Dynamic Docking of Conformationally Constrained Macrocycles: Methods and Applications

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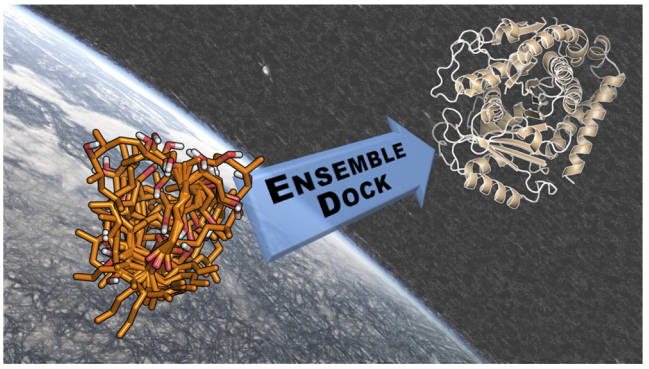
ABSTRACT: Many natural products consist of large and flexible macrocycles that engage their targets via multiple contact points. This combination of contained flexibility and large contact area often allows natural products to bind at target surfaces rather than deep pockets, making them attractive scaffolds for inhibiting protein−protein interactions and other challenging therapeutic targets. The increasing ability to manipulate such compounds either biosynthetically or via semisynthetic modification means that these compounds can now be considered as *starting points* for medchem campaigns rather than solely as ends. Modern medchem benefits substantially from rational improvements made on the basis of molecular docking. As such, docking methods have been enhanced in recent years to deal with the complicated binding modalities and flexible scaffolds of macrocyclic natural products and natural product-like structures. Here, we comprehensively review methods for treating and docking these large macrocyclic scaffolds and discuss some of the resulting advances in medicinal chemistry.



In recent years, there has been increasing interest in naturally occurring and synthetic or engineered biosynthetic macrocycles as potential therapeutics to target the large swath of human proteins that do not display an obvious small molecule binding pocket, i.e., drugging the undruggable.[1,2] This enthusiasm for macrocycles has been led by such successful examples as cyclosporin A, rapamycin, and FK506,[3] but even more recently, FDA approved Eribulin (Halaven)[4] and "stapled" α-helix mimetics like the NOTCH inhibitor SAHM1[5] have provided evidence, sometimes controversial,[6,7] that macrocycles represent promising scaffolds for drug development. Advances in synthetic chemistry[8,9] as well as new and novel biochemical techniques such as mRNA display and DNA-templated synthesis[10,11] have made it easier to synthesize, test, and optimize large libraries of structurally complex macrocycles. Though the interest in macrocycles is certainly very high, there is still much development that must be done to turn this class of molecules into a reliable platform for rational drug development.

Cyclization, especially of peptides, imparts enhanced stability and, in some cases, cell permeability.[12−15] In theory, cyclization can decrease the conformational freedom of side chains, predisposing them to the favorable orientation for binding of a single target with high affinity and selectivity.[1,16,17] The introduction of this conformational constraint lowers the entropic barrier to obtaining an active conformation,[18,19] enhancing the ability of the compound to target the challenging surfaces involved in protein−protein interactions.

One key area for macrocycle therapeutics that has seen recent improvements is the computational treatment and molecular docking of macrocycle scaffolds against therapeutic targets.[17,20] Modeling and docking simulations, which have been essential to drug discovery campaigns focused on classical small molecules, are at least equally if not more important when dealing with macrocycles. Macrocycles, with manifold conformers constrained by cyclization, present unique challenges for computational simulation. In the case of acyclic ligands, all possible conformations can theoretically be accessed through systematic scan of all rotatable bonds. However, the transition from one macrocycle conformer to another typically requires the concerted rotation of multiple dihedrals. Docking analyses that systematically scan predetermined rotamer libraries for side chain and acyclic ligand conformations are unable to resolve the concerted motions required for macrocycle conformation sampling. Instead, each individual torsion perturbation of the macrocycle returns a structure with aberrant bond lengths or angles and is often discarded. Poor poses resulting from a poor initial conformation can propagate through subsequent simulations, especially if access to a more bioactive conformer requires transversal of a high energy barrier or if that conformer is not accessible on the time scale of a simulation. Unfortunately, many standard docking packages still do not rigorously sample macrocycle conformations.

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Table 1. Summary of Conformational Search Techniques Used in Studies Cited Herein

General technique	Specific Applications	Software	Refs in this work
Monte Carlo or Stochastic Search			
	MCMM	MacroModel (Schrödinger)	57, 58, 59, 60, 63, 64, 65, 66, 67, 68, 71, 73, 86, 89
	MC/SD	MacroModel (Schrödinger)	54, 55
	MC	Spartan08	39
	Stochastic Search	MOE	94, 113
	MC with scaled potentials	FlexPepDock (Rosetta)	121
Low Mode			
	LMOD	MacroModel (Schrödinger)	93
	LowModeMD	MOE	61, 62, 95
Mixed Modes			
	MCMM/LMOD	Schrödinger	84
Molecular Dynamics			
	Constant Temperature	AMBER GROMACS CHARMM Desmond	99, 100, 101, 102, 103, 105
	High Temperature MD		72, 74, 75, 91, 97, 109, 110, 111
	REMD		76
	Constant T with increasing non-bonded interactions		122
	Metadynamics		125
NMR Deconvolution			
	NAMFIS[a]		84, 86, 89
No energy function			
	Based on Ramachandran		127, 129
	Based on Substructure topography		133
QM geometry opt			
	B3LYP/6-31G(d)		39, 40, 41
Other			
	Genetic Algorithm	AutoDock	51
	Distance-based Geometry		69, 70

[a]Since NAMFIS begins with an exhaustive conformation search, the references cited in the NAMFIS section are also included in the categories by the manner in which the conformation searches were conducted.

Robust treatment of macrocycle conformation is increasingly regarded as an essential step prior to docking efforts. Integrated prioritization and use of multiple conformers in rigorous ensemble docks have become the gold standard as more and more groups enter this field. We have sought here to emphasize such examples of robust computational handling of macrocycle conformational searches, where those searches are accompanied by detailed biological or biophysical data, such that the interested chemical biologist may have a ready approximation of the state of the art. A number of excellent reviews have been written on the detailed description of algorithms for docking and conformational searching for the computational specialist; we have sought to cite these appropriately. Figures of docked poses were created in PyMOL[21] using structure files supplied by the original authors of the cited works.

1.1. DEFINITIONS AND FILTERS

In order to provide a focused review, several criteria were employed to filter relevant examples. First, in our definition, a macrocycle refers to ring systems of 12 atoms or greater, a seemingly generally accepted number in the literature. Second, we have included only papers that use an ensemble of conformers in the docking simulation. This means that we have excluded many reports employing a single minimum energy structure, a single NMR solution-phase structure, or an X-ray-derived structure as the sole conformer. There are, in fact, many applications of macrocycle docking that employ such a single conformer strategy; however, the majority of data suggests that these approaches are limited and that rigorous assessment of molecular flexibility should be performed before docking of macrocycles. Importantly, studies have shown that the binding of many ligands to protein structures results in the ligand adopting an enthalpically unfavorable conformation in order to maximize favorable contacts with the receptor.[22−24] A single conformer approach may therefore miss the relevant pose. With respect to NMR structures, while the data provides an assessment of the *average solution structure* of a given compound, the biologically relevant conformer may lie outside that range.[25] Similarly, seeking to understand structure−activity relationships by using a single NMR or X-ray derived conformer can downplay the dramatic effect that a side chain alteration (A-values) can have on macrocycle conformation and binding geometry.

1.2. METHODS

Methods for the generation of conformation ensembles for macrocycle docking include those typically associated with large software packages, such as low mode vibrational analysis and Monte Carlo, as well as those associated with molecular dynamics simulations, including high-temperature MD simulations and replica-exchange MD (REMD). Here, we provide a general description of these techniques; a brief summary is given in Table 1.

In each step of a Monte Carlo (MC) conformation search, a set number of torsion angles is randomly perturbed and the resulting structure is energy-minimized.[26] MC searches are computationally inexpensive, but they are most successful when a small number of random torsion angles can greatly change the molecular structure. This is not always the case in macrocycles, which may require a specific set of torsions to be modified in order to identify a new conformer. In order to accommodate macrocyclic compounds, most MC search programs define one bond as a ring closure bond.[27] This bond is opened during the MC conformation search, and the resulting conformations in which the bond is impossibly strained are discarded.

In low mode vibrational analysis, a frequency calculation is conducted that identifies vibrational modes that correspond to conformational changes. These vibrational modes are followed to two extremes, and the geometry is minimized and compared to previously obtained conformers.[28,29] Versions of Low-ModeMD, available in MOE and LMOD, available in Schrödinger are distinguishable by the way the programs follow the identified modes: the former uses short molecular dynamics simulations, whereas the latter simply optimizes the two structures on either side of the vibrational mode. Low mode conformer generation is best for collective conformational changes (i.e., conformational changes that require concerted rotation of multiple torsions), but it can be more computationally expensive than MC simulations. Search methods that combine these two methods (mixed mode searches) are available in some commercial packages and are generally recommended over any one method.[30]

Constant-temperature molecular dynamics simulations are also used for generating conformers. These simulations are among the simplest to run and can be performed in implicit solvent, which can greatly reduce computation time. (For those attempting MD for the first time, the authors recommend one of the three packages with the largest user base and troubleshooting communities: AMBER,[31] CHARMM,[32] and GROMACS;[33] even faster simulations can be achieved with discrete molecular dynamics.[34]) MD simulations at a constant temperature can be useful when a lot of data is known about the most likely docked conformation, and they are particularly useful when experimental data, typically X-ray or NMR, is available. However, it is very easy to become trapped in a local minimum, which may mislead one to assume that there is only one conformer available. Running one simulation at a very high temperature followed by energy minimization of frames along that trajectory can allow for the identification of more local minima. However, this method can also lead to the identification of unnaturally high-energy minima. Transversal of the energy barriers between local minima can be facilitated with the softening of potentials or with enhanced sampling techniques such as metadynamics or REMD, but these simulations can be more computationally expensive.[35,36] Force field parametrization is often recommended prior to running MD simulations; there are many good tutorials for parametrization available online.[37,38]

Structures generated from quantum mechanical (QM) calculations are generally more reliable and not as subject to force field-dependent errors in geometry compared to the above methods, but the computational cost scales severely with the size of the ligand and the number of conformations. Thus, exclusively QM-based calculations are generally avoided when an exhaustive search of conformers is required.[39−41]

Methods for computational docking simulations are legion; the pros and cons of individual methods and software packages[42−44] as well as current challenges in the field[45,46] have been extensively reviewed elsewhere.[47,48] A number of excellent commercial and free (primarily for academia) docking packages are available; these programs differ primarily in the force field used to treat the ligand as well as the degree of flexibility allowed in ligand and receptor pocket. At the time of writing, most docking software has little to no capacity for the sampling of macrocycle conformer ensembles concurrent to the dock, which necessitates pregeneration of macrocycle conformations prior to the docking simulation. Once the conformers are generated, the choice of software for the ensuing molecular docking simulation is usually dependent on previously validated models as well as personal preference and license availability.

Recent progress has been made in incorporating macrocycle conformer searches in several popular docking suites, though they are generally still not as rigorous as the stand-alone conformational search methods. For example, the ConfGen module of Schrödinger now contains a library of over 770 ring templates, many of which include multiple conformations for a given ring, though not all are macrocyclic.[49] Additionally, AutoDock includes a utility for the generation of macrocycle conformers,[50] though this adaptation for macrocyclic ligands has been underutilized since its incorporation in AutoDock 4.0.1 and official implementation in version 4.2.[51]

2.1. WHEN CYCLIZATION IS NOT ENOUGH: THE NEED FOR SEARCHING MACROCYCLE CONFORMATIONS

Macrocycles have achieved attention because of their potential to constrain the bioactive pose of the pharmacophore and thereby lower the entropy barrier to inhibition. This is an extremely appealing concept: although entropy costs can sometimes be dismissed in small molecule binding events, they are of substantial importance when targeting larger surfaces such as protein−protein interfaces. Constraining pharmacophores, even with small tethers, is nontrivial and can potentially yield closely matched and unanticipated conformers. A docking simulation preceded by conformer generation has frequently been used in this situation to identify how likely a macrocycle is to maintain a desired bioactive conformer.

Nowhere have the effects of conformational constraint been more exhaustively studied than in the α_v class of integrins. Structural studies have shown that binding to integrin $\alpha_v\beta_3$ is enhanced when residues in the key RGD binding motif are in an extended conformation and the C_β of the two polar residues are separated by ∼9 Å.[52] The discovery of loop mimetic cilengitide (*cyclo*[RGDf(N-Me)V], where f = (*D*)-Phe and Val is N-methylated) by Kessler has spurred furious research into new classes of linkers that restrain the three-residue "warhead" into a bioactive conformation (Figure 1A).[53] Researchers in this field have often used NMR-NOESY cross-peaks to conduct constrained conformational searches. Although the majority of these efforts use only a single conformer in the dock, there are several reports of robust ensemble docks.

Mingozzi and co-workers applied a single NMR constraint, a NOESY signal between two amide protons, in their study of a number of *iso*RGD peptidomimetics with diketopiperazine bridges.[54] Two conformation searches with differing initial conformations were conducted for each macrocyclic ligand, and

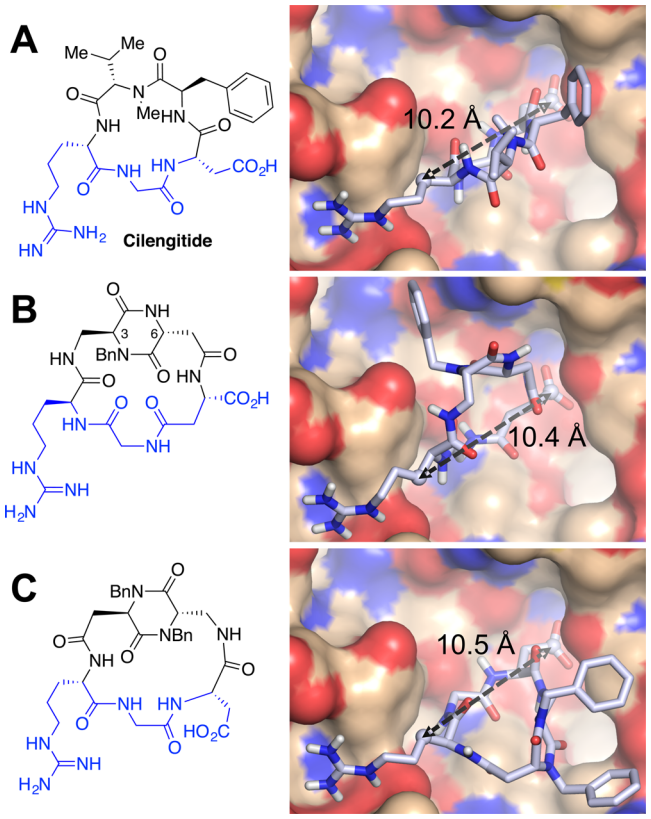

Figure 1. Successful macrocycles docked to the RGD binding pocket in $\alpha_v\beta_3$ integrin typically demonstrate a linear geometry linking the guanidinium and carboxylate "warheads". (A) Crystal structure of cilengitide (PDB code 1L5G) bound to $\alpha_v\beta_3$ integrin.[52] (B) Docked pose of monobenzylated RG(iso)D peptidomimetic.[54] (C) Docked pose of bisbenzylated DKP-cyclized RGD peptidomimetic.[55]

the NOESY datum was represented as a distance constraint of 2.0 ± 0.5 Å with a force constant k = 100 kJ/mol·Å^2. The MC/SD macrocycle conformation search showed that the (3*R*,6*S*) diastereomer adopts the desired extended *iso*DGR conformation for only 50% of the simulation, with an average C_β(Arg)−COO^-(Asp) distance of 8.8 Å. Conversely, the (3*S*,6*R*) diastereomer adopted two main conformations over the course of the simulation and demonstrated an extended conformation for approximately 80% of the simulation, with an average C_β(Arg)−COO^-(Asp) distance of 10.7 Å. In the subsequent docking study, the (3*R*,6*S*) diastereomer was not able to make key hydrogen-bond contacts with the β subunit of the receptor, whereas the (3*S*,6*R*) isomer docked more similarly to the native ligand (Figure 1B). The docking results agreed with the experimental results: the (3*S*,6*R*) diastereomer was 5-fold more effective than the (3*R*,6*S*) diastereomer and demonstrated greater selectivity for integrin $\alpha_v\beta_3$ over integrin $\alpha_v\beta_5$.

Similar results were seen in the docking study of bisbenzylated diketopiperazine (DKP)-cyclized RGD peptidomimetics.[55] Interestingly, the dibenzyldiketopiperazine diastereomer shown in Figure 1C demonstrated subnanomolar inhibition of integrin $\alpha_v\beta_3$, whereas its atropo-diastereomer, derived from hindered rotation about the diketopiperazine, was 1000-fold less effective. The conformation study of the less effective diastereomer showed a nonideal average C_β(Arg)−C_β(Asp) distance of just 6.6 Å, whereas the high-affinity diastereomer demonstrated an average distance of 9.0 Å. The molecular docking simulation of the effective diastereomer showed remarkable similarity to the docked pose of cilengitide with respect to the positioning of the key guanidinium and carboxylate functionalities. The binding epitope of a monobenzylated diketopiperazine-bridged RGD peptide was confirmed using STD-NMR techniques with integrin $\alpha_v\beta_3$ from the ECV304 bladder cancer cell line and integrin $\alpha_v\beta_3$ from human platelets.[56]

A study of *cyclo*[RGDf(Mor)] analogues of cilengitide (Mor = (*R*)- or (*S*)-morpholine-3-carboxylate) demonstrated the importance of a rigorous MCMM conformational search prior to the docking simulation.[57] For the (*S*)-Mor isomer, two interconverting structures were obtained, differing in rotation about the morpholino amide. This compound demonstrated markedly worse activity than its (*R*)-Mor diastereomer, for which only one rotamer was observed. Recognition of this effect was possible only through the use of a search capable of exploring rotations about the *N*,*N*-disubstituted amide.

In their search for compounds to inhibit the cysteine protease calpain, toward the prevention of cataractogenesis, Stuart et al. designed constrained bridged peptides with the goal of holding the ligand in a β-strand-like conformation.[58,59] The designed compounds were then subjected to an MCMM conformational search in order to determine the likelihood that the ligands would maintain the desired conformation, indicated by the percentage of conformers with a characteristic β-strand shape. Interestingly, though macrocyclic ligands with a 16-member macrocycle possessed nearly 100% β-strand character, the most potent compound in the series, CAT811, displayed only 8% β-strand character. Subsequent efforts yielded a different compound bridged through a 1,4-disubsituted imidazole, exhibiting 76% β-strand character, which was 3-fold more potent.[60] The authors speculate the difference in potency arises from energetically accessible inactive poses that allow the compound to access the active site.

Saupe and Steinmetzer had a similar result in the design of macrocycle-based plasmin inhibitors. The researchers found that increasing the length of a linker on a peptidomimetic ligand by two methylenes increased the number of conformers from 87 to 275 in the ensuing conformational search with LowModeMD, demonstrating that a seemingly minor change in structure can give rise to a significant increase in conformational flexibility.[61,62] While both ligands adopted similar docked poses, the larger (and more flexible) ring displayed the stronger affinity (Figure 2). In this case, the dock demonstrates that the macrocycle itself provides extended contacts with the target.

In the end, the benefits of combining macrocyclization with rigorous conformational modeling can be tangible: the Poulsen lab has turned the results of a high-throughput screen (HTS) into multiple clinical candidates through such efforts.[63] In this case, the HTS identified a semicircular pharmacophore scaffold for the Aurora A kinase ATP-binding site. Poulsen et al. used molecular docking simulations to identify a linker that could constrain the ligand in the active conformation of the original HTS hit.[64] Following conformational searches using MCMM in the MacroModel module of Schrödinger, the authors docked the simulation against the crystal structures of Aurora A, JAK2, FLT3, and the CDK kinase using Glide. The compounds demonstrated appreciable inhibitory activity against the JAK2, FLT3, and CDK kinases, which led to identification of SB1387 for Phase I clinical trials against multiple myeloma and advanced/refractory hematologic malignancies,[65] SB1518, currently in Phase III clinical trails for myelofibrosis,[66,67] and

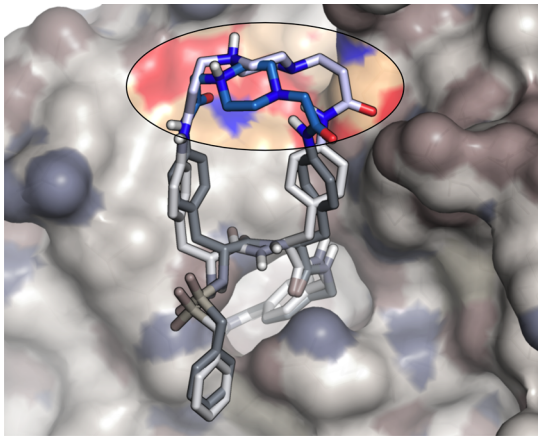

Figure 2. Increasing the number of methylene groups (highlighted) led to a great increase in the number of discrete conformers in the study of peptidomimetic plasmin inhibitors.[61]

SB1578, which has completed Phase I clinical trials for rheumatoid arthritis.[68] Likewise, a group at Merck identified a new class of P2−P4-tethered HCV NS3/4a protease inhibitors via inspection of the binding mode of a P1−P3-cyclized analogue.[69,70] Conformer generation with an in-house distance geometry algorithm preceded a docking study that identified the ideal tether structure and length. This work eventually led to the development of Grazoprevir (MK-5172), currently in Phase III clinical trials for treatment of hepatitis C.

2.2. DIFFERENCES BETWEEN THE LOWEST ENERGY CONFORMER AND THE BEST DOCKED POSE

Though we are long past believing that the preferred docking pose of an acyclic ligand is the lowest energy conformation in solution, it is alarming to think that this notion still stands in the case of macrocyclic compounds.[22,24] In the case of bryostatin bound to the cys2 domain of PKCδ, Keck and co-workers found that the lowest-energy macrocycle conformation is, in fact, the best docked pose. However, this conclusion was arrived at through a rigorous conformation search,[71] and the study reaffirmed results previously obtained by Itai.[72] There are still many instances in which the best docked pose is not the lowest energy solution conformer and the free energy cost of entering the bioactive pose greatly impacts relative activity. Two recent reports from Wiest and Karplus highlight the importance of considering the relative energies of free and bound conformers.

Wiest et al. demonstrated the effect that relative conformational energy has on the biological activity of macrocyclic histone deacetylase (HDAC) inhibitors FK228 and largazole.[73] Both compounds act by binding the active site zinc of mammalian HDACs through the side chain thiol of a heavily modified depsipeptide macrocycle. HDAC inhibition assays comparing the natural products to their amide isosteres showed that the largazole isostere was 9-fold less active than largazole itself, whereas the FK228 isostere was 40-fold less active than native FK228. A rigorous model and dock was undertaken in order to understand the structural basis of this difference in activity. MCMM conformer generation was performed starting from multiple structures, followed by clustering of similar conformers; the centroid of each cluster was then included in an ensemble dock (Figure 3). The docking shows that for largazole thiol the lowest energy conformation is also the

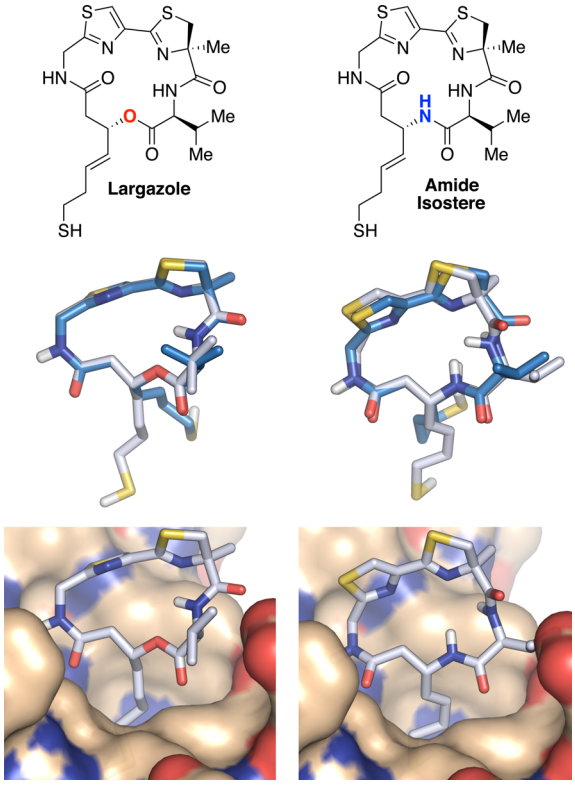

Figure 3. Best docked conformation of largazole (bottom left) is also the lowest energy solution-state conformation (overlay in middle left); the two structures differ in the amide isostere (right).[73] Note that in the overlay the blue structure represents the lowest energy conformer in solution; the white structure is the lowest energy docked pose.

docked conformation, suggesting that no additional energy is required for the molecule to achieve maximum interaction with the protein. However, for the largazole amide isostere thiol, the bioactive conformation is 48 kJ/mol higher in energy than the lowest energy solution phase cluster, suggesting that the difference in bioactivity may be related to the energy required to organize the molecule into the bioactive state. Moreover, the lowest energy bound (proposed bioactive state) pose differed from the most stable solution phase cluster by a root-mean-squared deviation (RMSD) of only 1.41 Å. Docking of a pose from the latter cluster suggests that it may also suffice as an (albeit lower affinity) inhibitor, thereby rationalizing the lower overall observed activity. On the other hand, of the seven clusters within 50 kJ/mol of the lowest energy conformation of FK228, none came within an RMSD of 2 Å of the bioactive conformation, indicating that the binding conformation of FK228 amide isostere is not energetically accessible from the most stable cluster and that allowable solution-phase conformations are incapable of the native potency.

In a series of studies, Karplus and co-workers exploited a similar conformational preference to identify improved resorcylic lactone inhibitors of Hsp90.[74] The resorcylic lactone radicicol is known to bind Hsp90 in an ATP-binding pocket; despite being one of the most potent Hsp90 inhibitors, radicicol lacks activity *in vivo* due to metabolic instability of its epoxide and conjugated enone groups.[75] A molecular docking study of several radicicol analogues was used to quantify the energy required for the molecules to adopt a bioactive conformation. High-temperature MD simulations, clustering, and energy minimization identified three main conformational

groups: L-shaped, P (planar), and L′-shaped (Figure 4). For all of the resorcylic lactones examined, the docking study showed

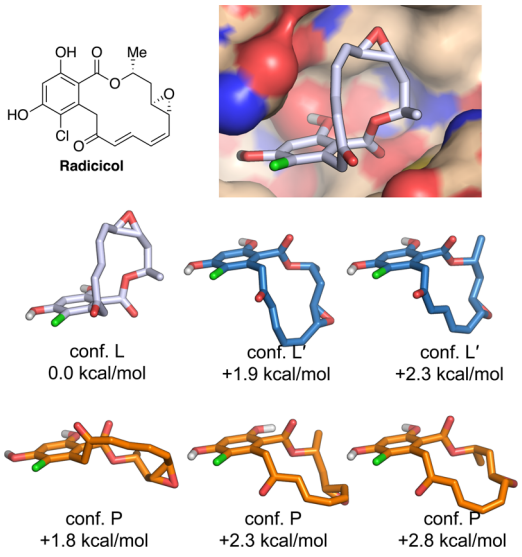

Figure 4. Six discrete conformations of radicicol were identified in a conformation search; the lowest-energy conformation also represented the best pose upon docking.[74]

that the L-shaped conformer was the most favorable pose for interaction with the receptor. What differentiated active and inactive, however, was preference for the same L-shaped conformer in solution. In the case of radicicol itself, the L-shaped conformer is also the most stable conformer in solution, whereas in the case of the studied analogues, they preferentially adopted the L′-shaped conformer in solution. Although the energy differences between the L- and L′-shaped conformers are small (0.4 to 2.7 kcal/mol), linear free energy relationship of calculated and experimental $\Delta G_{binding}$ suggested that hydrophobic interactions within the pocket were key to the binding of these compounds. Importantly, these efforts identified pochonin D, a recently discovered radicicol analogue with a slight preference for the L-shaped conformer in solution, which was confirmed to be potent in Hsp90 affinity assays. The trend could further be extended to known active analogues containing an *E*-oxime functionality.[76]

The depsipeptide isosteres and resorcylic lactones provide examples of how solution-phase (lowest energy) conformation may differ from the best fit (active) binding pose and the degree to which that difference may impact the activity of a compound. In the latter case of the lactones, this difference allowed inference of new structures, minimizing the different conformational preference and improving overall activity. Clearly, the relative conformational energy of macrocyclic ligands, not necessarily accounted for in many docking simulations, can play a role in determining bioactivity and should be strongly considered when evaluating the docking results.

2.3. THE ROLE OF SOLVENT WHEN GENERATING MACROCYCLE CONFORMERS

Backbone solvation is strongly impacted by the target binding site and can profoundly alter conformer stability. Therefore, it is worth considering the role of solvent when generating macrocycle conformers for a docking simulation. In general, conformation searches are usually performed in *implicit solvent of water*, though conformation generation with MD techniques can often include *explicit solvation* as well. Yet, a conformation search conducted in a polar implicit solvent may miss important conformers that are stabilized in a hydrophobic environment,[77] and a search in hydrophobic solvent may result in conformers with exaggerated hydrogen-bond contacts.[78]

A few studies have directly compared the effects of conformation searching under different solvent conditions. Kessler et al. observed changes in preferred solution-state conformation of cilengitide when MD simulations were run separately in methanol or water: in methanol, a key N-methyl amide rotated out of the bioactive conformation, demonstrating the impact of solvent on the ability to identify biologically relevant macrocycle conformers.[79] In contrast, Peach et al. saw minimal difference in MCMM conformation searches of

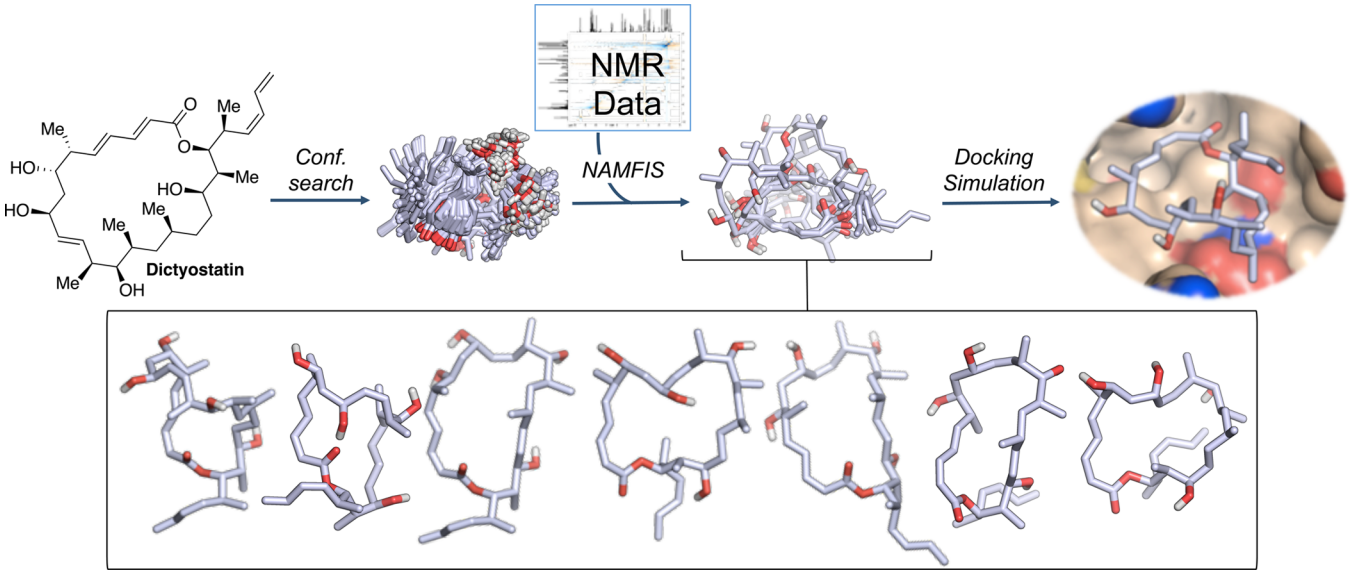

Figure 5. In the NAMFIS workflow, exhaustive conformer generation is followed by a fitting of conformer structures to NMR data to identify conformers that contribute most to the observed spectra.[81] Here, the dictyostatin study is shown as an example.[84]

bryostatin and its C9-deoxy analogue when the structures were treated in implicit water or octanol.[71] The conformation search in the two solvents served to determine whether an intramolecular hydrogen-bonding network, which had previously been observed in the crystal structure and solution-phase NMR structure, was a result of the crystal lattice or the low-polarity NMR solvent. These efforts confirmed that the lowest energy conformation of bryostatin 1 closely resembles that from the NMR structures and single-crystal structures.[80] Ultimately, the choice of solvent may be relevant on a case-by-case basis, and it may be prudent to conduct searches in two implicit solvents. Although many studies have examined the effects of solvation/desolvation on membrane transduction of macrocycles,[13−15] this remains an area for potential improvement in conformational searching against target engagement.

2.4. THE MEANING OF NMR CONSTRAINTS IN MACROCYCLE CONFORMATION

The conversion of NOE signals and $^3J_{HH}$ couplings to positional restraints can be a useful method of gleaning conformational information from experimental techniques. However, the conversion of this data to a single NMR solution-state conformer may be a flawed endeavor, as these signals can also be attributed to the rapid interconversion of multiple conformer states on a time scale faster than NMR.[25] The 2D NMR analysis technique NAMFIS (NMR analysis of molecular flexibility in solution), developed by Bazzo and co-workers, can be used to deconvolute NMR data by identifying conformers with high enough populations to contribute significantly to the observed NMR data.[81] In the NAMFIS method, an exhaustive conformation is performed to create a pool of possible conformers, and theoretical coupling constants and NOE signals are calculated for each conformer.[82,83] A least-squares fit of the experimental and calculated NMR signals is calculated for each conformer and compared to all other conformers to identify a set of conformers for which the Boltzmann-weighted sum of the NMR signals of the ensemble best matches the empirical data.

A molecular docking study of the macrolide dictyostatin to the taxane-binding site of β-tubulin was preceded by conformer generation with NAMFIS (Figure 5).[84] Mixed low mode/Monte Carlo searches in MacroModel generated 2053 unique conformations. Application of the NAFMIS method using NMR data collected in both methanol and DMSO identified 16 and 15 conformers, respectively. A previous report from Canales et al. had identified a single bound conformer based on transferred NOE data,[85] and re-evaluation of this NOE data using the ensembles generated from solution-state NMR led to the identification of a conformer present in the DMSO ensemble that was a comparable fit. Docking simulations conducted using these two conformations showed that the previously reported conformer bound in a promiscuous fashion, with similarly scored binding poses, whereas the best pose identified by NAMFIS bound in just one high-scoring pose. Additionally, the pose identified in the NAMFIS search better matches previously reported SAR data. For example, the C6-epi-methyl diastereomer exhibits similar activity to dictyostatin; the NAMFIS-identified pose puts the C2−C6 diene portion in a solvent-exposed position, whereas the previously reported binding pose has the moiety in a deep hydrophobic pocket.

The interactions between the ligands geldanamycin and radicicol with Hsp90 were also probed using the NAMFIS method.[86] Both receptor-bound and solid-state crystal structures of geldanamycin and radicicol were available prior to the docking study. Conformations of the two compounds were obtained using a MCMM calculation in MacroModel, starting from random conformers and using an implicit solvent model to match the $CDCl_3$ NMR solvent. The conformation search identified 1246 independent conformations for geldanamycin and 382 for radicicol. NAMFIS analysis using coupling constants and ROE-derived distances identified 12 conformations for geldanamycin and 6 for radicicol, which were then utilized in a molecular docking simulation with Glide. Interestingly, the NAMFIS-generated ensembles of both molecules contained the bioactive conformation, within at least 1.6 Å. Though the bioactive conformation was not the highest scoring pose according to the Glide dock, rescoring with the MM-GBSA (molecular mechanics−generalized Born surface area)[87] method in Prime easily identified the bioactive pose for both molecules. The MM-GBSA method for rescoring docking results has shown mixed results and is most successful when docking is not accompanied by large conformational changes in the ligand and/or receptor.[88]

The NAMFIS method was applied to the generation of conformers of laulimalide using NMR data obtained in DMSO-d_6 to identify 15 main conformers separated into 5 families.[89] These 15 conformers were then loaded into MarvinSketch, and 100 additional conformers were generated from each, to give a total of 1515 conformers of laulimalide that were submitted to docking with AutoDock4.[90] The docking site on β-tubulin, identified by mass shift perturbation mapping, is similar to that identified by Nguyen et al.,[91] though the binding conformation of laulimalide proposed here is substantially different. Crystal structures of laulimalide and peloruside A bound to β-tubulin published in 2014 confirmed the binding site but, unfortunately, not the proposed binding conformation/orientation.[92]

2.5. STARTING WITHOUT A STARTING POINT

Though relying too heavily on NMR data to generate an initial conformation can lead to errors, generating an initial structure in the absence of relevant structure data (besides chemical intuition) to support the solution-phase or bound-state conformation of a compound can also lead to mistakes in predicting binding poses. In these cases, a conformation search that effectively samples multiple ring conformations is absolutely necessary. Starting a conformational search from an X-ray structure can pose additional concerns, especially when using a conformation generation method that does not allow transversal of energy barriers. For example, a recent study of the archetypal integrin $\alpha_v\beta_3$ inhibitor cilengitide has shown that the ligand adopts a very different conformation in a single-crystal structure compared to the structure it adopts when bound to integrin $\alpha_v\beta_3$ due to the formation of intermolecular hydrogen bonds in the crystal lattice.[79]

Kimura and co-workers found, through a rigorous conformational search and docking simulation, that the single-crystal structure conformation of bryostatin 1 is also the most likely bioactive conformation.[72] Conformers of a model bryostatin 1 macrocycle structure were generated using high-temperature MD; the conformer ensemble was then subjected to a docking simulation using an in-house program, which showed that, in fact, the crystal structure conformation of the bryostatins was also the most stable binding pose. The computed docking model is very similar to that shown in ref 71.

In their study of brunsvicamide A and a series of analogues with varying substitution and stereochemistries, Walther et al.

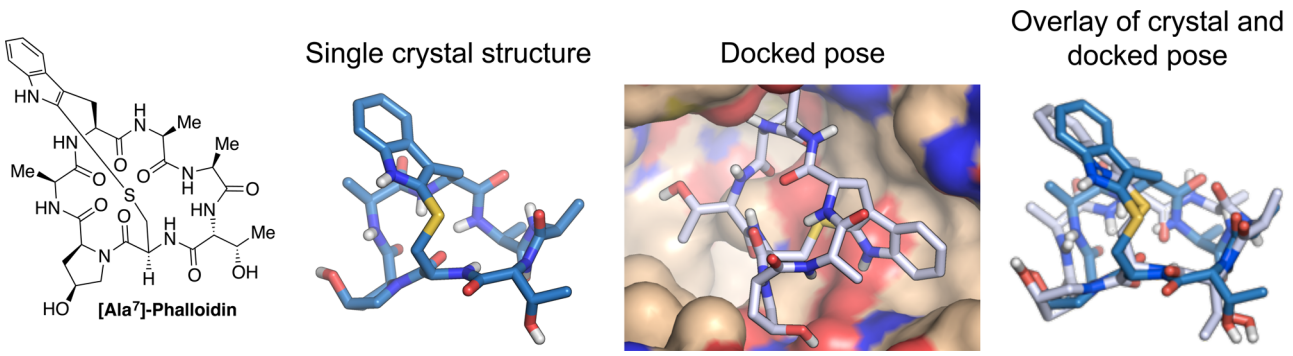

Figure 6. Overlay of the single-crystal structure[114] of [Ala^7]-phalloidin over the best docked pose[113] suggests a significant difference between the solid-state and protein-bound conformations.

performed low mode conformational searches on the native diastereomer and its ε-amido (L)-lysine epimer. The docking study showed that the two epimers bound to the protein in wholly different macrocycle conformations and that only the native ε-amido (D)-lysine epimer was able to make the key contacts between the side chain carboxylate and a zinc atom in the binding site.[93]

Weinrich et al. employed an MCMM conformational search to generate conformers of efomycin M and a series of analogues prior to a dock against a shallow pocket on E-sellectin.[94] Similarly, a LowModeMD conformation search was used prior to the docking of liverwort component marchantin E against the PA subunit of the H1N1 endonuclease, generating 250 distinct conformers.[95]

CDOCKER, a module in the Accelrys DiscoveryStudios molecular modeling package, is one of the few docking programs also able to sample macrocycle conformations,[96] which can be useful when no conformation data is available. However, this integrated pregeneration of ligands may be a double-edge sword since the ligand conformers cannot be inspected prior to the docking step. This method was employed in the molecular docking of tetrazole-substituted largazole analogues to a homology model of human HDAC1.[97]

Generation of 3D structures from 2D drawings can be a useful starting point for a macrocycle conformation search, but the output from this single-structure calculation should be subjected to a conformation search that allows transversal of high energy barriers, which is not always guaranteed in constant-temperature MD simulations of macrocyclic compounds.[20,98] If the initial structure is located in an energy well, then only this local minimum will be sampled. The initial structures for rediocides A and G, which were examined for inhibition of α-cobratoxin, were generated with Chem3D without empirical data.[99] These structures were then submitted to a 10 ps MD simulation using the MM2 force field as implemented in Chem3D, with structures extracted every 0.1 ps. Similar methods were employed in the generation of conformers of 1,3-dione-linked RGD peptidomimetics.[100] Constant-temperature MD simulations are best applied in situations where empirical data is available to describe the conformation in solution. For example, Vilaça et al. used NMR constraints to build an initial conformation of a constrained RGD integrin inhibitor, from which a constant-temperature MD simulation generated the conformation ensemble used in subsequent docking simulations.[101] Similarly, Yoshikawa et al. used simulated annealing with NMR constraints to generate initial conformers of cyclic pentapeptide FC131[102,103] and analogues in a docking study against CXCR4.[104]

Unrestrained constant-temperature MD was used effectively in the generation of conformers for a molecular docking study of macrocyclic bis-intercalators that bind DNA duplexes with mismatched thymine base pairs.[105] Initial conformations of the ligands were generated using MarvinSketch, followed by an initial conformer search with ChemAxon's Calculator plugin, which also protonated the aliphatic amines, and an MD simulation with Desmond. The structures minimized in the gas phase showed extended conformations, but semiclosed conformations dominated the MD simulation. Docking was performed with GOLD 4.1 using an ensemble of semiclosed conformations extracted from the MD simulation and an 11-mer DNA oligomer containing a mismatched thymine base pair (T−T). The docking results confirmed the experimental results, namely, that the ligand 2,7-BisNP binds well to T−X mismatches but not to matched T−A base pairs. The docked pose was later confirmed using NMR data.[106]

A particularly difficult challenge arises when both the bioactive conformation of the ligand *and* the binding site on the protein are unknown. Such a dilemma preceded the study of the interactions of laulimalide and peloruside A with β-tubulin, which necessitated a conformation study of the ligands and the docking site.[91] For the ligands, a 1 ns MD simulation at 1500 K was performed, followed by minimization and selection of 5 initial conformations of low energy and high hydropathy.[107] A previously hypothesized binding site[108] was optimized with a manual induced-fit procedure in which two loops blocking the pocket were removed, the ligands were docked with Glide, and the loops were replaced and iteratively refined.

The same approach was utilized in the docking of halichondrin B and eribulin to β-tubulin.[109] Interestingly, as was seen in the case of cilengitide,[79] the structure of halichondrin B built from the single-crystal structure of a congener was unable to be docked in the protein. Instead, molecular dynamics simulations starting from the crystal structure-like pose generated folded conformers that were able to be used in the docking procedure. An undocking procedure was also conducted in order to simulate how the loops blocking the binding site move to accommodate the ligand.

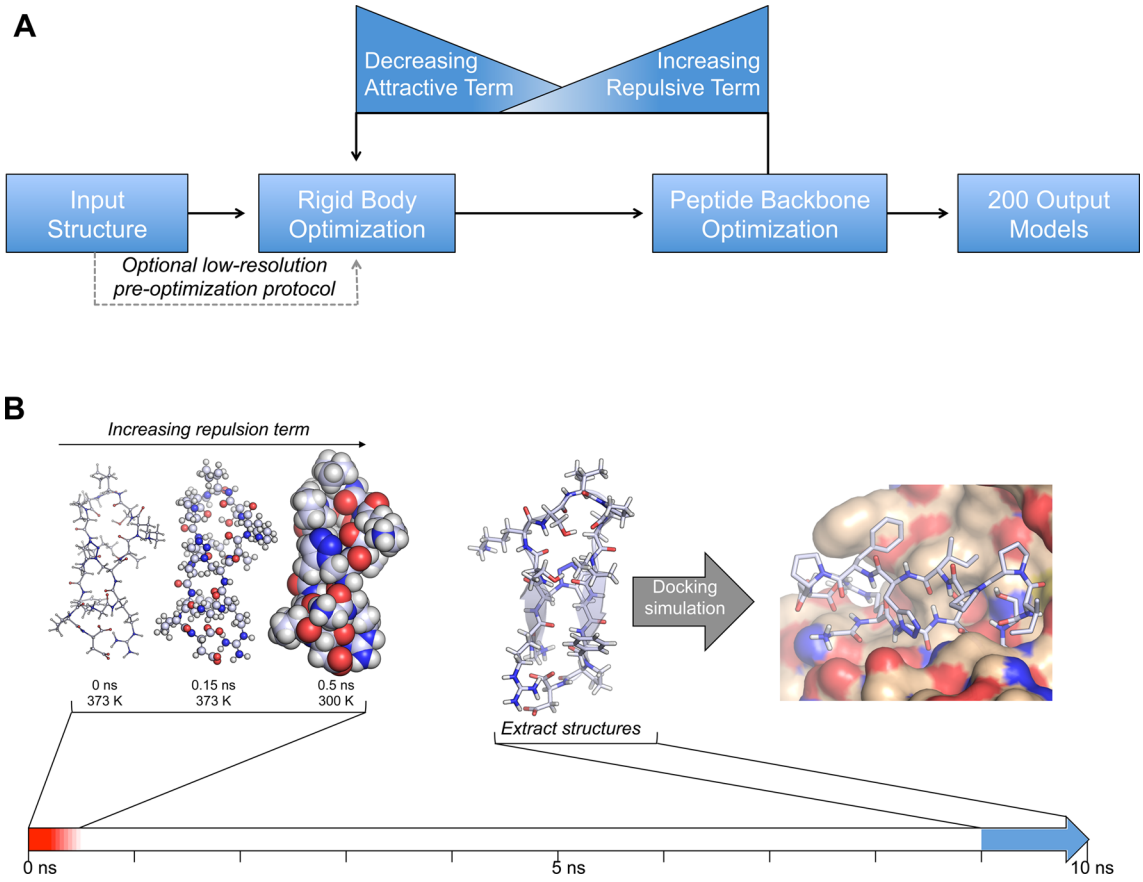

Figure 7. Two peptide-based methods applied to macrocycle conformer generation and computational docking simulations. (A) Workflow for FlexPepDock. (B) MD workflow employed in the study of SFTI-1 and analogues vs trypsin and matriptase. Increasing van der Waals potentials in the first 0.5 ns are represented as scaled spheres.

2.6. THE IMPACT OF SIDE CHAINS ON MACROCYCLE CONFORMATION

When a crystal structure of a similar compound is available, it can be tempting to simply exchange side chains and use that structure in the docking simulation. However, epimerization of a single stereocenter, a seemingly minor change in structure, can have a profound effect on binding conformation that can be lost if a proper conformation search is not conducted. For example, even though a crystal structure of chitinase bound by the cyclopentapeptide argifin was available, Gouda et al. ran conformational searches for a number of analogues. The researchers used CAMDAS (conformational analyzer with molecular dynamics and sampling), a program that performs high-temperature MD simulations, followed by energy minimization and clustering.[110] The roughly 4000 conformers thus generated were docked to chitinase B using Glide, and the top 100 poses were energy-minimized and rescored with MM-GBSA, leading to the identification of a novel pentapeptide with improved activity over argifin.[111,112] These efforts helped identify a derivative, in which D-Ala(5) of argifin was replaced with D-Leu and a 4-benzylpiperdine was attached to L-Asp(4), exhibiting 28-fold more inhibition than argifin itself.

Similarly, Arndt and co-workers ran a docking simulation preceded by conformational searches for [Ala^7]-phalloidin; this, despite the availability of single-crystal structure for [Ala^7]-phalloidin (Figure 6).[113] Interestingly, the phalloidin search yielded 56 relevant conformational states, whereas a parallel search with the less constrained macrocycle chondramide C generated 2648 conformers. In the event, the best docked conformation of [Ala^7]-phalloidin showed a RMSD of 1.1 Å from the single-crystal structure, a large departure for a small macrocycle. More importantly, all available SAR data on phalloidin, with its extended side chain, are in agreement with this new binding geometry, which indicates access to a hydrophobic cavity. In both instances, the impact of side chains was validated through rigorous conformational searches, despite the availability of structures that could easily have caused researchers to avoid the extra computational expense.

2.7. BLURRING THE LINES BETWEEN PEPTIDE MACROCYCLES AND PROTEIN MACROMOLECULES

Given the recent upturn in loop mimetics and cyclic peptides[115] as well as recent studies on improving cell permeability,[13,116] new macrocyclic compounds are often peptide-like, and the methods for molecular docking simulations of macrocycles and peptides are becoming intertwined. Though computational design of peptide inhibitors of protein−protein interactions is well-reviewed elsewhere,[117,118] it should be noted that FlexPepDock, a program designed for the modeling of peptide−protein interactions within the Rosetta framework, performs a Monte Carlo calculation as part of its generation of peptide conformations.[119] In this simulation, the attractive van der Waals interaction are increased by 225% while the repulsive interactions are decreased to 2% to maintain contact between peptide and protein. A Monte Carlo simulation with energy

minimization at each step is first applied to rigid body rotation and translation of the peptide, followed by backbone perturbation, which is accompanied by a gradual decrease of attractive van der Waals interactions and a complementary increase in repulsive interactions (Figure 7A). The FlexPepDock protocol was applied to a number of linear peptides from the CAPRI database (critical assessment of prediction of interactions),[120] which is used to benchmark docking simulation software. Of the 29 targets tested, a near-native pose was identified for 72% of the protein−protein interactions.[121]

The FlexPepDock method was applied to the docking of a cyclic peptide with a disulfide bridge, with the goal of designing an inhibitor of a globular protein−protein interaction.[121] In particular, a peptide was designed to inhibit the EphB4−EphrinB2 interaction, implicated in angiogenesis, by creating a loop mimetic from the G−H loop of EphrinB2 (sequence: KFQEFSPNLWGLE). Residues 2 and 12 of the original loop were mutated *in silico* to cysteine to create a ring-closing disulfide bond, which did not appear to have any negative effect on the FlexPepDock protocol. The resulting macrocycle was subjected to the FlexPepDock protocol described above, and the binding energy of the loop suggested that this motif might be an effective binder to EphB4.

A novel method was used in the conformer generation for the 14-residue polypeptide sunflower trypsin inhibitor-1 (SFTI-1) and analogues for docking against trypsin and matriptase (Figure 7B).[122] To allow the ligands to transverse energetic boundaries caused by macrocyclization, an MD simulation starting from a solution-phase NMR structure was conducted. The simulation began at an initial temperature of 373 K, with scaled-down nonbonded interactions. Over the course of the simulation, the temperature was brought down to 298 K with an incremental increase in the strength of nonbonding interactions; conformers were sampled from the last 1 ns of the simulation. This method was applicable to both monocyclic polypeptide constructs, linked through residues 3 and 11 via a disulfide or a triazole ring, and bicyclic constructs with additional head-to-tail cyclization. Analysis of the ensemble docking simulation using generated conformers showed excellent agreement between the experimental and calculated ΔG_{bind}.

Metadynamics simulations present a useful alternative to standard molecular dynamics simulations, particularly in cases where large energy barriers separate local minima on the potential energy surface (PES). Metadynamics simulations are steered along two variables, called collective variables, or CVs.[123,124] The history-dependent algorithm prevents the simulation from revisiting previously accessed values of the CVs, and one can obtain a plot of the PES of the system as a function of the CVs employed. Spitaleri and co-workers applied this technique in their exploration of the conformational flexibility of cilengitide and two disulfide-linked cyclopentapeptide RGD inhibitors, CDRGC and CisoDGRC, using the psi and phi angles of the central glycine as the CVs.[125] Population analysis of the resulting PES showed 97% of cilengitide structures are in the bioactive conformer, compared to just 42% for CisoDGRC. Interestingly, acetylation of the N-terminal amine of CisoDGRC increased that fraction to 83%. Subsequent ensemble docking and an integrin $\alpha_v\beta_3$ binding assay confirmed the increased potency of the acetylated congener.

2.8. CONFORMATION SEARCHES WITHOUT ENERGY FUNCTIONS

The heavy computational cost of exhaustive conformational searches makes simplified analytical approaches extremely valuable in accelerating the overall process of molecular docking. For example, studies of cyclic pentapeptides have shown that these compounds adopt backbone conformations that match the allowed regions of Ramachandran plots of linear peptides.[126] Thus, the complexity associated with a systematic scan of torsional space for these macrocycles can be reduced by restricting the Φ- and Ψ-angles to values allowed according to the respective residues. Våbenø and co-workers exploited this simplification in study of cyclopentapeptides with inhibitory activity against CXCR4 (Figure 8A).[127] Three different combinations of (Φ, Ψ) were included for proline, eight for glycine, and six for all other residues; except in the case of proline and N-methyl residues, only the *s-trans* (ω = 180) amide linkage was allowed. The resultant backbone structures were then pared to remove all redundant conformations and conformations in which cyclization was not possible, affording

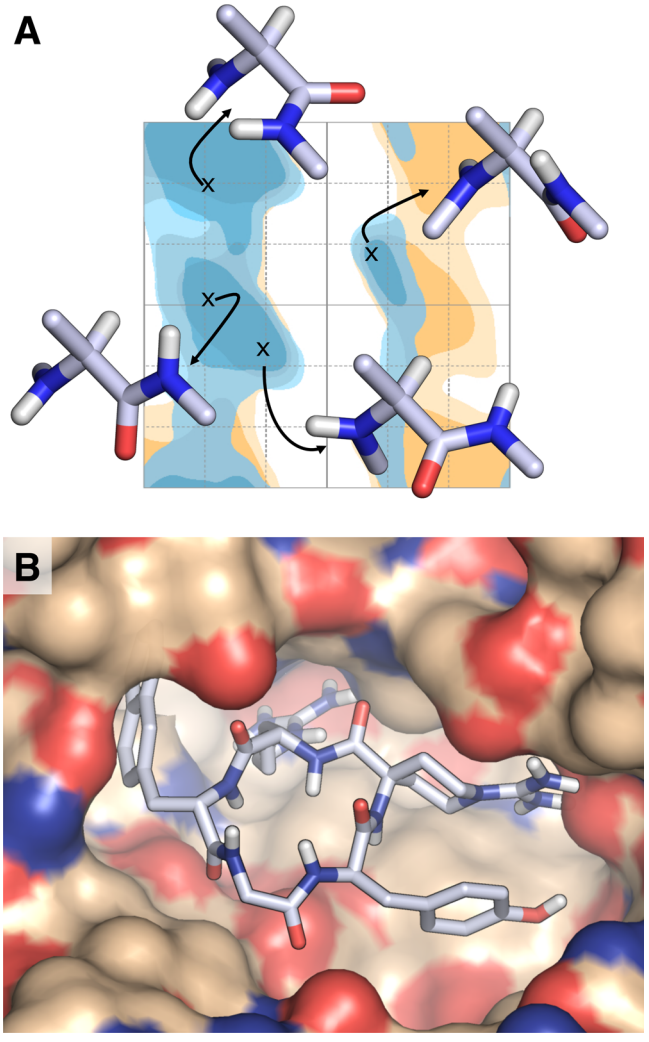

Figure 8. Conformer search absent an energy function was utilized in the conformer generation and subsequent docking of FC131.[127] (A) Conformers of FC131 analogues were constructed from amino acid rotamers in the allowed region of (Φ, Ψ) space. (B) Best docked pose of a FC131 analogue with an achiral cyclohexyl arginine.

50−500 ring conformers depending on the number of prolines and N-methylated residues. Side chains were added, and conformations were energy-minimized with a computationally inexpensive united-atom force field,[128] subjected to a 10 kcal/mol cutoff, and subsequently minimized with the all-atom OPLS_AA force field. The conformations identified in the study were applied in a molecular docking simulation of the CXCR4 antagonist FC131 and several analogues.[129] Docking showed that modifying an Arg residue to an achiral α,α-disubstituted moiety improved the inhibitory activity; the docked pose of the compound showed that key salt bridges are established by this residue (Figure 8B). The binding mode identified in this study matched a pose obtained by Yoshikawa et al.,[104] and QuikChange mutagenesis of the FC131 binding site validated the proposed binding pose.[130]

A similar search protocol is employed by the conformation generator ROCK (rigidity-optimized conformational kinetics),[131] which uses bond length, angles, and torsional constraints identified by the program FIRST (floppy inclusion and rigid substructure topography)[132] to conduct a systematic search of macrocycle torsional space. No energy function is employed in the conformation search, which precludes the quantitative determination of relative conformer stability. ROCK has been employed in generation of conformers of zearalenone and cyclosporin, targeting estrogen receptor and peptidyl prolyl isomerase cyclophilin A, respectively. The cyclophilin dock suggests that a significant amount of flexibility is tolerated in the CypA−cyclosporin complex.[133] Importantly, in both the Våbenø work and with ROCK, the absence of an energy calculation until the last step greatly reduces the computational cost of this exhaustive conformation search. A similar search could be applied to an acyclic system, but without the constraint of cyclization, the number of conformers would be astronomically high.

3. CONCLUSIONS

Macrocyclic drug candidates have seen something of a renaissance in recent years, owing in great part to renewed interest in inhibiting protein−protein interactions and related studies of their unexpected bioavailability and the entropic and energetic benefits of macrocyclization. Chemical synthesis has kept pace, with advances in macrocycle synthesis and library-based screening methods making macrocycle design much more facile. Though computational chemistry tools are becoming increasingly available and are often employed to support structural hypotheses, the application of molecular docking simulations to macrocyclic compounds is often beset by imperfect treatment of macrocycle conformation dynamics. Over-reliance on NMR and X-ray techniques to generate a single macrocycle conformation can belie the significant role that relative conformation energies of macrocycle conformers can play on binding affinity. With scientifically rigorous treatment of macrocycle docking, computational investigations of macrocyclic compounds can continue to serve an important complementary role to biological and synthetic studies.

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Notes

The authors declare no competing financial interest.

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KEYWORDS

CAMDAS: conformational analyzer with molecular dynamics and sampling
CAPRI: critical assessment of prediction of interactions
CDK: cyclin-dependent kinase
CVs: collective variables
CXCR4: C−X−C chemokine motif receptor 4
DMSO: dimethyl sulfoxide
FIRST: floppy inclusion and rigid substructure topography
FLT3: FMS-like tyrosine kinase 3
HCV NS3: hepatitis C virus, nonstructural protein 3
HDAC: histone deacetylase
Hsp90: heat shock protein 90
HTS: high-throughput screen
JAK2: janus kinase 2
LMOD: low mode
MC: Monte Carlo
MC/SD: Monte Carlo/stochastic dynamics
MCMM: Monte Carlo multiple minimum
MD: molecular dynamics
MM-GBSA: molecular mechanics, generalized Born surface area (or, solvent accessibility)
MOE: molecular operating environment
NAMFIS: NMR analysis of molecular flexibility in solution
NMR: nuclear magnetic resonance
NOE: nuclear Overhauser effect
NOESY: nuclear Overhauser effect spectroscopy
PES: potential energy surface
PKCδ: protein kinase C, delta-type
QM: quantum mechanics
REMD: replica exchange molecular dynamics
RGD: arginine-glycine-aspartic acid
RMSD: root-mean-square deviation
ROCK: rigidity-optimized conformational kinetics
ROE: rotating-frame Overhauser enhancement
SAR: structure−activity relationship
SFTI: sunflower trypsin inhibitor
STD-NMR: saturation-transfer difference nuclear magnetic resonance

REFERENCES

(1) Driggers, E. M., Hale, S. P., Lee, J., and Terrett, N. K. (2008) The exploration of macrocycles for drug discovery–an underexploited structural class. *Nat. Rev. Drug Discovery 7*, 608−624.

(2) Heinis, C. (2014) Drug discovery: Tools and rules for macrocycles. *Nat. Chem. Biol. 10*, 696−698.

(3) Sigal, N. H., and Dumont, F. J. (1992) Cyclosporin A, FK-506, and Rapamycin: Pharmacologic Probes of Lymphocyte Signal Transduction. *Annu. Rev. Immunol. 10*, 519−560.

(4) Yu, M. J., Zheng, W., Seletsky, B. M., Littlefield, B. A., and Kishi, Y. (2011) Case History: Discovery of Eribulin (HALAVEN), a

Halichondrin B Analogue That Prolongs Overall Survival in Patients with Metastatic Breast Cancer, in *Annual Reports in Medicinal Chemistry* (Macor, J. E., Ed.) Vol. *46*, pp 227−241, Academic Press.
(5) Moellering, R. E., Cornejo, M., Davis, T. N., Del Bianco, C., Aster, J. C., Blacklow, S. C., Kung, A. L., Gilliland, D. G., Verdine, G. L., and Bradner, J. E. (2009) Direct inhibition of the NOTCH transcription factor complex. *Nature 462*, 182−188.
(6) DeLorbe, J. E., Clements, J. H., Teresk, M. G., Benfield, A. P., Plake, H. R., Millspaugh, L. E., and Martin, S. F. (2009) Thermodynamic and Structural Effects of Conformational Constraints in Protein−Ligand Interactions. Entropic Paradoxy Associated with Ligand Preorganization. *J. Am. Chem. Soc. 131*, 16758−16770.
(7) Okamoto, T., Zobel, K., Fedorova, A., Quan, C., Yang, H., Fairbrother, W. J., Huang, D. C., Smith, B. J., Deshayes, K., and Czabotar, P. E. (2012) Stabilizing the pro-apoptotic BimBH3 helix (BimSAHB) does not necessarily enhance affinity or biological activity. *ACS Chem. Biol. 8*, 297−302.
(8) Yu, X., and Sun, D. (2013) Macrocyclic drugs and synthetic methodologies toward macrocycles. *Molecules 18*, 6230−6268.
(9) Marsault, E., and Peterson, M. L. (2011) Macrocycles Are Great Cycles: Applications, Opportunities, and Challenges of Synthetic Macrocycles in Drug Discovery. *J. Med. Chem. 54*, 1961−2004.
(10) Takahashi, T. T., Austin, R. J., and Roberts, R. W. (2003) mRNA display: ligand discovery, interaction analysis and beyond. *Trends Biochem. Sci. 28*, 159−165.
(11) Li, X., and Liu, D. R. (2004) DNA-Templated Organic Synthesis: Nature's Strategy for Controlling Chemical Reactivity Applied to Synthetic Molecules. *Angew. Chem., Int. Ed. 43*, 4848−4870.
(12) Brandt, W., Joachim Haupt, V., and Wessjohann, L. A. (2010) Chemoinformatic Analysis of Biologically Active Macrocycles. *Curr. Top. Med. Chem. 10*, 1361−1379.
(13) Rezai, T., Yu, B., Millhauser, G. L., Jacobson, M. P., and Lokey, R. S. (2006) Testing the Conformational Hypothesis of Passive Membrane Permeability Using Synthetic Cyclic Peptide Diastereomers. *J. Am. Chem. Soc. 128*, 2510−2511.
(14) Rezai, T., Bock, J. E., Zhou, M. V., Kalyanaraman, C., Lokey, R. S., and Jacobson, M. P. (2006) Conformational Flexibility, Internal Hydrogen Bonding, and Passive Membrane Permeability: Successful in Silico Prediction of the Relative Permeabilities of Cyclic Peptides. *J. Am. Chem. Soc. 128*, 14073−14080.
(15) Hewitt, W. M., Leung, S. S. F., Pye, C. R., Ponkey, A. R., Bednarek, M., Jacobson, M. P., and Lokey, R. S. (2015) Cell-Permeable Cyclic Peptides from Synthetic Libraries Inspired by Natural Products. *J. Am. Chem. Soc. 137*, 715−721.
(16) Villar, E. A., Beglov, D., Chennamadhavuni, S., Porco, J. A., Kozakov, D., Vajda, S., and Whitty, A. (2014) How proteins bind macrocycles. *Nat. Chem. Biol. 10*, 723−731.
(17) Mallinson, J., and Collins, I. (2012) Macrocycles in new drug discovery. *Future Med. Chem. 4*, 1409−1438.
(18) Fairlie, D. P., Abbenante, G., and March, D. R. (1995) Macrocyclic Peptidomimetics - Forcing Peptides into Bioactive Conformations. *Curr. Med. Chem. 2*, 654−686.
(19) Khan, A. R., Parrish, J. C., Fraser, M. E., Smith, W. W., Bartlett, P. A., and James, M. N. (1998) Lowering the entropic barrier for binding conformationally flexible inhibitors to enzymes. *Biochemistry 37*, 16839−16845.
(20) Yudin, A. K. (2015) Macrocycles: lessons from the distant past, recent developments, and future directions. *Chem. Sci. 6*, 30−49.
(21) *The PyMOL Molecular Graphics System*, version 1.5.0.3, Schrödinger, LLC.
(22) Boström, J., Norrby, P.-O., and Liljefors, T. (1998) Conformational energy penalties of protein-bound ligands. *J. Comput.-Aided Mol. Des. 12*, 383−383.
(23) Sitzmann, M., Weidlich, I. E., Filippov, I. V., Liao, C., Peach, M. L., Ihlenfeldt, W.-D., Karki, R. G., Borodina, Y. V., Cachau, R. E., and Nicklaus, M. C. (2012) PDB Ligand Conformational Energies Calculated Quantum-Mechanically. *J. Chem. Inf. Model. 52*, 739−756.
(24) Perola, E., and Charifson, P. S. (2004) Conformational analysis of drug-like molecules bound to proteins: an extensive study of ligand reorganization upon binding. *J. Med. Chem. 47*, 2499−2510.
(25) Nevins, N., Cicero, D., and Snyder, J. P. (1999) A Test of the Single-Conformation Hypothesis in the Analysis of NMR Data for Small Polar Molecules: A Force Field Comparison. *J. Org. Chem. 64*, 3979−3986.
(26) Chang, G., Guida, W. C., and Still, W. C. (1989) An internal-coordinate Monte Carlo method for searching conformational space. *J. Am. Chem. Soc. 111*, 4379−4386.
(27) Lipton, M., and Still, W. C. (1988) The multiple minimum problem in molecular modeling. Tree searching internal coordinate conformational space. *J. Comput. Chem. 9*, 343−355.
(28) Kolossváry, I., and Guida, W. C. (1996) Low mode search. An efficient, automated computational method for conformational analysis: Application to cyclic and acyclic alkanes and cyclic peptides. *J. Am. Chem. Soc. 118*, 5011−5019.
(29) Labute, P. (2010) LowModeMD—Implicit Low-Mode Velocity Filtering Applied to Conformational Search of Macrocycles and Protein Loops. *J. Chem. Inf. Model. 50*, 792−800.
(30) Chen, I.-J., and Foloppe, N. (2013) Tackling the conformational sampling of larger flexible compounds and macrocycles in pharmacology and drug discovery. *Bioorg. Med. Chem. 21*, 7898−7920.
(31) Salomon-Ferrer, R., Case, D. A., and Walker, R. C. (2013) An overview of the Amber biomolecular simulation package. *WIREs Comput. Mol. Sci. 3*, 198−210.
(32) Brooks, B. R., Brooks, C. L., III, Mackerell, A. D., Jr., Nilsson, L., Petrella, R. J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A. R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R. W., Post, C. B., Pu, J. Z., Schaefer, M., Tidor, B., Venable, R. M., Woodcock, H. L., Wu, X., Yang, W., York, D. M., and Karplus, M. (2009) CHARMM: The biomolecular simulation program. *J. Comput. Chem. 30*, 1545−1614.
(33) Pronk, S., Pall, S., Schulz, R., Larsson, P., Bjelkmar, P., Apostolov, R., Shirts, M. R., Smith, J. C., Kasson, P. M., van der Spoel, D., Hess, B., and Lindahl, E. (2013) GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics 29*, 845−854.
(34) Proctor, E. A., Ding, F., and Dokholyan, N. V. (2011) Discrete molecular dynamics. *WIREs Comput. Mol. Sci. 1*, 80−92.
(35) Lei, H., and Duan, Y. (2007) Improved sampling methods for molecular simulation. *Curr. Opin. Struct. Biol. 17*, 187−191.
(36) Earl, D. J., and Deem, M. W. (2005) Parallel tempering: Theory, applications, and new perspectives. *Phys. Chem. Chem. Phys. 7*, 3910−3916.
(37) AMBER Tutorials. http://ambermd.org/tutorials/.
(38) A terrific tutorial for parameterizing dihedrals is available: Ruiz, S. *Small Molecule Dihedrals Parameterization*. http://www.ub.edu/cbdd/?q=content/small-molecule-dihedrals-parametrization.
(39) Terracciano, S., Chini, M. G., Bifulco, G., D'Amico, E., Marzocco, S., Riccio, R., and Bruno, I. (2010) Synthesis of new mono and bis amides projected as potential histone deacetylase (HDAC) inhibitors. *Tetrahedron 66*, 2520−2528.
(40) Villanueva, H. E., and Setzer, W. N. (2010) Cembrene Diterpenoids: Conformational Studies and Molecular Docking to Tubulin. *Rec. Nat. Prod. 4*, 115−123.
(41) Sliwa, A., Dive, G., Zervosen, A., Verlaine, O., Sauvage, E., and Marchand-Brynaert, J. (2012) Unprecedented inhibition of resistant penicillin binding proteins by bis-2-oxoazetidinyl macrocycles. *MedChemComm 3*, 344−351.
(42) Warren, G. L., Andrews, C. W., Capelli, A.-M., Clarke, B., LaLonde, J., Lambert, M. H., Lindvall, M., Nevins, N., Semus, S. F., Senger, S., Tedesco, G., Wall, I. D., Woolven, J. M., Peishoff, C. E., and Head, M. S. (2006) A Critical Assessment of Docking Programs and Scoring Functions. *J. Med. Chem. 49*, 5912−5931.
(43) Huang, S.-Y., and Zou, X. (2010) Advances and Challenges in Protein-Ligand Docking. *Int. J. Mol. Sci. 11*, 3016−3034.

(44) Kroemer, R. T. (2007) Structure-based drug design: docking and scoring. *Curr. Protein Pept. Sci. 8*, 312−328.
(45) Moitessier, N., Englebienne, P., Lee, D., Lawandi, J., and Corbeil, C. R. (2008) Towards the development of universal, fast and highly accurate docking/scoring methods: a long way to go. *Br. J. Pharmacol. 153*, S7−S26.
(46) (a) Yuriev, E., Agostino, M., and Ramsland, P. A. (2011) Challenges and advances in computational docking: 2009 in review. *J. Mol. Recognit. 24*, 149−164. (b) Yuriev, E., and Ramsland, P. A. (2013) Latest developments in molecular docking: 2010−2011 in review. *J. Mol. Recognit. 26*, 215−239.
(47) Sliwoski, G., Kothiwale, S., Meiler, J., and Lowe, E. W. (2014) Computational Methods in Drug Discovery. *Pharmacol. Rev. 66*, 334−395.
(48) (2011) *Virtual Screening: Principles, Challenges, and Practical Guidelines* (Sotriffer, C., Ed.) Wiley-VCH Verlag & Co. KGaA, Weinheim.
(49) Watts, K. S., Dalal, P., Murphy, R. B., Sherman, W., Friesner, R. A., and Shelley, J. C. (2010) ConfGen: A Conformational Search Method for Efficient Generation of Bioactive Conformers. *J. Chem. Inf. Model. 50*, 534−546.
(50) Forli, S., and Botta, M. (2007) Lennard-Jones Potential and Dummy Atom Settings to Overcome the AUTODOCK Limitation in Treating Flexible Ring Systems. *J. Chem. Inf. Model. 47*, 1481−1492.
(51) Canales, J., Fernández, A., Rodrigues, J. R., Ferreira, R., Ribeiro, J. M., Cabezas, A., Costas, M. J., and Cameselle, J. C. (2009) Hydrolysis of the phosphoanhydride linkage of cyclic ADP-ribose by the Mn2+-dependent ADP-ribose/CDP-alcohol pyrophosphatase. *FEBS Lett. 583*, 1593−1598.
(52) Xiong, J.-P., Stehle, T., Zhang, R., Joachimiak, A., Frech, M., Goodman, S. L., and Arnaout, M. A. (2002) Crystal structure of the extracellular segment of integrin alpha Vbeta3 in complex with an Arg-Gly-Asp ligand. *Science 296*, 151−155.
(53) Auzzas, L., Zanardi, F., Battistini, L., Burreddu, P., Carta, P., Rassu, G., Curti, C., and Casiraghi, G. (2010) Targeting avB3 Integrin: Design and Applications of Mono- and Multifunctional RGD-Based Peptides and Semipeptides. *Curr. Med. Chem. 17*, 1255−1299.
(54) Mingozzi, M., Dal Corso, A., Marchini, M., Guzzetti, I., Civera, M., Piarulli, U., Arosio, D., Belvisi, L., Potenza, D., Pignataro, L., and Gennari, C. (2013) Cyclic isoDGR Peptidomimetics as Low-Nanomolar α vβ 3Integrin Ligands. *Chem. - Eur. J. 19*, 3563−3567.
(55) Marchini, M., Mingozzi, M., Colombo, R., Guzzetti, I., Belvisi, L., Vasile, F., Potenza, D., Piarulli, U., Arosio, D., and Gennari, C. (2012) Cyclic RGD Peptidomimetics Containing Bifunctional Diketopiperazine Scaffolds as New Potent Integrin Ligands. *Chem. - Eur. J. 18*, 6195−6207.
(56) Guzzetti, I., Civera, M., Vasile, F., Araldi, E. M., Belvisi, L., Gennari, C., Potenza, D., Fanelli, R., and Piarulli, U. (2013) Determination of the binding epitope of RGD-peptidomimetics to $\alpha v\beta 3$ and αIIbβ3 integrin-rich intact cells by NMR and computational studies. *Org. Biomol. Chem. 11*, 3886−3893.
(57) Cini, N., Trabocchi, A., Menchi, G., Bottoncetti, A., Raspanti, S., Pupi, A., and Guarna, A. (2009) Morpholine-based RGD-cyclopentapeptides as $\alpha v\beta 3/\alpha v\beta 5$ integrin ligands: Role of configuration towards receptor binding affinity. *Bioorg. Med. Chem. 17*, 1542−1549.
(58) Stuart, B. G., Coxon, J. M., Morton, J. D., Abell, A. D., McDonald, D. Q., Aitken, S. G., Jones, M. A., and Bickerstaffe, R. (2011) Molecular Modeling: A Search for a Calpain Inhibitor as a New Treatment for Cataractogenesis. *J. Med. Chem. 54*, 7503−7522.
(59) Abell, A. D., Jones, M. A., Coxon, J. M., Morton, J. D., Aitken, S. G., McNabb, S. B., Lee, H. Y. Y., Mehrtens, J. M., Alexander, N. A., Stuart, B. G., Neffe, A. T., and Bickerstaffe, R. (2009) Molecular Modeling, Synthesis, and Biological Evaluation of Macrocyclic Calpain Inhibitors. *Angew. Chem., Int. Ed. 48*, 1455−1458.
(60) Chen, H., Jiao, W., Jones, M. A., Coxon, J. M., Morton, J. D., Bickerstaffe, R., Pehere, A. D., Zvarec, O., and Abell, A. D. (2012) New tripeptide-based macrocyclic calpain inhibitors formed by N-alkylation of histidine. *Chem. Biodiversity 9*, 2473−2484.
(61) Saupe, S. M., Leubner, S., Betz, M., Klebe, G., and Steinmetzer, T. (2013) Development of New Cyclic Plasmin Inhibitors with Excellent Potency and Selectivity. *J. Med. Chem. 56*, 820−831.
(62) Saupe, S. M., and Steinmetzer, T. (2012) A New Strategy for the Development of Highly Potent and Selective Plasmin Inhibitors. *J. Med. Chem. 55*, 1171−1180.
(63) Poulsen, A., William, A., Lee, A., Blanchard, S., Teo, E., Deng, W., Tu, N., Tan, E., Sun, E., Goh, K. L., Ong, W. C., Ng, C. P., Goh, K. C., and Bonday, Z. (2008) Structure-based design of Aurora A & B inhibitors. *J. Comput.-Aided Mol. Des. 22*, 897−906.
(64) Poulsen, A., William, A., Blanchard, S., Nagaraj, H., Williams, M., Wang, H., Lee, A., Sun, E., Teo, E.-L., Tan, E., Goh, K. C., and Dymock, B. (2013) Structure-based design of nitrogen-linked macrocyclic kinase inhibitors leading to the clinical candidate SB1317/TG02, a potent inhibitor of cyclin dependant kinases (CDKs), Janus kinase 2 (JAK2), and Fms-like tyrosine kinase-3 (FLT3). *J. Mol. Model. 19*, 119−130.
(65) William, A. D., Lee, A. C. H., Goh, K. C., Blanchard, S., Poulsen, A., Teo, E.-L., Nagaraj, H., Lee, C. P., Wang, H., Williams, M., Sun, E. T., Hu, C., Jayaraman, R., Pasha, M. K., Ethirajulu, K., Wood, J. M., and Dymock, B. W. (2012) Discovery of Kinase Spectrum Selective Macrocycle (16 E)-14-Methyl-20-oxa-5,7,14,26-tetraazatetracyclo-[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene (SB1317/TG02), a Potent Inhibitor of Cyclin Dependent Kinases (CDKs), Janus Kinase 2 (JAK2), and Fms-like Tyrosine Kinase-3 (FLT3) for the Treatment of Cancer. *J. Med. Chem. 55*, 169−196.
(66) William, A. D., Lee, A. C. H., Blanchard, S., Poulsen, A., Teo, E.-L., Nagaraj, H., Tan, E., Chen, D., Williams, M., Sun, E. T., Goh, K. C., Ong, W. C., Goh, S. K., Hart, S., Jayaraman, R., Pasha, M. K., Ethirajulu, K., Wood, J. M., and Dymock, B. W. (2011) Discovery of the macrocycle 11-(2-pyrrolidin-1-yl-ethoxy)-14,19-dioxa-5,7,26-triaza-tetracyclo[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8,10,12(27),16,21,23-decaene (SB1518), a potent Janus kinase 2/fms-like tyrosine kinase-3 (JAK2/FLT3) inhibitor for the treatment of myelofibrosis and lymphoma. *J. Med. Chem. 54*, 4638−4658.
(67) Poulsen, A., William, A., Blanchard, S., Lee, A., Nagaraj, H., Wang, H., Teo, E., Tan, E., Goh, K. C., and Dymock, B. (2012) Structure-based design of oxygen-linked macrocyclic kinase inhibitors: discovery of SB1518 and SB1578, potent inhibitors of Janus kinase 2 (JAK2) and Fms-like tyrosine kinase-3 (FLT3). *J. Comput.-Aided Mol. Des. 26*, 437−450.
(68) William, A. D., Lee, A. C. H., Poulsen, A., Goh, K. C., Madan, B., Hart, S., Tan, E., Wang, H., Nagaraj, H., Chen, D., Lee, C. P., Sun, E. T., Jayaraman, R., Pasha, M. K., Ethirajulu, K., Wood, J. M., and Dymock, B. W. (2012) Discovery of the Macrocycle (9 E)-15-(2-(Pyrrolidin-1-yl)ethoxy)-7,12,25-trioxa-19,21,24-triaza-tetracyclo-[18.3.1.1(2,5).1(14,18)]hexacosa-1(24),2,4,9,14(26),15,17,20,22-nonaene (SB1578), a Potent Inhibitor of Janus Kinase 2/Fms-Like-Tyrosine Kinase-3 (JAK2/FLT3) for the Treatment of Rheumatoid Arthritis. *J. Med. Chem. 55*, 2623−2640.
(69) Liverton, N. J., Holloway, M. K., McCauley, J. A., Rudd, M. T., Butcher, J. W., Carroll, S. S., DiMuzio, J., Fandozzi, C., Gilbert, K. F., Mao, S.-S., McIntyre, C. J., Nguyen, K. T., Romano, J. J., Stahlhut, M., Wan, B.-L., Olsen, D. B., and Vacca, J. P. (2008) Molecular Modeling Based Approach to Potent P2−P4Macrocyclic Inhibitors of Hepatitis C NS3/4A Protease. *J. Am. Chem. Soc. 130*, 4607−4609.
(70) Harper, S., McCauley, J. A., Rudd, M. T., Ferrara, M., DiFilippo, M., Crescenzi, B., Koch, U., Petrocchi, A., Holloway, M. K., Butcher, J. W., Romano, J. J., Bush, K. J., Gilbert, K. F., McIntyre, C. J., Nguyen, K. T., Nizi, E., Carroll, S. S., Ludmerer, S. W., Burlein, C., DiMuzio, J. M., Graham, D. J., McHale, C. M., Stahlhut, M. W., Olsen, D. B., Monteagudo, E., Cianetti, S., Giuliano, C., Pucci, V., Trainor, N., Fandozzi, C. M., Rowley, M., Coleman, P. J., Vacca, J. P., Summa, V., and Liverton, N. J. (2012) Discovery of MK-5172, a Macrocyclic Hepatitis C Virus NS3/4a Protease Inhibitor. *ACS Med. Chem. Lett. 3*, 332−336.

(71) Keck, G. E., Poudel, Y. B., Rudra, A., Stephens, J. C., Kedei, N., Lewin, N. E., Peach, M. L., and Blumberg, P. M. (2010) Molecular Modeling, Total Synthesis, and Biological Evaluations of C9-Deoxy Bryostatin 1. *Angew. Chem., Int. Ed. 49*, 4580−4584.
(72) Kimura, K., Mizutani, M. Y., Tomioka, N., Endo, Y., Shudo, K., and Itai, A. (1999) Docking Study of Bryostatins to Protein Kinase C Cys2 Domain. *Chem. Pharm. Bull. 47*, 1134−1137.
(73) Bowers, A. A., Greshock, T. J., West, N., Estiu, G., Schreiber, S. L., Wiest, O., Williams, R. M., and Bradner, J. E. (2009) Synthesis and Conformation−Activity Relationships of the Peptide Isosteres of FK228 and Largazole. *J. Am. Chem. Soc. 131*, 2900−2905.
(74) Karthikeyan, G., Zambaldo, C., Barluenga, S., Zoete, V., Karplus, M., and Winssinger, N. (2012) Asymmetric Synthesis of Pochonin E and F, Revision of Their Proposed Structure, and Their Conversion to Potent Hsp90 Inhibitors. *Chem. - Eur. J. 18*, 8978−8986.
(75) Moulin, E., Zoete, V., Barluenga, S., Karplus, M., and Winssinger, N. (2005) Design, synthesis, and biological evaluation of HSP90 inhibitors based on conformational analysis of radicicol and its analogues. *J. Am. Chem. Soc. 127*, 6999−7004.
(76) Spichty, M., Taly, A., Hagn, F., Kessler, H., Barluenga, S., Winssinger, N., and Karplus, M. (2009) The HSP90 binding mode of a radicicol-like E-oxime determined by docking, binding free energy estimations, and NMR 15N chemical shifts. *Biophys. Chem. 143*, 111−123.
(77) Kolář, M., Fanfrlík, J., Lepšík, M., Forti, F., Luque, F. J., and Hobza, P. (2013) Assessing the accuracy and performance of implicit solvent models for drug molecules: conformational ensemble approaches. *J. Phys. Chem. B 117*, 5950−5962.
(78) Urban, J. J., Cramer, C. J., and Famini, G. R. (1992) A computational study of solvent effects on the conformation of dopamine. *J. Am. Chem. Soc. 114*, 8226−8231.
(79) Marelli, U. K., Frank, A. O., Wahl, B., La Pietra, V., Novellino, E., Marinelli, L., Herdtweck, E., Groll, M., and Kessler, H. (2014) Receptor-Bound Conformation of Cilengitide Better Represented by Its Solution-State Structure than the Solid-State Structure. *Chem. - Eur. J. 20*, 14201−14206.
(80) Kamano, Y., Zhang, H.-P., Morita, H., Itokawa, H., Shirota, O., Pettit, G. R., Herald, D. L., and Herald, C. L. (1996) Conformational analysis of a marine antineoplastic macrolide, bryostatin 10. *Tetrahedron 52*, 2369−2376.
(81) Cicero, D. O., Barbato, G., and Bazzo, R. (1995) NMR Analysis of Molecular Flexibility in Solution: A New Method for the Study of Complex Distributions of Rapidly Exchanging Conformations. Application to a 13-Residue Peptide with an 8-Residue Loop. *J. Am. Chem. Soc. 117*, 1027−1033.
(82) Karplus, M. (1959) Contact Electron-Spin Coupling of Nuclear Magnetic Moments. *J. Chem. Phys. 30*, 11−15.
(83) Haasnoot, C. A. G., de Leeuw, F. A. A. M., and Altona, C. (1980) The relationship between proton-proton NMR coupling constants and substituent electronegativities—An empirical generalization of the Karplus equation. *Tetrahedron 36*, 2783−2792.
(84) Jogalekar, A. S., Damodaran, K., Kriel, F. H., Jung, W.-H., Alcaraz, A. A., Zhong, S., Curran, D. P., and Snyder, J. P. (2011) Dictyostatin Flexibility Bridges Conformations in Solution and in the β-Tubulin Taxane Binding Site. *J. Am. Chem. Soc. 133*, 2427−2436.
(85) Canales, A., Matesanz, R., Gardner, N. M., Andreu, J. M., Paterson, I., Díaz, J. F., and Jiménez-Barbero, J. (2008) The Bound Conformation of Microtubule-Stabilizing Agents: NMR Insights into the Bioactive 3D Structure of Discodermolide and Dictyostatin. *Chem. - Eur. J. 14*, 7557−7569.
(86) Thepchatri, P., Eliseo, T., Cicero, D. O., Myles, D., and Snyder, J. P. (2007) Relationship Among Ligand Conformations in Solution, in the Solid State, and at the Hsp90 Binding Site: Geldanamycin and Radicicol. *J. Am. Chem. Soc. 129*, 3127−3134.
(87) Rapp, C., Kalyanaraman, C., Schiffmiller, A., Schoenbrun, E. L., and Jacobson, M. P. (2011) A molecular mechanics approach to modeling protein-ligand interactions: relative binding affinities in congeneric series. *J. Chem. Inf. Model. 51*, 2082−2089.
(88) Graves, A. P., Shivakumar, D. M., Boyce, S. E., Jacobson, M. P., Case, D. A., and Shoichet, B. K. (2008) Rescoring Docking Hit Lists for Model Cavity Sites: Predictions and Experimental Testing. *J. Mol. Biol. 377*, 914−934.
(89) Thepchatri, P., Cicero, D. O., Monteagudo, E., Ghosh, A. K., Cornett, B., Weeks, E. R., and Snyder, J. P. (2005) Conformations of Laulimalide in DMSO- d6. *J. Am. Chem. Soc. 127*, 12838−12846.
(90) Bennett, M. J., Barakat, K., Huzil, J. T., Tuszynski, J., and Schriemer, D. C. (2010) Discovery and Characterization of the Laulimalide-Microtubule Binding Mode by Mass Shift Perturbation Mapping. *Chem. Biol. 17*, 725−734.
(91) Nguyen, T. L., Xu, X., Gussio, R., Ghosh, A. K., and Hamel, E. (2010) The Assembly-Inducing Laulimalide/Peloruside A Binding Site on Tubulin: Molecular Modeling and Biochemical Studies with [3H]Peloruside A. *J. Chem. Inf. Model. 50*, 2019−2028.
(92) Prota, A. E., Bargsten, K., Northcote, P. T., Marsh, M., Altmann, K.-H., Miller, J. H., Díaz, J. F., and Steinmetz, M. O. (2014) Structural Basis of Microtubule Stabilization by Laulimalide and Peloruside A. *Angew. Chem., Int. Ed. 53*, 1621−1625.
(93) Walther, T., Renner, S., Waldmann, H., and Arndt, H.-D. (2009) Synthesis and Structure-Activity Correlation of a Brunsvicamide-Inspired Cyclopeptide Collection. *ChemBioChem 10*, 1153−1162.
(94) Wienrich, B. G., Krahn, T., Schön, M., Rodriguez, M. L., Kramer, B., Busemann, M., Boehncke, W. H., and Schön, M. P. (2006) Structure−Function Relation of Efomycines, a Family of Small-Molecule Inhibitors of Selectin Functions. *J. Invest. Dermatol. 126*, 882−889.
(95) Iwai, Y., Murakami, K., Gomi, Y., Hashimoto, T., Asakawa, Y., Okuno, Y., Ishikawa, T., Hatakeyama, D., Echigo, N., and Kuzuhara, T. (2011) Anti-Influenza Activity of Marchantins, Macrocyclic Bisbibenzyls Contained in Liverworts. *PLoS One 6*, e19825.
(96) Wu, G., Robertson, D. H., Brooks, C. L., and Vieth, M. (2003) Detailed analysis of grid-based molecular docking: A case study of CDOCKER−A CHARMm-based MD docking algorithm. *J. Comput. Chem. 24*, 1549−1562.
(97) Li, X., Tu, Z., Li, H., Liu, C., Li, Z., Sun, Q., Yao, Y., Liu, J., and Jiang, S. (2013) Biological Evaluation of New Largazole Analogues: Alteration of Macrocyclic Scaffold with Click Chemistry. *ACS Med. Chem. Lett. 4*, 132−136.
(98) Inman, W. D., and Crews, P. (2011) Unraveling the Bastarane and Isobastarane Oximo Amide Configurations and Associated Macrocycle Conformations: Implications of Their Influence on Bioactivities. *J. Nat. Prod. 74*, 402−410.
(99) Utsintong, M., Kaewnoi, A., Leelamanit, W., Olson, A. J., and Vajragupta, O. (2009) Rediocides A and G as Potential Antitoxins Against Cobra Venom. *Chem. Biodiversity 6*, 1404−1414.
(100) Gentilucci, L., Cardillo, G., Spampinato, S., Tolomelli, A., Squassabia, F., De Marco, R., Bedini, A., Baiula, M., Belvisi, L., and Civera, M. (2010) Antiangiogenic Effect of Dual/Selective $\alpha_5\beta_1/\alpha_v\beta_3$ Integrin Antagonists Designed on Partially Modified Retro-Inverso Cyclotetrapeptide Mimetics. *J. Med. Chem. 53*, 106−118.
(101) Vilaça, H., Ferreira, P. M. T., and Micaelo, N. M. (2014) New cyclic RGD peptides: synthesis, characterization, and theoretical activity towards $\alpha_v\beta_3$ integrin. *Tetrahedron 70*, 5420−5427.
(102) Tamamura, H., Hiramatsu, K., Ueda, S., Wang, Z., Kusano, S., Terakubo, S., Trent, J. O., Peiper, S. C., Yamamoto, N., Nakashima, H., Otaka, A., and Fujii, N. (2005) Stereoselective Synthesis of [l-Arg- l/d-3-(2-naphthyl)alanine]-Type (E)-Alkene Dipeptide Isosteres and Its Application to the Synthesis and Biological Evaluation of Pseudopeptide Analogues of the CXCR4 Antagonist FC131. *J. Med. Chem. 48*, 380−391.
(103) Tamamura, H., Tsutsumi, H., Masuno, H., Mizokami, S., Hiramatsu, K., Wang, Z., Trent, J. O., Nakashima, H., Yamamoto, N., Peiper, S. C., and Fujii, N. (2006) Development of a linear type of low molecular weight CXCR4 antagonists based on T140 analogs. *Org. Biomol. Chem. 4*, 2354−2357.
(104) Yoshikawa, Y., Kobayashi, K., Oishi, S., Fujii, N., and Furuya, T. (2012) Molecular modeling study of cyclic pentapeptide CXCR4

antagonists: New insight into CXCR4−FC131 interactions. *Bioorg. Med. Chem. Lett. 22*, 2146−2150.
(105) Granzhan, A., Largy, E., Saettel, N., and Teulade-Fichou, M.-P. (2010) Macrocyclic DNA-Mismatch-Binding Ligands: Structural Determinants of Selectivity. *Chem. - Eur. J. 16*, 878−889.
(106) Jourdan, M., Granzhan, A., Guillot, R., Dumy, P., and Teulade-Fichou, M. P. (2012) Double threading through DNA: NMR structural study of a bis-naphthalene macrocycle bound to a thymine-thymine mismatch. *Nucleic Acids Res. 40*, 5115−5128.
(107) Kellogg, G. E., and Abraham, D. J. (2000) Hydrophobicity: is LogP o/w more than the sum of its parts? *Eur. J. Med. Chem. 35*, 651−661.
(108) Huzil, J. T., Chik, J. K., Slysz, G. W., Freedman, H., Tuszynski, J., Taylor, R. E., Sackett, D. L., and Schriemer, D. C. (2008) A Unique Mode of Microtubule Stabilization Induced by Peloruside A. *J. Mol. Biol. 378*, 1016−1030.
(109) Bai, R., Nguyen, T. L., Burnett, J. C., Atasoylu, O., Munro, M. H. G., Pettit, G. R., Smith, A. B., III, Gussio, R., and Hamel, E. (2011) Interactions of Halichondrin B and Eribulin with Tubulin. *J. Chem. Inf. Model. 51*, 1393−1404.
(110) Gouda, H., Terashima, S., Iguchi, K., Sugawara, A., Saito, Y., Yamamoto, T., Hirose, T., Shiomi, K., Sunazuka, T., Ōmura, S., and Hirono, S. (2009) Molecular modeling of human acidic mammalian Chitinase in complex with the natural-product cyclopentapeptide Chitinase inhibitor argifin. *Bioorg. Med. Chem. 17*, 6270−6278.
(111) Gouda, H., Sunazuka, T., Iguchi, K., Sugawara, A., Hirose, T., Noguchi, Y., Saito, Y., Yanai, Y., Yamamoto, T., Watanabe, T., Shiomi, K., Ōmura, S., and Hirono, S. (2009) Computer-aided rational molecular design of argifin-derivatives with increased inhibitory activity against Chitinase B from Serratia marcescens. *Bioorg. Med. Chem. Lett. 19*, 2630−2633.
(112) Tsujishita, H., and Hirono, S. (1997) Camdas: An automated conformational analysis system using molecular dynamics - Springer. *J. Comput.-Aided Mol. Des. 11*, 305−315.
(113) Waldmann, H., Hu, T.-S., Renner, S., Menninger, S., Tannert, R., Oda, T., and Arndt, H.-D. (2008) Total Synthesis of Chondramide C and Its Binding Mode to F-Actin. *Angew. Chem., Int. Ed. 47*, 6473−6477.
(114) Zanotti, G., Falcigno, L., Saviano, M., D'Auria, G., Bruno, B. M., Campanile, T., and Paolillo, L. (2001) Solid state and solution conformation of [Ala7]-phalloidin: a synthetic phallotoxin analogue. *Chem. - Eur. J. 7*, 1479−1485.
(115) Hao, J., Serohijos, A. W. R., Newton, G., Tassone, G., Wang, Z., Sgroi, D. C., Dokholyan, N. V., and Basilion, J. P. (2008) Identification and rational redesign of peptide ligands to CRIP1, a novel biomarker for cancers. *PLoS Comput. Biol. 4*, e1000138.
(116) Bockus, A. T., McEwen, C. M., and Lokey, R. S. (2013) Form and Function in Cyclic Peptide Natural Products: A Pharmacokinetic Perspective. *Curr. Top. Med. Chem. 13*, 821−836.
(117) (2015) *Computational Peptidology* (Zhou, P., and Huang, J., Eds.) Springer, New York.
(118) Discrete molecular dynamics simulations are showing potential in peptide−protein docking: Dagliyan, O., Proctor, E. A., D'Auria, K. M., Ding, F., and Dokholyan, N. V. (2011) Structural and Dynamic Determinants of Protein-Peptide Recognition. *Structure 19*, 1837−1845.
(119) Raveh, B., London, N., and Schueler-Furman, O. (2010) Sub-angstrom modeling of complexes between flexible peptides and globular proteins. *Proteins: Struct., Funct., Genet. 78*, 2029−2040.
(120) Janin, J. (2005) Assessing predictions of protein-protein interaction: The CAPRI experiment. *Protein Sci. 14*, 278−283.
(121) London, N., Raveh, B., Movshovitz-Attias, D., and Schueler-Furman, O. (2010) Can self-inhibitory peptides be derived from the interfaces of globular protein-protein interactions? *Proteins: Struct., Funct., Genet. 78*, 3140−3149.
(122) Avrutina, O., Fittler, H., Glotzbach, B., Kolmar, H., and Empting, M. (2012) Between two worlds: a comparative study on in vitro and in silico inhibition of trypsin and matriptase by redox-stable SFTI-1 variants at near physiological pH. *Org. Biomol. Chem. 10*, 7753−7762.
(123) Laio, A., and Parrinello, M. (2002) Escaping free-energy minima. *Proc. Natl. Acad. Sci. U. S. A. 99*, 12562−12566.
(124) Laio, A., and Gervasio, F. L. (2008) Metadynamics: a method to simulate rare events and reconstruct the free energy in biophysics, chemistry and material science. *Rep. Prog. Phys. 71*, 126601.
(125) Spitaleri, A., Ghitti, M., Mari, S., Alberici, L., Traversari, C., Rizzardi, G.-P., and Musco, G. (2011) Use of Metadynamics in the Design of isoDGR-Based $\alpha v\beta 3$ Antagonists To Fine-Tune the Conformational Ensemble. *Angew. Chem., Int. Ed. 50*, 1832−1836.
(126) Viles, J. H., Mitchell, J. B. O., Gough, S. L., Doyle, P. M., Harris, C. J., Sadler, P. J., and Thornton, J. M. (1996) Multiple Solution Conformations of the Integrin-Binding Cyclic Pentapeptide Cyclo(-Ser-d-Leu-Asp-Val-Pro-). Analysis of the (phi,psi) Space Available to Cyclic Pentapeptides. *Eur. J. Biochem. 242*, 352−362.
(127) Våbenø, J., Nikiforovich, G. V., and Marshall, G. R. (2006) A minimalistic 3D pharmacophore model for cyclopentapeptide CXCR4 antagonists. *Biopolymers 84*, 459−471.
(128) Nemethy, G., Pottle, M. S., and Scheraga, H. A. (1983) Energy parameters in polypeptides. 9. Updating of geometrical parameters, nonbonded interactions, and hydrogen bond interactions for the naturally occurring amino acids. *J. Phys. Chem. 87*, 1883−1887.
(129) Mungalpara, J., Thiele, S., Eriksen, Ø., Eksteen, J., Rosenkilde, M. M., and Våbenø, J. (2012) Rational Design of Conformationally Constrained Cyclopentapeptide Antagonists for C-X-C Chemokine Receptor 4 (CXCR4). *J. Med. Chem. 55*, 10287−10291.
(130) Thiele, S., Mungalpara, J., Steen, A., Rosenkilde, M. M., and Våbenø, J. (2014) Determination of the binding mode for the cyclopentapeptide CXCR4 antagonist FC131 using a dual approach of ligand modifications and receptor mutagenesis. *Br. J. Pharmacol. 171*, 5313−5329.
(131) Lei, M., Zavodszky, M. I., Kuhn, L. A., and Thorpe, M. F. (2004) Sampling protein conformations and pathways. *J. Comput. Chem. 25*, 1133−1148.
(132) Rader, A. J., Hespenheide, B. M., Kuhn, L. A., and Thorpe, M. F. (2002) Protein unfolding: Rigidity lost. *Proc. Natl. Acad. Sci. U. S. A. 99*, 3540−3545.
(133) Zavodszky, M. I., Lei, M., Thorpe, M. F., Day, A. R., and Kuhn, L. A. (2004) Modeling correlated main-chain motions in proteins for flexible molecular recognition. *Proteins: Struct., Funct., Genet. 57*, 243−261.