*Bioinformatics*, YYYY, 0–0
doi: 10.1093/bioinformatics/xxxxx
Advance Access Publication Date: DD Month YYYY
Application Note

*Structural bioinformatics*

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

Daniel K. Weber[1], Songlin Wang[1], John L. Markley[2], Gianluigi Veglia[1,3*] and Woonghee Lee[2*]

[1]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA, [2]National Magnetic Resonance Facility at Madison, Biochemistry Department, University of Wisconsin-Madison, Madison, WI 53706, USA, [3]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA

*To whom correspondence should be addressed.

Associate Editor: XXXXXXX

Received on XXXXX; revised on XXXXX; accepted on XXXXX

## Abstract

**Motivation:** Two-dimensional [$^{15}$N-$^{1}$H] separated local field (SLF) solid-state 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:** *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.

**Contact:** veglia001@umn.edu or wlee44@wisc.edu

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

## 1 Introduction

Oriented sample solid-state NMR (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 (MAS) 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 (2D) [$^{15}$N-$^{1}$H] 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), have 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 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 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).

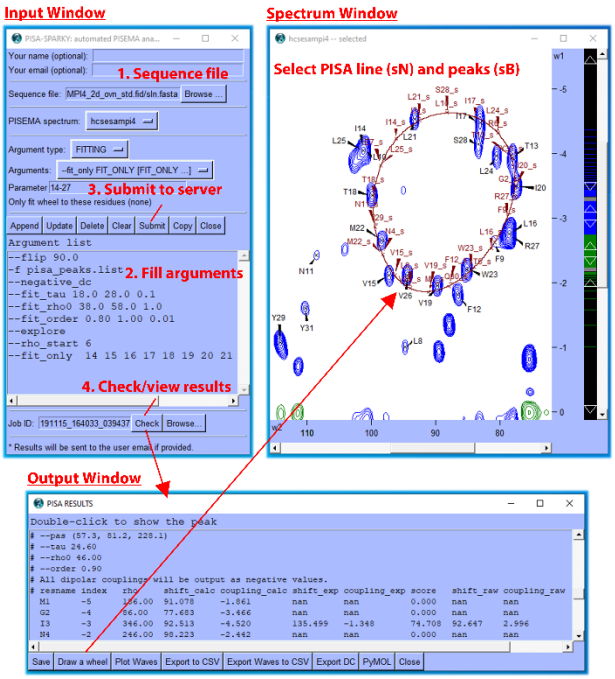
## 2 Methods

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 **Fig. S1** along with all parameters and their associated default values (**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 **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 a 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 (**Fig. S3**), exhaustive




fitting to assignments selected within NMRFAM-SPARKY (**Fig. S2**), basic PISA wheel simulation (**Fig. S4A**), error analysis (**Fig. S4B**) and *PyMOL* integration (**Fig. S5**).

## Acknowledgements

We thank Dimitri Maziuk for maintenance of the NMRFAM computing server, which hosts the PISA webserver.

## Funding

This work has been supported the National Science Foundation (DBI 1902076 to J.L.M. & W.L.), and partially by the National Institutes of Health (R01 GM 64742 and HL 144130 to G.V.; P41 GM 103399 to J.L.M.) and the American Heart Association (19POST34420009 to D.K.W.). Part of this work was carried out using hardware provided by the University of Minnesota Supercomputing Institute.

*Conflict of Interest:* none declared.

## References

Denny, J.K., *et al.* PISEMA powder patterns and PISA wheels. *J. Magn. Reson.* 2001;152(2):217-226.

Dürr, U.H.N., Gildenberg, M. and Ramamoorthy, A. The Magic of Bicelles Lights Up Membrane Protein Structure. *Chem. Rev.* 2012;112(11):6054-6074.

Hester, R.K., *et al.* Separated Local Field Spectra in NMR: Determination of Structure of Solids. *Phys. Rev. Lett.* 1976;36(18):1081-1083.

Lee, W., *et al.* Integrative NMR for biomolecular research. *J. Biomol. NMR* 2016;64(4):307-332.

Lee, W., Stark, J.L. and Markley, J.L. PONDEROSA-C/S: Client-server based software package for automated protein 3D structure determination. *J. Biomol. NMR* 2014;60(2-3):73-75.

Lee, W., Tonelli, M. and Markley, J.L. NMRFAM-SPARKY: enhanced software for biomolecular NMR spectroscopy. *Bioinformatics* 2015;31(8):1325-1327.

Marassi, F.M. and Opella, S.J. A Solid-State NMR Index of Helical Membrane Protein Structure and Topology. *J. Magn. Reson.* 2000;144(1):150-155.

Matthews, E.E., Zoonens, M. and Engelman, D.M. Dynamic Helix Interactions in Transmembrane Signaling. *Cell* 2006;127(3):447-450.

Nevzorov, A.A. and Opella, S.J. Selective averaging for high-resolution solid-state NMR spectroscopy of aligned samples. *J. Magn. Reson.* 2007;185(1):59-70.

Opella, S.J. and Marassi, F.M. Structure Determination of Membrane Proteins by NMR Spectroscopy. *Chem. Rev.* 2004;104(8):3587-3606.

Park, S.H.*, et al.* Nanodiscs versus macrodiscs for NMR of membrane proteins. *Biochemistry* 2011;50(42):8983-8985.

Sanders, C.R. and Landis, G.C. Reconstitution of Membrane Proteins into Lipid-Rich Bilayered Mixed Micelles for NMR Studies. *Biochemistry* 1995;34(12):4030-4040.

Wang, J.*, et al.* Imaging Membrane Protein Helical Wheels. *J. Magn. Reson.* 2000;144(1):162-167.

Wang, S., Gopinath, T. and Veglia, G. Improving the quality of oriented membrane protein spectra using heat-compensated separated local field experiments. *J. Biomol. NMR* 2019(0123456789).

Weber, D.K. and Veglia, G. A Theoretical Assessment of the Structure Determination of Multi-Span Membrane Proteins by Oriented Sample Solid-State NMR Spectroscopy. *Aust. J. Chem.* 2019.

Wu, C.H., Ramamoorthy, A. and Opella, S.J. High-Resolution Heteronuclear Dipolar Solid-State NMR Spectroscopy. *J. Magn. Reson., Ser A* 1994;109(2):270-272.