Macrocycle and Constrained Peptide Simulations
Macro cyclic drug-like molecules are being explored in many active drug development programs. Although larger than small drug-like molecules, these structures are much smaller than proteins, which raises unique challenges both experimentally and computationally.

Schrödinger provides a large body of applications that can solve most of the challenges these molecules will bring. In this paper we highlight particular advances in molecular modeling, molecular dynamics, ligand-receptor docking, and biologics that were designed to handle these challenges. Our software also has methods for analyzing the hydrophobicity of these structures, which will provide a better understanding of their true conformation and detailed information on the structure's properties.

**Conformational Analysis**

The utility of macrocyclic molecules as drug candidates stems, in part, from their diversity and flexibility, and also from the benefits of restricting conformational

A few of the macrocycles from the PDB (4 letter codes) used to validate macrocycle protocols.
space of these large molecules by cyclizing them. A key component of computational studies of macrocycles involves determining what low-energy conformations such macrocycles can adopt. The restriction on conformational space can cause it to be subdivided into regions separated by high energy barriers. Since macrocyclic drug-like molecules are larger than typical small-molecule drugs, they have considerable flexibility within the rings. In addition, extended non-cyclic flexible branches can be bonded to these rings, which means that these molecules provide difficult challenges to most search methods due to the size and segmented nature of their conformational ensembles.

Our conformational analysis software’s conformational search protocol for macrocycles consists of a unique combination of large scale low mode search steps with simulation cycles composed of alternating stochastic dynamics and minimization calculations, which permits both broad and intensive local exploration of conformational space. This method has been carefully calibrated to reproduce the conformations of a diverse, yet representative, collection of 100 drug-like macrocyclic compounds from the Cambridge Structural Database (CSD) and the Protein Data Bank with molecular weights up to 1700 Daltons, containing up to 40 atoms in a ring, with on average 8 non-ring rotatable bonds and consisting of natural and unnatural peptides as well as non-peptidic components. This protocol excels at reproducing the ring conformations of these molecules giving a median RMSD value of 0.36 Å for the ring atoms with respect to the crystal structure and outperforms competing methods on a publicly available dataset of macrocycles.

While this protocol handles the macrocyclic portions of these molecules very well, the conformations of the large non-ring portions of some of these molecules can be sensitive to the environment (e.g. in a protein-macrocycle complex). Follow-on calculations with ligand-receptor docking, induced-fit docking, or protein structure prediction software, using the ring conformations generated by this protocol may be key to a successful study. The breadth of Schrödinger’s industry-leading applications uniquely enables such solution-directed studies of these systems.

Hydrophobicity can be of crucial importance for understanding the specificity and activity of peptides, particularly macrocyclic oligopeptides. Hydrophobicity can be calculated using a variety of our methods:

**Rapid ADME prediction** software uses topological descriptors to very rapidly calculate hydrophobicity, which can be useful for calculating changes in hydrophobicity when small localized changes to molecular structure are made for large flexible molecules like macrocycles.

**Molecular modeling and protein prediction** software can be used to generate sample conformations and solvation free energies using implicit solvent calculations.

**Molecular Dynamics** software can perform simulations of macrocyclic molecules in explicit solvents, such as water and octanol. The simulation event analysis tool can analyse the resulting trajectories to build predictive models for hydrophobicity using a number of applications.
**Molecular Dynamics**

Our software includes a high-performance molecular dynamics simulation engine for biomolecular systems that combines speed and accuracy to create a powerful way to sample conformations of macrocycles. This approach can provide an accurate representation of the atomic movements of macrocycles, which can be used to understand shape, stability, and energetics. Our software provides powerful and intuitive graphical interfaces for system setup, running simulations, and analyzing trajectories.

We used our molecular dynamics simulations software to study a series of stabilized stapled α-helical peptides over a range of temperatures in solution. The predicted α-helical propensities derived from the simulations were in good agreement with the experimentally observed circular dichroism melting curves. The local flexibility of key residues could be related to differences in affinity of the stapled peptides binding to MDM2. These simulations provide new insights into the design of α-helical stapled peptides and the development of potent inhibitors of α-helical protein–protein interfaces. A video highlighting this work can be found here: http://youtu.be/WScPbvUwDno

**Ligand-Receptor Docking**

Efficient sampling of ring conformations is essential to reproduce experimental binding modes in docking experiments, particularly for macrocycles where there can be hundreds of low energy conformations.

Our ligand-receptor docking software relies on an extensive database of ring conformations to sample low energy states and otherwise does not sample macrocycle ring systems. The default ring templates for macrocycles considerably improve native pose prediction.

A representative example for the macrocycle from 2QZK is shown in this figure where the native ligand buries the macrocycle ring into a mostly enclosed pocket. To bind in this pocket requires a particular ring conformation that forms key interactions to ligand carbonyl and protonated amine moieties. With standard ring templates, a suitable macrocycle conformation is found and the docked pose has a low RMSD of 0.22 Å. Without the ring templates, even with a visually similar ring conformation with RMSD=0.71 Å for macrocycle ring atoms, our software positions the ring outside the pocket leading to a very large RMSD of 10.23 Å.

Pose accuracy can be further improved by docking multiple low-energy macrocycle conformations and selecting the best-docked pose by GlideScore. By docking
structures generated by our molecular modeling software's macrocyclic sampling procedure, the median RMSD was 1.54 Å compared to 2.76 Å docking only a single conformation from LigPrep with ring templates active. Our software finds poses with RMSD < 2.0 Å that are suitable for structural analysis and SAR work in 61% of the macrocycles. By augmenting the default set of ring templates with ring templates for these macrocycles virtually eliminated instances where these macrocycles failed to dock.

Comparison of the 1JFF protein with the crystallographic ligand conformation (green) overlaid with our ligand-receptor docking software's top-ranked docked conformation (blue). On the right is the top-ranked docked conformation without a template and on the left is the much more accurate top-ranked docked conformation with a template.

**Biologics**

Our biologics software can vary peptidic residues within proteins and other molecules, such as macrocycles, containing amino acid residues as well as non-peptidic components to rapidly generate variations in the sequence for general use. Biologics can assist in the selection of promising sequences by optimizing intermolecular interactions between the scanned species and another molecule. For instance, one can scan the amino acid residues in a macrocycle and prioritize the variations generated based upon how well the resulting molecule interacts with a given protein.

With the exploration of macrocyclic drug-like molecules as an effective therapeutic agent, the tools to study and predict the properties of these compounds are developing rapidly. Here at Schrödinger, we are continuously expanding and refining our tools to make more accurate predictions on pharmaceutically-relevant molecules.

