

SOURSOP: A Python Package for the Analysis of Simulations of Intrinsically Disordered Proteins

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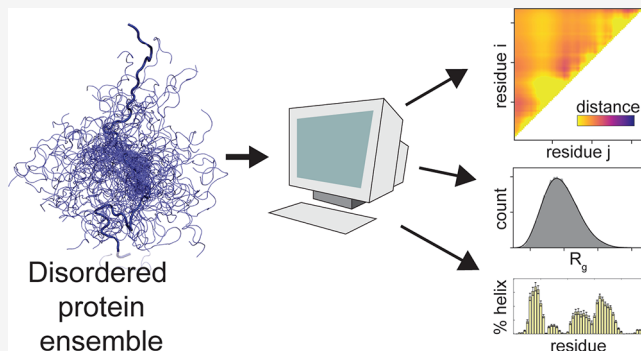


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ABSTRACT: Conformational heterogeneity is a defining hallmark of intrinsically disordered proteins and protein regions (IDRs). The functions of IDRs and the emergent cellular phenotypes they control are associated with sequence-specific conformational ensembles. Simulations of conformational ensembles that are based on atomistic and coarse-grained models are routinely used to uncover the sequence-specific interactions that may contribute to IDR functions. These simulations are performed either independently or in conjunction with data from experiments. Functionally relevant features of IDRs can span a range of length scales. Extracting these features requires analysis routines that quantify a range of properties. Here, we describe a new analysis suite simulation analysis of unfolded regions of proteins (SOURSOP), an object-oriented and open-source toolkit designed for the analysis of simulated conformational ensembles of IDRs. SOURSOP implements several analysis routines motivated by principles in polymer physics, offering a unique collection of simple-to-use functions to characterize IDR ensembles. As an extendable framework, SOURSOP supports the development and implementation of new analysis routines that can be easily packaged and shared.



1. INTRODUCTION

Natively unfolded proteins or intrinsically disordered proteins and regions (collectively referred to as IDRs) are a ubiquitous class of proteins and domains that regulate various molecular functions and cellular phenotypes.^{1–4} Unlike folded domains, which are well described by a few structurally similar microstates, IDRs are defined by their conformational heterogeneity.^{4,5} As a result, the accurate description of IDRs in the solution state necessitates a statistical description of the underlying conformational ensembles.⁶ These ensembles, which are affected by changes to solution conditions and the types of components present in the solvent, are distributions of energetically accessible protein conformations that capture the sequence-encoded conformational biases associated with a given IDR.^{4,7,8} Several studies have established direct connections between sequence–ensemble relationships of IDRs and the molecular functions of these conformationally heterogeneous regions.^{8–10} Accordingly, there is a need for facile, ready-to-use methods to uncover the molecular grammars that underlie sequence–ensemble–function relationships of IDRs.⁹

Measurements of IDR ensembles in solution allow for quantitative mapping of sequence–ensemble relationships. Techniques that obtain statistical information on molecular conformation without pre-supposing the existence of a single dominant state are well equipped to characterize IDR ensembles. These techniques include static and dynamic

multiangle light scattering (SLS and DLS, respectively), small-angle X-ray scattering (SAXS), circular dichroism (CD), infrared spectroscopies, electron paramagnetic resonance (EPR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, multiparameter fluorescence spectroscopies, and other single-molecule techniques.^{7,11–16} While these experimental techniques offer a window into conformational behaviors, they typically probe a single type of conformational characteristic (e.g., global ensemble-average dimensions, distances between specific positions along the chain, etc.). Accordingly, alongside these experimental approaches, all-atom and coarse-grained molecular simulations are routinely deployed to make predictions or interpret data obtained from experimental measurements. The joint application of experimental and computational methods enables the integration of multiple conformational inputs, thus affording a holistic assessment of sequence–ensemble relationships.^{17–23}

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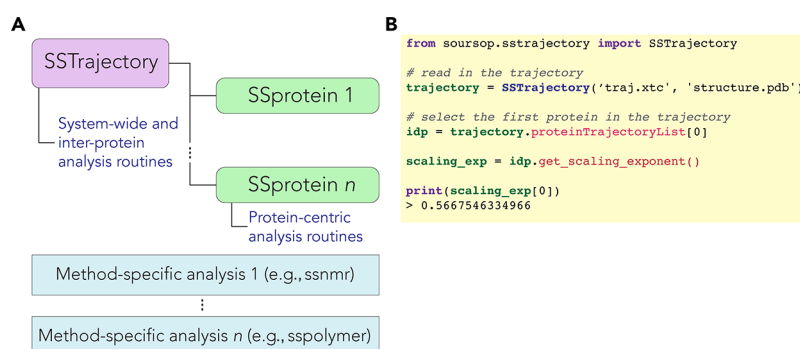


Figure 1. Architecture and example code for SOURSOP. (A) Trajectory files are read into an SSTRajjectory object. This object automatically parses each polypeptide chain into separate SSprotein objects. Each SSprotein object has a set of object-based analyses associated with them. Each trajectory must have between 1 and n protein chains in it. In addition, various stateless method-specific analysis modules exist for certain types of analysis. Additional stateless methods can be extended to allow new analysis routines to be incorporated in a way that does not alter the SSprotein or SSTRajjectory code. (B) Example code illustrating how the apparent scaling exponent can be calculated from an ensemble.

Simulations of all stripes, but specifically all-atom simulations based on explicit or implicit representations of the solvent, are especially useful for describing sequence-specific conformational ensembles of IDRs.^{24,25} If a simulation can fully explore the conformational landscape and the force field being used is accurate enough, then all-atom molecular simulations enable the direct prediction of ensembles from sequence. These computationally derived ensembles can be compared directly or indirectly with experiments or used in isolation to understand functional and evolutionary constraints on IDRs.^{19,20,25} Consequently, there has been substantial interest in developing and applying molecular dynamics (MD) and Monte Carlo (MC) simulations to study IDRs.^{26–33} It is worth noting that the length of an IDR is not an intrinsic limitation on the generation of converged results from all-atom simulations, especially MC simulations based on implicit solvent models such as ABSINTH. The limitation is invariably the ruggedness of the free energy landscape, which is generally a challenge for IDRs characterized by a diverse range of energy scales.

As all-atom simulations have become increasingly routine, various software packages have emerged to perform and analyze molecular simulations. Major packages for performing all-atom simulations (so-called simulation engines) include, but are not limited to, Amber, CAMPARI, CHARMM, Desmond, GROMACS, LAMMPS, OpenMM, and NAMD.^{30,34–40} Alongside the development of simulation engines, there has also been an emergence of stand-alone packages for simulation analysis. Although most simulation engines contain their analysis routines, stand-alone analysis packages provide an alternative that, in principle, can be relatively lightweight, customizable, and unburdened by coding practices or conventions of the inevitably larger simulation engines. General-purpose analysis packages include Bio3D, CPPTRAJ, ENSPARA, LOOS, MDAnalysis, MDtraj, ST-Analyzer, VMD, and others^{41–43} (see Table S1 for a more extensive list). While some packages are general-purpose libraries for analyzing simulation trajectories, others are developed with a specific goal in mind.^{44–46} Decoupling analysis from performing simulations allows for ease of use, installation, and portability to be prioritized in analysis packages, while performance can be prioritized in simulation engines. It also enables familiarity with a single-analysis framework that can be applied across different simulation engines.

All-atom simulations of IDRs are becoming increasingly common.^{17,19,47} Despite this, there is a lack of stand-alone analysis packages specifically catering to the analysis of IDR conformational ensembles. Given their inherently heterogeneous ensembles and the lack of a relevant single reference structure, many of the structure-centric analyses commonly employed in the context of folded may be poorly suited for characterizing IDR ensembles. In contrast, concepts and principles from polymer physics have been taken and applied to interpret and understand disordered and unfolded proteins to great effect.^{6,11,19,48–51}

Here, we introduce SOURSOP (simulation analysis of unfolded regions of proteins), a Python-based software package for the analysis of all-atom simulations of disordered and unfolded proteins. SOURSOP combines analysis routines commonly found for folded proteins with a range of IDR-centric analyses that have been used extensively across many publications over the last half-decade. In the remainder of this article, we lay out the software architecture of SOURSOP, provide several analysis examples that can be performed, and offer a discussion of practical and conceptual features associated with the software.

2. METHODS

SOURSOP is written in Python 3.7+ and built atop the general-purpose simulation analysis package MDtraj.⁴¹ SOURSOP uses MDtraj as a backend for parsing simulation trajectories and can accept trajectories in a wide variety of file formats. Although trajectory files are parsed into SOURSOP-specific objects, the underlying mdtraj.topology and mdtraj-trajectory objects remain user-facing and accessible. In this way, any analysis written to work with MDtraj is directly applicable to SOURSOP objects.

SOURSOP reads a simulation trajectory into an SSTRaj object. The SSTRaj object automatically extracts individual protein chains into their SSprotein objects. SSprotein objects are the base object upon which single-chain analysis routines are applied as object functions. In addition, peripheral modules that include ssnmr and sspre provide modular, protein-independent analyses that work in conjunction with an SSprotein object. In this way, SOURSOP abides by the software principle of loose coupling, facilitating maintainability and future extension. The overall architecture of SOURSOP is shown in Figure 1A.

Where possible and appropriate, SOURSOP engages in memoization, a dynamic programming approach where expensive calculations are saved after being executed once.⁵² This offers a general strategy that avoids repeated recalculation of (for example) the same sets of distances. In addition to intramolecular analysis codified in the SSProtein object, intermolecular and multi-chain analysis routines are included in the SSTraj object. In this way, a simple and standardized interface for working with protein ensemble data is provided. Ensembles to be analyzed could be generated through standard all-atom simulations, but PDB ensembles from NMR or ensemble selection procedures are also directly analyzable.

A major goal in developing SOURSOP is to make simulation analysis easy and intuitive, for both users and developers. For example, Figure 1B offers a simple example of computing a protein's apparent scaling exponent (ν^{app}) for a protein in a simulation trajectory. While a straightforward user experience is an obvious goal for any software package, providing a consistent, well-defined, and accessible software architecture is essential for long-term maintenance and extensibility. Well-structured software is also necessary to enable productive and sustainable open-source contributions.

The current working version can be found at <https://github.com/holehouse-lab/soursop>, with documentation at <https://soursop.readthedocs.io/>. SOURSOP uses PyTest (<https://docs.pytest.org/en/stable/>) for unit testing, Sphinx (<https://www.sphinx-doc.org/en/master/>) and readthedocs (<https://readthedocs.org/>) for documentation, and Git (<https://git-scm.com/>) and GitHub (<https://github.com/>) for version control. The original repository structure was generated using cookiecutter (<https://github.com/cookiecutter/cookiecutter>). Explicit dependencies include MDTraj,⁴¹ SciPy,⁵³ NumPy,⁵⁴ Pandas,⁵⁵ and Cython.⁵⁶

In addition to the analyses shown here, we provide a collection of Jupyter notebooks along with the full trajectories (where possible) that offer examples of more general IDR-centric analysis that can be performed on the ensembles studied here (https://github.com/holehouse-lab/supportingdata/tree/master/2023/lalmansingh_2023). The SOURSOP code is consistent and heavily commented. The documentation also provides specific guidance for the development and integration of new analysis routines into SOURSOP.

3. RESULTS

To demonstrate the analyses available in SOURSOP, we have analyzed a collection of ensembles generated by various methods. The trajectories analyzed were generated using CAMPARI (an all-atom Monte Carlo simulation engine) or Desmond (an all-atom MD simulation engine).^{26,57,58} The analyses performed here are offered as convenient examples of the types of analyses and insight enabled by SOURSOP. All of the analyses described in this section are based on functions defined in the documentation at <https://soursop.readthedocs.io/>, and we note in-line the associated function names the first time a specific analysis is referenced. In addition, examples and tutorials for SOURSOP are available at <https://soursop.readthedocs.io/en/latest/usages/examples.html>.

3.1. IDR Global Dimensions Show Extensive Sequence-Dependent Conformational Biases. A challenge in studying IDRs is the absence of an obvious reference state. While folded proteins are typically associated with a native conformation, which can serve as a reference point for further

analysis, the structural heterogeneity of an IDR means that no single state serves this purpose. Conveniently, polymer physics offers analytical tools that can serve as reference states for disordered and unfolded protein ensembles.^{30,48,59–63} As a result, dimensionless polymeric parameters can be computed, which allows the conformational behavior of very different proteins to be quantitatively and directly compared. SOURSOP implements the calculation of many of these parameters, facilitating ensemble analysis.

We reanalyzed a series of conformational ensembles using two such dimensionless reference parameters. Specifically, we computed instantaneous asphericity (δ^*) (`get_asphericity()`), which measures the shape of a given conformation.⁶⁴ The instantaneous asphericity is defined as

$$\delta^* = 1 - 3 \frac{L_1 L_2 + L_2 L_3 + L_3 L_1}{(L_1 + L_2 + L_3)^2} \quad (1)$$

where L_1 , L_2 , and L_3 are the eigenvalues of the gyration tensor (\mathbf{T}), which in turn is defined as

$$\mathbf{T} = \frac{1}{n} \sum_{i=1}^n (r_i - r_c) \otimes (r_i - r_c) \quad (2)$$

where \mathbf{T} is calculated for every conformation, n is the number of atoms in the system, r_i is the position vector of atom i in the conformation of interest, r_c is the centroid of all atom positions in that conformation, and \otimes reflects the tensor product (also known as the dyadic product). The gyration tensor itself is also accessible via the `get_gyration_tensor()` method.

In addition to the instantaneous asphericity, we can calculate t (`get_t()`), a dimensionless parameter that quantifies global dimensions effectively via a normalized radius of gyration as originally defined by Vitalis and Pappu as

$$t = f_1(f_2(R_g/L_c))^{f_3/N^{0.33}} \quad (3)$$

where N is the number of residues in the sequence, L_c is the contour length of the polypeptide in Angstroms ($3.6 \times N$), and f_1 , f_2 , and f_3 are parameters used to ensure that t remains in an interval of 0–1.⁶⁵ f_1 , f_2 , and f_3 are defined as 2.5, 1.75, and 4.0, respectively.⁶⁵ By generating two-dimensional density plots that quantify the simultaneous evaluation of δ^* and t for each conformation, a quantitative and length-normalized representation of IDR global conformational preferences can be easily visualized.

Both t and δ^* are transformations of the eigenvalues from the gyration tensor \mathbf{T} . They represent global order parameters to describe the size and shape of a given conformation. An alternative normalization approach is using polymer models as reference states or considering additional polymeric parameters that report on the global chain dimensions (e.g., hydrodynamic radius, end-to-end distance, or the apparent polymer scaling exponent).^{66–69} All of these can be calculated in SOURSOP (`get_radius_of_gyration()`, `get_hydrodynamic_radius()`, `get_end_to_end_distance()`, and `get_scaling_exponent()`). For convenience, we focus here on t and on the normalized radius of gyration, although other metrics would likely report similar conclusions. To normalize the radius of gyration, we make use of an analytical model (the analytical Flory random coil, AFRC) that provides the expected radius of gyration if the chain behaved as a polymer in a theta solvent.⁶⁶

We analyzed conformational ensembles with over 3×10^4 distinct conformers obtained from previously published

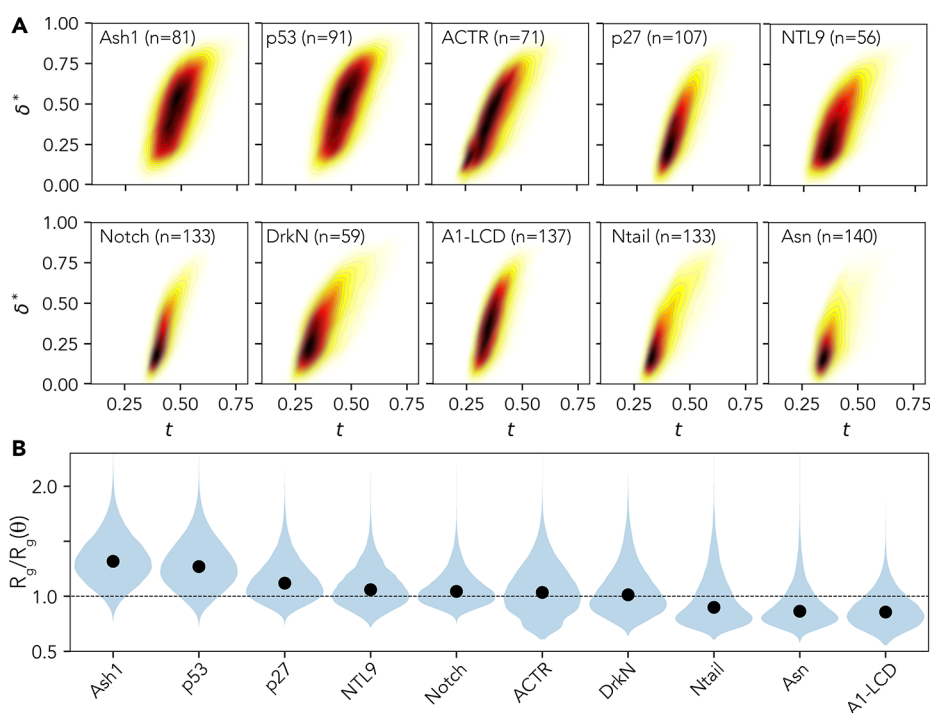


Figure 2. Global conformational analysis of 10 disordered protein ensembles analyzed with SOURSOP. (A) The two-dimensional density plots for instantaneous asphericity (δ^*) and normalized dimensions (t) reveal a broad range of conformational landscapes. Ash1,⁵⁸ p53,⁷³ p27,⁷² NTL9,⁶⁸ Notch,⁷⁰ and A1-LCD⁵⁷ are ensembles generated by Monte Carlo ensembles with the ABSINTH implicit solvent model.⁶⁰ ACTR, drkN, Ntail, and Asn (alpha synuclein) are ensembles generated by molecular dynamics simulations with the Amber99-disp force field.²⁶ Note that NTL9 is not an IDR, but the ensemble reported here represents an unfolded-state ensemble obtained under native conditions.⁶⁸ (B) Normalized chain dimensions were calculated by normalizing the instantaneous radius of gyration from ensembles by the expected radius of gyration from a sequence-matched chain in the theta state, where chain–chain and chain–solvent interactions are counterbalanced.^{6,62,73}

simulations that have been directly benchmarked against experiments to compare how ensemble size and shape vary across different IDRs (Figure 2A and Table S1).^{26,57,58,68,70–72} This analysis revealed a wide array of global conformational behaviors for IDRs. Our observations highlight properties ranging from heterogeneous compact ensembles to highly expanded self-avoiding random chains commensurate with polypeptides under strongly denaturing conditions. To contextualize these global dimensions, we also calculated normalized radii of gyration using the dimensions of a sequence-matched chain under conditions in which chain–chain and chain–solvent interactions are counterbalanced, with similar results (Figure 2B).⁶⁶

The diversity in global IDR properties (size and shape), as illustrated in Figure 2A, is often masked by ensemble-average properties. As a result, two IDRs may appear, on average, to be highly similar. The simulation analysis uncovers differences using the full distribution of conformations, which is evidently even for relatively simple order parameters such as δ^* and t , in agreement with prior work, showing that ensemble-average properties can mask complexities in the underlying conformational ensemble.^{74–76}

3.2. Aromatic Residues, Charged Residues, and Proline Play an Outsized Role in Dictating the Conformational Behavior of Disordered Proteins. Next, we applied SOURSOP to identify key sequence determinants of the attractive and repulsive intramolecular interactions that determine global and local conformational biases in IDR ensembles. To evaluate local chain interactions, we computed the radius of gyration over a sliding window of 14 residues to generate a linear profile of local density, normalizing for steric

effects via an atomistic excluded volume (EV) model (Figure 3, [get_local_collapse()]) see the Supporting Information). We note that the window size was chosen to reflect approximately 3 times the polypeptide blob length, but the window size is a free parameter that can be passed to the function.⁷⁷ To assess long-range interactions, we computed scaling maps (Figure 4, [get_distance_map()]). Scaling maps report inter-residue distances (distance maps) normalized by the expected distances from some reference polymer model, in this case, the EV model. The use of scaling maps accounts for the intrinsic contribution that chain connectivity has to inter-residue distances. While we use numerical simulations of EV polymers to generate the reference state for our scaling maps here, scaling maps can, more broadly, involve any convenient reference state, which may include theoretical models or even sequences of different compositions.^{66,78} Moreover, SOURSOP also provides the ability to fit an ensemble to an apparent homopolymer model and then calculate deviations from that model across intramolecular distances [get_polymer_scaled_distance_map()].

Our analysis here across the set of simulations confirmed prior observations made by many groups that charged, aromatic, and proline residues emerge as key determinants of IDR local and global interactions irrespective of the force field or simulation approach being used (Figures 3 and 4).

While our analysis is necessarily retrospective and correlative, it is in line with prior experimental work.^{57,79–82} To explore this observation further, we performed all-atom simulations using the ABSINTH implicit solvent model of the p53^{1–91} with three phosphomimetic mutations (S15E, T18E, and S20E) and compared the result to previous simulations of

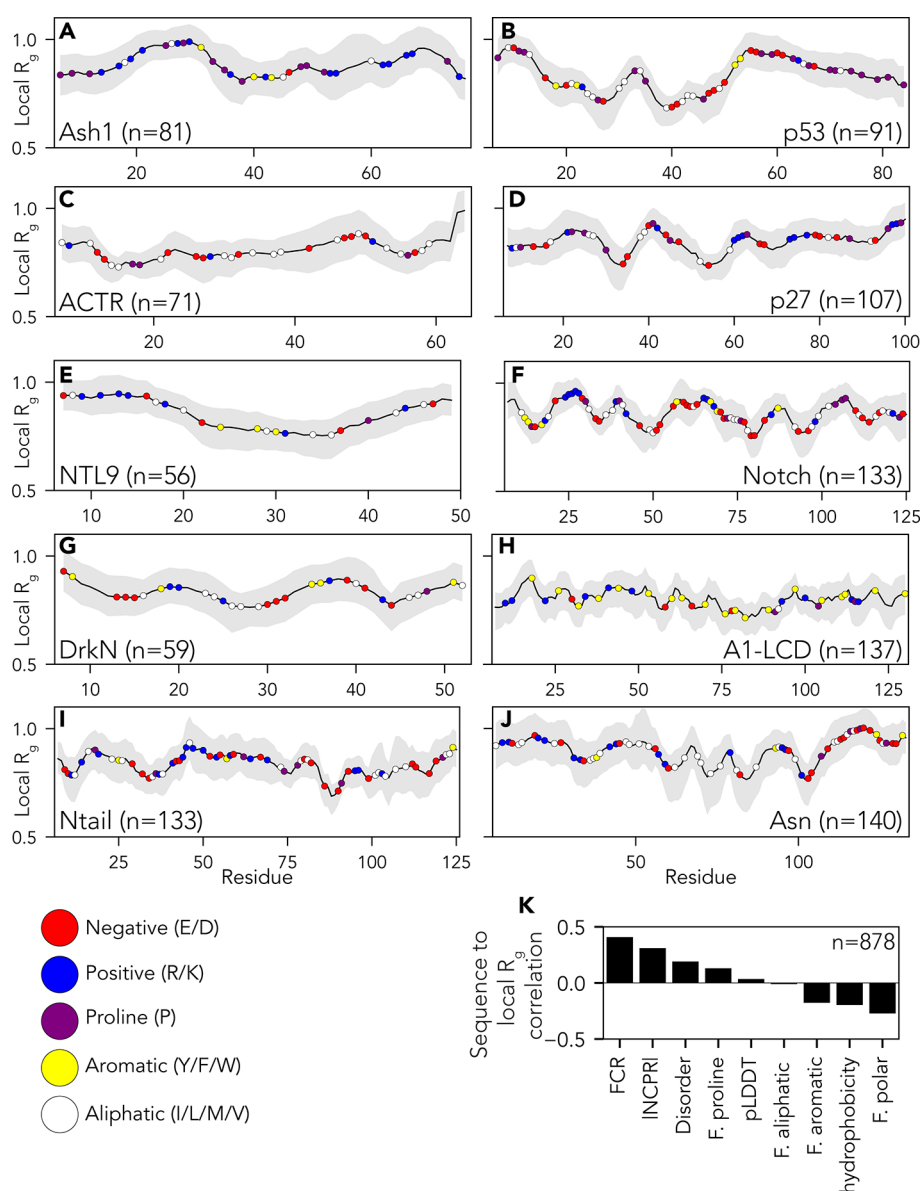


Figure 3. Local chain compaction with residue chemistry superimposed over the local radius of gyration (R_g). (A–J) Individual plots showing analysis for each protein ensemble, as introduced in Figure 2. Local R_g is calculated using a 14-residue sliding window. Colored circles on each plot represent different amino acid chemistry groups, highlighted in the legend below panel (I). (K) Pearson's correlation coefficient between local R_g obtained for each windowed fragment reported in panels (A–J) and the amino acid chemistry within the window in question (see also Figure S2). Specific sequence properties reported are the fraction of charged residues (FCR), absolute net charge per residue (INCPRI), mean disorder score as predicted by metapredict (Disorder), fraction of proline residues (F: proline), mean predicted local distance difference test (pLDDT - a measure of predicted AlphaFold2 structure confidence), fraction of aliphatic residues (F: aliphatic), fraction of aromatic residues (F: aromatic), Kyte Doolittle hydrophobicity (hydrophobicity), and fraction of polar residues (F: polar). The fraction of charged residues (FCR) is the strongest positive determinant of expansion, closely followed by the absolute net charge per residue (INCPRI). While polar residues, in principle, correlate as negative determinants of expansion, the negative correlation is driven by subregions deficient in charged residues and enriched in only polar residues.

the wildtype sequence (Figure 5A).⁷¹ While glutamic acid is an imperfect analogue for the phosphate group, the results revealed that relatively modest changes in linear charge density can cause local and long-range changes in the conformational ensemble. Despite substantial local conformational rearrangement, this leads only to a modest change of 0.5 Å in the mean radius of gyration (Figure 5B). Charge effects leading to seemingly minor changes in global dimensions while altering local networks of intramolecular interactions mirror prior work on the multi-phosphorylated proteins Ash1 and Sic1 and a region of the RNA polymerase CTD.^{58,78,83,84} Taken together, these results suggest that while local changes in charge density

can induce local conformational changes in ensemble behavior, compensatory changes in attractive (and repulsive) interactions that act on different or overlapping length scales can mask the effects of large-scale changes when global chain dimensions are examined.

3.3. Molecular Accessibility Is Context-Dependent in IDRs. It is often convenient to imagine IDRs as being uniformly solvent-accessible. While appealingly simple, given the complex conformational behavior observed in our analyses here and elsewhere, it may not be a given that every residue is equally accessible.^{58,75,85–88} To examine this idea further, we computed local accessibility across an eight-residue sliding

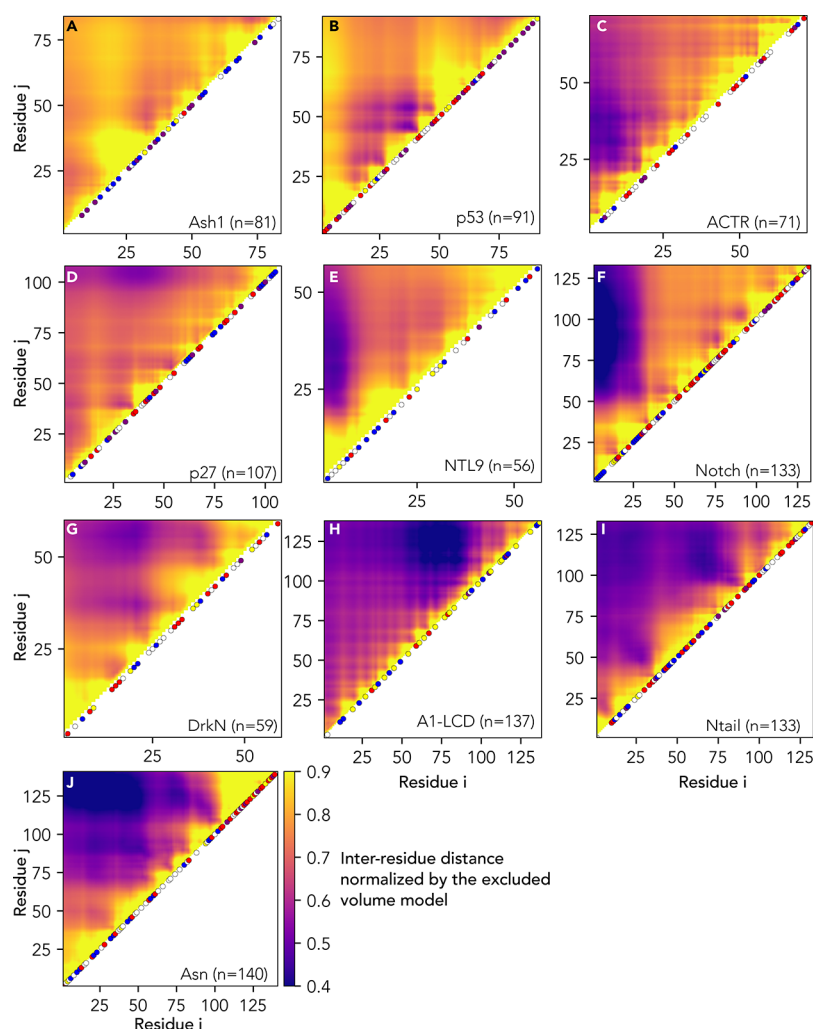


Figure 4. Preferential attraction and repulsion quantified via scaling maps that report the normalized distance between every pair of residues in the protein. (A–J) Individual plots of analysis for each protein ensemble, as introduced in Figure 2. Normalized distances are calculated by dividing the ensemble-average inter-residue distance by the distance obtained for the EV model. Attractive interactions emerge as darker colors, while repulsive interactions are lighter. Along the diagonal, subsets of residues are colored using the same color scheme used in Figure 3.

window for each IDR using a 10 Å spherical probe ([get_regional_SASA()]; Figure 6). Solvent accessibility here was calculated using the Shrake–Rupley algorithm.⁸⁹ We chose eight residues here to provide information on local conformational accessibility only (i.e., slightly larger than the blob size), although the window size used can be varied as an input parameter to the analysis function. This analysis allows us to assess how accessibility varies as a function of local sequence position.

Our analysis reveals substantial variation in molecular accessibility, suggesting that two residues of the same type may be differentially accessible depending on their broader sequence context (Figure 6). Clearly, the local sequence environment offers a mechanism to control the effective concentration of a local binding motif. The importance of local sequence context on molecular interactions can be further expanded if sequence-encoded chemistry provides partner-specific attractive and repulsive interactions. Taken together, despite the lack of a fixed 3D structure, it seems reasonable to speculate that the binding of motifs from IDRs should be considered not only both in terms of molecular sterics and shape complementarity (as is the conventional view for rigid-body molecular recognition) but also in terms of if and how

the local chain context influences their accessibility and chemical context.^{90,91}

4. DISCUSSION

Here, we introduce SOURSOP, an integrative Python-based software package for the analysis of all-atom ensembles extracted from simulations of intrinsically disordered proteins. SOURSOP is easy to install and use and is accompanied by extensive documentation and unit tests. Here, we have shown how SOURSOP can be applied to analyze all-atom ensembles extracted from two types of simulations (Monte Carlo simulations and molecular dynamics simulations) of different IDRs. SOURSOP contains a range of additional routines not explored in this work but have been applied to various systems under a range of contexts, including the local residual structure, intraresidue contacts, and the interaction between folded and disordered regions (Figure S1).^{57,58,92–94}

4.1. SOURSOP as a Stand-Alone Package. SOURSOP was developed as a stand-alone analysis package built on the existing general-purpose simulation analysis package MDTraj.⁴¹ The decision to develop SOURSOP as an independent package, as opposed to expanding the functionality of MDTraj, was motivated by several factors.

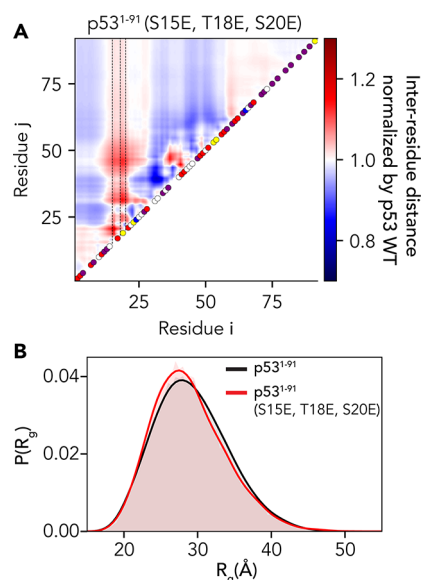


Figure 5. Comparison of changes in local and global dimensions for wildtype vs phosphomimetic versions of p53. (A) Scaling maps where inter-residue distances for the phosphomimetic version of the p53 N-terminal domain (p53^{1–91}) are normalized by distances for the wildtype protein. Despite differing by only three residues in the N-terminal quarter of the protein, the phosphomimetic version of p53 shows substantial differences in long-range and local dimensions, as shown by the emergence of both attractive (blue) and repulsive (red) interactions. (B) Despite these rearrangements, a relatively small change in overall global dimensions is observed. While the wildtype ensemble-average R_g is 29.4 Å, the phosphomimetic variant is 29.1 Å, a difference below the statistical detection limits for most experimental techniques.

First, many of the analysis routines built into SOURSOP are of limited value for the analysis of well-folded proteins. At this juncture, MDTraj is a stable and mature software package that functions as the backend to a range of tools associated with molecular simulations.^{44,95–99} To add features into MDTraj would unavoidably lead to additional technical debt—more features to keep track of, manage, and test for. Technical debt adds viscosity, risks the introduction of new bugs, and can hamper future development if several coding styles are combined.¹⁰⁰ Accordingly, the drawbacks of integrating the analysis routines into MDTraj were judged to be substantially greater than the possible benefits.

Second, our goal is for SOURSOP to provide a general platform where novel analysis routines appropriate for disordered proteins can be implemented by the burgeoning community of labs performing simulations of disordered proteins. This requires our ability to maintain control over a consistent programmatic interface, which can be achieved via an interface layer between MDTraj and SOURSOP but becomes challenging if analysis routines are implemented directly inside of MDTraj. For this reason, providing SOURSOP as a loosely coupled software component that works with MDTraj, as opposed to within MDTraj, enables the best of both worlds.

Finally, applying principles from polymer physics to analyze disordered proteins is not new. Several of the analysis routines provided by SOURSOP are also available in extant software, notably in the simulation engine CAMPARI (<http://campari.sourceforge.net/>).^{6,30,60,61,101} SOURSOP provides a lightweight toolkit that is simple to install, simple to use, and

interoperable with MDTraj and the collection of existing analysis tools therein. Therefore, while some overlap exists, we do not see SOURSOP as replacing the analysis routines in MDTraj or CAMPARI. Instead, SOURSOP is a complement to extant routines and packages. Furthermore, it makes it relatively straightforward for groups to publish scripts or Jupyter notebooks that enable full reproduction of their analysis workflow.

4.2. SOURSOP in the Broader Ecosystem of Simulation Software. Molecular simulations enable the characterization of biophysical properties that may be inaccessible to direct experimental measurement. Simulations typically involve either molecular dynamics (MD)- or Monte Carlo (MC)-based approaches. In addition, the underlying molecular system must be represented in some way, be that at all-atom resolution or some degree of coarse-graining. All-atom models represent every atom in a system explicitly. In contrast, coarse-grain models combine two or more atoms into groups and may include united-atom models (where typically two to four atoms are combined together) or lower resolution models in which residues or whole domains are represented as single beads.

As model resolution decreases, longer and larger simulations become increasingly accessible, yet assumptions and caveats made to justify the loss of details become increasingly broad. While it is tempting to expect all-atom models to be the gold standard, if simulations are unable to adequately sample a phenomenon of interest, then they may be inappropriate for a given question. In contrast, while coarse-grained simulations may seem appealing given their comparative simplicity when compared to all-atom simulations, the many assumptions that underlie their simplicity can be, inadvertently, misleading. As such, the appropriate model depends entirely on the question of interest.

The emergence and refinement of molecular simulations have coincided with significant advancements in computational resources in the past 30 years. As a result, simulations are now commonly used in the generation of hypotheses, to aid in the interpretation of experimental data, and to build molecular models of complex biological processes. In parallel, as molecular simulations have become more common-place, there has been a veritable Cambrian explosion of APIs, toolkits, plugins, and frameworks for analyzing molecular simulations (see Table S1 for a survey of ~100 analysis packages). These tools help make a complex, integrative analysis routine and lower the barrier for scientific discovery.

4.3. SOURSOP Analysis of Coarse-Grained Simulations. While SOURSOP was built to analyze all-atom simulations, many of the routines provided can be directly applied when working with coarse-grained simulations. If an input topology (PDB) file defines beads as “CA” (alpha carbon) as their atom type, then SOURSOP will correctly parse individual chains into individual residues, and the majority of the analyses described here are applicable for the analysis of coarse-grained simulations. It is important to emphasize that while we have used SOURSOP extensively to analyze coarse-grained simulations, certain functionality may not make sense or may fail (e.g., secondary structure analysis, sidechain bond vector analysis, etc.).^{102–104} As such, while SOURSOP does not officially support the analysis of coarse-grained simulations at this juncture, unofficially, it works relatively well in this capacity.

4.4. SOURSOP Is an Extendable Platform for Novel Analysis Routines. Analyzing IDR ensembles to reveal clear

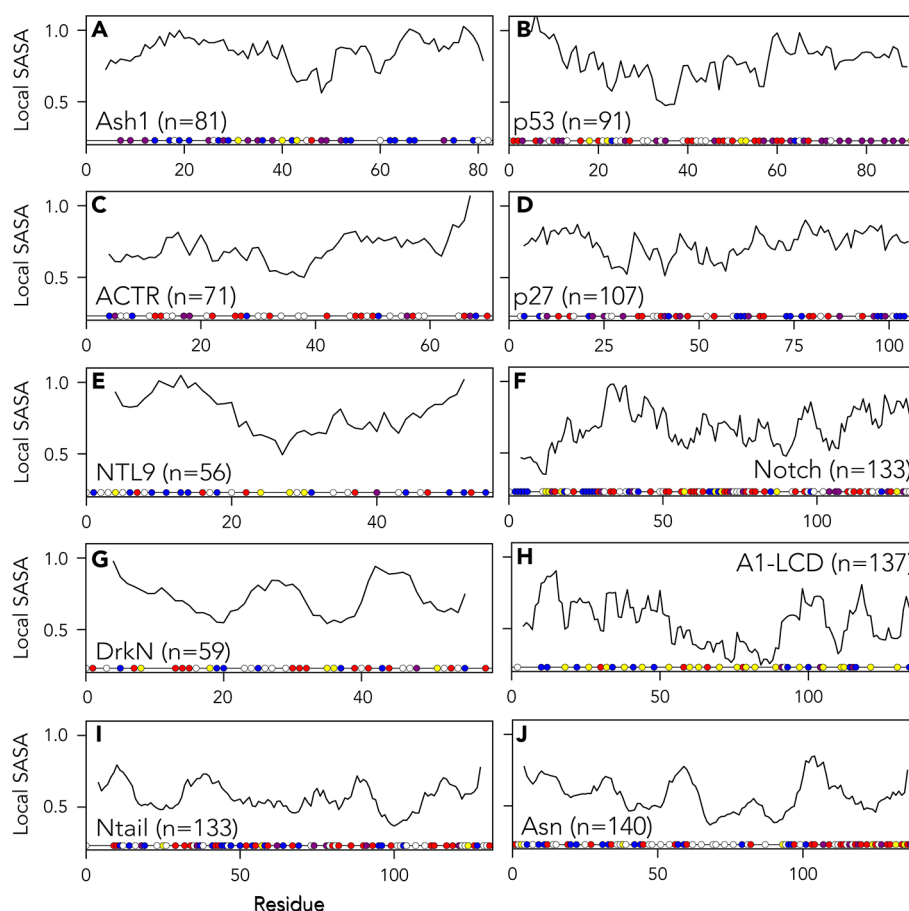


Figure 6. (A–J) Normalized local solvent-accessible surface area (SASA) using an eight-residue sliding window and a 10 Å probe size. Normalization is done using excluded volume (EV) reference simulations to account for side-chain-dependent differences in solvent accessibility. Amino acid residues are colored, as shown in Figure 3. Distinct patterns of accessibility are observed across different proteins, indicating that long- and short-range intramolecular interactions can influence the accessibility of local binding sites.

and interpretable conclusions remains challenging. Absent a native reference state, it can be difficult to generate informative and visually coherent representations that fully capture the inherent high dimensionality of an IDR ensemble. While various “standard” analyses have emerged for folded proteins (e.g., contact maps, per-residue RMSF, and the fraction of native contacts), there is less consensus on what the standard analyses should be when assessing IDR ensembles.

Rather than a problem, this raises an opportunity, where novel analysis and visualization approaches are needed. With this in mind, we hope that new analysis routines can be integrated into SOURSOP, facilitating distribution and packaging. Considering this objective, SOURSOP includes a well-defined style guide for new analysis routines and a collection of utility functions that provide automatic sanity checking and defensive programming for input data. We also provide documentation on how best to introduce a new routine and how to integrate it into the main codebase. These features, combined with the broad reach of the Python programming language, will lower the barrier to open-source and community-driven scientific development.

5. CONCLUSIONS

SOURSOP is an open-source Python toolkit for the general analysis of ensembles of disordered proteins. In addition to analyzing disordered protein ensembles, SOURSOP can also be used to analyze folded protein trajectories or individual

PDB files. As such, SOURSOP offers a general interface for calculating molecular properties, polymeric parameters, and the development of new IDR-centric analysis routines.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jctc.3c00190>.

Details on the simulations performed for this manuscript, along with a detailed description of the modern ecosystem of simulation analysis packages and several supplementary figures (PDF)

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Notes

The authors declare no competing financial interest. SOURSOP was previously called CTraj and CAMPARITraj. We renamed the package SOURSOP to avoid confusion and the incorrect implication that this is a CAMPARI-specific analysis package.

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