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Comparative structural analysis of protein complexes with SPICE

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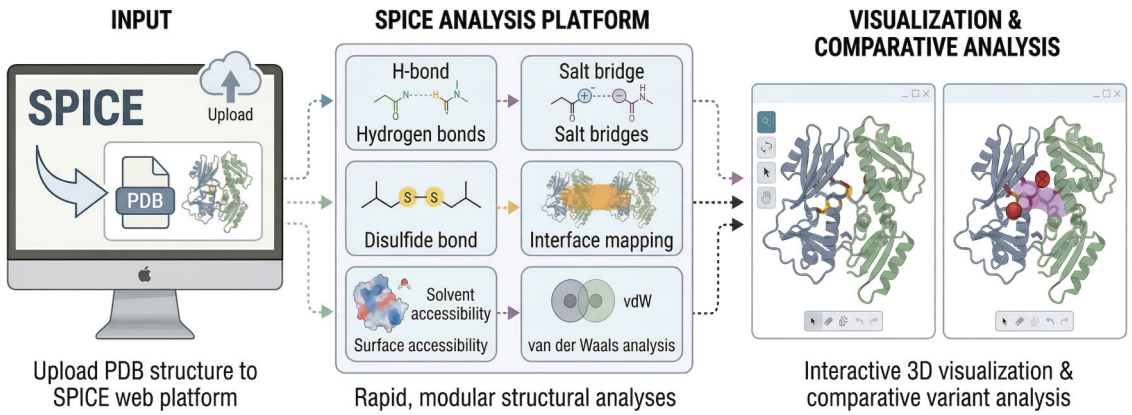
Abstract

Computational tools for studying the structure of protein complexes are essential for providing mechanistic insights into protein–protein interactions and therapeutic drug design. Here, we present SPICE (Structural Protein Interaction Complex Evaluator), a web-based platform that allows structural biologists to perform rapid, modular analyses of protein complexes directly from Protein Data Bank (PDB) structures. SPICE allows users to define and execute analysis workflows via an intuitive web interface, reducing analysis times from minutes to seconds. The platform offers a broad range of analytical capabilities, including (i) detection of hydrogen bonds, salt bridges, and disulfide bonds; (ii) protein–protein interface mapping; and (iii) computation of solvent accessibility, van der Waals energetics, and other key geometric descriptors. SPICE further provides interactive 3D visualization and supports comparative analyses across multiple complexes, enabling the study of mutational effects and binding variants. The tool is freely available at https://spice.cs.ucr.edu (no registration required).

Graphical abstract



Introduction

Protein–protein interactions act as the fundamental mechanism for nearly all cellular processes, including signal transduction, gene expression, metabolic regulation, and immune response, among others [1]. The structural analysis of protein complexes can provide critical insights into the molecular mechanisms of protein-protein interactions and can enable therapeutic drug design.

The structure of all known protein complexes is stored in the Protein Data Bank [2]. The analysis of these complexes requires specialized computational tools, several of which are available in the literature. For instance, PDBePISA [3] and PISA [4] provide thermodynamic analysis of protein complexes but the provided pipelines cannot be customized according to the users' needs. PIC [5] offers interaction cataloging but requires local installation, while PRODIGY [6] focuses specifically on binding affinity prediction with limited interface characterization. MAPIYA [7] provides accessible interface analysis but lacks modular selection and comparative capabilities. Overall, these tools suffer from the following limitations: (i) rigid pipelines that are difficult to customize based on users' interest; (ii) lack of comparative capabilities (i.e. comparing multiple complexes requires manual post-processing); (iii) limited interactive and intuitive exploration of the results.

SPICE is web-based tool that addresses these limitations by providing a comprehensive and flexible platform for the structural analysis of protein complexes. SPICE's key

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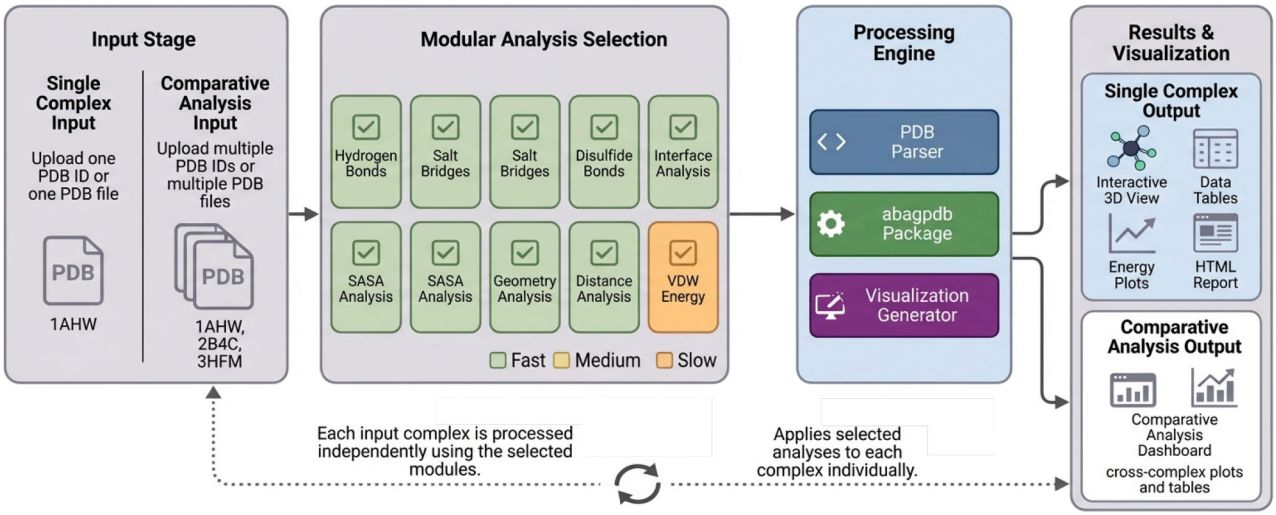

Figure 1. General workflow of SPICE. Users submit a protein complex either by providing a PDB ID or upload a PDF-formatted file; then they select any number of structural analyses (e.g., hydrogen bonds, SASA, interface geometry, and energetics); the requested analyses are executed in the background with real-time progress updates producing interactive 3D visualization, data tables, plots, and downloadable reports.

innovations include (i) a modular framework that allows users to customize their analyses; (ii) a multi-complex comparative tool for systematic analysis of structural variants; (iii) an interactive 3D visualization that enables the exploration of interface residues; and (iv) the generation of publication-ready figures for comparing multiple variants or mutation effects. By combining these features, SPICE makes advanced structural analysis accessible to users with limited computational expertise.

Materials and methods

Implementation and architecture

SPICE is a Flask-based web application powered by a new stand-alone Python library for structural bioinformatics that we developed. Our package PyPDBcomplex can also be used as stand-alone library for Python programmers (see https://github.com/fbabd/PyPDBcomplex for examples of usage). The platform was deployed on Google Cloud platform with a filesystem-based session management to accommodate large datasets. Rate limiting is used to ensure fair resource allocation. The modular architecture separates the interface from analytical functions, allowing checkbox-based analysis selection, reducing typical processing time from a few minutes to a few seconds. Users can either provide the PDB identifiers (in which case, the structures are automatically fetched from RCSB) or upload PDB/mmCIF files. For multi-complex comparisons, uploaded structures are automatically 3D-aligned to the reference structure using Biopython [8] to enable a synchronized a side-by-side 3D visualization (optional). The general workflow of the tool is illustrated in Fig. 1.

Analysis types

Atomic contact analysis

SPICE can identify three types of atomic contacts critical for protein-protein recognition. To detect hydrogen bonds, we use the following geometric criteria: (i) donor–acceptor distance within 3.5 Å and (ii) donor–H-acceptor angle of at least 120°. SPICE considers all potential hydrogen bond donors (N–H, O–H) and acceptors (O, N) across the interface, accounting for both backbone and side-chain interactions. Salt bridges are identified between oppositely charged residues (Asp/Glu and Arg/Lys/His) where any charged atom pair falls within 4.0 Å. This distance threshold captures both direct ion pairs and water-mediated electrostatic interactions commonly observed in protein interfaces [9]. Disulfide bonds are detected between cysteine residues with sulfur-sulfur distances between 2.0 and 2.2 Å, the typical range for stable covalent S-S bonds in protein structures [10].

Interface characterization

Interface residues are atoms within a certain minimum distance to partner chains (typically 5.0 Å), capturing direct contacts and proximal residues. For each interface residue, SPICE calculates contact frequency (number of atomic contacts with the partner chain) and categorizes contacts by contact type. This residue-level analysis enables the identification of binding hot-spots and comparison across related complexes.

Energetic analysis

Solvent Accessible Surface Area (SASA) calculations employ FreeSASA [11], which implements the Lee-Richards algorithm with a 1.4 Å probe radius. SPICE computes SASA for the selected chains in both bound (complex) and unbound (isolated) states. The buried surface area (BSA), calculated as the difference between unbound and bound SASA, quantifies the interface size and provides insights into binding strength, as BSA correlates with binding affinity across diverse protein complexes [12]. The classification of interface residues based on the relative solvent accessible surface area (RSA) using the criteria proposed in [13] is also available. The van der Waals (VDW) energy calculations employ the Lennard–Jones 6–12 potential to estimate non-bonded interactions across the interface. Due its higher computational cost, the VDW analysis is optional and users are informed of longer processing times. The energy contributions are computed at the residue level, enabling identification of energetically favorable and unfavorable interactions. While these calculations provide relative

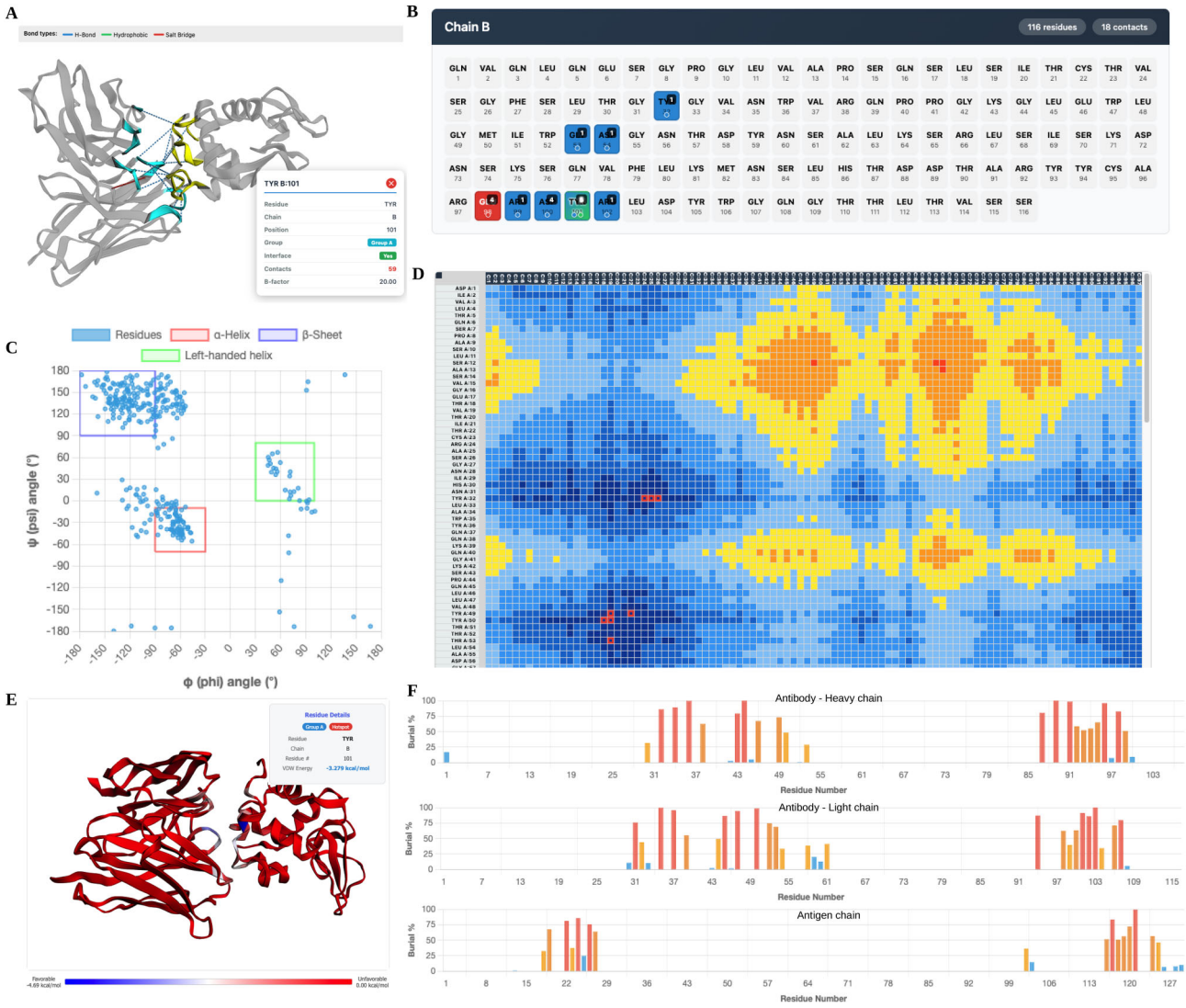

Figure 2. Outputs generated by SPICE for the 1A2Y antibody–antigen complex. (**A**) Detection of the antibody–antigen interface highlighting interacting residues. (**B**) Residue-level contact profiling (antibody light chain) showing multiple interaction types and per-residue contact counts with interactive inspection. (**C**) Ramachandran plot summarizing backbone conformational distributions of the complex. (**D**) Inter-residue distance matrix (between antibody and antigen) with interface cutoff highlighting. (**E**) Per-residue van der Waals energy mapped onto the structure, indicating favorable binding regions. (**F**) Per-residue burial fraction, highlighting buried antibody CDRs and antigen epitope regions.

rather than absolute binding energies, they effectively highlight regions of favorable packing and potential steric clashes.

Geometric and distance measurements

SPICE provides structural quality assessment through geometric analysis. Ramachandran plots use ϕ/ψ backbone dihedrals to classify 2D regions as favored, allowed, or disallowed. SPICE highlights the interface residues in the Ramachandran plots. Chi angle analysis evaluates side-chain rotameric states ($\mathcal{X}_1 - \mathcal{X}_4$) comparing interface versus non-interface conformations. Bend angles measure backbone curvature identifying flexible and rigid regions. Distance analysis computes inter-residue measurements using minimum atomic distances. Interactive distance heatmaps display color-coded matrices highlighting close contacts with a tuneable cut-off distance.

Multi-complex comparative analysis

A distinguishing feature of SPICE is the comparative analysis of multiple protein complexes, which enables the systematic comparison of wild-type versus mutant structures, different binding partners, or evolutionary variants. The comparison module performs alignment-free analysis, computing each complex independently then aggregating results into comparative tables and differential visualizations.

For multi-complex comparison, SPICE generates comprehensive side-by-side comparisons with residue-level resolution. Comparative visualizations include per-residue metrics such as buried surface fraction, VDW energy contributions, bond counts by interaction type (hydrogen bonds, salt bridges, and disulfide bonds), and total interface contacts. Statistical summaries identify top contributing residues across variants and quantify interaction type frequencies. Mutation impact analysis correlates structural and energetic changes through ΔVDW versus ΔSASA scatter plots, revealing whether mutations affect binding through energetic or geometric mechanisms. Synchronized 3D visualization enables direct structural comparison with residues color-coded by respective properties. This multi-dimensional comparison proves particularly valuable for structure-based mutagenesis

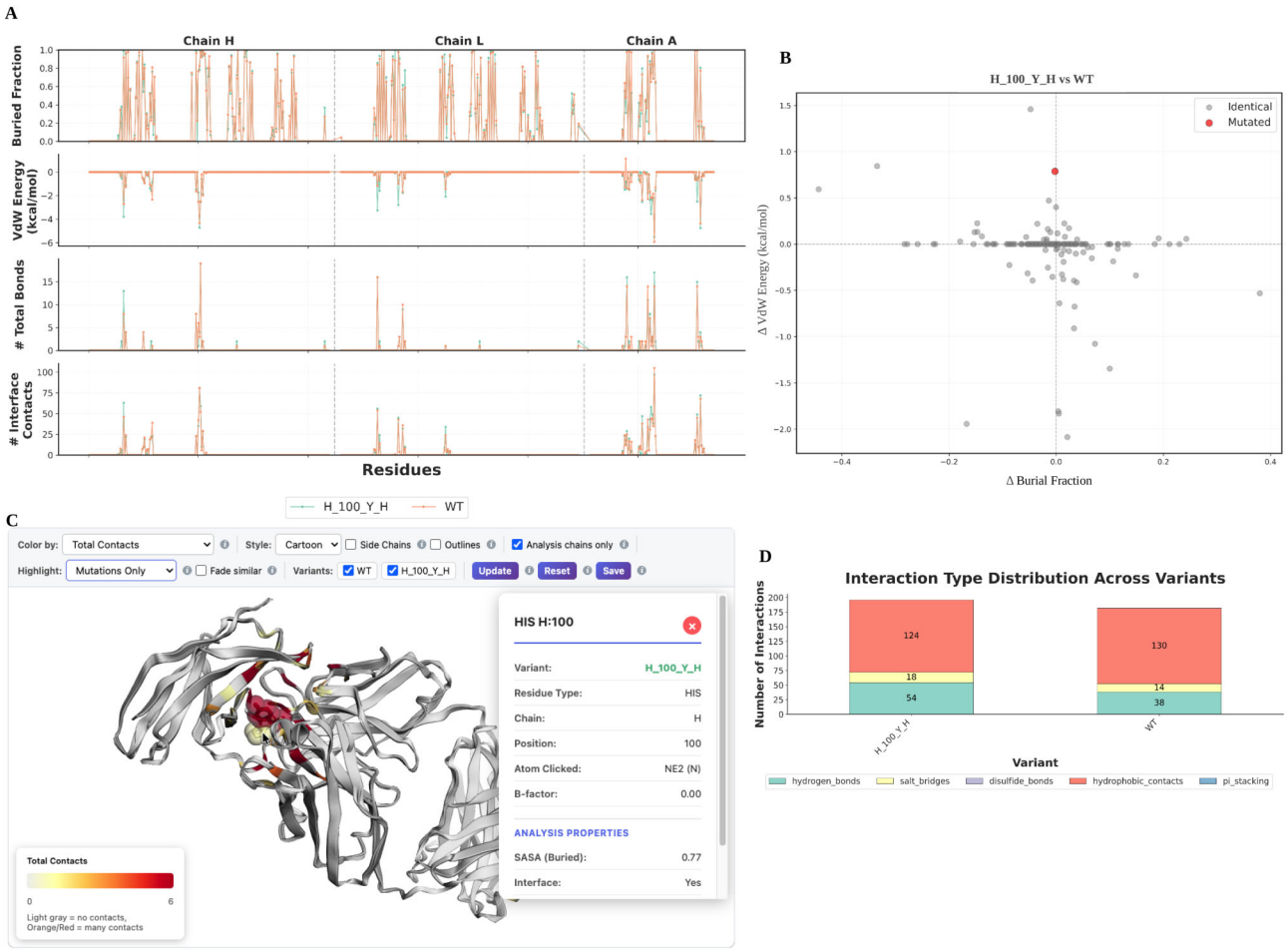

Figure 3. Outputs generated by SPICE for the comparative analysis of the 5GGS antibody–antigen complex. The wild-type complex (5GGS) is compared against a single-mutation variant (5GGS_H_100_Y_H). (**A**) Per-residue property profiles across all chains, comparing burial fraction, van der Waals energy, total bonds, and interface contacts between wild-type and mutant. (**B**) Residue-level mutation impact showing changes in burial fraction and van der Waals energy relative to the wild-type. (**C**) Interactive 3D visualization with mutation-aware highlighting and interactive structural and energetic annotations. (**D**) Distribution of interaction types, illustrating changes in bond counts between the wild-type and mutated complexes.

studies and antibody engineering where systematic variant evaluation is essential.

Visualization and output

SPICE integrates 3Dmol.js [14] for interactive molecular visualization, enabling users to explore protein complexes with residues highlighted and color-coded by their respective properties. Users can rotate structures, zoom to specific regions, and toggle display of different interaction classes. Clicking interface residues in the 3D viewer show all the precomputed properties of the residues, which is particularly useful in multi-complex analysis.

Quantitative results are presented through as high-quality graphical plots and comprehensive HTML tables, which support sorting and filtering. All results are packaged in downloadable HTML reports that can be archived or shared with collaborators. The interface design prioritizes clarity and scientific rigor while remaining accessible to users with limited computational expertise.

Results

We demonstrate the capabilities of SPICE through two case studies that highlight both single complex analysis and multi-complex comparison. Using experimentally resolved complex structures, we show how SPICE integrates structural, energetic, and interaction-based analyses to characterize binding interfaces at residue level, and how they change due to mutation events.

Case study 1: egg white lysozyme in complex with mouse monoclonal antibody D1.3

To demonstrate single-complex analysis in SPICE, we analyzed the egg white lysozyme complex with the mouse monoclonal antibody D1.3 (PDB ID: 1A2Y). SPICE automatically identified the antibody–antigen interface and interacting residues across the heavy and light chains, and the antigen (Fig. 2A). Residue-level contact profiling further resolved the types and frequencies of intermolecular interactions, enabling rapid identification of contact rich regions and potential binding hotspots (Fig. 2B).

SPICE also provided structural and conformational assessments, including Ramachandran analysis (Fig. 2C) to evaluate the backbone geometry, and distance matrix (Fig. 2D) visualizations to examine spatial proximity patterns that define the interface. Energetic and solvent-accessibility analyses complemented these structural views. Per-residue van der Waals energy profiles highlighted energetically favorable interface

Table 1. Comparing SPICE with existing tools for the analysis of protein complexes

Feature	SPICE [this work]	PDBePISA [3]	PISA [4]	MAPIYA [7]	PIC [5]	PRODIGY [6]
Modular selection	✓	–	–	–	–	–
Multi-complex comparison	✓	–	–	–	–	–
H-bonds/salt bridges	✓	✓	✓	✓	✓	–
SASA analysis	✓	✓	✓	✓	–	✓
VDW energetics	✓	–	–	–	–	–
Interactive 3D	✓	✓	–	✓	–	–
Web-based	✓	✓	✓	✓	–	✓

regions (Fig. 2E), while burial fraction measurements revealed strong burial of antibody CDRs and corresponding antigen epitope residues upon complex formation (Fig. 2F).

Figure 2 is a small representative subset of the visualizations that SPICE can produce on each major analysis category. SPICE can generates additional quantitative summaries, alternative visualizations, and downloadable reports for each analysis module, allowing users to explore protein-protein interactions at multiple scales within a single, unified framework.

Case study 2: PD1–pembrolizumab complex–wildtype versus mutated comparison

To demonstrate SPICE's multi-complex comparative analysis capabilities, we analyzed the PD1–pembrolizumab complex (PDB ID: 5GGS) and a single-mutation variant (5GGS_H_100_Y_H). SPICE enabled side-by-side comparison of structurally aligned complexes and systematically quantified mutation-induced changes across structural, energetic, and interaction-based properties.

Per-residue property profiles (Fig. 3A) revealed how the mutation altered burial fraction, van der Waals energy, total intermolecular bonds, and interface contacts across all chains, allowing direct localization of mutation-driven effects along the sequence. Residue-level differential analysis further summarized changes in burial and energetic contributions relative to the wild-type, highlighting residues most impacted by the mutation (Fig. 3B). Interactive 3D visualization (Fig. 3C) integrated these quantitative results with structural context, enabling mutation-aware highlighting and interactive inspection of residue-specific properties. An aggregate interaction statistics across complexes to compare distributions of hydrogen bonds, salt bridges, hydrophobic contacts, and other interaction types between variants is shown in Fig. 3D.

Again, Fig. 3 illustrate a small representative subset of outputs from each comparative analysis module; SPICE provides additional summaries, visualizations, and downloadable reports to support comprehensive assessment of mutation effects on protein–protein interaction.

Discussion

SPICE addresses key limitations in existing protein complex analysis tools through modular analysis selection, integrated multi-complex comparison, and interactive visualization. Table 1 illustrates the featured offered by SPICE compared to existing tools for protein complex analysis. Unlike most of other tools which execute rigid analysis pipelines, SPICE empowers users to select only the analyses relevant to their scientific investigation, optimizing computational time and resources while retaining access to a broad range of structural and energetic metrics. Its native support for multi-complex comparison further automates variant- and homolog-level analysis, eliminating the manual post-processing typically required to evaluate mutation effects or structural differences across complexes. By unifying flexible analysis, comparative workflows, and publication-ready visualization within a single platform, SPICE lowers the barrier to systematic, reproducible interrogation of protein–protein interfaces and provides a practical foundation for both exploratory and hypothesis-driven studies in structural biology, protein engineering, and drug design.

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Conflict of interest

None declared.

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Data availability

The Python package underlying SPICE is publicly available via Zenodo at https://doi.org/10.5281/zenodo.18509535 and https://github.com/fbabd/PyPdbComplex.

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