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 [¹⁵N-¹H] 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 Polar Index Slant Angle (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 (OSS-NMR) 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 [¹⁵N-¹H] separated local field (SLF) experiments (Hester et al., 1976) of uniformly ¹⁵N-labeled samples, such as PISEMA (Wu et al., 1994) and SAMP14 (Nevzorov and Opella, 2007), provide residue-specific orientational restraints by correlating amide ¹⁵N chemical shifts and ¹⁵N-¹H 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 Polar Index Slant Angle (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...
3 Results

The plugin is demonstrated in the Supplementary Data using an hcSe-SAMPI spectrum of sarcoplasmic 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 PSA-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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