Structural bioinformatics

CHESPA/CHESCA-SPARKY: automated NMR data analysis plugins for SPARKY to map protein allostery

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Abstract

Motivation: Correlated Nuclear Magnetic Resonance (NMR) chemical shift changes identified through the CHEMical Shift Projection Analysis (CHESPA) and CHEMical Shift Covariance Analysis (CHESCA) reveal pathways of allosteric transitions in biological macromolecules. To address the need for an automated platform that implements CHESPA and CHESCA and integrates them with other NMR analysis software packages, we introduce here integrated plugins for NMRFAM-SPARKY that implement the seamless detection and visualization of allosteric networks.

Availability and implementation: CHESCA-SPARKY and CHESPA-SPARKY are available in the latest version of NMRFAM-SPARKY from the National Magnetic Resonance Facility at Madison (http://pine.nmrham.wisc.edu/download_packages.html), the NMRbox Project (<https://nmrbox.org>) and to subscribers to the SBGrid (<https://sbgrid.org>). The assigned spectra involved in this study and tutorial videos using this dataset are available at <https://sites.google.com/view/chescachespa-sparky>.

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Supplementary information: [Supplementary data](#) are available at *Bioinformatics* Online.

1 Introduction

Protein allostery is one of the primary mechanisms for regulating biological function. It relies on long-range couplings between orthosteric and remote allosteric sites (Boulton and Melacini, 2016). Such couplings typically arise from transitions between macromolecular states with distinct structural and/or dynamic profiles. When allostery relies on subtle conformational and/or dynamical changes, the underlying allosteric networks often remain elusive to traditional structural determination methods. Protein NMR chemical shift perturbations offer a robust approach to mapping otherwise elusive allosteric networks. Specifically, variations in ¹H and ¹⁵N resonance frequencies are exquisitely sensitive not only to nearest-neighbor binding contacts but also to subtle, yet functionally relevant, allosteric transitions underlying long-range effects. Both CHESPA and CHESCA assume that the dynamic conformational equilibria underlying the allosteric couplings occur in the fast chemical-exchange regime. In this case, the observed NMR chemical shifts are linear,

population-weighted averages of conformer-specific values. Hence, it is possible to utilize peak positions to monitor shifts in local conformational equilibria.

The growing cohort of CHESPA and CHESCA applications (Akimoto *et al.*, 2013; Axe *et al.*, 2014; Chen *et al.*, 2017; Selvaratnam *et al.*, 2011, 2012; Shao *et al.*, 2020; VanSchouwen *et al.*, 2015; Walker *et al.*, 2019; Wang *et al.*, 2019; Xu *et al.*, 2019) illustrates the potential of these methods. To fully leverage this potential, both methods require a seamless integration with the PINE-SPARKY.2 and the I-PINE webserver (Lee and Markley, 2018; Lee *et al.*, 2019) for semi-automated protein chemical shift assignment and validation through 3D NMR triple-resonance experiments for the same protein in multiple bound or mutated forms. To enable such integration, we developed plugins and a webserver for the implementation and visualization of CHESPA and CHESCA through one of the most popular biomolecular NMR analysis suites: NMRFAM-SPARKY (Lee *et al.*, 2015) (Fig. 1 and Supplementary Fig. S1).

The CHESPA (two-letter-code cH; [Supplementary Fig. S2](#), left panel) and CHESCA (two-letter-code ch; [Supplementary Fig. S2](#), right panel) plugins enable the semi-automatic visualization of allosteric networks starting from unassigned NMR spectra ([Supplementary Methods](#)). To execute CHESPA-SPARKY, both reference and perturbation vectors need to be defined as well as parameters, such as ^{15}N versus ^1H weights, between-state and inter-peak chemical shift cutoffs. CHESCA-SPARKY set up is similar but relies on a library of differentially perturbed protein samples, along with additional parameters, such as correlation coefficient cutoff values. Both methods are executed locally with agglomerative clustering and singular value decomposition run by the NMRFAM server. Results are plotted with the integrated NDP-PLOT module and can be highlighted through PyMOL molecular visualization if a PDB structure is provided.

Both CHESPA- and CHESCA-SPARKY plugins are illustrated through the analysis of a set of ^1H , ^{15}N HSQC NMR spectra of the RI α subunit 119–244 construct of the cAMP-dependent protein kinase (protein kinase A, PKA) and its four complexes with cAMP and analogs. The results of the demonstration are available in the [Supplementary Figures S3–S8](#).

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