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[f001]

Host-Guest Dynamics studied by Fluorescence Correlation Spectroscopy

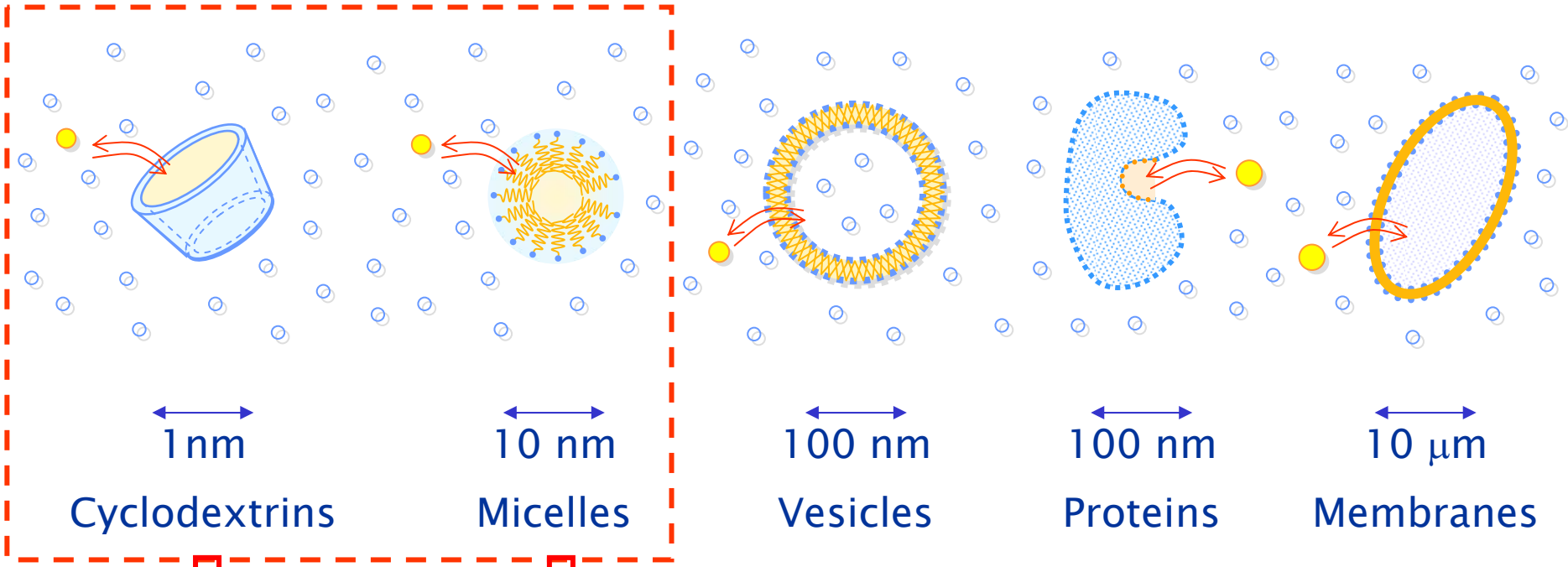
Wajih Al-Soufi[1], Mercedes Novo[1], Belén Reija[1], Suren Felekyan[2] and Claus A. M. Seidel[2]

[1]Dpto. Química Física, Facultade de Ciencias, Universidade de Santiago de Compostela, E-27001 Lugo, Spain.

[2]Lehrstuhl für Molekulare Physikalische Chemie, Heinrich-Heine Universität Düsseldorf, D-40225 Düsseldorf, Germany.

Dynamics of matter exchange in supramolecular structures

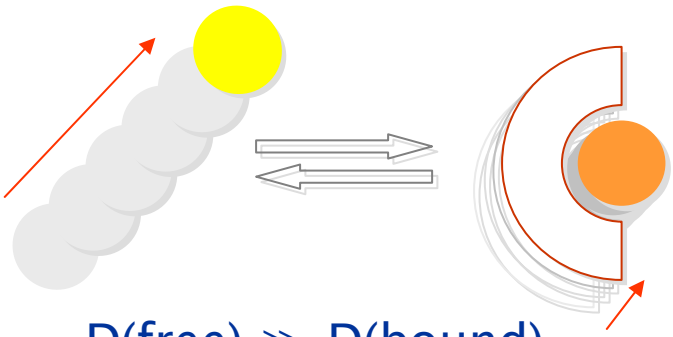
- Most of the studies on supramolecular structures deal with their stability and their structural properties, but few are focused on their dynamics.
- We are interested in the study of host-guest dynamics in structures of increasing complexity, as for example:



- In this work we compare the host-guest dynamics of two types of cages.

Dynamics in Supramolecular Structures

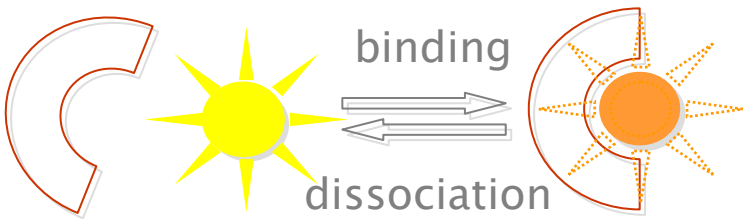
- We analyze two different dynamic processes in the supramolecular structures:



D(free) ≫ D(bound)

Diffusional dynamics (time scale $>10^{-4}$ s):

- change in the diffusion coefficient D of free and bound guest
- allows one to detect binding
- is used to determine the equilibrium binding constant



Spectral properties

Free guest ≠ Bound guest

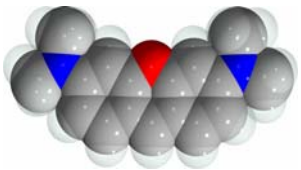
Binding dynamics (time scale $<10^{-3}$ s):

- given by the rate constants of binding and dissociation
- observed when the spectral properties of the guest change upon binding
- allows to study the structure dependence of binding and the heterogeneity of binding sites

Dynamics in Supramolecular Structures

- Association of a fluorescent guest with a host induces changes in the fluorescence intensity which allows to study the binding dynamics using the technique of Fluorescence Correlation Spectroscopy.

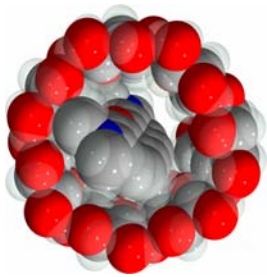
$$\text{Free Guest} \underset{k_-}{\overset{k_+[\text{Host}]}{\rightleftharpoons}} \text{Bound Guest}$$



Pyronine

k_+ [CD]

k_-

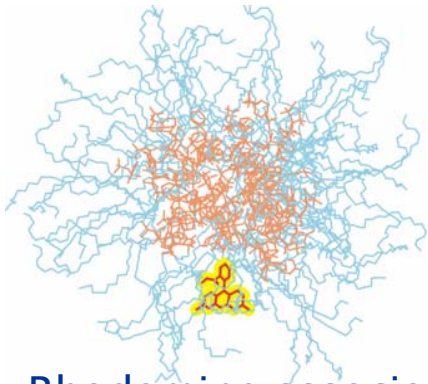


Inclusion complex
Pyronine:Cyclodextrin

Rhodamine

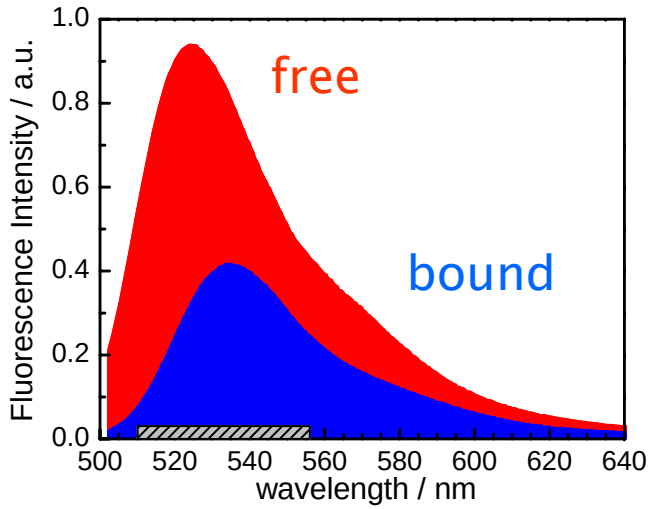
k_+ [M]

k_-



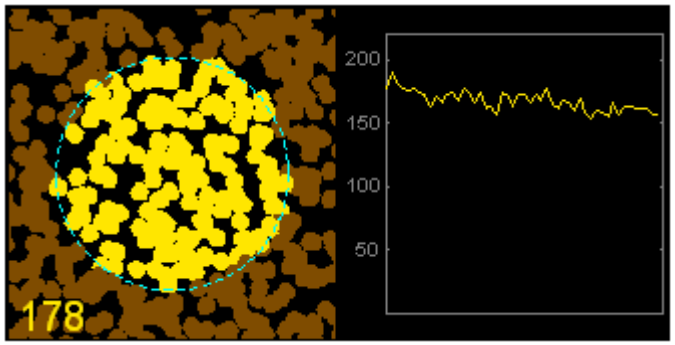
Rhodamine associated
to a neutral micelle

Fluorescence Emission Spectrum



Fluorescence Correlation Spectroscopy (FCS)

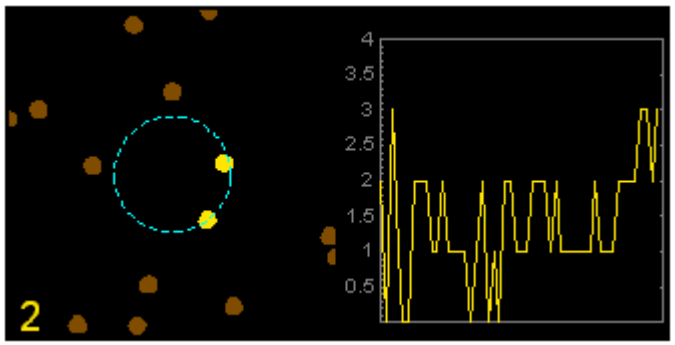
- FCS analyses spontaneous fluctuations in fluorescence intensity provoked by molecular processes of systems at thermodynamic equilibrium.
- The amplitude of the fluctuation is inversely proportional to the number of molecules N under observation, so that very diluted solutions (≈ 1 nM) and very small observation volumes (≈ 1 fl) are used.



bulk

$N \gg 1$

Amplitude of fluctuation $\sim 1/\sqrt{N}$

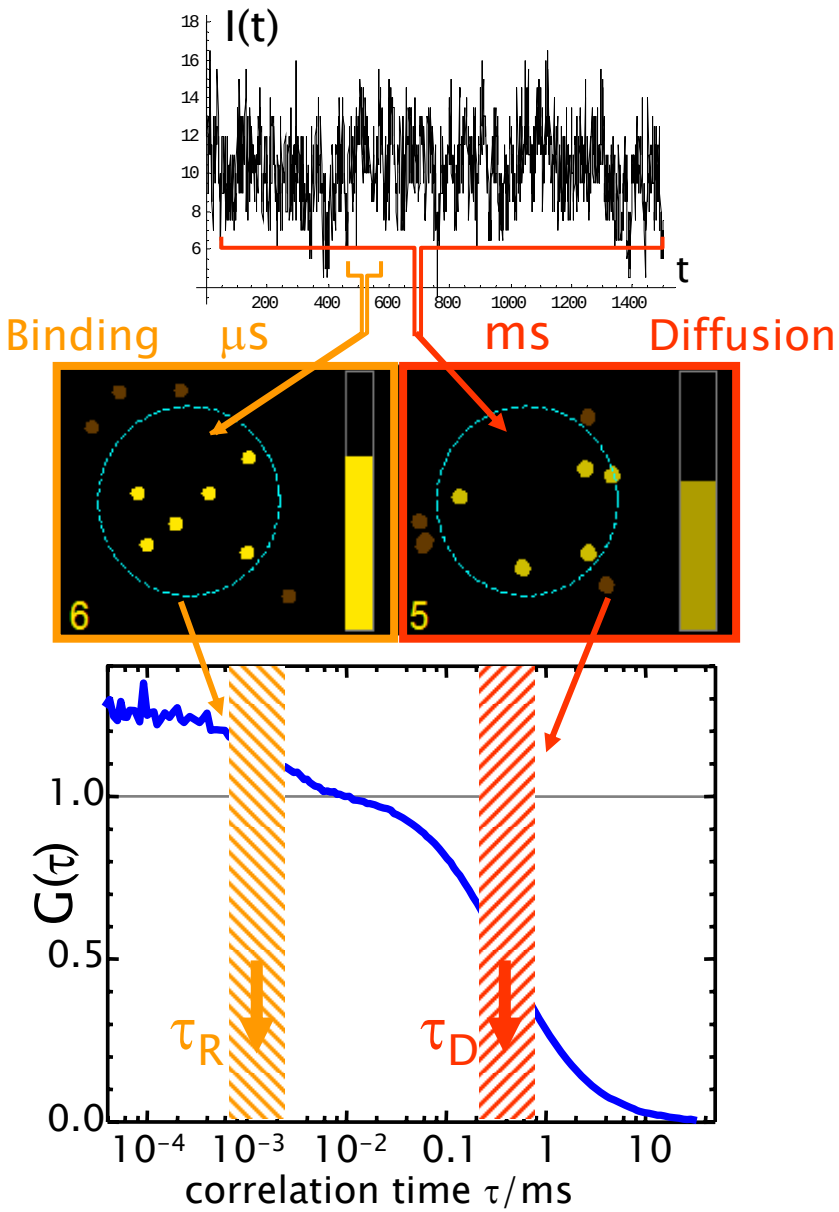


FCS

$N \approx 1\text{–}10$

- The correlation function is calculated to obtain the characteristic times of the fluctuations which reveal the dynamic processes.

Fluorescence Correlation Spectroscopy (FCS)



- The correlation function calculated from the fluctuations of fluorescence intensity with time shows at least two correlation times due to the two dynamic processes taking place:

Diffusion time (at fast exchange):

$$\overline{\tau}_D = \frac{w_{xy}^2}{4(X_G D_G + X_{G:H} D_{G:H})}$$

→ W_{xy}: Geometrical parameter
→ D: Diffusion coefficients
→ X: Molar fraction
→ G: Free guest, GH: bound guest

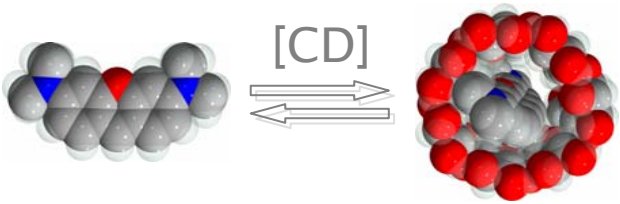
Reaction time (Binding / Dissociation):

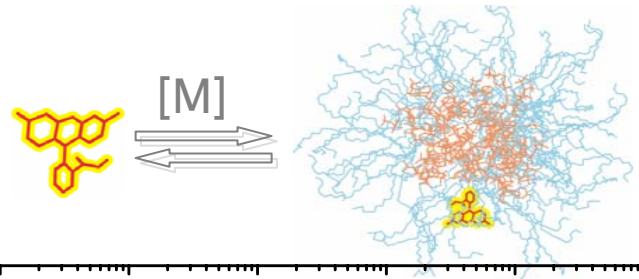
$$\tau_R = (k_+[H] + k_-)^{-1}$$

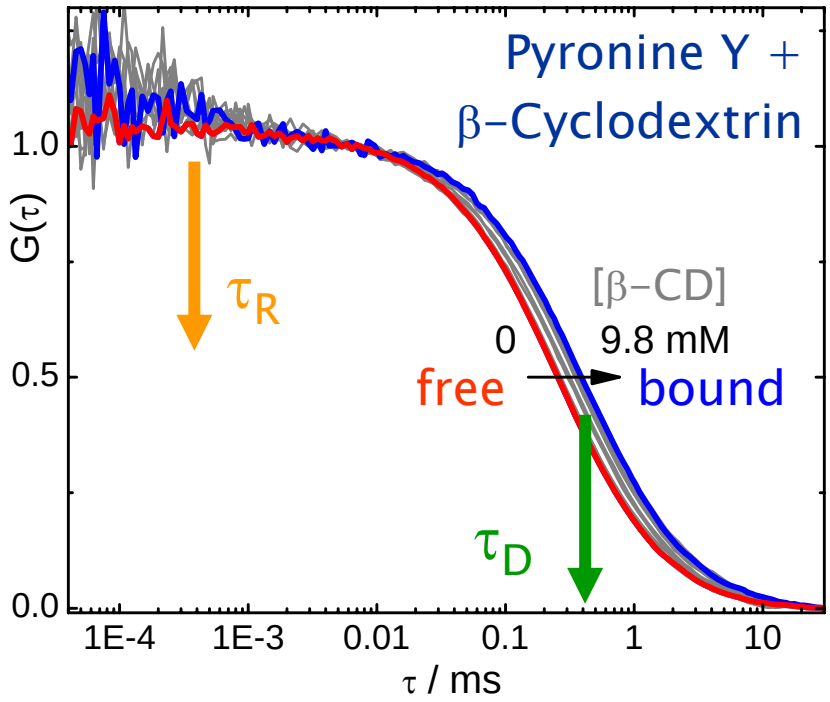
→ Rate constants k_+, k_-
(Binding constant: $K = k_+/k_-$)

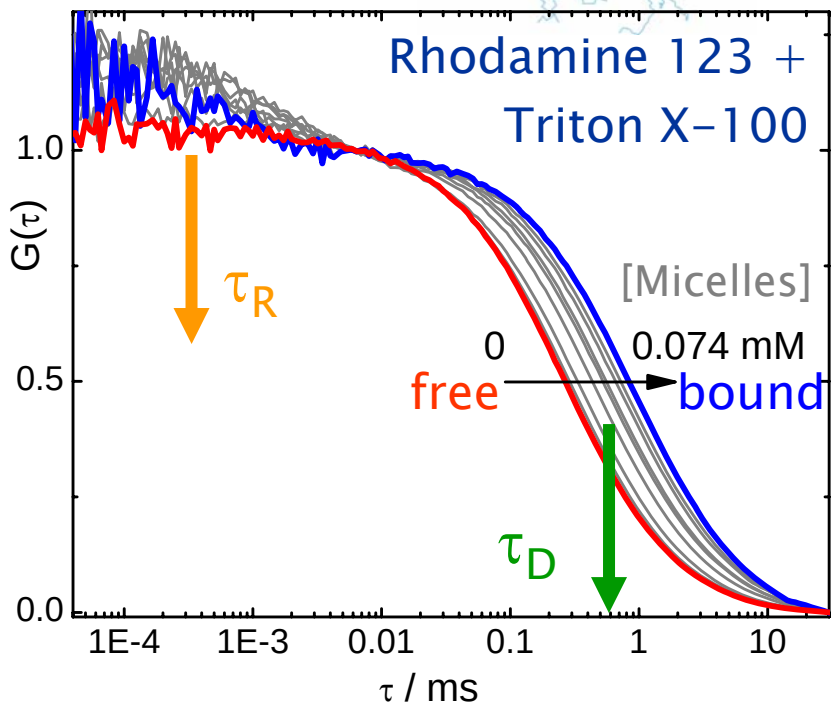
FCS: Correlation curves

- The correlation times τ_D and τ_R vary with the concentration of host due to the change in the equilibrium concentrations of free guest and bound guest and the increase in the binding rate.





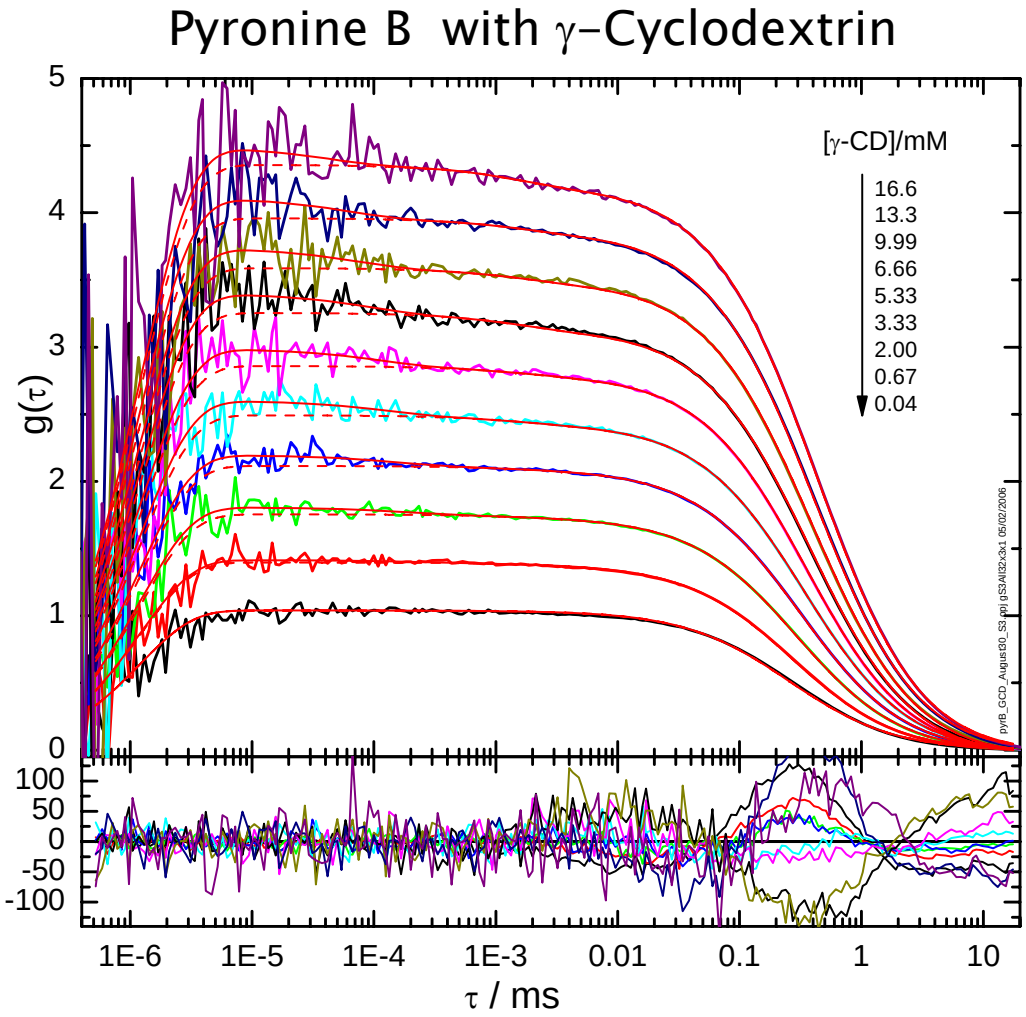




- Additional correlation times can be observed due to photophysical dynamic processes, such as triplet formation and fluorescence deactivation.

FCS curves: Pyronine + Cyclodextrin

- Analysis of series of correlation curves at increasing host concentration yields precise values for the individual limiting diffusion times and for the binding/dissociation rate constants:



Diffusion times:

$\tau_G = 0.26 \pm 0.02$ ms

$\tau_{GH} = 0.49 \pm 0.02$ ms

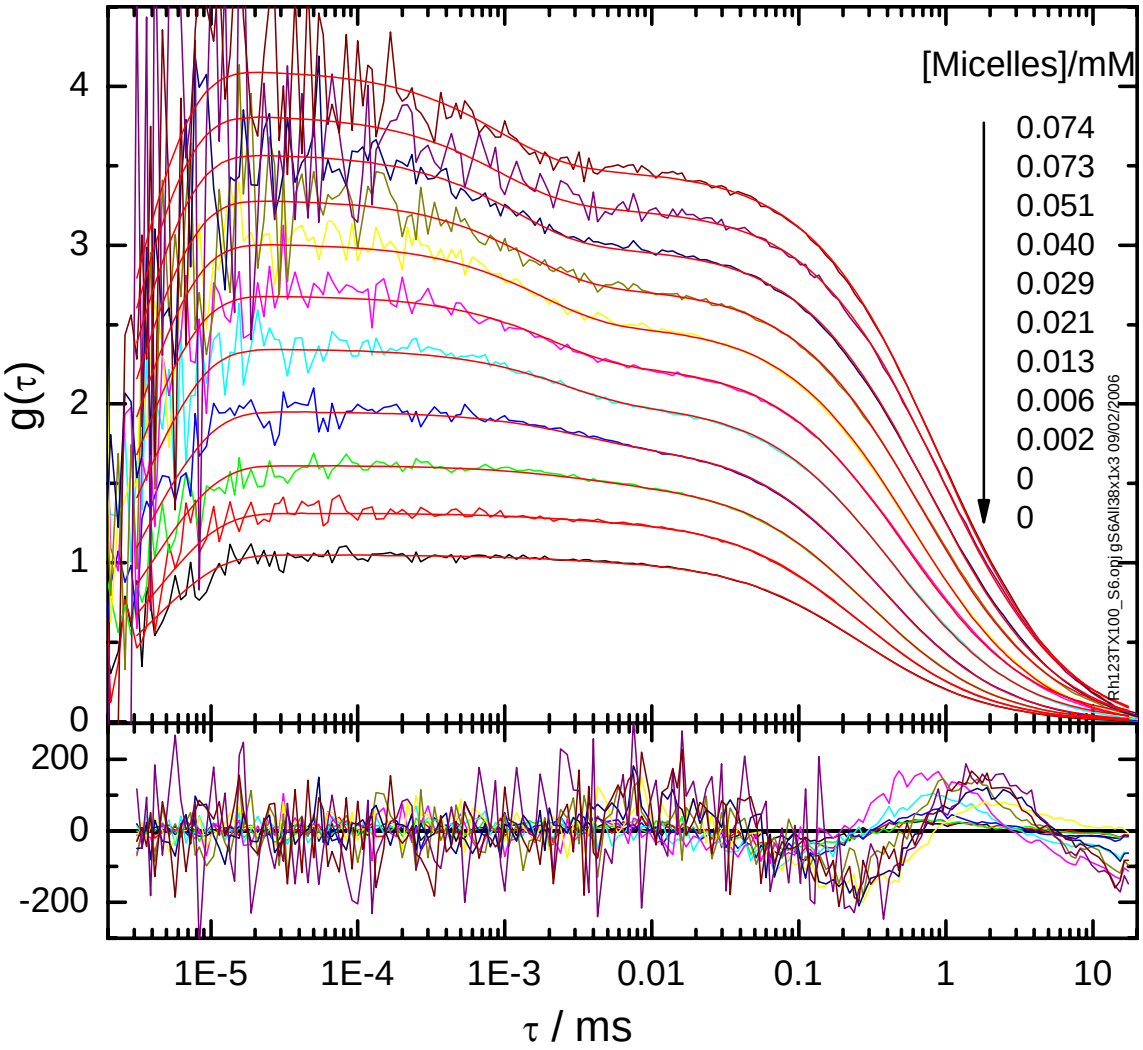
Rate constants:

$k_+ = (0.8 \pm 0.1) \times 10^9$ $M^{-1}s^{-1}$

$k_- = (40 \pm 10) \times 10^5$ s^{-1}

FCS curves: Rodamine + Neutral Micelle

Rhodamine 123 with Triton X-100 micelles



Diffusion times:

$\tau_G = 0.263 \pm 0.002$ ms

$\tau_{GH} = 1.66 \pm 0.05$ ms

Rate constants:

$k_+ = (14 \pm 1) \times 10^9\ M^{-1}s^{-1}$

$k_- = (2.2 \pm 0.1) \times 10^5\ s^{-1}$

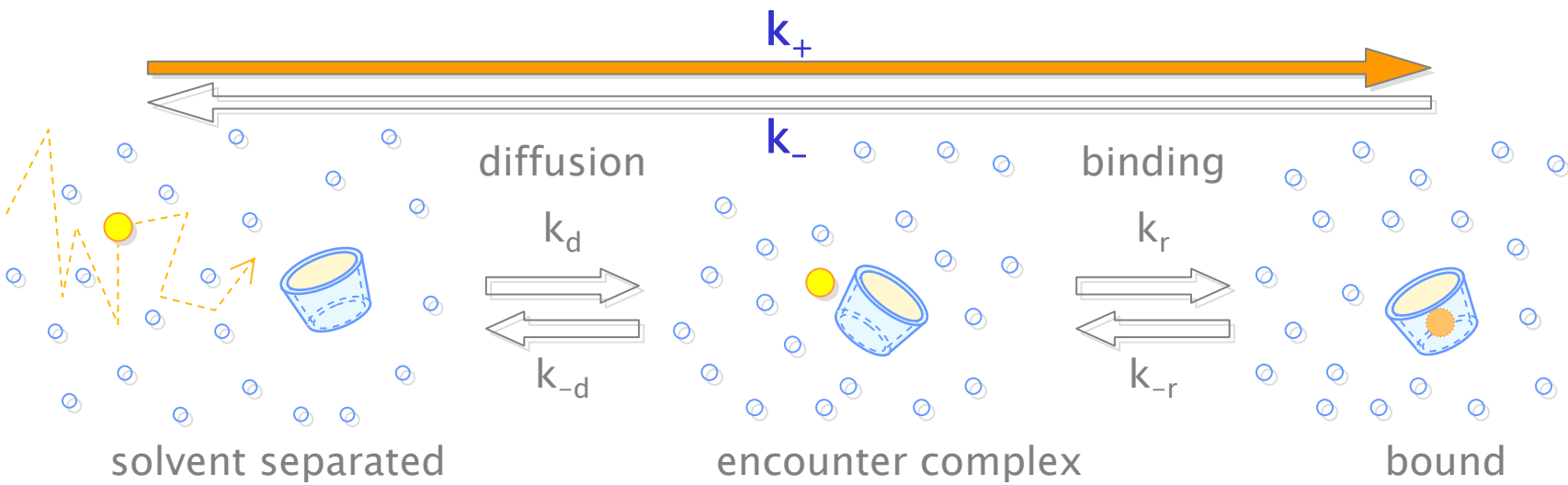
Diffusion Coefficients

- The diffusion times of free and bound guest can be converted to the corresponding translational diffusion coefficients (D).
- The diffusion coefficients give information about the size and the hydrodynamic behavior of the supramolecular systems.
- The hydrodynamic behavior of the pironine-cyclodextrin inclusion complexes is very close to that of compact spheres, with the typical dependence of the diffusion coefficient with $M^{1/3}$.

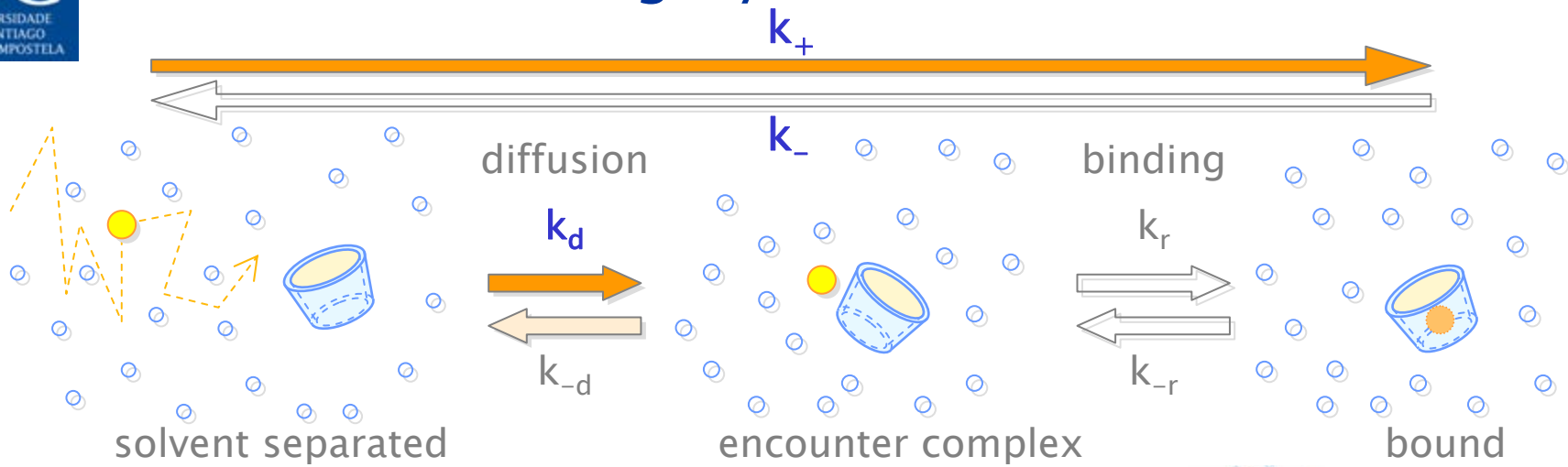
	D / $10^{-10}\ m^2 s^{-1}$	M_W / Da
Free PY	4.2 ± 0.3	267
Free PB	3.5 ± 0.3	324
Complex PY·β–CD	2.4 ± 0.3	1403
Complex PB·β–CD	2.6 ± 0.3	1459
Complex PY·γ–CD	2.2 ± 0.2	1564
Complex PB·γ–CD	2.2 ± 0.1	1621
Free R123	3.5 ± 0.2	267
R123 in TX100 micelles	0.57 ± 0.04	>40000

Host-Guest Binding Dynamics

- A host-guest binding process can be seen as a two step process, the first step being the diffusion of guest and host together to form an encounter complex (diffusion) and the second step being the binding itself to yield the supramolecular complex (binding).
- In order to compare the binding dynamics of the different systems under study the diffusion limited binding rate constant (k_d) which determines the diffusion step is estimated using the hydrodynamic properties obtained from FCS itself.



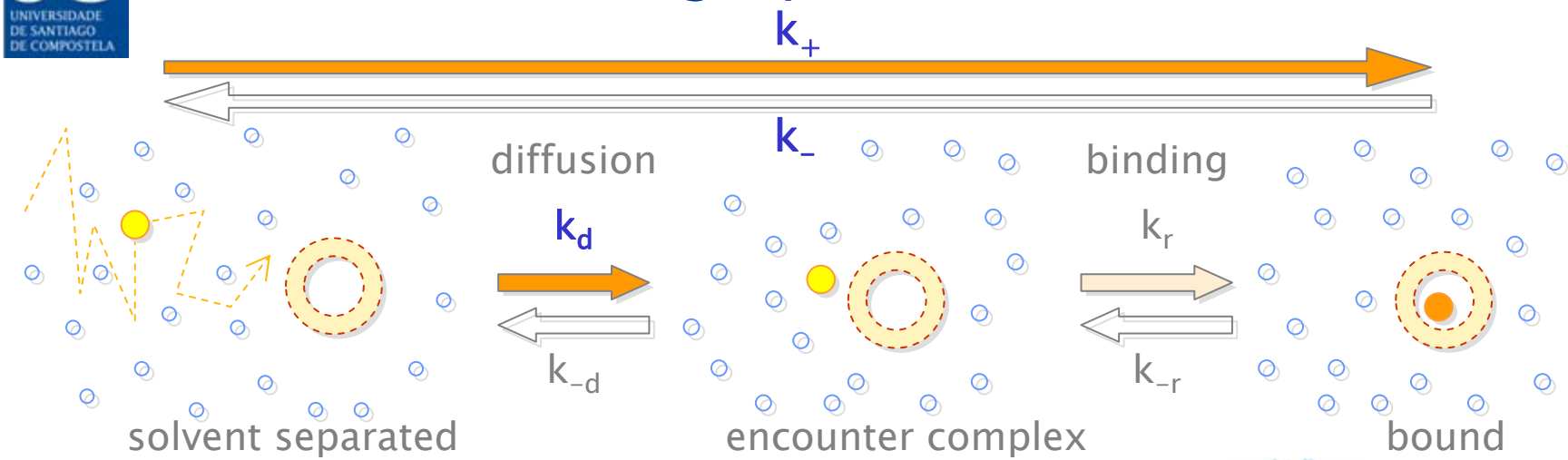
Host–Guest Binding Dynamics



	PY:β–CD	PB:β–CD	PB:γ–CD	R123:TX100
k_d /10^9 $M^{-1}s^{-1}$	8	8	8	15
k_+/ 10^9 $M^{-1}s^{-1}$	0.2	0.15	0.8	14
k_-/ 10^5 s^{-1}	5	0.75	40	2.2

- With cyclodextrins as hosts the binding rate constant k_+ is much lower than the diffusion-controlled rate constant k_d ⇒ diffusion is not the rate limiting step of the binding process.
- This shows that geometry constraints determine binding ⇒ cyclodextrins behave as *hard cages.*

Host–Guest Binding Dynamics



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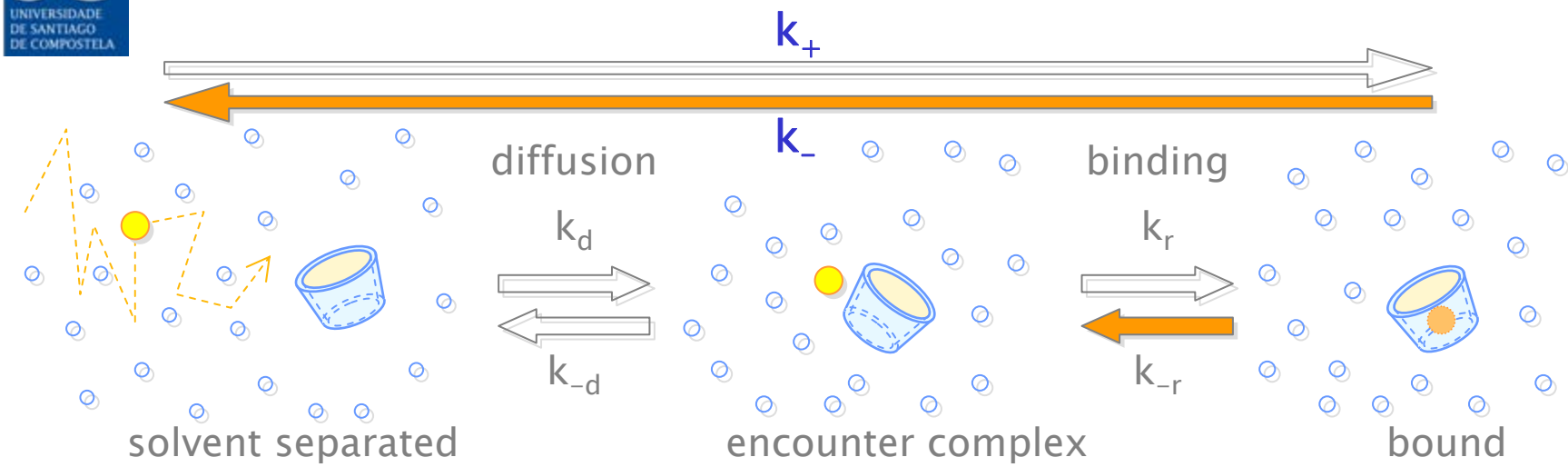
k_-/ 10^5 s^{-1}	5	0.75	40	2.2

- In the case of micelles the binding process is determined by the diffusion step ($k_+ \approx k_d$), indicating that there are no geometry constraints.
- In conclusion, there is a great difference in the host–guest binding dynamics of hosts which behave as *hard cages* (as cyclodextrins) and those which behave as *soft cages* (as micelles).

Host–Guest Binding Dynamics



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k_d /10^9 $M^{-1}s^{-1}$	8	8	8	15
k_+/ 10^9 $M^{-1}s^{-1}$	0.2	0.15	0.8	14
k_-/ 10^5 s^{-1}	5	0.75	40	2.2
K /10^3 M^{-1}	0.4	2	0.2	65

- For the two types of hosts the dissociation rate constant k_- depends on the specific host–guest interactions and determines the thermodynamic stability of the supramolecular complex.

Related Literature

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