Interaction of disubstituted 1,2,3-triazole- and β-aminoacid-containing peptides with phospholipid bilayers

Final report

1 Short summary of the achievements

The main results achieved during the term of this project are summarized below.

1.1 Development of a Molecular Mechanics Force-Field for the Simulation of β-peptides and Mixed α/β Peptides

The first task of the project was to develop a molecular mechanics force field for the simulation of β-peptides. I chose CHARMM36m, a force-field optimized for biomolecules, with special emphasis on the folding properties of natural proteins and peptides, including the correct treatment of intrinsically disordered ones (J. Huang et al. 2016). As the most important feature of β-peptides is their readiness of folding into various secondary structures, I optimized the dihedral potential energy terms of the β-backbone. First I chose four simple β-amino acids representing each common class of substitution:

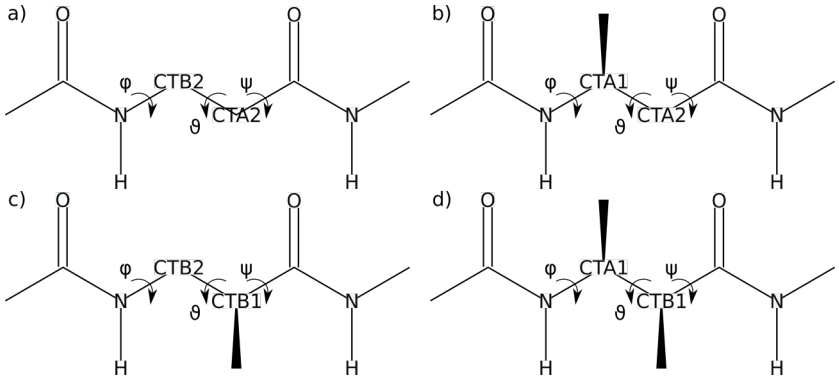

Figure 1: The four simple diamides used for determining the optimal backbone dihedral potential terms. The additional atom types CT{A,B}{1,2} assigned are also shown.

1. β-homo-glycine, or β-alanine, e.g. a simple β-backbone without any side-chain
2. β^2-homo-ananine, with a methyl group attached to the α-carbon
3. β^3-homo-alanine, with a methyl group attached to the β-carbon
4. $\beta^{2,3}$-homo-alanine, where both the α- and the β-carbon have a methyl side-chain.

All of the above four were capped with acetyl on the N-terminal and N-methylamine on the C-terminal.

Using Gaussian 09 at MP2/6-31G(d) level of theory, I have performed potential energy scans on each of the three backbone dihedral angles. These served as the reference of the optimization.

Next I selected the independent variables to be optimized. The CHARMM functional form of the torsion terms is

$$V_{torsion} = \sum_i \sum_j K_{ij}\left(1+\cos\left(n_{ij}\,\chi_i - \chi_{0,ij}\right)\right),$$

where i indexes the dihedral angles (χ_i)in the molecule. For each dihedral angle more than one set of parameters can be given (hence the j index), corresponding to a Fourier-like expansion. The parameter K_{ij} or "force constant" controls the "strength" of the potential energy term, while n_{ij}, is the multiplicity (an integer) and $\chi_{0,ij}$ is the zero phase (either 0 or π). Because of the geometry of the molecules, I allowed only n =1, 2, 3 and 6. Considerations of molecular symmetry led me to constrain some independent variables to be equal or negative of other variables, or even set them to zero.

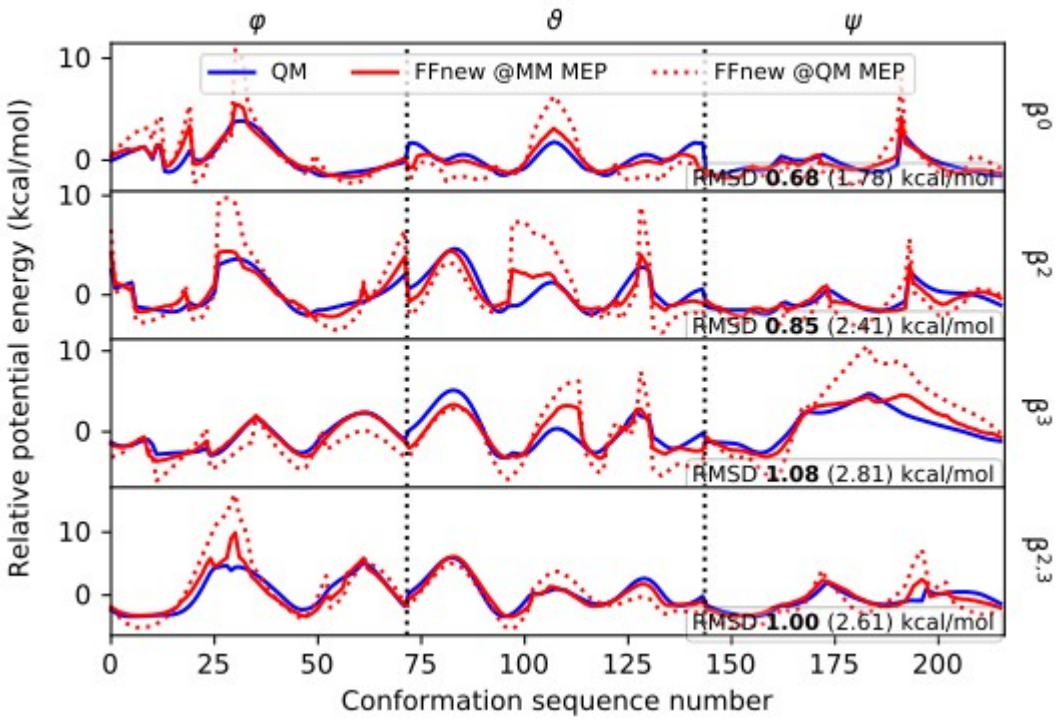

Figure 2: Matching of potential energy surfaces calculated from ab initio method (blue curve) and by using the new force field (continuous red). The dotted red curve indicates the potential energy surface without MM relaxation. The horizontal axis corresponds to the scan in the selected dihedral angle.

I determined the best-fitting values of the reduced free parameter set by calculating the potential energy according to the MM force field (other parameters than the optimizable ones were taken from the original FF according to chemical analogy). The details of the procedure are given in the published paper (Wacha, Beke-Somfai, and Nagy 2019).

The performance of the resulting extended force field was checked on three model peptides with extensive literature available on their folding properties. I have shown that the careful optimization of the backbone torsions resulted in better reconstruction of the folding dynamics of the structures, compared to the un-optimized CHARMM36m force field and to a previous attempt at the same problem by Zhu et. al. (Zhu et al. 2010). The three model peptides of choice include "VALXVAL", a β-peptide

known for folding into a 3_{14} helix in methanol, a hexapeptide with a proven "hairpin" structure in methanol and a longer, amphiphilic peptide which was designed to adopt the 3_{14} helix in water. The structure of these is shown in Figure 3.

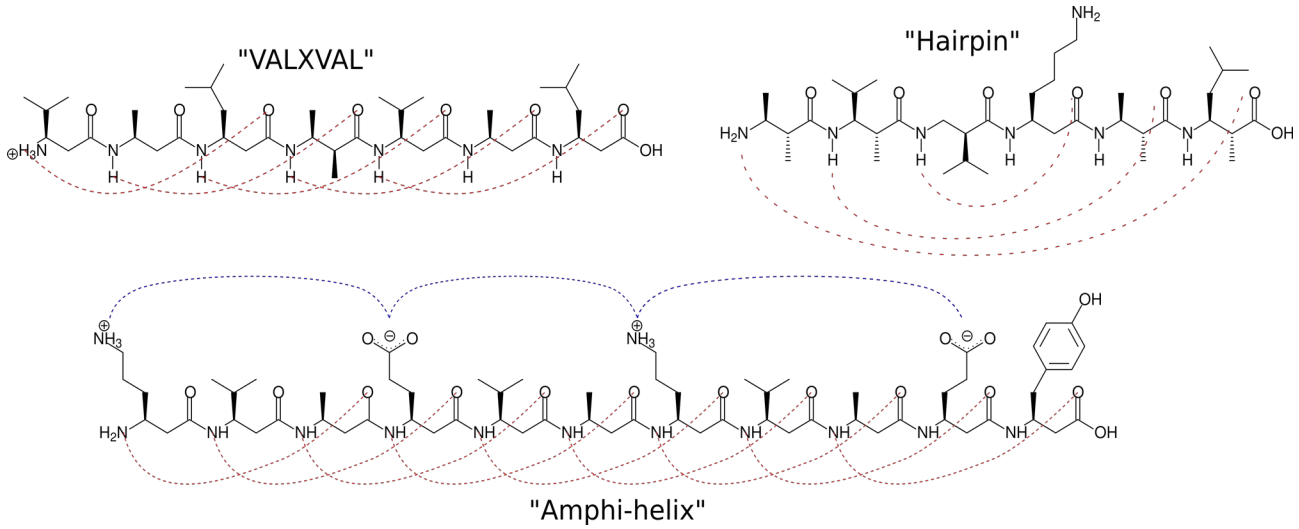

Figure 3: Test peptides for asseesing the performance of the new force field. Red dashed lines indicate hydrogen bonds corresponding to the primary fold of the peptides. Blue dashed lines indicate salt bridges

As an illustration of the structural stability of the peptides under the three different force fields, the root mean square deviation from the initial, helical structure over time is shown in the case of the "VALXVAL" peptide in Figure 4.

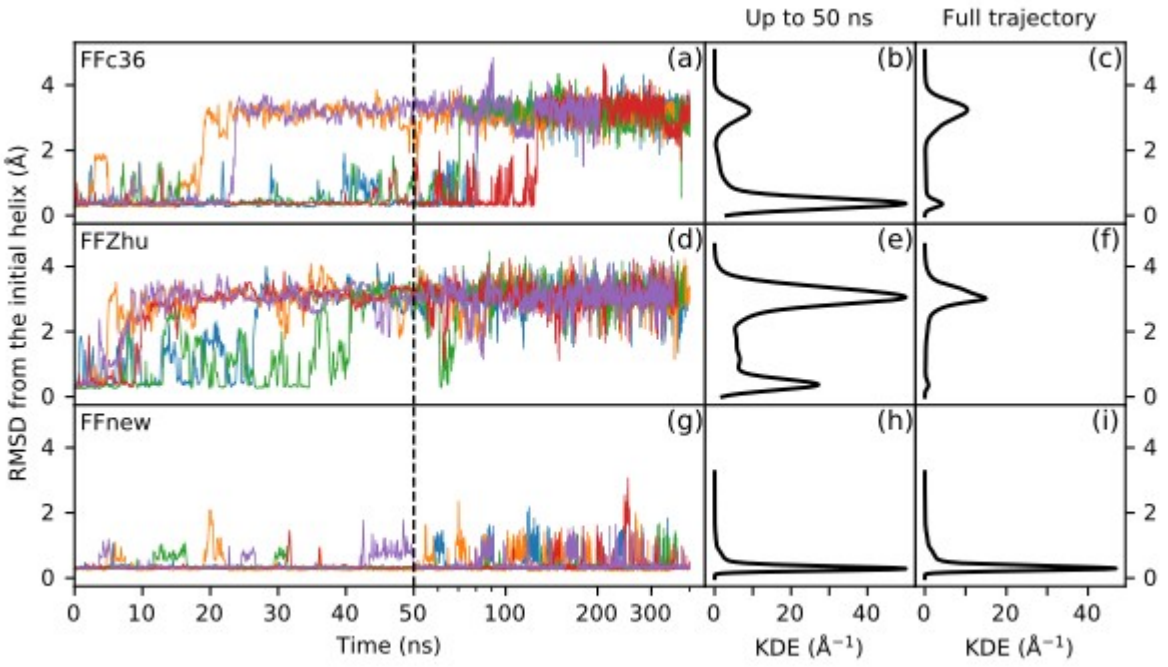

Figure 4: Time evolution of the root mean square deviation from the initial 3_{14} helical structure of the VALXVAL peptide under different parametrizations of the CHARMM36m force field: original parameter set (FFc36), previous attempt of Zhu et. al. (FFZhu) and the new parameter set (FFnew) obtained in this project

As seen from the figure, both the un-optimized CHARMM36 FF as well as the attempt of Zhu et. al. results in the unfolding of the peptide from the prepared helical structure, while the new parametrization conserves the fold, while allowing for some expectable dynamic fluctuations.

In addition to starting the simulation from a prepared helical state, I did a parallel study when the peptide was prepared in an extended, "straight" conformation. Figure 5 shows the evolution of the number of $i \rightarrow i+2$ hydrogen bonds which is characteristic to the hydrogen bonds stabilizing the 3_{14} helix.

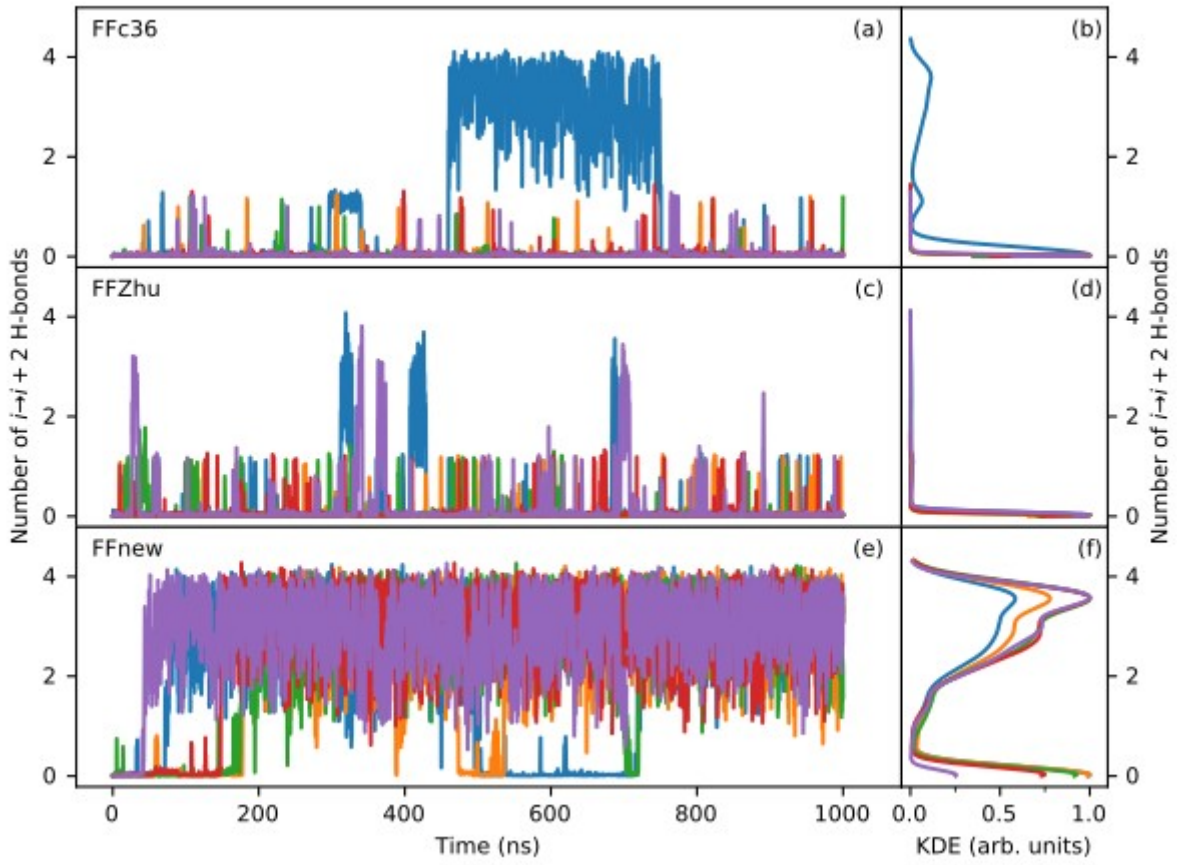

Figure 5: The evolution of the number of the hydrogen bond network corresponding to the 3_{14} helical structure ($i \rightarrow i+2$) in the case of the VALXVAL peptide started from an extended conformation under the three different force field parametrizations.

Maybe not so surprisingly, the first two force fields fail to fold the peptide. More surprising is that the new force field parametrization finds the helical structure in all five independent simulation runs at relatively short time scales.

Moreover, in contrast to previous attempts, the new parametrization can handle both singly (β^2 and β^3) and doubly ($\beta^{2,3}$) substituted β-amino acids with all possible absolute conformations of the backbone. I also generated a large library of β-amino acid topologies with various proteinogenic side-chain combinations, which can be used by other investigators to set up molecular dynamics simulations of β- and mixed α/β-peptides as well.

The force field is distributed free of charge at https://gitlab.com/awacha/charmm-beta.ff, with on-line documentation on how to use it at https://charmm-betaff.readthedocs.io/en/latest/.

1.2 Explaining the Self-assembed Structure of a Designed β-peptide

The first application of the above described force field for β-peptides was a collaboration with the Research Group of Biomolecular Self-Assembly, led by Tamás Beke-Somfai (Cs. Szigyártó et al. 2020). They have designed and synthesized several β-peptides with alternating chirality, an example of which is shown in Figure 6. They were found experimentally to self-assemble under aqueous conditions into aggregates observable with transmission electron-microscopy and polarized light spectroscopy methods (circular and linear dichroism). The amphipatic character of the monomers and their aggregates enables it to penetrate phospholipid bilayers, making it membrane-active.

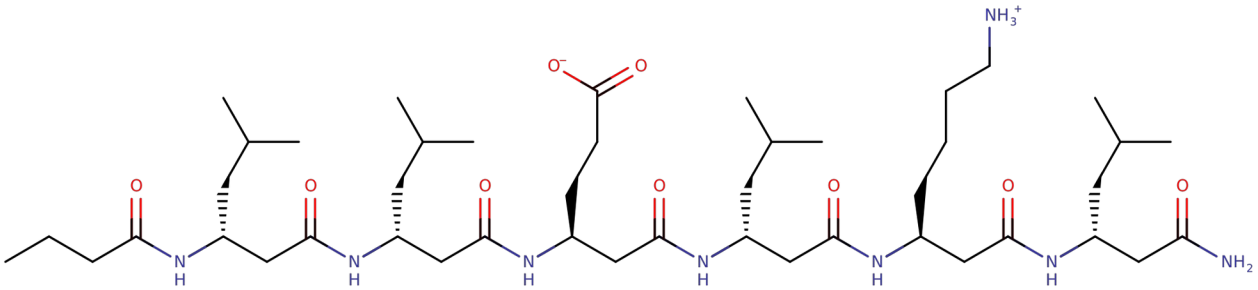

Figure 6: An example of a self-assembling β-peptide

Using the above described extension for the CHARMM force field, it was possible to explain the nature of the oligomers. It was found that even isolated monomers, while inherently flexible, prefer a relatively stable zig-zag conformation (shown in Figure 7) due to the steric effects of the side-chains, thus no hydrogen bonding is needed to stabilize this fold.

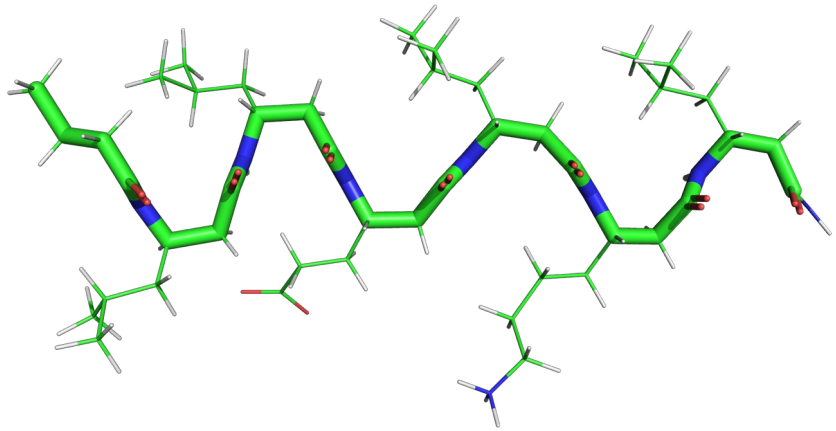

Figure 7: The zig-zag shape adopted by the designed β-peptide

The relatively flexible conformation of these chains is stabilized when they aggregate into sheets very much like the β-sheets observed in α-peptides and proteins. The sheets are held together by inter-chain hydrogen bonds lying parallel with the plane of the sheets. When these sheets are formed, all hydrophilic (lysine an glutamate) side-chains end up on the same side of the sheet while all leucines are on the other, making one side of the sheet of hydrophilic, while the other of hydrophobic character. In aqueous milieu, two sheets make a sandwich structure while hiding the leucine side-chains between them Figure 8. I have followed the process of this self-assembly using a molecular dynamics simulation with the above described extended force field. Eight chains of the peptide were placed into a cubic

box, each in a fully extended conformation, at random orientation, their centers at the eight corners of a cube. The stable octamer was formed after 200 nanoseconds (Figure 8, panel D), afterwards only small internal reorganizations were observed.

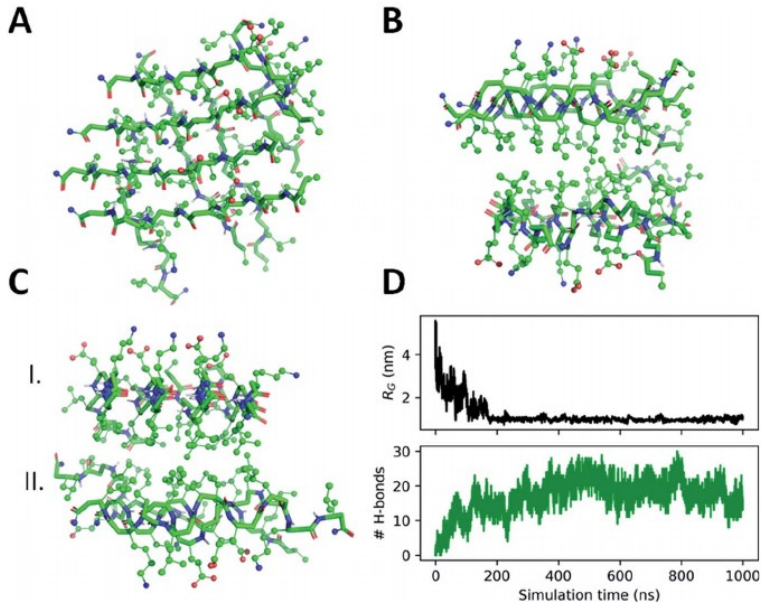

Figure 8: The self-assembled structure of the designed hexapeptide from top (A), from one side (B) and from the other (C). Panel D shows the time-evolution of the radius of gyration of the system and the number of inter-chain hydrogen bonds when 8 identical chains were started from extended conformation, placed well apart from each other.

The hydrophobic compartment between the layers can be used to host small guest molecules. We have tried several molecules experimentally, including Thioflavin-T (ThT) and 8-anilinonaphtalene-1-sulfonic acid (ANS), two common fluorescence probes, and pyrene, a small, planar molecule commonly used as a marker molecule for linear dichroism. The incorporation of the latter into the above described aggregate has been also studied with molecular dynamics. An example of the β-peptide octamer and the incorporated pyrene is shown in Figure 9.

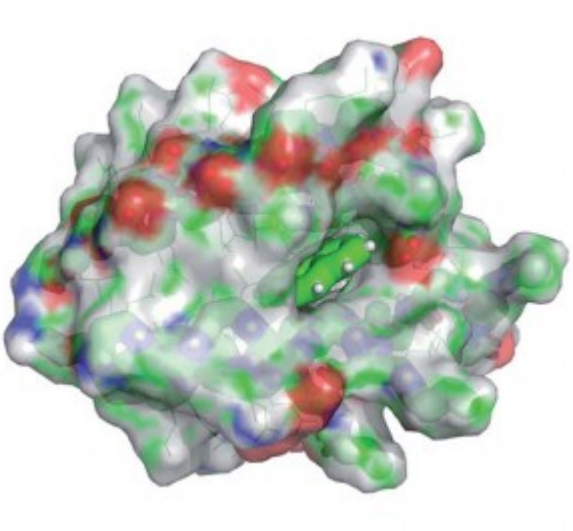

Figure 9: A single pyrene molecule incorporated into an octamer of a designed β-hexapeptide

The amphiphilic character of the aggregate led us to surmise that if turned inside out, it can also be stable in hydrophobic milieu, e.g. in the carbon chain region of a lipid bilayer. In the published work, Gergely Kohut did simulations on an inverse sandwich, i.e. where the leucine side-chains pointed outwards and the hydrophilic ones inwards. He also tried various the monomeric and tetrameric form. The depth distribution of the aggregates inside the bilayer over time and a snapshot of the octamer inside the bilayer are shown in Figure 10.

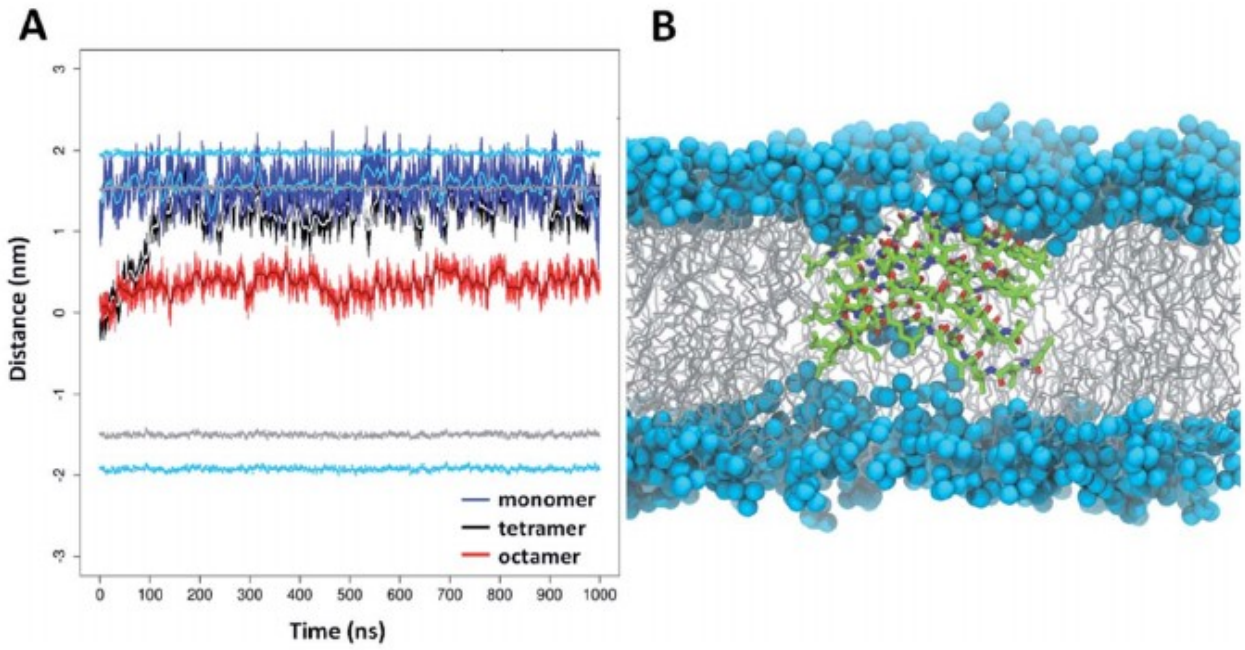

Figure 10: Depth distribution of oligomers in DOPC lipid bilayer based on MD simulations. (A) Distances of the monomeric (blue line), tetrameric (black line) and octameric (red line) forms of the peptide compared to the center of mass (COM) of the lipid bilayer (moving averages are displayed by cyan, grey and brown lines, respectively). The positions of the COM of phosphorus atoms (light blue) and carbonyl groups of the acyl chains(grey) are illustrated relative to the COM of the bilayer. (B) MD snapshot of the lipid bilayer containing the octameric form of 5 (color code: carbon: green; nitrogen: blue; oxygen: red). The DOPC phosphorus atoms are shown as light blue balls and the acyl chains in grey.

1.3 Development of a Graphical Interface for the Design and Analysis of α/β-peptides

For natural α-peptides and proteins well-developed and powerful tooling exists for constructing, handling and visualizing molecular models. Probably the most frustrating problem in the study of β-peptides is that the available procedures and methods are rendered unusable by the additional methylene group in the backbone, which hampers even the first step of the computational study of these molecules, i.e. building *in silico* models of these molecules. Based on the topology library I developed for β-amino acids with various proteinogenic side-chains, I created a plug-in for the PyMOL molecular graphics system. I chose this platform because it is one of the *de facto* standard utilities for drawing publication-quality graphs of molecular models and also because of its extendability using the Python programming language.

The plug-in is focused on a single PyMOL command, "betafab2", which can build various peptides from α- and β-amino acids, including cyclic ones such as aminocyclopentanecarboxylic acid (ACPC) and aminocyclohexanecarboxylic acid (ACHC) in user-defined conformation. The user can input the desired sequence using a simple syntax, which allows the declaration of Cα and Cβ absolute conformations (in the R/S notation) and even backbone dihedral angles. This can also be thought of as a generalization of the "fab" command available out-of-the-box in PyMOL.

I also created a graphical user interface to the above command for editing the sequence and building the folded peptide structure. The layout of the window as well as an example structure built is shown in Figure 11.

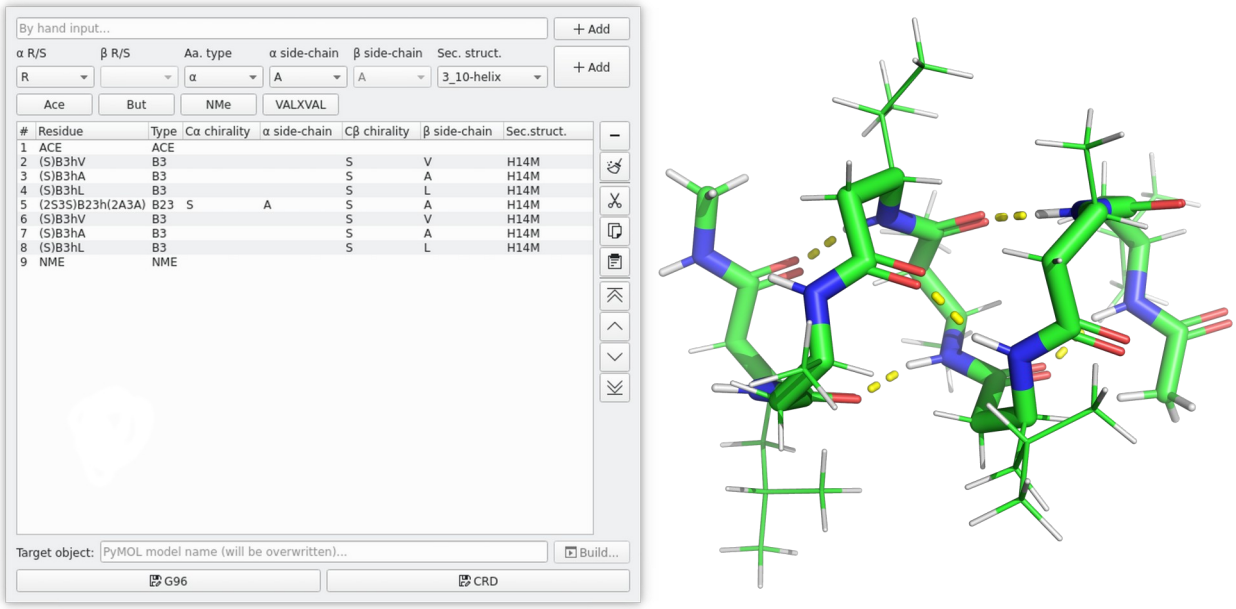

Figure 11: The graphical sequence editing window for the "betafab2" program (left) and the structure built using the parameters given (right).

In addition to the main functionality of building molecular models of peptides, the package contains other useful commands and facilities for handling models of artificial peptides. The complete list of commands and the user manual is found at https://pmlbeta.readthedocs.io/en/latest/, from where the plugin can also be downloaded and installed free of charge. The source code is available at https://gitlab.com/awacha/pmlbeta.

A journal article detailing the above described PyMOL extension has been accepted for publication by the journal SoftwareX recently (Wacha and Beke-Somfai 2021).

1.4 Comparison with Other Force Fields (ongoing)

When the β-peptide extension to the CHARMM36 force field was developed, its performance was compared to two other variants of the CHARMM force field: the unmodified CHARMM36m parameter set (J. Huang et al. 2016) where atom types and interactions were assigned on chemical analogy and the previous, similar attempt to parametrize the CHARMM22 force field for β^3-peptides (Zhu et al. 2010). The obvious next step is to compare results with other force fields where β-peptide studies were already performed: the GROMOS (W. Huang, Lin, and van Gunsteren 2011; Lin and van Gunsteren 2013; D. Wang et al. 2012) and the AMBER force fields (Németh, Hegedüs, and Martinek 2014). In an ongoing study, I have chosen seven different peptides from the available literature (see Figure 12) and performed molecular dynamics simulations with four force fields: the above described CHARMM36m extension, GROMOS 54A7 and 54A8 and the Amber force field using the parametrization given by Németh et. al. (Németh, Hegedüs, and Martinek 2014).

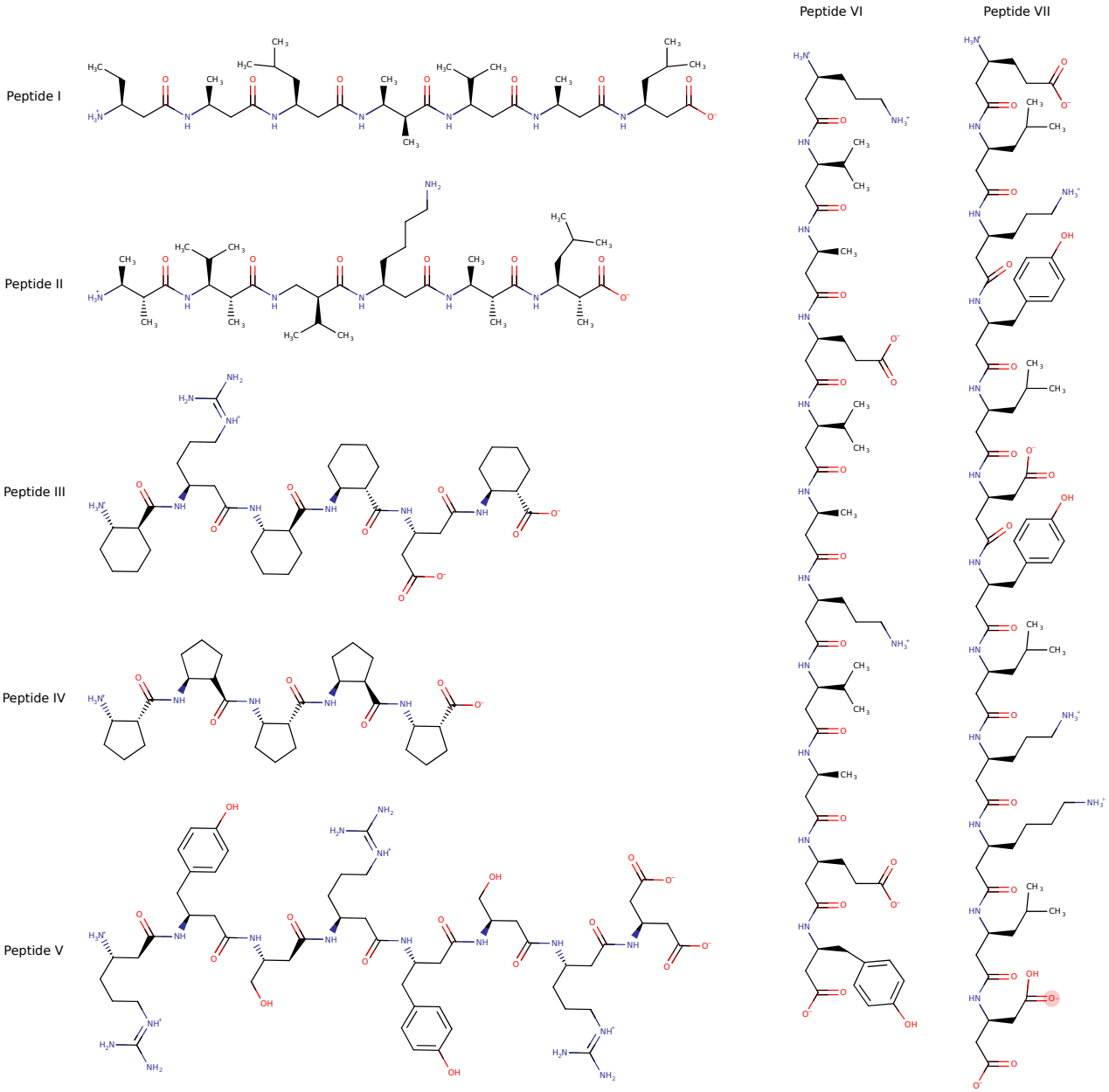

Figure 12: Seven peptides for comparing the performance of the CHARMM36m force field extension, GROMOS 54A7 and 54A8 and the Amber force field.

Peptide I is a common benchmark for force fields. It has been reported to fold into a 314 helical structure in methanol, and has been extensively studied by both experimental methods (NMR) and molecular dynamics (MD) (Seebach et al. 1996; Daura et al. 1997; 1998; Daura, van Gunsteren, and Mark 1999; Zhu et al. 2010; Wacha, Beke-Somfai, and Nagy 2019). Peptide II has been designed to adopt a hairpin-like conformation in aqueous solution (Seebach et al. 1999; Daura et al. 2001; Zagrovic et al. 2008; W. Huang, Lin, and van Gunsteren 2011; Wacha, Beke-Somfai, and Nagy 2019). Peptides III-V were used as test cases by Németh et.al. for Amber-compatible MM parameter derivation of β-amino acids (Németh, Hegedüs, and Martinek 2014). Peptide III prefers a 3_{14} helical conformation in aqueous media, and was found to bind to synaptotoxic amyloid-β oligomers. Peptide IV was found to form isolated, elongated strands in DMSO (Martinek et al. 2006) and assemble into nanostructured sheet-minicking fibres in methanol and water (Martinek et al. 2002). Peptide V is disordered in water,

without any long-range contact between residues (Németh, Hegedüs, and Martinek 2014). Peptide VI belongs to the first β-peptides which adopt stable 14-helical conformation in water, intended to act as inhibitors of protein-protein interactions (Kritzer et al. 2005). Finally, peptide VII, while also forming 14-helices in water, is reported to form stable octamers in water in the shape of two cupped hands (Craig, Goodman, and Schepartz 2011; P. S. P. Wang and Schepartz 2016) .

As preliminary results, I show the evolution of the hairpin structure of Peptide II. The "hairpinity" value is the number of existing hydrogen bonds characteristic to the hairpin structure reported in the literature (joining residues 1-6, 2-5 and 3-4) in Figure 13.

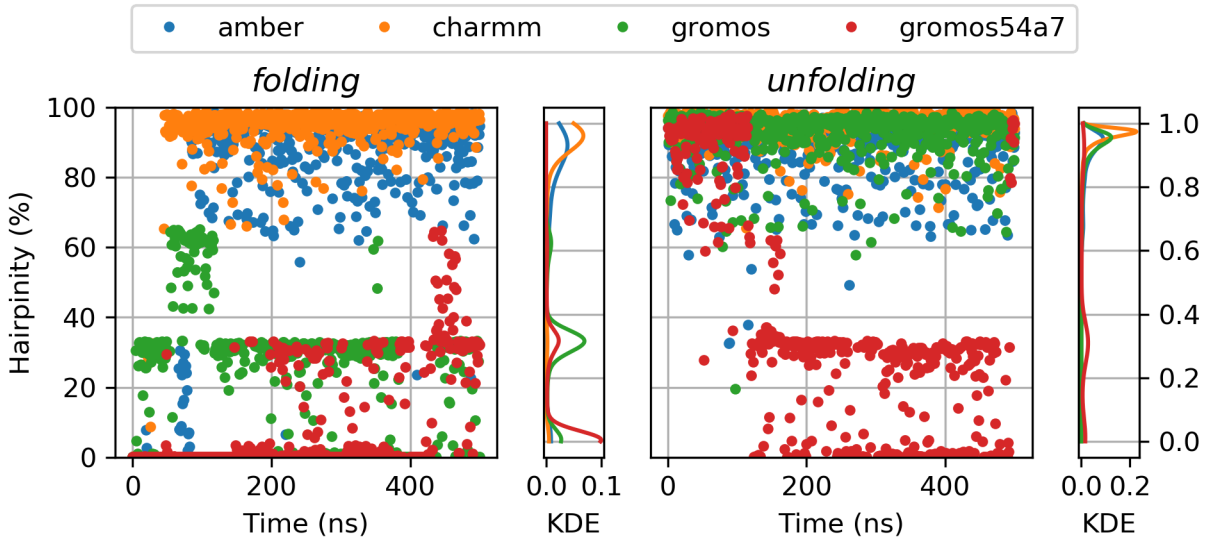

Figure 13: The evolution of the hairpin conformation in peptide II, started from extended (left) and folded (right) conformation. For the sake of visibility, the maximum values of the Gaussian kernel density estimates are normalized to 1. The bandwidth was chosen as 0.182% using Scott's rule of thumb (Scott 2014).

From the figure it is clear that Amber and CHARMM fold the peptide in its desired configuration while the two GROMOS versions do not. When prepared in the folded state, CHARMM, Amber and GROMOS 54A8 do not unfold the peptide while GROMOS 54A7 does it.

Figure 14 shows the evolution of Peptide VII, prepared in the octameric state reported by Craig et. al. (Cambridge Crystallographic Database, deposition number 804687) (Craig, Goodman, and Schepartz 2011). It is seen that Amber and CHARMM preserves the structure while both GROMOS versions unfold it. Our CHARMM force field extension has a larger number of interchain H-bonds than Amber. It must be noted that all simulations were carried out using GROMACS version 2019.5 (Lindahl et al. 2019), and recent studies reported incompatibilities between the non-physical twin-range approach used by the GROMOS program, and thus inherently in the parametrization of the GROMOS force fields (Silva et al. 2018; Gonçalves et al. 2019; Hess et al. 2019), which might impact the performance of the GROMOS force fields here.

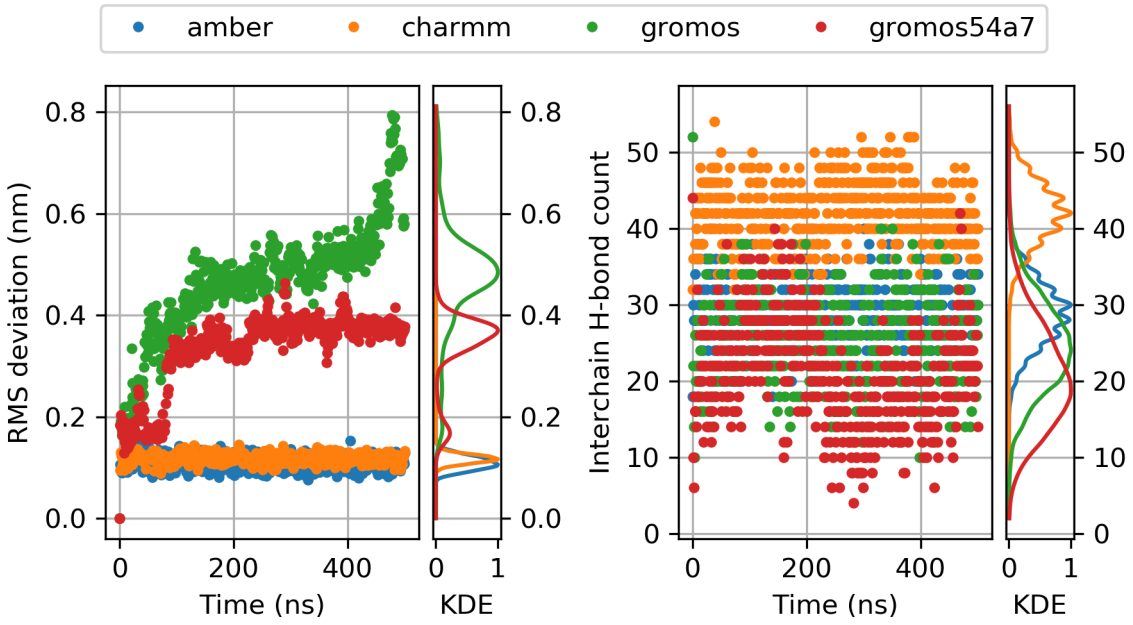

Figure 14: Root mean square deviation of the reference structure over time (left) and the number of inter-chain hydrogen bonds (right) in the octamer of Zwit-EYYK. The system was prepared in the state reported by Craig et. al, deposited in the Cambridge Crystallographic Database (deposition number 804687)

These results need more repetitions and verifications before publishing, though.

1.5 References

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2 Exploitability of the results

In the frame of this project I have created two tools which are readily usable by other researchers in the study of peptides containing (not exclusively) β-amino acids:

1. An extension to the CHARMM force field which makes it capable of correctly reproducing the folding and self-association dynamics of β-peptides. This has already been successfully employed in a collaboration with another research group for explaining the self-association behavior of an amphiphilic β-peptide with alternating chirality.

2. A plug-in for the PyMOL molecular graphics engine for user-friendly construction of β-amino acids. As model construction is the first step of *in silico* studies, it is expected to aid theoretical researchers in β-peptides by making possible the construction of computational models of not just pure β-peptides in any given folded/unfolded state but α-peptides / proteins and mixed α/β-peptides as well. Model building / analysis is also an important tool for more experimentally oriented researchers, as a visual, three-dimensional model can be an indispensable help for molecule design.

3 Differences from the original research plan and their justification

Due to circumstances unforeseen when writing the proposal, I had to divert from the original research plan. The three main reasons to do this were:

1. The second year of the proposal would have been dedicated to simultaneous molecular dynamic (MD) and small-angle X-ray scattering (SAXS) studies of β-peptides in phospholipid membranes. Due to problems with the synthesis in the Research Group of Molecular Self-Assembly, there was not enough β-peptide sample of controllable purity for SAXS experiments, making the planned parallel study impossible.

2. The third year of the proposal would have covered the parametrization of the CHARMM36m force field for disubstituted 1,2,3-triazoles as amino acid-like building blocks in a similar fashion as with β-peptides, and a similar joint MD-SAXS study in lipid membranes. However, just after the start of the project, Marion et. al. (2018) did a successful force field parametrization of the same molecule class in Amber. Additionally, as the Research Group of Molecular Self-Assembly, from which I expected to get samples for SAXS experiments, continued to work on β-pep-

tides instead of turning to triazoles, I decided that extending my work on β-peptides would come with more benefits for both the Research Group and me.

Instead of the originally planned work, I did the following additional tasks:

1. Contributed to the work of the Research Group by explaining the self-assembly of their designed β-hexapeptides with MD simulations
2. Created an extension for the PyMOL molecular graphics system for building and handling molecular models of β-peptides, which proved to be an indispensable tool
3. Started a comparative study of three different β-peptidic MD force fields: CHARMM, GROMOS and Amber. The study is still ongoing
4. There are two more projects running jointly with the Research Group involving β-peptide design, where I contribute with molecular dynamics simulations

4 Publications Pertaining to this Project

Al-Khafaji, Mohammed A., Anikó Gaál, András Wacha, Attila Bóta, and Zoltán Varga. 2020. "Particle Size Distribution of Bimodal Silica Nanoparticles: A Comparison of Different Measurement Techniques." *Materials* 13 (14): 3101. https://doi.org/10.3390/ma13143101.

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Szentirmai, Veronika, András Wacha, Csaba Németh, Diána Kitka, Anita Rácz, Károly Héberger, Judith Mihály, and Zoltán Varga. 2020. "Reagent-Free Total Protein Quantification of Intact Extracellular Vesicles by Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) Spectroscopy."

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