COMPUTATIONAL PREDICTION OF THE SUPRAMOLECULAR SELF-ASSEMBLING PROPERTIES OF ORGANIC MOLECULES: FLEXIBILITY *vs* RIGIDITY

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Laura Le Bras*

Unité de Chimie Physique
Théorique et Structurale,
Chemistry Department,
Namur Institute of Structured Matter,
University of Namur, Belgium
`laura.lebras@unamur.be`

Yves L. Dory

Laboratoire de Synthèse Supramoléculaire,
Département de Chimie,
Institut de Pharmacologie,
Université de Sherbrooke,
Sherbrooke, Québec, Canada

Benoît Champagne*

Unité de Chimie Physique
Théorique et Structurale,
Chemistry Department,
Namur Institute of Structured Matter,
University of Namur, Belgium
`benoit.champagne@unamur.be`

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ABSTRACT

Two families of organic molecules with different backbones have been considered. The first family, composed by a substituted central phenyl is considered as flexible. The second one, based on a macrolactam-like unit, is considered as rigid. They have however a common feature, three amide moieties (as substituents for the phenyl and within the cycle for the macrolactam-like molecule) that allow hydrogen bonding when molecules are stacked. In this study we propose a computational protocol to unravel the ability of the different families to self-assemble into organic nanotubes. Starting from the monomer and going towards larger assemblies like dimers, trimers, and pentamers we applied different theoretical protocols to rationalize the behavior of the different assemblies. Both structures and thermodynamics were investigated to give a complete picture of the process. Thanks to the combination of a quantum mechanics approach and molecular dynamics simulations along with the use of tailored tools (non covalent interaction visualization) and techniques (umbrella sampling), we have been able to differentiate the two families and highlight the best candidate for self-assembling purposes.

1 Introduction

Supramolecular chemistry is a key concept for many edifices or mechanisms that are essential for life [1]. So it is for some applications going from material sciences to medicine through information storage[2, 3, 4, 5, 6]. It has been defined by Lehn, one of its founders, as the chemistry of intermolecular bonds and interactions[7]. Supramolecular chemistry involves a wide variety of weak interactions of different strength. Metal-ligand interactions are the strongest one with an interaction energy of about 80 kcal.mol^{-1} . Ionic, ion-dipole and dipole-dipole interactions are slightly less stabilizing with an interaction energy ranging from 15 to 50 kcal.mol^{-1} . Aromatic interactions, encompassing π - π , π -cation and π -hydrogen bond, lead to a bounding of 3 kcal.mol^{-1} . Finally, hydrogen bonds and van der Waals forces are the less energetic ones with interactions energies of $1\text{-}10 \text{ kcal.mol}^{-1}$ and lower than 1 kcal.mol^{-1} , respectively. As the previously mentioned interactions are non-covalent, it means that the interactions can be reversible and thus confer a kind of flexibility to the system. Nevertheless, increasing the number or combining weak interactions can lead to really stable assemblies and one can cite for example the structure of ice, DNA [8] or the synthetic supramolecular polymer, namely the nylon[9]. In both cases, hydrogen bonds (H-Bonds) are at the origin of the large stability of the supramolecular assemblies (SMA). The association and dissociation processes within large SMA is ensured by the inter- and intramolecular H-Bonds, the best example being the secondary and tertiary structures of proteins that are dictated by those weak interactions [10, 11, 12]. In particular, H-Bonds

based on an amide pattern ($\text{N-H} \cdots \text{O=}$) are frequently found. The low to medium strength of this type of H-Bond, around 2 kcal.mol^{-1} , along with its flexibility, allow SMA to assemble and disassemble easily. One of the specificity of the amide-based H-Bonds is their directionality. It has been used to build SMA that are characterized by a stacking of the molecules leading to a one-dimensional columnar SMA [13, 14]. Many studies have reported SMA structures where the building block was a substituted benzene with various number of amide moieties for the formation of organogels or liquid crystals [15, 16, 17, 18]. The number and the orientation of the amide moieties have been shown to be crucial for the effectiveness of the formation of the stacking[13].

Based on the idea of an amide-substituted benzene for the formation of columnar stacking, we have designed four benzenetricarboxamide molecules (**B4s** family, Figure 1). Amide moieties are perpendicular to the plane of the benzene. A difference in the orientation of the three amide moieties was observed for **B4s** family, while two of them were oriented in the same direction, the last one was found antiparallel (see Figure S3 in ESI†). The remaining positions of the central benzene were substituted by $-\text{CH}_3$ (**B4**, **B4c**, **B4p**) or $-\text{I}$ (**B4pI**) groups to maintain the amide moieties perpendicular to the plane of the molecule. In addition to the substitution of the central benzene, amides were also substituted by $-\text{C}_6\text{H}_{13}$ alkane chain (**B4**), $-\text{C}_6\text{H}_{11}$ cyclic group (**B4c**), and $\text{CH}_2\text{-Ph}$ moiety for both **B4p** and **B4pI**. A different backbone, based on a macrolactam-like unit, has been considered (**B9s** family, Figure 1). It also encompassed three amide moieties that are conjugated to an alcene to avoid their rotation. Two substitution patterns have been added, a $-\text{CH}_3$ for **B9m** and a $-\text{CH}_2\text{OCOCH}_3$ for **B9**. The global idea is to compare those two apparently C_3 -symmetric backbones for an efficient formation of columnar stacking involving amide-based H-Bonds.

To address this challenge we propose a computational approach. Recent studies have already shown that a fully theoretical approach, based on molecular dynamics simulations and quantum chemical calculations, was able to unravel hydrogen bond interactions between organic molecules within supramolecular assemblies[19]. Hence, the multiscale approach that will be developed in this study will address for the different small organic molecules (See Figure 1) their self-assembling property and the stability of the possible columnar stackings, considered as organic nanotubes. The multiscale approach will involve calculations based on quantum mechanics but also classical molecular dynamics simulations combined with tailored tools to characterize the H-Bonds. Umbrella sampling is particularly relevant for the analysis of supramolecular interactions as it allows the calculation of a binding energy through the derivation of a potential of mean force[20, 21, 22, 23]. Non-covalent interactions (NCI) visualization is also a valuable tool that can illustrate the underlying weak interactions such as hydrogen bonds or van der Waals interactions within SMA[24, 25].

Figure 1: Representation of the structures of the four molecules of the **B4s** family, **B4**, **B4c**, **B4p** and **B4pl** and of the two molecules of the **B9s** family, **B9** and **B9m**. The three important dihedral angles are also defined.

2 Computational Details

2.1 Quantum Mechanics

2.1.1 Single point calculations

All the calculations have been performed using Gaussian16 package [26] within the density functional theory (DFT) framework. We used the ω B97X-D range-separated hybrid exchange correlation functional (XCF) combined with the 6-311+G(d,p) atomic basis set [27]. This XCF is known as one of the most efficient to consider structures and energies of assemblies involving hydrogen bond interactions[28]. The solvent (water) was modeled using an implicit solvation model, namely the polarizable continuum model (PCM) [29]. In order to describe large assemblies, we relied on a QM/QM' ONIOM approach. Within this model it is necessary to define 2 subsystems. The model system, composed of the central phenyl and the amide groups for **B4s** and the macrolactam-like unit for **B9s**, will be described at both the high level of theory (ω B97X-D/6-311+G(d,p)) and the low level (HF/3-21G(d)) of theory. The real system, encompassing the model system and the lateral groups of both families, will only be treated at the low level of theory. A charge embedding framework was also added to the hybrid QM/QM' ONIOM scheme [30].

2.1.2 Non-covalent interactions

It has been possible to visualize non-covalent interactions (NCI) through the use of NCIPLOT code[31]. NCI analysis gives rise to an index that is based on the calculated electronic density and its reduced gradient, represented as a two-dimensional plot. For a given system, there will be a drastic change in the reduced density gradient (RDG) between the atoms that are interacting, leading to density critical points. The latter can be represented on the molecular structure as an isosurface to indicate the region where a weak interaction is occurring. Nevertheless, both attractive (H-Bonds, van der Waals) and repulsive (steric repulsion) interactions can be spotted thanks to this index. By looking at the second derivative of the density and to the sign of its eigenvalue it is possible to distinguish attractive and repulsive interactions. Hence the density and the sign of the eigenvalue of the density second derivative give information about the strength and the type of interaction respectively and one can visualize them via isosurfaces. The electronic density used to compute the NCI index is the one calculated at the same level of theory as the one presented in Section 2.1.1.

2.2 Molecular Dynamics

2.2.1 Classical Molecular Dynamics

Molecular dynamics (MD) simulations were run with the generalized AMBER force field (GAFF) [32] within GROMACS 2018.3 package[33]. As GAFF is not directly implemented in GROMACS, we used acpypi script [34, 35, 36] to convert the files from AMBER to GROMACS formalism. For each molecule, the atomic charges were derived following the parametrization procedure in GAFF, that is to say using HF/6-31G(d) RESP charges. The validity of the force field was checked by comparing the structures obtained after an optimization process in vacuum with GAFF and with DFT at the ω B97X-D/6-311+G(d,p) level. Results are provided in the Supporting Information section.

During the simulations, the system is composed by n organic molecules (n being equal to 1, 2, 3, 5, and 10 and the organic molecules being either from **B4s** or **B9s** families). The general philosophy of our molecular dynamics simulations is represented schematically on Figure 2. The size of the simulation box and the number of water molecules depend on the system under investigation. All those information for each system under investigation are gathered in Supporting Information.

To describe the electrostatic interactions, periodic boundary conditions were imposed along with a cut-off of 10 Å and the use of the Particle Mesh Ewald (PME) method [37, 38]. Following a steepest descents minimization, each system was equilibrated in two steps. For the first step, a simulation in the canonical ensemble (NVT) during 100 ps was carried out. The temperature was set to 310 K using the Berendsen weak

Figure 2: Schematic representation of our strategy for molecular dynamics simulations. Within a simulation box full of water molecules we will successively consider either monomers, dimers, pentamers or aggregates composed of ten monomers.

coupling method[39]. Organic molecules and solvent were coupled to separate temperature coupling baths. For the following second step, simulation under constant pressure (NPT) was performed. To maintain an isotropic pressure of 1 bar, we relied on the previously mentioned weak-coupling Berendsen method. The production phase was then carried out in the same NPT ensemble. Temperature was controlled thanks to the Nosé-Hoover thermostat while the isotropic character of the pressure was maintained via the Parinello-Rahman barostat. Combining this thermostat and barostat ensures the presence of a true NPT ensemble. Simulation time, if not explicitly precised, was set to 10 ns.

2.2.2 Umbrella sampling

Within the umbrella sampling (US) approach we have considered dimers, trimers and pentamers. The systems were placed in a rectangular box that can allow a pulling simulation (e.g a simulation box that is too small will lead to an interaction with the periodic images). In particular, the z length had to be large enough in order to satisfy the minimum image convention. The solvent molecules (water), were described through the TIP3P model. The first step consisted in an NPT equilibration of 100 ps, as it was described above. For the proper pulling simulation, restraints were applied to one of the monomers for dimers, to a dimer for trimers and to a tetramer in the case of the pentamer. The molecules that were restrained were thus considered as immobile references. The molecule that was not restrained was then pulled away from the immobile one, along the z -axis over 500 ps at a rate of 0.1 nm.ps^{-1} with a spring constant of $250 \text{ kJ.mol}^{-1}\text{nm}^{-2}$. The final COM (center of mass) distance between the two considered assemblies that was obtained was 4 nm. Snapshots were extracted from this pulling simulation in order to be as many starting points for the different umbrella sampling windows. For COM distances under 1 nm, a separation of 0.05 nm was considered between each window and

Figure 3: Schematic representation of the umbrella sampling approach. With (a) the starting configuration for a dimer. The dimer is positioned voluntarily on one side of the simulation box and the z -axis is voluntarily set to a large value to allow the next pulling step. (b) Pulling simulation. B is pulled away from A at a constant rate. (c) Along the pulling simulations, different configurations with different A-B distances (A-B₁, A-B₂) are extracted in order to be as many starting configuration for different sampling window simulations that will be centered on the A-B distance. (d) For each sampling window, we count the number of times that the A-B distance is equal to each value. (e) A Potential of Mean Force can be reconstructed and can be associated to the free binding energy, ΔG_{bind} .

then, for COM distances above 1 nm and up to 2.5 nm, the spacing between the windows was 0.1 nm. An example of the corresponding histogram is provided in Supporting Information. It allows a smoother and more accurate description of the interaction at small COM distances. This approach leads to around 25 windows.

For each window, a 10 ns simulation was performed, resulting in a total simulation time of 250 ns for the US approach, for each assembly of each molecule. The analysis of the results was done using the Weight Histogram Analyzis Method (WHAM), implemented in the GROMACS 2018.3 package[40]. A schematic representation of the umbrella sampling approach is provided in Figure 3.

3 Results and discussion

3.1 Monomers

Key structural parameters (see Figure 1 for their definitions) have been selected to study the different monomers. For both **B4s** and **B9s** families, three dihedral angles were defined (α , β , and γ), illustrating the relative position of the amide groups with respect to the plane of the molecule. We followed their evolution along the 10 ns MD simulations. The average values are reported in Table 1.

Table 1: Average values of the selected dihedral angles (in degrees) defined on Figure 1 along with the RMSD value (in Å) for each of the molecules. Standard deviations are provided in parentheses.

	α	β	γ	RMSD
B4	-90 (13)	91 (14)	88 (13)	2.33 (0.48)
B4c	-90 (13)	89 (14)	89 (13)	2.08 (0.48)
B4p	-89 (13)	90 (13)	90 (13)	3.07 (0.56)
B4pI	-88 (14)	90 (12)	89 (13)	3.12 (0.51)
B9	95 (7)	95 (6)	96 (8)	0.44 (0.12)
B9m	83 (7)	83 (7)	84 (8)	0.80 (0.13)

For the **B4s** family, as previously mentioned, all the amide moieties are not oriented in the same direction. As illustrated by α that is negative, there is one amide moiety that is antiparallel to the two other ones for **B4s**. This feature will thus induce a difference for the stacking behavior of the next steps. For the **B9s** family, all the amide groups are oriented in the same direction. During the MD simulations, all the dihedral angle values are close to ideal value of 90° and no complete rotation of the amide moieties was observed. It indicates that all the amide moieties are and stay perpendicular to the plan of the molecule. Considering the standard deviation associated to those values, **B9** and **B9m** show a smaller flexibility with deviations around 7° while this value increases up to 14° for **B4s** family. To confirm this hypothesis, we computed the root mean square deviation (RMSD) of the different structures along the simulation, the reference structure being the ideal one with perfectly perpendicular amide groups. One has to notice that for the RMSD calculation, the hydrogen atoms were not considered. The results are gathered in Figure 4 and Table 1.

Figure 4: Evolution of the RMSD values, in Å, for the considered molecules along the simulation time. Hydrogen atoms were not considered for the calculation of the RMSD.

The two families can be clearly distinguished. RMSD values are lower than 1 Å for **B9** and **B9m** while they are systematically larger than 1.5 Å for **B4s** family. It indicates that during the simulations, **B4s** molecules are more flexible than the **B9s**. Due to the inclusion of the amide groups within the macrolactam-like backbone of **B9s**, there is almost no degree of freedom for the molecule, as illustrated by the low average RMSD value for this family (0.44 and 0.80 Å for **B9** and **B9m** respectively). Fluctuations are also small with deviations equal to 0.12 and 0.13 Å for **B9** and **B9m** respectively. On the other hand, for **B4s** molecules, the average RMSD is ranging from 2.08 Å to 3.12 Å for **B4c** and **B4pl** respectively, indicating quite large structural modifications during the simulation. Standard deviations confirm this trend with values around 0.50 Å for the four molecules of **B4s** family. Studying the monomers of the different families has highlighted the fact that one family can be considered as rigid (**B9s**) while the other one appears to be more flexible (**B4s**). This feature may have an impact if one wants to build larger supramolecular assemblies as it will ease, or not, the formation of hydrogen bonds. The next sections are dedicated to the comparison of the two families for the formation and the stabilities of different SMA.

3.2 Dimers

3.2.1 Qualitative approach

To study the possible self-assembling behavior of the different molecules, we decided to consider the smallest and thus simplest supramolecular entity, namely a dimer. The interaction of the two monomers is ensured by the amide moieties. Indeed it is possible to form a hydrogen bond between the N-H part of the amide of one molecule and the C=O bond of another amide. As previously observed during the study of the monomers, the

amide groups are perpendicular to the plan of the molecule and it is thus possible to stack monomers via a network of three hydrogen bonds. The dimers we built are represented on Figure 5.

Figure 5: Representation of the stacking of the dimers for the (a) **B4s** and (b) **B9s** families. Key distances are also identified for both stackings. (c) Representation of a particular stacking observed for **B4s** and definition of a new hydrogen bond, h' .

MD simulations were performed for each dimer. Three new key structural parameters were introduced, namely h_1 , h_2 , and h_3 , which illustrate the three hydrogen bonds and d , that illustrates the distance between the centroids of the central phenyl for **B4s** and of the macrolactam-like unit for **B9s**. The average values for each dimer are gathered in Table 2.

By looking at the average value of d along the MD simulations it is already possible to have a qualitative idea of the stability of the different assemblies. If all the dimers involving monomers from the **B4s** family are stable, it is not the case for the **B9s** one. Indeed, the d values that are reported in Table 2 illustrate the fact that the two monomers involved within a dimer are remaining close to each other. If we go deeper in the analysis, we observe that the average value of d , ranging from 3.77 Å to 4.23 Å for **B4s**, is close to the crystalline characteristic value (4.8 Å) for such a stacking involving amide-based H-Bonds[41, 42, 43]. One has to notice that the standard deviations presented in Table 2, around 0.2-0.3 Å, illustrate the fact that the dimers are not "broken" along the simulation. The same conclusion can be drawn for **B9m** with an average value of 3.82 Å. As the dimer involving **B9** monomers were not stable, it was not relevant to measure d along the simulation. One has to notice that if h_1 , h_2 , and h_3 are equivalent for **B9s** family, they are not for **B4s** dimers. Because of the asymmetry in the orientation of the amide moieties, only h_2 and h_3 are equivalent while h_1 is unique.

Table 2: Average values, in Å, for key distances of the different dimers. The mean deviations are also reported. The corresponding simulation time, in ns, during which the averages have been calculated is also provided in parentheses.

	B4	B4c	B4p	B4pI	B9	B9m
d	4.23 $\pm$ 0.30 (10)	4.12 $\pm$ 0.23 (10)	4.11 $\pm$ 0.30 (10)	3.77 $\pm$ 0.27 (10)	- -	3.82 $\pm$ 0.23 (10)
h ₁	2.05 $\pm$ 0.23 ^a (10)	2.05 $\pm$ 0.34 (10)	1.99 $\pm$ 0.24 (10)	2.01 $\pm$ 0.20 (10)	- -	2.03 $\pm$ 0.22 (10)
h ₂	2.49 $\pm$ 0.66 ^a (8.4)	2.81 $\pm$ 0.61 (7.2)	2.49 $\pm$ 0.58 (8.9)	2.59 $\pm$ 0.71 (3.0)	- -	2.01 $\pm$ 0.18 (10)
h ₃	2.54 $\pm$ 0.64 ^a (3.1)	2.08 $\pm$ 0.35 (1.1)	2.62 $\pm$ 0.63 (3.2)	2.64 $\pm$ 0.67 (6.4)	- -	2.02 $\pm$ 0.21 (10)
h'	2.44 $\pm$ 0.60 ^a (1.2)	3.17 $\pm$ 0.66 (0.5)	2.24 $\pm$ 0.45 (9.3)	2.26 $\pm$ 0.49 (6.3)	- -	- -
h''	2.39 $\pm$ 0.53 ^a (7.7)	2.20 $\pm$ 0.50 (8.8)	- -	2.36 $\pm$ 0.61 (2.3)	- -	- -

^a For the calculation of the average value and the associated standard deviation, only the areas highlighted on Figure 6 are considered. The same procedure is used for the other molecules, averages are made when the bonds are effective during the corresponding time.

Looking at those different values gives insights into the interactions involved in the stability of the dimers. We have represented on Figure 6 the evolution of h_i distances for **B4**.

Figure 6: Evolution for **B4** dimer of the distances (a) h₁, (b) h₂, (c) h₃, (d) h', (e) h''. On (f), h₂, h₃, h', h'' distances have been gathered. All the distances are in Å.

It is clear from Figure 6(a) that the hydrogen bond h_1 is always effective during the 10 ns simulation. Moreover, with an average value of 1.99 ± 0.24 Å this hydrogen bond is within the range (1.5-3.5 Å) of a hydrogen bond with a medium strength (4 to 15 kcal.mol⁻¹). The same conclusion can be drawn for all the dimers of the **B4s** family. The analysis is quite trickier for h_2 and h_3 . As represented on Figure 6b and c, those H-Bonds are not always effective. The area that are colored represent the moment where h_2 and h_3 distances are within the 1.5-3.5 Å range. The time corresponding to the colored area are given in Table 2. If h_2 is effective during almost the 10 ns simulation (8.4 ns), it is not the case for h_3 , that is only effective transiently for 3.1 ns. Nevertheless, the dimer of **B4** is stable and the stacking is maintained during all the simulation (d distance gives this information). By looking closer to the structure along the trajectory it has been possible to highlight a particular structure (Figure 5(c)). During the simulation the two monomers do not stay aligned. There is a tilt of one monomer with respect to the other one, inducing an hybrid hydrogen bonding. The keto part of one amide points between two amino moieties of two other amides of the other monomer. One expected H-bond is thus presents (h_2 or h_3), even if it is longer than a perfectly aligned H-bond, while the other one is new. We have thus introduced two new H-bonds, namely h' and h'' (Figure 5(c)). Their evolutions are represented on Figure 6d and e. One can observe that either h' or h'' is effective during the simulation. Their average values along with their effective time are gathered in Table 2. It is possible to say that the H-Bonds complement each other. When h_2 is not effective, one can observe that h' is. When h_3 is not effective, h'' is taking over. We decided to combine those four H-Bonds on the same graphics (Figure 6(e)). It appears that there is always at least one but most of the time two hydrogen bonds (h_2 , h_3 , h' , or h'') that is present within the dimer, ensuring its stability along with h_1 . The Boltzmann population ratio for the normal (with h_1 , h_2 and h_3) and the hybrid (with h_1 and h_2/h'' or h_3/h') dimer (see Figure S4 in ESI†) is always around 40/60 for all the dimers of the **B4s** family. Indicating that the hybrid dimer is more favorable than the normal one. For the other dimers of the **B4s** family, the same behavior is observed with a compensation of h_2 and h_3 by h' and h'' to ensure the global stability of the dimers. For **B9m** dimer, h_1 , h_2 , and h_3 are effective during all the trajectory but no hybrid H-Bond can be observed as there is no sufficient flexibility for the amide moieties.

To summarize the findings about dimers, we have been able to highlight (i) the (non-)stability of **B4s** family and **B9m** (**B9**), (ii) the effectiveness of the hydrogen bond network to build supramolecular assemblies and (iii) an hybrid H-bond pattern allowed by the flexibility and the possibility for **B4s** dimers to orient their amide moieties.

3.2.2 Quantitative approach: Umbrella Sampling

To go further in assessing the stability of the different dimers, we have undertaken US simulations. It allows us to retrieve the binding free energy, ΔG , along a reaction coordinate, x , that represents the preferential direction for the stacking pattern. Using around 25 sampling windows along this axis, one can construct a one-dimensional potential of mean force (PMF) profile for each system under study, leading to a binding energy, E_{bind} . US simulations for larger assemblies, namely a trimer and a pentamer, were also performed. For each PMF profile, the minimum energy is associated to a particular distance, d_{com} , that can be roughly compared to the d distance discussed in the previous section as it is the distance between the center of masses of the different assemblies. All those values, E_{bind} and d_{com} , for each system and for each molecule, are gathered in Table 3. For the following discussion, the comparison and evolution of E_{bind} will be made on the absolute value.

Table 3: Computed binding free energy, E_{bind} , in kcal.mol⁻¹ for different assemblies and the corresponding optimal interaction distance, d_{com} , in Å.

	Dimer		Trimer		Pentamer	
	E_{bind}	d_{com}	E_{bind}	d_{com}	E_{bind}	d_{com}
B4	17	4.2	21	4.3	15	4.3
B4c	24	4.2	17	4.0	12	4.1
B4p	12	4.2	12	3.9	8	4.1
B4pI	21	3.6	23	3.9	22	3.8
B9	7	3.8	9	3.8	7	4.2
B9m	12	4.2	6	4.0	4	4.1

The binding energy that are obtained for the **B4s** dimers are globally higher than the one for the **B9s**. They are equal to 17, 24, 12, and 21 kJ.mol⁻¹ for **B4**, **B4c**, **B4p**, and **B4pI** respectively while they are equal to 7 and 12 kJ.mol⁻¹ for **B9** and **B9m**. It indicates it is more stabilizing for **B4s** to form dimers than for **B9s**. One can notice that the US d_{com} values are consistent with the MD average values, with a distance around 4.0 Å between the two monomers, corresponding to a classic stacking distance for this kind of molecules.

The question that motivated the new simulations involving trimers and pentamers is the following: How is the binding energy evolving when the supramolecular assembly is getting larger? More specifically, is it getting harder to add a monomer to a dimer, a tetramer? The answer to this question will help us to understand the self-supramolecular assembling behavior of **B4s** and **B9s** into larger assemblies. The same umbrella sampling approach presented before was used to study first the interaction within a trimer. Two subsystems were considered, a dimer and a monomer and we were looking for the binding energy of the monomer with the dimer. For the pentamer, the binding energy is computed for the interaction of a tetramer and a monomer. The PMF profile for trimers and pentamers, for each molecule, is provided on Figure 7. For **B4s** family, the

Figure 7: Potential of mean force (PMF) curves for each of the molecules in different configurations: dimer (bold line), trimer (dotted line) and pentamer (dashed line).

three possible different behavior are observed, a slight increase of E_{bind} for **B4** (+4) and **B4pl** (+2), a decrease for **B4c** (-7) and no evolution for **B4**. One can nevertheless notice that the formation of trimers still remain favorable in all the cases. For **B9** and **B9m**, E_{bind} is slightly increasing (+2) and decreasing (-6) respectively. In conclusion, adding a monomer to an already formed dimer is more favorable for **B4s** than for **B9s**. Trimers of **B4s** appear even more stable than dimers of **B9s**. Going further and considering pentamers for both families leads to a unique conclusion. The binding energy is always decreasing when compared to the energies obtained for dimers and trimers. Nevertheless, the values obtained for **B4s** (15, 12, 8, and 22 kcal.mol⁻¹ for **B4**, **B4c**, **B4p**, and **B4pl** respectively) are still higher than the ones obtained for **B9s** (7 and 4 kcal.mol⁻¹ for **B9** and **B9m** respectively), indicating that adding a monomer to a small oligomer of **B9s** family will be less favorable than for the **B4s** family.

3.3 Pentamers

Classical molecular dynamics simulations involving pentamers have been performed for each of the molecules. Two behaviors were observed for the two families. For **B9s**, almost no H-bond interactions were maintained throughout the trajectory leading to a non-stability for both of the assemblies. Though, dimers were observed ponctually (see Figure S8 and S9 in ESI†). The conclusions that were drawn in the previous sections are thus confirmed with (1) a low but still possible stability for dimers and (2) an unfavorable binding energy for systems involving a large number of monomers.

Figure 8: (a) Representation of the pentamer structure of **B4p**. h_1 bonds are also highlighted by the blue frames to illustrate the tilt of the amide moieties. Schematic representation of the H-Bond framework between the monomers with (b) the expected framework with perfectly stacked monomers and (c) the framework that is observed during the simulations with the formation and alternation of hybrid bonds.

For **B4s** family the conclusions are different with respect to **B9s**. Indeed, for the entire **B4s** family, the pentamers appeared as stable along the trajectory (Figure 8 for **B4p** and Figure S5, S6, and S7 in ESI† for **B4**, **B4c**, **B4pI** respectively). The H-Bond network is at the origin of this stability. As for the dimers, h_1 is always effective and can thus be considered as the backbone of the entire supramolecular assembly. Within the pentamer, amide moieties are no longer perpendicular to the plane of the molecules, leading on average to a tilt of 15° (8(a)). The fact that the amide moieties are quite flexible also allows the formation of the previously mentioned hybrid bonds. During the simulation, there is an alternation of h_2/h' and h_3/h'' bonds (Figure 8(c)) which implies that the monomers are stacked in staggered rows. The interactions between each pair of monomers and the stability of this interaction is provided by the formation of one expected H-Bond (h_1) and an hybrid scheme composed of two H-Bonds (h_2/h' or h_3/h''). This finding confirms the importance

of the slight flexibility of the amide moieties for the global stability of SMA. It also highlights the fact that the stability of the SMA is dynamic as there is a constant compensation of the H-bonds.

3.4 Aggregation mode

Once the strength of the different supramolecular assemblies (dimers to pentamers) have been considered from both a qualitative and quantitative point of view, we decided to have a look at the formation of those assemblies. In the previous sections, we reported data about already formed supramolecular assemblies, in this section, we will study the aggregation and association process, aiming at answering the question: How do we go from monomers to larger assemblies? Different simulations with similar starting configurations were set up as follows to address different problems:

- Non-interacting monomers (no H-Bond between them) placed in the center of the simulation box for a long (100 ns) simulation time.
- Non-interacting monomers (no H-Bond between them) placed in the center of the simulation box for 100 short (1 ns) simulation times.

The first type of simulation will allow us to know if SMA can self-assemble spontaneously and if this SMA will be stable along a long simulation time. The second type of simulation will provide information on the frequency of formation of such SMA and more precisely the frequency of formation of H-Bonds. For simplicity, results and detailed analysis on **B4p** are presented here and in Supporting Information for the others.

3.4.1 One long simulation

We used PACKMOL[44] to generate a starting configuration encompassing 10 monomers that are loosely compacted, meaning they are relatively close to each other but with no hydrogen bonds or particular interaction between them. They were then placed in the center of a simulation box for a 100 ns long simulation time. To ensure that the monomers are not interacting at the beginning of the simulation and more particularly that no hydrogen bond is effective for the starting configuration, we represented the radial distribution function (RDF) for the oxygen and hydrogen atoms involved in those interactions (see Figure 9(a)). The atoms O₁, O₂ and O₃ and H₁, H₂ and H₃ are the ones associated to h₁, h₂ and h₃ hydrogen bond identified on Figure 5. One can observe on Figure 9(a) that for each oxygen atom (O₁, O₂ and O₃) there is no peak indicating a possible hydrogen bond with either H₁, H₂ or H₃ for the starting configuration. The first and most intense peak corresponds to an intramolecular peak at around 3.0 nm corresponding to oxygen and hydrogen atoms of the same amide moiety. The following peaks, appearing at distances larger than 3.0 nm, are indicating the relative spacing of the different O and H atoms and thus of the different monomers. We have then represented the same

Figure 9: Radial distribution function for a **B4p** aggregate containing 10 monomers. (a) For the starting configuration ($t=0s$) and (b) for a 100 ns long simulation. The attention has been focused on the oxygen and hydrogen atoms involved in possible H-Bond (see Figure 5).

RDF but for a 100 ns long simulation, differentiating the three possible H-Bond that can be formed between two monomers (Figure 9(b)). One can observe that the O_1-H_1 is predominantly formed when compared to possible O_1-H_2 and O_1-H_3 H-Bonds. Looking at the two other graphics, one can say that O_2 is almost not implied in H-Bond while some O_3-H_2 and O_3-H_3 are effective. If O_3-H_3 is an expected H-Bond in the case of a perfectly aligned dimer, O_3-H_2 H-Bond corresponds to the hybrid H-Bond already mentioned in the previous section, proving once again the importance of such interaction.

On Figure 10 we have provided a representative structure of a SMA of **B4p**, involving 8 monomers, that has been formed during the simulation. One has to notice that the two remaining monomers are involved in a dimer that is not interacting with the octamer. To illustrate the interactions between the different monomers we also provided interaction surfaces extracted from the NCI analysis for each pair of monomers. Various

Figure 10: Representative structure of **B4p** aggregate extracted from the 100 ns long simulation along with the NCI analysis. Isosurfaces ($s=0.5$ a.u) in dark green represent strong and attractive interactions (e.g H-Bonds), whereas weak interactions such as van der Waals interactions are depicted in light green. For the sake of clarity for the structure on the left, hydrogen atoms have not been represented, except those of the amide moieties.

interactions have been highlighted among which the predominant ones are:

- h_1 H-Bond (Mono2-Mono3, Mono6-Mono7)
- h_2 (h_3) associated with h' (h'') hybrid H-Bond (Mono3-4/5/6)
- N-H $\cdots \pi$ bond (Mono3-Mono4/5/6, Mono7-Mono8)
- H-Bond involving O_1 atoms and H_2/H_3 atoms (Mono1-Mono2, Mono7-Mono8)

One has to notice that other interactions can be found within the SMA, for example π - π interactions between the phenyls of different monomers and C-H $\cdots \pi$ interactions. Those interactions are nevertheless more labile. If we go deeper in the analysis of the different interactions, it is possible to observe that all the interactions are not formed simultaneously but sequentially. Indeed the first interaction that is effective within the SMA is the h_1 bond, with a formation at the nanosecond timescale (see Figure 11). We then have the formation of the previously mentioned interactions (h_2 and h') at a slightly higher timescale (few nanoseconds), followed by N-H $\cdots \pi$ interactions and O_1 - H_2/H_3 after tens of nanosecond. If h_1 is the first interaction being observed, it is also the most stable one as it is effective during almost the entire trajectory (Figure 11(b)). For other molecules of the **B4s** family, there is always the formation of a SMA involving at least five monomers (**B4** and **B4pI**). **B4p**, with 8 monomers involved is the most efficient one while **B4c** forms a seven-members SMA (see Figure S10, S11, and S12 in ESI[†]). The same kind of interactions are present within all the SMA with a predominance for the h_1 bond, completed with the previously mentioned interactions.

On the opposite, when considering **B9** and **B9m** molecules, no large SMA were detected and only poorly stable dimers were observed. As a conclusion one can say that the self-assembling process is (1) efficient for **B4s** family but not for the **B9s** one, (2) quite fast, of the order of the nanosecond, (3) can be a long process as the interactions are added sequentially, and (4) dynamic in the sense that some interactions (N-H $\cdots \pi$, O_1 - H_2 ,

Figure 11: (a) Evolution for **B4p** octamer of the distances h_1 , h_2 , h' , $N-H \cdots \pi$, O_1-H_2 , O_1-H_3 during a 100 ns long simulation. All the distances are in Å. (b) Schematic representation of the formation time and effectiveness of the previously mentioned interactions.

O_1-H_3) are labile but also compensated by other interactions ($C-H \cdots \pi$, $\pi-\pi$), ensuring the global stability of the SMA.

3.4.2 Many short simulations

We observed in the previous section that, when a H-Bond is formed, it is then quite stable along the trajectory. The analysis we propose to perform in this section aims at retrieving the frequency of formation of the H-Bond. To do so, we generated 100 different starting configurations encompassing 10 monomers close enough to each other but with no H-Bond between them and performed a 1 ns simulation. We then extracted the final structure of each simulation and counted the H-Bonds between all the monomers. One has to notice that no distinction was made between all the possible H-Bonds (9 in total). On Figure 12 we have represented, for the 100 simulations and for **B4p** and **B9m** only, the total number of H-Bonds that have been observed between each pair of dimers. The results for **B4**, **B4c**, **B4pI** and **B9** are also provided in ESI† (Figure S13 and S14).

Figure 12: Schematic representation of the occurrence of the formation of H-Bonds between all the monomers (M_i with $i=1,\dots,10$) for **B4p** (bottom left, green) and **B9m** (up right, gray) for a total of 100 simulations. The structures that are considered for the count are the final ones obtained at the end of the 1 ns simulation. The size of the dots is proportionnal to the number of H-Bonds that have been detected. For the largest dots, we provided the corresponding exact number of interactions.

One can observe immediately on Figure 12 that the number of H-Bonds present within **B4p** aggregate is larger than the ones of **B9m**. Due to the particular arrangement of the molecules, some preferential interactions are observed. For example, for **B4p**, the formation of H-Bond between monomer 1 and monomer 2 is almost systematic. Indeed, the count reveals 143 H-bonds between those 2 monomers for a maximum of 300. Nevertheless, one can also observe that monomer 1 is also involved in interactions with monomer 5 (52) and in a lesser extent with monomer 6 (31) and 7 (18). So does monomer 2 with monomer 7 (17), 8 (43) and 10 (29). For **B4p**, there are six main interactions involving M1-M2, M3-M4, M4-M10, M10-M7, M5-M8, and M6-M9. For information, all the monomers are staying close to each other, as a loose aggregate, during all the simulations. This can be explained by the fact that other interactions ($C-H \cdots \pi$ and $N-H \cdots \pi$) are formed and thus enforce the stability of the entire supramolecular assembly. For **B9m**, no systematic interaction was observed with a maximum count observed for a M6-M9 interaction. This result may also be due to the fact that **B9m** monomers are not staying "packed" during the simulation. The loose aggregate is thus not even stable for **B9m** molecules.

3.5 Conclusion

By defining a complete theoretical protocol based mainly on molecular dynamics simulations and aiming at studying the ability of organic molecules to form supramolecular assemblies and their resulting stabilities, we have been able to provide some hints for an effective self-supramolecular assembling process. A total of 6 molecules (**B4**, **B4c**, **B4p**, **B4pI**, **B9**, and **B9m**) divided into 2 families (**B4s** and **B9s**), bearing a different backbone but with three amide moieties in common have been considered. The study of the monomers allowed us to validate our molecular dynamics approach and also to understand the properties of the molecules when they are isolated. Considering the dimers has permitted to define the hydrogen bonds network. Expected hydrogen bonds (h_1 , h_2 , and h_3) were always observed for both families. Nevertheless, they were not always effective all together and an hybrid scheme (h' and h'') has been highlighted. This hybrid H-bond network was observed only for one of the two families, namely **B4s**. The calculation of the binding energies clearly showed that dimers and even trimers or pentamers of **B4s** family were more favorable than those of the **B9s**. Nevertheless, the evolution of the binding energy going from dimers to pentamers indicates that small oligomers (e.g trimers) may be more stabilized than larger SMA (e.g pentamers). Looking at the the formation of the assemblies starting from a loose aggregate allowed us to observe that not only H-bonds can ensure the stability of the aggregate but also $N-H \cdots \pi$ or $C-H \cdots \pi$ in a lesser extent. Finally, the most important point to consider for the self-assembling process is the dynamical behavior of the stability. If SMA of **B4s** family are more stable than those of **B9s** it is because they have a relative flexibility. One can also notice that the stability is not something that is frozen but that is also dynamical. We have shown within this study that considering relatively flexible molecules, instead of rigid ones, is a better strategy for the conception of supramolecular assemblies.

Supporting Information

See supporting information for: Validation of GAFF parameters; General parameters for molecular dynamics simulations; Parameters for the umbrella sampling approach; Particular structure of **B4s** and **B9s** families; Hybrid hydrogen bond network; Pentamers for **B4**, **B4c**, **B4pI**, **B9**, and **B9m**; Interaction maps for **B4**, **B4c**, **B4pI** and **B9**.

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