Accurate Measurement of RDC and RCSA for Small Molecule Configuration Analysis Using a Gel-Stretching Device

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Introduction

Stereochemical determination of small drug-like compounds represents a frequent challenge in the pharma industry. Conventionally, we have relied on NOE/ROE and *J* coupling data in combination with DFT (Density Functional Theory) modeling for such work. However, the limit of this approach manifests when the chiral centers are separated beyond the scope of these short-ranged data. This limitation prompted us to develop techniques that provide long-range structural information. While both RDC and RCSA are known to serve this purpose, RCSA is particularly attractive for proton-deficient molecules in which measurable RDCs are scarce. However, the application of RCSA for small molecules is rare. The main hurdle for RCSA is the lack of a convenient and reliable approach to eliminate isotropic chemical shift changes upon molecular alignment. Here we describe a simple gel-stretching device that provides accurate and clean measurement of RCSA data and allows RDC measurement as well. The utility is demonstrated for several natural products including strychnine, estrone, and retrorsine.

Design of the Gel-Stretching NMR Tube

The stretching device is an NMR tube with different inner diameters (ID) on two open ends. This design is adopted from a previous report that used a two-stage NMR tube for RCSA measurement in proteins[1] but with improvement for better durability. Isotropic data are collected with the gel in the wide segment (4.2mm ID) while anisotropic data are collected with the gel stretched in the narrow segment (3.0-3.4 mm ID) of the tube. Shimming is easy and good field homogeneity can be obtained. We used the chloroform-compatible PMMA gels[2] for all studies.

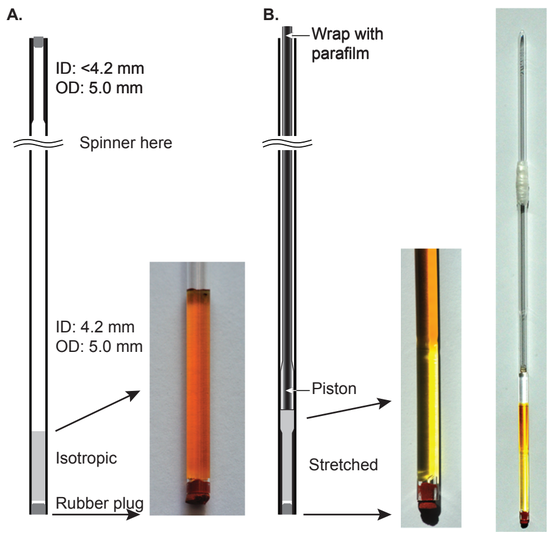

Figure 1: The design of the 2-stage NMR tube for gel stretching and measurement of RDC and RCSA.

A. The setup for the isotropic measurement. **B.** The setup for the anisotropic measurement. The gel is artificially colored for visualization.

Representative Spectral Data and Configuration Analysis Results for Strychnine

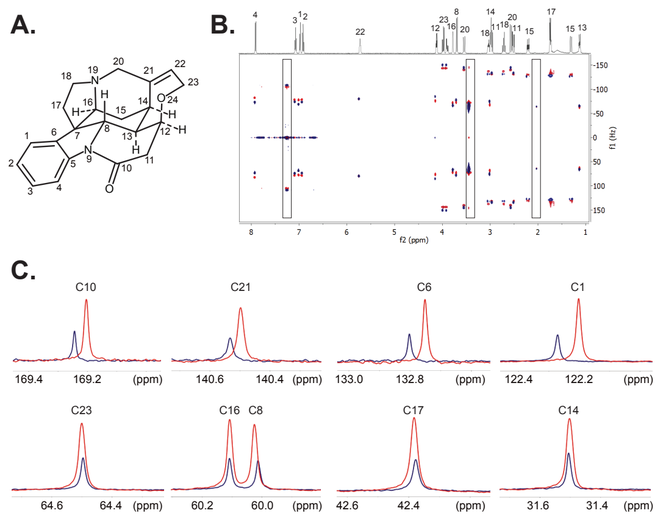

Figure 2: Experimental RDC and RCSA data

A. Strychnine structure. **B.** Overlay of *J*-resolved BIRD-HSQC experiments collected under isotropic (red) and anisotropic (blue) conditions. PMMA signals are enclosed in rectangles. **C.** Overlays of ^{13}C spectra under isotropic (red) and anisotropic (blue) conditions, showing RCSA effects at representative carbons; carbonyl and aromatic carbons (top row) tend to have larger RCSA values than aliphatic carbons (bottom row).

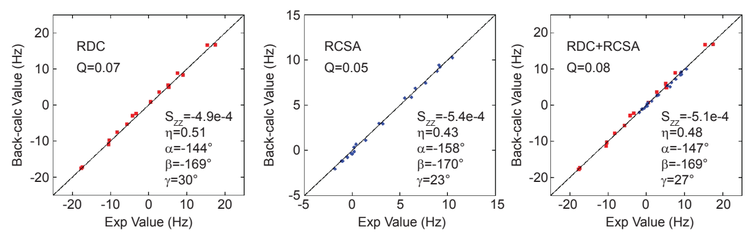

Figure 3: Evaluation of data quality, showing correlations between experimental values and SVD back-calculated values, showing results for RDC data only (left), RCSA data only (center), and RDC-RCSA data combined (right). The asymmetry parameter η is defined as $(|S_{yy}|-|S_{xx}|)/|S_{zz}|$.

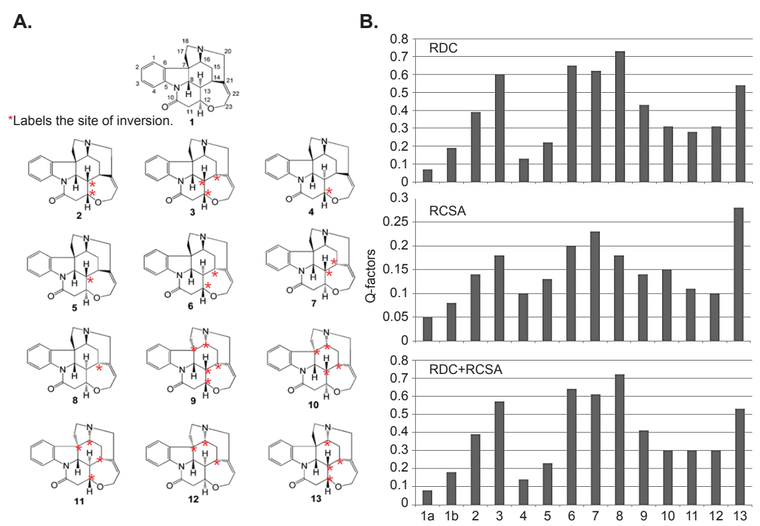

Figure 4: Using RDC and RCSA data for stereochemistry determination.

A. Structures of strychnine (1) and its 12 diastereoisomers with inverted stereochemistry. **B.** Q-factors of RDC alone, RCSA alone, and RDC-RCSA combined are calculated for lowest-energy DFT structures.

Enhancing Stereochemistry Discrimination by "Maximally Superimposable Differentiation" (MSD)

Maximally superimpose two candidates and only use RDC/RCSA data of the superimposable parts for alignment tensor determination. Then back-calculate RDC/RCSA values for the non-superimposed parts and obtain Q_{free} factors and correlation plots. The subscript "free" means these data were not used in alignment tensor determination. The difference in Q_{free} is expected to be more significant than the difference in Q. More importantly, the numeric differences in Q_{free} factors can be more readily rationalized from a structure perspective by identifying outliers in the correlation plot.

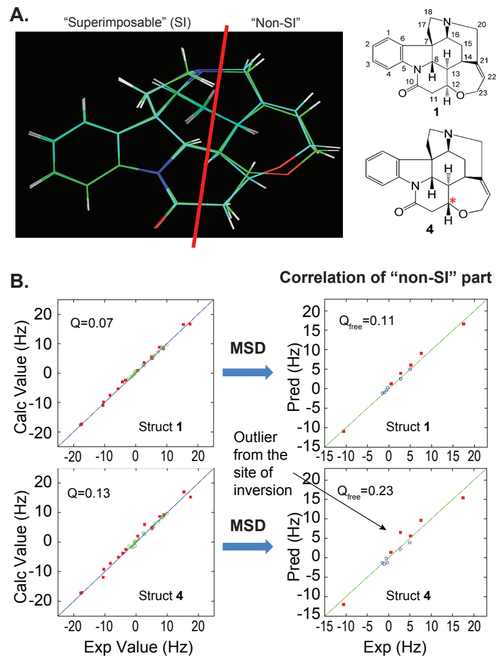

Figure 5: MSD analysis for strychnine diastereomers 1 (correct) and 4 (incorrect).

Red dots: RDC; green/blue dots: RCSA. **A.** Maximal superposition of 1 and 4. **B.** Correlation plots from SVD fail to reveal logical outliers in 4. MSD clearly reveals the RDC at the center of chiral inversion as an outlier.

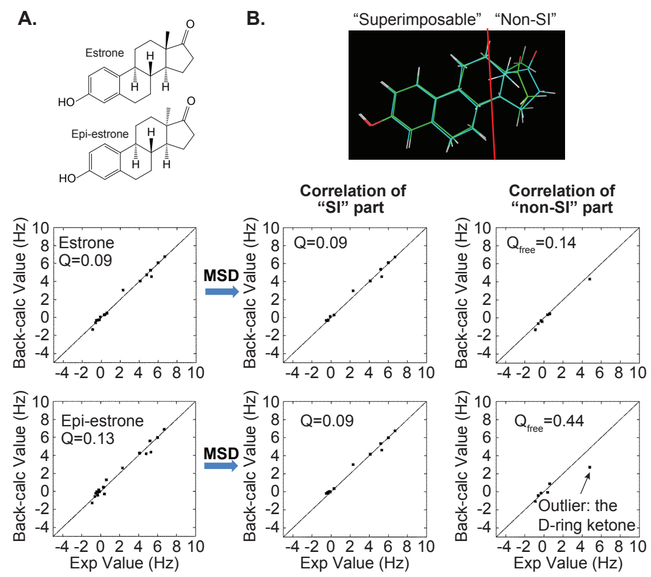

Figure 6: MSD analysis for estrone and epi-estrone using RCSA data.

A. RCSA only slightly favors estrone over epi-estrone. **B.** By MSD, the correct isomer (Q_{free}=0.14) has a much better correlation than the incorrect isomer (Q_{free}=0.44). The outlier from D-ring ketone is easily rationalized from the structure differences.

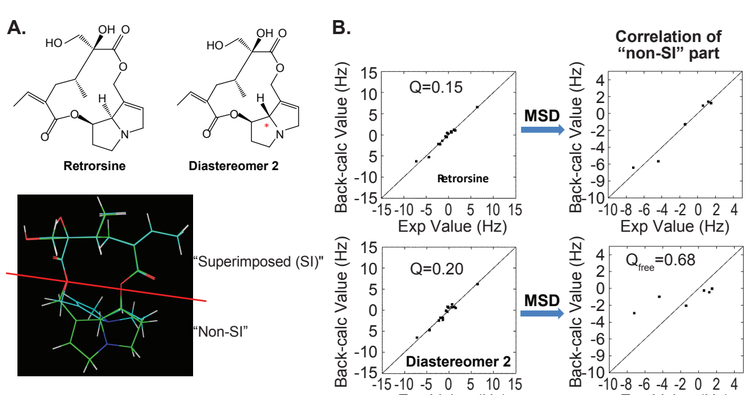

Figure 7: MSD analysis for retrorsine using RCSA data.

A. RCSA only slightly favors the correct retrorsine structure over the incorrerct one (diastereomer 2). **B.** By MSD, the correct isomer (Q_{free}=0.18) has a much better correlation than the incorrect isomer (Q_{free}=0.68).

References:

1. Liu and Prestegard, *J Biomol NMR.* (2010), 47, 249-258.

2. Gayathri, Tsaresvsky, and Gil, *Chemistry.* (2010), 16, 3622-6.

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