Resolving the Structural Adaptation and Resulting Bioactivity Challenge of Cyclic Peptides by Multi-Scale Molecular Dynamics Methods.

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An incisive study of biological processes requires sufficient knowledge about protein-protein interactions. These interactions are vastly diverse in the biochemical domain, and this diversity makes protein-protein interactions difficult to study and analyze mechanistically. One critical approach uses modifiers of the proteins' potential binding region.[1] However, this approach is challenging because of the ever-changing structural conformations of the peptides such as cyclic peptides. Here the complexities in modeling cyclic peptides that can act as diverse modulators of protein-protein interactions due to their structural selectivity and binding affinities will be explored. The effectiveness of cyclic peptides has proven beneficial in therapeutics with a range of uses including antibiotics, anticancer drugs, and antiviral drugs as well as being essential to vasopressin and oxytocin signaling pathways.[1] When modeling cyclic peptides, obstacles that remain in their use include the primary source of the peptides and their structural integrity; they are flexible in different environments and this ultimately results in the complexity of their characterization by spectroscopic techniques such as NMR.[2] With the advancement of molecular dynamics techniques, advanced algorithms and simulations can be implemented to resolve the conformational space problem with the goal of mapping their structural resolution to their underlying bioactivity. Several computational designs and structural resolution platforms that have been implemented to study and analyze cyclic peptide structures and functions include Rosetta[3], EGSCyP[4], and PEPFOLD[5]. Computational methods that can be implemented include Replica Exchange Molecular Dynamics (REMD*)*, implemented by Sugita and Okamoto, which replicates several copies of the peptide and simulates them simultaneously and independently over a range of temperatures, and the replica exchange (each with a specified temporal environment) at neighboring temperatures are maintained by the Metropolis criterion.[6]

The problem with this technique is that neighboring temperature potential energy distributions must overlap adequately enough to maintain a sufficient exchange rate. This is because N represents the number of particles in the system and $\sqrt{N}$ is proportional to the potential energy distribution.[1] Other molecular dynamics methods include Metadynamics which is essential for enhancing MD sampling. This method uses collective variables which describe conformational space degrees of freedom dihedral backbone angles and provide an essential description of the simulated system. An application of the Metadynamics method is used to study the Coupled Two-Dihedral Motions of Cyclic Peptides. The transition of interest and slow degrees of freedom represent the selected coordinates. McHugh et al. investigated numerous cyclic conformations, particularly cyclo- (GGGGGG) and several other cyclic peptide switch conformations.[7] Well-Tempered Metadynamics decreases the height of the gaussian with simulation time which smooths the convergence allowing a smooth convergence of V_G. However, one setback is that this method relies on a history-dependent potential bias. Accelerated Molecular

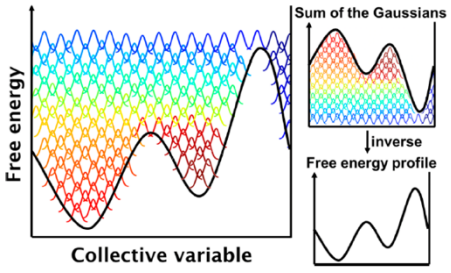

Figure 1: Metadynamics schematic. Gaussian potential distribution. Color code highlights that the system should not revisit the same place twice

Dynamics (aMD) increases how frequent transitions cross the energy barriers.[8] This is done by raising the potential surface near the minima (increase bias potential). Kamenik et al. investigated the aMD approach on cyclo-(PSlDV), cyclo-(RGDfV), and cyclo-(RRWWRF). The researchers used the AMBER-14SB force field TIP3P water model to investigate the aMD simulation where the unbiased results were recovered by dihedral component analysis. Kamenik et al. used conventional MD to show the limited sampling of cyclo-(PSlDV) while aMD shows the cis and trans isomers bond between the two amino acid residues: Pro1 and Val5.[9]

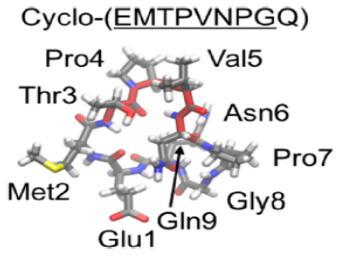

Figure 2: Structure of cyclo-(ETPVNPGQ) during REMD simulation. Red highlight shows type-I beta turn at TPVN

Another method, Complementary-coordinates molecular dynamics (CoCo-MD) was developed by Shkurti et al. and is often used as a conformational sampling method. This is done by integrating the "complementary coordinates" (CoCo) method alongside MD simulations. This method was used by Shkurti et al. to investigate cyclosporin A (N-methylated cyclic peptide). Earlier research using conventional MD done by Witek et al. and Shkurti et al. also investigate the structure of cyclosporin by using cMD, with CoCo-MD and accelerated MD (aMD), and loter the results to that of Witek et al.'s and it was discovered the cMD and aMD failed to replicate the sample conformation results of Witek et al., but the data of the CoCo-MD provided even more conformation space. The results also showed rarely accessible thermal states that can be observed. The conformational states dihedral was labeled φ (g+, t, or g−), ψ (g+, t, or g−), and ω was used to represent cis or Trans, results showed that CoCo-MD was able to characterize 9822 different conformational States in comparison to cMD 2224 identified by cMD and 5912 states identified by aMD.[10]

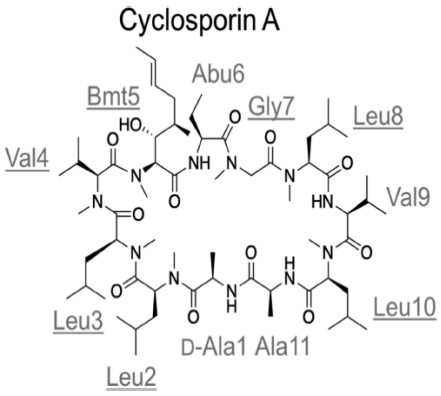

Figure 3: MD of "CoCo" validated 9822 different conformational states while aMD anc conventional MD simulated less

One application for the REMD MD method is to analyze specific force fields of cyclic peptides of specific amino acid residue. Due to the conformational flexibility of cyclic peptides, the accuracy to determine the free energy of the system must be high which is essential in understanding the mechanistic bioactivity of the cyclic peptide. Geng et al. investigated the effectiveness of four force fields by using REMD. Each amino acid type has a specific rotamer-dependent Ramachandran plot and Geng et al. were able to replicate the results by adding specific residual modifications.[11] After the cyclic peptides sampling backbones were compared to the linear counterpart, the researcher discovered that there is a higher probability for the sample's least likely value of φ and ψ. The researcher also discovered that the sampling backbone of the cyclic peptide is more relatable to those found in globular proteins. The solution structure of cyclic peptides

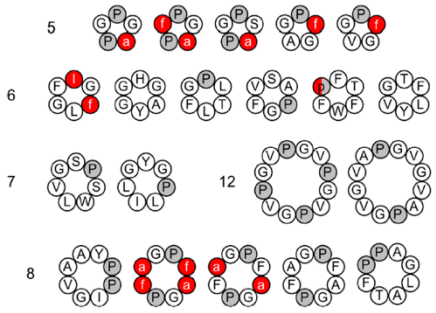

Figure 4: REMD Simulation with four force field- 20 cyclic peptides simulated and force field 2 results best verified X-ray structure

can be also elucidated by MD simulations as demonstrated by REMD in the study of α-Fetoprotein-Derived Cyclic Peptides. This is because REMD can help to better understand the structure which can ultimately aid in the design of peptides with antiestrogenic properties.[11] These derived peptides could play a role in inhibiting breast cancer that occurs as a result of estrogen. Researchers also previously discovered that the small sequence EMTPVNPG (octapeptide), a residue of human α-fetoprotein allows for antiestrogenic activity.[12] However, there was not much structural information available about the peptide sequence. Similar cyclic residues EMTPVNPG, cyclo-(EKTPVNPGQ), cyclo-(EMTPVNPGQ), EMTPTNPG, and cyclo-(EKTPVNPGN) were all reported to have a similar inhibitory effect but with little structural information known.[12] As a result, to tackle the structural design, researchers resorted to the use of REMD simulations to resolve the structure.

The dynamical behavior of the proposed drug candidate can also be simulated which can further facilitate the future design of other drug candidates. This can also be done using REMD as shown in the structure of LapD-Derived Cyclic Peptides.Razavi et al. implemented REMD simulations and successfully sample 20 cyclic peptidomimetics designs in order to mimic the β-hairpin structure≈ (from bacteria LapD).[13] The β-hairpin of the LapD is essential in biofilm formation (interaction between LapD and LapG).[14] The researchers designed the two scaffolds to mimic the salt bridges and H-bonding pattern between amino acid residues Arg and Glu. The simulation was done using the AMBER-99SB-ILDN force field and TIP3P water model. The Markov State models also verified that the design mimics conformation that is similar to the native population.[15] consequently, the advancements in computational methods are also constantly being updated.

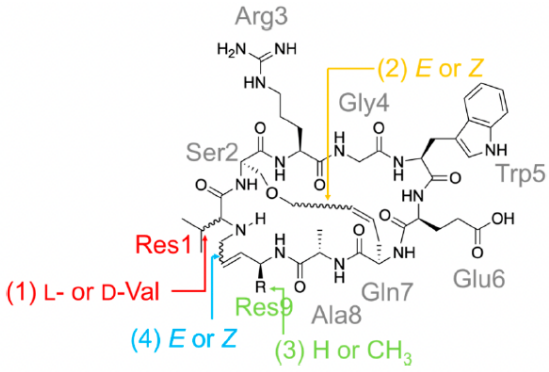

Figure 5: REMD Simulation: Derivative of 16 peptide designs from LapD. Best mimicking target β-hairpin structure: L-Val-E-Z-H, L-Val-E-Z-CH_3, D-Val-E-ZCH_3, and D-Val-E-Z-H

In addition to the vast amount of experimentally verified results that were already predicted by computation models, computational methods can provide a high-accuracy prediction for effective drug design that is not yet imagined experimentally.

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