



Macromolecular modeling and design in Rosetta: recent methods and frameworks

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. Its advantages are its performance and interoperability between broad modeling capabilities. Here we review tools developed in the last 5 years, including over 80 methods. We discuss improvements to the score function, user interfaces and usability. Rosetta is available at <http://www.rosettacommons.org>.

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. Schrödinger¹, the Molecular Operating Environment² and Discovery Studio³ 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 HHSuite⁴ includes tools for bioinformatics, sequence alignments, structure prediction and modeling. The BioChemicalLibrary⁵ (BCL) includes tools for structure prediction and drug discovery, and several sequence-to-structure methods using machine learning approaches. The Integrative Modeling Platform⁶ (IMP) models large macromolecular complexes by incorporating various types of experimental data. OpenBabel⁷ is a ChemInformatics toolbox supporting molecular mechanics calculations, being most heavily used for interconversion of file formats.

Molecular dynamics packages like CHARMM⁸, AMBER⁹, GROMACS¹⁰ 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. OpenMM¹¹ is an API (application programming interface) for setting up molecular simulations and can be used as a library or stand-alone application.

Many other tools are available for more specialized tasks — for instance, for de novo modeling (AlphaFold^{12,13}, QUARK¹⁴, RaptorX¹⁵), homology modeling (Modeller¹⁶, SwissModel¹⁷), fold recognition (iTasser¹⁸), protein–protein docking (HADDOCK¹⁹, Zdock²⁰, ClusPro²¹), ligand docking (AutoDock²², FlexX²³, Glide²⁴) and many 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.

Development of Rosetta started in the mid-1990s; it was initially aimed at protein structure prediction and protein folding²⁵. Over time, the number of applications grew to address diverse modeling tasks, from protein–protein or protein–small molecule docking to incorporating nuclear magnetic resonance (NMR) data, loop

modeling, protein design, and interaction with peptides and nucleic acids (Fig. 1 and Tables 1 and 2). 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 worldwide²⁶. The software has undergone several transitions, including in programming language and implementation, with the latest protocols based on Rosetta3, first released in 2008²⁷. The score function has been continuously improved and was described in refs. 28,29. As part of our sustained focus on accessibility, usability and scientific reproducibility, we developed several interfaces (PyRosetta³⁰, RosettaScripts³¹, Foldit³²) and emphasized publishing protocol captures³³ to accompany manuscripts. As those interfaces have grown more versatile and modular, development has accelerated and branched in many directions. However, the 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 5 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.

General overview and challenges

A typical Rosetta protocol is outlined in Fig. 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:

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

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

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 “Rosetta's score function” below), or technical challenges. Many protocols suffer from undersampling³⁴, 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 because of the size and combinatorics of the torsion space (see “Modeling nucleic acids and their interactions with proteins” below), membrane proteins because of their size, and carbohydrates because of branching and flexibility.

Some Rosetta applications suffer from technical challenges in implementation; a lack of documentation, protocol captures or support; and 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.

Rosetta's score function

Rosetta's score function has been continuously improved over many years³⁵ 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 respectively van der Waals energies, hydrogen bonds, electrostatics, disulfide bonds, residue solvation, backbone torsion angles, sidechain rotamer energies, and an average unfolded state reference energy (Fig. 2b):

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

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 recently²⁸.

The newest score function²⁹, REF2015, reproduces thermodynamic observables (such as liquid-phase properties³⁶ and liquid-to-vapor transfer free energies³⁷) in addition to structure-based³⁸ 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 space³⁹. The cartesian term³⁹ is also the basis for a cartesian_ddG method, which has been used to calculate $\Delta\Delta G$ values of mutations (where ΔG is the free energy of folding) to assess changes in protein stability. Only the backbones and side chains of residues near the mutation site are allowed to move⁴⁰. Due to the local optimization, this protocol is much faster than the previous gold-standard ddg_monomer⁴¹ 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 metalloproteins⁴². Score functions that enable simultaneous modeling of protein and RNA are being explored⁴³. REF2015 is now thread-safe and fully mirror symmetric; that is, 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 protein⁴⁶.

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 protocol⁴⁷ has been used to design de novo coiled coils with interaction specificity mediated by designed hydrogen bond networks, including homo-oligomers⁴⁷, membrane proteins⁴⁸ and large sets of orthogonal heterodimers⁴⁹. An improvement to HBNet uses a Monte Carlo search to sample hydrogen bond networks with notably improved performance⁵⁰. We further developed a statistical potential to place highly coordinated water molecules on the surface of biomolecules. On a dataset 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 positions⁵¹. The potential is accessible through the ExplicitWaterMover (formerly WaterBoxMover) in RosettaScripts.

There are still several limitations to the score function. (1) It does not directly estimate entropy⁵², which has been shown to improve sampling efficiency⁵³. 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 (in comparison 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, although more accurate, over time. (5) The solvation model is implicit, and 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.

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 respectable⁵⁴, 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 CASP13⁵⁴. Another highly ranking method is the Zhang server, built on iTasser¹⁴ and QUARK¹⁴.

Homology modeling was improved by using multiple templates in RosettaCM⁵⁵ (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 novo⁵⁵. 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 GREMLIN⁵⁸) has led to enormous improvements in model quality. An iterative hybridization 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 lead 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 models⁵⁹ as well as homology models⁶⁰. Model refinement or generating ensembles of structures (useful for design) can be accomplished by several algorithms in Rosetta: FastRelax⁶¹, Backrub⁶² or vicinity sampling⁶³ using kinematic closure (KIC) or next-generation-KIC (NGK) loop

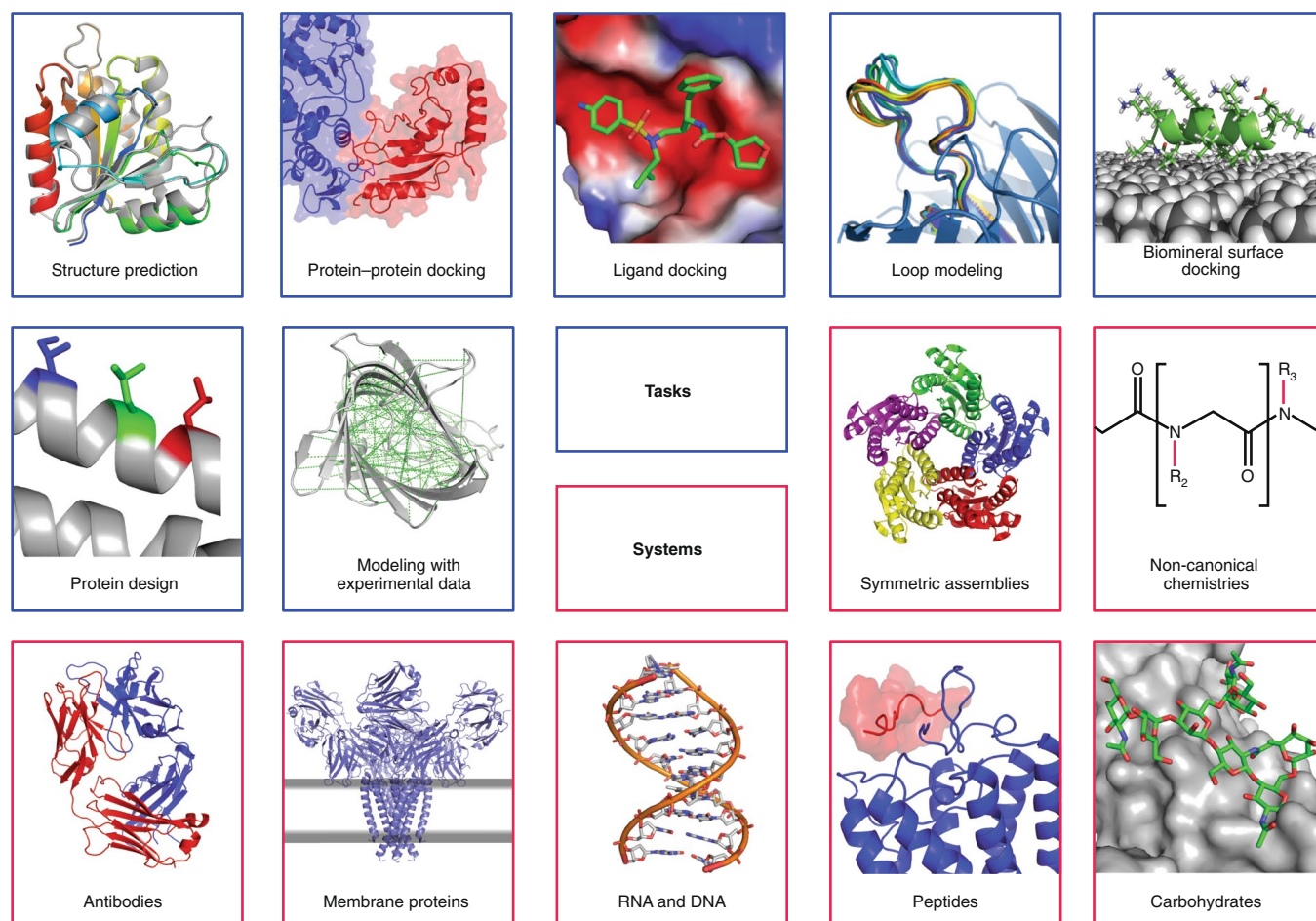


Fig. 1 | Capabilities of the Rosetta macromolecular modeling suite. Some popular tasks that can be addressed in Rosetta (blue) and major systems that can be modeled (red). Note that this is an incomplete list of Rosetta's broad modeling capabilities.

modeling⁶⁴. Loop modeling⁶⁵ was implemented early in Rosetta^{66,67}, with initial approaches relying on fragment sampling and iterative cyclic coordinate descent (CCD)⁶⁸ for chain closure. Later, a KIC approach relied on polynomial resultants to analytically solve for closed conformations, producing more native-like loops^{69,70}. Next-generation KIC⁶⁴ is an innovation that improves sampling by employing diversification (that is, wider range of conformations) and intensification (that is, focus around previously generated conformations), substantially increasing the fraction of near-native models⁶⁴ and modeling longer loops. A related method, GeneralizedKIC⁴⁴ (GenKIC), samples loop geometries between fixed endpoints, including non-standard peptide chemistries or chemistries that conventional loop-modeling 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, RosettaDock4.0⁷¹, incorporates protein flexibility from pre-generated protein ensembles, mimicking conformer selection. This has improved sampling efficiency by automatically adjusting the sampling rate on the basis of 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 complexes⁷¹.

For symmetric homomers, Rosetta SymDock2⁷² 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 framework⁷³. An induced-fit-based all-atom refinement step 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, respectively⁷². These accuracies can be markedly 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. RosettaLigand⁷⁴ has demonstrated success in predicting small molecule-protein interactions. Later in the drug development process, medicinal chemists optimize ligands on the basis of structure-activity relationships by synthesizing different ligands that share a core chemical scaffold and are assumed to bind to their target in a similar fashion⁷⁵. RosettaLigandEnsemble⁷⁶ improves sampling during ligand docking by taking advantage of ligand similarities and docking a congeneric series of ligands simultaneously, allowing a placement that works for all considered ligands while optimizing the binding interface for each ligand independently. Experimental structure-activity relationships can help identify preferred binding modes. Small-molecule ligands can also

Table 1 | Overview of recent methods developed in Rosetta

Method	Developing laboratory
Score function	
REF2015 score function ^{28,29}	Frank DiMaio, David Baker
cartesian_ddG ²⁹	Frank DiMaio, Phil Bradley
HBNNet ^{47,50}	David Baker, Brian Kuhlman
HBNNetEnergy ⁴⁷	Richard Bonneau, David Baker ^a
AACompositionEnergy	Richard Bonneau, David Baker ^a
AARepeatEnergy	Richard Bonneau, David Baker ^a
VoidsPenaltyEnergy	Richard Bonneau, David Baker ^a
NetChargeEnergy	Richard Bonneau, David Baker ^a
BuriedUnsatPenalty	Richard Bonneau, David Baker ^a
Protein structure prediction	
fragment picker ¹⁹⁰	Dominik Gront ^{a,b}
RosettaCM ⁵⁵	David Baker
iterative hybridize ^{59,60}	David Baker, Sergey Ovchinnikov ^a
Loop modeling	
NGK (next-generation KIC) ⁵⁴	Tanja Kortemme
GenKIC (generalized KIC) ⁴⁴	Richard Bonneau, David Baker ^a
LoopHashKIC	Tanja Kortemme
Consensus_Loop_Design ^{101,191}	David Baker
Protein–protein docking	
RosettaDock4.0 ⁷¹	Jeffrey Gray
Rosetta SymDock2 ⁷²	(Ingemar André) ^c , Jeffrey Gray
Small molecule ligand docking	
RosettaLigand ^{74,192,193}	Jens Meiler
RosettaLigandEnsemble ⁷⁶	Jens Meiler
pocket optimization ^{77,78}	John Karanicolas
DARC ^{194–196}	John Karanicolas
Modeling of antibodies and immune system proteins	
RosettaAntibody ^{80–83}	Jeffrey Gray
AbPredict ^{89,90}	Sarel Fleishman
RosettaMHC ¹⁹⁷	Nik Sgourakis
TCRModel ¹⁹⁸	Brian Pierce
SnugDock ⁹¹	Jeffrey Gray
Design of antibodies and immune system proteins	
RAbD ⁹³ (RosettaAntibodyDesign)	Bill Schief, Roland Dunbrack
Epitope removal ^{195,96}	David Baker, Cyrus Biotechnology
AbDesign ^{97,98}	Sarel Fleishman
Protein design	
SEWING ^{103,104}	Brian Kuhlmann
RosettaRemodel ¹⁰⁶	Possu Huang ^{a,b}
LooDo ¹⁹⁹	Sagar Khare
RECON ¹⁰⁸	Jens Meiler
curved β -sheet design ¹⁰¹	David Baker
biased forward folding ¹⁰¹	David Baker
fold_from_loops ¹¹¹	Bruno Correia ^{a,b}
FunFolDes ¹¹²	Bruno Correia
Protein interface design	
FlexDDG ¹¹⁷	Tanja Kortemme
Coupled Moves ²⁰⁰	Tanja Kortemme, DSM Biotechnology Center
Parametric design ^{48,120}	Richard Bonneau ^a

^aThe main developer(s) in this lab were formerly in the lab of David Baker when this application was developed. ^bThe main developer(s) now have their own labs. ^cNames in parentheses were either initial developers or previously involved in development but are no longer involved in development and maintenance of this part of the code.

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^{77,78}. The sampled conformations match 'druggable' alternative conformations observed in ligand-bound structures^{77,78}, 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 screening⁷⁹.

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 immunoglobulin G, T-cell receptors, displayed antigens of the major histocompatibility complex (MHC), and other soluble antigens and immunogens. RosettaAntibody^{80–83} is a protocol for modeling of antibodies. It identifies homologous templates, assembles them into a single structure and then models complementarity-determining region (CDR) H3 loops de novo while refining the orientation of the variable domain of the heavy and light chains⁸⁴. Recent advances use multiple templates⁸⁴, incorporate key structural constraints^{85,86} into CDR H3 modeling, and model camelid antibodies⁸² and antibodies on the scale of the human repertoire^{87,88}. AbPredict⁸⁹ 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 webserver⁹⁰. SnugDock⁹¹ is a related method for antibody–antigen docking, taking as input a plausible starting conformation and optionally an ensemble of antibodies and antigens. SnugDock then runs local docking to refine both the antibody–antigen interface and the heavy chain–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^{85,86} and docking camelid antibodies⁹². 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.

RosettaAntibodyDesign⁹³ (RAbD) is based on RosettaAntibody⁸² 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 clustering⁹⁴, 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 stability⁹⁵. The approach implements machine-learning-based epitope prediction for 28 different alleles, restricts design to select 15-mer epitope regions, and uses greedy stepwise protein design⁹⁶ 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

Table 2 | Overview of additional recent methods developed in Rosetta

Method	Developing laboratory
Peptides and peptidomimetics	
FlexPepDock ^{123,201}	Ora Schueler-Furman
PIPER-FlexPepDock ¹²¹	Ora Schueler-Furman
PeptiDerive ²⁰²	Ora Schueler-Furman
simple_cycpep_predict ^{44,45,120}	Richard Bonneau, David Baker ^a
MFPred ²⁰³	Sagar Khare
RosettaSurface ^{124,125,204}	Jeffrey Gray
Modeling with experimental data	
cryo-EM de novo ²⁰⁵	Frank DiMaio, David Baker
cryo-EM: RosettaES ¹²⁶	Frank DiMaio
cryo-EM: iterative refinement ^{206,207}	Frank DiMaio ^{a,b}
cryo-EM: automated refinement ¹²⁷	Frank DiMaio
NMR: CS-Rosetta ¹³⁰	Nik Sgourakis
NMR: PCS-Rosetta, GPS-Rosetta ^{132,133}	Thomas Huber
RosettaNMR framework ¹⁴⁸ : using RDC/PRE/PCS/NOE/CS for ab initio protein-protein docking, ligand docking, symmetric assembly	Jens Meiler, Richard Bonneau, (Jeffrey Gray) ^c
mass-spec: HRF hydroxyl radical footprinting ^{149,150}	Steffen Lindert
mass-spec: PyTXMS ¹⁵¹	Lars Malmström
RNA modeling	
SWA (stepwise assembly) ^{153,154}	Rhiju Das
SWM (stepwise Monte-Carlo) ¹⁵²	Rhiju Das
FARFAR (fragment assembly medium resolution structure prediction) ^{157,208,209}	Rhiju Das
ERRASER (refinement into EM density maps) ^{155,156}	Rhiju Das
CS-Rosetta-RNA (modeling with NMR data) ²¹⁰	Rhiju Das
RECCES (Reweighting of Energy-function Collection with Conformational Ensemble Sampling) ²¹¹	Rhiju Das
DRRAFTER (de novo modeling of protein-RNA complexes into EM densities) ¹⁵⁸	Rhiju Das
Membrane proteins	
RosettaMP framework ¹⁷² : mp_ddg, mp_dock, mp_relax, mp_symdock	Jeffrey Gray, Richard Bonneau
RosettaMP toolkit ¹⁷⁴ : mp_score, mp_transform, mp_mutate_relax, helix_from_sequence	Jeffrey Gray, Richard Bonneau
mp_lipid_acc ¹⁷⁵	Richard Bonneau
mp_domain_assembly ¹⁷⁶	Richard Bonneau
RosettaCM for membrane proteins ³³	Jens Meiler
Carbohydrates	
RosettaCarbohydrate framework ^{128,129}	Jeffrey Gray, William Schief
User interfaces	
PyRosetta ^{30,182,212}	Jeffrey Gray
RosettaScripts ^{31,33}	Sarel Fleishman ^{a,b}
InteractiveRosetta ¹⁸³	Chris Bystroff
Foldit Standalone ^{32,184,185,213}	Seth Cooper ^{a,b} , Firas Khatib ^{a,b} , Justin Siegel, Scott Horowitz, David Baker
ROSIE server ^{186,187}	Jeffrey Gray
Miscellaneous	
Metalloproteins ⁴²	David Baker, Richard Bonneau ^a
Waters ⁵¹	Frank DiMaio
SimpleMetrics	William Schief
AmbRose	Sagar Khare
RosettaRC	William Schief

^aThe main developer(s) in this lab were formerly in the lab of David Baker when this application was developed. ^bThe main developer(s) now have their own labs. ^cNames in parentheses were either initial developers or previously involved in development but are no longer involved in development and maintenance of this part of the code.

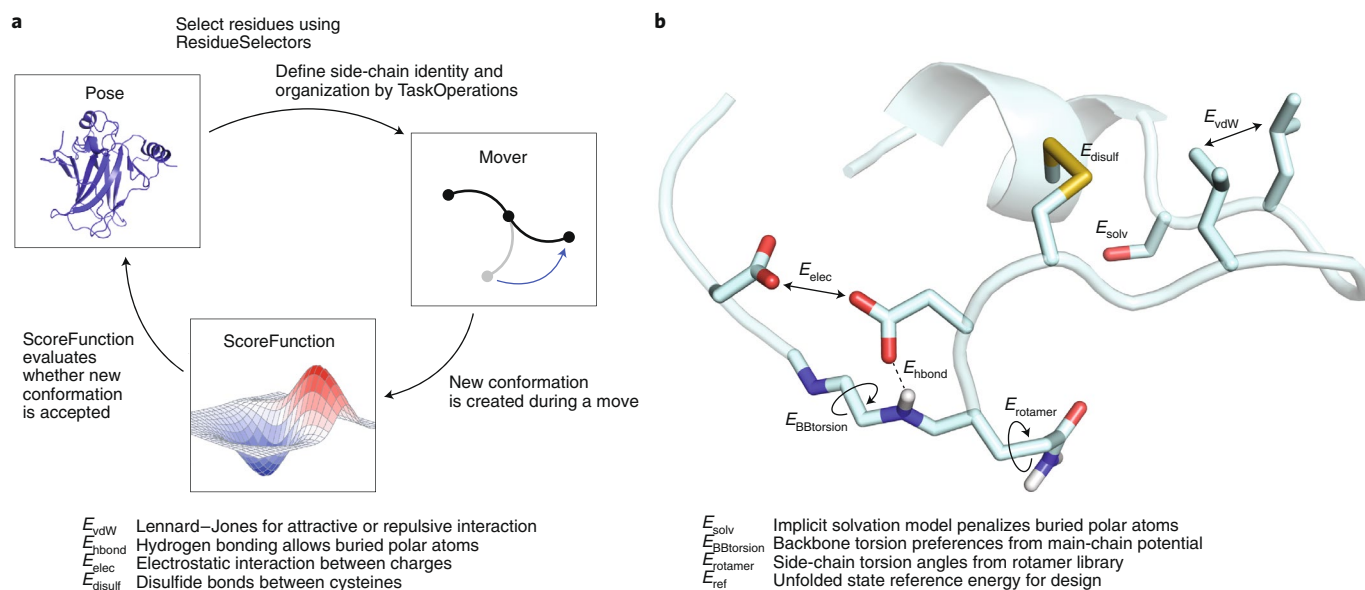


Fig. 2 | Main elements of Rosetta are scoring and sampling. a, Three main elements are required in a Rosetta protocol. The Pose is the biomolecule, such as a protein, RNA, DNA, small molecule, or glycan, in a specific conformation. Residues in the Pose can be selected via ResidueSelectors and the behavior 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 according to 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 in the text.

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 comprising rigorous backbone sampling and sequence design for improving model stability and binding affinity.

Designing new proteins and functions. Protein design⁹⁹ 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 principle. For example, this synergy is well illustrated by the biased forward folding method: during de novo protein design¹⁰⁰, 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 folding¹⁰¹ uses 3 (instead of 200) fragments per residue position, with fragments being chosen on the basis of the r.m.s. deviation 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 such as thermostability¹⁰². 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 toward 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 blocks¹⁰³. 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 design¹⁰⁴. A similar algorithm was implemented for antibody design (AbDesign, see above), which was generalized for enzyme design¹⁰⁵. A more general approach is RosettaRemodel, performing protein design by rebuilding parts or all of the structure¹⁰⁶ from fragments of known protein 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 toward a certain goal (positive design), but design away from undesired features (negative design). Such a multi-state design¹⁰⁷ approach evaluates strengths and weaknesses of a single sequence on multiple backbones — for instance, binding to one but not another protein partner. REstrained CONvergence¹⁰⁸ (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 multi-state design) for large multi-state design problems, such as antibody affinity maturation or the prediction of evolutionary sequence profiles of flexible backbones^{109,110}.

Protein function can be designed by motif grafting—that is, 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 design¹¹¹ 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 that includes multi-segment motif grafting, different residue length motif insertion, the incorporation of restraints, and folding in the presence of a binding target¹². 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 between proteins and proteins or small-molecule ligands and prediction of $\Delta\Delta G$ values of mutation (for example, alanine scanning). Predicting $\Delta\Delta G$ values of mutations for protein stability or protein–protein interactions is difficult with low correlation coefficients (0.5–0.7)¹¹³ 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 residues into alanine), methods that use a ‘soft-repulsive’ score function without modeling backbone flexibility^{114,115} typically outperform methods that allow protein flexibility and use hard-repulsive score functions¹¹⁶. FlexDDG¹¹⁷ improves protein–protein interface $\Delta\Delta G$ predictions and generalizes them to residues other than alanine. The protocol creates conformational ensembles using Backrub sampling¹¹⁸, then repacks sidechains, minimizes torsions and computes the change in protein–protein interaction $\Delta\Delta G$ by averaging across the ensembles. On 1,240 interface mutants, FlexDDG outperformed 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 superhelical coiled coils that are well described by geometric equations using Crick parameters¹¹⁹, including variables for the radius of the bundle, major helical twist and minor helix rotation about the primary axis. Several Movers, such as MakeBundle, PerturbBundle and BundleGridSampler, allow one to design helical bundles^{48,120} and β -barrels on the basis of predefined 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—for example, docking—the increase in conformational space makes the modeling task challenging by any method. PIPER-FlexPepDock¹²¹ is Rosetta’s global peptide docking protocol. It rigid-body docks fragments using PIPER FFT-based docking¹²² and refines the complex using FlexPepDock¹²³. PIPER-FlexPepDock can generate peptide–protein complexes from a peptide sequence and a free receptor structure (Fig. 3f). Performance decreases in cases of receptor flexibility.

Cyclic peptide conformations can be sampled with *simple_cycpep_predict*, restricting the conformational search space through cyclization^{44,45,120} via the GenKIC algorithm (see “Predicting protein structures” above). *Simple_cycpep_predict* does not rely on protein fragments and can model non-canonical chemistries (Fig. 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. RosettaSurface¹²⁴ samples protein conformations *ab initio* in both the solution and adsorbed states (Fig. 3d) to account for adsorption-induced conformational changes. Experimental data can be incorporated¹²⁵ 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 (cryo-EM) 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, RosettaES¹²⁶ 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 of low resolution, refinement into density maps is necessary. Several methods have been developed for density maps in the 3.0–4.5 Å resolution range. More recently, an automated fragment-guided refinement pipeline¹²⁷ 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 on the basis of 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 modeling¹²⁸ (below) were connected¹²⁹, 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 (CS) were used for fragment picking using CS-Rosetta¹³⁰, which could be used with nuclear Overhauser enhancements (NOEs), residual dipolar couplings (RDCs)¹³¹, pseudo-contact shifts (PCSs)^{132–134} and paramagnetic relaxation enhancement (PRE) data. Improvements—for instance through RASREC resampling¹³⁵—allowed the use of sparse¹³⁶ or unassigned data¹³⁷; the use of easier-to-obtain data (backbone-only¹³⁸); the modeling of larger and more complex proteins¹³⁹, membrane proteins¹⁴⁰ and symmetric systems¹⁴¹; and combination with data from small-angle X-ray scattering (SAXS)¹⁴², cryo-EM¹⁴³, distance restraints from homologous proteins¹⁴⁴ and evolutionary couplings¹⁴⁵. CS-Rosetta also has the AutoNOE^{146,147} module for automated assignment of NOE 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 and NOE) in one consistent framework¹⁴⁸ for structure prediction, protein–protein docking, protein–ligand docking and symmetric assemblies.

Covalent-labeling mass spectrometry data provide information on relative solvent exposure of residues, yielding information on protein tertiary structure. A low-resolution score term that allows use of hydroxyl radical footprinting has been implemented that can improve model quality in structure prediction^{149,150}. Moreover, data from chemical cross-linking mass spectrometry has been incorporated into an automated workflow to identify protein–protein interactions. The PyTXMS¹⁵¹ protocol combines the sensitivity of mass spectrometry for analyzing complex samples with the power of Rosetta structural modeling and protein–protein docking to efficiently sample the vast conformational space and identify interactions (Fig. 3c). A machine-learning algorithm based on high-resolution first-stage mass spectrometry (MS1) data guides the potential binding interface selection, being validated and adjusted by a repository of structural models and second-stage mass spectrometry (MS2, data-dependent acquisition) 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 underdeveloped score functions, low quality alignments, and a much larger sampling

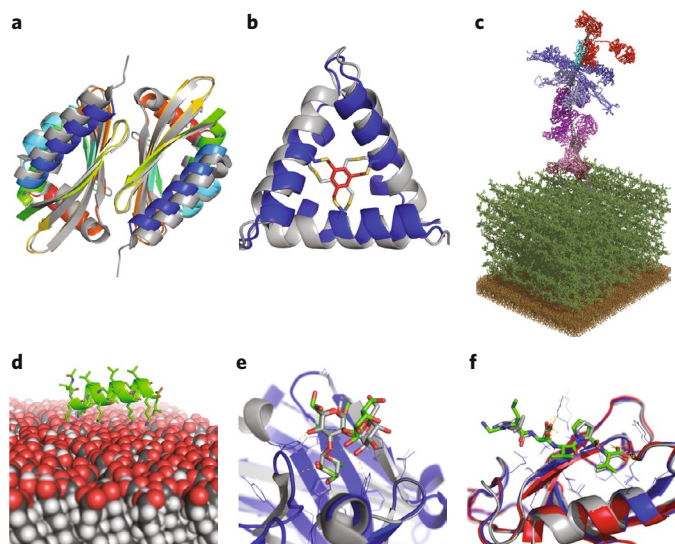


Fig. 3 | Rosetta can successfully address diverse biological questions.

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 (PDB 5U35). The protein is designed de novo and features a curved β -sheet, a large pocket and a homodimer interface¹⁰¹. **b**, Parametric design: overlay of the de novo designed macrocycle 3H1 in blue and the NMR structure in gray (PDB 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)¹²⁰. **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; membrane, brown). This 1.8-MDa structure contains over 200 chemical cross-links¹⁵¹ and is measured in a complex mixture of intact bacteria and human plasma. All models are provided by Rosetta: M1 protein (gray), immunoglobulin G (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 α -1-antitrypsin (Serpina1; plum). **d**, RosettaSurface: model of an LK- α peptide (LKKLLKLLKLLKLL, with a periodicity of 3.5 under the assumption of a helical conformation) on a hydrophilic self-assembled monolayer surface. The peptide is unstructured in solution and assumes helical structure¹²⁵ when on the surface, as experiments show. **e**, RosettaCarbohydrate: flexible docking of a carbohydrate antigen to an antibody. The crystal structure is in gray (PDB 1MFA) and the model in blue, with the carbohydrate in green. Antibody coordinates were taken from PDB and glycan coordinates started from a randomized backbone conformation and rigid-body orientation¹²⁸. **f**, PIPER-FlexPepDock: high-resolution model of a peptide–protein complex (model, blue; solved structure, gray; PDB 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, PDB 2H3L; red)¹²¹.

torsion space than for proteins (that of a 70-residue RNA being comparable to that of a 200-residue protein). In contrast to protein helices, where side chains display sequence information on the helix exterior, helical RNA side chains 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 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 prediction reaching atomic accuracy¹⁵²; the approach provides an acceleration over the original enumerative StepWise Assembly (SWA) method^{153,154}. 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 ERRASER (Enumerative Real-space Refinement Assisted by Electron Density under Rosetta)^{155,156}.

The most recent advances in RNA tools expand the fragment assembly protocol to support modeling RNA–protein complexes through simultaneous folding and docking¹⁵⁷. RNA–protein interactions are handled via 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 $\Delta\Delta G$ protocol⁴³. 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)¹⁵⁸. Redesign and prediction of protein–DNA interfaces^{159,160} have been accomplished with flexible protein backbones¹⁶¹, genetic algorithms^{159,161,162} and motif-biased rotamer sampling^{163,164}. A potential limitation is the reliance on fixed DNA backbone conformations, as DNA backbone conformations can be flexible. Key to successful protein–DNA design is a score function optimized^{164,165} for these highly charged and solvated interfaces. Rosetta supports prediction of specificity and affinity¹⁶⁶, the prediction of DNA binding preferences of homologous proteins, and multi-template modeling in RosettaCM^{55,167}.

Modeling membrane proteins. Membrane proteins constitute about 30% of all proteins and are targets for over 60% of pharmaceuticals on the market¹⁶⁸. However, experimental difficulties have limited our understanding of their structures¹⁶⁹. Previously, Yarov-Yarovoy¹⁷⁰ and Barth¹⁷¹ implemented tools for low- and high-resolution structure prediction of membrane proteins, termed RosettaMembrane. These tools were re-engineered for compatibility with Rosetta3²⁷ into a platform called RosettaMP¹⁷². 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, $\Delta\Delta G$ prediction¹¹³, PyMOL visualization¹⁷³ and assembly of symmetric proteins. Additionally, a set of basic modeling tools¹⁷⁴ allows scoring, transformation of a membrane protein into the membrane coordinate frame, modeling of single-transmembrane-span helices de novo, introduction of mutations, and visualization in the membrane. RosettaMP has enabled rapid development of new tools, including those for structure-based detection of lipid-exposed residues in the membrane¹⁷⁵ and domain assembly of full-length protein models from structures of transmembrane and soluble domains¹⁷⁶. The RosettaCM protocol for multi-template homology modeling has also been adapted to membrane proteins³³.

Describing membrane protein energetics is challenging as these proteins reside in an anisotropic environment and bury polar solvent molecules (for example, water and 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 structures¹⁷⁷. 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 than 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 thicknesses¹⁷⁸, yet many effects remain to be acknowledged, such as measurement-specific or observed membrane geometries (micelles, bicelles, nanodiscs, vesicles, different pore types, and fusion and fission of multiple membranes) and macroscopic physical phenomena such as membrane tension and fluidity. Challenges in including these effects are experimental

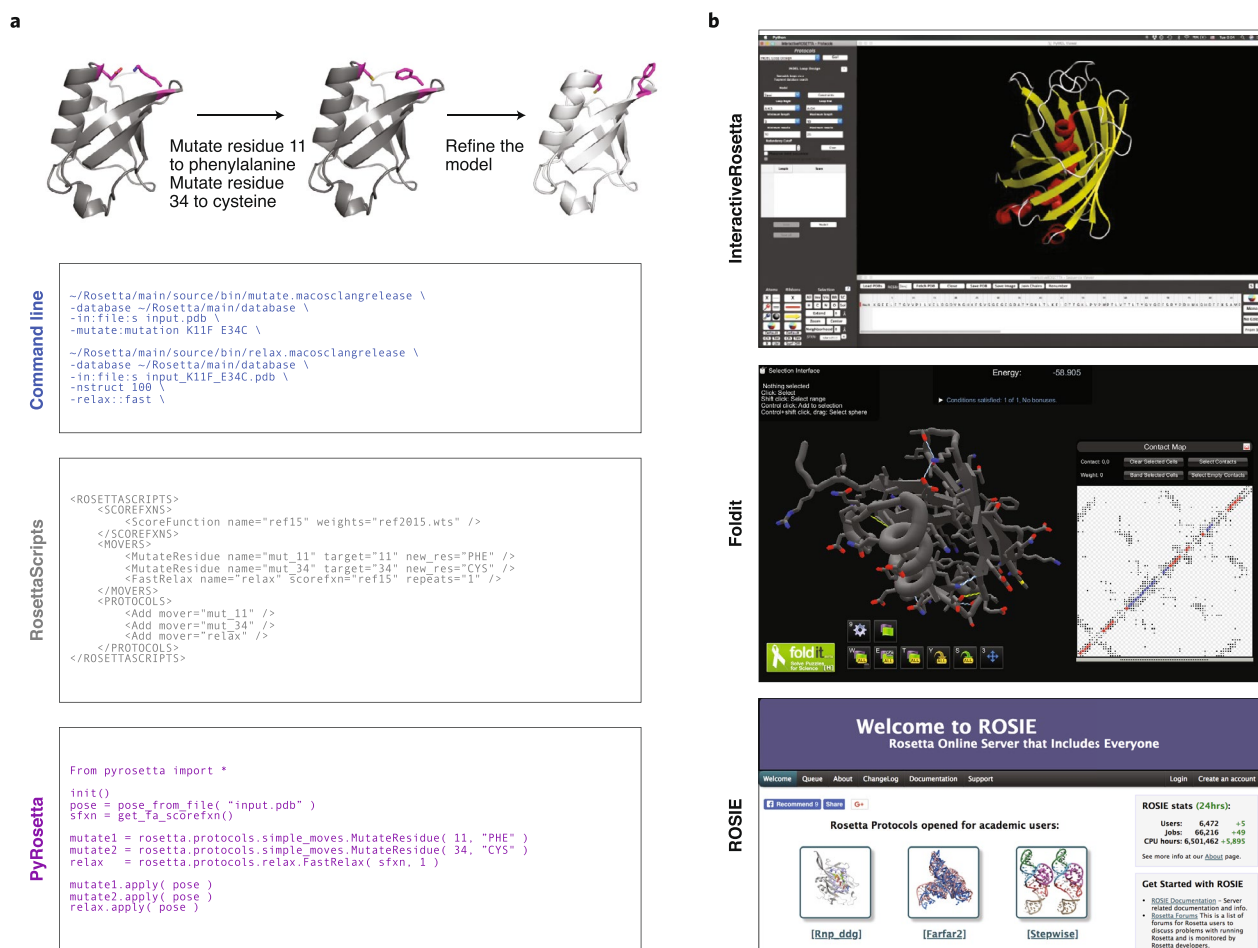


Fig. 4 | User interfaces to the codebase. **a**, Rosetta can be run from a terminal and offers three interfaces to the codebase. The top panel outlines the task to be accomplished: making two mutations in a protein and then refining the structure. The panels underneath show how this task can be accomplished 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 video game primarily used to crowd-source real-world scientific puzzles and is also usable 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 25 Rosetta methods. (InteractiveRosetta and Foldit panels reprinted from refs.^{184,214} under Creative Commons licenses.).

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^{179,180}, but because of challenges in experimental characterization and computational sampling and scoring, their structures have been historically under-studied. The RosettaCarbohydrate framework¹²⁸ models carbohydrate structures and complexes such as glycosylated proteins or protein–sugar complexes (Fig. 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 term¹⁸¹. 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 maps¹²⁹. Limitations in the carbohydrate framework include the increased sampling space due to carbohydrate flexibility and branching, and the need to model many different chemistries with possible branching and cyclization. Developments in this area have only recently started, and much work remains.

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 (Fig. 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. RosettaScripts³¹ uses Extensible Markup Language (XML) to build complex protocols using core machinery²⁷, without requiring knowledge of the codebase. PyRosetta^{30,182} is a collection of Python bindings to the source code, allowing flexible and

Home

Getting Started
 Build Documentation
 Rosetta Tutorials
 Rosetta Basics
 Rosetta Applications
 Rosetta Scripting Interfaces
 Development Documentation
 FAQ
 Glossary
 Encyclopedia
 Options List
 Release Notes

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 1996, 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.

Fig. 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 improved the user experience of the software.

fast custom protocol development, but requires familiarity with the underlying codebase. Other interfaces are InteractiveRosetta¹⁸³ and the gaming interface Foldit Standalone^{184,185} (Supplementary Note).

We devoted an enormous effort to rewriting and adding documentation (Fig. 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. Web servers 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 web servers, ROSIE (Rosetta Online Server that Includes Everyone)^{186,187} (<http://rosie.rosettacommons.org/>) implements a simple framework for 'serverification' of protocols. ROSIE currently contains 25 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 5 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 scientific

reproducibly protocols. This was motivated by the growth of the software and the developer community, the various user interfaces, the diversity of the community²⁶ 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 and facilitate user support for the various interfaces (command line, RosettaScripts, PyRosetta, and so forth). Over the years, these applications have moved beyond tackling basic science questions (that is, 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), the 11 spin-off companies that were created from RosettaCommons²⁶, and the ever-increasing adoption by 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, influencing score function development and aiding in the development of therapeutic interventions¹⁸⁸; restructuring the software for massively parallel computing architectures (for example, GPUs and TPUs) and quantum computers¹⁸⁹; greater use of machine-learning (for example, deep-learning) approaches (for example, 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 also to inform experiments that will galvanize the engineering of industrial enzymes, enable the creation of novel biomaterials, and accelerate the discovery of potent new therapeutics.

Code availability.

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

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41592-020-0848-2>.

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J.K.L. wrote the manuscript with help from B.D.W. 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 R.B.

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. D.B., L.M., D.G., J.M., O.S.-F., J.J.G., N.G.S., S.L., J.K., R.B., T.K. and P.B. are unpaid board members of the RosettaCommons. As members of the Scientific Advisory Board of Cyrus Biotechnology, D.B. and J.J.G. are granted stock options. Y.S., I.C.K., S.M.L., B.F., K.R.K. and R.E.P. are employed at Cyrus Biotechnology with granted stock options. Cyrus Biotechnology distributes the Rosetta software. B.D.W. and S.E.B. hold equity in Lyell Immunopharma. V.K.M. is a cofounder of and shareholder in Menten Biotechnology Labs, Inc. The content of this manuscript is relevant to work performed at Lyell and Menten. J.B.S. is a cofounder and shareholder of Digestiva, Inc. and Pvp Biologics Inc. D.B. is a cofounder of, shareholder in, or advisor to the following companies: ARZEDA, Pvp Biologics, Cyrus Biotechnology, Cue Biopharma, Icosavax, Neoleukin Therapeutics, Lyell Immunotherapeutics, Sana Biotechnology and A-Alpha Bio.

Additional information

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