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Applications Note

Structural bioinformatics

PISA-SPARKY: an interactive SPARKY plugin to analyze oriented solid-state NMR spectra of helical membrane proteins

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Abstract

Motivation: Two-dimensional [^{15}N-^{1}H] separated local field solid-state nuclear magnetic resonance (NMR) experiments of membrane proteins aligned in lipid bilayers provide tilt and rotation angles for α-helical segments using *P*olar *I*ndex *S*lant *A*ngle (PISA)-wheel models. No integrated software has been made available for data analysis and visualization.

Results: We have developed the *PISA-SPARKY* plugin to seamlessly integrate PISA-wheel modeling into the *NMRFAM-SPARKY* platform. The plugin performs basic simulations, exhaustive fitting against experimental spectra, error analysis and dipolar and chemical shift wave plotting. The plugin also supports *PyMOL* integration and handling of parameters that describe variable alignment and dynamic scaling encountered with magnetically aligned media, ensuring optimal fitting and generation of restraints for structure calculation.

Availability and implementation: *PISA-SPARKY* is freely available in the latest version of *NMRFAM-SPARKY* from the National Magnetic Resonance Facility at Madison (http://pine.nmrfam.wisc.edu/download_packages.html), the NMRbox Project (https://nmrbox.org) and to subscribers of the SBGrid (https://sbgrid.org). The *pisa.py* script is available and documented on GitHub (https://github.com/weberdak/pisa.py) along with a tutorial video and sample data.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Oriented sample solid-state nuclear magnetic resonance (OS-ssNMR) spectroscopy enables the acquisition of highly resolved spectra of membrane proteins aligned in lipid bilayers (Opella and Marassi, 2004). In contrast to solution NMR and magic-angle spinning ssNMR, anisotropic contributions dominate chemical shifts and dipolar couplings of OS-ssNMR spectra, leading to enhanced spectral dispersion, especially for α-helices. These parameters provide invaluable topological restraints for structure determination and potentially provide highly sensitive probes that capture subtle signal transduction mechanisms that conventional structural techniques miss (Matthews *et al.*, 2006).

Two-dimensional [^{15}N-^{1}H] separated local field (SLF) experiments (Hester *et al.*, 1976) of uniformly ^{15}N-labeled samples, such as PISEMA (Wu *et al.*, 1994) and SAMPI4 (Nevzorov and Opella, 2007), provide residue-specific orientational restraints by correlating amide ^{15}N chemical shifts and ^{15}N-^{1}H dipolar couplings. The introduction of magnetically aligned media, such as bicelles (Sanders and Landis, 1995) and macrodiscs (Park *et al.*, 2011), has substantially improved the quality of these experiments. These lipid-mimetic systems provide high hydration levels and high lipid-protein ratios, which help stabilize membrane proteins structure and function (Dürr *et al.*, 2012). These improvements yield resolution sufficient for studies of larger multi-spanning systems (Weber and Veglia, 2019). For α-helical proteins, SLF spectra produce circular patterns of the resonances, reflecting the periodic nature of secondary structures, described accurately by the *P*olar *I*ndex *S*lant *A*ngle (PISA)-wheel model (Marassi and Opella, 2000; Wang *et al.*, 2000). These phenotypical models predict cross-peak positions for each residue as a function of the tilt (or slant) and rotational angles of the overall helical segment (Denny *et al.*, 2001), and they are commonly used in conjunction with selective labeling and unlabeling schemes for resonance assignments; while simultaneously determining

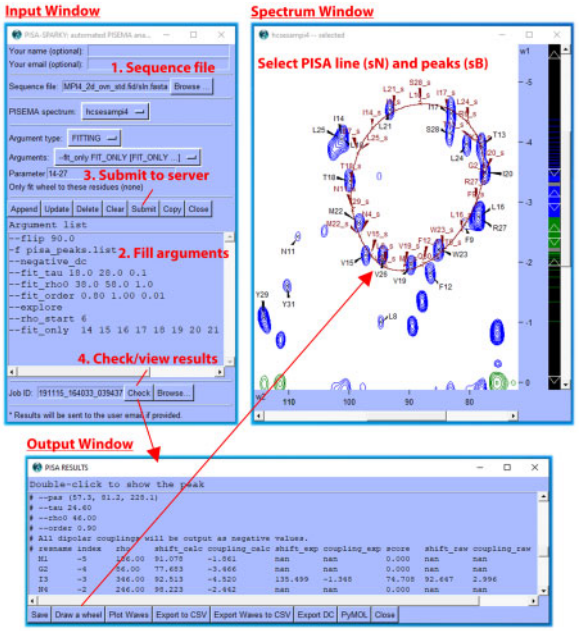

Fig. 1. Partial screenshot and workflow of the *PISA-SPARKY* plugin

descriptive topological parameters without complete structural calculations.

Although PISA models have been integral to OS-ssNMR for almost two decades, no software has been developed for widespread use by the ssNMR community for quantitative data analysis. We have, therefore, built the *PISA-SPARKY* plugin into *NMRFAM-SPARKY* (Lee *et al.*, 2015) as part of our development of an integrative platform for biomolecular NMR research (Lee *et al.*, 2016).

2 Materials and methods

The user loads the plugin *Input Window* by entering the two-letter code 'PS'. This sets up simulation and fitting calculations for submission to the *PISA-SPARKY* webserver. The plugin was written in *Python 2.7* using the *Tkinter* GUI module. The PISA simulation method is detailed in Supplementary Data and Supplementary Fig. S1 along with all parameters and their associated default values (Supplementary Table S1). Job ID is in date/time format YYMMDD_HHmmSS followed by a random number. Results are returned to the user's *SPARKYHOME/PISA/Job ID* directory (The two-letter code 'RD' is used to set SPARKYHOME) and read using the *Check* button, which displays the *Output Window* (see Fig. 1 or Supplementary Fig. S2 for full screenshot and workflow) showing all information contained in the *log file* with visualization and export options. Simulated peaks and a line taken from interpolated PISA data points in the accompanying *wave file* are displayed in the *Spectral Window* by the *Draw a Wheel* button. The *Plot Waves* button, which utilizes the *NDP-PLOT* module (Lee *et al.*, 2016), is used to prepare chemical shift and dipolar coupling wave plots. Peaks selected in the *Spectral Window* may be highlighted in *PyMOL* by the associated button. The *Export DC* button is used to export scaled dipolar coupling restraints as input into *PONDEROSA-C/S* (Lee *et al.*, 2014) for automated structure calculation.

Fittings are executed by the *pisa.py* script, which, if desired, may be used as a standalone tool (see Supplementary Data for additional information) on a local machine in a shell environment. The webserver provides an installation-free environment for computation-intensive jobs. The webserver consists of an AMD 48-core Opteron 2.6 GHz, 128 GB RAM, running the 64-bit *Linux CentOS* and *Python 3* virtual environment. The *pisa.py* script was written in *Python 3.7* using the *Numpy* library for numerical calculations and the *concurrent* library to parallelize fitting routines over multiple cores. Interactive communication between the user's local computer and the remote webserver is handled using *HTML*, *CGI/Perl*, *Bash* and *SSH*.

3 Results

The plugin is demonstrated in the Supplementary Data using an hcSE-SAMPI4 spectrum of sarcolipin reconstituted into an unflipped bicelle (Wang *et al.*, 2019). Examples describe usage of assignment-free fitting against manually specified spectral boundaries (Supplementary Fig. S3), exhaustive fitting to assignments selected within NMRFAM-SPARKY (Supplementary Fig. S2), basic PISA-wheel simulation (Supplementary Fig. S4A), error analysis (Supplementary Fig. S4B) and *PyMOL* integration (Supplementary Fig. S5).

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Conflict of Interest: none declared.

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