EXPERIMENTAL INVESTIGATION OF THE EFFECT OF MACROMOLECULAR CROWDING ON PROTEIN CONFORMATION

Josef HAMACEK, Francesco PIAZZA,
Claire MADELEINE-PERDRILLAT, Salvatore MAGAZU

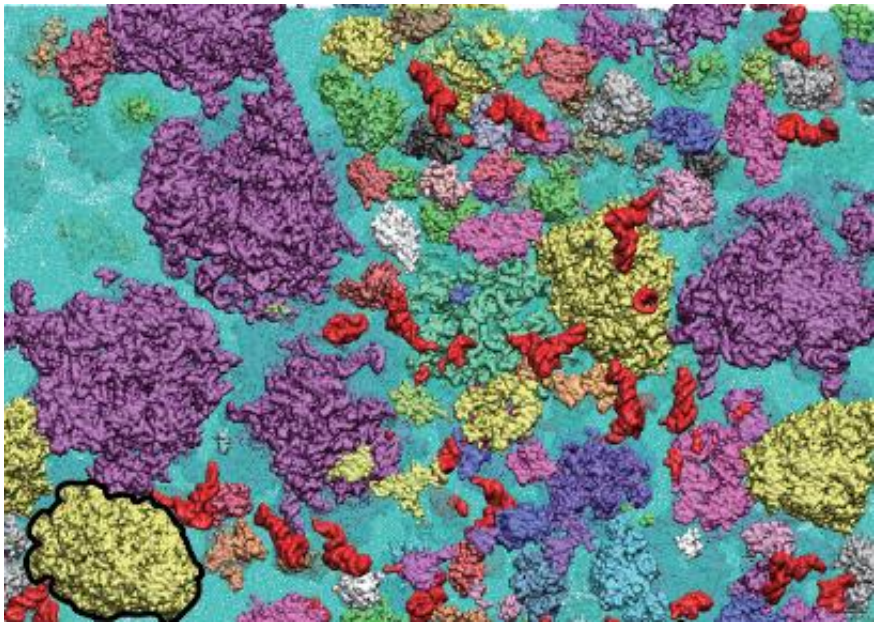
Center of Molecular Biophysics CNRS / University of Orléans, Rue Charles Sadron, 45071 Orléans Cedex 2, France

Warsaw, March 2018

UNIVERSITE D'ORLEANS

CBM – Center for Molecular Biophysics (CNRS)

Crowded Media and Living Systems



- High concentrations of different macromolecules (excluded volume)
- Limited diffusion to reaction centres, impact on kinetics

Aims:

- Evaluate enzymatic activity in artificial media models
- Understand dynamic behaviour and excluded volume effects

Characterisation of Reaction Systems

- "Crowding" medium - sugar-derived molecules and polymers

  Concentration, density, viscosity, solution structure

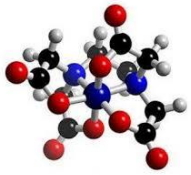
- Active components – substrate, enzyme, product(s)

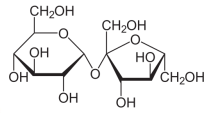
  Concentration, activity, diffusion, conformation

- Self-organisation

  Specific and nonspecific interactions in solution

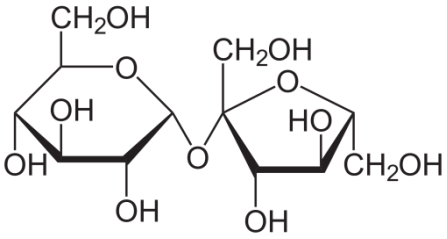
Investigating Dynamics and Structure

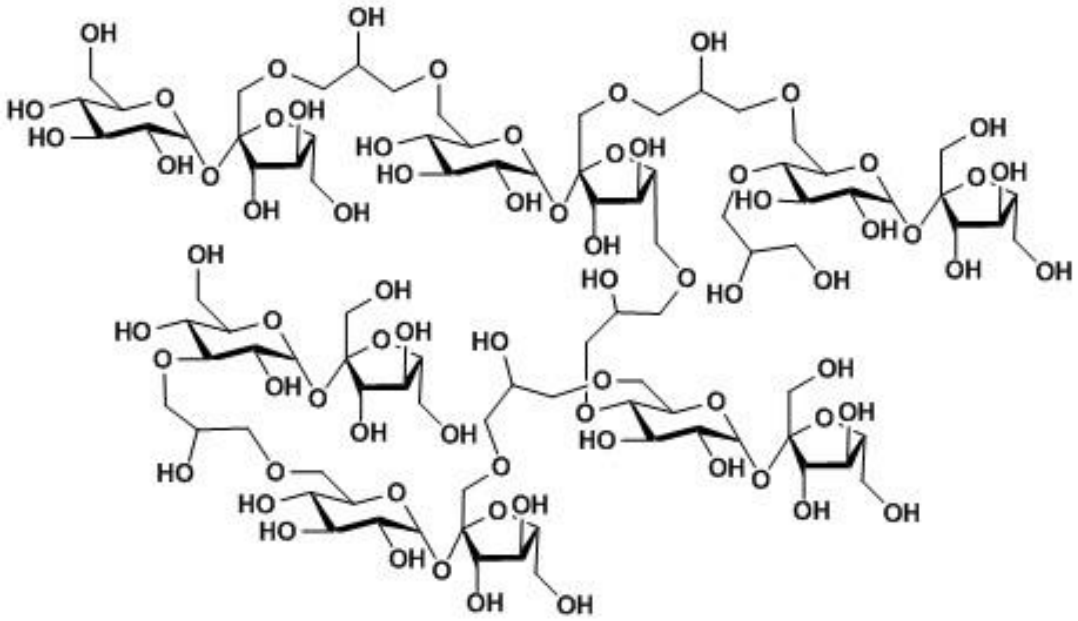




- DLS
- SAXS / SANS
- Optical spectroscopy (IR, Raman, fluorescence)
- NMR spectroscopy
- Other methods

Ficoll 70

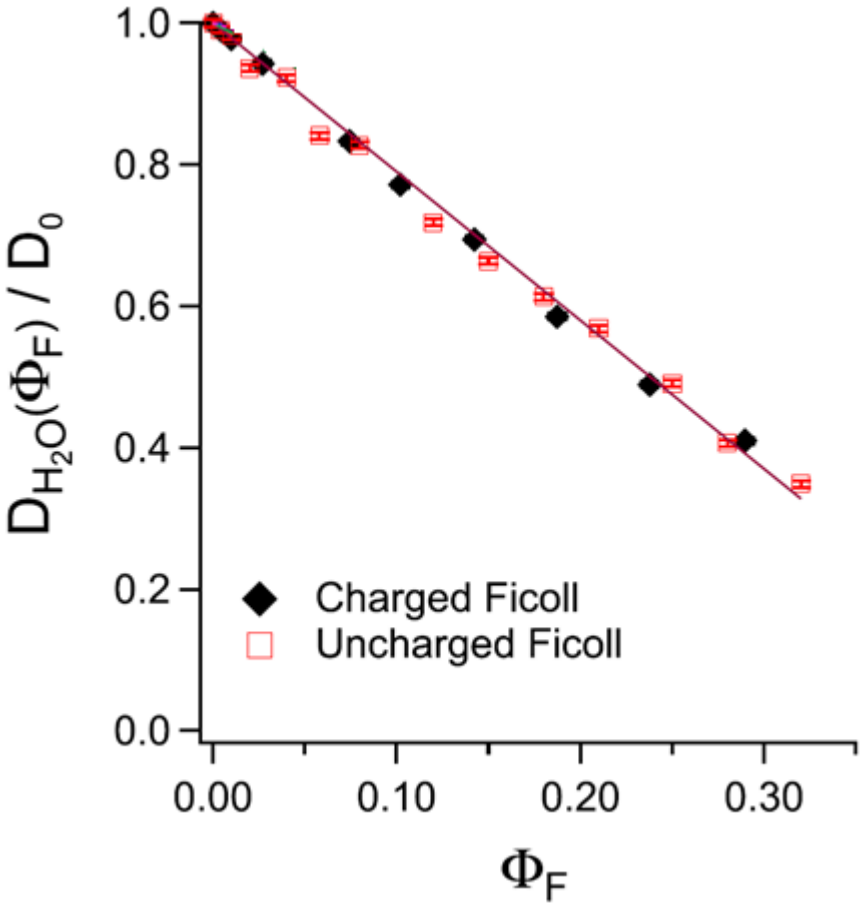


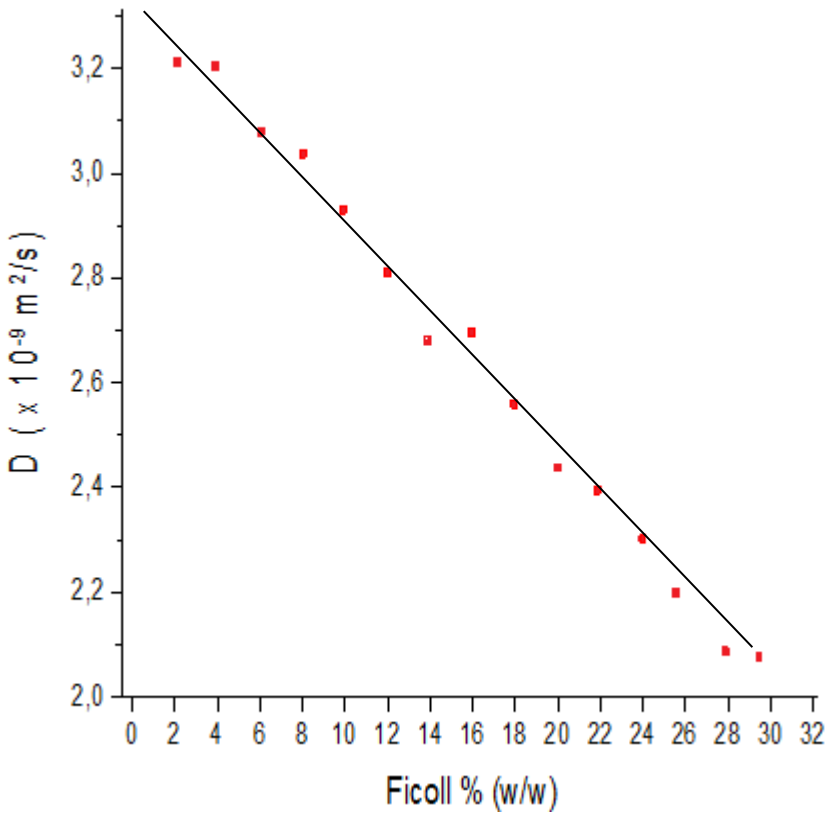


~ 70 kDa

- Sucrose branched polymer, uncharged, relatively inert
- Supposed spherical structure (5.1 Å)

Diffusion of Water in Ficoll 70

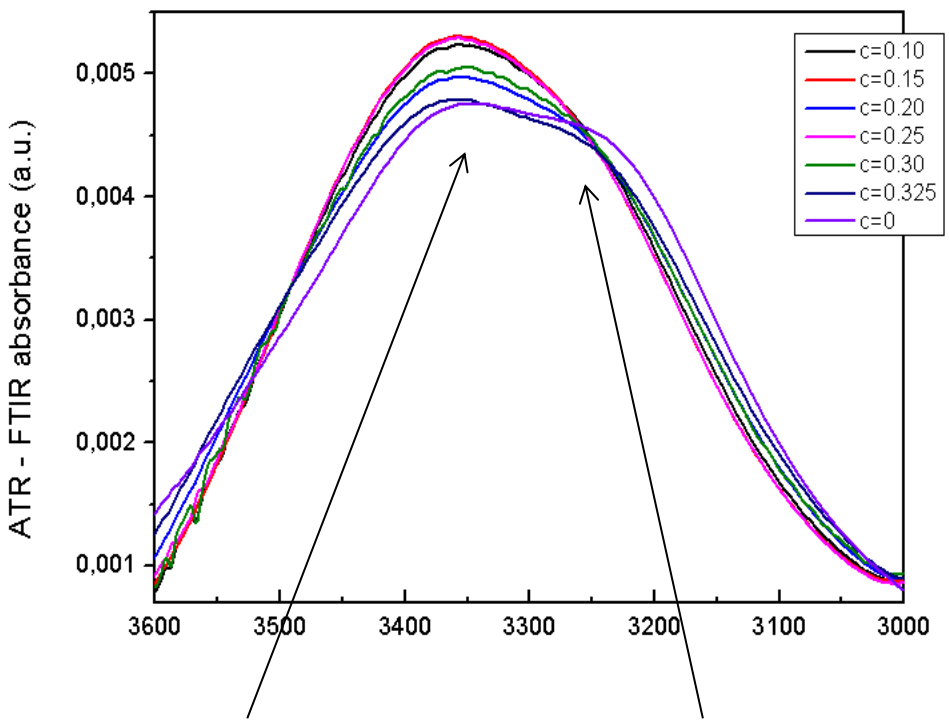




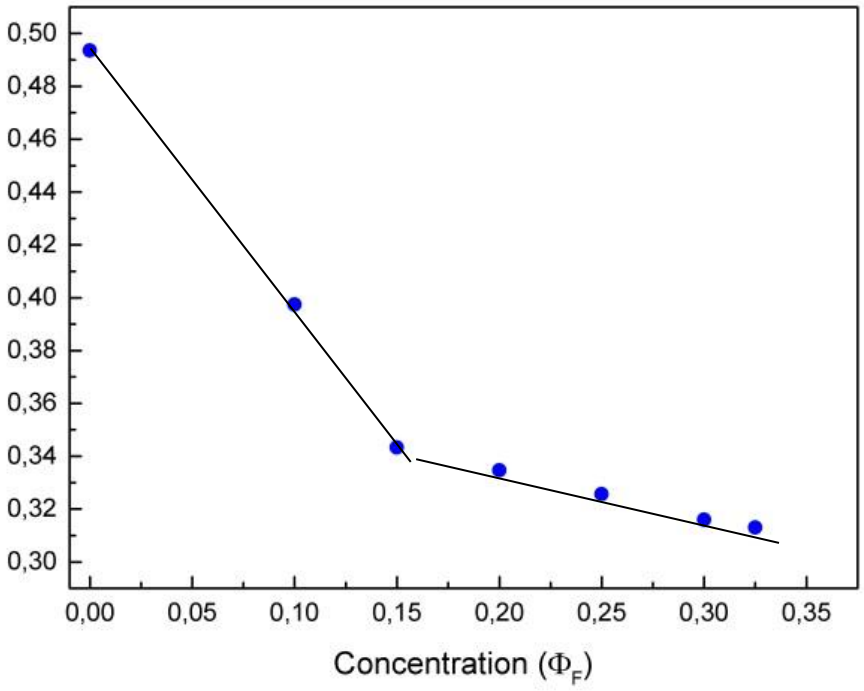
S. Palit, A. Yethiraj, *J. Chem. Phys*, 149, **2017**

Infrared Spectra of H_2O in Ficoll 70

OH-stretching band

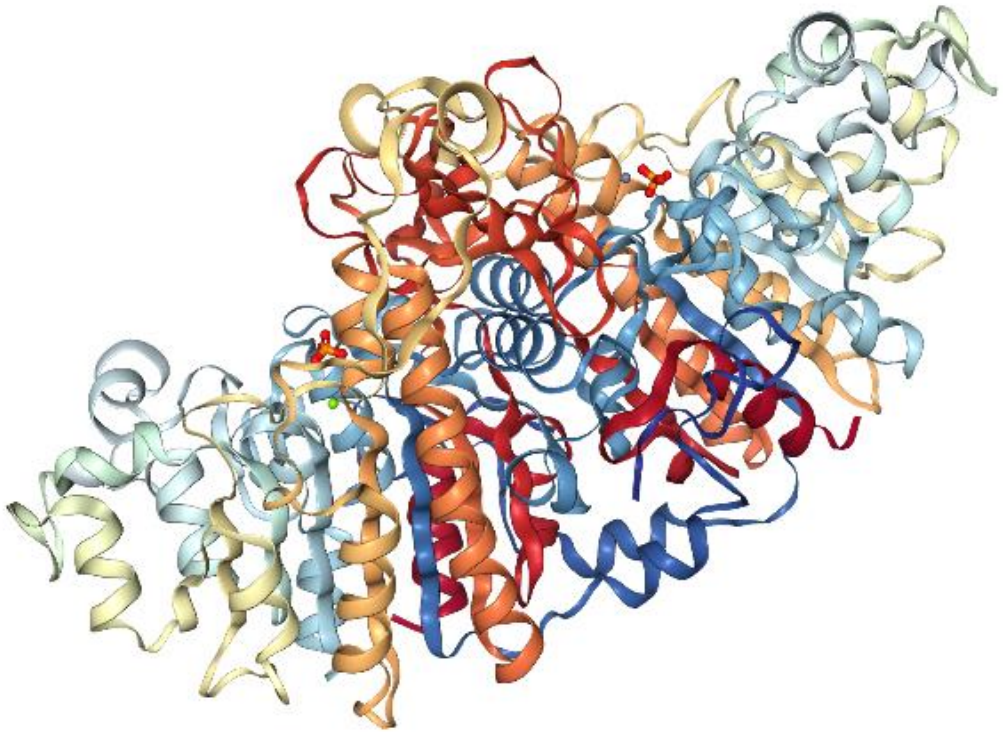


bulk water + H-bonded water



- Apparent break at 10-15 % of F70
- Fraction of bulk water significantly decreases
- Structural rearrangement in the F70 matrix

Alcaline Phosphatase



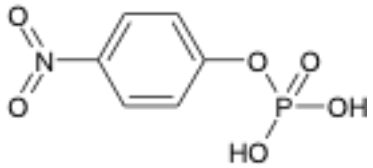
~160 kDa

R_{cr} ~ 31Å

- Dimeric membrane-derived glycoprotein
- Dephosphorylation of proteins or DNA/RNA
- BIOSAXS (SOLEIL) with sucrose and sorbitol: R_{SAXS} ~ 31(2) Å

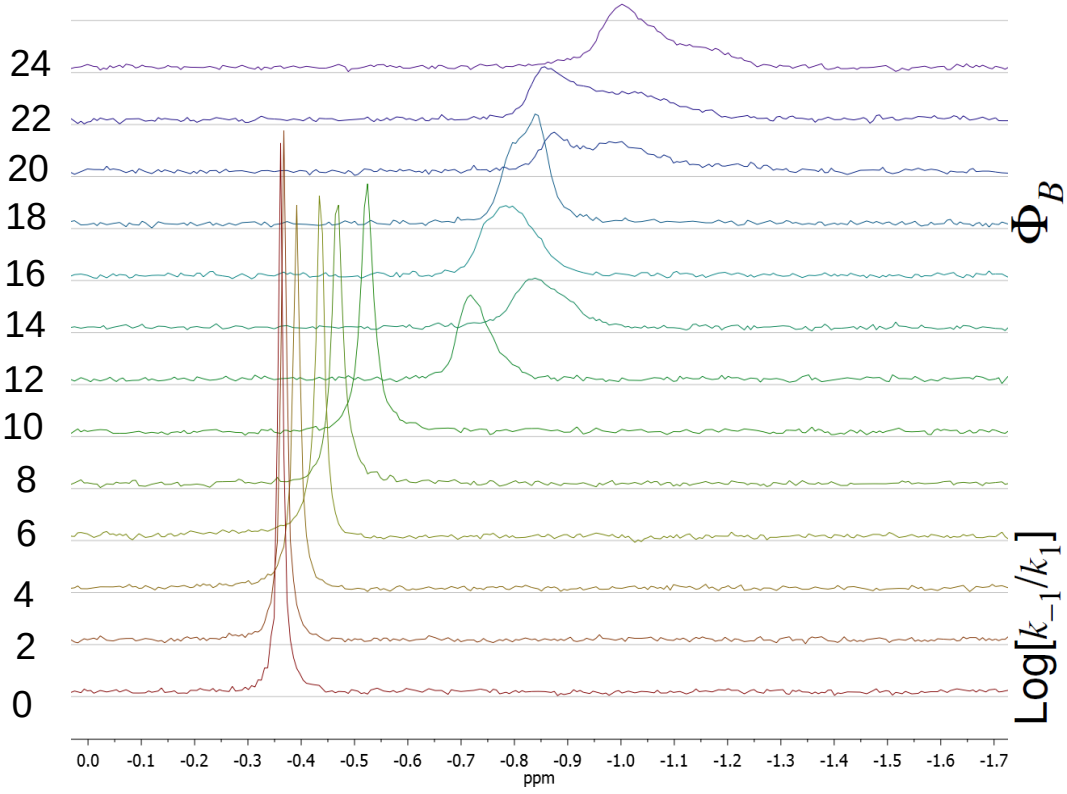
^{31}P NMR Investigations

- PNPP in Ficoll 70 media

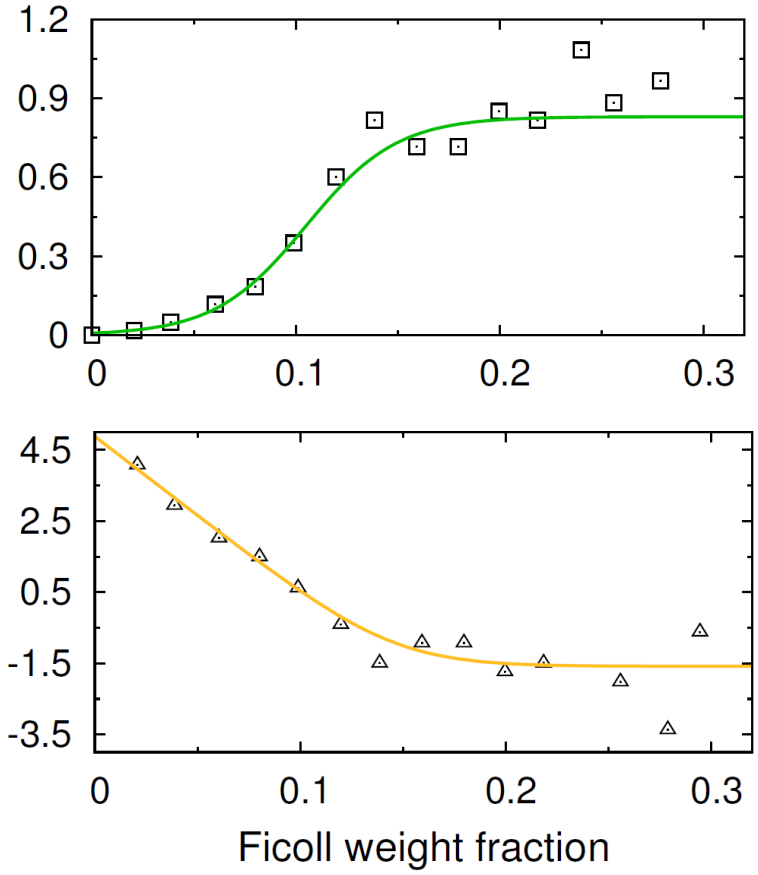


PNPP (A) $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ PNPP* (B)

$$k_{ex} = k_1 + k_1 = \pi \Delta\nu_o^2 / 2((W_{1/2})_e - (W_{1/2})_o)$$



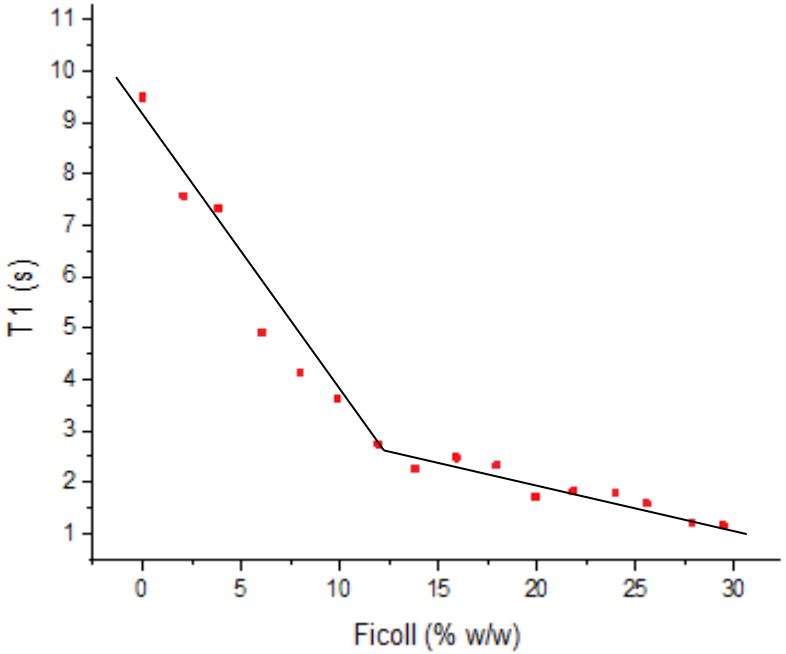
Bruker Avance 400 MHz, 310K



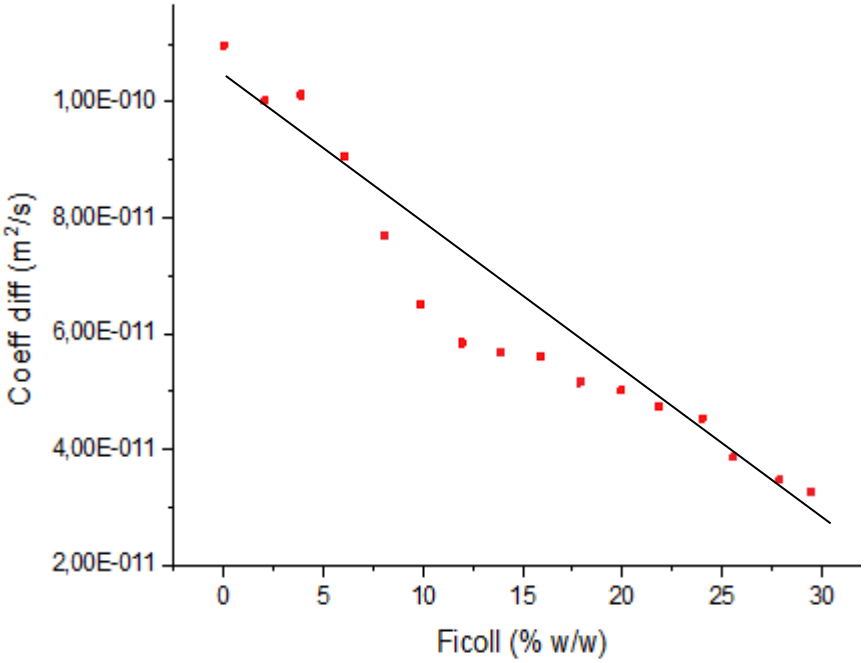
^{31}P NMR Investigations

PNPP (A) $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ PNPP* (B)

- T1 Relaxation



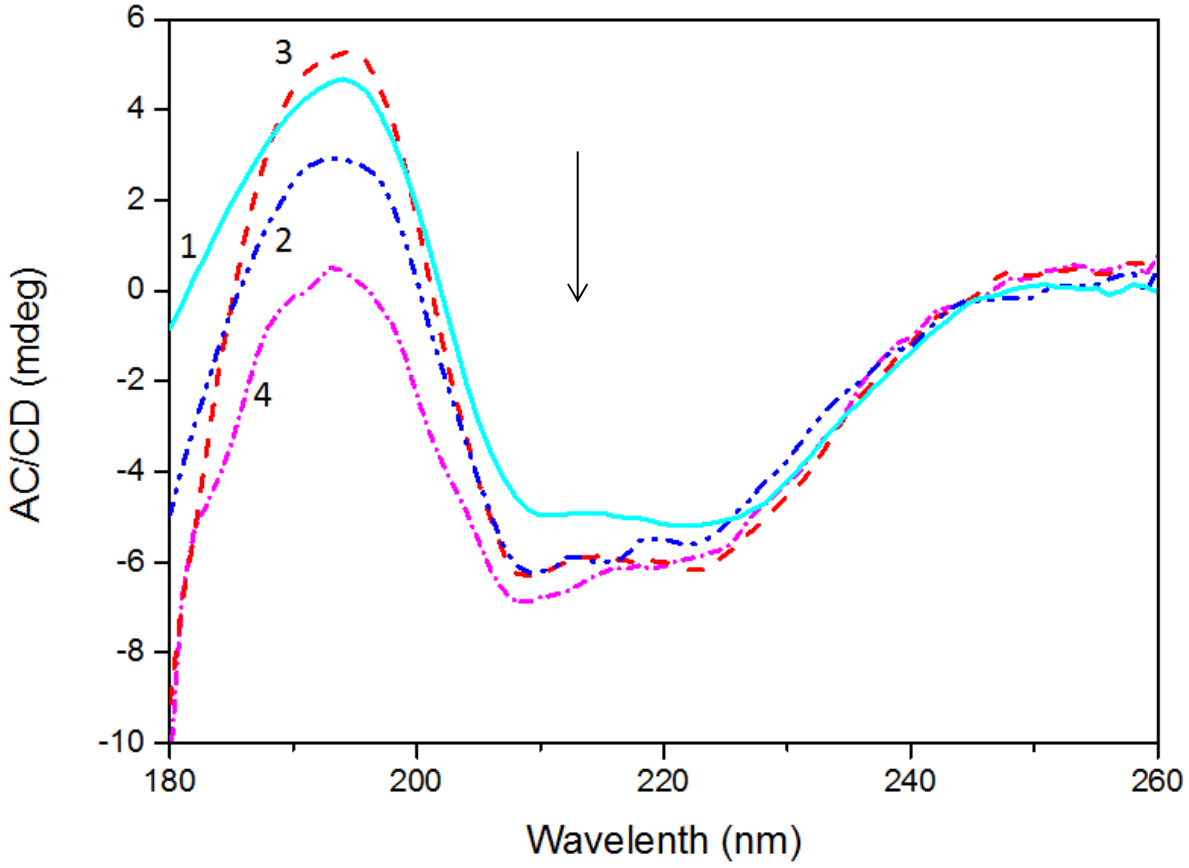
- DOSY



- NMR confirm the Ficoll rearrangement observed in IR

Synchrotron Radiation Circular Dichroism (SRCD)

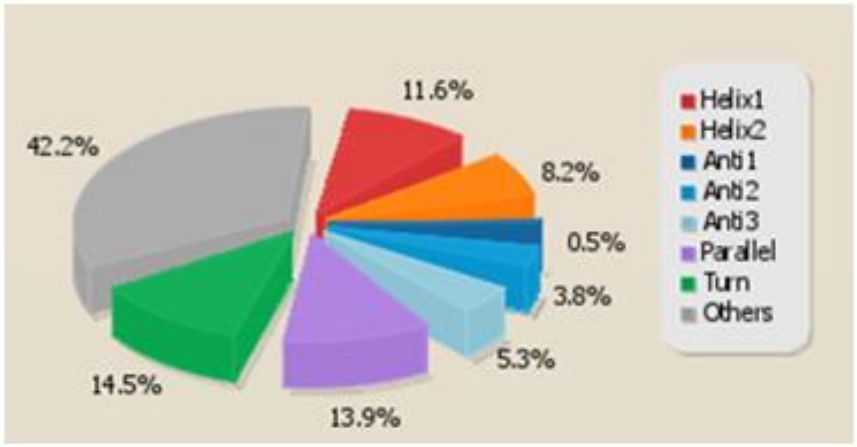
- Synchrotron SOLEIL, DISCO beamline (Gif-sur-Yvette, France), Dr Franck Wien
- Alcaline phosphatase in Ficoll 70 (3 mg/ml)



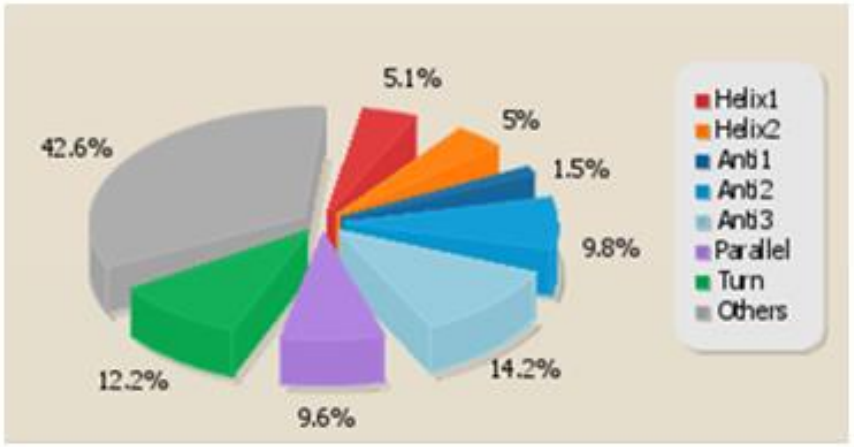
1 – 0% F70
2 – 4% F70
3 – 12% F70
4 – 20% F70

Synchrotron Radiation Circular Dichroism (SRCD)

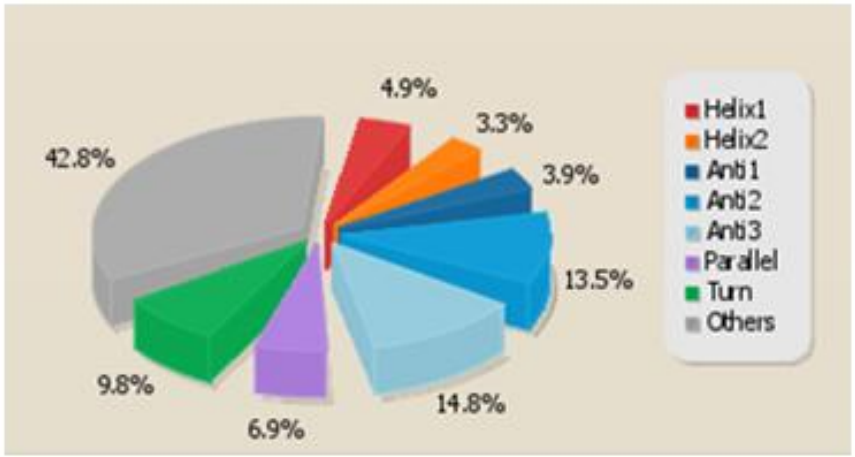
- BeStSel Algorithm *(Micsonai et al. PNAS 112:E3095-103 (2015))*



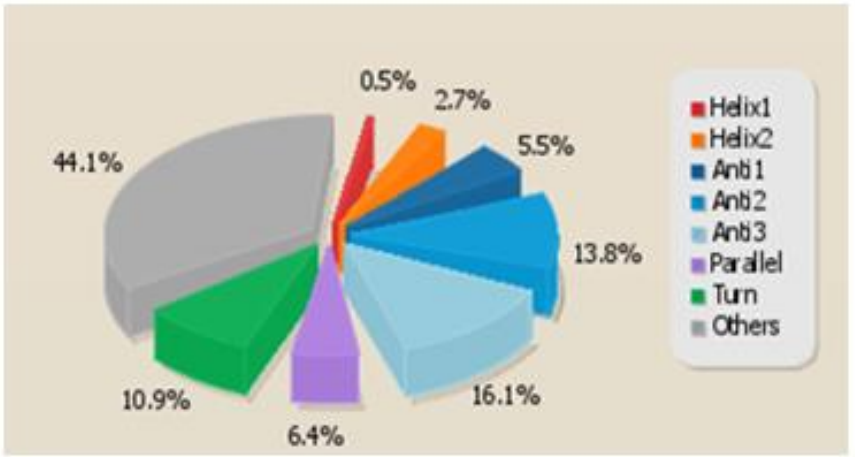
Alkaline phosphatase in solution of buffer without ficoll



Alkaline phosphatase in solution of buffer with 4% ficoll



Alkaline phosphatase in solution of buffer with 12% ficoll



Alkaline phosphatase in solution of buffer with 20% ficoll

Conclusions

- Combination of biophysical approaches for a better fundamental understanding of crowding effects
- Correlation of experiments with theoretical models and simulations
- Parameterization for kinetics studies (see Poster 22)
- Rational predictions and design for applications in cosmetics, pharmaceutics and medicine

Acknowledgement

CBM Orléans

Pr Francesco Piazza, Poster No. 22

Pr Salvatore Magazu

Dr Claire Madelaine-Perdrillat

Dr Sandrine Villette

Sandra Henri

Synchrotron Soleil and ILL Grenoble

Dr Javier Perez

Dr Franck Wien

Dr Viviana Cristiglio

CBM
Centre de Biophysique Moléculaire

UNIVERSITE D'ORLEANS

cnrs
dépasser les frontières

LE STUDIUM
Loire Valley
Institute for Advanced Studies

LA LIGUE
CONTRE LE CANCER

Cosmeto sciences
Recherche & Industrie, innovons ensemble

Région Centre-Val de Loire

Région Centre

ARBRE MOBIEU
BETWEEN ATOM AND CELL

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY