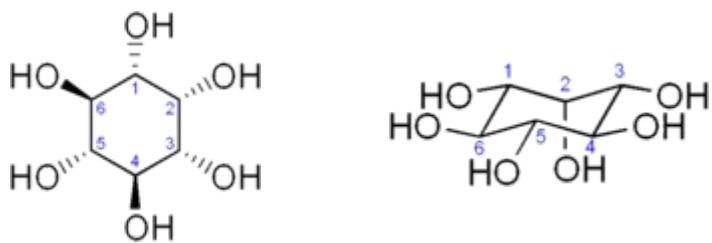


INOSITOL

Inositol, (of which the most prominent naturally-occurring form is myo-inositol, cis-1,2,3,5-trans-4,6-cyclohexanehexol), is a carbocyclic polyol that plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells, including inositol phosphates, phosphatidylinositol (PI) and phosphatidylinositol phosphate (PIP) lipids. It is found in many foods, particularly in cereals with high bran content, nuts, beans, and fruit, especially cantaloupe melons and oranges. It is also noticeably present in popular "boost" drinks such as Red Bull. It can be synthesised by the human body.

Other naturally occurring isomers (though in minimal quantities) are scylo-, chiro-, muco-, and neo-inositol. Other possible isomers are allo-, epi- and cis-inositol.

Myo-Inositol is classified as a member of the vitamin B complex (often referred to as vitamin B8), though it is not considered a vitamin itself.

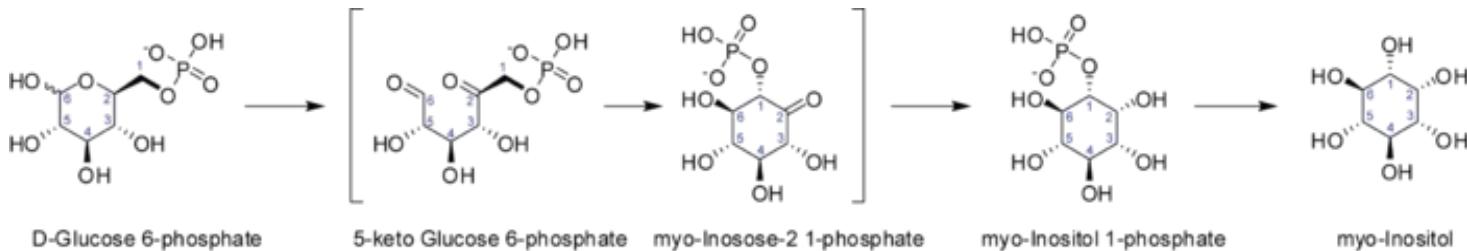


Structure:

The chemical formula of myo-inositol is C₆H₁₂O₆. In its most stable geometry, the inositol ring is in the chair conformation. There are nine stereoisomers, all of which may be referred to as inositol; however, the natural isomer has a structure in which the 1st, 3rd, 4th, 5th and 6th hydroxyls are equatorial, while the 2nd hydroxyl group is axial.

Synthesis:

myo-Inositol is synthesized from glucose-6-phosphate (G-6-P) in two steps. First, G-6-P is isomerised by INYNA1 to myo-inositol 1-phosphate, which is then dephosphorylated by IMPA1 to give myo-inositol.



Function:

Inositol as the basis for a number of signalling and secondary messenger molecules, is involved in a number of biological processes, including:

insulin signal transduction
cytoskeleton assembly

nerve guidance (Epsin)
intracellular calcium (Ca^{2+}) concentration control
cell membrane potential maintenance
serotonin activity modulation
breakdown of fats and reducing blood cholesterol
gene expression

Clinical implications:

Some preliminary results of studies on inositol supplements show promising results for people suffering from problems such as bulimia, panic disorder and bipolar depression.

D-chiro-inositol (DCI) has been found in two double-blind studies to be an effective treatment for many of the clinical hallmarks of polycystic ovary syndrome (PCOS), including insulin resistance, hyperandrogenism and oligo-/amenorrhea. The impetuses for these studies were the observed defects in DCI metabolism in PCOS and the implication of DCI in insulin signal transduction.

myo-Inositol has been found in double-blind studies to be an effective treatment for obsessive-compulsive disorder (OCD). It is equal in effectiveness to SSRIs and is virtually free from side effects.

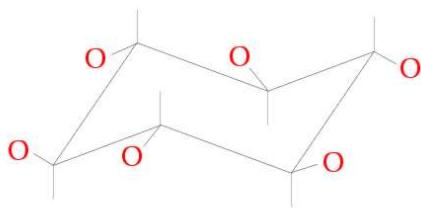
Animal studies suggest inositol reduces the severity of the osmotic demyelination syndrome if given prior to rapid correction of chronic hyponatraemia. Further study is required prior to its application in humans for this indication.

Studies from in vitro experiments, animal studies, and limited clinical experiences, claim that inositol may be used effectively against some types of cancer, particularly when used in combination with phytic acid.

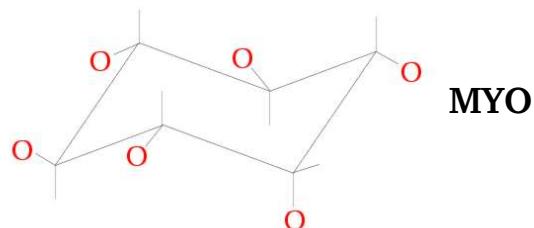
Ref: <http://en.wikipedia.org/wiki/Inositol>

The isomers

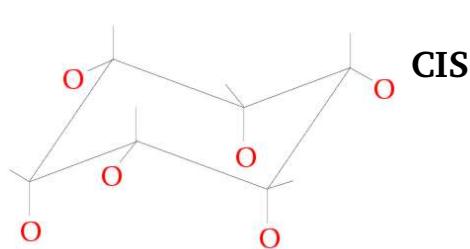
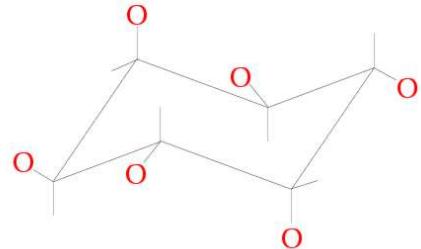
SCYLLO



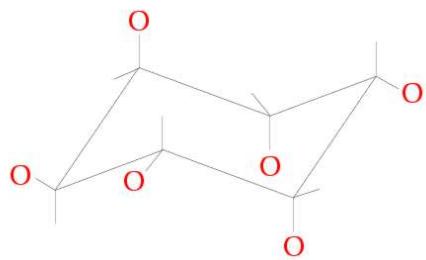
MYO



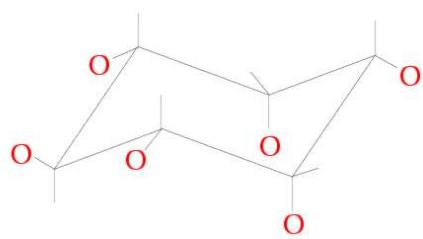
NEO



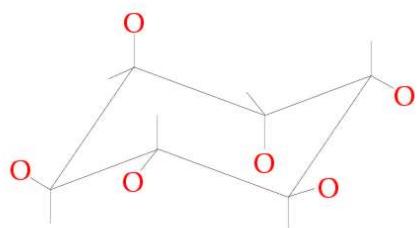
ALLO



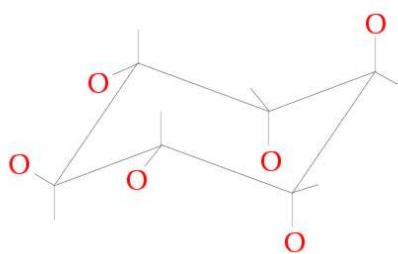
EPI



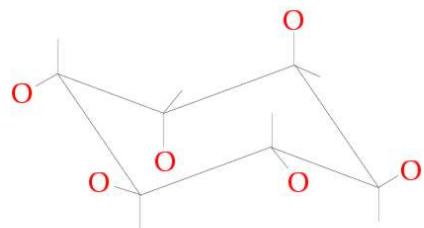
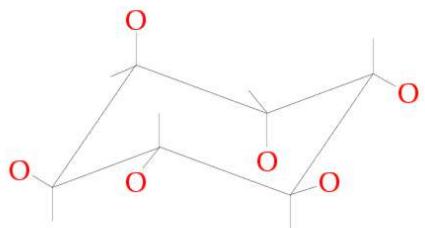
CHIRO



MUCO



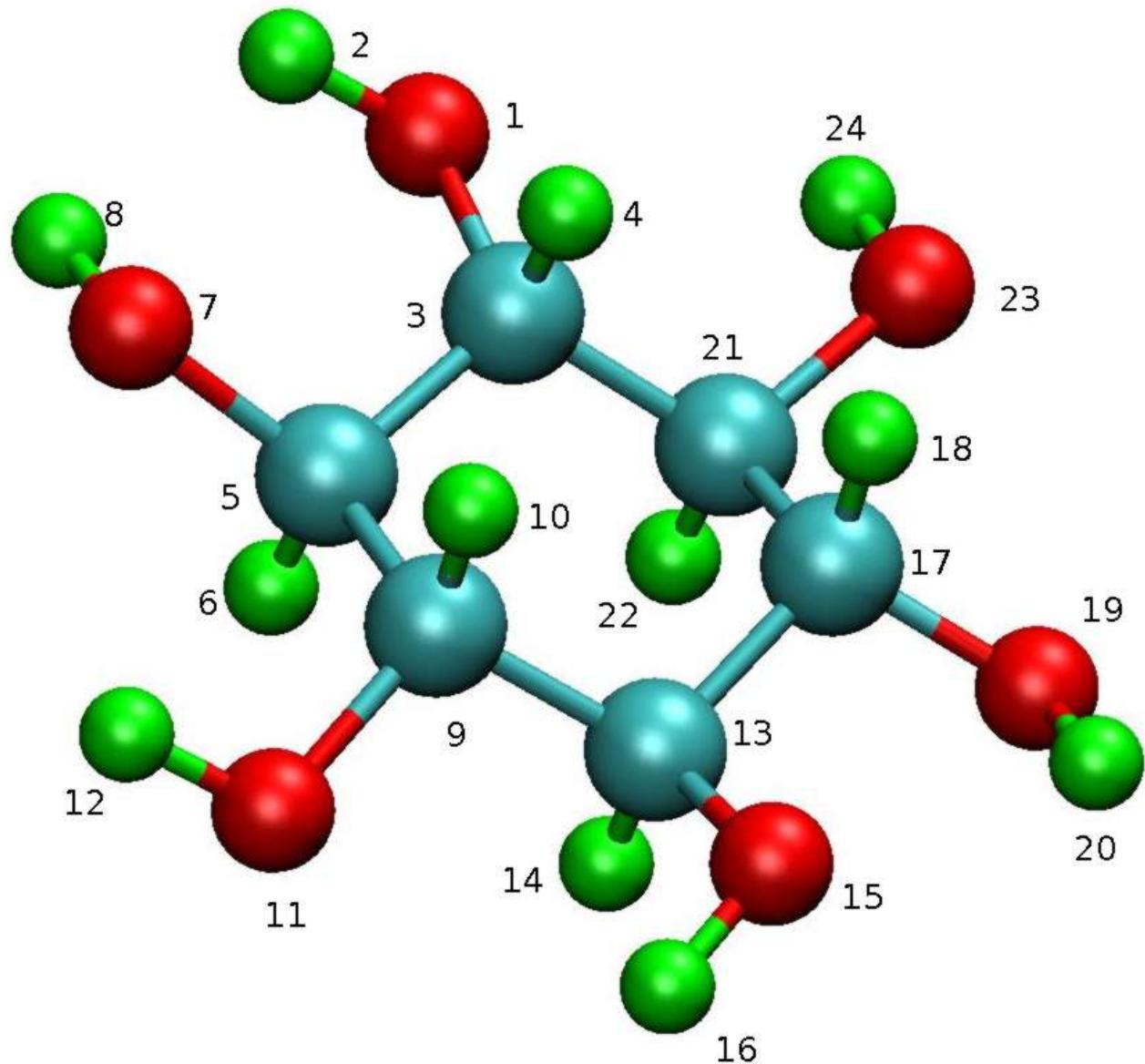
CHIRO



INOSITOL and its interaction with peptides

Modelisation of the molecule of inositol

Until now, the simulations we did on the different elastin-like and amyloid-like peptides were performed using the oplsaa force field. We want to describe the molecule of inositol using this same force field. In order to create the topology file necessary to describe the interactions of the inositol in a solvent and with other compounds, we use the carbohydrates parameters (W. Damm et al, Journal of Computational Chemistry. Vol 18, 16, 1955-1970 (1997)).



To describe the molecule of scyllo-inositol, we need to characterize 24 bonds, 36 angles, 72 dihedrals and 72 pairs.

The bonds

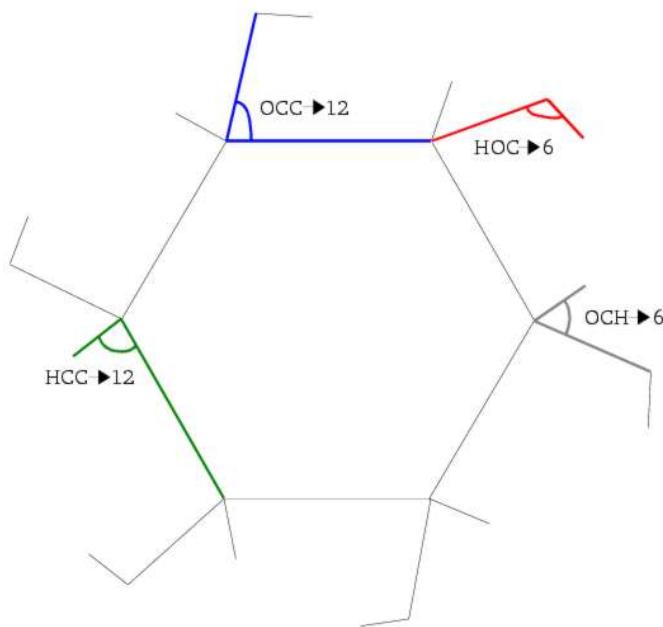
There are four types of bonds: C-C, C-O, O-H and C-H.
With the numbers of the pictures :

C-H	3-4	5-6	9-10	13-14	17-18	21-22
C-O	3-1	5-7	9-11	13-15	17-19	21-23
O-H	1-2	7-8	11-12	15-16	19-20	23-24

C-C	3-5
	5-9
	9-13
	13-17
	17-21

The angles

There four types of angles: OCH, HOC, OCC and HCC

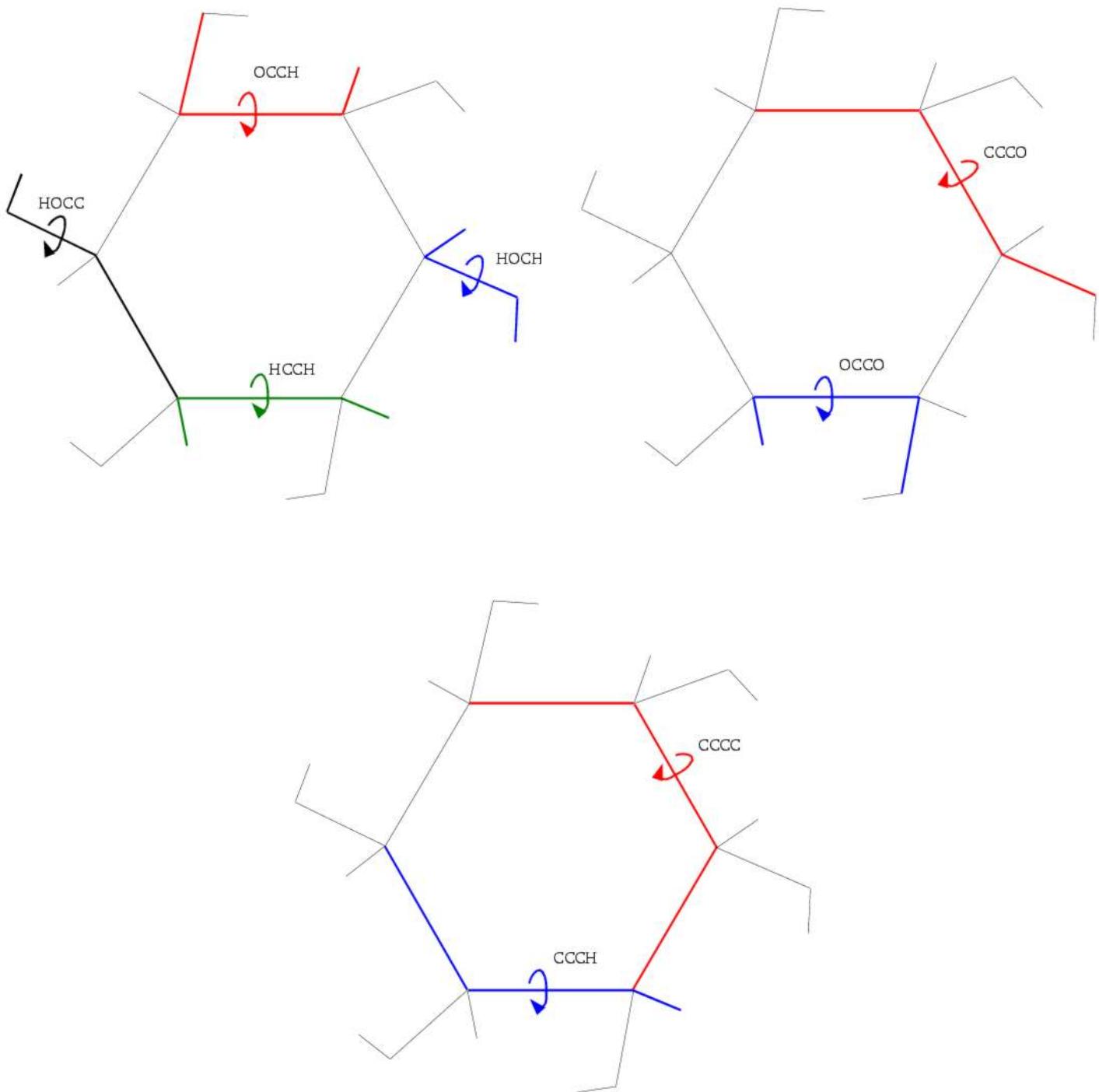


OCH	1-3-4 7-5-6 11-9-10 15-13-14 19-17-18 23-21-22	HOC	2-1-3 8-7-5 12-11-9 16-15-13 20-19-17 24-23-21
-----	---	-----	---

OCC	1-3-21 1-3-5 7-5-3 7-5-9 11-9-5 11-9-13 15-13-9 15-13-17 19-17-13 19-17-21 23-21-17 23-21-3	HCC	4-3-21 4-3-5 6-5-3 6-5-9 10-9-5 10-9-13 14-13-9 14-13-17 18-17-13 18-17-21 22-21-17 22-21-3
-----	--	-----	--

The dihedrals

There eight types of dihedrals: OCCO, HOCH, HCCH, CCCC, CCCH, HOCC, CCCO and OCCH



OCCO

1-3-5-7
7-5-9-11
11-9-13-15
15-13-17-19
19-17-21-23
23-21-3-1

HOCH

2-1-3-4
8-7-5-6
12-11-9-10
16-15-13-14
20-19-17-18
24-23-21-22

HCCH

4-3-5-6
6-5-9-10
10-9-13-14
14-13-17-18
18-17-21-22
22-21-3-4

CCCC	3-5-9-13 5-9-13-17 9-13-17-21 13-17-21-3 17-21-3-5 21-3-5-9	CCCH	3-5-9-10 5-9-13-14 9-13-17-12 13-17-21-22 17-21-3-4 21-3-5-6 3-21-17-18 21-17-13-14 17-13-9-10 13-9-5-6 9-5-3-4 5-3-21-22	HOCC	2-1-3-5 8-7-5-9 12-11-9-13 16-15-13-17 20-19-17-21 24-23-21-3 2-1-3-21 8-7-5-3 12-11-9-5 16-15-13-9 20-19-17-13 24-23-21-17
CCCO	3-5-9-11 5-9-13-15 9-13-17-19 13-17-21-23 17-21-3-1 21-3-5-7 3-21-17-19 5-3-21-23 9-5-3-1 13-9-5-7 17-13-9-11 21-17-13-15	OCCH	1-3-5-6 7-5-9-10 11-9-13-14 15-13-17-18 19-17-21-22 23-21-3-4 1-3-21-22 7-5-3-4 11-9-5-6 15-13-9-10 19-17-13-14 23-21-17-18		

From the paper cited previously, the parameters describing the bonds, the angles and the dihedrals are the following:

<i>atom</i>	$\sigma(\text{\AA})$	ϵ	<i>charge</i>	<i>opls atom type</i>
CT	3.500	0.066	0.205	158
OH	3.070	0.170	-0.700	23
HO	0.000	0.000	0.435	24
HC	2.500	0.030	0.060	140

<i>bond</i>	$r_{eq} (\text{\AA})$	$K_r (\text{kcal/mol\AA}^2)$
CT-CT	1.529	268
CT-OH	1.410	320
CT-HC	1.090	340
OH-HO	0.945	553

<i>angle</i>	Θ_q ($^{\circ}$)	K_{θ} (kcal/molrad 2)
CT-CT-CT	112.7	58.35
CT-CT-HC	110.7	37.5
CT-CT-OH	109.5	50
CT-OH-HO	108.5	55
OH-CT-HC	109.5	35

For the alkanes, the following proper dihedral potential is often used:

$$V_{rb}(\phi_{ijk}) = \sum_{n=0}^5 (\cos \Psi)^n$$

<i>dihedrals</i>	V_1 (kcal/mol)	V_2 (kcal/mol)	V_3 (kcal/mol)
HC-CT-CT-HC	0	0	0.318
HC-CT-CT-CT	0	0	0.366
CT-CT-CT-CT	1.740	-0.157	0.279
HO-OH-CT-HC	0	0	0.450
HO-OH-CT-CT	2.674	-2.883	1.026
OH-CT-CT-OH	9.066	0	0
OH-CT-CT-CT	-1.336	0	0
HC-CT-CT-OH	0	0	

The Ryckaert-Bellemans functions can also be used to include the OPLS dihedral potential. The OPLS potential function is given as the first four terms of a Fourier series:

$$V_{rb}(\phi_{ijk}) = V_0 + \frac{1}{2} [V_1(1 + \cos \Psi) + V_2(1 - \cos 2\Psi) + V_3(1 + \cos 3\Psi)]$$

$$C_0 = V_0 + V_2 + 0.5*(V_1 + V_3)$$

$$C_1 = 0.5*(3V_3 - V_1)$$

$$C_2 = -V_2$$

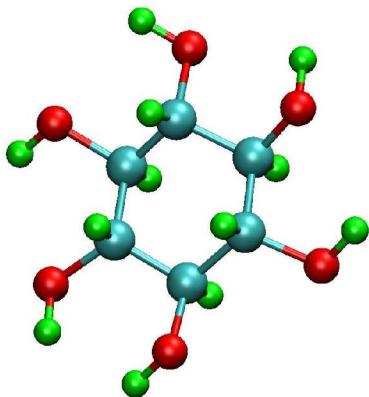
$$C_3 = -2V_3$$

$$C_4 = 0$$

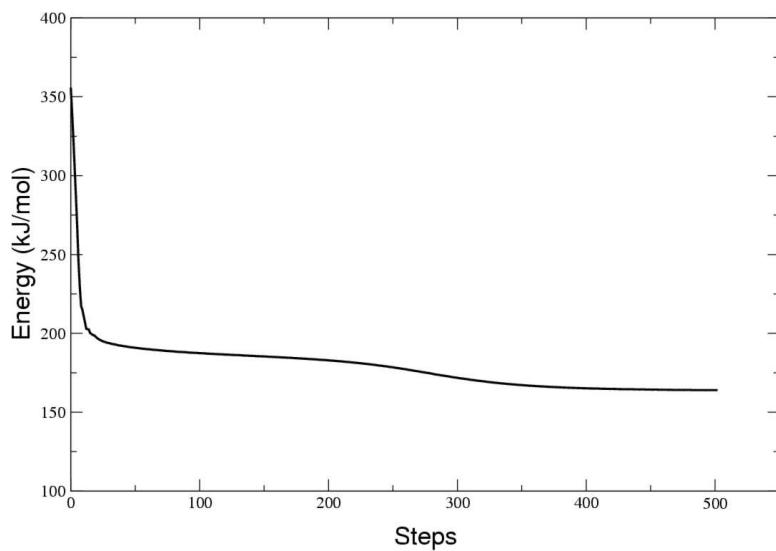
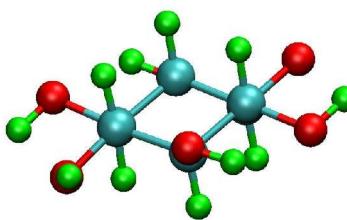
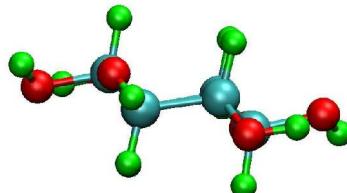
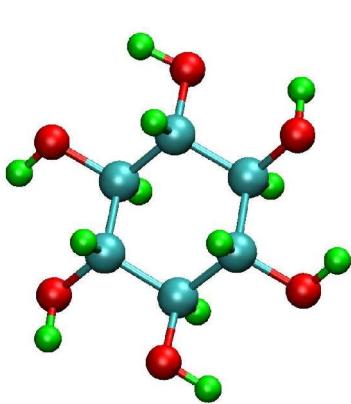
$$C_5 = 0$$

Simulations with INOSITOL

We start with simulations in vacuo (both energy minimisation (EM) and molecular dynamics (MD)).



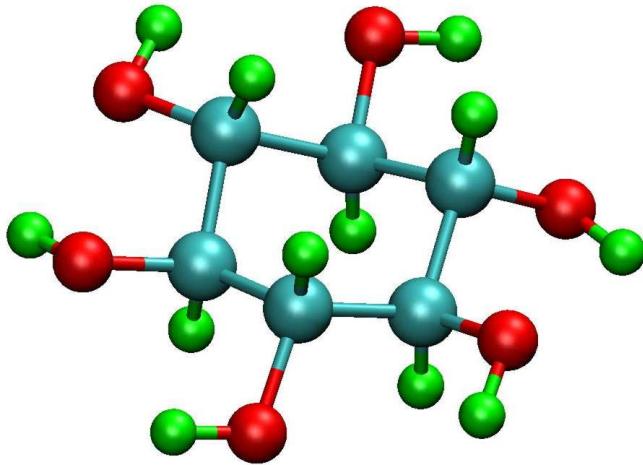
After 525 steps of EM (steep integrator, no pbc and cut-off coulombtype):



Variation of the potential energy
during the EM in vacuo

Using the energy minimized structure, we performed 2ns of MD in vacuo.

SD integrator
cut-off coulombtype
T=300K
no pbc



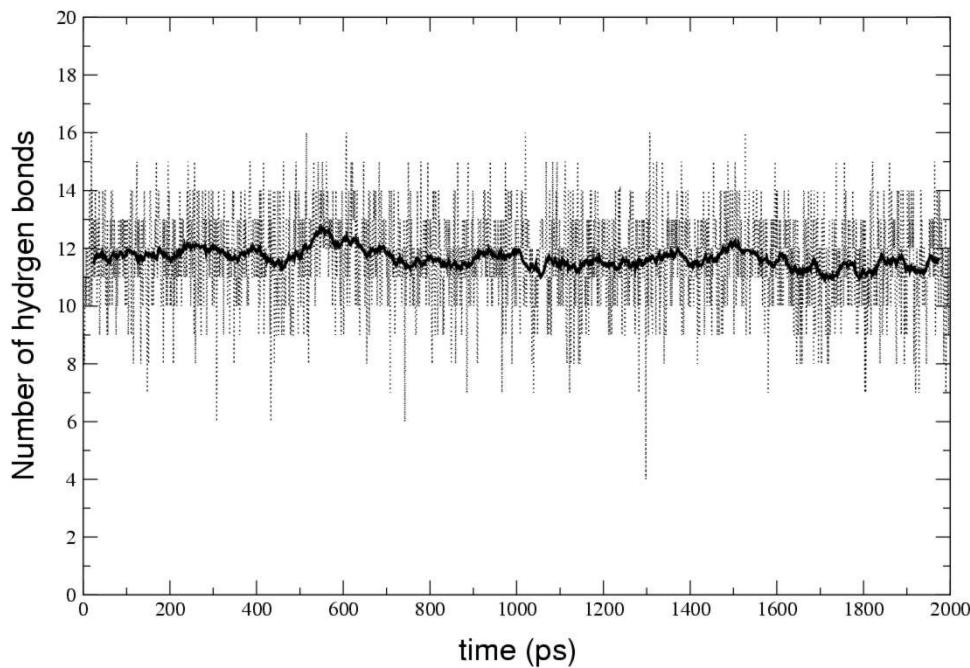
Structure obtained after the MD simulation in vacuo.

MD Simulations in water

We consider a cubic box: $30 \times 30 \times 30 \text{ \AA}^3$. One molecule of inositol is placed in this box with 885 water molecules. Without the inositol, there would be ideally 903 water molecules.

NVT simulation: $T=300\text{K}$ $\tau_T = 2.0$
 md integrator
 pbc
 PME

Along the 2ns of simulation there are on average 11.6 hydrogen bonds between inositol and the water molecules.

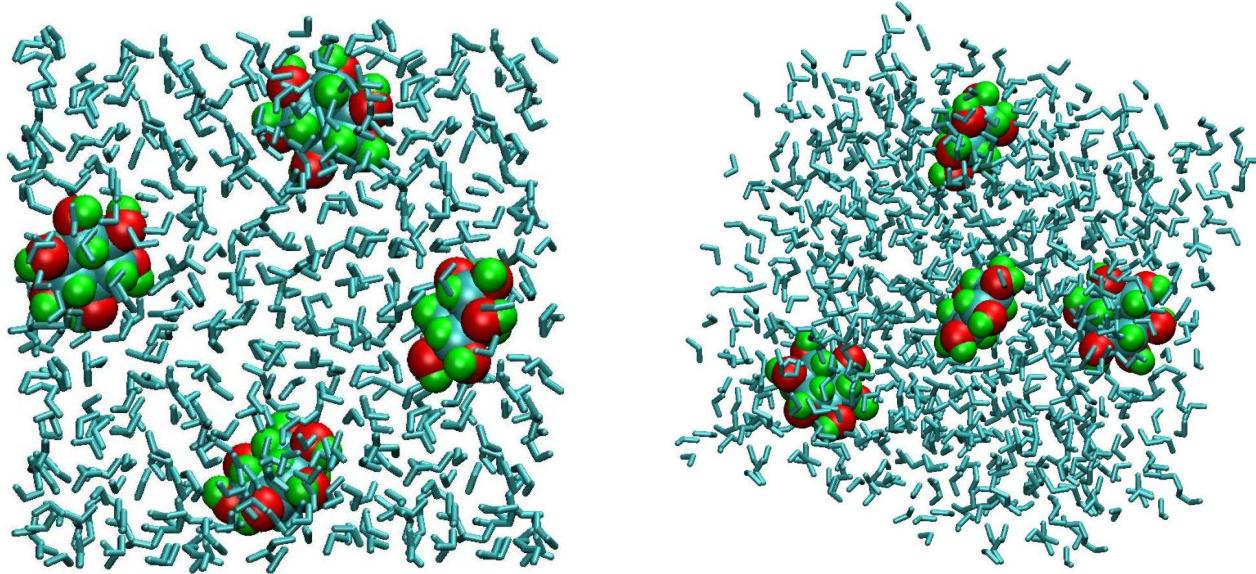


Variation of the number of HB between inositol and water along the MD simulation.

With this system, we could simulate $\sim 6.6\text{ns}$ per day.

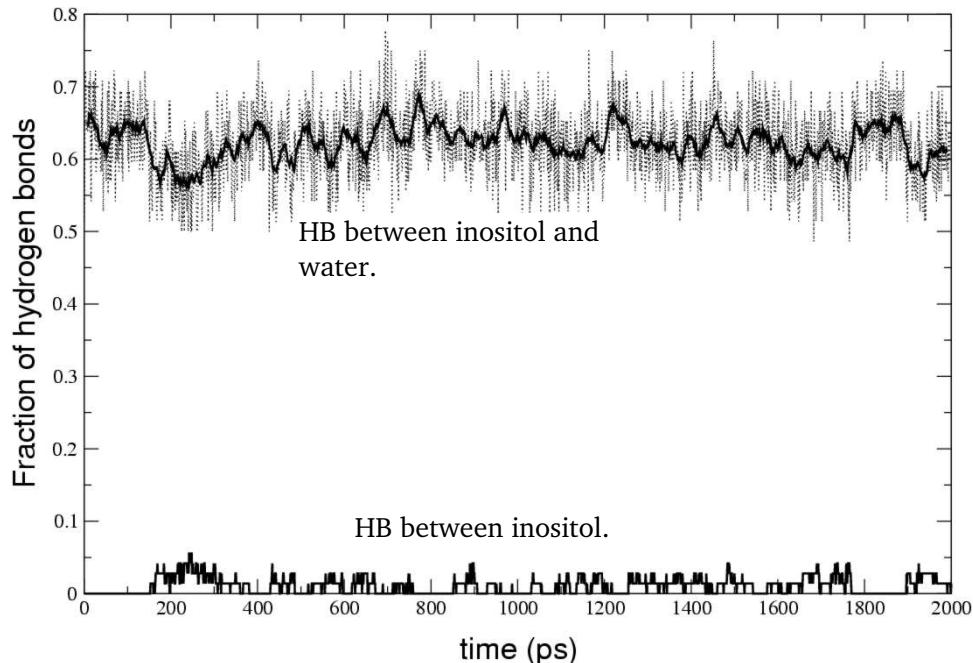
4 inositol in a $30 \times 30 \times 30 \text{ \AA}^3$ box

The four configurations were picked from the 2ns simulation of isolated inositol in water. Snapshots at 100 ps, 150ps, 1050ps and 1620ps were taken. These four molecules are hydrated with 861 water molecules.



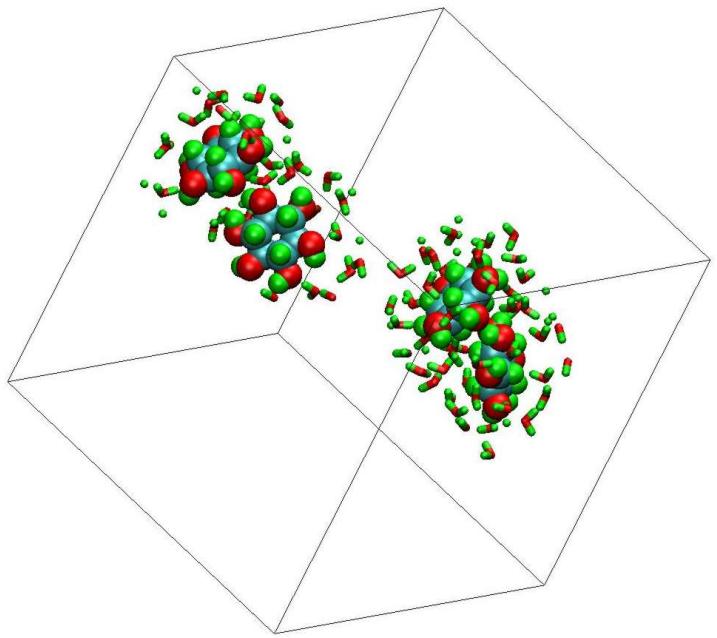
Views of the box at the beginning of the simulation.

On average there are 44.8 HB between inositol and water molecules.

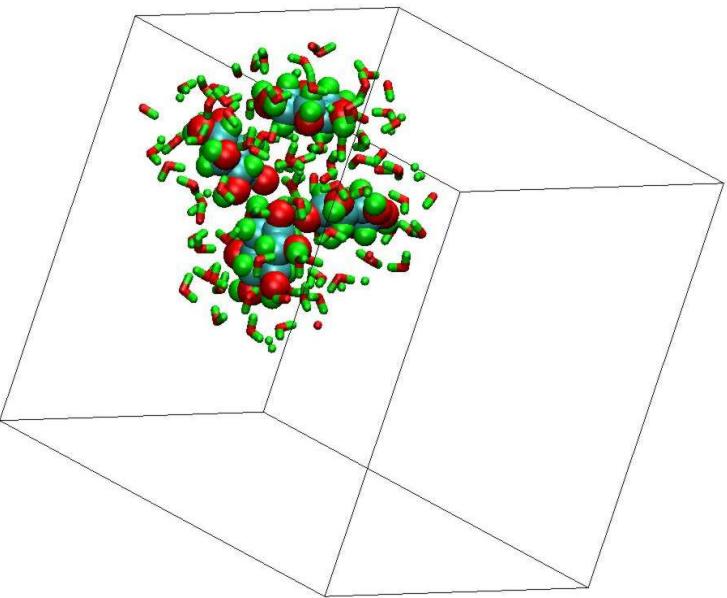


Evolution of the normalized number of HB between inositol molecules and between inositol and water molecules. The normalization is done dividing the number of HB by 72 (4x18) because one OH group of inositol can ideally bind to three other groups (2 HB for O and 1 for H)

Along the simulations it is possible to observe clusters of 2 or 4 inositol molecules.



$t = 332$ ps. Water molecules
within 4\AA of INS.



$t = 1445$ ps. Water molecules
within 4\AA of INS.

Concentration of the inositol: $\text{C}_6\text{O}_6\text{H}_{12} \rightarrow 180 \text{ g.mol}^{-1}$

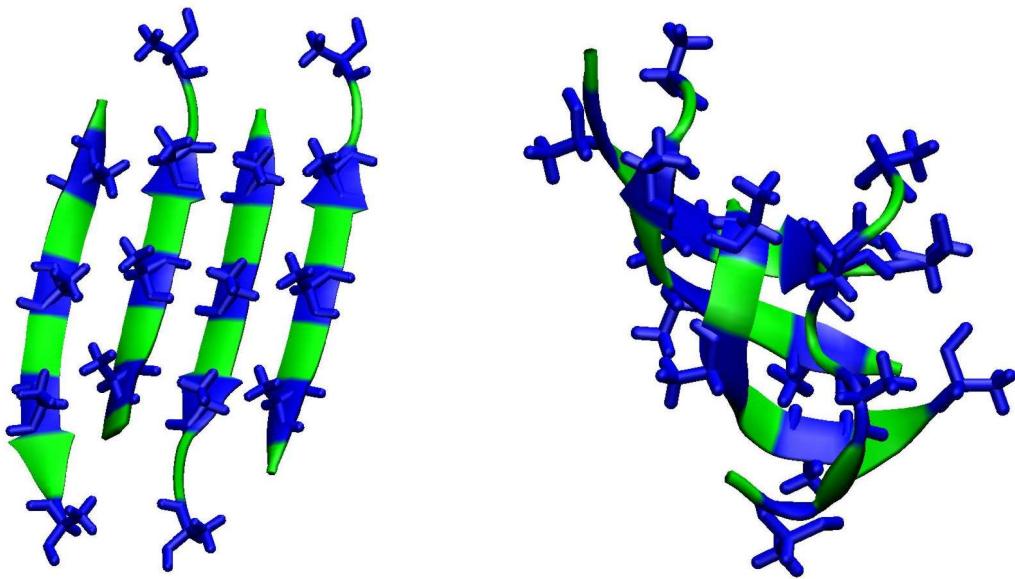
We have 4 INS in $(30.10^{-9})^3 \text{ m}^3 = 2.7 10^{-23} \text{ L}$

In 1L there are $1.481.10^{23}$ inositol molecules or 0.246 moles.

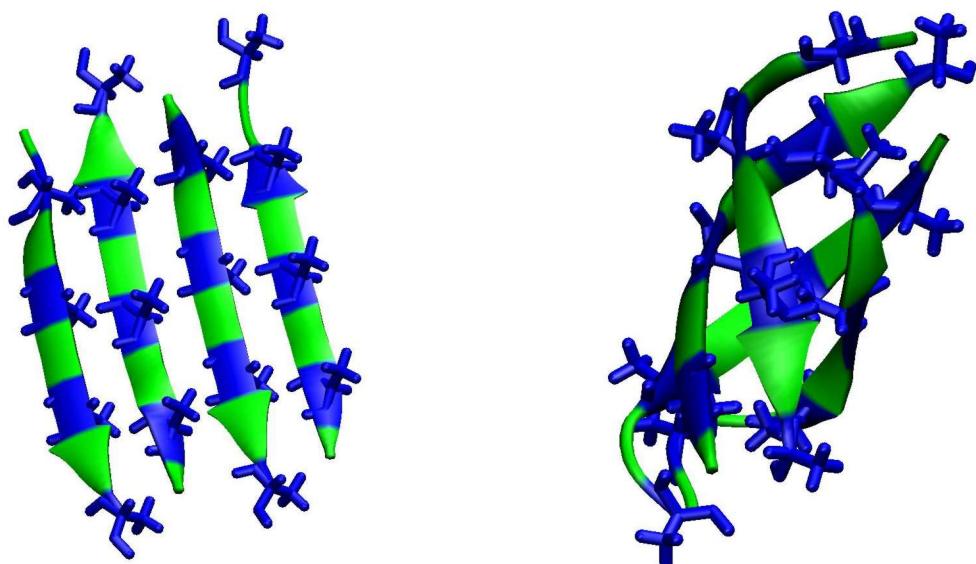
With this kind of system, it is possible to reach ~ 5.9 ns per day.

Preparation of the β -sheets

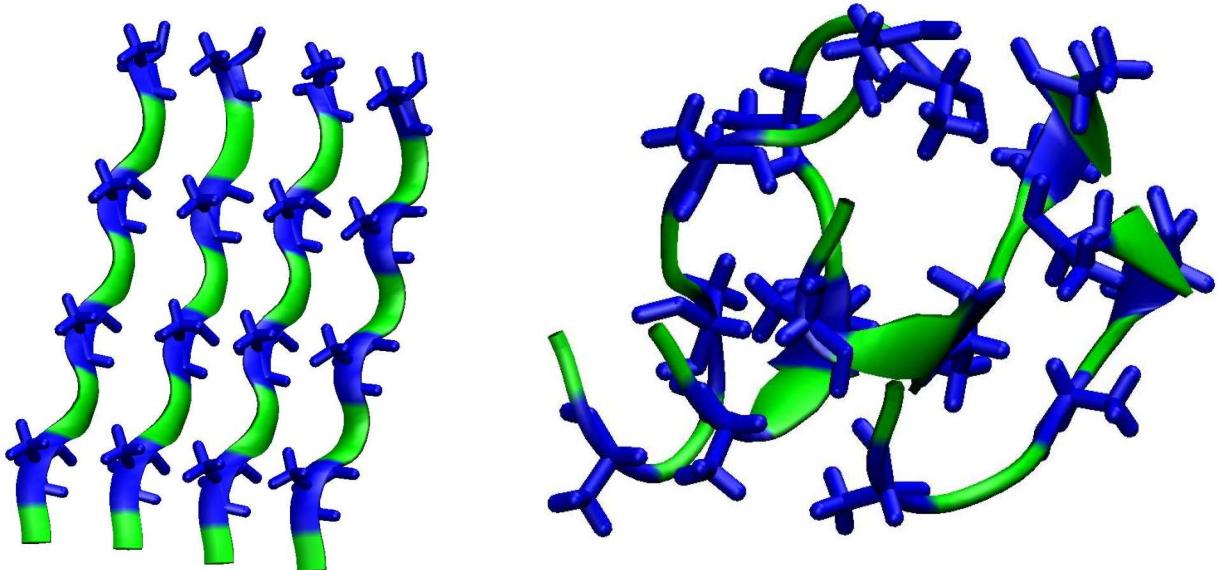
We use the octameric sequence GAGAGAGA which contains 77 atoms. It is possible to create parallel or anti-parallel sheets with alanine sidechains pointing all in the same plane or in different plane when going from one peptide to another.



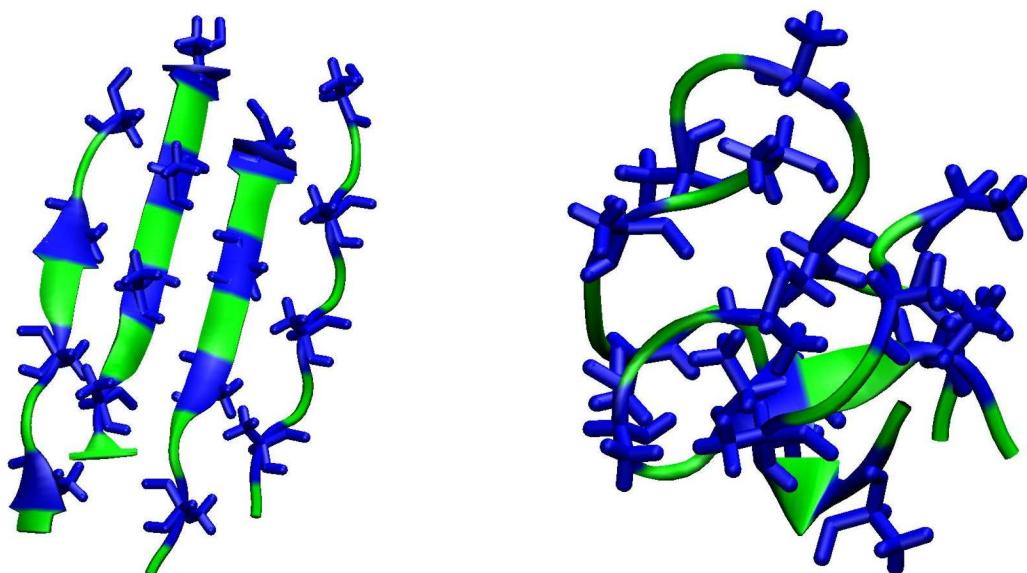
Anti-parallel β -sheets after the EM (left) and 20 ns of MD simulation in vacuo (right). All the alanine sidechains are situated on the same side of the peptides.



Anti-parallel β -sheets after the EM (left) and 20 ns of MD simulation in vacuo (right). The alanine sidechains are situated on different side of the peptides going from one peptide to the next.

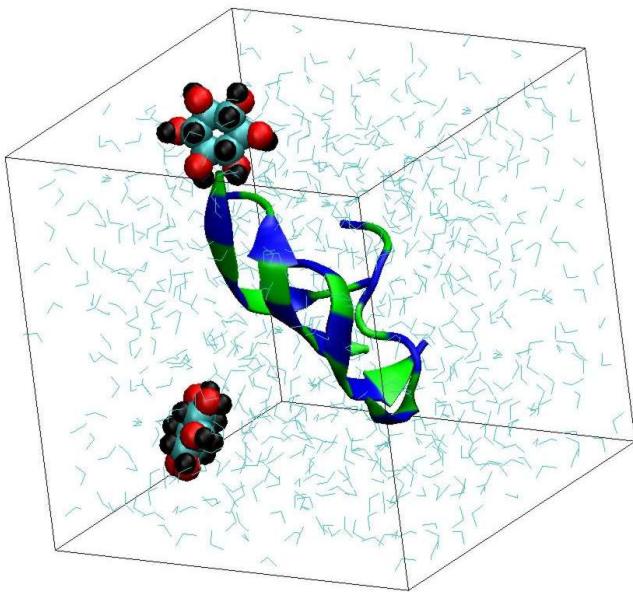


Parallel β -sheets after the EM (left) and 20 ns of MD simulation in vacuo (right). All the alanine sidechains are situated on the same side of the peptides.

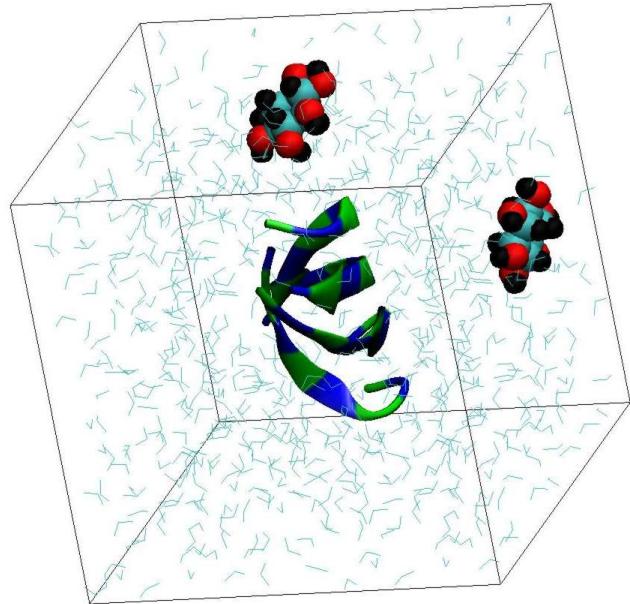


Parallel β -sheets after the EM (left) and 20 ns of MD simulation in vacuo (right). The alanine sidechains are situated on different side of the peptides going from one peptide to the next.

For the runs with inositol, we just use the anti-parallel conformations since they are the one staying in β -sheets conformations.



AP_1F setup for MD in water



AP_2F setup for MD in water

Size of the box: $30 \times 30 \times 30 \text{ \AA}^3$

NPT simulations

PME

pbc

vdw switch

rwdw = 1.4 nm

rlist = rcoulomb = 1.45 nm

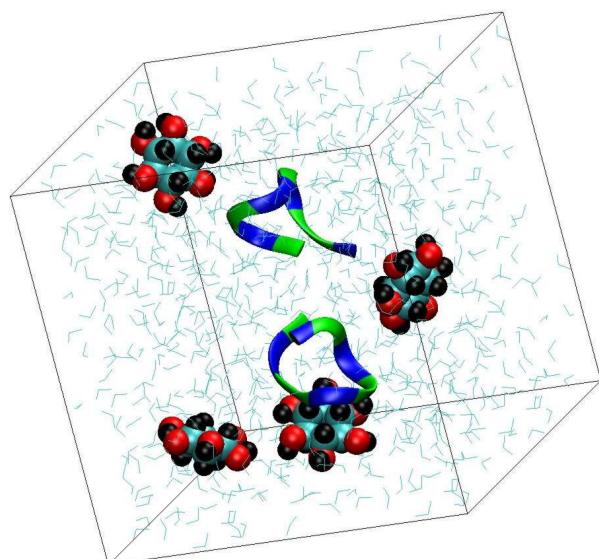
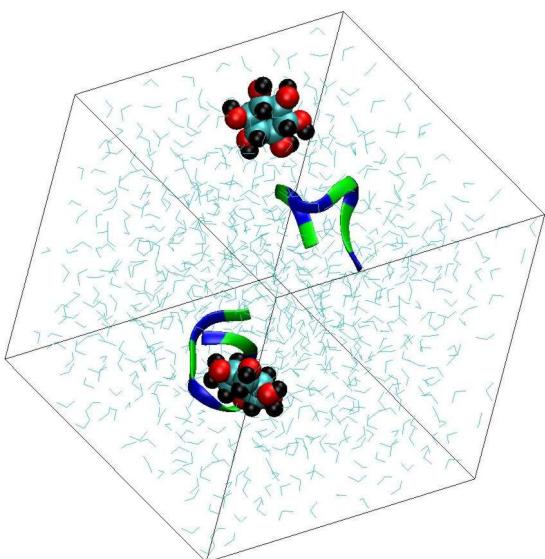
T = 300K $\tau_T = 0.1$

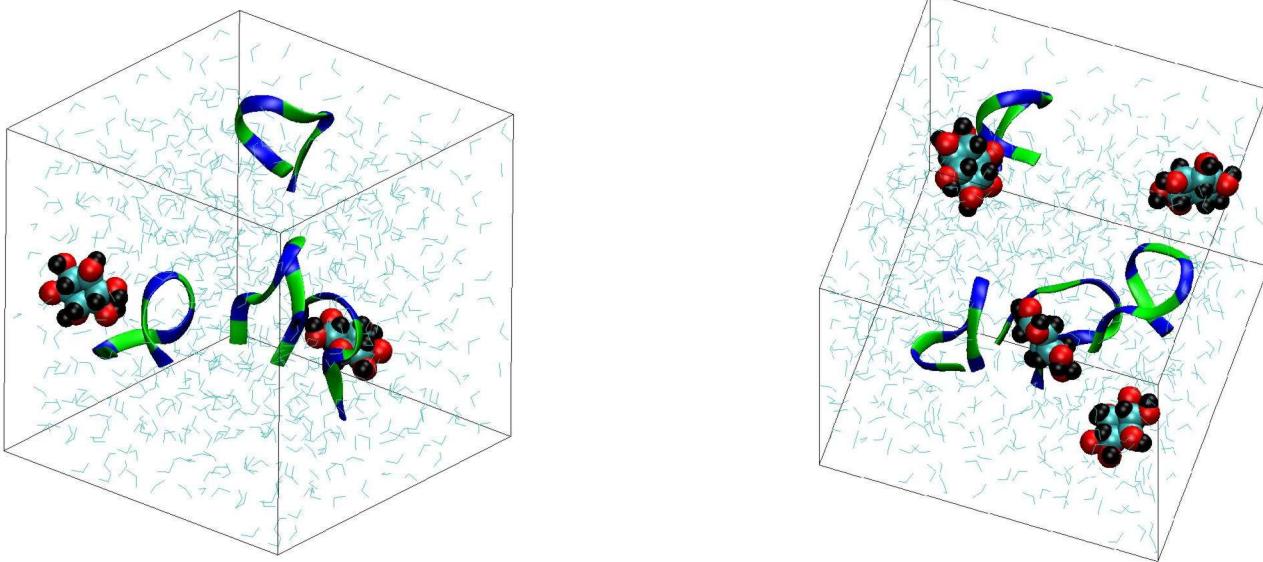
P = 1 atm $\tau_P = 4.0$ $\chi = 4.5 \cdot 10^{-5}$

shake

As controls we also run 2 MD simulations without iniositol molecules. This should allow us to check the stability of the β -sheets.

Another kind of control systems are boxes of the same size containing 2 or 4 inositol molecules as well as 2 or 4 GAGAGAGA chains extracted from single chain MD simulations in water.

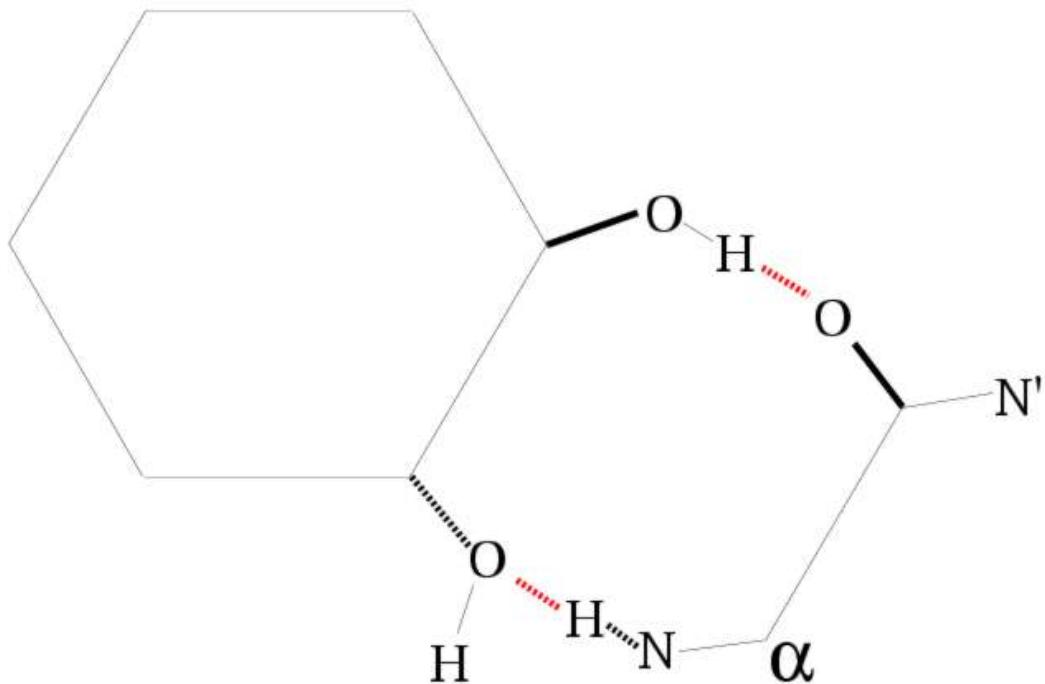




<i>System</i>	<i>Number of peptides</i>	<i>Number of inositol</i>	<i>Number of water</i>
ap2fwat	4	0	782
ap1fwat	4	0	778
ap2fino	4	2	767
ap1fino	4	2	760
2oct2ino	2	2	826
2oct4ino	2	4	806
4oct2ino	4	2	763
4oct4ino	4	4	742

First analysis of the results

We are specifically interested in the way that inositol will bind the peptide. Given the fact that in water inositol makes a lot of HB with water molecules, we expect that with GAGAGAGA the O and H of inositol will make HB with the C=O and N-H of the backbone. Referring to "Crystal Growth & design" vol 6 number 10, 2301-2307 (2006), we expect some binding configurations where inositol will have created 2 HB with 2 groups of the backbone.



From the observation of the movies of the different trajectories, it is possible to observe different binding modes of the inositol molecules. Most of the configurations seem to present a single hydrogen bond per inositol: these are designed as monodentate modes or conformations; a lower number of conformations consist of inositol molecules with 2 hydrogen bonds: these are the bidentate modes or conformations.

Method used to characterize the conformations of the inositol molecules:

loop on the inositol molecule

```
ind = 0  
DO l=1,12  
    pos(l) = 0  
    typ(l) = 0  
    pair(l) = 0  
    pairc(l) = 0
```

END DO

loop on peptide chain

loop on groups of inositol (1 to 6)

loop on residues

search for HB between O_{ino} and NH_{pep} --> IF HB stateO = 1

search for HB between H_{ino} and CO_{pep} --> IF HB stateH = 1

IF stateO = 1 or stateH = 1, we store the type of binding and the conjugate of the group on the peptide chains. We also increment the ind variable: ind = ind + 1

end loop

```

    end loop
end loop

```

Depending on the value of ind, we know if the molecule of inositol is monodentate, bidentate, or eventually if it possesses more than 2 HB. Writing of the informations in different files.

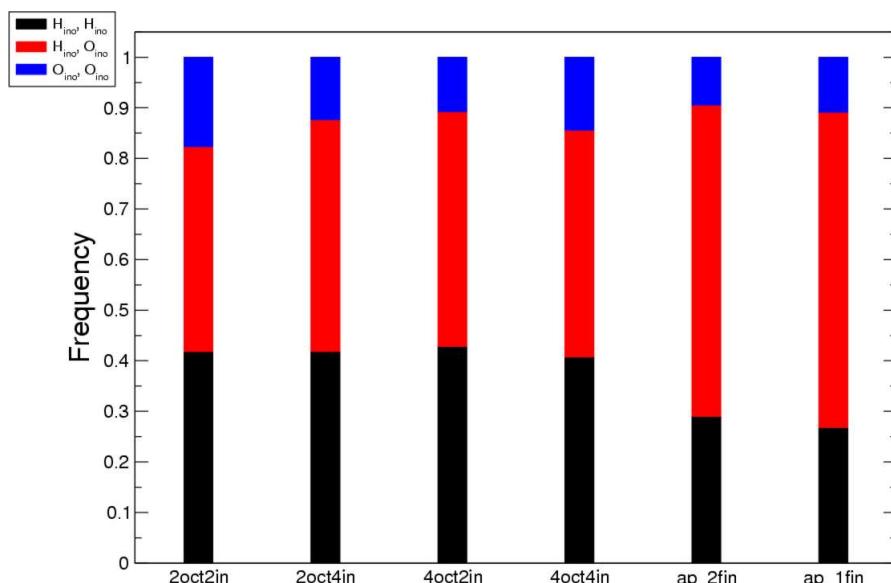
end loop

July 6th 2007

Systems	Time (ns)	Total HB	Monodentate conf	Bidentate conf	Conf with more than 2HB
ap_2f_ino	52	91599	19044	14061	13275
ap_1f_ino	36	38337	15151	6887	2961
2oct_2in	46	26687	12948	4768	1332
2oct_4in	40	53215	22330	8696	4020
4oct_2in	52	81899	22113	11491	10012
4oct_4in	52	109043	41356	17666	9698

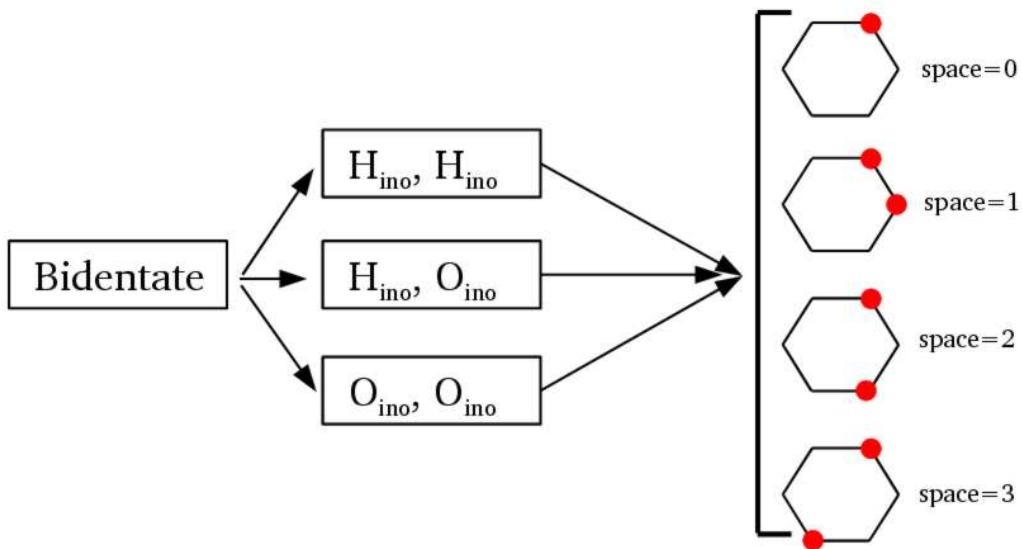
The different analysis are performed on trajectories where snapshots are saved every ps.

Systems	HB per ino per snapshot	Monodentate conf per ino per snapshot	Bidentate conf per ino per snapshot
ap_2f_ino	0.881	0.183	0.135
ap_1f_ino	0.532	0.210	0.096
2oct_2in	0.290	0.141	0.052
2oct_4in	0.332	0.140	0.054
4oct_2in	0.787	0.213	0.110
4oct_4in	0.524	0.199	0.085

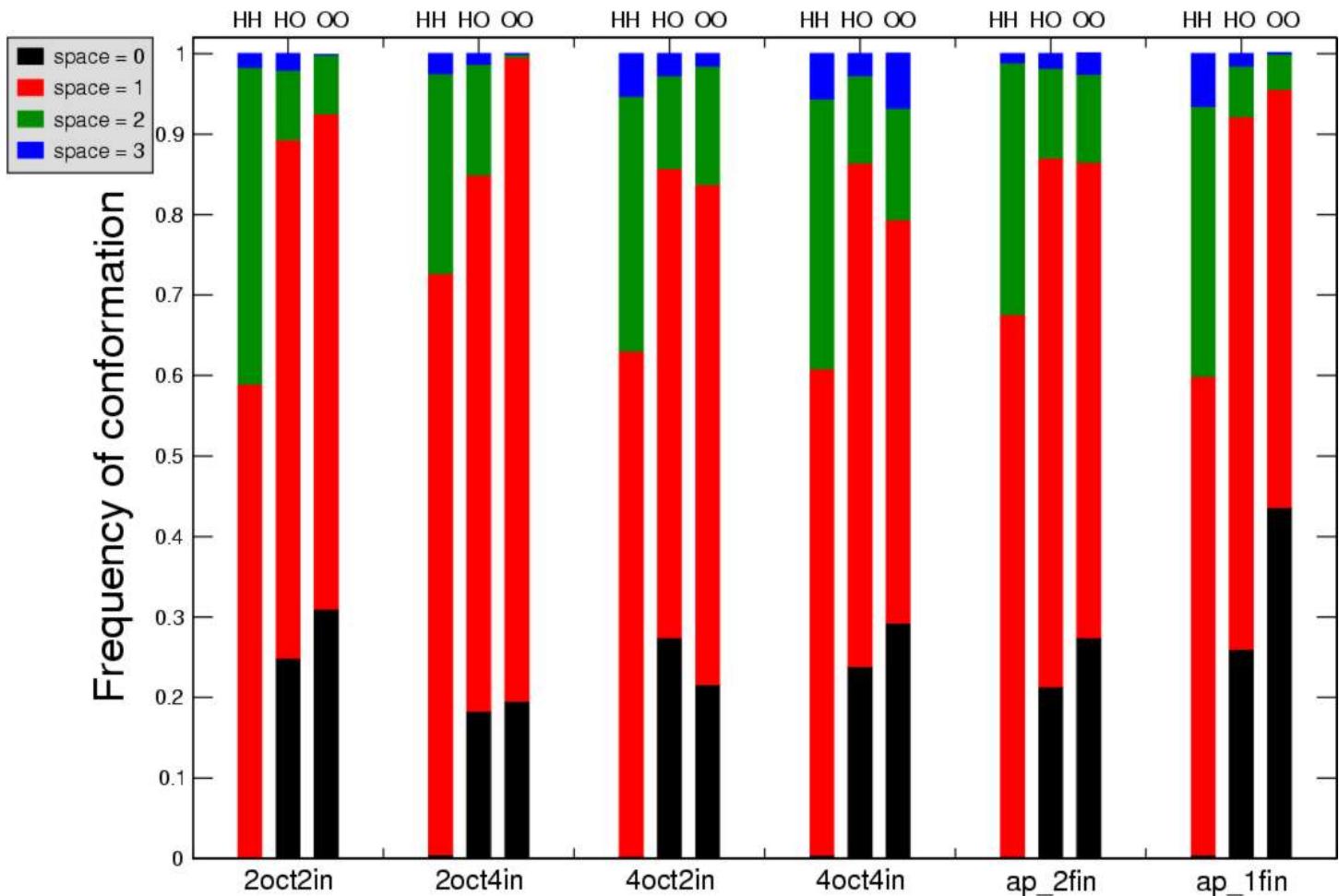


Frequency of the bidentate conformations in terms of the nature of the groups engaged in the HB.

Caracterisation of the “bidentate” state:



Space = 1, 2 and 3 correspond to the true bidentate states. Space = 0 corresponds to inositol molecule with 2HB on the same site.



Frequency of the bidentate conformations in terms of the spacing between groups engaged in the HB.

HH configuration

Systems	Total	Space = 0	Space = 1	Space = 2	Space = 3
2oct_2in	1990	0.05	58.84	39.34	1.76
2oct_4in	3632	0.41	72.19	24.86	2.53
4oct_2in	4916	0.22	62.73	31.69	5.35
4oct_4in	7190	0.37	60.42	33.52	5.69
ap_2f_ino	4069	0.22	67.31	31.28	1.18
ap_1f_ino	1841	0.38	59.48	33.51	6.63

Percentage of each conformation.

HO configuration

Systems	Total	Space = 0	Space = 1	Space = 2	Space = 3
2oct_2in	1931	24.81	64.42	8.65	2.12
2oct_4in	3982	18.26	66.57	13.79	1.38
4oct_2in	5332	27.38	58.27	11.53	2.81
4oct_4in	7914	23.79	62.55	10.85	2.81
ap_2f_ino	8651	21.27	65.71	11.12	1.9
ap_1f_ino	4293	25.95	66.15	6.27	1.63

Percentage of each conformation.

OO configuration

Systems	Total	Space = 0	Space = 1	Space = 2	Space = 3
2oct_2in	847	30.93	61.51	7.32	0.12
2oct_4in	1082	19.5	79.94	0.37	0.18
4oct_2in	1243	21.56	62.11	14.72	1.61
4oct_4in	2562	29.16	50.12	13.86	6.87
ap_2f_ino	1341	27.37	59.06	10.96	2.61
ap_1f_ino	753	43.56	51.92	4.52	0

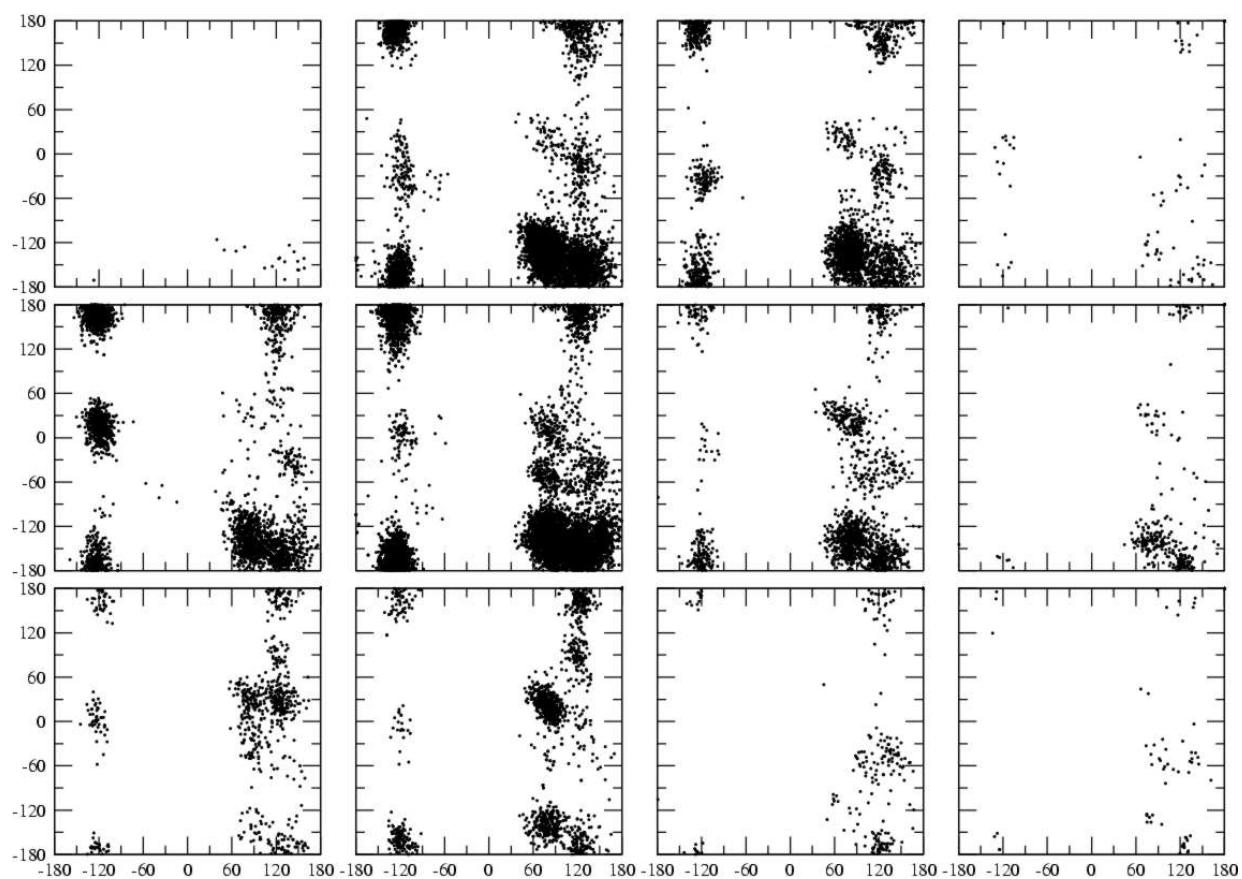
Percentage of each conformation.

Ramachandran plots of the (ϕ, ψ) of the residues forming HB with the OH groups of inositol.

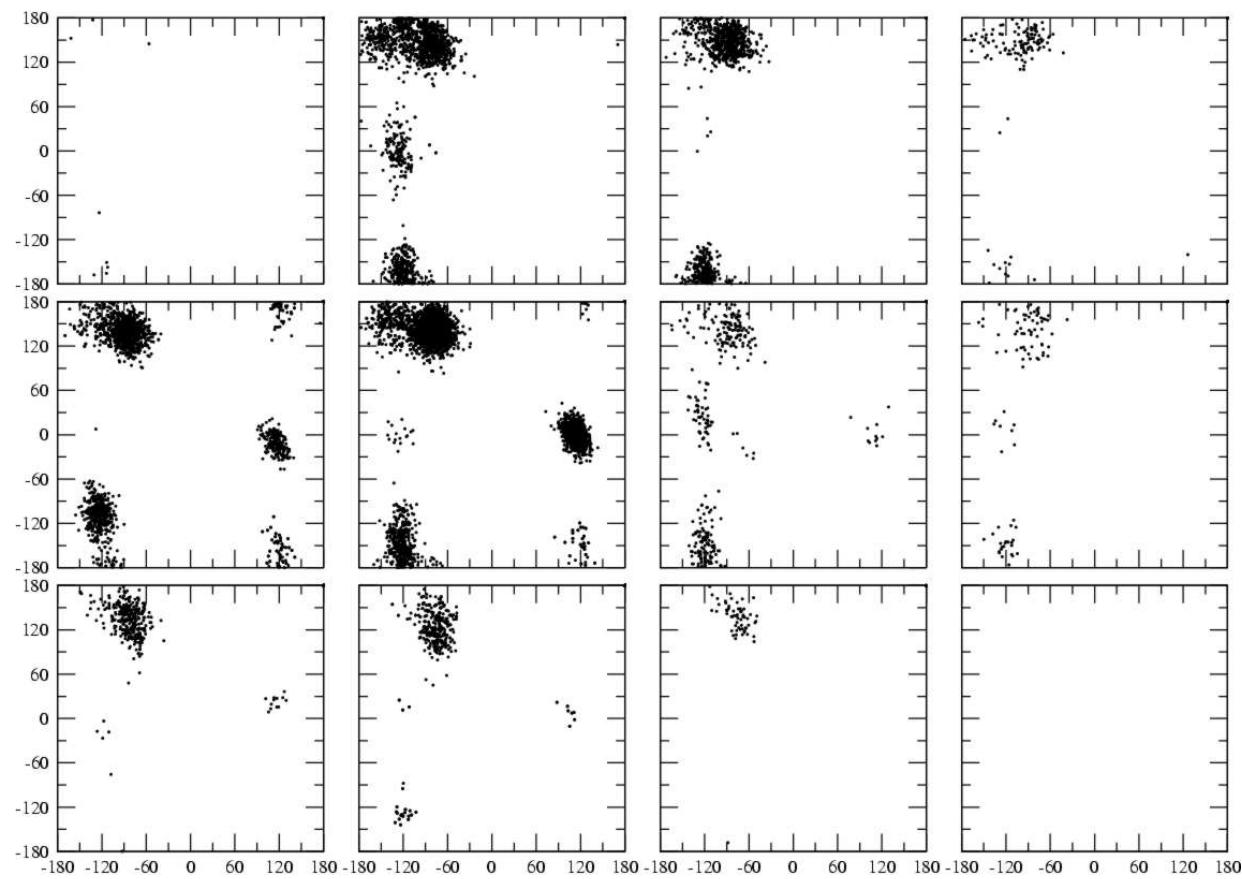
	Space = 0	Space = 1	Space = 2	Space = 3
H, H				
H, O				
O, O				

Schematic of the array of graphs displaying the ramachandran plot

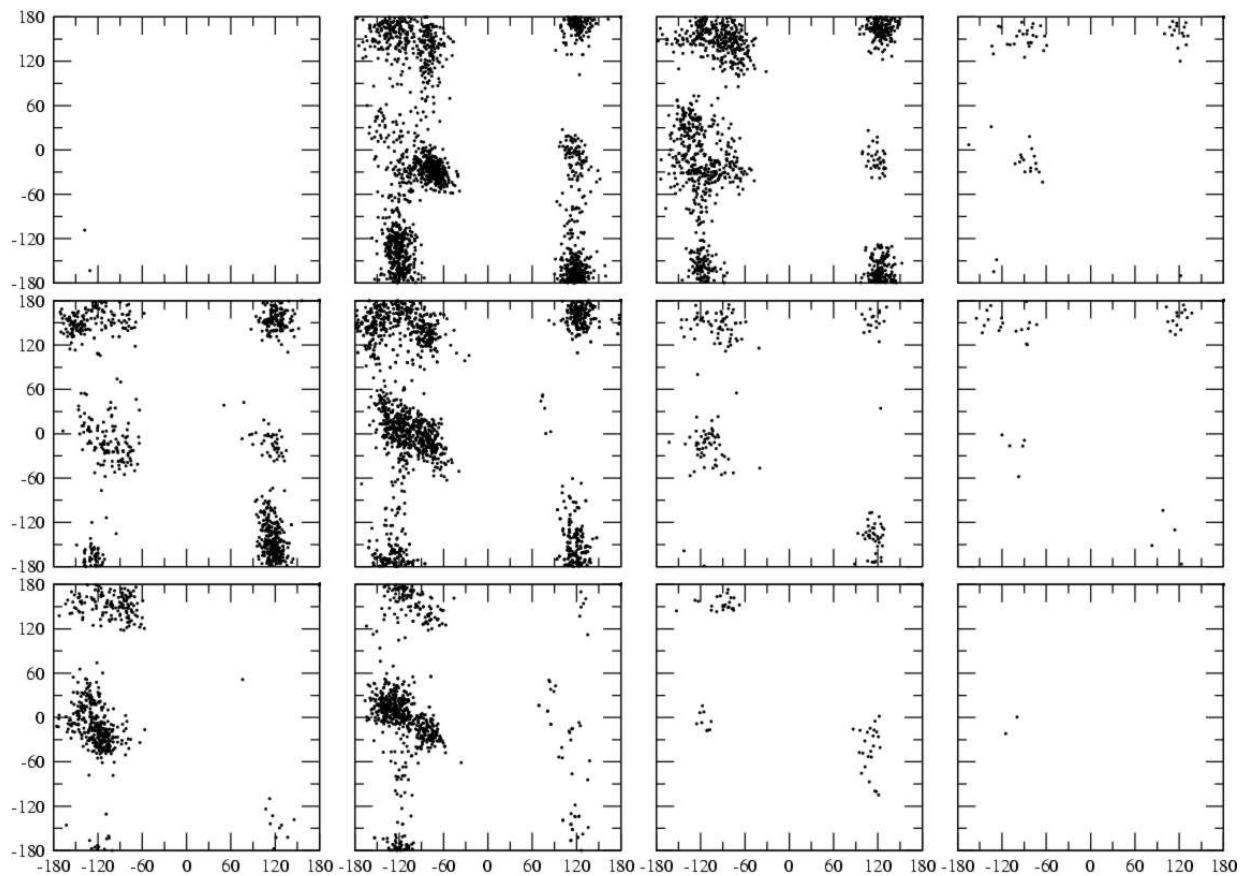
AP2FIN



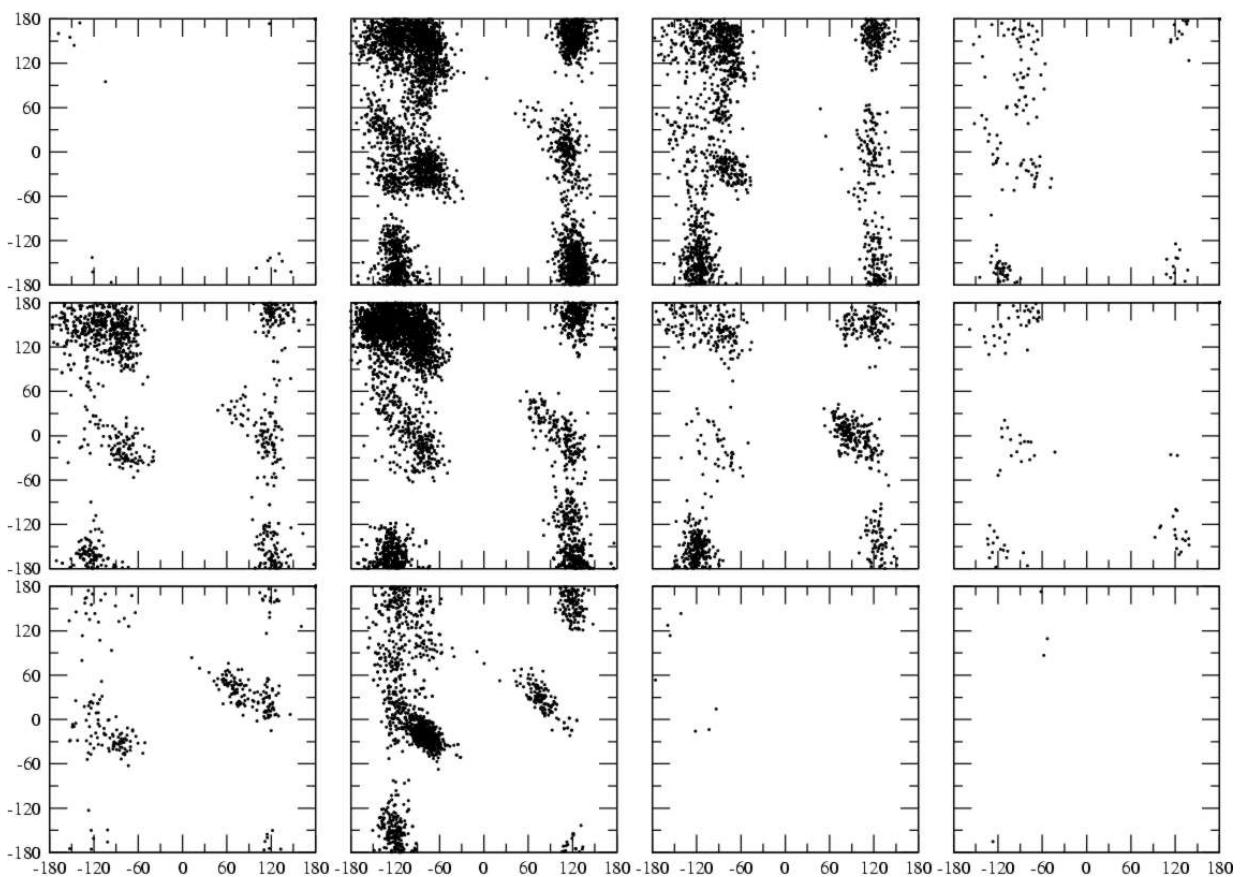
AP1FIN



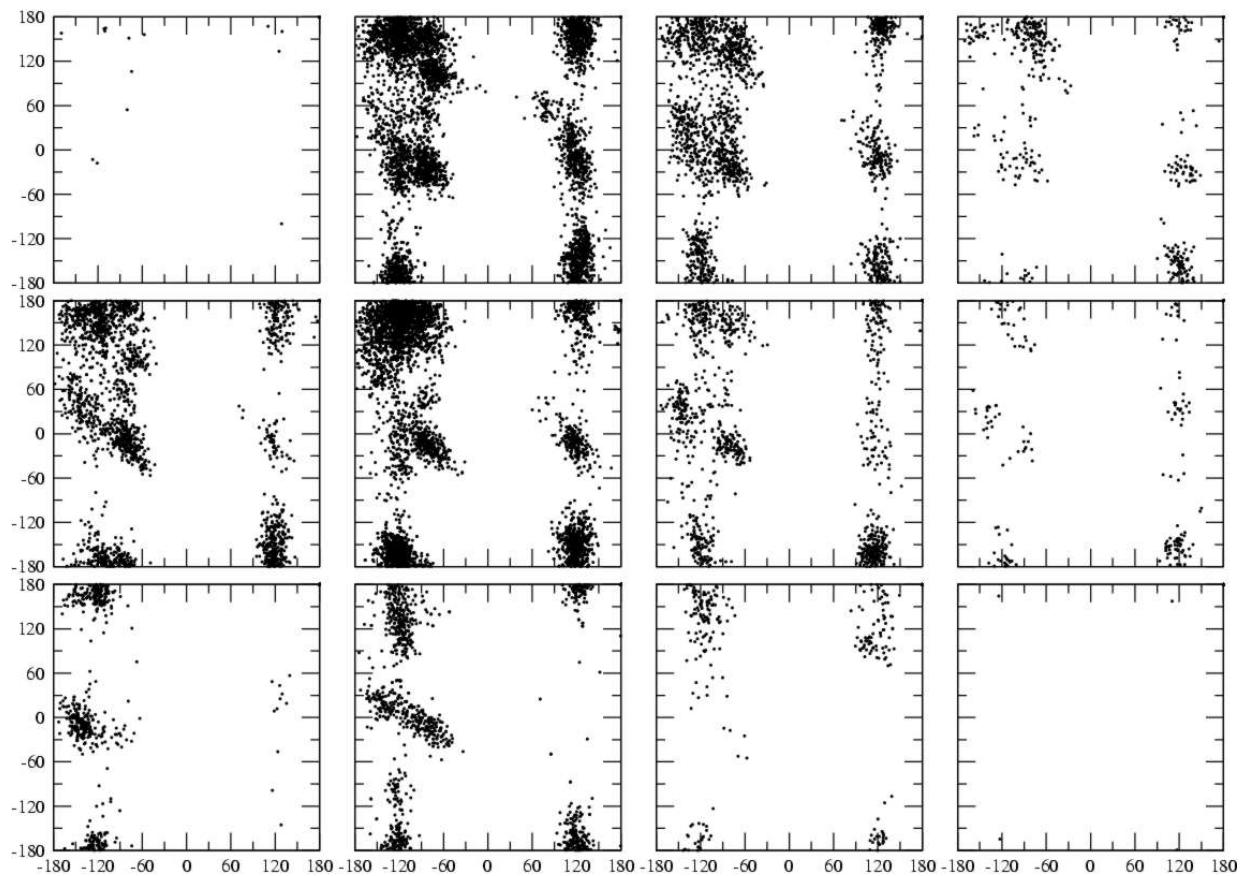
2OCT2IN



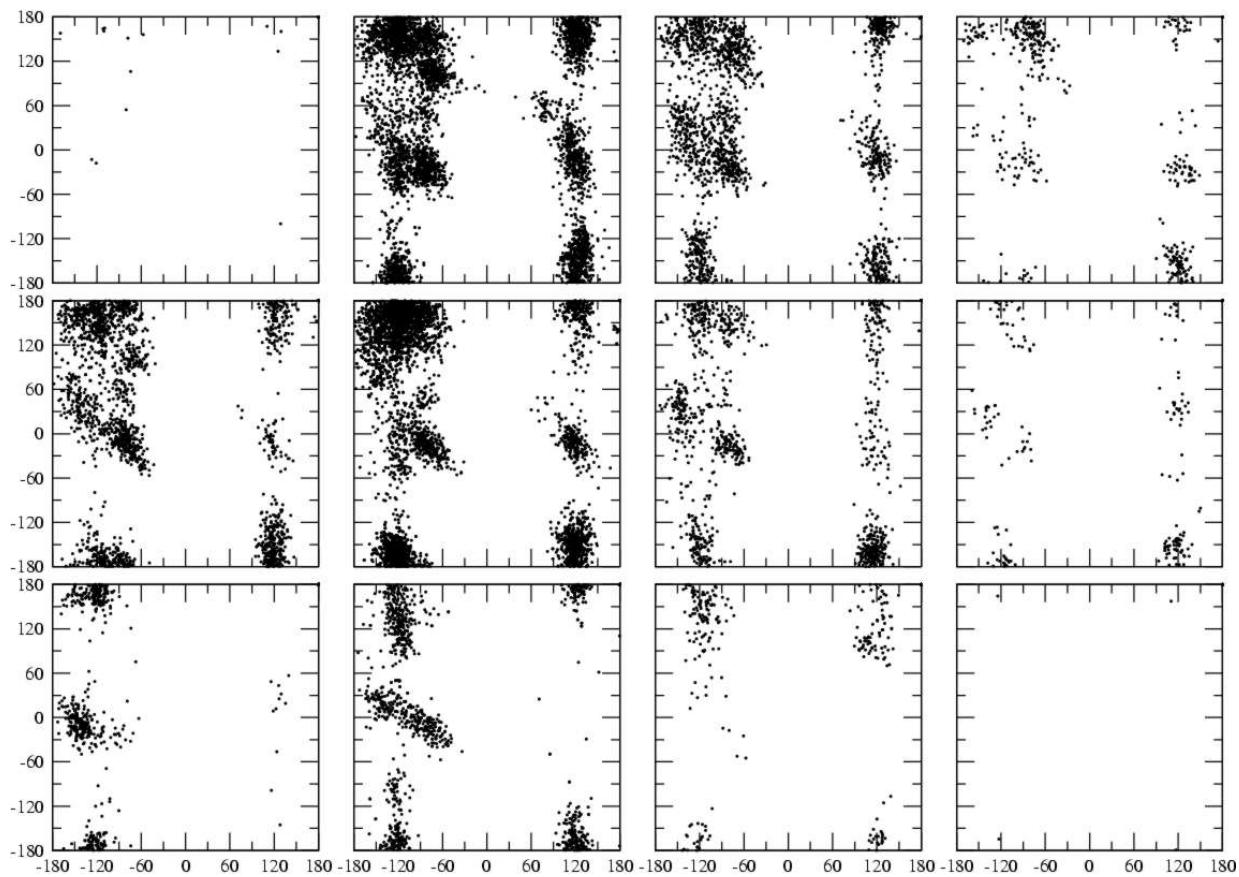
2OCT4IN

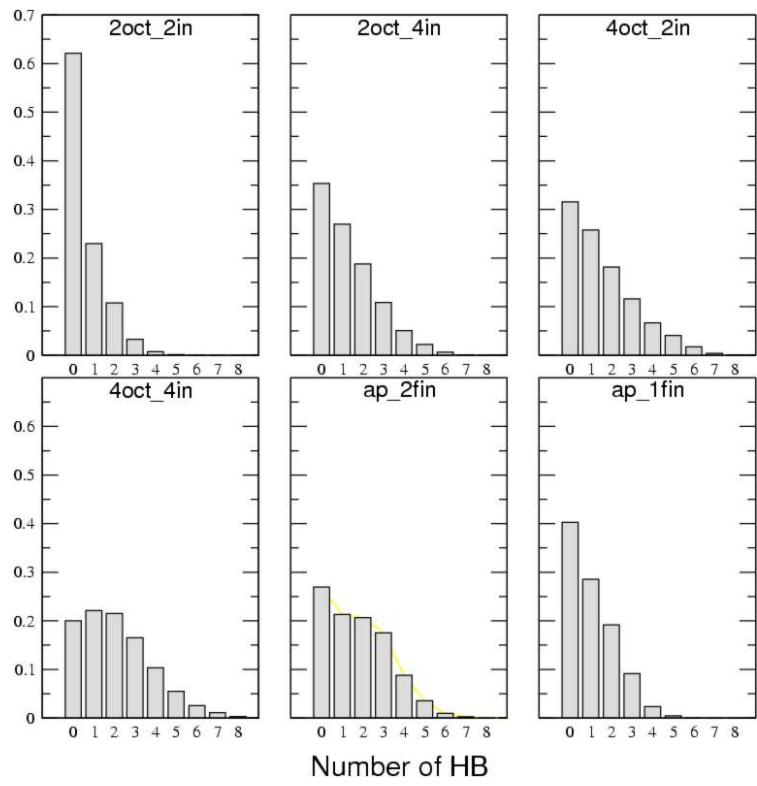
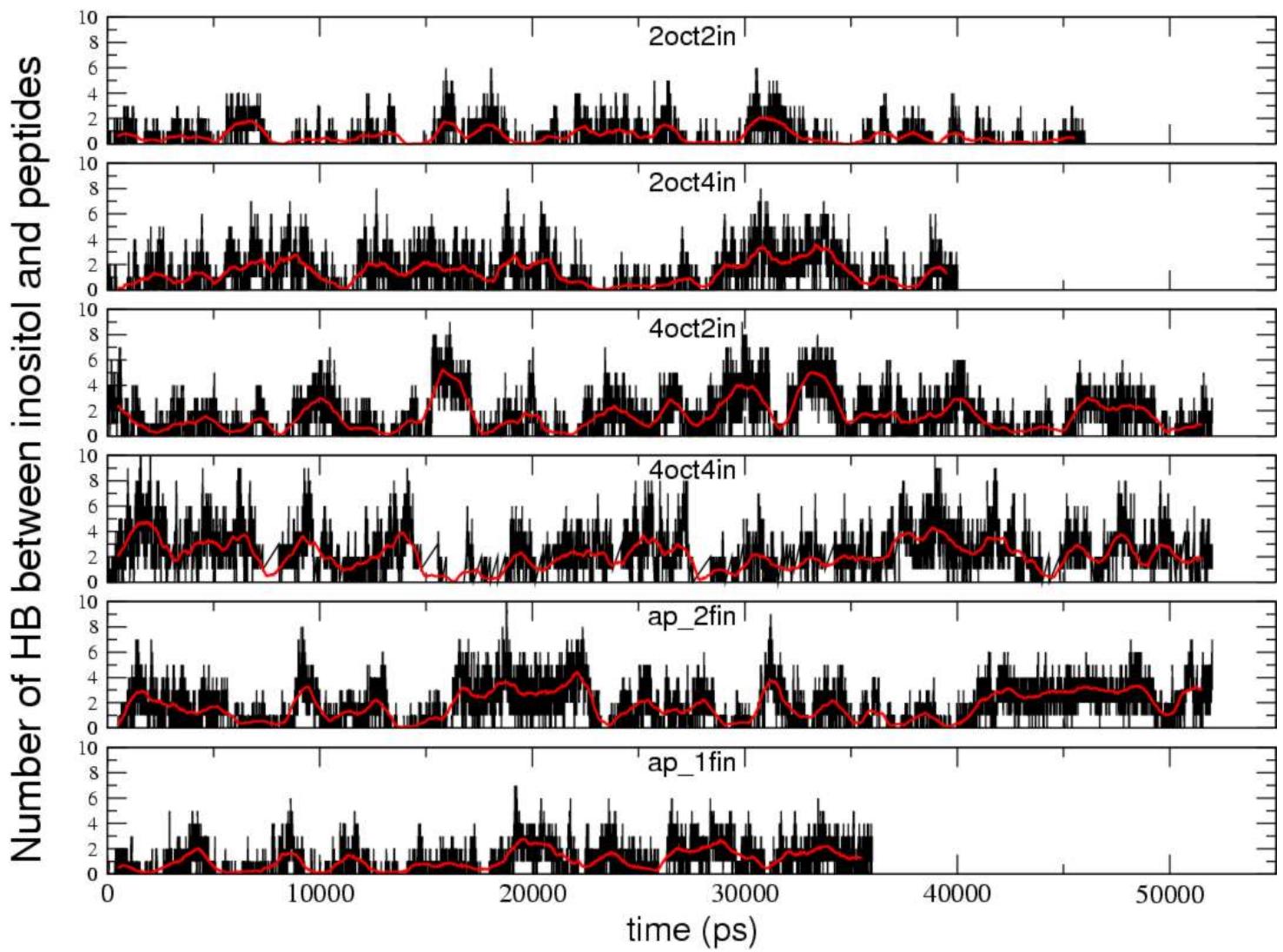


4OCT2IN



4OCT4IN



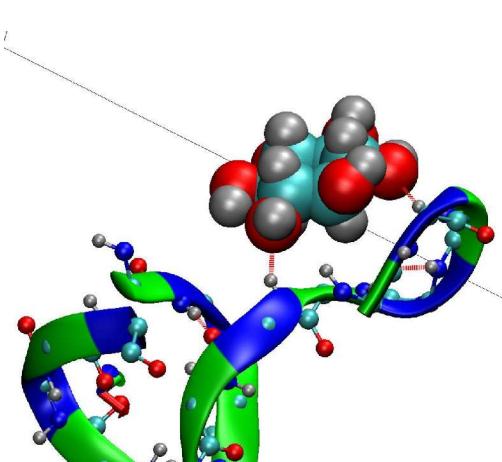
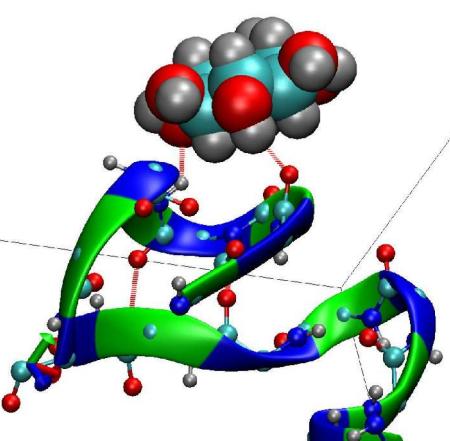
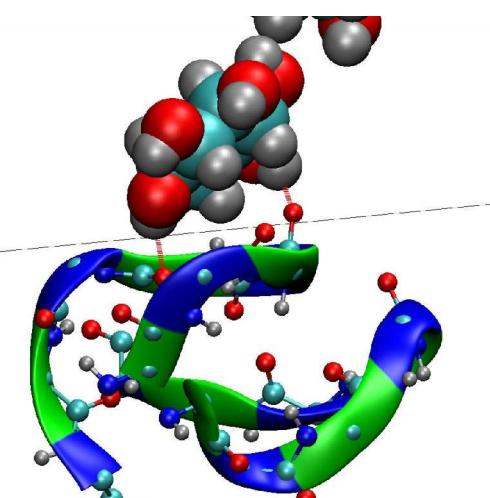
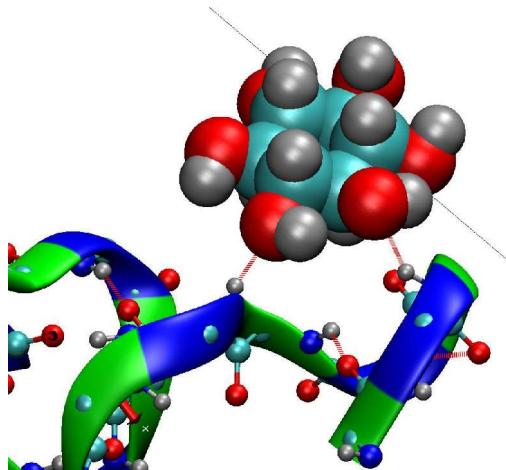
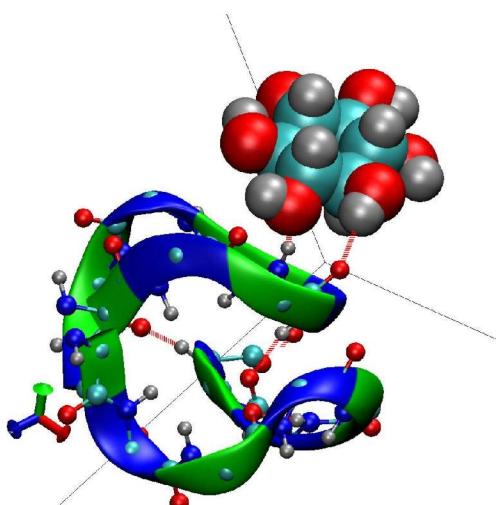
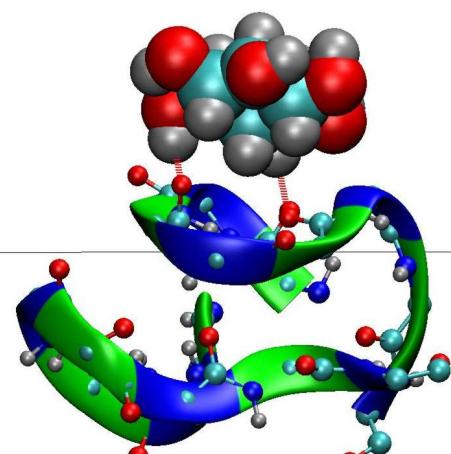
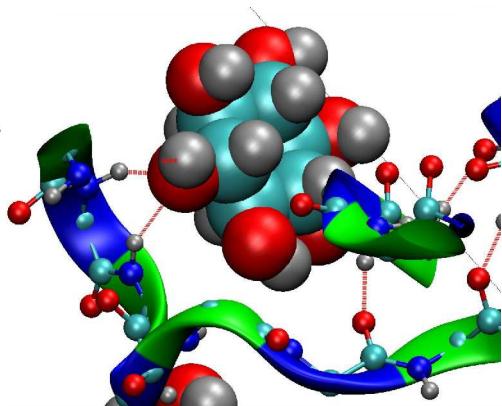
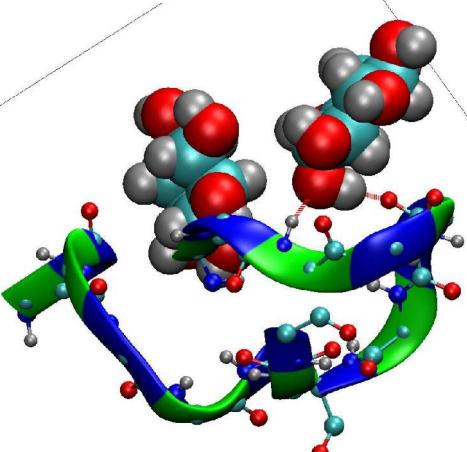
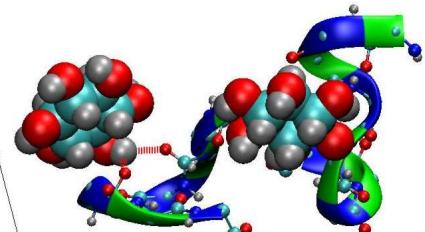


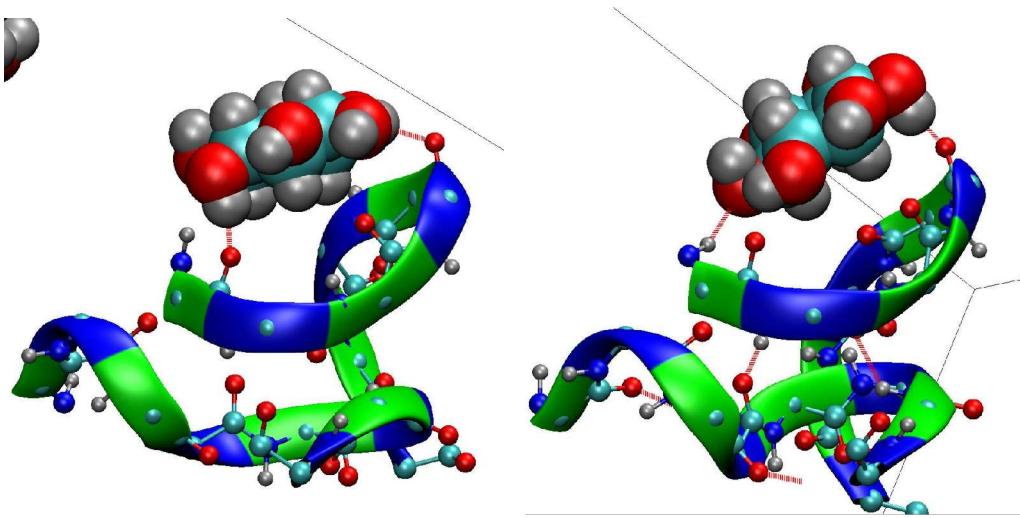
Distribution of the
number of hydrogen
bonds per snapshots for
the 6 systems.

HH

HO

OO





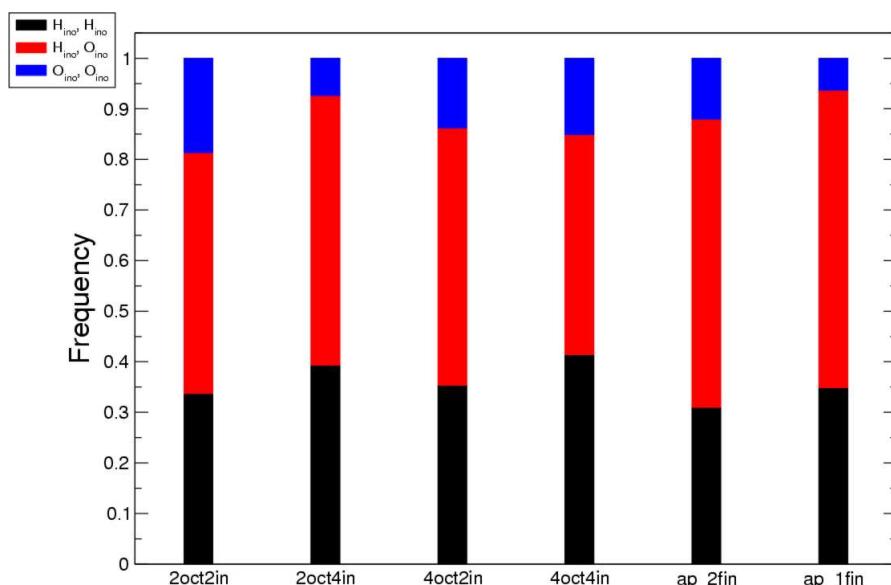
The 11 eleven snapshots presented above are extracted from the trajectory of the 2oct_2in system.

July 19th 2007

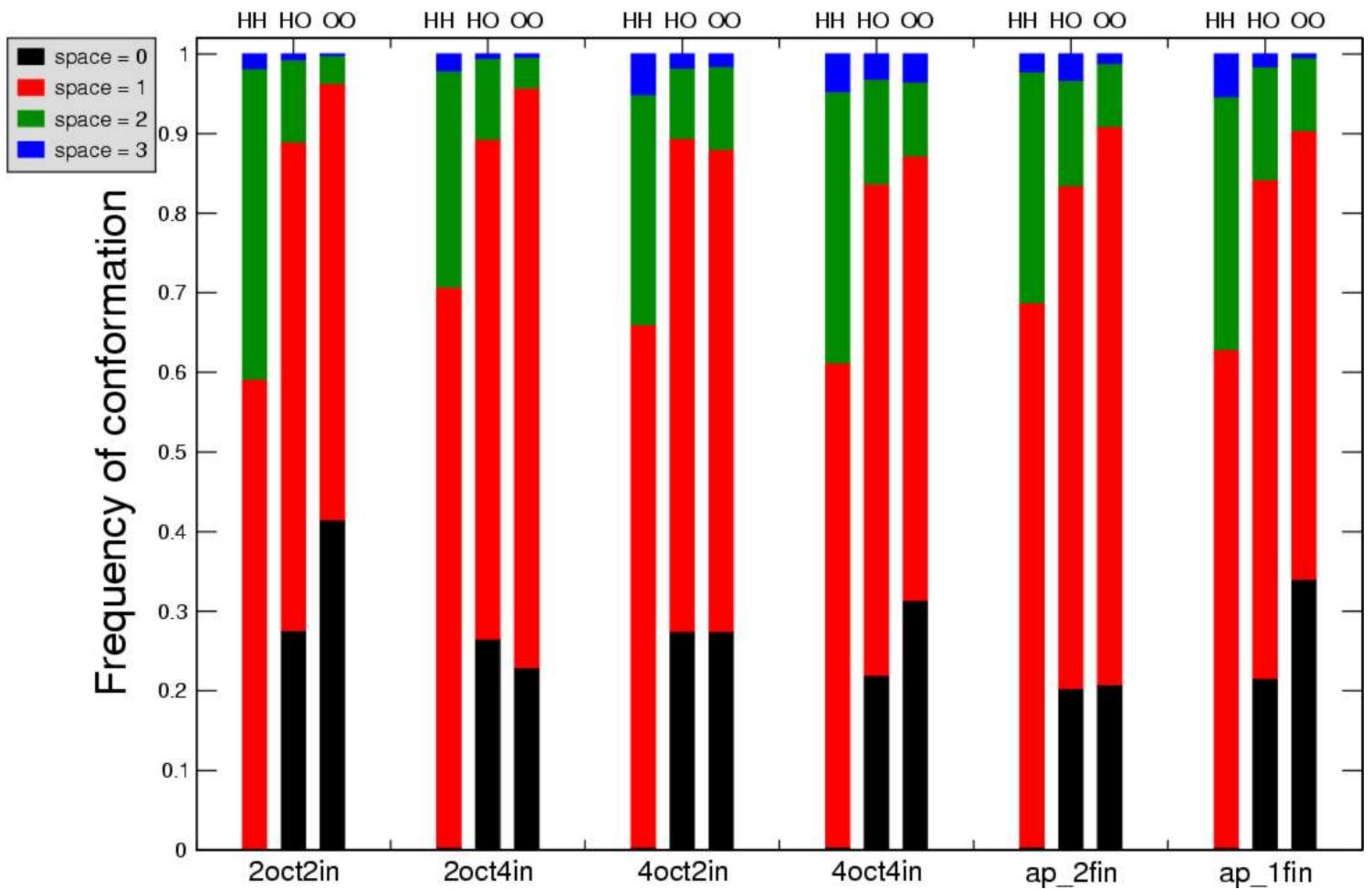
Systems	Time (ns)	Total HB	Monodentate conf	Bidentate conf	Conf with more than 2HB
ap_2f_ino	100	141726	41002	22858	16573
ap_1f_ino	100	139893	43191	19843	16475
2oct_2in	100	78595	32790	13909	5526
2oct_4in	100	159046	55420	27181	14987
4oct_2in	100	146546	42571	22853	16466
4oct_4in	100	273013	76955	39816	32904

The different analysis are performed on trajectories where snapshots are saved every ps.

Systems	HB per ino per snapshot	Monodentate conf per ino per snapshot	Bidentate conf per ino per snapshot
ap_2f_ino	0.709	0.205	0.114
ap_1f_ino	0.699	0.216	0.099
2oct_2in	0.393	0.164	0.069
2oct_4in	0.398	0.139	0.068
4oct_2in	0.733	0.213	0.114
4oct_4in	0.682	0.192	0.100



Frequency of the bidentate conformations in terms of the nature of the groups engaged in the HB.



Frequency of the bidentate conformations in terms of the spacing between groups engaged in the HB.

HH configuration

Systems	Total	Space = 0	Space = 1	Space = 2	Space = 3
2oct_2in	4678	0.19	58.87	38.99	1.95
2oct_4in	10669	0.3	70.35	27.15	2.19
4oct_2in	8065	0.3	65.62	28.9	5.18
4oct_4in	16450	0.32	60.81	34.05	4.82
ap_2f_ino	7071	0.32	68.35	29	2.32
ap_1f_ino	6905	0.28	62.52	31.75	5.45

Percentage of each conformation.

HO configuration

<i>Systems</i>	<i>Total</i>	<i>Space = 0</i>	<i>Space = 1</i>	<i>Space = 2</i>	<i>Space = 3</i>
2oct_2in	6626	27.51	61.32	10.37	0.8
2oct_4in	14488	26.45	62.76	10.17	0.62
4oct_2in	11622	27.41	61.92	8.84	1.83
4oct_4in	17333	21.87	61.75	13.14	3.24
ap_2f_ino	13020	20.22	63.16	13.23	3.38
ap_1f_ino	11672	21.51	62.62	14.19	1.68

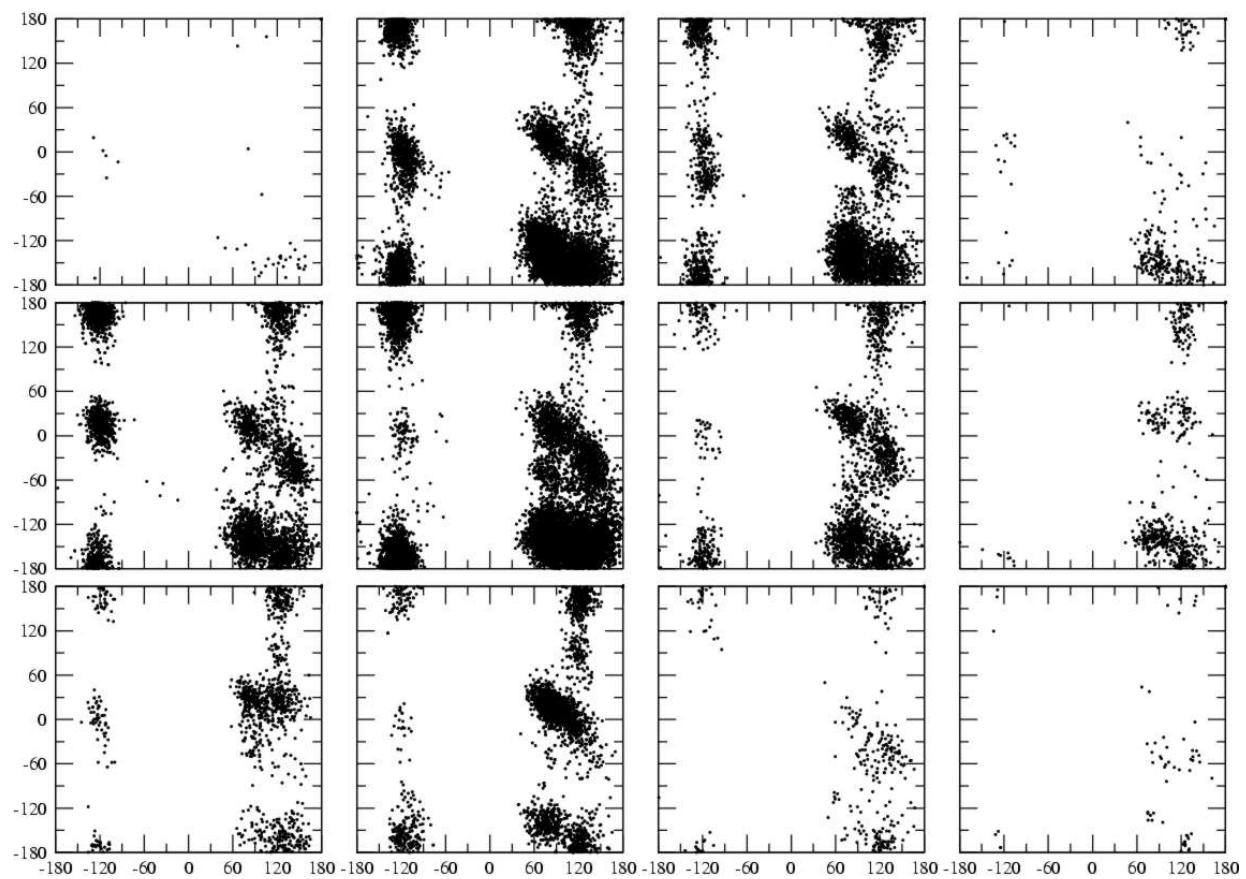
Percentage of each conformation.

OO configuration

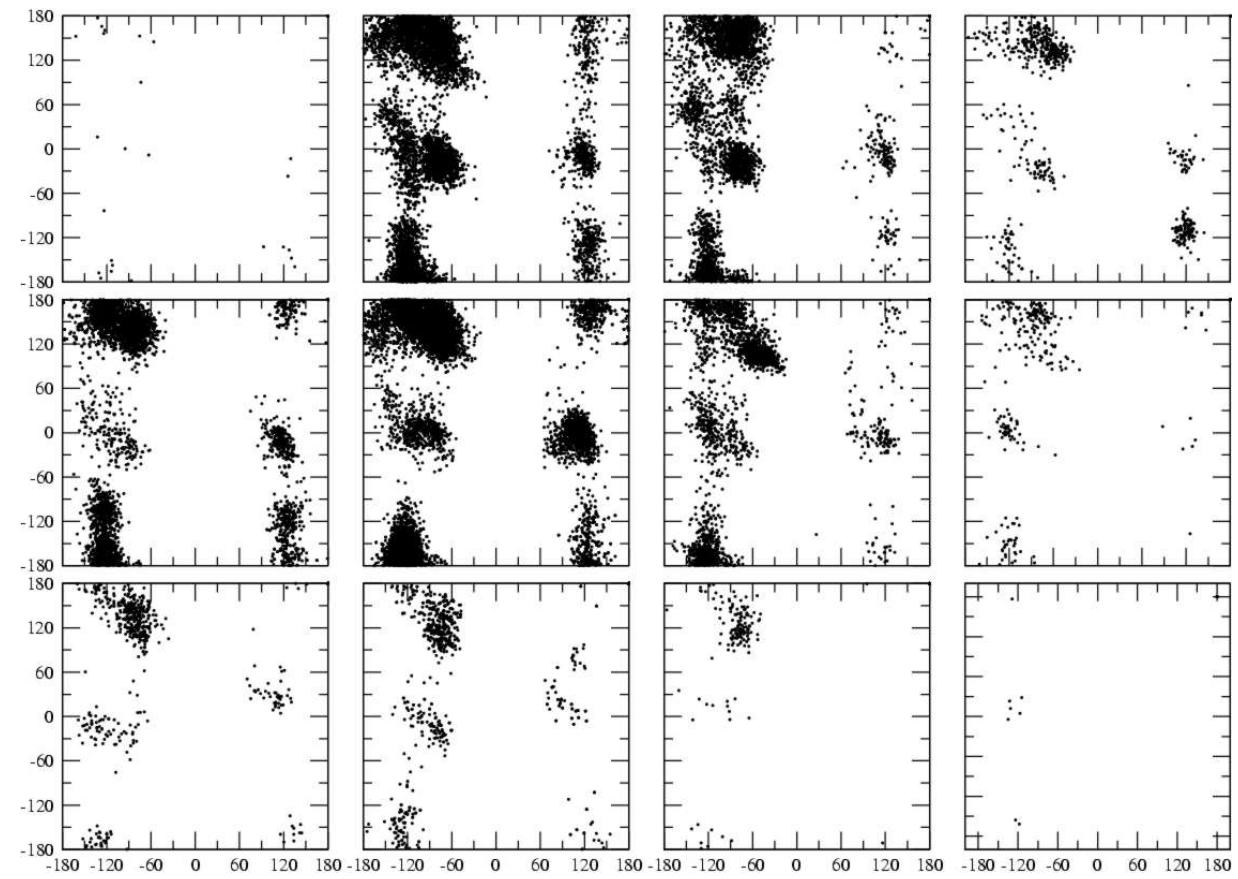
<i>Systems</i>	<i>Total</i>	<i>Space = 0</i>	<i>Space = 1</i>	<i>Space = 2</i>	<i>Space = 3</i>
2oct_2in	2605	41.42	54.81	3.49	0.27
2oct_4in	2024	22.87	72.78	3.85	0.49
4oct_2in	3166	27.42	60.52	10.42	1.64
4oct_4in	6033	31.28	55.84	9.28	3.6
ap_2f_ino	2767	20.67	70.15	7.91	1.26
ap_1f_ino	1266	33.96	56.32	9.16	0.55

Percentage of each conformation.

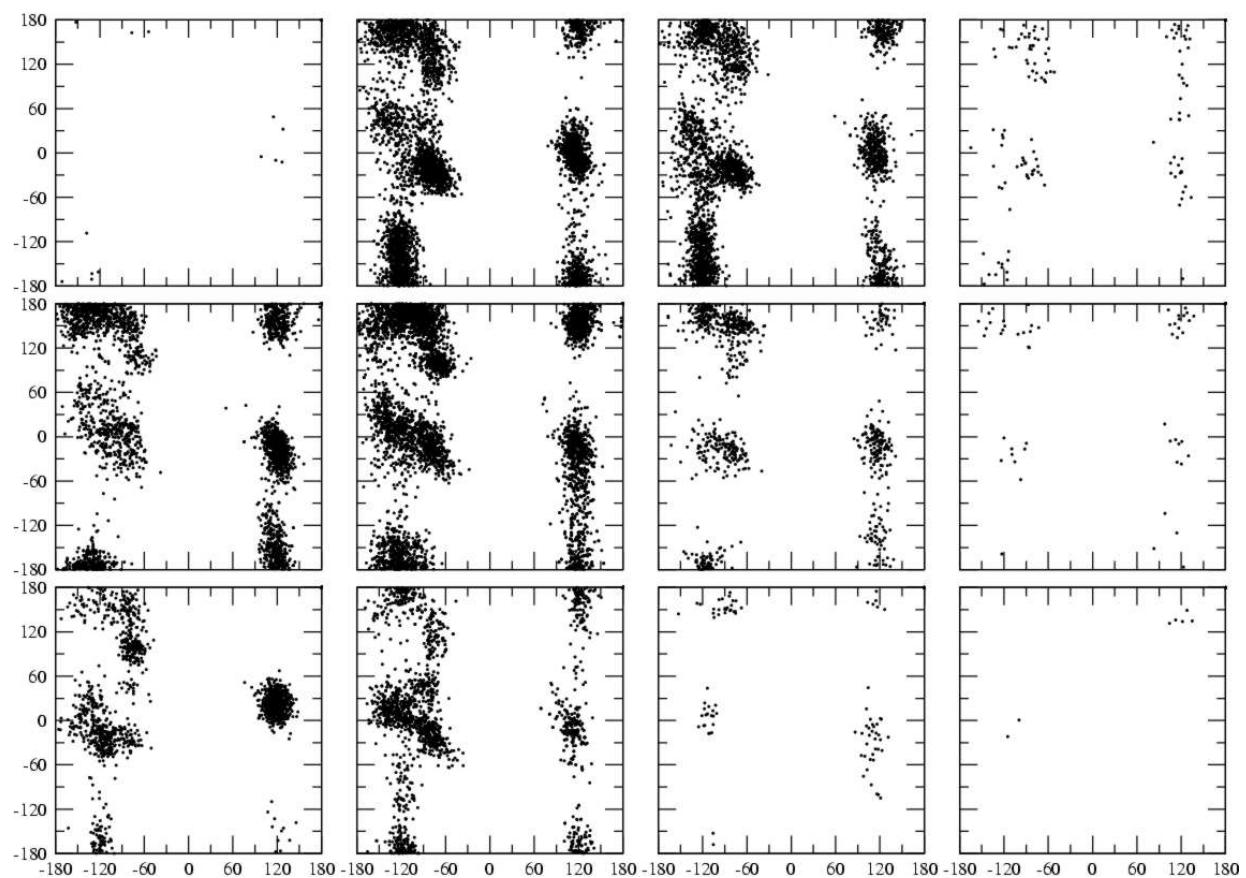
AP2FIN



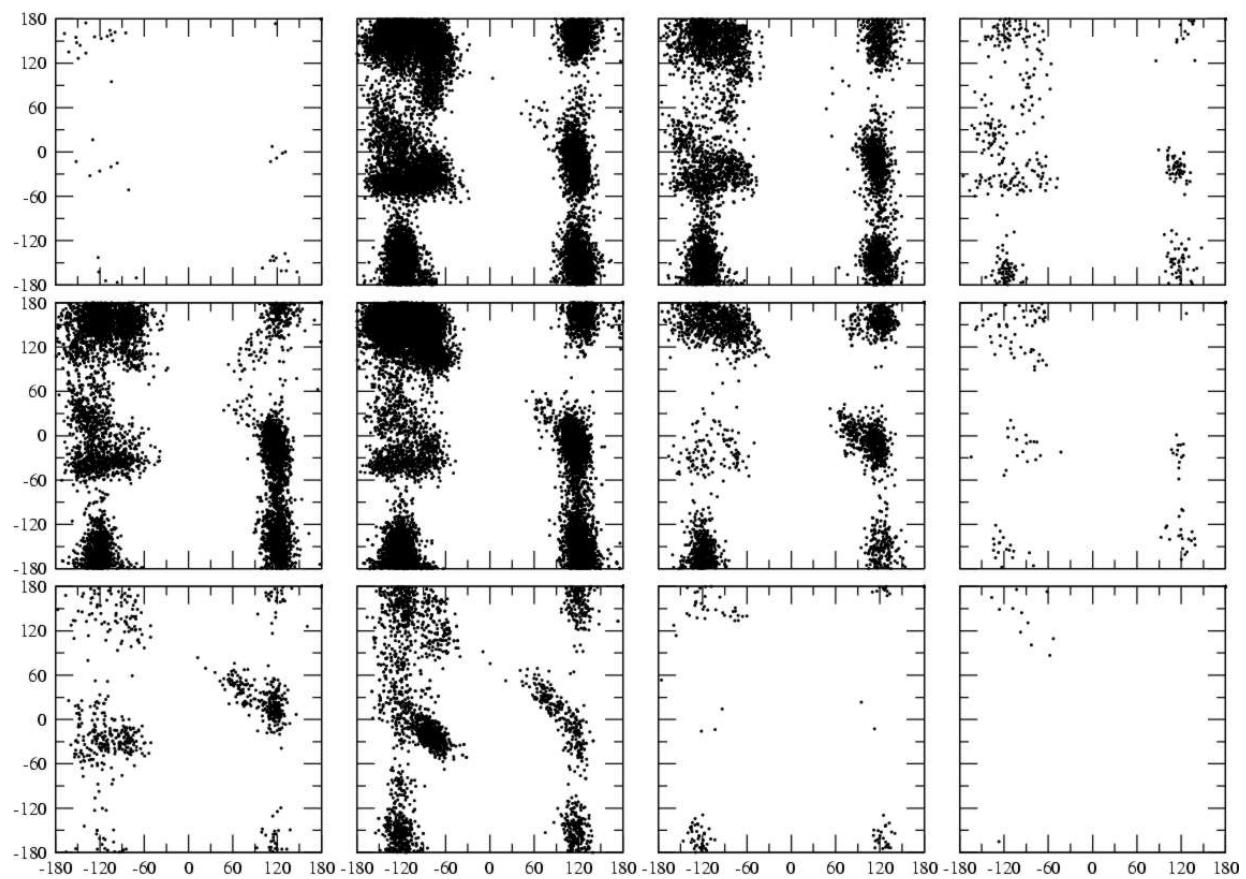
AP1FIN



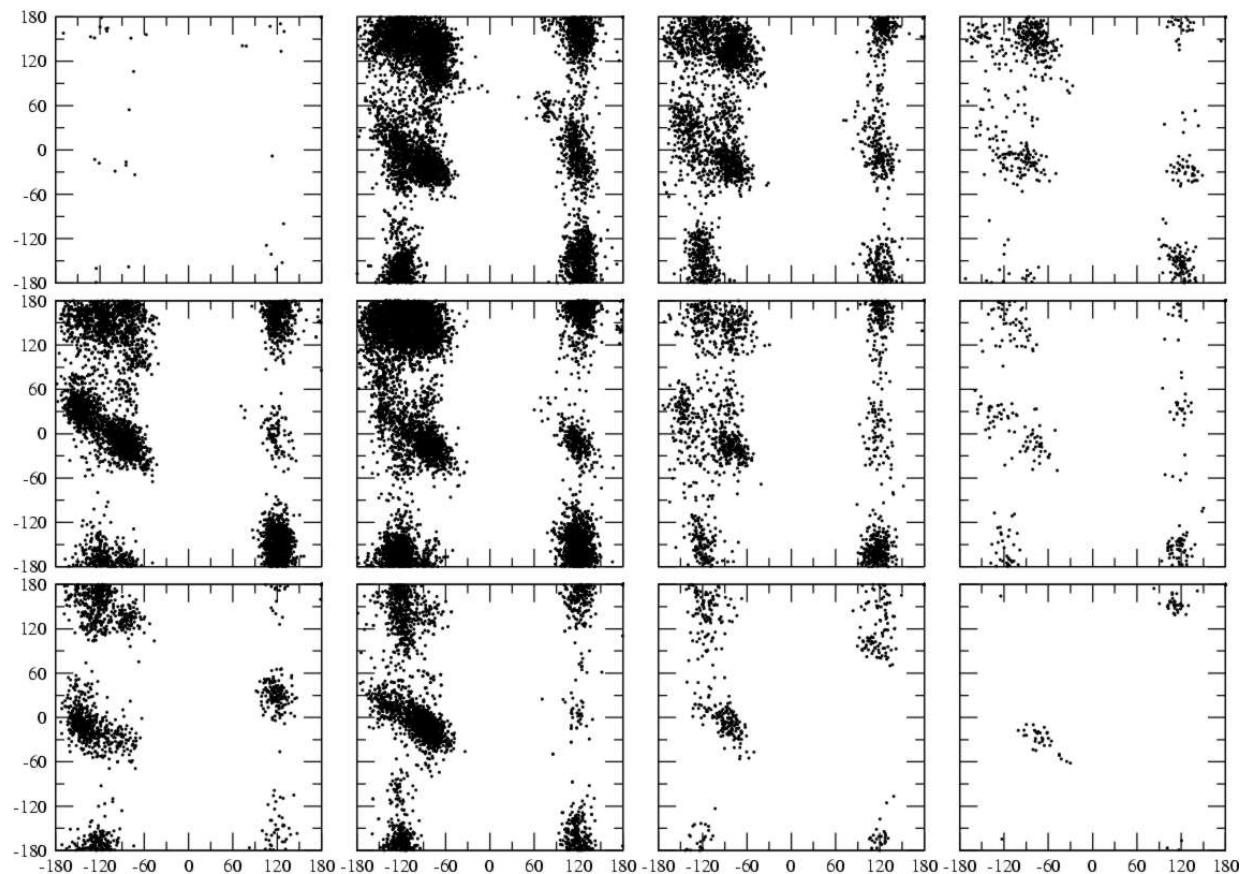
2OCT2IN



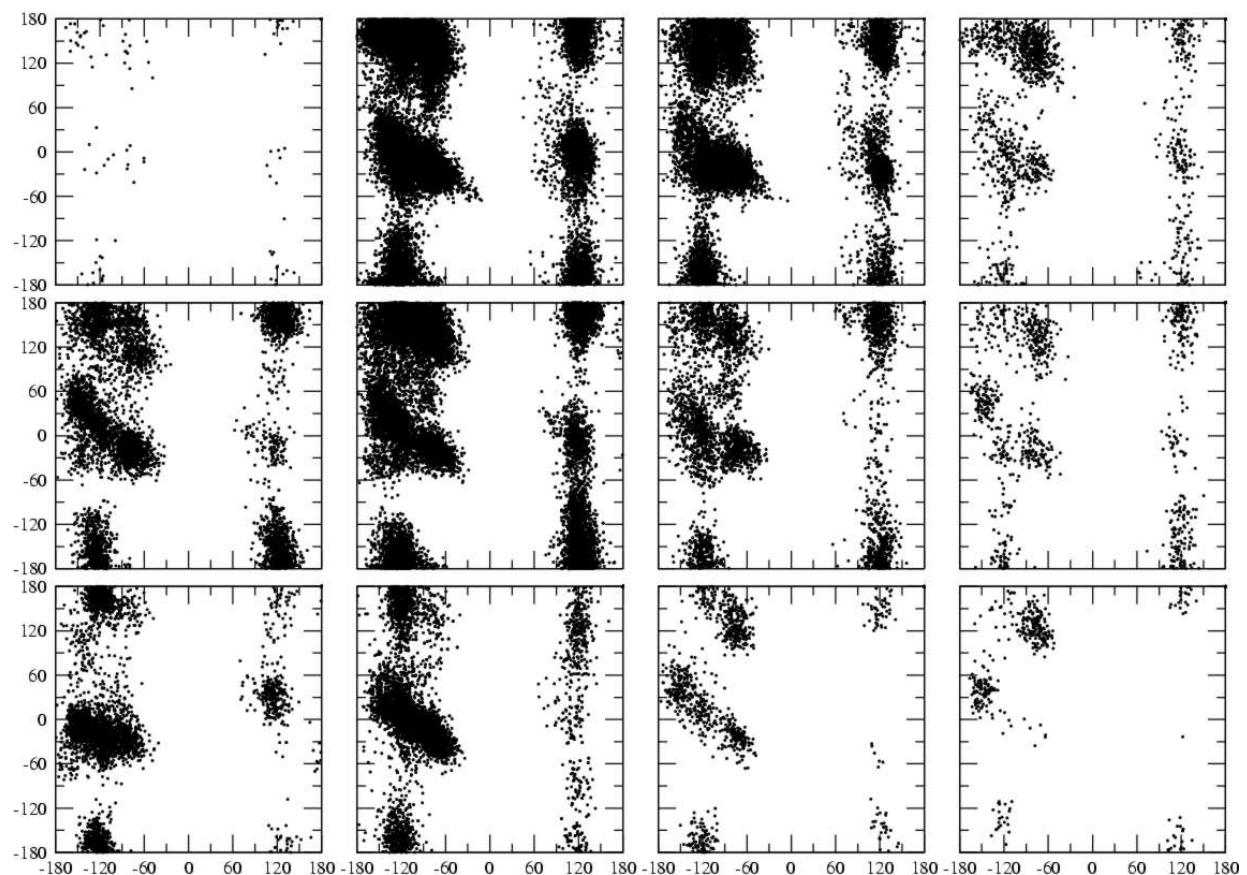
2OCT4IN

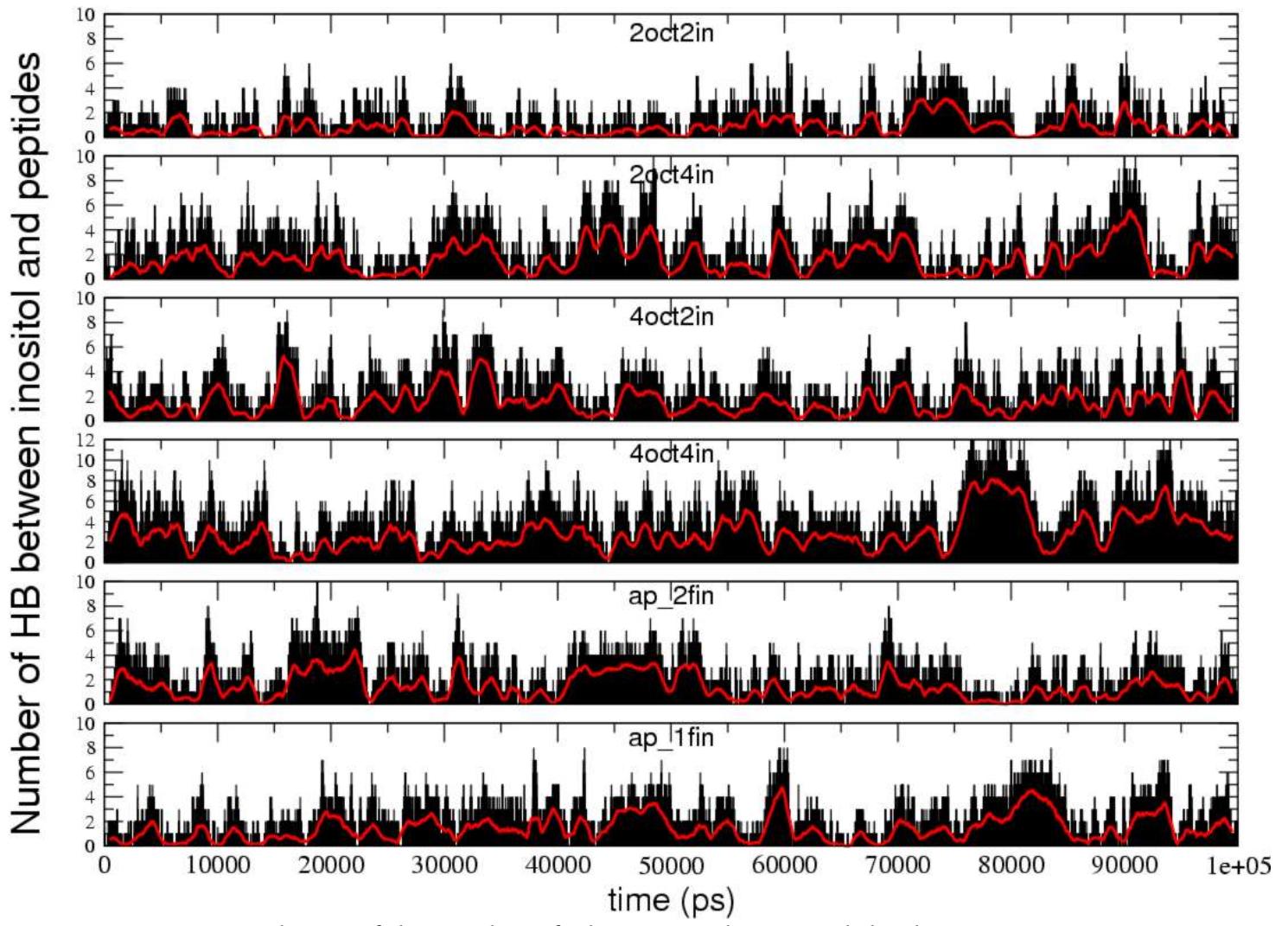


4OCT2IN

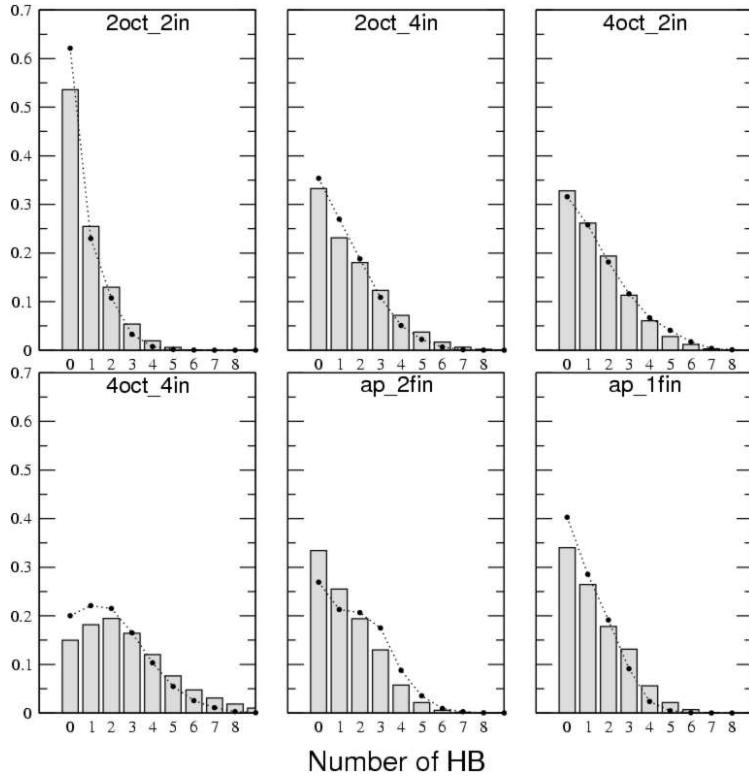


4OCT4IN





Evolution of the number of Hb per snapshot. In red the data are averaged over 1000 snapshots.



Distribution of the number of hydrogen bonds per snapshots for the 6 systems. The bars correspond to the distribution computed from 100ns simulations. The circles and the dashed line correspond to the distributions computed previously (36 to 52ns depending on the system).

		2oct2ino						2oct4ino					
		HH		HO		OO		HH		HO		OO	
0	0	0	66.67	15.36	62.86	0	100	0	53.13	31.78	49.77	0	99.57
	1	33.33		35.78		99.17		5.88		16.99		93.28	
	2	33.33		23.21		0.74		5.88		40.69		6.72	
	3	0		5.32		0		28.41		5.93		0	
	4	0		6.28		0		0		2.15		0	
	5	0		1.05		0.09		52.94		1.57		0	
	6	16.67		0		0		5.88		0.68		0	
	7	16.67		13		0		0		0.21		0	
1	0	12.77	71.64	45.82	68.82	43.14	92.37	12.83	52.84	60.34	80.96	32.77	80.99
	1	35.12		13.23		42.99		28.19		10.69		57	
	2	41.1		25.18		3.41		39.81		22.59		4.02	
	3	5.42		1.14		2.73		8.17		4.7		5.62	
	4	2.74		0.21		1.29		3.15		0.27		0.59	
	5	0.61		1.79		6.44		6.03		1.14		0	
	6	0.41		0.93		0		1.66		0.22		0	
	7	1.82		11.7		0		0.15		0.05		0	
2	0	0	74.45	0.36	40.47	0	36.26	0	50.09	0.33	61.44	0	37.18
	1	38.88		53.6		0		22.33		49.39		0	
	2	45.8		10.79		0		50.31		6.3		0	
	3	8.84		5.76		78.79		8.55		21.88		96.55	
	4	2.5		7.55		3.03		6.55		3.09		0	
	5	2.21		0.36		18.18		6.2		10.06		3.45	
	6	1.18		2.88		0		5.65		8.73		0	
	7	0.59		18.71		0		0.41		0.22		0	
3	0	0	45.05	0	50.94	0	0	0	20.51	0	32.22	0	0
	1	0		3.7		0		0		0		0	
	2	14.63		66.67		0		12.5		6.9		0	
	3	48.78		11.11		0		27.08		17.24		0	
	4	2.44		0		0		39.58		34.48		0	
	5	0		0		0		8.33		41.38		0	
	6	31.71		11.11		0		12.5		0		0	
	7	2.44		7.41		0		0		0		0	

		4oct2ino							4oct4ino						
		HH		HO		OO			HH		HO		OO		
0	0	0	50	24.37	59.38	0	99.19	0	59.62	29.8	59.68	0	98.89		
	1	33.3		37.68		99.3		9.68		30.86		97.82			
	2	50		18.29		0.7		22.58		29		2.12			
	3	0		4.07		0		45.16		6.5		0.05			
	4	0		0.85		0		12.9		2.48		0			
	5	16.67		1.32		0		9.68		1.06		0			
	6	0		10.15		0		0		0.13		0			
	7	0		3.28		0		0		0.18		0			
1	0	13.54	51.23	73.12	78.15	42.64	73.07	10.74	66.65	63.62	70.86	26.15	85.57		
	1	34.82		10.76		54.64		23.5		8.56		68.05			
	2	30.84		9.67		2.07		53.17		22.2		4.41			
	3	4.72		2.76		0.57		8.23		4.23		1.04			
	4	3.02		0.55		0		1.05		0.63		0.17			
	5	4.54		0.18		0		1.74		0.4		0.14			
	6	3.61		2.01		0		0.28		0.28		0.03			
	7	4.91		0.96		0.07		1.27		0.08		0			
2	0	0	43.2	0	35.15	0	16.06	0	49.7	0.16	27.61	0	10		
	1	22.74		36.57		0		22.23		14.63		0			
	2	38.03		22.99		0		53.7		36.41		0			
	3	4.47		7.76		3.77		7.72		10.81		60.71			
	4	1.19		5.54		1.89		5.85		13.99		32.14			
	5	8.44		13.02		1.89		2.73		16.69		7.14			
	6	13.41		6.93		7.55		0.83		6.52		0			
	7	11.72		7.2		84.91		6.93		0.79		0			
3	0	0	38.52	0	19.25	0	0	0	16.52	0	21.71	0	1.84		
	1	0		0		0		0		0.82		0			
	2	6.21		12.2		0		32.82		54.1		0			
	3	1.24		2.44		0		46.56		31.97		25			
	4	1.86		53.66		0		13.74		10.66		75			
	5	52.17		7.32		0		2.29		2.46		0			
	6	11.18		2.44		0		3.82		0		0			
	7	27.33		21.95		0		0.76		0		0			

			Ap2fino						ap1fino					
			HH		HO		OO		HH		HO		OO	
0	0	0	21.74	39.49	68.29	0	99.13	0	21.05	25.36	63.12	0	100	
	1	40		8.18		87.3		50		39.87		99.77		
	2	0		43.16		12.52		50		19.5		0		
	3	40		8.18		0		0		5.93		0		
	4	20		0.33		0		0		0		0		
	5	0		0.17		0		0		0		0		
	6	0		0.22		0		0		8.71		0.23		
	7	0		0.28		0.18		0		0.63		0		
1	0	31.11	37.58	79.39	66.26	37.47	80.58	20.56	55.87	47.8	63.17	57.32	90.04	
	1	22.03		2.83		58.25		31.3		23.15		40.19		
	2	24.67		14.35		2.37		22.72		22.48		0.62		
	3	17.24		1.52		0		9.37		1.97		0.31		
	4	1.05		0.18		0		5.47		0.52		0		
	5	3.19		0.48		0.19		1.95		0.02		0		
	6	0.66		1.19		0		8.21		3.47		1.56		
	7	0.06		0.06		1.73		0.41		0.58		0		
2	0	0	30.42	0	34.63	0	25.57	0	32.07	0	59.24	0	18.1	
	1	28.04		33.84		0		31.86		81.27		0		
	2	37.66		9.72		0		16.22		6.45		19.05		
	3	8.17		15.91		5.36		12.38		4.47		23.81		
	4	8.49		18.93		8.93		37.13		1.14		57.14		
	5	15.87		14.41		73.21		1.85		0		0		
	6	1.6		6.37		12.5		0.14		8.53		0		
	7	0.16		0.84		0		0.43		0.21		0		
3	0	0	12.8	0	35	0	57.14	0	17.24	0	17.35	0	42.86	
	1	0		0.65		0		0		47.06		0		
	2	0		2.6		0		3.08		5.88		0		
	3	28.57		18.18		0		47.69		32.35		0		
	4	23.81		16.23		60		20		5.88		100		
	5	28.57		56.49		15		0		2.94		0		
	6	14.29		5.19		25		29.23		5.88		0		
	7	4.76		0.65		0		0		0		0		