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Introduction to Services

The HMS East Quad Bio-NMR Core Facility (EQNMR), and the DFCI NMR Core have formed a consortium to provide state-of-the-art NMR services to investigators at HMS, DFCI, affiliated institutions, and local bio-technology companies. Offered services and resources include:

- **700, 600, 500, and 400MHz systems plus access to 800MHz**
- Protein NMR data collection and backbone assignment
- Non-uniform sampling and Optimal Control Theory optimized expts.
- Structure-guided lead discovery/fragment screening/ligand binding
- Structure elucidation of natural products/mass limited samples
- Medicinal chemistry and organic synthesis support
- Metabolomics and metabolic profiling

Natural Products/Med Chem Support

The facility has dedicated hardware and software for structure elucidation of natural products, NMR of mass limited samples, and support of Med Chem as detailed below:

- Highest sensitivity for mass limited samples
- 5mm, 3mm, and 1.7mm tube sizes for maximum mass sensitivity
- Non-uniformly sampled heteronuclear correlation experiments
- Ultra-high sensitivity for ¹³C direct detection with TXO cryoprobe
- Primary structure verification for Med Chem/Natural products
- Full automation capabilities for data collection and processing

Fragment-based Lead Discovery

The core has a robust Fragment-based Lead Discovery (FBLD) program that offers the following services to the user community:

- Property-based fragment screening of in-house or user's libraries
- Traditional/specialized ligand and protein detected methods available
- SAR by NMR using multiple approaches
- Access to products of HMS-based research for FBLD
- Primary, secondary, and follow-up screens (proton and fluorine NMR)
- NMR analysis of hits from high throughput screening
- Med-Chem services via collaboration with Academic partners

Protein NMR Services

The facility is especially equipped for protein NMR data collection and rapid resonance assignment using novel methodologies

- High and mid-field systems with high sensitivity cryogenic probes
- Optimal use of non-uniform sampling acquisition schemes
- Rapid backbone assignments via HNCA-based line shape analysis
- Non-standard isotope labeling schemes
- Assigned proteins integrated into fragment-based platform
- QC studies of biologic drug preparations

700MHz NMR System



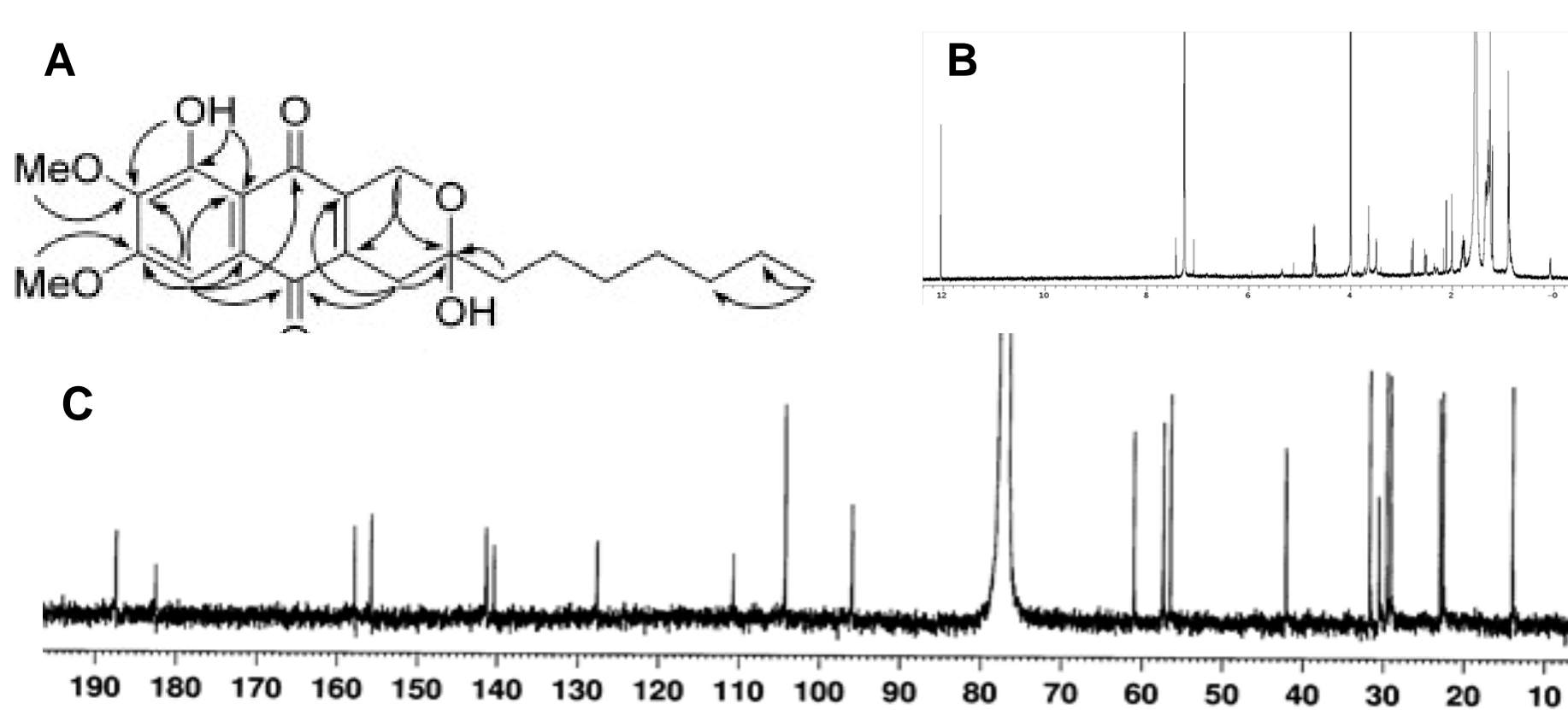
- Modern, automated 4-channel system allows complete analysis
- 5mm {H,C,N} cryoprobe delivers highest proton sensitivity
- Optimized for protein NMR and mass limited samples
- "Salt tolerant" probe design increases S:N for salty samples
- BioPack environment allows NMR-Pipe real time data workup
- Excellent RF power handling for ¹⁵N relaxation dispersion expts.

600 and 400 MHz NMR Systems



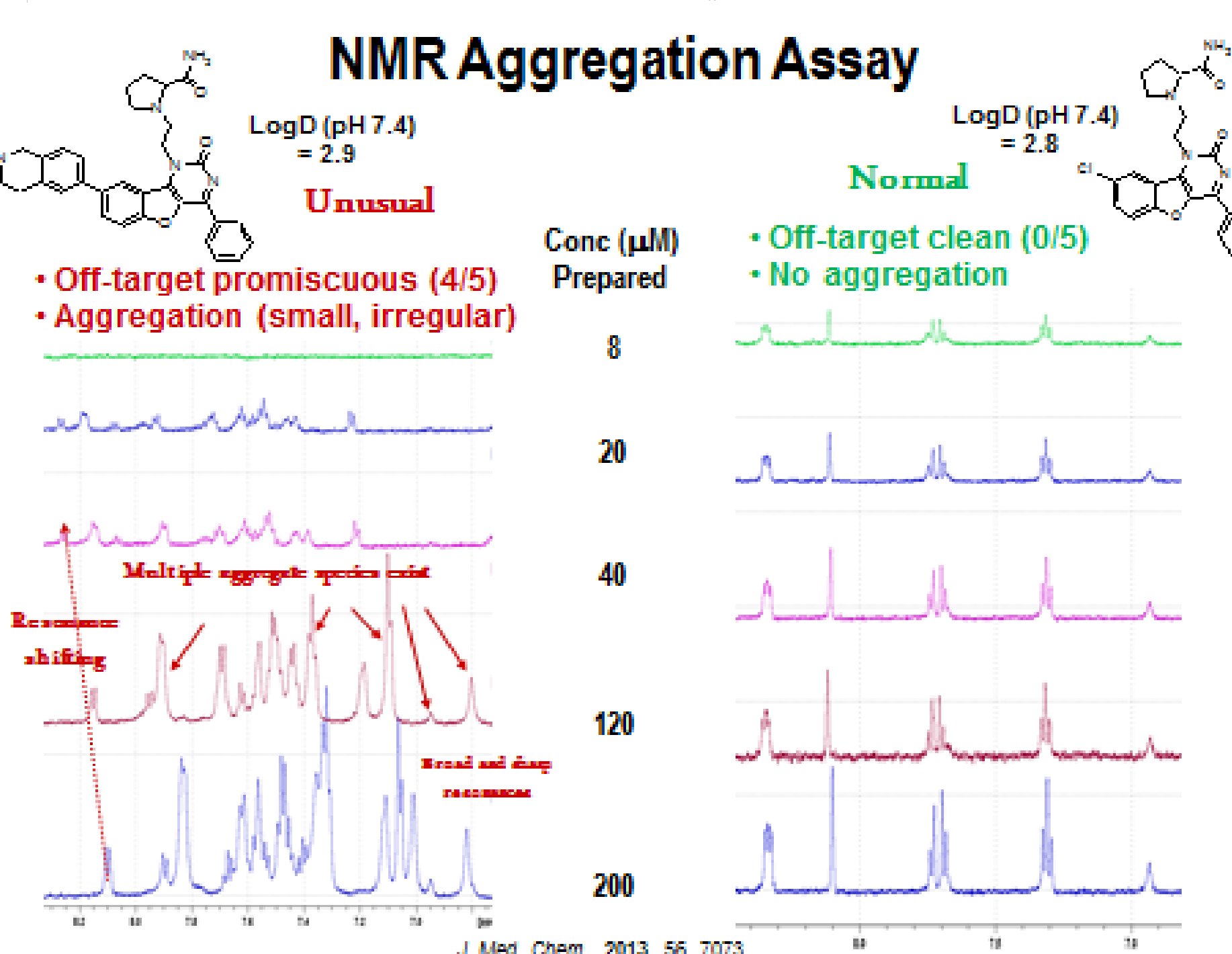
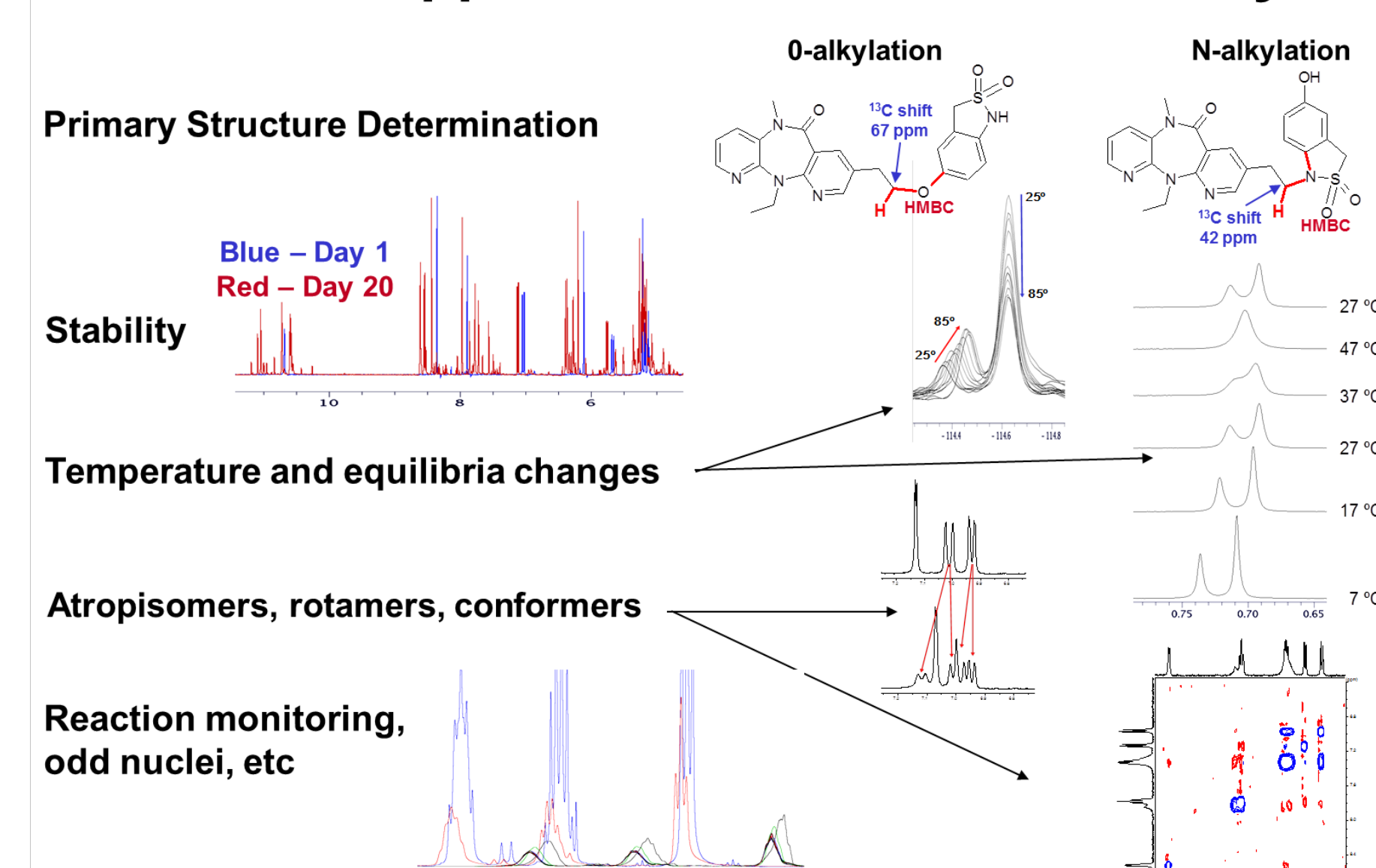
- Automated 4 channel system
- 1H/19F, 13C, 15N cryoprobe
- 480 tube SampleJet robot
- Quantification tools
- Fragment screening
- Ligand binding studies
- Autotune on all channels
- Automated 2 channel system
- 1H/X-BB One Probe
- 96 sample changing robot
- Quantification tools
- Routine chemistry applications
- Walkup access at all times
- Autotune on all channels

Structure Elucidation of Natural Products



(A) HMBC correlations for deltichianone A, isolated from *Deltichia wintarii*, an endophytic fungus from Costa Rica. (B) Proton NMR. (C) Carbon NMR.

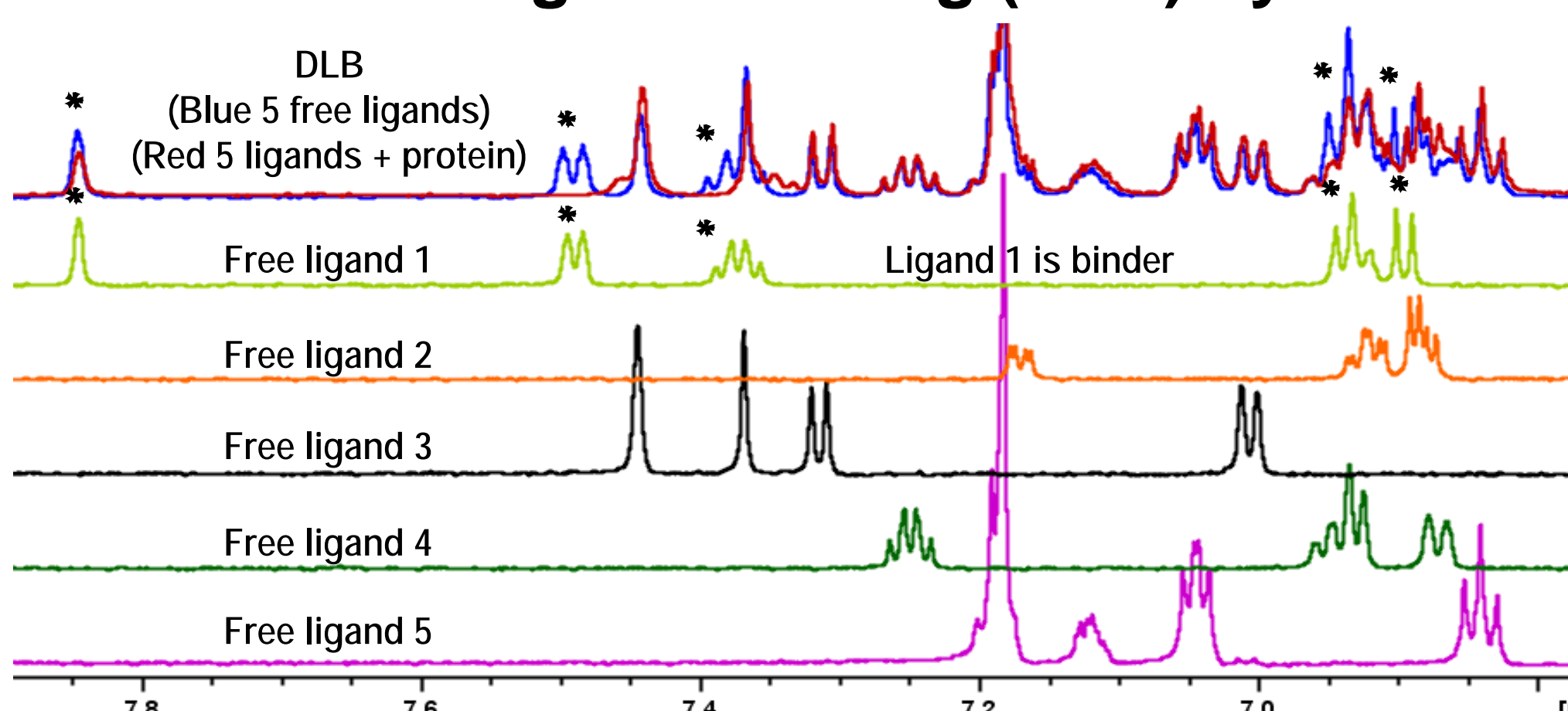
NMR Support for Medicinal Chemistry



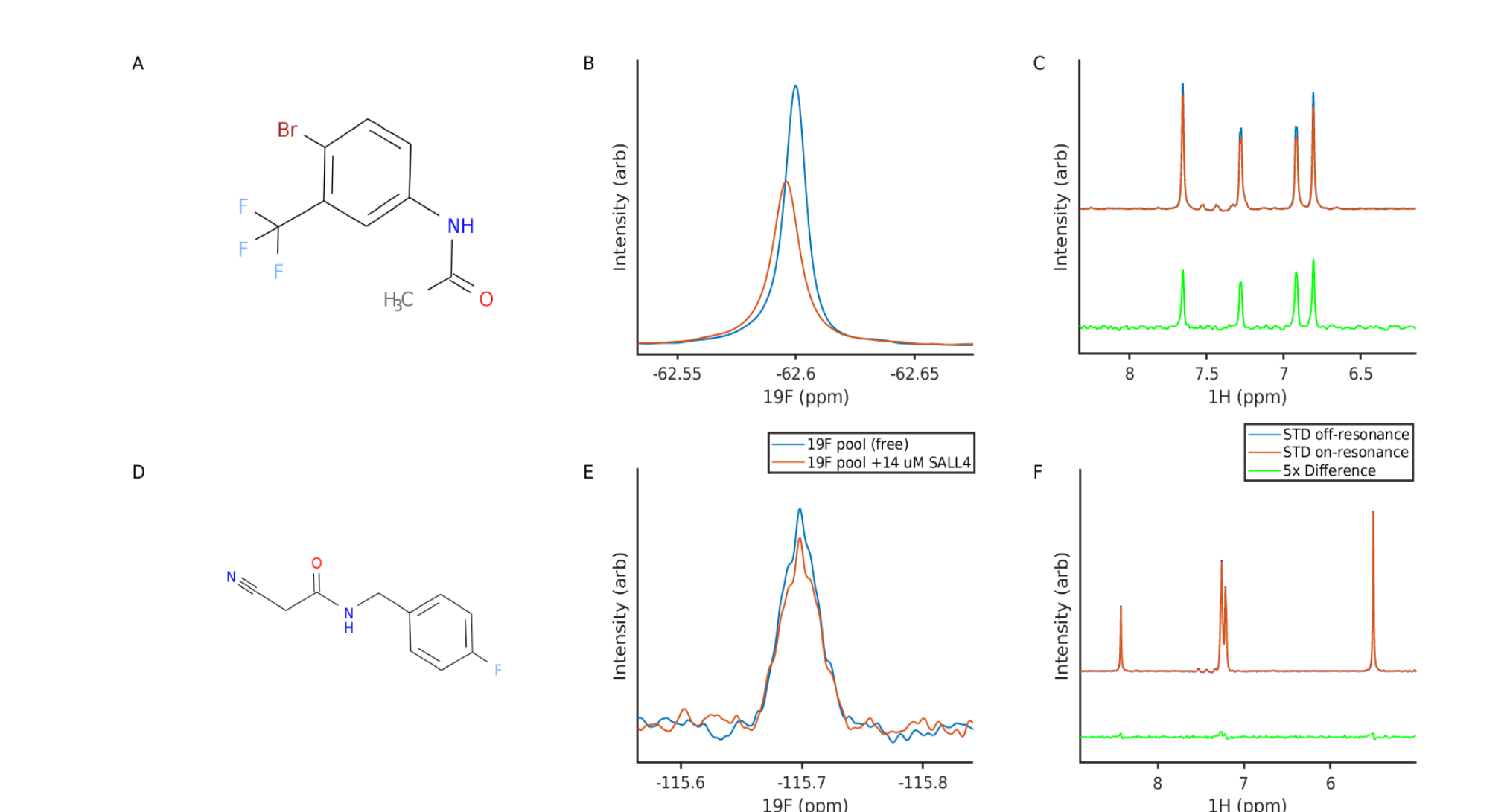
NMR Analysis of HTS hits

- Free state compound analysis (solubility and aggregation)
- Ligand-detected confirmation of binding to target
- Protein-detected confirmation of binding to target
- Competition binding assays to identify proximity binders
- Automated and quantitative analysis of binding data
- Novel in-house routines for data workup and analysis
- SAR by catalog follow-up using direct binding assays

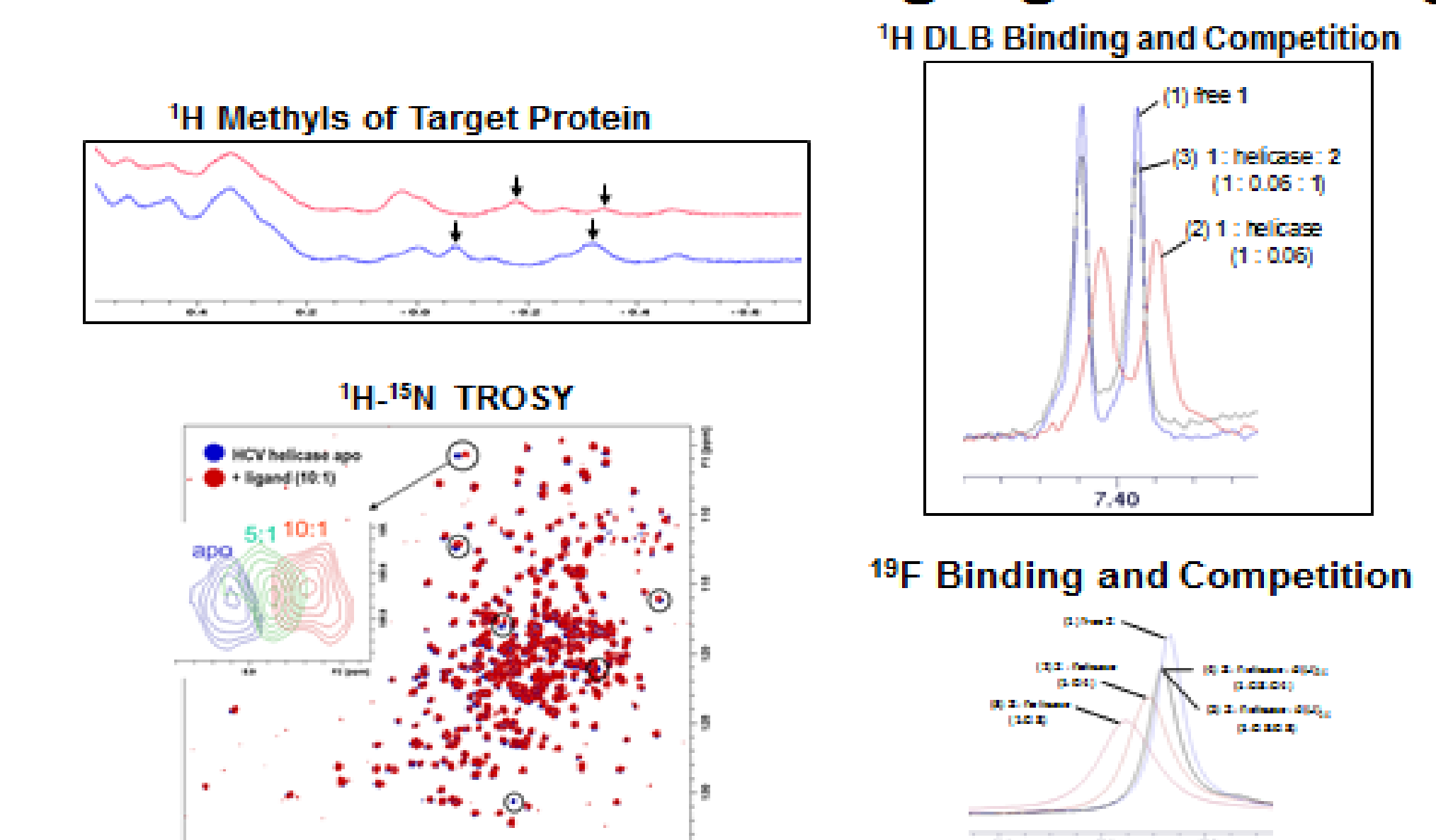
Detection of Ligand Binding (DLB) by ¹H NMR



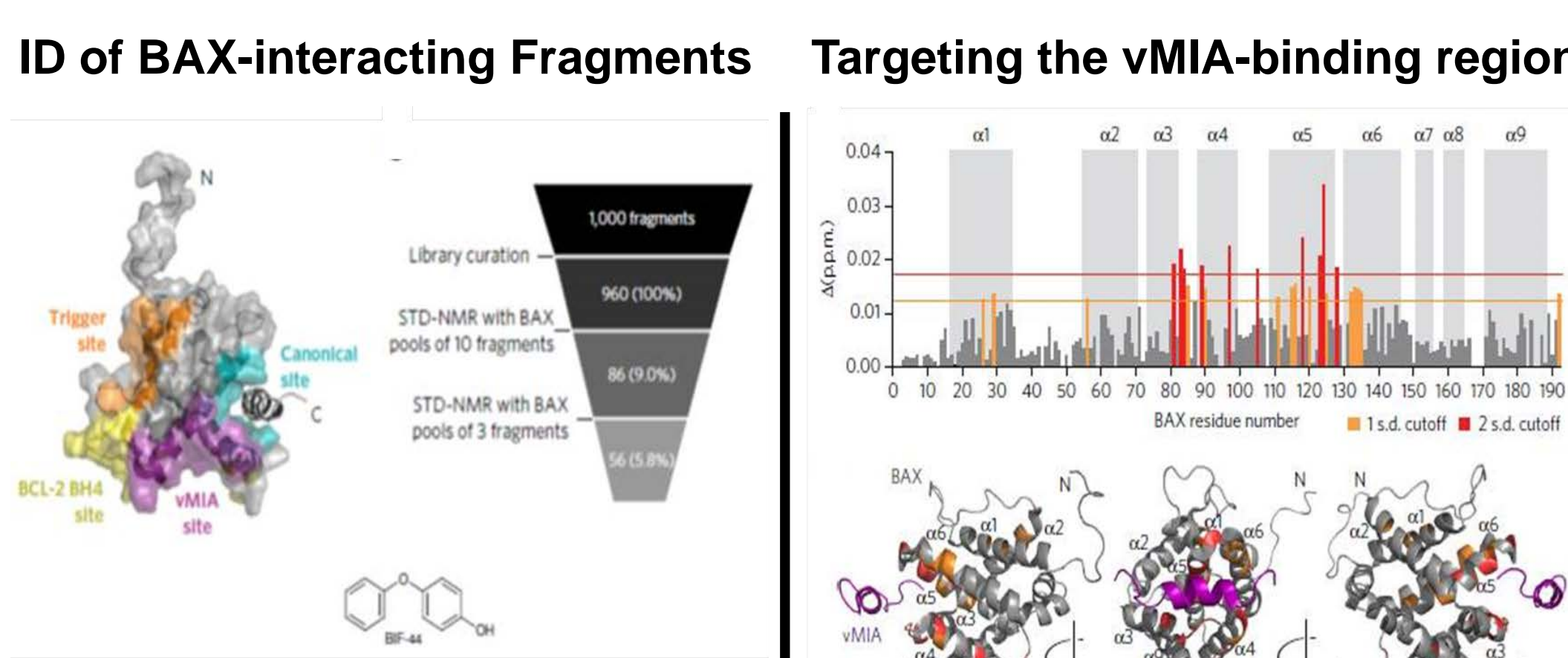
Identification of Bound Ligands by ¹⁹F NMR



NMR Methods for Detecting Ligand Binding

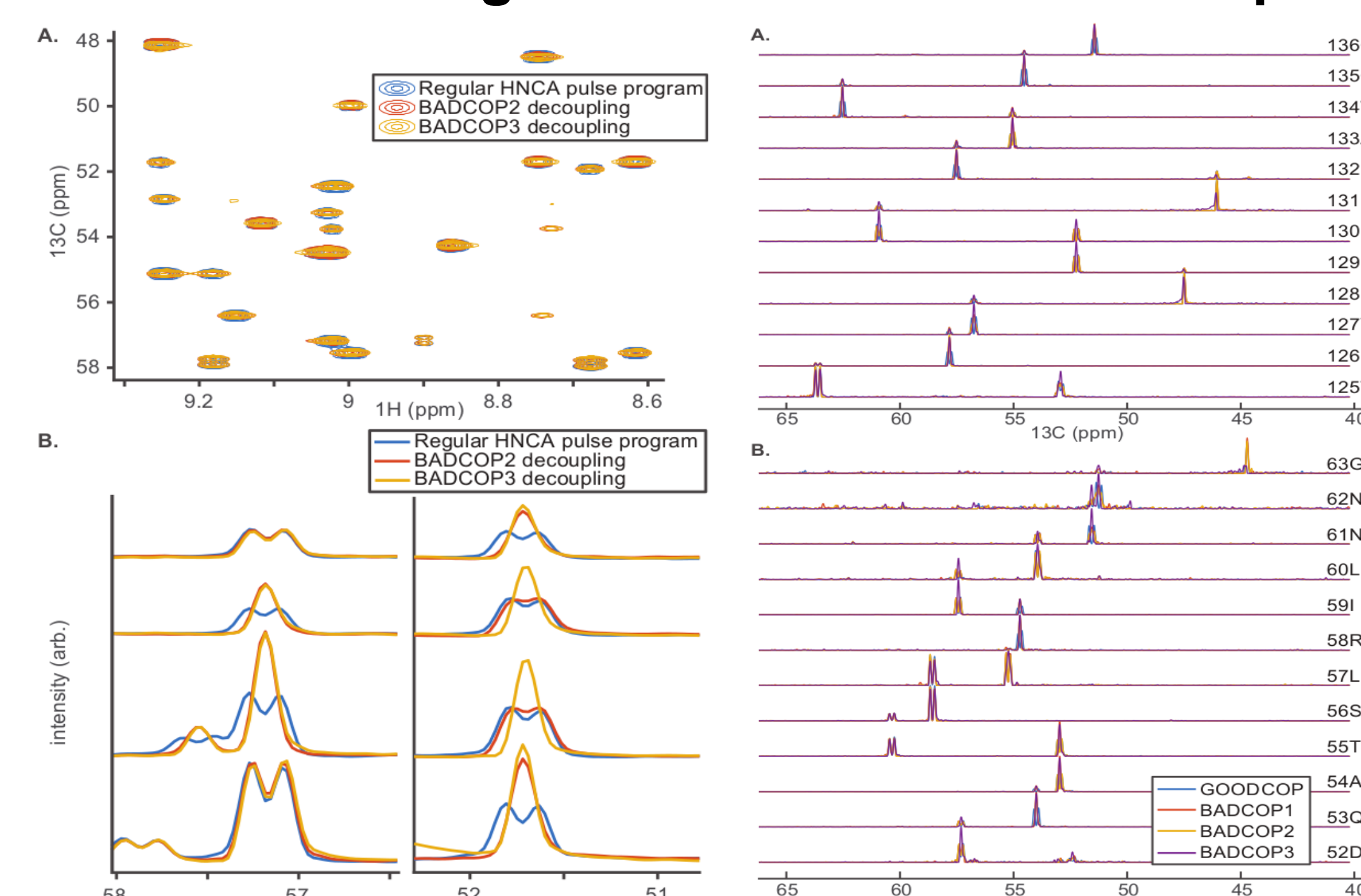


HMS/DFCI Project – Allosteric Sensitization of BAX



Nature Chemical Biology, Vol. 13, September, 2017, pg. 961

Backbone Assignment from HNCA Lineshapes

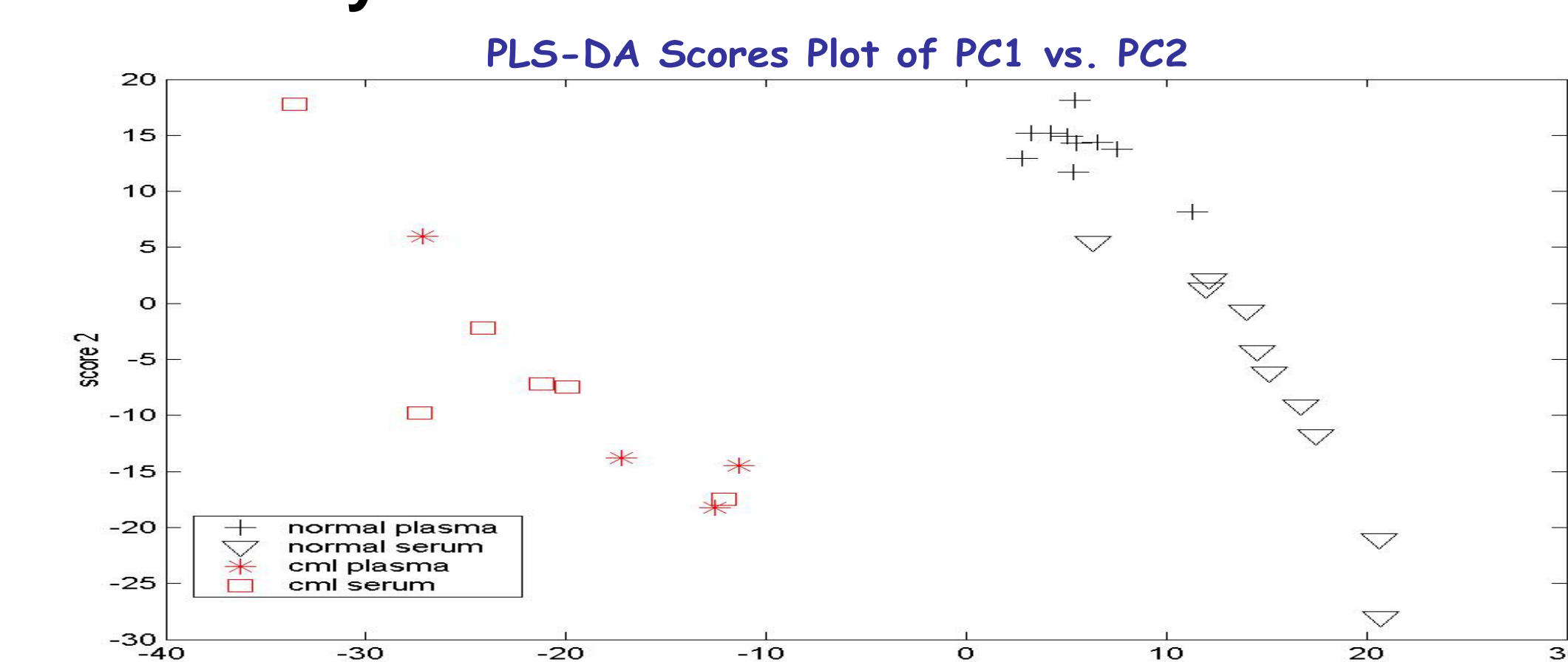


- Based only on HNCA expts. with selective beta-carbon decoupling
- Uses decoupling pulses from optimal control theory
- Amino acid-specific alpha carbon line-shapes allow assignments
- Employs NUS to achieve high resolution in carbon dimension
- Requires only ~4 HNCA experiments (~6 days NMR time)
- Bloch-Siegert shifts are eliminated by nature of decoupling pulses

Nature Communications, volume 9, Article number: 3014 (2018)

Metabolomics of biofluids/Cell Extracts

Analysis of Human CML Patient Plasma



Conclusions

- EQNMR is available to support your Bio-NMR needs
 - Medicinal chemistry and natural products support
 - Protein NMR data collection and backbone assignment
 - Fragment screening/fragment-based lead discovery platform
 - Proton and Fluorine NMR for screening/ligand binding studies
 - Metabolomics
 - **Project-based full service upon request**
- State-of-the-art systems are available
 - 700, 600, and 500MHz cryoprobes – 800 or 900MHz if needed
 - Liquid handler is available for sample prep
- To become a user, visit <https://eqnmr.med.harvard.edu/user-authorization> or contact Charles_Sheahan@hms.harvard.edu