Besançon, 29 april 2016

Presentation of the Laboratory of Molecular Biophysics at the Interfaces

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IAP/Belspo P7/44 project : Integrative Protein Science (iPROS)
Bourse d'excellence Wallonie Bruxelles International (WBI)

UNIVERSITÉ DE LIÈGE
Gembloux Agro-Bio Tech

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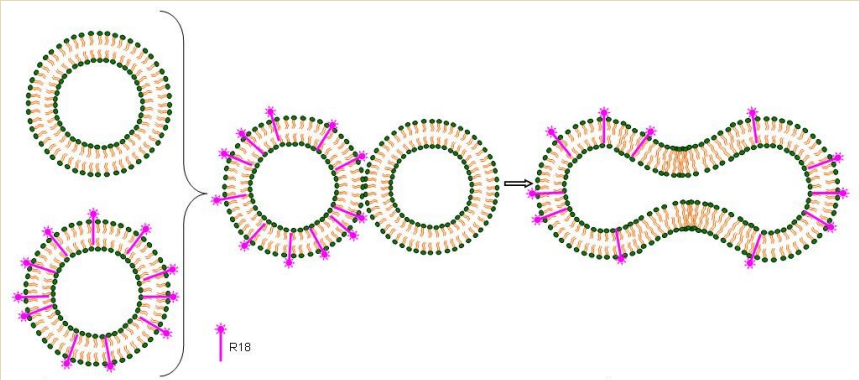
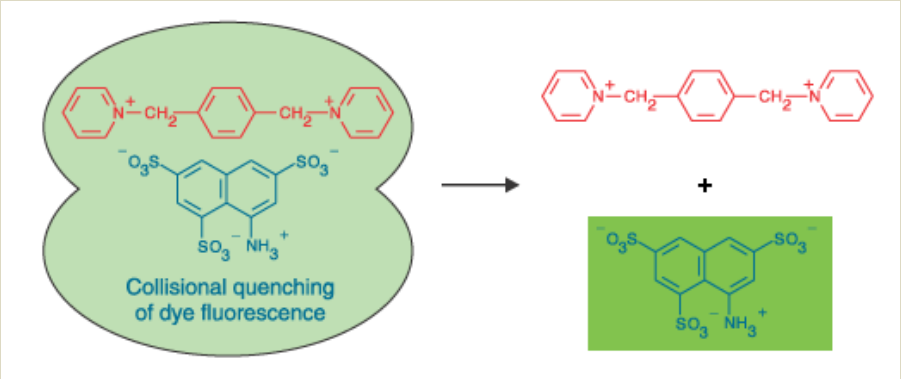
Belgian Science Policy Office
belspo

Experimental techniques

Fluorospectroscopy

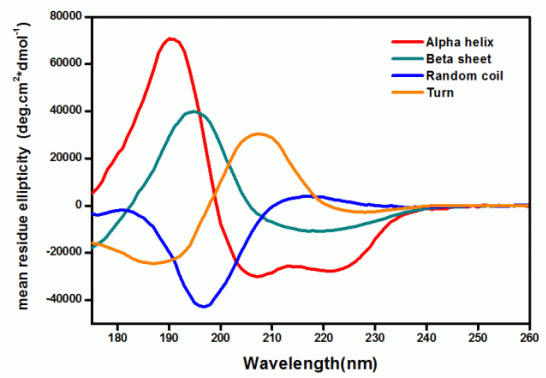
Lipid mixing and leakage of liposome contents experiments





Circular dichroism

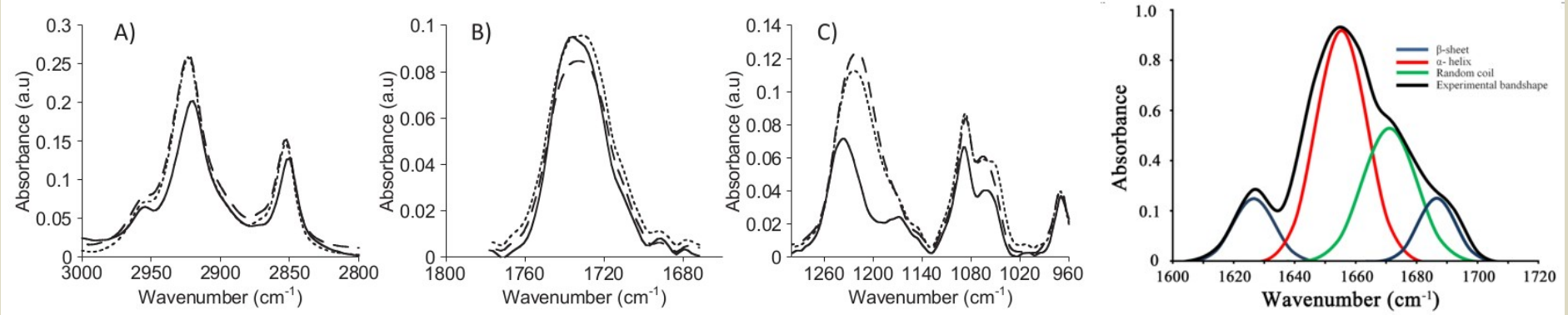
Secondary structure of peptides



Experimental techniques

- Infrared spectroscopy

  Peptides secondary structures and membrane interactions

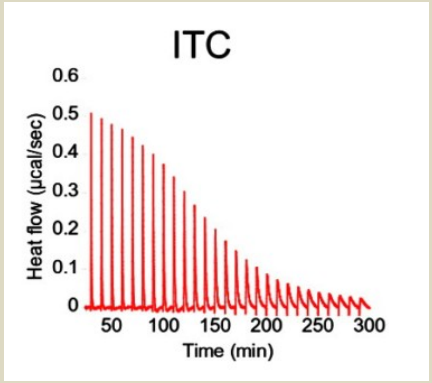


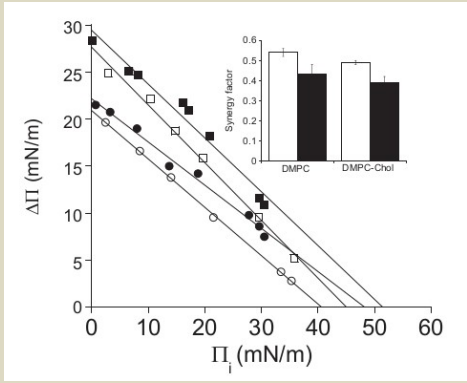
- Langmuir trough

  Adsorption experiments at constant surface area

- Isothermal Titration Calorimetry

  Calculation of thermodynamic parameters

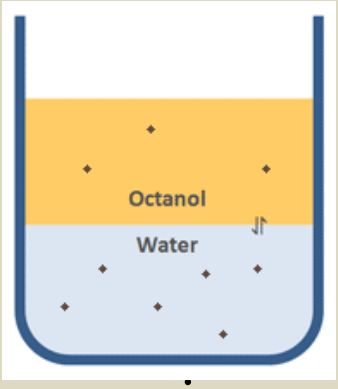




Modeling through hydrophobicity scales

- Partition coefficient have been used to define hydrophobocity scales (Eisenberg *et al*. 1982)

$$E_{tr} = -2{,}3\,RT \log \frac{[Mol]_{Octanol}}{[Mol]_{Water}}$$



Acides aminés	Hydrophobicité
Cystéine	0,29
Sérine	-0,18
Thréonine	-0,05
Proline	0,12
Alanine	0,62
Glycine	0,48
Asparagine	-0,78
Aspartate	-0,90
Glutamate	-0,74
Glutamine	-0,85
Histidine	-0,40
Arginine	-2,50
Lysine	-1,50
Méthionine	0,64
Isoleucine	1,40
Leucine	1,10
Valine	1,10
Phénylalanine	1,20
Tyrosine	0,26
Tryptophane	0,81

- For simple organic molecules, transfer energies are proportionnal to the number of carbon atomes in the molecule
- Atomic Etr (Brasseur *et al*. 1991)

  Hypothesis: Additive properties of transfer energies

C Sp2 = -1.503
C sp3 = -2.436
H(q=0) = -0.537
H(q<0) = 1.030
O = 2.833
S = -2.751
N = 3.035

Molecular orientation at the interface

- Tammo (Brasseur *et al*. 1991)

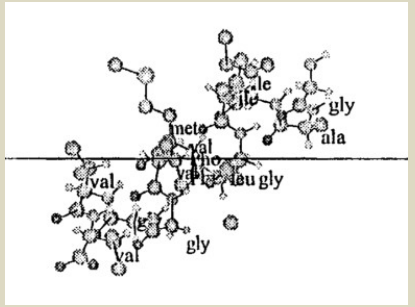
  Alignement of hydrophobic ΣEtr_{pho} and hydrophilic ΣEtr_{phi} centers along Z

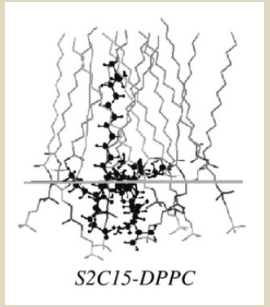
- Hypermatrice (Brasseur *et al*. 1991)

  Monte Carlo for the interaction with lipids

- IMPALA (Ducarme *et al*. 1998)

  Monte Carlo for the interaction with the membrane

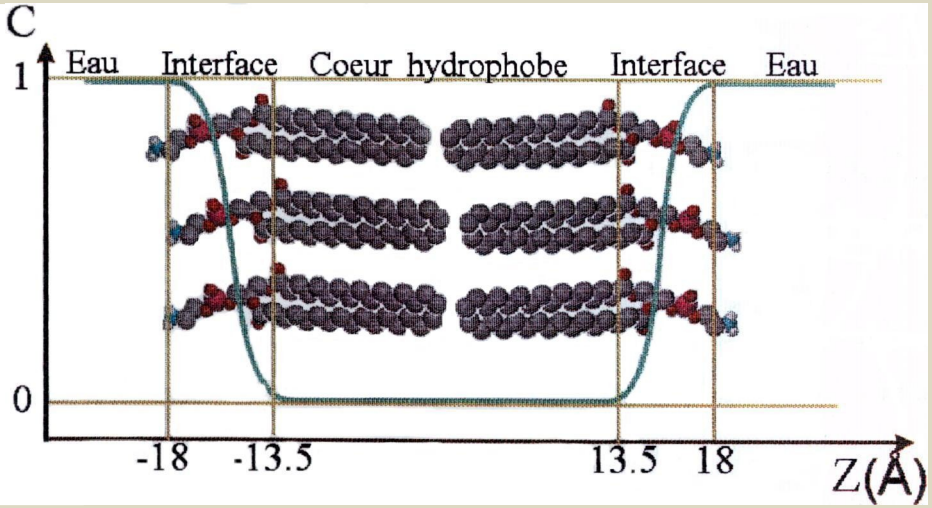




$$C_{(z)} = 1 - \left(\frac{1}{1 + e^{\alpha(z - z_0)}} \right)$$

$$E_{lip} = 0.018 \sum_{i=1}^{N} S_{(i)} \left(1 - C_{(z_i)}\right)$$

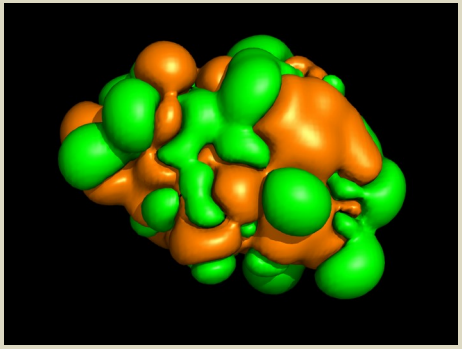
$$E_{\text{int}} = -\sum_{i=1}^{N} S_{(i)} E_{tr(i)} C_{(z_i)}$$



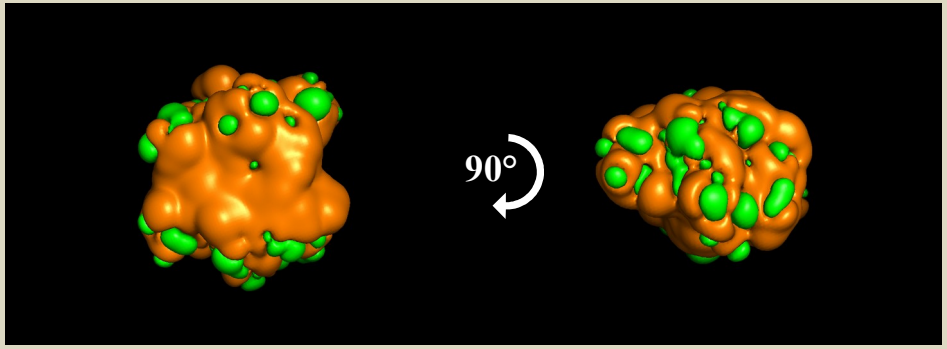
Hydrophobicity representation

- Mean Hydrophobicity Potential (Brasseur *et al*. 1991) $MHP=\Sigma_i Etr_i e^{(ri-di)}$

Lyzozyme



MraY



The accessible surface of soluble proteins is 50 % hydrophobic (Lins *et al*. 2003)

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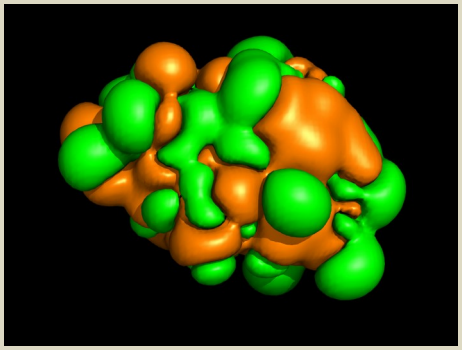
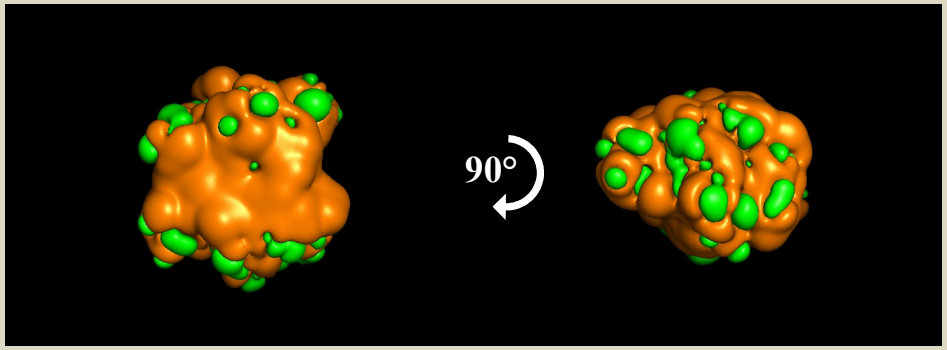
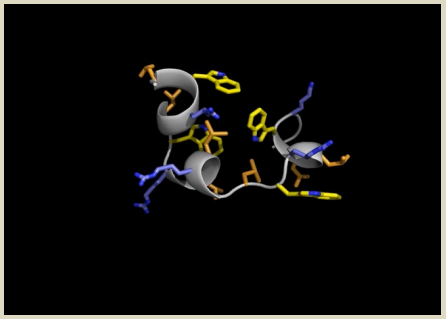


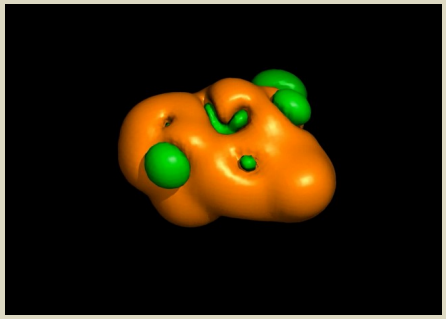
MraY



The accessible surface of soluble proteins is 50 % hydrophobic (Lins *et al*. 2003)

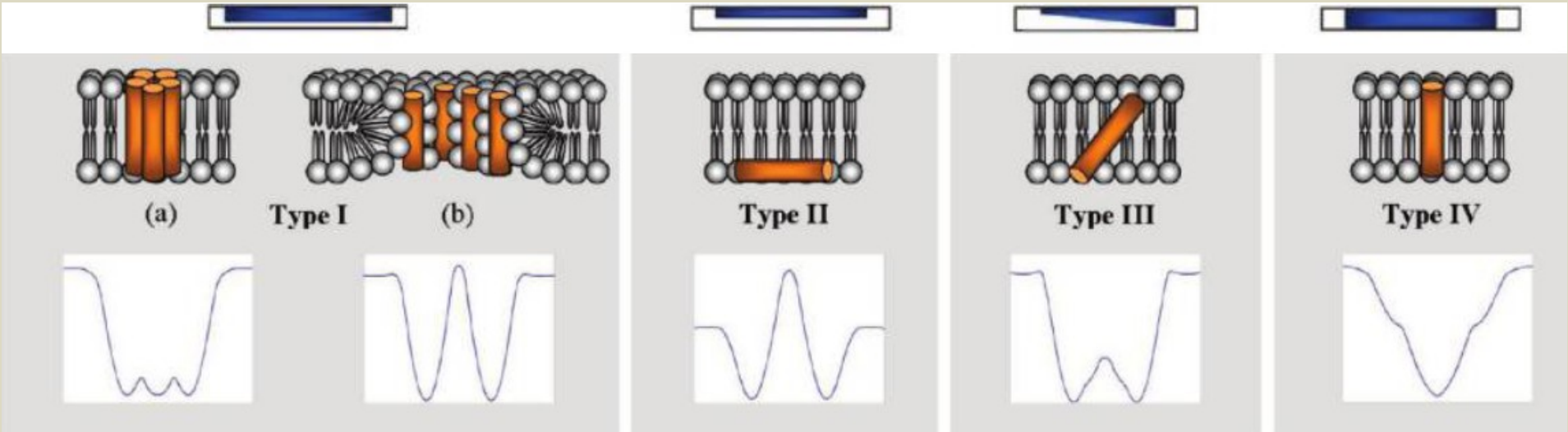
- MHP can be visualized during MD trajectories (Crowet *et al*. 2009)





Classes of amphiphilic alpha-helical peptides

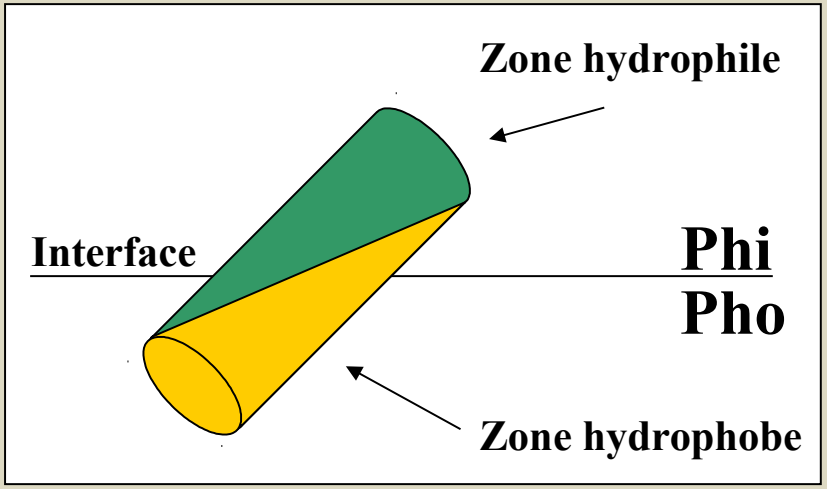
- MHP have been used to define different membrane peptide categories based on their hydrophobicity profiles (Brasseur *et al*. 1991)
- Gkeka *et al.* have recently tested characteristic peptides of these categories by CG MD (2010)

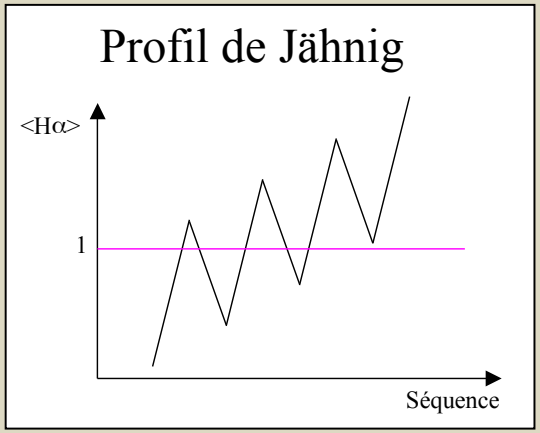


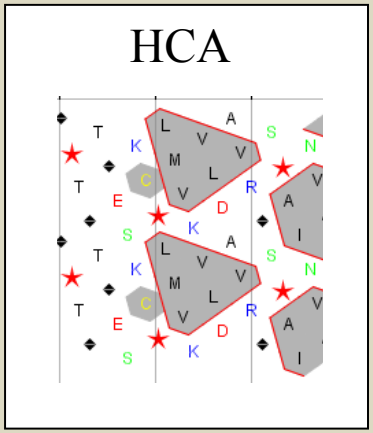
Tilted peptides

- Peptides of 11-19 residues with <H> between 0.2 and 0.9 and that presents an hydrophobicity gradient
- These peptides can be identified by a sequence analysis

HCA (Gaboriaud *et al.* 1987), Jahnig (Jähnig 1990)





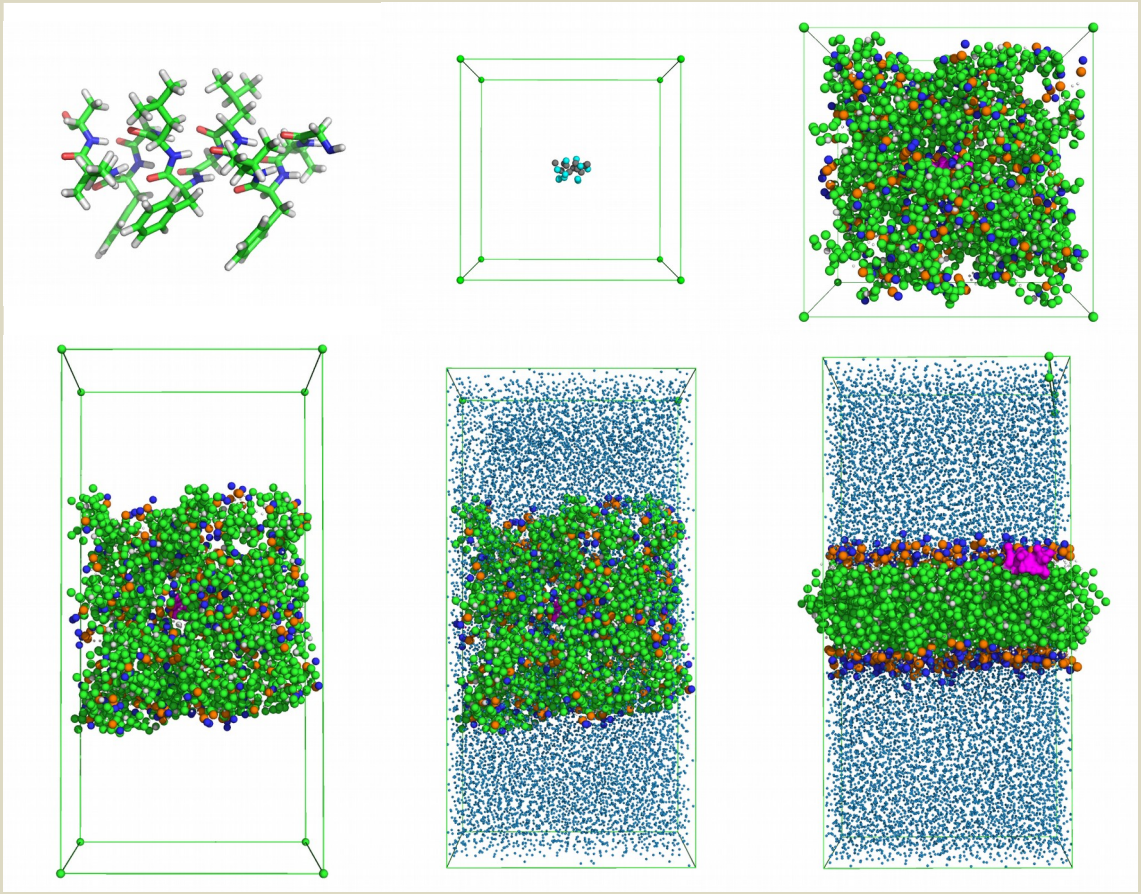


- Tilted peptides have been identified since 1988 from fusion peptides of different viruses (SIV, NDV, Influenza, …) as well as in other protein types and have a destabilizing effect on membranes (Horth *et al.* 1991, Vonèche *et al.* 1992, ...)

Bilayer formation by self assembly

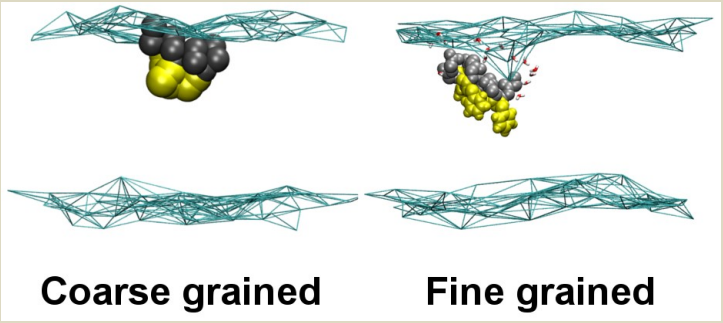
Methodology

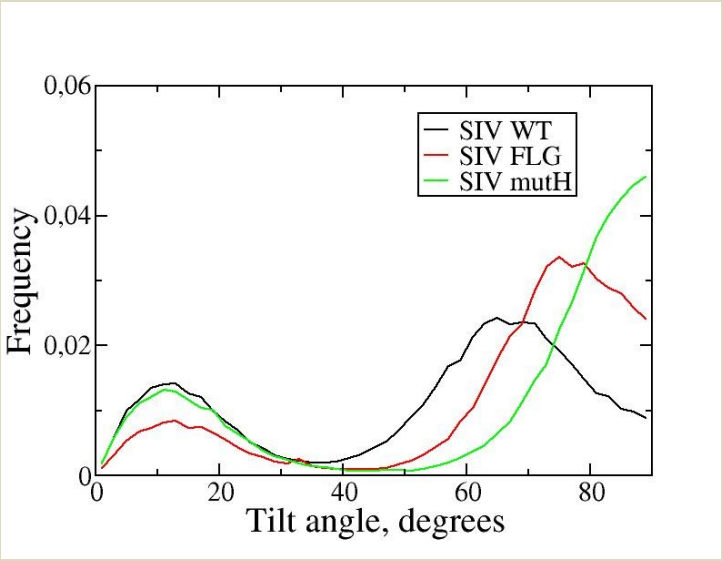
Bond *et al*. 2006, Hall *et al*. 2011



Application to the SIV fusion peptide

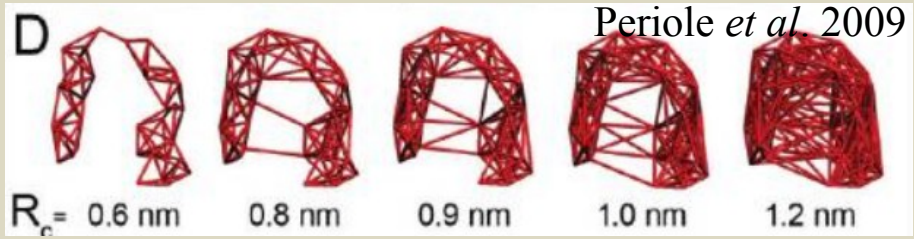
Crowet *et al*. 2012



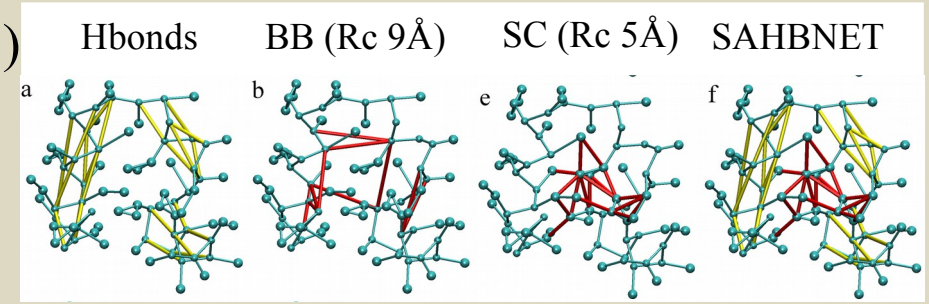


The protein structures have to be maintained in CG

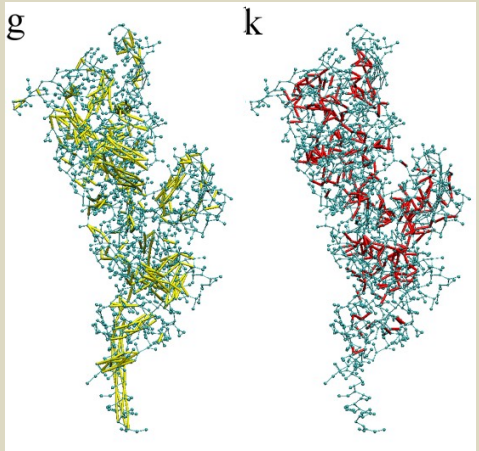
- ELNEDYN use an elastic network based on a cut-off

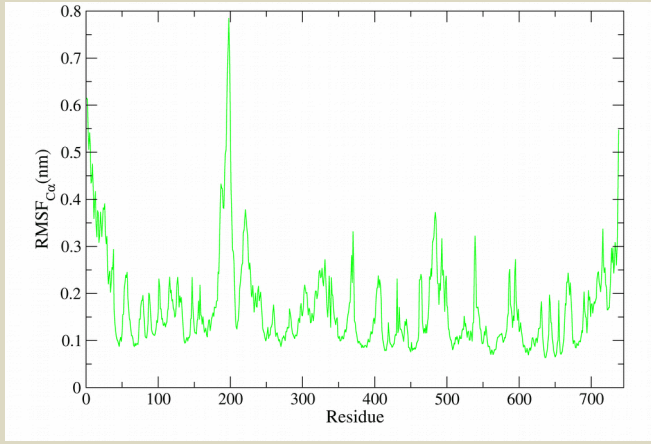


- SAHBNET use an elastic network based on H bonds and the accessible surface (Dony *et al.* 2013)



- PBP1b, a monotopic membrane protein of *E. coli*
  - 3FWM ; 738 residues
  - 453 hbonds
  - 345 SA (SAc 30 %, Rc 5Å)
  - ELNEDYN = 3428

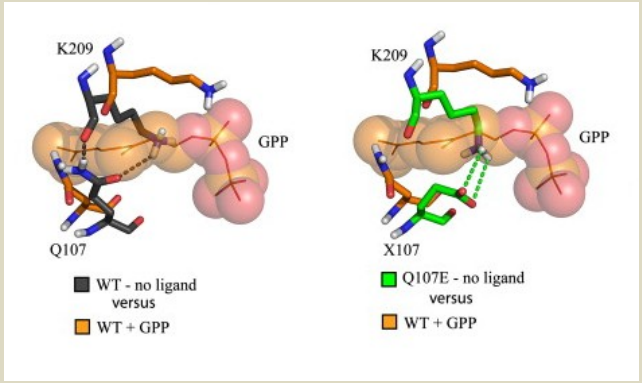




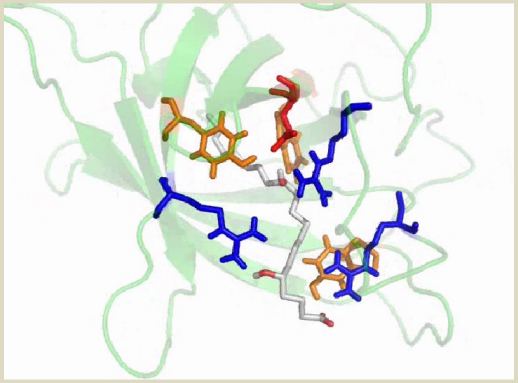
Molécular dynamics

Protein-ligand simulations

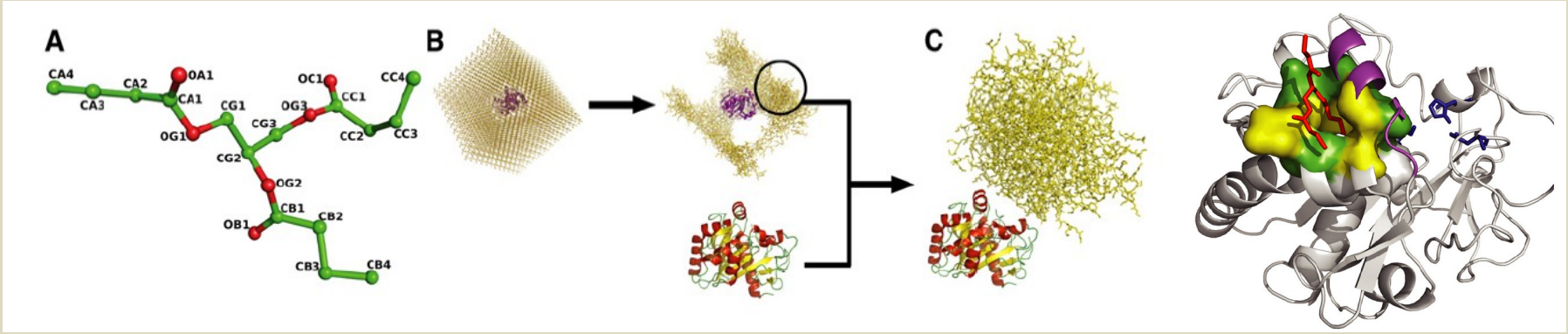
Double enzymatic activity of a aphid phenyltransferase (Vandermoten *et al*. 2009)



Interaction between the lipocaline and the LTB4 (Beaufays *et al*. 2008)



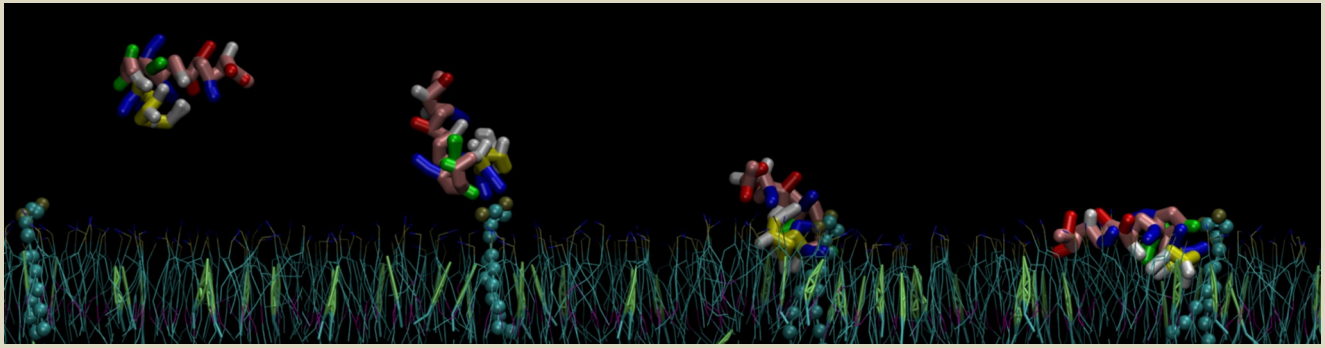
Interaction between a lipid globule and a lipase (Santini *et al*. 2009)



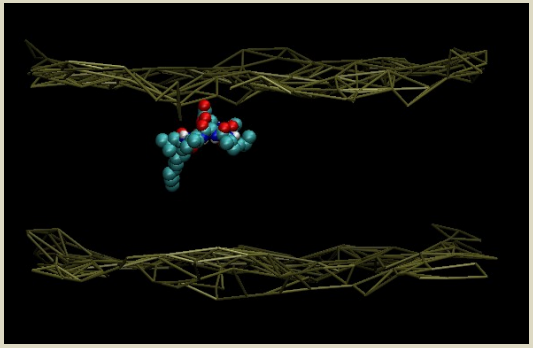
Molecular Dynamics

Simulations of peptide membrane interactions

The Remorin C terminal domain

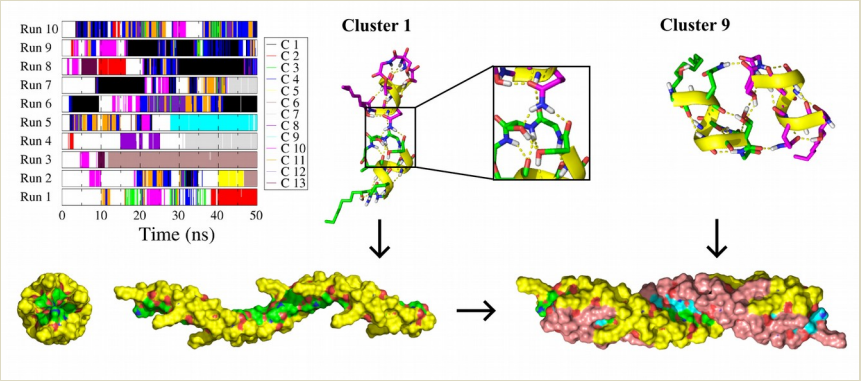


The surfactin

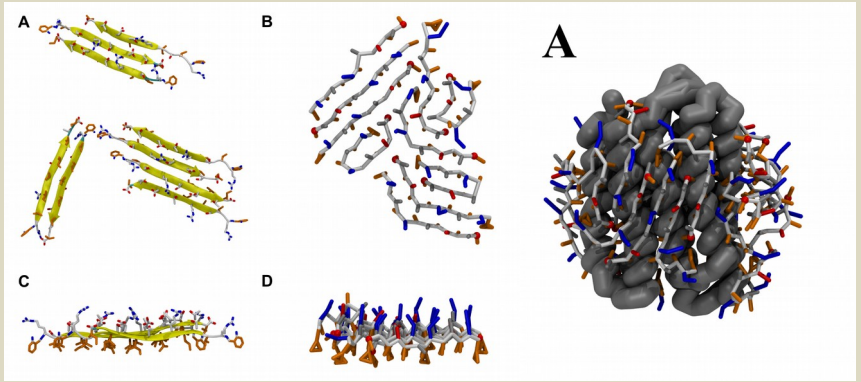


Other simulations

Pseudodesmin fibrils



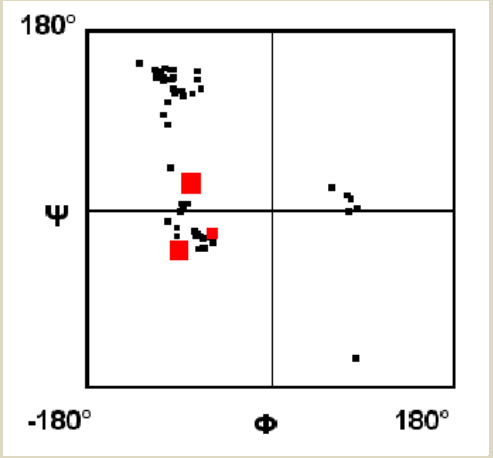
Beta amphiphile agregation



PepLook, a tool to predict the 3D structure of peptides

(Thomas et al. 2006)

- Etchebest *et al.*, 2005 => 64 couples Φψ/aa



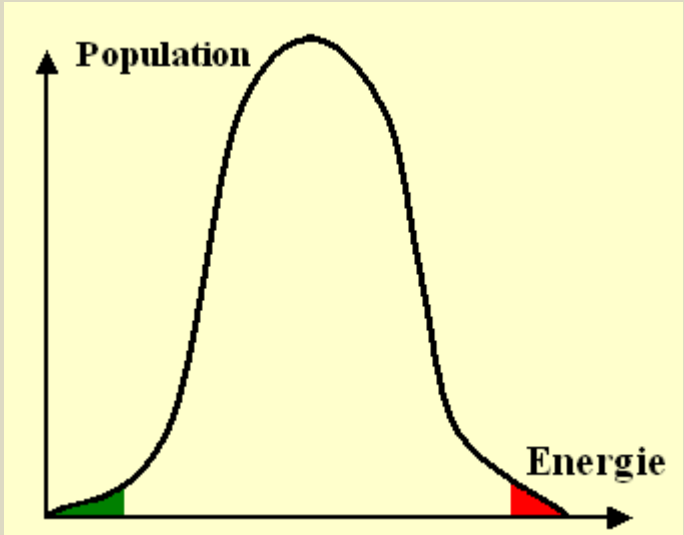
10.000 à Random structures

E_{vdW}
+ E_{elec}
+ E_{pho_intra}
+ E_{pho_out}
E_{tot}

200x

Probability Φ and ψ ⇧ or ⇩

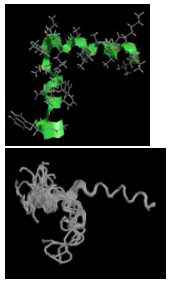
Energy Distribution



Prime

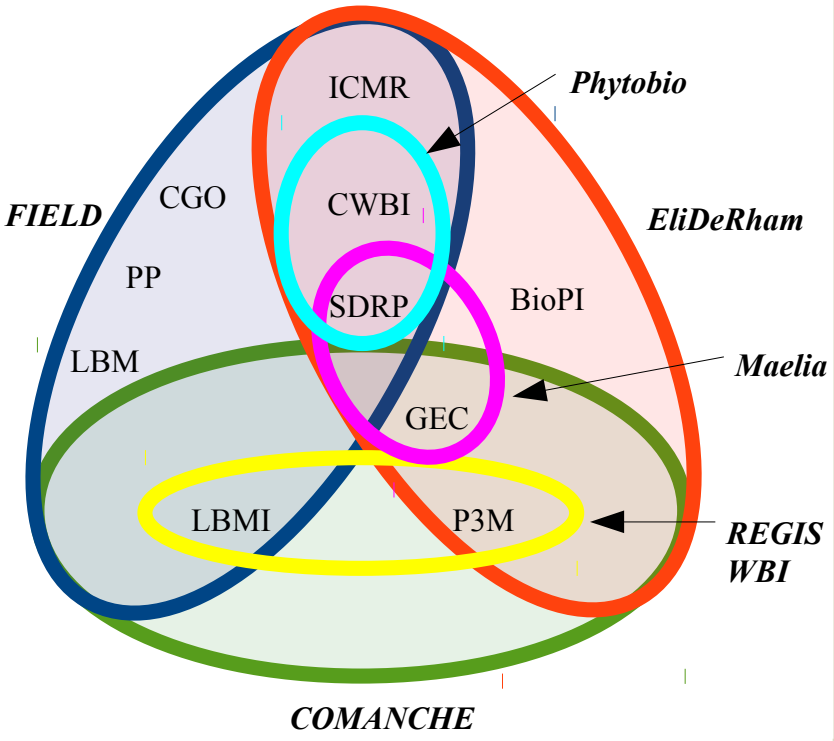
+

98 structures



Study of elictors of the plant defense responses

- COMANCHE :
  COmparative Membrane Adapted from Numerical CHaracterizations using Energy
- REGIS :
  Reims Et Gembloux Innovent par Simulations
- EliDeRham :
  ELiciteurs DErivés de RHAMnolipides : synthèses, modélisations et activités biologiques
- FIELD :
  New alternatives to chemical pesticides : deciphering the action mechanism of lipid based plant elicitors via complementary biophysical and biological approches
- PHYTOBIO :
  Développement et promotion de nouveaux produits phytosanitaires pour la lutte biologique contre les maladies des plantes
- MAELIA :
  Etude du mode d'action et de perception d'éliciteurs amphiphiles stimulant l'immunité innée des végétaux



Université de Liège
CGO, LBMI, CWBI, PP

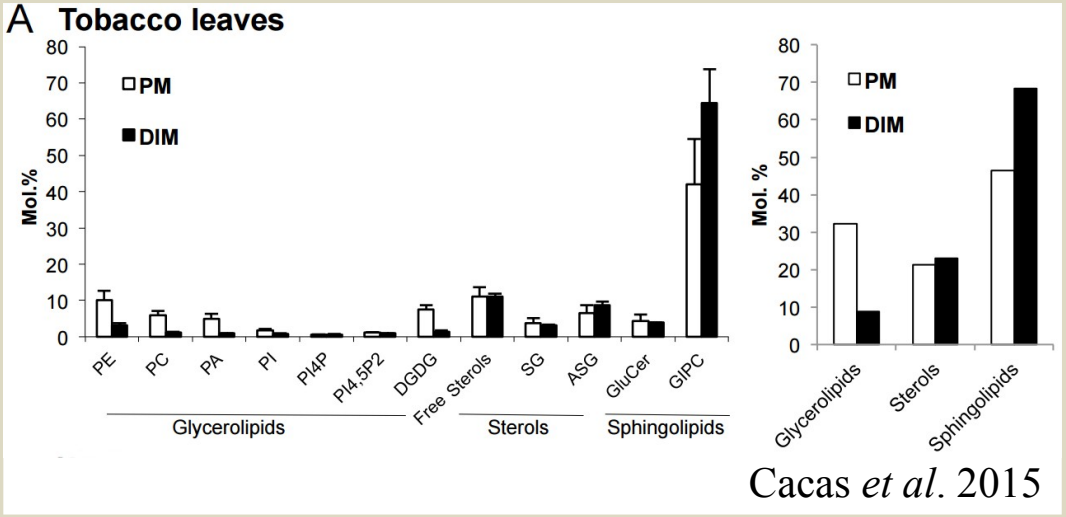
Université de Reims Champagne Ardenne
ICMR, P3M, SDRP

Université de Picardie Jules Verne
GEC, BioPI

Université Bordeaux Segalen
LBM

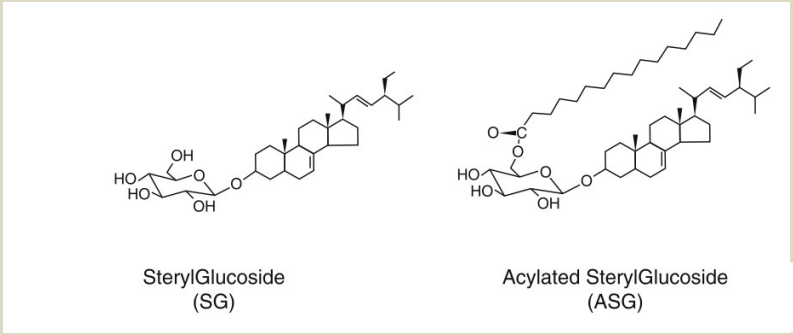
Topologies of several important plant lipids are not yet available

- The GIPC represents up to 40 % of plant plasma membranes

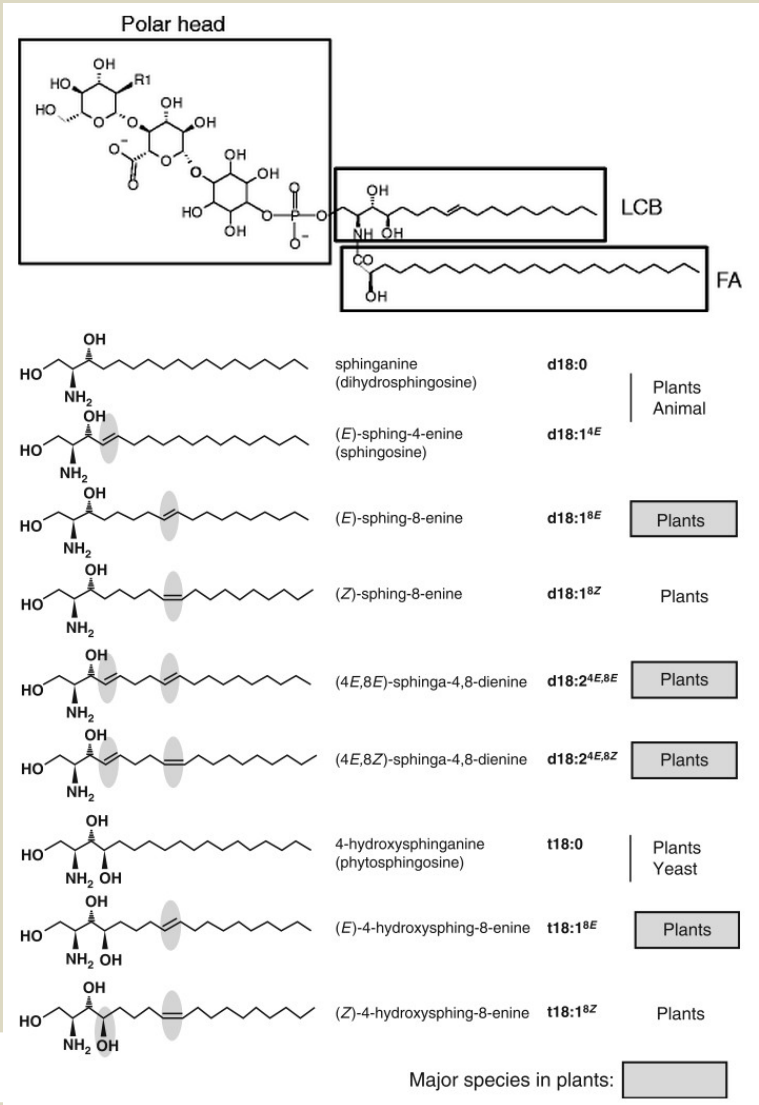


Cacas *et al*. 2015

- Sterols are more diversified



Furt *et al*. 2011



Modeling of membranes and membrane interactions in a biological context tend to a more detailed description

- MD of membranes usually use 1 or 2 lipids
- Cellular membranes involve hundreds of lipids
- There should be up to 100k lipidic species and the LIPID MAPS consortium has already identified more than 40k
- The number of lipidic topologies increase (Charmm et Martini)

Table 1. Lipid classification and the number of lipid types in *Membrane Builder*.

Classification	Sterols	PA lipids	PC lipids	PE lipids	PG lipids	PS lipids
# lipid types	2	14	14	16	14	14
Classification	**PI lipids**	**CL lipids**	**PUFA lipids**	**SM lipids**	**Bacterial lipids**	
# lipid types	46	23	15	15	9	

Wu *et al*. 2014

Lipid classification and the number of lipids in Martini

Classification	PC lipids	PG lipids	PE lipids	PS lipids	Glycoglycero lipids	Other glycerolipids
# lipid types	22	23	23	23	11	15
Classification	**PI lipids**	**CL lipids**	**PA lipids**	**SM lipids**	**Sterols**	**Glycosphingo lipids**
# lipid types	14	3	21	9	3	11

Martini website (http://cgmartini.nl/)

- Specific tools are developped : Insane, Backwards, …
- The first simulations that intend to reproduce the lipidic complexcity are published : Ingolfsson 2014, Van Eerden et al. 2015, Koldso 2015, Reddy 2015, Flinner 2015

Aims of the modeling efforts

« To Develop a molecular modeling platform dedicated to the building of complex lipidic membranes and to caracterize their interactions with bioactive molecules »

- To gather teams specialized in modeling techniques
  - Synergy through sharing of expertise, methodologies and tools
  - Provision of the university community via P3M

- Development of a tool of construction and comparison of lipid membranes of plant and animal models through a web interface
  - To propose model membranes for various organisms and organels
  - With an automated construction and use of a lipid database
  - To evaluate the interaction of amphiphilic molecules with a focus on the composition
  - To Improve the modeling of plant membranes that remains poorly documented

The LBMI team

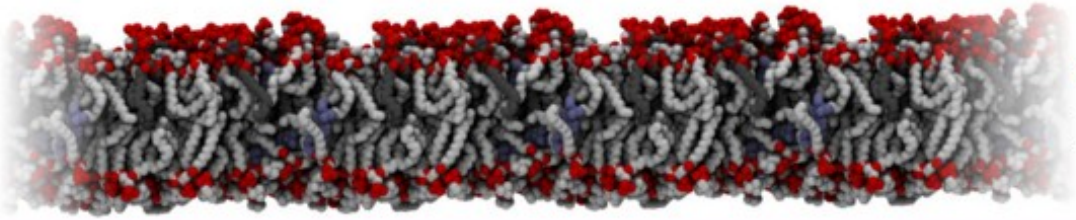
Laurence LINS
Senior scientific researcher
at FNRS

Magali DELEU
Senior scientific researcher
at FNRS

Mehmet Nail NASIR
PhD

Comanche

Membrane Builder and Repository



Welcome, jmcrowet!

Log out | Change your users info

Home | Membrane builder | Libraries | Jobs

System options

Name: | Forcefield: Martini

Membranes: Ingolfsson_plasma_membrane | Temperature: 297 | Nb lipids: 4096

Lipids

Lipids Up		Lipids Down	
676	CHOL	551	CHOL
377	PIPX	172	PIPC
257	POPX	121	POPE
130	DPSM	117	POPC
81	PNSM	111	PAPE
60	PAPC	98	PAPS
57	XNSM	81	PIPE

Interacting molecules

PDB ID | Or | PDB Structure ... | Topology ... | Nb prot | Delete

Add

N-cap: NH3+ | Sequence | C-cap: COO- | Structure: Helix | Nb prot | Delete

Add

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