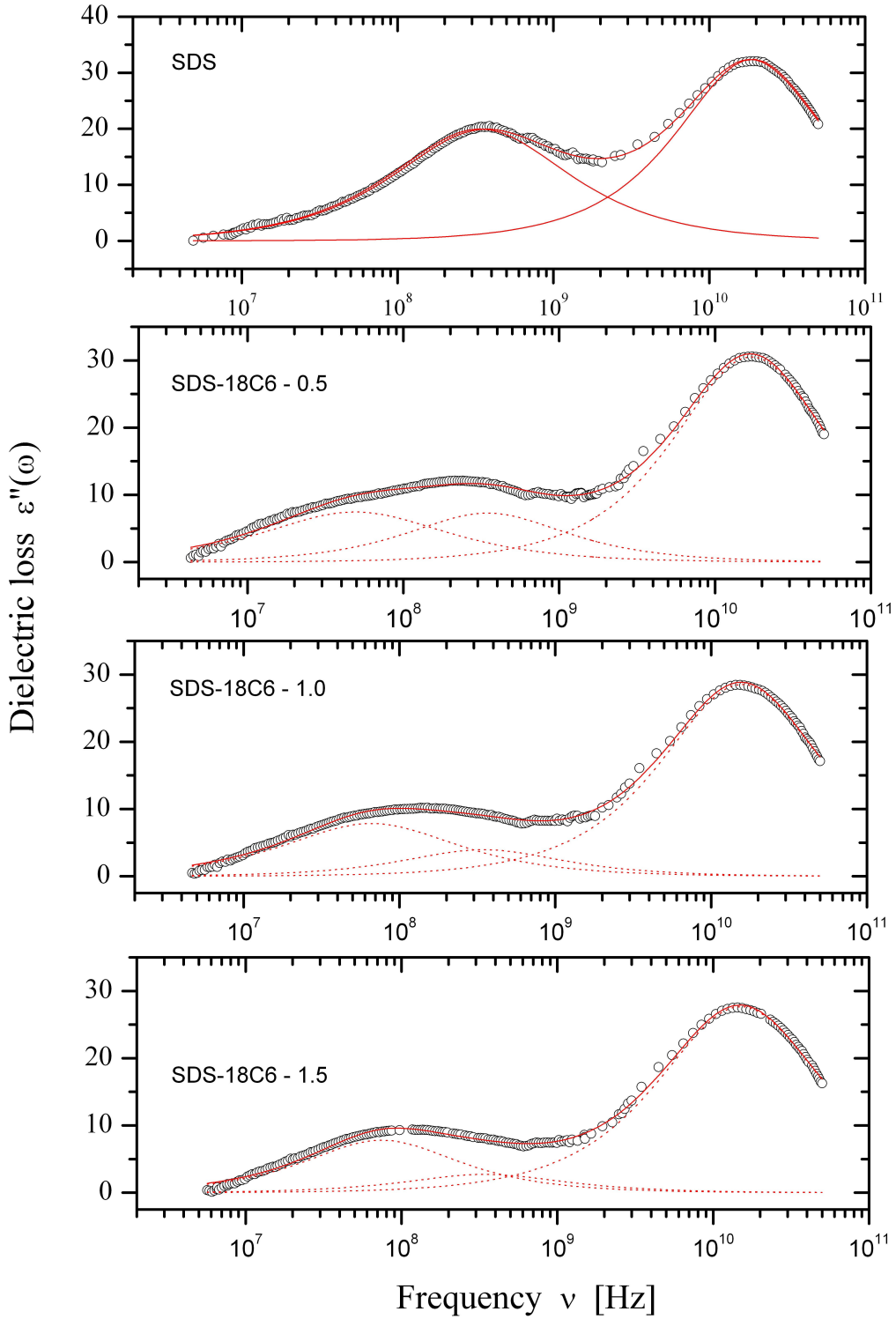
1) 400-900 ps DUE TO THE MOTION OF COUNTERION BOUND TO THE INTERFACE OF THE MICELLAR SOLUTION (LATERAL MOTION);

2) 100-130 ps DUE TO BOUND WATER AT THE MICELLAR INTERFACE.

** Leandro Lanzi, M. Carla`, Leonardo Lanzi, C. M. C. Gambi "A new insight on the dynamics of sodium dodecyl sulfate aqueous micellar solutions by dielectric spectroscopy" J. Colloid Interf. Sci. 330, 156 (2009).

** Leandro Lanzi, M. Carlà, M.C.C. Gambi, Leonardo Lanzi, "Differential and double-differential dielectric spectroscopy to measure complex permittivity in transmission lines", Rev. Sci. Instrum. 73 (2002) 3085-3088.


SDS
SDS-K222 - 0.5
SDS-K222 -1.0
SDS-K222 - 1.5
Dielectric loss ε''(ω)
Frequency ν [Hz]


SDS
SDS-18C6 - 0.5
SDS-18C6 - 1.0
SDS-18C6 - 1.5
Dielectric loss ε''(ω)
Frequency ν [Hz]

Raman Scattering on "Cluster Phases of Patchy Micelles"

Collaboration: Dep. of Physics, University of Trento

ADDITION OF LIGAND INDUCES ROD-LIKE SHAPE ON THE MICELLES THAT STAY CLOSER EACH OTHERS LEADING TO AGGREGATION OF MICELLES IN CLUSTERS

K222 SCREANS BETTER THE CHARGE THAN 18C6

FOR BOTH LIGANDS C-C AND CH2 STRECHING DOES NOT CHANGE VS. CONCENTRA-TION INCREASE

STRETCHING IN POLAR HEAD S-OC AND C-C GROUPS GIVES AGGREGATION OF MICELLES FOR K222 MICELLES

.....

Articoli 2007-2010

C. Ziparo, R. Eramo, C.M.C. Gambi and R. Torre "Percolation transition in water-AOT-decane microemulion investigated by transient grating measurement"
Philos. Mag. 87, 759 (2007).

C.M.C. Gambi, R. Giordano, A. Chittofrati, R. Pieri, M. Laurati, P. Baglioni, J. Teixeira, "Small-Angle Neutron Scattering of Mixed Ionic Perfluoropolyether Micellar Solutions", J. Phys. Chem. B, 111, 1348 (2007)

S. Marchetti, G. Onori, C. Cametti: Ethanol-induced compaction of DNA molecules:a viscosimetry and light scattering study,
Philosophical Magazine, 87, 525 (2007).

David Dolci, Giovanni Aloisi, Leonardo Lanzi and Marcello Carla` -
A study of the ionic conduction of mica surface by admittance spectroscopy -
J. Chem. Phys. 127, 74701 (2007).

D. Senatra, C. Ziparo, Gambi C.M.C., L. Lanzi - Energy balance in dense microemulsions - J. Therm. Anal. Cal., v. 92, p. 535 (2008).

S. Marchetti, F. Sbrana, R. Raccis, L. Lanzi, C. M. C. Gambi, M. Vassalli, B. Tiribilli, A. Pacini, A. Toscano "Dynamic light scattering and atomic force microscopy imaging on fragments of beta-connectin from human cardiac muscle"
Phys. Rev. E, 77, 021910 (2008).

G. Aloisi, F. Bacci, M. Carla', D. Dolci and L. Lanzi -
Implementation on a desktop computer of the real time feedback control
loop of a scanning probe microscope -
Rev. Sci. Instrum. 79, 113702 (2008).

Leandro Lanzi, M. Carla`, Leonardo Lanzi, C. M. C. Gambi
"A new insight on the dynamics of sodium dodecyl sulfate micellar solutions by dielectric spectroscopy",
J. Colloid and Interf. Sci., 330, 156-162, (2009).

V. Pini, B. Tiribilli, C.M.C. Gambi, M. Vassalli
“Dynamical characterization of vibrating AFM cantilevers forced by photothermal excitation”
Phys. Rev. B, 81, 054302 (2010).

M. Laurati, C. M. C. Gambi, R. Giordano, P. Baglioni, J. Teixeira
"Small-angle neutron scattering of percolative perfluoropolyether water-in-oil microemulsions"
J. Phys. Chem. B, 114, 3855 (2010).

S. Marchetti, F. Sbrana, E. Fratini, M. Carla`, M. Vassalli, B. Tiribilli, A. Pacini, A. Toscano, C. M. C. Gambi
“Beta-connectin studied by SAXS and AFM spectroscopy” (to be submitted).

FINE

Risultati di scattering di luce su “Cluster Phases”

θ angular degrees	micelles diameter nm	%	aggreg. medium nm	%	aggreg. large nm	%
18C6/SDS=1.5						
150	1-2	15	8-15	30	300-600	55
135	1-2	60	-	-	400-700	40
120	1-4	90	-	-	200-500	10
90	1-3	100	-	-	-	-
40	1-2	80	-	-	300-600	20
18C6/SDS=1.0						
150	1-2	82	10-25	18	-	-
135	1-2	68	-	-	100-200	32
90	1-2	63	-	-	100-200	37
50	1-2	73	-	-	300-500	15
40	1-2	78	-	-	60-130	22
30	1-2	67	-	-	200-400	33
15	1-2	51	-	-	200-400	49

Risultati di scattering di luce su “Cluster Phases”

θ angular degrees	micelles diameter nm	%	aggreg. medium nm	%	aggreg. large nm	%
18C6/SDS=0.5						
150	4.9	5	second cumulant			
135	5.0	14	second cumulant			
	1-3	90	Contin		100-200	10
90	4.7	6	second cumulant			
50	1-2	67	Contin		200-400	33
40	1-2	66	Contin		200-400	34
30	1-2	32	Contin		200-400	68

Risultati di scattering di luce su “Cluster Phases”

θ angular degrees	micelles diameter nm	%	aggreg. medium nm	%	aggreg. large nm	%
18C6/SDS=1.0						
20^oC						
135	1-2	68	-	-	100-200	32
90	1-2	63	-	-	100-200	37
30	1-2	67	-	-	200-400	33
15	1-2	51	-	-	200-400	49
30^oC						
135	1-2	56	4-8	44	-	-
90	1-3	100	-	-	-	-
15	1-2	8	-	-	110-230	92

Risultati di scattering di luce su “Cluster Phases”

θ angular degrees	micelles diameter nm	%	aggreg. medium nm	%	aggreg. large nm	%
40^oC						
150	1-2	68	11-24	32	-	-
135	1-3	100	-	-	-	-
90	1-5	100	-	-	-	-
30	1-2	68	-	-	110-230	32
20^oC						
135	1-2	42	7-14	58	-	-
90	1-2	100	-	-	-	-
30	1-2	74	-	-	200-400	26
15	1-2	37	110-230	56	480-850	8

SAXS ANALYSIS OF PROTEINS IN SOLUTION

CHARGE	-13
ASSE MAGGIORE (A)	33
ASSE MINORE (A)	13
DIAMETRO EFFETTIVO (A)	49

		Dilute	*Diafiltered*
Charge	Z	-13.0	-13.0
Major semiaxis (Å)	a	33.0	33.6
Minor semiaxis (Å)	b	13.6	12.9
Axial Ratio	a/b	2.4	2.6
Ratio	ρ_{sh}/ρ_s	1.06	1.09
Effective hard-core diameter (Å)	σ_{HC}	49.1	48.8
Volume fraction (%)	η	0.47	17.50
Reduced screening parameter	κ	3.24	8.80
Debye Length(Å)	l_D	15.2	5.5
Contact potential	γe^{-1}	1.74	0.63