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Macromolecular modeling and design in Rosetta: recent methods and frameworks

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Abstract

The Rosetta software for macromolecular modeling, docking, and design is extensively used in laboratories worldwide. During two decades of development by a community of laboratories at more than 60 institutions, Rosetta has been continuously refactored and extended. Here we review tools developed in the last five years, including over 80 methods. We discuss improvements to the score function, user interfaces, and usability. Rosetta is available at www.rosettacommons.org.

Editorial summary

Tools developed over the past five years in the macromolecular modeling, docking and design software Rosetta are reviewed in this Perspective.

Introduction

The understanding that molecular structure determines biological function has motivated decades of experimental determination of protein structure and function. Many computational packages have been developed to guide experimental methods and elucidate macromolecular structure, including Rosetta. Rosetta offers capabilities spanning many bioinformatics and structural-bioinformatics tasks. Computational structural biology frameworks with similarly comprehensive scope are few, but key to progress in biology. Schrodinger[1], the Molecular Operating Environment[2], and Discovery Studio[3] are computational chemistry platforms for advanced modeling and design for structural biology, drug discovery and material science, based on molecular mechanics, molecular dynamics and quantum mechanics calculations. The HHSuite[4] includes tools for bioinformatics, sequence alignments, structure prediction and modeling. The BioChemicalLibrary[5] (BCL) includes tools for structure prediction, drug discovery, and several sequence-to-structure methods using machine learning approaches. The Integrative Modeling Platform[6] (IMP) models large macromolecular complexes by incorporating various types of experimental data. OpenBabel[7] is a ChemInformatics toolbox supporting molecular mechanics calculations, being most heavily used for interconversion of file formats.

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JKL wrote the manuscript with help from BDW. All authors edited and approved the manuscript and were substantially involved in developing the methods described, either by conception of the ideas or by implementing the methods into Rosetta. The idea for this paper was conceived by RB.

Molecular dynamics packages like CHARMM[8], AMBER[9], GROMACS[10] and others simulate most atoms explicitly with a physics-based energy function that relies on solving Newton's equation of motion. These methods can be used for folding small proteins, model refinement, modeling phenomena such as ion flow through membrane channels, and modeling interactions with small molecules and are therefore highly complementary to Rosetta. OpenMM[11] is an API (application programming interface) for setting up molecular simulations and can be used as a library or standalone application.

Many other tools are available for more specialized tasks, for instance for *de novo* modeling (AlphaFold[12,13], QUARK[14], RaptorX[15]), homology modeling (Modeller[16], SwissModel[17]), fold recognition (iTasser[18]), protein-protein docking (HADDOCK[19], Zdock[20], ClusPro[21]), ligand docking (AutoDock[22], FlexX[23], Glide[24]) and numerous other tasks requiring molecular modeling. As the focus here is on Rosetta developments, a comprehensive list of related methods is listed in the Supplementary Note.

One of Rosetta's advantages is inter-operability of its large number of applications; however, this makes it challenging to track the scope of functionality available to scientists who wish to use the software. This Perspective is meant to guide new, returning, or seasoned users; to help them find the right protocol hiding in the Rosetta haystack.

Development of Rosetta started in the mid-1990s; it was initially aimed at protein structure prediction and protein folding[25]. Over time, the number of applications grew to address diverse modeling tasks, from protein–protein or –small molecule docking to incorporating NMR data, loop modeling, protein design, and interaction with peptides and nucleic acids (Figure 1). Over more than 20 years, the community of developers and scientists, the RosettaCommons, grew from a single academic laboratory to laboratories at over 60 institutions wordwide[26]. The software has undergone several transitions, including in programming language and implementation, with the latest protocols based on Rosetta3, first released in 2008[27]. The score function has been continuously improved and has been described in [28] and [29]. As part of our sustained focus on accessibility, usability, and scientific reproducibility, we developed several interfaces (PyRosetta[30], RosettaScripts[31], Foldit[32]), and emphasized publishing protocol captures[33] to accompany manuscripts. As those interfaces have grown more versatile and modular, development has accelerated and branched in many directions. However, this interoperability, extensibility and modularity enable scientists to combine modules in a wide variety of combinations, making it difficult to keep up with all the developments within the software and the scientific community. Here we have compiled the latest method developments in Rosetta from the past five years, divided into several categories; we provide direction on where to find further information for specific modeling problems. The Supplementary Note contains more details on the protocols with extensive links to documentation, resources on the web, limitations, and competitors.

1. General overview and challenges

A typical Rosetta protocol is outlined in Figure 2A: the conformation of a biomolecule (the *Pose*) is altered, either deterministically or stochastically, via a *Mover* and the resulting conformation is evaluated by a *ScoreFunction*. The *Move* is accepted based on the

Metropolis criterion and the energy difference between the original and the new conformation:

$$\text{if } E_{new} < E_{orig} \quad \text{accept}$$

$$\text{if } E_{new} \geq E_{orig} \quad \text{accept with probability } P = e^{-\left(\left(E_{new} - E_{orig}\right)/T\right)}$$

Many independent trajectories are generated, and the final models are evaluated based on the scientific objective. This setup highlights common limitations in Rosetta protocols involving sampling, scoring (discussed in the score function section), or technical challenges. Many protocols suffer from under-sampling[34], especially when flexibility is involved. Sampling is a limitation for structure prediction (especially for large structures), protein design and unconstrained global protein-protein docking. For example, even with local docking we are limited by backbone flexibility and performance deteriorates with larger flexibility in the binding interface. Small molecule docking similarly relies on correct identification of the binding interface and is limited by flexibility between unbound and bound states. Enormous conformational search spaces are also prohibitive for RNA modeling due to the size and combinatorics of their torsion space (see RNA section), membrane proteins due to their size, and carbohydrates because of branching and flexibility.

Some Rosetta applications suffer from (1) technical challenges in implementation, (2) a lack of documentation, protocol captures, or support, and (3) a need for more diverse chemistries for biomolecules. Technical challenges are either historical or due to lack of interest in the community to develop and advance methods in these unique areas.

2. Rosetta's score function

Rosetta's score function has been continuously improved over many years[35] with guiding principles including: improving speed of computation, increasing extensibility, and improving accuracy across multiple tasks. The main score function is a linear combination of weighted score terms that balances physics-based and statistically derived potentials describing *van der Waals* energies, hydrogen bonds, electrostatics, disulfide bonds, residue solvation, backbone torsion angles, sidechain rotamer energies, and an average unfolded state reference energy (Figure 2B):

$$E = E_{vdW} + E_{hbond} + E_{elec} + E_{disulf} + E_{solv} + E_{BBtorsion} + E_{rotamer} + E_{ref}$$

Some energy terms are decomposed into several components to parameterize each of them separately. For instance, the *van der Waals* energy is split into attractive and repulsive terms between different residues, in addition to an intra-residue repulsive term. A detailed account of the all-atom score function was published recently[28].

The newest score function REF2015[29] reproduces thermodynamic observables (such as liquid-phase properties[36] and liquid-to-vapor transfer free energies[37]) in addition to structure[38]-based tests. It also utilizes a new, derivative-free optimization technique, which is suitable for robust optimization of >100 parameters. Further, a new energy term was added that takes into consideration non-ideality of bond lengths and angles in cartesian space[39]. The cartesian term[39] is also the basis for a *cartesian_ddG* method that has been used to calculate ΔΔGs of mutations to assess changes in protein stability. Only the backbones and side chains of residues near the mutation site are allowed to move[40]. Due to the local optimization, this protocol is much faster than the previous gold-standard *ddg_monomer*[41], while retaining the same level of accuracy. REF2015 is now compatible with an expanded palette of chemical building-blocks: canonical and non-canonical L-α-amino acids and their D-amino acid counterparts, exotic achiral amino acids, peptoids, and oligoureas, and can model metalloproteins[42]. Score functions that enable simultaneous modeling of protein and RNA are being explored[43]. REF2015 is now thread-safe and fully mirror symmetric, i.e. enantiomers in mirror conformations score identically. Guidance energy terms for design have been added to encourage certain features, such as specific amino acid compositions[44,45], hydrogen bonding networks, or global or local net charges, and discourage others, such as repeat sequences that hinder NMR assignments, buried unsatisfied hydrogen bond donors and acceptors, or voids within the protein[46].

Hydrogen bond networks are important for biomolecular structure and catalysis but have been challenging to design because of pairwise interactions that have multi-body, cooperative properties. The HBNet protocol[47] has been used to design *de novo* coiled coils with interaction specificity mediated by designed hydrogen bond networks, including homo-oligomers[47], membrane proteins[48], and large sets of orthogonal heterodimers[49]. An improvement to HBNet uses a Monte Carlo search to sample hydrogen bond networks with drastically improved performance[50]. We further developed a statistical potential to place highly-coordinated water molecules on the surface of biomolecules. On a data set of 153 high-resolution protein-protein interfaces, the method predicts 17% of native interface waters with 20% precision within 0.5 Å of the crystallographic water positions[51]. The potential is accessible through the ExplicitWaterMover (former: WaterBoxMover) in RosettaScripts.

There are still several limitations to the score function: (1) it does not directly estimate entropy[52], which has been shown to improve sampling efficiency[53]. However, rotamer bond angles, solvation, fragments and pair terms all implicitly model this component of the free energy, which at these temperatures and solvation densities account for more than half of the entropy. (2) In most cases, knowledge-based score terms are derived from high-resolution crystal structures, representing a single state on the energy landscape and do not represent flexibility well (compared to solution NMR); (3) knowledge-based terms are less interpretable and transferable than physics-based terms; (4) scoring performance scales with the number of score terms and has become slower, yet more accurate, over time; (5) the solvation model is implicit, hence fast, but hinders explicit modeling of ions, water molecules, or lipid environments; (6) several score functions for specific applications (RNA, membrane proteins, carbohydrates, non-canonical amino acids) are still developing.

3. Major applications

Predicting protein structures—Rosetta was originally developed for *de novo* protein structure prediction, assembling fragments from known protein structures *via* a Monte Carlo procedure and evaluating the models with the score function. While the community's main goals have moved to macromolecular design over the past decade, performance in the CASP13 blind prediction challenge remains respectable[54], with ranking for refinement and prediction of multimeric complexes among the top three groups. Meanwhile, other groups have refined their tools exploiting evolutionary couplings and machine learning, for instance Google's DeepMind developed AlphaFold[12,13] (which uses Rosetta for refinement) with outstanding performance in the recent CASP13[54]. Another highly ranking method is the Zhang server built on iTasser[14], and QUARK[14].

Homology modeling was improved by using multiple templates in RosettaCM[55] (now available on the new Robetta[56,57] server), which hybridizes the most homologous portions from multiple templates into a single model, while modeling missing residues *de novo*[55]. Without a template, predicting protein structures *de novo*, remains one of the most challenging tasks in structural biology, even though the incorporation of evolutionary coupling constraints (for instance from GREMLIN[58]) has led to enormous improvements in model quality. An iterative hybridize approach improves sampling and uses a genetic algorithm that recombines models from an input pool to create models that have features from their parents but are also distinct. Creating several child models in each iteration, updating the input pool, and performing 30–50 iterations led to improved model accuracy because features that are scored favorably are repeatedly used in the recombination, such that the models in the pool converge over time. Iterative hybridization has been used to improve model quality of *de novo* predicted models[59] as well as homology models[60]. Model refinement or generating ensembles of structures (useful for design) can be accomplished by several algorithms in Rosetta: *FastRelax*[61], *Backrub*[62], or vicinity sampling using KIC/Next-Generation-KIC loop modeling [63,64]. Loop modeling[65] was implemented early in Rosetta[66,67], with initial approaches relying on fragments sampling and iterative Cyclic Coordinate Descent (CCD)[68] for chain closure. Later, a kinematic closure (termed "KIC") approach relied on polynomial resultants to analytically solve for closed conformations, producing more native-like loops[69,70]. Next-Generation KIC (NGK)[64] is a recent innovation that improves sampling by employing diversification (i.e. wider range of conformations) and intensification (i.e. focus around previously generated conformations), substantially increasing the fraction of near-native models[64] and modeling longer loops. A related method, GeneralizedKIC[44] (GenKIC) samples loop geometries between fixed endpoints including non-standard peptide chemistries or chemistries that conventional loop-modelling algorithms do not typically handle.

Modeling protein–protein complexes—Another early expansion of Rosetta's functionality was RosettaDock, a method for predicting the structure of protein-protein complexes. The latest version, RosettaDock4.0[74] incorporates protein flexibility from pre-generated protein ensembles, mimicking conformer selection. This has improved sampling efficiency by automatically adjusting the sampling rate based on the diversity of the input ensembles. Scoring has been improved by a six-dimensional coarse-grained scoring scheme called *motif_dock_score*, employing score grids generated from known complexes in the Protein Data Bank (PDB). In local docking benchmarks with backbone deviations of up to 2.2 Å, RosettaDock4.0 successfully docked ~50% of complexes[74]. For symmetric homomers, Rosetta SymDock2[75] uses the same six-dimensional scoring scheme as RosettaDock. Symmetry information can be extracted from a homologous complex, or from a global docking search for a given point symmetry using our symmetry framework[152]. An induced-fit based all-atom refinement relieves clashes in tightly-packed complexes to give physically realistic models. On a benchmark set of 43 complexes with different cyclic and dihedral symmetries, global docking on homology models had accuracies of 61% and 42% for cyclic and dihedral symmetries, respectively[75]. These accuracies can be dramatically improved when adding restraints.

Docking small molecule ligands into proteins—Structure-based drug design has become a key drug optimization tool and leverages the vast array of knowledge contained in the increasing numbers of deposited structures in the PDB. RosettaLigand[76] has demonstrated success in predicting small molecule-protein interactions. Later in the drug development process, medicinal chemists optimize ligands based on structure-activity relationships (SAR) by synthesizing different ligands that share a core chemical scaffold and are assumed to bind to their target in a similar fashion[153]. RosettaLigandEnsemble[79] improves sampling during ligand docking by taking advantage of ligand similarities and docking a congeneric series of ligands simultaneously, allowing for a placement that works for all considered ligands while optimizing the binding interface for each ligand independently. Experimental SARs can help identify preferred binding modes. Small molecule ligands can also be used as competitive inhibitors of protein-protein interactions. However, a protein’s inhibitor-bound conformation often differs from the unbound or protein-protein bound conformation, thus Rosetta’s ability to model protein conformational flexibility is key. Rosetta’s pocket optimization approach identifies protein surface pockets and uses their volume as an additional scoring term: this allows the user to start from an unbound protein structure and bias sampling such that low-energy pocket-containing states are preferentially explored[80,81]. The sampled conformations match “druggable” alternate conformations observed in ligand-bound structures[80,81], making these states excellent starting points for virtual screening. Pockets sampled on a protein surface can then be matched to complementary ligands by using the pocket as the starting point for pharmacophore-based screening[154].

Modeling and designing antibodies and immune system proteins—Due to the therapeutic significance of antibodies, several antibody-specific and immune-specific protocols have been developed for structure prediction, docking and design (with specific protocols targeting IgG, T-cell receptors, displayed antigens of the Major Histocompatibility Complex (MHC) and other soluble antigens and immunogens). RosettaAntibody[85–88] is a protocol for modeling of antibodies[88]. It identifies homologous templates, assembles them into a single structure and then models CDR H3 loops *de novo* while refining the VH-VL orientation[155]. Recent advances use multiple templates[155], incorporate key structural constraints[156,157] into CDR H3 modeling, model camelid antibodies[87] and antibodies on the scale of the human repertoire[158,159]. AbPredict[89] predicts antibody structures without homologous templates. Instead, it samples backbone fragments and rigid-body orientations from known antibody structures, without relying on sequence homology, therefore accurately modeling cases with sequence identity as low as 10%. AbPredict2 is available as a webserver[90]. SnugDock[93] is a related method for antibody-antigen docking, taking as input a plausible starting conformation and optionally an ensemble of antibodies/antigens. SnugDock then runs local docking to refine both the antibody–antigen interface and the heavy–light chain interface (within the antibody) and re-models the CDR H2/H3 loops at the interface. Recent advances include a CDR H3 structural constraint[156,157] and docking camelid antibodies[160]. Limitations in antibody modeling depend on the task: docking is limited by knowledge of the binding site (global vs. local docking); structure prediction, design and refinement are limited by protein flexibility, and modeling of CDRs or other loops is challenging if they are longer than 12 to 15 residues.

RosettaAntibodyDesign[94] (RAbD) is based on RosettaAntibody[87] (see below) and allows design of specific CDRs of different clusters and lengths, sequence design using cluster-based CDR profiles or conservative mutations, or *de novo* design of whole antibodies. RAbD uses North-Dunbrack CDR clustering[161], reducing deleterious sequence mutations, and was benchmarked on 60 diverse antibody-antigen interfaces from complexes including both λ and κ light chains. Experimental benchmarking of two antibody-antigen complexes showed affinity improvements between 10 and 50-fold. Rosetta has been integrated with experimental immunogenic epitope data, MHC epitope prediction tools, and host genomic data to design proteins with reduced immunogenicity while retaining function and stability[95]. The approach implements machine learning-based epitope prediction for 28 different alleles, restricts design to select 15mer epitope regions, and uses a greedy stepwise protein design[96] to eliminate the most immunogenic epitopes with the least mutations, avoiding disruptive core mutations likely to destabilize the protein. Another method, AbDesign, splits experimentally determined antibody structures along conserved positions to create interchangeable segments and then recombines them to produce a diverse set of novel antibody models[97,98]. The models are docked to a target of interest, either locally to a specific epitope, or globally, followed by an optimization step comprised of rigorous backbone sampling and sequence design for improving model stability and binding affinity.

Designing new proteins and functions—Protein design[162] relies on several of the same core functionalities needed for structure prediction, and synergy and interoperability between design and prediction models has always been a core Rosetta design principle. For example, this synergy is well illustrated by the biased forward folding method: During *de novo* protein design[163], a test for the consistency of the designed sequence is whether *ab initio* structure prediction will yield the same structure that was used as a starting point for the design. However, computationally testing a large number of designs is prohibited by the vast conformational search space for *ab initio* structure prediction. To limit that space and test more designs, biased forward folding[72] uses three (instead of 200) fragments per residue position with fragments being chosen based on the RMSD to the native structure used to instantiate the design process. Protein design is easier when starting from known structures and when redesigning for well understood objectives like thermostability [164]. More difficult design objectives include *de novo* design (without a template structure) and design for novel folds or functions. Successes in these cases require sampling of enormous conformational spaces, depending on the protein size. Another simplification of *de novo* design is thermostabilization of the protein, essentially creating rigid structures that are mostly non-functional, by expanding the energy gap between folded and unfolded designs to facilitate structural characterization. To date, novel functional designs mostly exploit known structures and the next frontier is the design of novel functions onto *de novo* scaffolds. Moreover, nature typically does not design for the global minimum energy conformation (in terms of stability) because proteins require flexibility to carry out their functions.

Design of novel protein structures and functions towards therapeutic intervention is addressed by various methods in Rosetta: SEWING creates *de novo* designs by recombining parts of protein structures from randomly-selected helical building blocks[99]. SEWING's requirement-driven approach allows users to specify features that should be incorporated into their designs during backbone generation without requiring a certain size or three-dimensional fold. New features include incorporation of functional motifs such as protein-binding peptides for protein interface design and ligand binding sites for ligand-binding protein design[100]. A similar algorithm has been implemented for antibody design (AbDesign, see above), which was generalized for enzyme design[165]. A more general approach is RosettaRemodel, performing protein design by rebuilding parts or all of the structure[101] from fragments of known proteins structures. RosettaRemodel uses a blueprint file in which the user defines secondary and supersecondary structure of the desired fold. Remodel interfaces with various Rosetta protocols and allows *de novo* modeling, fixed-backbone sequence design, refinement, loop insertion, deletion, and remodeling, disulfide engineering, domain assembly, and motif grafting.

A common task is not only design towards a certain goal (positive design), but additionally, design away from undesired features (negative design). Such a *Multi-State* Design[166] (MSD) approach evaluates strengths and weaknesses of a single sequence on multiple backbones, for instance binding to one but not another protein partner. REstrained CONvergence[103] (RECON) allows each state to sample multiple sequences during the design process, which is iteratively applied by increasing the restraint weight to encourage sequence convergence. RECON achieves on average 70% sequence recovery (a 30% increase compared to MSD) for large multi-state design problems, such as antibody affinity maturation or predicting evolutionary sequence profiles of flexible backbones[167,168].

Protein function can be designed by *motif grafting*, i.e. grafting a known motif or predicted active- or binding-site from a template structure onto a new protein. This approach has been used for antibodies and vaccine design[104] using the *fold_from_loops* application, where the functional motif is used as a starting point of an extended structure that is folded following the constraints of a target topology. Iterative refinement is carried out via sequence design and structural relaxation before filtering and human-guided optimization. This protocol has been extended into the *Functional Folding and Design* (FunFolDes) protocol, including multi-segment motif grafting, different residue length motif insertion, incorporating restraints, and folding in the presence of a binding target[105]. Performance of the folding stage can be improved by selecting fragments according to the target topology via the *StructFragmentMover*.

Designing interfaces between proteins and interaction partners—Protein design problems include interface design of proteins with proteins or small molecule ligands and predicting ΔΔGs of mutation (e.g. alanine scanning). Predicting ΔΔGs of mutations for protein stability or protein-protein interactions is difficult with low correlation coefficients (0.5–0.7)[169], because the effect of the mutation is small compared to the total energy in the system, and because protein flexibility adds noise to the energies that can mask the effect of mutations. In alanine scanning (mutating into Ala), methods that use a "soft-repulsive" score function without modeling backbone flexibility[170,171] typical outperform methods that allow protein flexibility and use hard-repulsive score functions[172]. FlexDDG[106] improves protein-protein interface ΔΔG predictions and generalizes them to residues other than Ala. The protocol creates conformational ensembles using backrub sampling[173], then repacks sidechains, minimizes torsions and computes change in protein-protein interaction ΔΔG by averaging across the ensembles. On 1240 interface mutants, FlexDDG outperforms the earlier *ddg_monomer* application, which was created to predict changes in stability upon mutation, not interfaces.

Symmetric protein assemblies modeled using parametric design. Nature created super-helical coiled-coils that are well-described by geometric equations using Crick parameters[174], including variables for the radius of the bundle, major helical twist, minor helix rotation about the primary axis, etc. Several Movers such as *MakeBundle*, *PerturbBundle*, and *BundleGridSampler* allow designing helical bundles[48,108] and β-barrels based on pre-defined or sampled parameters. These parametric methods do not rely on fragments libraries and can be applied to non-canonical coiled-coil heteropolymers.

Modeling peptides and peptidomimetics—The inherent flexibility of peptides imparts a large conformational search space to them, leading to challenging modeling problems; when peptide modeling is combined with another simulation, e.g. docking, the increase in conformational space makes the modeling task quite challenging by any method. PIPER-FlexPepDock[111] is Rosetta's global peptide docking protocol. It rigid-body docks fragments using PIPER FFT-based docking[175], and refines the complex using FlexPepDock[109]. PIPER-FlexPepDock can generate peptide-protein complexes from a peptide sequence and a free receptor structure (Figure 3F). Performance decreases in case of receptor flexibility.

Cyclic peptide conformations can be sampled with *simple_cycpep_predict*, restricting the conformational search space through cyclization[44,45,108] via the Generalized Kinematic Closure (GenKIC) algorithm (see "loop modeling" above). *Simple_cycpep_predict* does not rely on protein fragments and can model non-canonical chemistries (Figure 3B), being a generalization of earlier protocols. Experimental protein structure determination is challenging for proteins on solid surfaces such as biominerals, self-assembled monolayers, inorganic catalysts, and nanomaterials. RosettaSurface[114] samples protein conformations *ab initio* in both the solution and adsorbed states (Figure 3D) to account for adsorption-induced conformational changes. Experimental data can be incorporated[115] to improve scoring.

Using experimental data to direct modeling—Using experimental data in modeling can vastly restrict the conformational space, allowing the modeling of larger, more complex biomolecules to greater accuracy. Electron density maps generated by cryo-electron microscopy (cryoEM) or X-ray crystallography have improved in quality and become substantially more available in the past decade and methods to incorporate them can produce high-resolution structures. To deal with variations in the resolution of these methods RosettaES[118] samples enumeratively, not requiring initial assignment of densities; it gradually extends the model one residue at a time until all residues are assigned. At each iteration, short fragments are used to sample the nearby conformational space of the growing model, while undergoing a series of clustering and filtering steps based on the energy and fit to the density. If assignment is complete but the data are low-resolution, refinement into density maps is necessary. Several methods have been developed for density maps in the 3.0–4.5Å resolution range. More recently, an automated fragment-guided refinement pipeline[121] splits the density map into independent training and validation maps. It finds regions with poor density fit, iteratively rebuilds them with fragments using the training map, filters the models based on their fit to the validation map, model geometry from MolProbity and fit to the full map, and then optimizes against the full map. Further, the frameworks for electron density maps and carbohydrate modeling[143] (below) were connected[144], allowing refinement of carbohydrates into low-resolution density maps.

NMR data were incorporated into *de novo* structure prediction early on, embodied in RosettaNMR. Chemical shifts were used for fragment picking using CS-Rosetta[122], which could be used with Nuclear Overhauser Enhancements (NOEs), Residual Dipolar Couplings (RDCs)[176], Pseudo-Contact Shifts (PCSs)[123,124,177] and Paramagnetic Relaxation Enhancement (PRE) data. Improvements, for instance through RASREC resampling[178] allowed the use of sparse[179] or unassigned data[180], easier-to-obtain data (backbone-only[181]), modeling larger and more complex proteins[182], membrane proteins[183], symmetric systems[184], and combination with data from SAXS[185], cryoEM[186], distance restraints from homologous proteins[187] and evolutionary couplings[188]. CS-Rosetta also has the AutoNOE[189,190] module for automated assignment of NOESY data for use in structure calculations. RosettaNMR was recently overhauled and reconciled with CS-Rosetta and PCS-Rosetta to seamlessly integrate several types of NMR restraints (CS, RDC, PCS, PRE, NOE) in one consistent framework[191] for structure prediction, protein-protein docking, protein-ligand docking, and symmetric assemblies.

Covalent labeling mass spectrometry data provides information on relative solvent exposure of residues, yielding information on protein tertiary structure. A low-resolution score term that allows for use of hydroxyl radical foot-printing has been implemented that can improve model quality in structure prediction[126,127]. Moreover, data from chemical cross-linking mass spectrometry has been incorporated into an automated workflow to identify protein-protein interactions. The PyTXMS[128] protocol combines the sensitivity of mass spectrometry to analyze complex samples with the power of Rosetta structural modeling and protein-protein docking to efficiently sample the vast conformational space and identify interactions (Figure 3C). A machine learning algorithm based on high resolution MS1 data guides the potential binding interface selection, being validated and adjusted by a repository of structural models and MS2 (data-dependent acquisition (DDA)) samples.

Modeling nucleic acids and their interactions with proteins—DNA and RNA modeling requires addressing a multitude of challenges due to a lack of structures leading to under-developed score functions, low quality alignments, and a much larger sampling torsion space than for proteins (70 residue RNA comparable to 200 residue protein). In contrast to protein helices where side-chains display sequence information on the helix exterior, helical RNA sidechains point inwards, therefore hiding sequence information from the environment, making prediction of tertiary or non-local contacts more difficult. Non-local contacts are mediated by loops, challenging for prediction algorithms. Several advances have been made in the representation of nucleic acids in Rosetta. The *StepWise Monte Carlo* protocol (SWM) has achieved RNA structure predictions reaching atomic accuracy[131]; the approach provides an acceleration over the original enumerative *StepWise Assembly* (SWA) method[129,130]. A version of SWA that rebuilds one nucleotide at a time enables fine-grained correction of errors in RNA coordinates fit into crystallographic or cryo-EM maps by *Enumerative Real-space Refinement ASsisted by Electron density under Rosetta*[135,136] (ERRASER).

The most recent advances in RNA tools expand the fragment assembly protocol to support modeling RNA-protein complexes through simultaneous folding and docking[134]. RNA-protein interactions are handled via additional knowledge-based score terms that supplement the low-resolution RNA score function. Free energy perturbations from RNA or protein mutations can be modeled with the Rosetta-Vienna ΔΔG protocol[43]. Structure coordinates can further be built into cryo-EM density maps for large RNA-protein complexes with DRRAFTER (*De novo Ribonucleoprotein modeling in Real space through Assembly of Fragments Together with Experimental density in Rosetta*)[138]. Redesign and prediction of protein-DNA interfaces[192,193] has been accomplished with flexible protein backbones[194], genetic algorithms[192,194,195] and motif-biased rotamer sampling[196,197]. A potential limitation is the reliance on fixed DNA backbone conformations, which can be flexible. Key to successful protein-DNA design is a score function optimized[197,198] for these highly charged and solvated interfaces. Rosetta supports prediction of specificity and affinity[199], the prediction of DNA binding preferences of homologous proteins and multi-template modeling in RosettaCM[55200].

Modeling membrane proteins—Membrane proteins constitute about 30% of all proteins and are targets for over 60% of pharmaceuticals on the market[201]. However, experimental difficulties have limited our understanding of their structures[202]. Previously, Yarov-Yarovoy[203] and Barth[204] implemented tools for low- and high-resolution structure prediction of membrane proteins, termed RosettaMembrane. These tools were re-engineered for compatibility with Rosetta3[27] into a platform called RosettaMP[139]. RosettaMP implements core modules for representing, sampling, and scoring proteins in the context of an implicit membrane. RosettaMP is compatible with key modeling protocols including docking, design, $\Delta\Delta G$ prediction[169], PyMOL visualization[205], and assembly of symmetric proteins. Additionally, a set of basic modeling tools[140] allows scoring, transforming a membrane protein into the membrane coordinate frame, *de novo* modeling for single transmembrane span helices, introducing mutations, and visualization in the membrane. RosettaMP has enabled rapid development of new tools including structure-based detection of lipid exposed residues in the membrane[141] and domain assembly of full-length protein models from structures of transmembrane and soluble domains[142]. The RosettaCM protocol for multi-template homology modeling has also been adapted to membrane proteins[33].

Describing membrane protein energetics is challenging as these proteins reside in an anisotropic environment and bury polar solvent molecules (e.g. water, ions) that stabilize the structure and participate in important conformational transitions. Implicit membrane models often fail to reliably model membrane protein interiors. The method SPaDES is based on a hybrid explicit-implicit solvent model that enhances the prediction and design of membrane protein structures[206]. Limitations to membrane protein modeling are similar but less severe than for RNA modeling: there are fewer structures in databases, fewer method developers in this field and hence fewer available tools. Consequently, the score function is less mature compared to the latest score functions for soluble proteins: the implicit solvent hydrophobic slab model is a coarse-gained representation of the membrane. Ongoing efforts expand this model by including pores, lipid specificity and different thicknesses[207], yet many effects remain to be acknowledged such as measurement-specific or observed membrane geometries (micelles, bicelles, nanodiscs, vesicles, different pore types, fusion and fission of multiple membranes) and macroscopic physical phenomena like membrane tension and fluidity. Challenges in including these effects are experimental measurements for parameterization of these models and adaptation of a multitude of score terms.

Adding carbohydrates to the modeling process—Carbohydrates are fundamental to life[208,209], but because of challenges in experimental characterization and computational sampling and scoring, their structures have been historically under-studied. The RosettaCarbohydrate framework[143] models carbohydrate structures and complexes such as glycosylated proteins or protein–sugar complexes (Figure 3F) with the same algorithms one would use for proteins. RosettaCarbohydrate can handle commonly studied and uncommon carbohydrate structures, including linear, cyclic, and branched structures, sugar modifications, and conjugations. Methods exist for sampling ring conformations, packing substituents, refining glycosidic linkages, sampling from linkage "fragments", and extending glycan chains. Scoring of saccharide-containing sugars includes a quantum-mechanically derived intrinsic backbone term[210]. Because saccharide residues are stored as distinct data structures, we can integrate bioinformatic and statistical data into these algorithms, opening the door for glycoengineering and design applications. RosettaCarbohydrate has been integrated with other frameworks, such as loop modeling (GenKIC and Stepwise Assembly), refinement (*GlycanTreeModeler*), symmetry, and RosettaScripts-accessible classes such as *MoveMaps* and *ResidueSelectors*. Linkages are automatically determined during PDB read-in. Carbohydrates work with Cartesian minimization, and can be refined into electron density maps[144]. Limitations in the carbohydrate framework include the increased sampling space due to carbohydrate flexibility and branching, and need to model many different chemistries with possible branching and cyclization. Developments in this area have only recently started and much work has yet to be done.

4. User interfaces and usability

Advances have also focused on improving usability of Rosetta through several user interfaces to suit different use cases and workflow styles (Figure 4). The command line was the first and is still the most-often used interface to Rosetta methods. Additionally, Rosetta features two popular scripting interfaces: RosettaScripts and PyRosetta. RosettaScripts[31] uses E**x**tensible **M**arkup **L**anguage (XML) to build complex protocols using core machinery[27], without requiring knowledge of the codebase. PyRosetta[30,145] is a collection of Python bindings to the source code, allowing flexible and fast custom protocol development, but requires familiarity with the underlying codebase. Other interfaces are InteractiveRosetta[146] and the gaming interface Foldit Standalone[147,149] (see Supplementary Note).

We devoted an enormous effort to rewrite and add documentation (Figure 5). A public-facing Gollum wiki (https://www.rosettacommons.org/docs/latest/Home) houses various levels of documentation, such as application documentation, tutorials for beginning users, and static protocol captures that accompany manuscripts for scientific reproducibility (see Supplementary Note for links). The Gollum wiki is easily editable by members of the RosettaCommons which has drastically improved the quantity and quality of documentation.

A limitation of Rosetta is the need for a local installation and compilation in a Unix-like environment. Webservers provide a user-friendly alternative and a number of independent servers have emerged in our community. However, implementing and maintaining such servers comes at a substantial cost. To make it easier to provide protocol webservers, ROSIE (Rosetta Online Server that Includes Everyone)[150,151] (http://rosie.rosettacommons.org/) implements a simple framework for “serverification” of protocols. ROSIE currently contains 24 webservers, with additional protocols continually being added.

Conclusion

The Rosetta software is developed by a large, global community aiming to solve complex problems through real-time collaborative code development. In the last five years, great strides have been made in our software. More protocols enable modeling a broader range of biological and chemical macromolecular systems. Prediction accuracies have improved through advances in the score function, which is a combination of physics-based and knowledge-based potentials that were fit against known structures and thermodynamic observables. Incorporating experimental data into modeling has been facilitated and improved. Further, our community now develops more general, reusable, user-friendly, and scientifically reproducible protocols. This was motivated by the growth of the software and the developer community, the various user interfaces, the diversity of the community[26], and the complexities of the protocols used to solve real-world problems. The improvements to documentation allow users to quickly start using or developing custom protocols, while facilitating user support for the various interfaces (command line, RosettaScripts, PyRosetta, etc.). Over the years, these applications have moved beyond tackling basic science questions (i.e. the protein folding and design challenges) to more application-based scientific developments. The myriad advances described above have made integration of Rosetta into existing experimental and computational scientific workflows increasingly useful and standard, as evidenced by the large number of licenses (~30,000 academic and ~70 commercial including most of the largest pharmaceutical companies), 11 spin-off companies that were created from the RosettaCommons[26], and the ever-increasing number of citations from labs beyond those affiliated with RosettaCommons.

Rosetta development is ongoing and will continue to focus on expanding the scope of protein design and modeling by integrating high-throughput experimental data with high-throughput computation, impacting score function development and aiding in developing novel therapeutic interventions[211]; restructuring the software for massively parallel computing architectures (e.g. GPUs, TPUs) and quantum computers[212]; greater use of machine-learning (e.g. deep-learning) approaches (e.g. for score function development); modeling more realistic cellular environments; and improving user interfaces to make Rosetta accessible to more scientists. The predictive powers that we have reviewed above can be leveraged not only to analyze and verify existing data but to inform experiments that will galvanize engineering industrial enzymes, enable the creation of novel biomaterials, and accelerate the discovery of new potent therapeutics.

Code availability

Rosetta is licensed and distributed through www.rosettacommons.org. Licenses for academic, non-profit and government laboratories are free of charge, there is a license fee for industry users.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Competing Interests:

Rosetta software has been licensed to numerous non-profit and for-profit organizations. Rosetta Licensing is managed by UW CoMotion, and royalty proceeds are managed by the RosettaCommons. Under institutional participation agreements between the University of Washington, acting on behalf of the RosettaCommons, their respective institutions may be entitled to a portion of revenue received on licensing Rosetta software including programs described here. Baker, Malmström, Gront, Meiler, Schueler-Furman, Gray, Sgourakis, Lindert, Karanicolas, Bonneau, Kortemme, and Bradley are unpaid board members of the RosettaCommons. As members of the Scientific Advisory Board of Cyrus Biotechnology, Baker and Gray are granted stock options. Yifan Song, Indigo C. King, Steven M. Lewis, Brandon Frenz, Karen Khar and Ryan Pavlovicz are currently employed at Cyrus Biotechnology with granted stock options. Cyrus Biotechnology distributes the Rosetta software. Brian D. Weitzner and Scott E. Boyken hold equity in Lyell Immunopharma. Vikram K. Mulligan is a co-founder of and shareholder in Menten Biotechnology Labs, Inc. The content of this manuscript is relevant to work performed at Lyell and Menten. Justin B. Siegel is a co-founder and shareholder of Digestiva, Inc. and PvP Biologics Inc. David Baker is a co-founder, shareholder, or advisor to the following companies: ARZEDA, PvP Biologics, Cyrus Biotechnology, Cue Biopharma, Icosavax, Neoleukin Therapeutics, Lyell Immunotherapeutics, Sana Biotechnology, and A-Alpha Bio.

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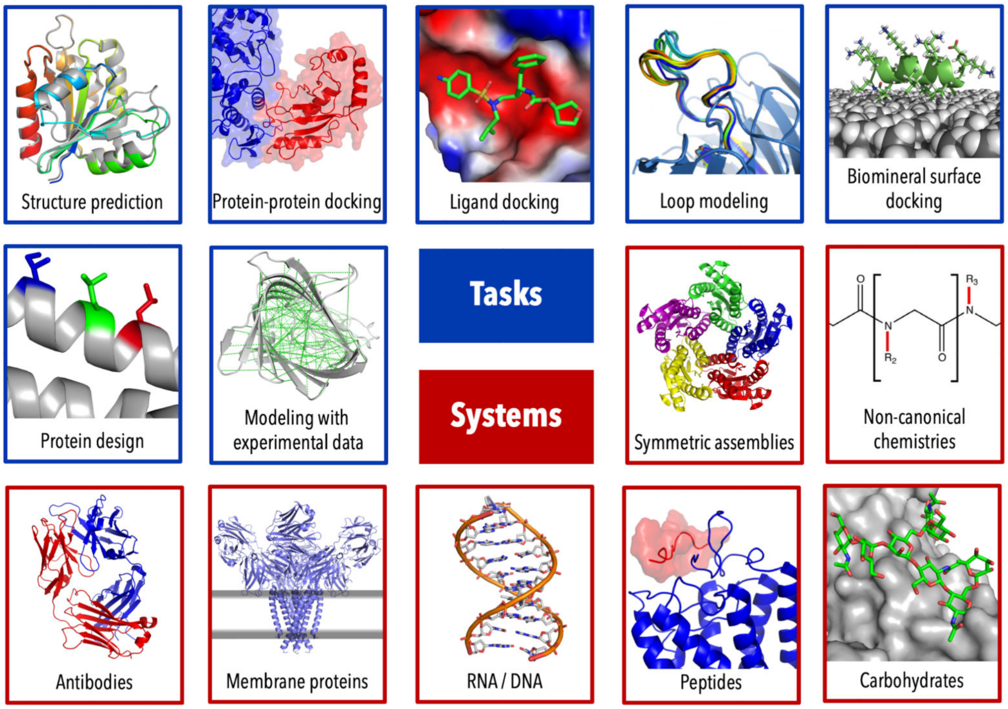
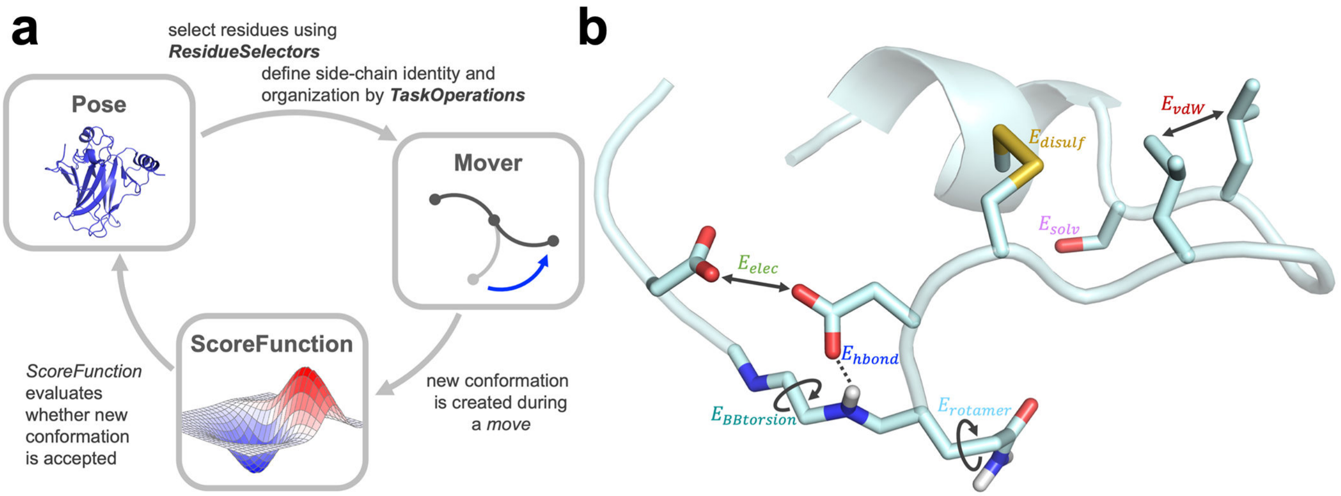


Figure 1: Capabilities of the Rosetta macromolecular modeling suite

Some popular tasks that can be addressed in Rosetta (blue) and major systems that can be modeled (red). Note this is an incomplete list of Rosetta's broad modeling capabilities.


a
select residues using *ResidueSelectors*
define side-chain identity and organization by *TaskOperations*
Pose
Mover
ScoreFunction
ScoreFunction evaluates whether new conformation is accepted
new conformation is created during a *move*
b
E_{vdW}
E_{disulf}
E_{solv}
E_{elec}
E_{hbond}
$E_{BBtorsion}$
$E_{rotamer}$

E_{vdW}	Lennard-Jones for attractive / repulsive interaction	E_{solv}	implicit solvation model penalizes buried polar atoms
E_{hbond}	hydrogen bonding allows buried polar atoms	$E_{BBtorsion}$	backbone torsion preferences from mainchain potential
E_{elec}	electrostatic interaction between charges	$E_{rotamer}$	sidechain torsion angles from rotamer library
E_{disulf}	disulfide bonds between cysteines	E_{ref}	unfolded state reference energy for design

Figure 2: Main elements of Rosetta are scoring and sampling

(A) Three main elements are required in a Rosetta protocol. The *Pose* is the biomolecule, such as a protein, RNA, DNA, small molecule, or glycan, in a specific conformation. Residues in the *Pose* can be selected via *ResidueSelectors* and the behavior for side-chain optimization or mutation can be defined by *TaskOperations*. Specific *Movers* then control how the conformation of the *Pose* is changed, and the new conformation is subsequently evaluated by a *ScoreFunction*. The Metropolis criterion decides whether the new conformation is accepted during sampling. Many independent sampling trajectories are generated, and the final models are evaluated based on the purpose of the protocol. (B) The score function consists of a weighted linear combination of various score terms, highlighted in the figure and described above.

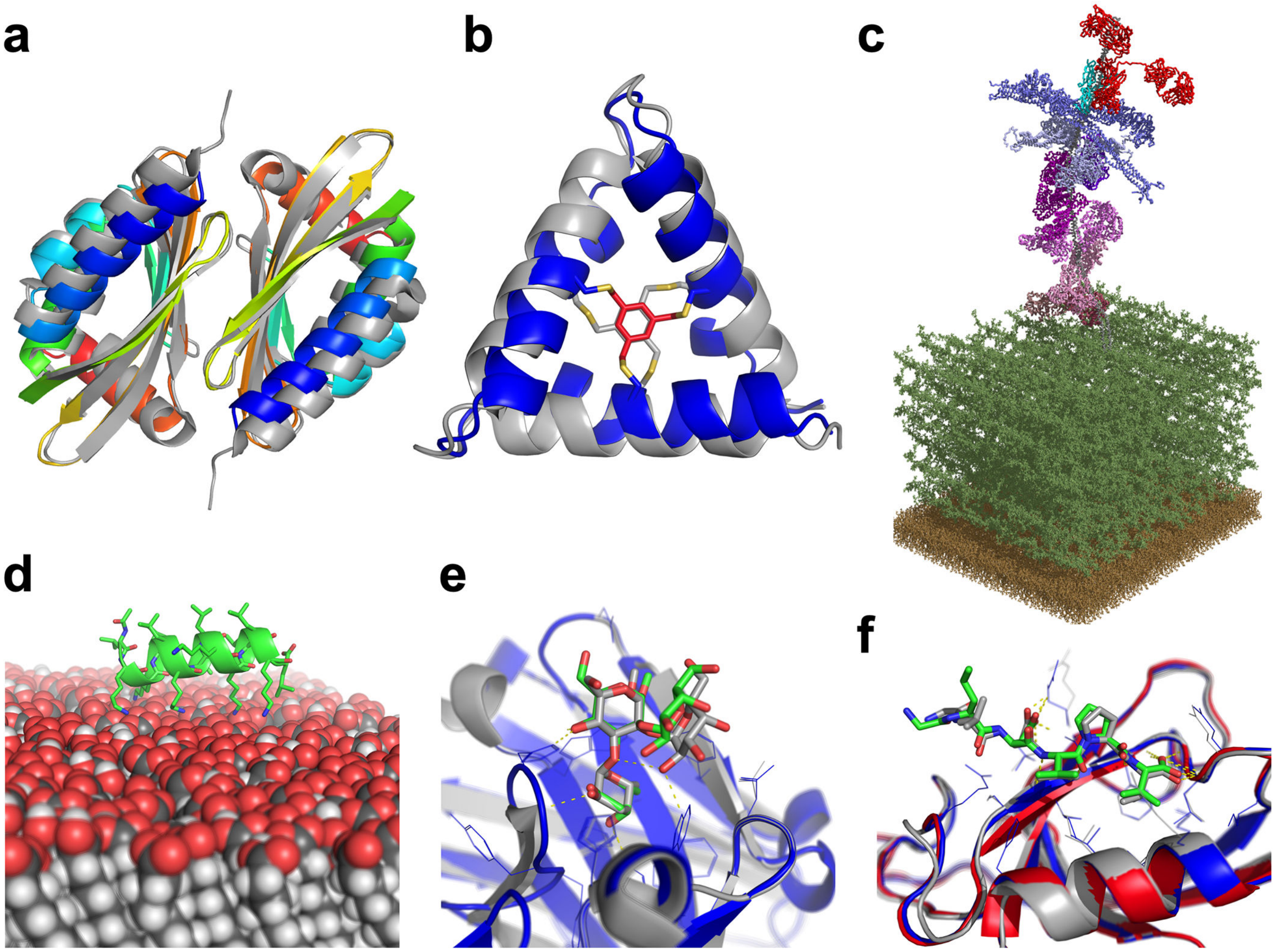
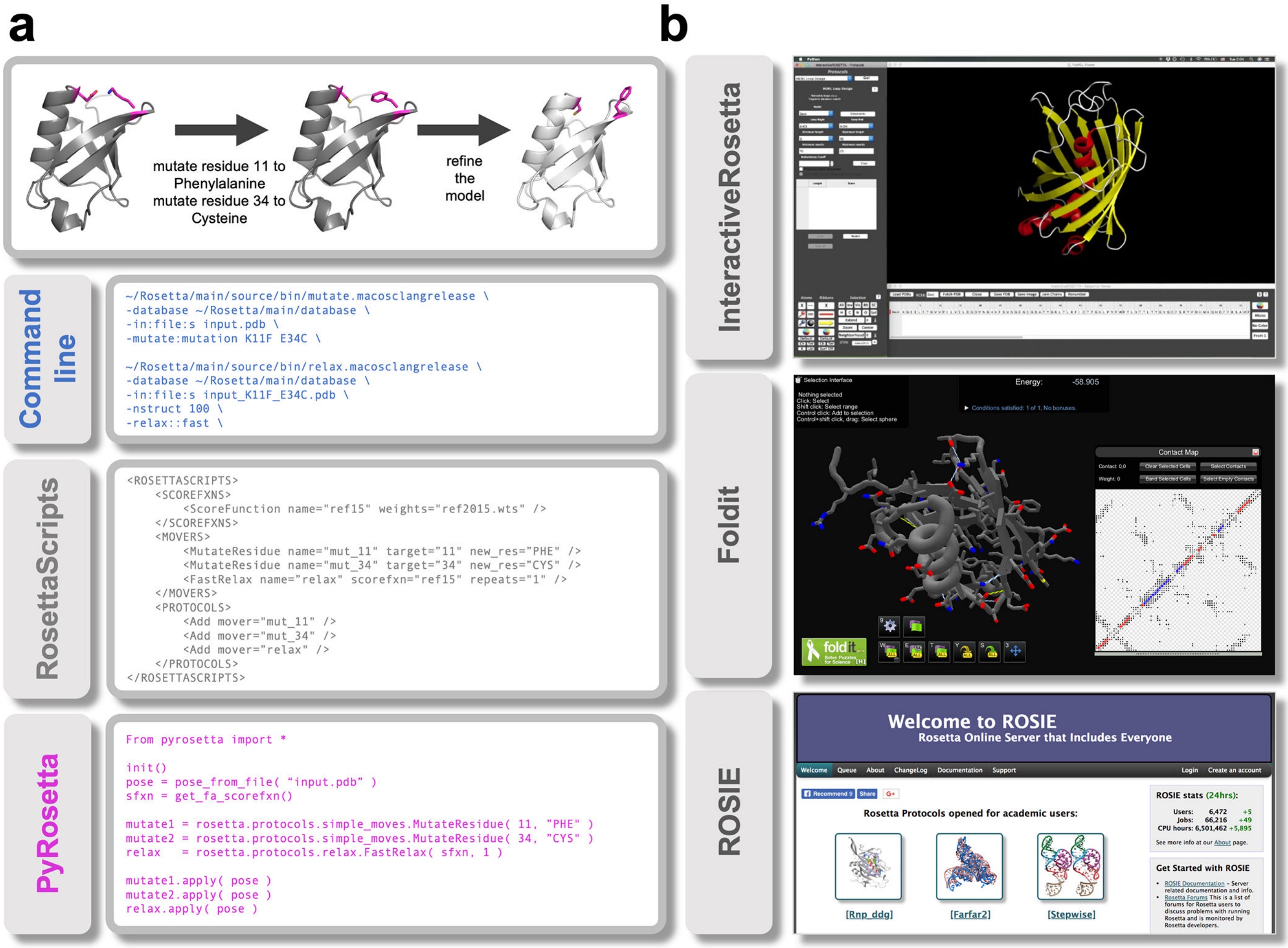


Figure 3: Rosetta can successfully address diverse biological questions

(A) Curved β-sheet design: overlay of the designed homo-dimeric curved β-sheet (dcs-E_4_dim_cav3) in rainbow and the crystal structure in gray (PDBID 5u35). The protein is designed *de novo* and features a curved β-sheet, a large pocket, and a homodimer interface[72]. (B) Parametric design: overlay of the *de novo* designed macrocycle 3H1 in blue and the NMR structure in gray (PDBID 5v2g). This “CovCore” (covalent core) miniprotein is held together covalently by a hydrophobic cross-linker at its core (in red for the design and gray for the NMR structure)[108]. (C) PyTXMS: the interactome of M1 protein (virulence factor of Group A *streptococcus*) and 15 human plasma proteins on the surface of bacteria (peptidoglycan layer (dark green), and the membrane (brown)). This 1.8MDa structure contains over 200 chemical cross-links[128] and is measured in a complex mixture of intact bacteria and human plasma. All models are provided by Rosetta: M1 protein (gray), IgG (red), four fibrinogens (dark to light blue), six albumins (dark to light pink), coagulation factor XIII A [F13A] (purple), C4bPa (cyan), haptoglobin [HP] (brown), and alpha-1-antitrypsin [SerpinA1] (plum). (D) RosettaSurface: model of an LK-α peptide (LKKLLKLLKKLLKL with a periodicity of 3.5 assuming a helical conformation) on a hydrophilic self-assembled monolayer surface. The peptide is unstructured in solution and assumes helical structure[115] when on the surface, as experiments show. (E) RosettaCarbohydrate: flexible docking of a carbohydrate antigen to an antibody. The crystal structure is in gray (PDBID 1mfa) and the model in blue, with the carbohydrate in green. Antibody coordinates were taken from the PDB and glycan coordinates started from a randomized backbone conformation and rigid-body orientation[143]. (F) PIPER-FlexPepDock: high-resolution model of a peptide-protein complex (model: blue; solved structure in gray, PDBID 1mfg). The model was generated from a peptide sequence (LDVPV, derived from the C-terminal tail of ErbB2R) and the unbound structure of the receptor (Erbin PDZ domain, PDBID 2h3l, colored in red)[111].



```
~/Rosetta/main/source/bin/mutate.macosclangrelease \
-database ~/Rosetta/main/database \
-in:file:s input.pdb \
-mutate:mutation K11F E34C \

~/Rosetta/main/source/bin/relax.macosclangrelease \
-database ~/Rosetta/main/database \
-in:file:s input_K11F_E34C.pdb \
-nstruct 100 \
-relax::fast \
```

```
<ROSETTASCRIPTS>
    <SCOREFXNS>
        <ScoreFunction name="ref15" weights="ref2015.wts" />
    </SCOREFXNS>
    <MOVERS>
        <MutateResidue name="mut_11" target="11" new_res="PHE" />
        <MutateResidue name="mut_34" target="34" new_res="CYS" />
        <FastRelax name="relax" scorefxn="ref15" repeats="1" />
    </MOVERS>
    <PROTOCOLS>
        <Add mover="mut_11" />
        <Add mover="mut_34" />
        <Add mover="relax" />
    </PROTOCOLS>
</ROSETTASCRIPTS>
```

```
From pyrosetta import *

init()
pose = pose_from_file( “input.pdb” )
sfxn = get_fa_scorefxn()

mutate1 = rosetta.protocols.simple_moves.MutateResidue( 11, "PHE" )
mutate2 = rosetta.protocols.simple_moves.MutateResidue( 34, "CYS" )
relax   = rosetta.protocols.relax.FastRelax( sfxn, 1 )

mutate1.apply( pose )
mutate2.apply( pose )
relax.apply( pose )
```

Selection Interface
Energy: -58.905
Nothing selected
Click: Select
Shift click: Select range
Control click: Add to selection
Control+shift click, drag: Select sphere
Conditions satisfied: 1 of 1, No bonuses.
Contact Map
foldit
Solve Puzzles for Science

Welcome to ROSIE
Rosetta Online Server that Includes Everyone

Rosetta Protocols opened for academic users:

[Rnp_ddg] [Farfar2] [Stepwise]

ROSIE stats (24hrs):

Users: 6,472 +5
Jobs: 66,216 +49
CPU hours: 6,501,462 +5,895

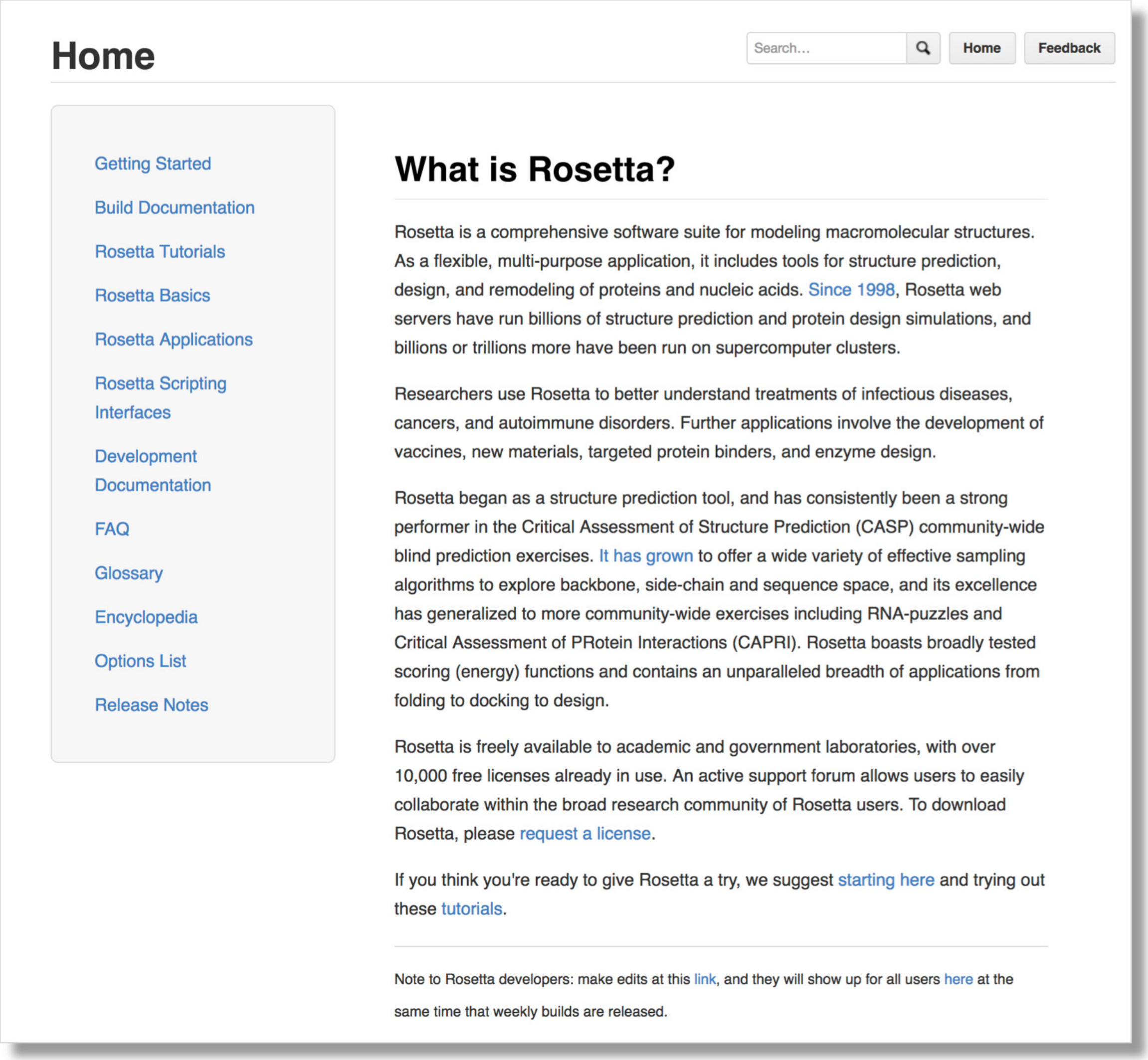
See more info at our About page.

Get Started with ROSIE

- ROSIE Documentation - Server related documentation and info.
- Rosetta Forums This is a list of forums for Rosetta users to discuss problems with running Rosetta and is monitored by Rosetta developers.

Figure 4: User interfaces to the codebase

(A) Rosetta can be run from a terminal and offers three interfaces to the codebase. The top panel outlines the task to be accomplished: making two mutations in a protein and then refining the structure. The panels underneath show how this task can be accomplished in the different interfaces. The command line panel shows the executable, input files and options to run two specific applications. RosettaScripts is an XML-based scripting language that offers more flexibility by combining *Movers* and *ScoreFunctions* into a custom *Protocol*. PyRosetta offers direct access to the underlying code objects but requires knowledge of the codebase. (B) Point-and-click interfaces to the codebase. InteractiveRosetta is a graphical user-interface (GUI) to PyRosetta. It offers controls to the most popular protocols, file formats and options. Foldit is a videogame primarily used to crowd-source real-world scientific puzzles but can also be used on custom proteins of interest. It can run some popular applications via a game interface. ROSIE hosts a multitude of servers each executing a particular protocol. It currently includes servers for 21 Rosetta methods. [The InteractiveRosetta and Foldit panels were originally published in [213] and [147] under Creative Commons licenses that allows reproduction as is.]



What is Rosetta?

Rosetta is a comprehensive software suite for modeling macromolecular structures. As a flexible, multi-purpose application, it includes tools for structure prediction, design, and remodeling of proteins and nucleic acids. Since 1998, Rosetta web servers have run billions of structure prediction and protein design simulations, and billions or trillions more have been run on supercomputer clusters.

Researchers use Rosetta to better understand treatments of infectious diseases, cancers, and autoimmune disorders. Further applications involve the development of vaccines, new materials, targeted protein binders, and enzyme design.

Rosetta began as a structure prediction tool, and has consistently been a strong performer in the Critical Assessment of Structure Prediction (CASP) community-wide blind prediction exercises. It has grown to offer a wide variety of effective sampling algorithms to explore backbone, side-chain and sequence space, and its excellence has generalized to more community-wide exercises including RNA-puzzles and Critical Assessment of PRotein Interactions (CAPRI). Rosetta boasts broadly tested scoring (energy) functions and contains an unparalleled breadth of applications from folding to docking to design.

Rosetta is freely available to academic and government laboratories, with over 10,000 free licenses already in use. An active support forum allows users to easily collaborate within the broad research community of Rosetta users. To download Rosetta, please request a license.

If you think you're ready to give Rosetta a try, we suggest starting here and trying out these tutorials.

Note to Rosetta developers: make edits at this link, and they will show up for all users here at the same time that weekly builds are released.

Figure 5: Main external documentation page

In 2015, our community performed a complete overhaul of our documentation. Documentation is now hosted on a Gollum wiki, which is version controlled and easily editable by members of our community. Accessibility and ability to edit the documentation has drastically improved the user-experience of the software.

Table 1:

Overview of recent methods developed in the Rosetta software

Method	**Lab developed**
Score function	
REF2015 score function[28,29]	Frank DiMaio, David Baker
cartesian_ddG[29]	Frank DiMaio, Phil Bradley
HBNet[47,50]	David Baker, Brian Kuhlman
HBNetEnergy[47]	Richard Bonneau, David Baker [*]
AACompositionEnergy	Richard Bonneau, David Baker [*]
AARepeatEnergy	Richard Bonneau, David Baker [*]
VoidsPenaltyEnergy	Richard Bonneau, David Baker [*]
NetChargeEnergy	Richard Bonneau, David Baker [*]
BuriedUnsatPenalty	Richard Bonneau, David Baker [*]
Protein structure prediction	
fragment picker[71]	Dominik Gront [*,**]
RosettaCM[55]	David Baker
iterative hybridize[59,60]	David Baker, Sergey Ovchinnikov [*]
Loop modeling	
NGK (next-generation KIC) [64]	Tanja Kortemme
GenKIC (generalized KIC) [44]	Richard Bonneau, David Baker [*]
LoopHashKIC	Tanja Kortemme
Consensus_Loop_Design[72,73]	David Baker
Protein-protein docking	
RosettaDock4.0[74]	Jeffrey Gray
Rosetta SymDock2[75]	(Ingemar André), Jeffrey Gray
Small molecule ligand docking	
RosettaLigand[76–78]	Jens Meiler
RosettaLigandEnsemble[79]	Jens Meiler
pocket optimization[80,81]	John Karanicolas
DARC[82–84]	John Karanicolas
Modeling of antibodies and immune system proteins	
RosettaAntibody[85–88]	Jeffrey Gray
AbPredict[89,90]	Sarel Fleishman
RosettaMHC[91]	Nik Sgourakis
TCRModel[92]	Brian Pierce
SnugDock[93]	Jeffrey Gray
Design of antibodies and immune system proteins	

Method	**Lab developed**
RAbD[94] (Rosetta AntibodyDesign)	Bill Schief, Roland Dunbrack
Epitope removal[95,96]	David Baker, Cyrus Biotechnology
AbDesign[97,98]	Sarel Fleishman
Protein design	
SEWING[99,100]	Brian Kuhlmann
RosettaRemodel[101]	Possu Huang*,**
LooDo[102]	Sagar Khare
RECON[103]	Jens Meiler
curved β-sheet design[72]	David Baker
biased forward folding[72]	David Baker
fold_from_loops[104]	Bruno Correia*,**
FunFolDes[105]	Bruno Correia
Protein interface design	
FlexDDG[106]	Tanja Kortemme
Coupled Moves[107]	Tanja Kortemme & DSM Biotechnology Center
Parametric design[48,108]	Richard Bonneau*
Peptides and peptidomimetics	
FlexPepDock[109,110]	Ora Schueler-Furman
PIPER-FlexPepDock[111]	Ora Schueler-Furman
PeptiDerive[112]	Ora Schueler-Furman
simple_cycpep_predict[44,45,108]	Richard Bonneau, David Baker*
MFPred[113]	Sagar Khare
RosettaSurface[114–116]	Jeffrey Gray
Modeling with experimental data	
cryoEM *de novo*[117]	Frank DiMaio, David Baker
cryoEM: RosettaES[118]	Frank DiMaio
cryoEM: iterative refinement[119,120]	(formerly David Baker) Frank DiMaio
cryoEM: automated refinement[121]	Frank DiMaio
NMR: CS-Rosetta[122]	Nik Sgourakis
NMR: PCS-Rosetta, GPS-Rosetta[123,124]	Thomas Huber
RosettaNMR framework[125]: using RDC/PRE/PCS/NOE/CS for ab initio, protein-protein docking, ligand docking, symmetric assembly	Jens Meiler, Richard Bonneau (Jeffrey Gray)
mass-spec: HRF hydroxyl radical footprinting[126,127]	Steffen Lindert
mass-spec: PyTXMS[128]	Lars Malmstroem
RNA modeling	
SWA (stepwise assembly) [129,130]	Rhiju Das
SWM (stepwise Monte-Carlo) [131]	Rhiju Das

Method	Lab developed
FARFAR (fragment assembly medium resolution structure prediction) [132–134]	Rhiju Das
ERRASER (refinement into EM density maps) [135,136]	Rhiju Das
CS-Rosetta-RNA (modeling with NMR data) [137]	Rhiju Das
RECCES (Reweighting of Energy-function Collection with Conformational Ensemble Sampling)	Rhiju Das
DRRAFTER (*de novo* modeling of protein-RNA complexes into EM densities) [138]	Rhiju Das
Membrane proteins	
RosettaMP framework[139]: mp_ddg, mp_dock, mp_relax, mp_symdock	Jeffrey Gray, Richard Bonneau
RosettaMP toolkit[140]: mp_score, mp_transform, mp_mutate_relax, helix_from_sequence	Jeffrey Gray, Richard Bonneau
mp_lipid_acc[141]	Richard Bonneau
mp_domain_assembly[142]	Richard Bonneau
RosettaCM for membrane proteins[33]	Jens Meiler
Carbohydrates	
RosettaCarbohydrate framework[143,144]	Jeffrey Gray, William Schief
User interfaces	
PyRosetta[30,145]	Jeffrey Gray
RosettaScripts[31,33]	Sarel Fleishman [*,**]
InteractiveRosetta[146]	Chris Bystroff
Foldit Standalone[32,147–149]	Seth Cooper [*,**], Firas Khatib [*,**], Justin Siegel, Scott Horowitz, David Baker
ROSIE server[150,151]	Jeffrey Gray
Miscellaneous	
Metalloproteins[42]	David Baker, Richard Bonneau [*]
Waters[51]	Frank DiMaio
SimpleMetrics	William Schief
AmbRose	Sagar Khare
RosettaRC	William Schief

[*] the main developer(s) in this lab was/were formerly in the lab of David Baker when this application was developed

[**] the main developer now has their own lab