Implementation of novel tools to facilitate fragment-based drug discovery by NMR:

Automated analysis of large sets of ligand-observed NMR binding data and ^{19}F methods

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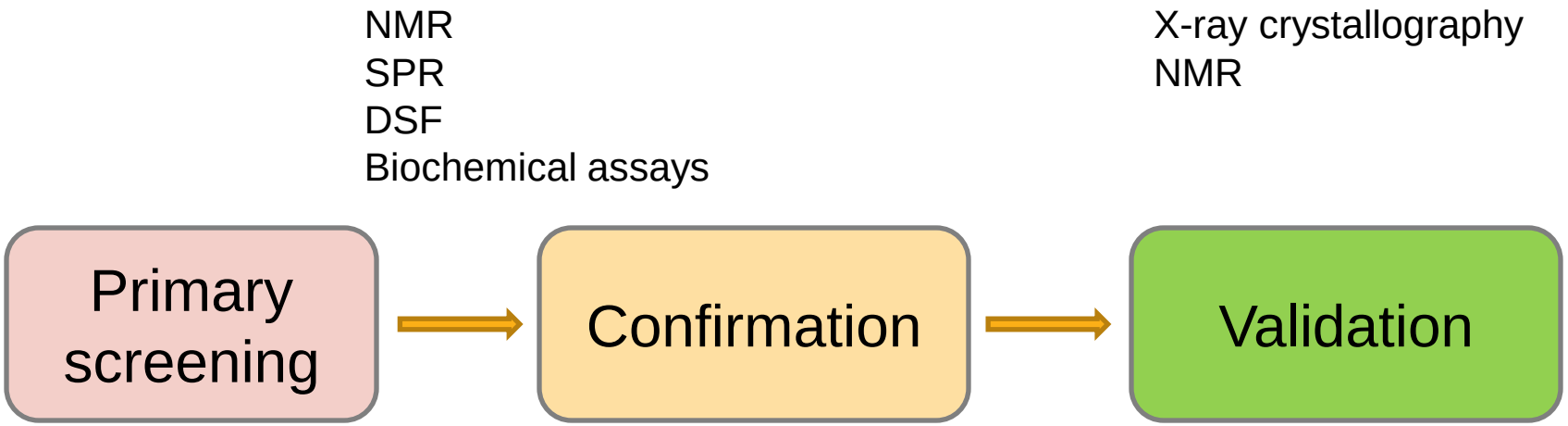
Outline

How to improve NMR-based, ligand-observed hit finding?

- Short introduction of workflow and main challenges
- Automated analysis of ligand-observed NMR binding data
- ^{19}F-based NMR applications

Several bottlenecks in target-based hit finding

Need for tools to facilitate screening and characterization



- Single concentration
- Yes/no assessment
- Hit calling based on thresholds

- Dose response
- Specificity
- K_D or IC_{50}
- Rank ordering

- Structural information
- Localized binding site
- Specificity
- Analoging

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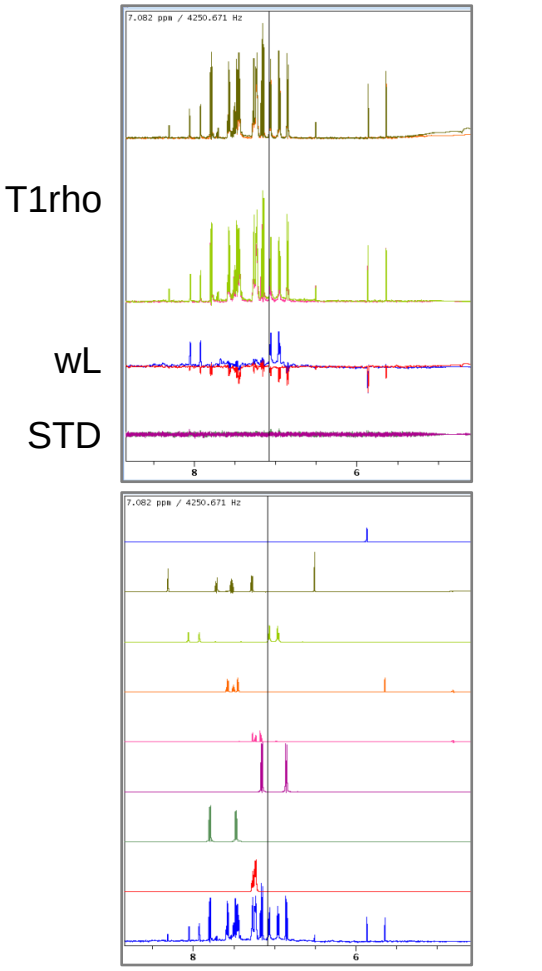
Several bottlenecks in target-based hit finding

Spectra are complex and manual analysis labor-intensive

^{1}H ligand-observed NMR experiments



Primary screening

- Single concentration
- Yes/no assessment
- Hit calling based on thresholds

- Compounds in mixtures
- Spectra are complex and signals overlap
- Multiple experiments available (T1rho, WaterLOGSY, STD)

- Comparison to single compound spectra
- Time consuming manual analysis

→ Automatic analysis of ligand-observed screening data

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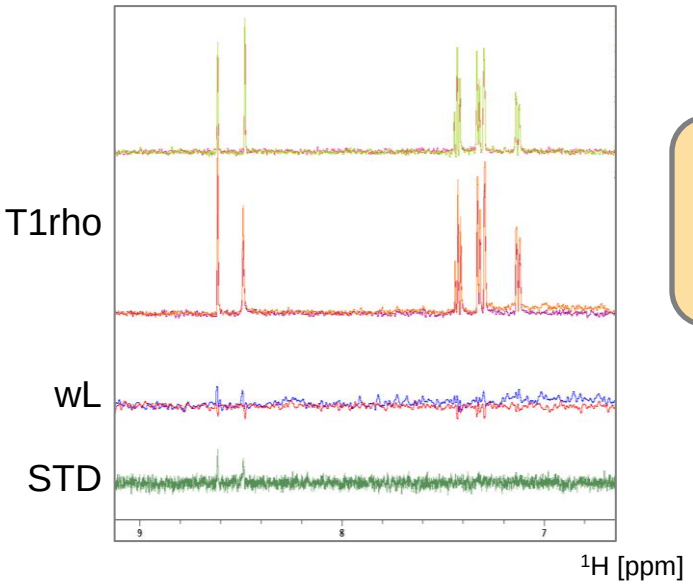
Several bottlenecks in target-based hit finding

Potential for robust, simplified and quantitative assays

^{1}H ligand-observed NMR experiments

Single compound confirmation



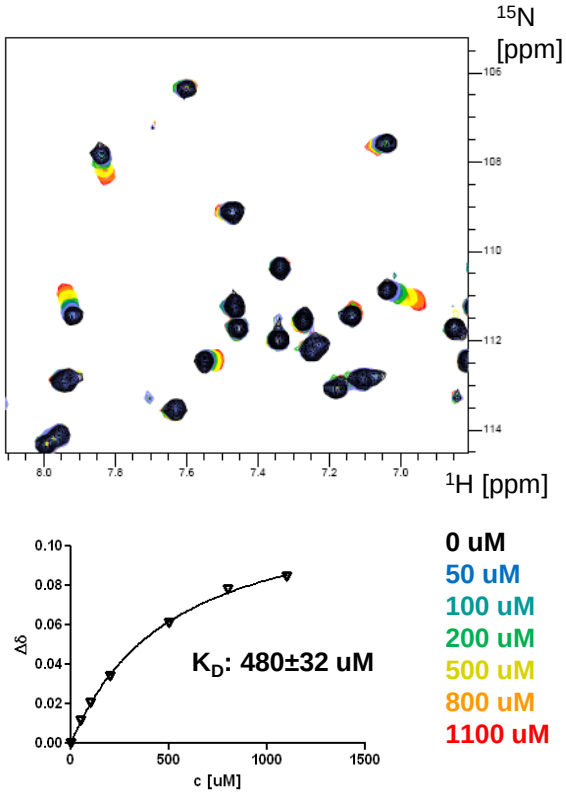
Confirmation

- Dose response
- Specificity
- K_D or IC_{50}
- Rank ordering

Automated analysis & simple, quantitative NMR assays

Protein-observed NMR experiments

Chemical shift perturbations



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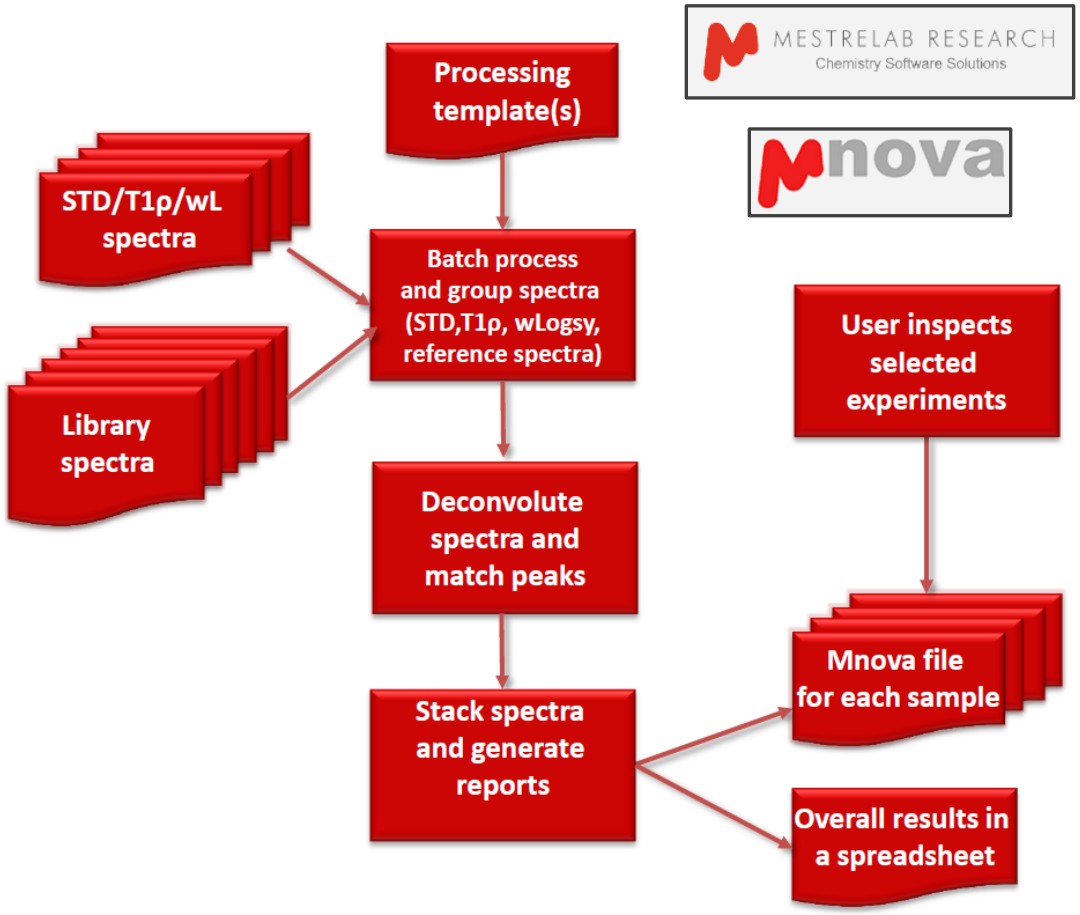
A novel software tool to process and analyze NMR-based binding data

Features

- Imports and collates spectra
- Data processing
- Alignment and/or normalization
- Peak picking using Global Spectral Deconvolution (GSD)
- Matching of library peaks with the mixture (STD, T1ρ or WaterLOGSY) peaks and comparing of intensity changes
- Reporting of hits in a spreadsheet with quantitative data, interface for visual inspection

Workflow



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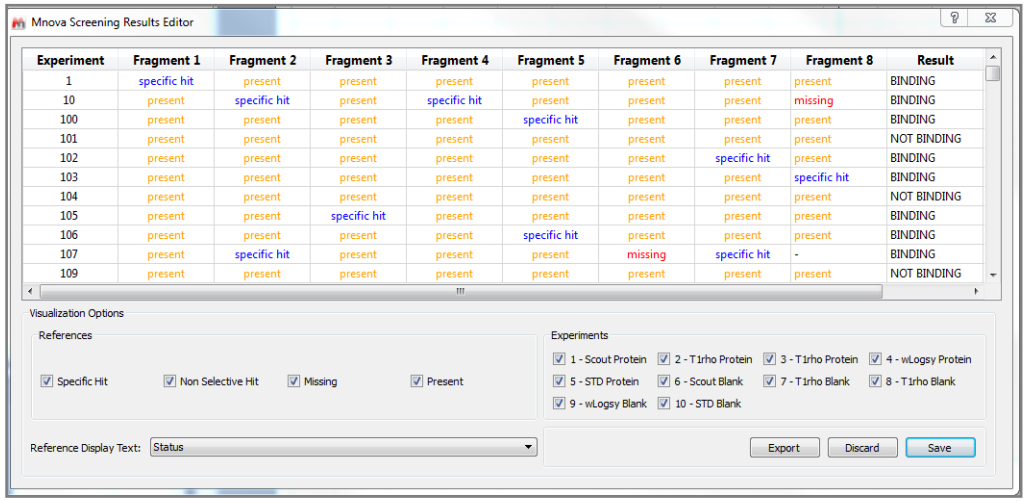
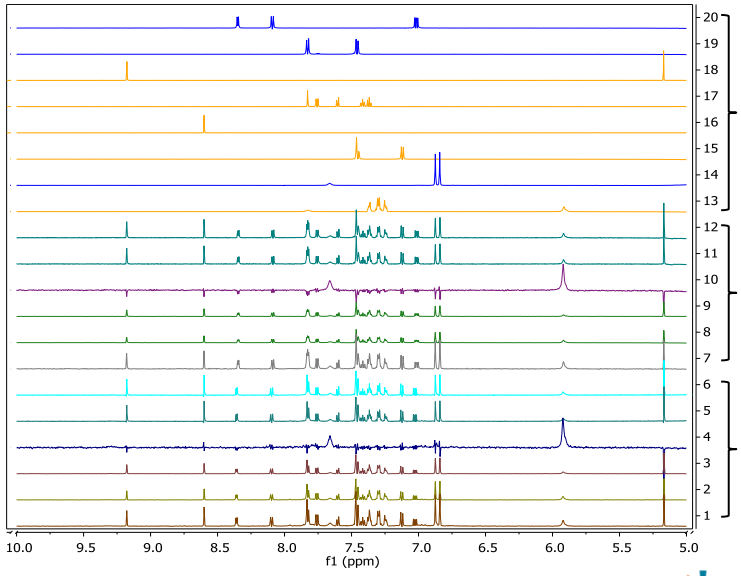


Experiment	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Fragment 6	Fragment 7	Fragment 8	Result
1	specific hit	present	present	present	present	present	present	present	BINDING
10	present	specific hit	present	specific hit	present	present	present	missing	BINDING
100	present	present	present	present	specific hit	present	present	present	BINDING
101	present	present	present	present	present	present	present	present	NOT BINDING
102	present	present	present	present	present	present	specific hit	present	BINDING
103	present	present	present	present	present	present	present	specific hit	BINDING
104	present	present	present	present	present	present	present	present	NOT BINDING
105	present	present	specific hit	present	present	present	present	present	BINDING
106	present	present	present	present	specific hit	present	present	present	BINDING
107	present	specific hit	present	present	present	missing	specific hit	-	BINDING
109	present	present	present	present	present	present	present	present	NOT BINDING

Results are easily accessible in table format, color coding for hits/no hits

Visual inspection:
Stacked spectra view within Mnova enables manual checking and modification of hit calling



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Performance against manually analyzed data

Optimization of mixture analysis on-going

- T1rho and STD spectra of single compound experiments tested, hits from automated analysis compare well with manually analyzed results (> 85%)

- Currently testing of mixture data, large dataset from fragment library screen
- Primary hit calling (adding to total): 1 out of 3 experiments positive

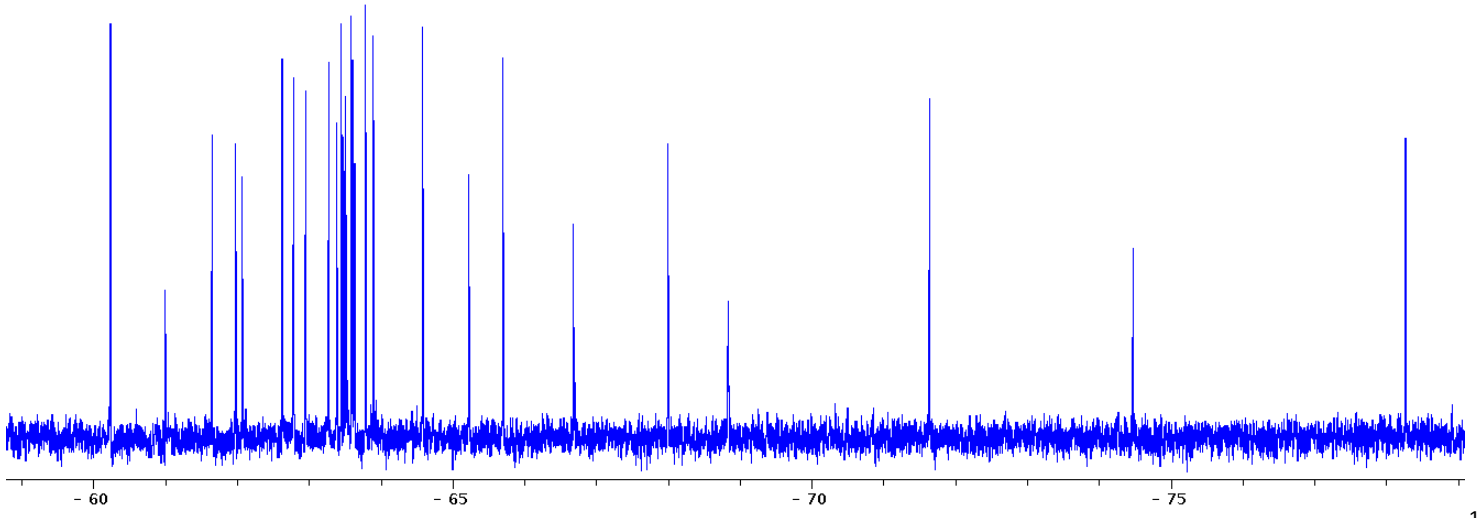
Example from a mixture analysis

	Manual analysis No. of hits	No. and % found by Mnova screen
T1rho	91	78 / 85%
STD	180	100 / 55%
WaterLOGSY	213	143 / 67%
Total	202	159 / 79%

^{19}F-detected NMR provides many advantages

Rapid and robust hit finding by ^{19}F NMR screening

- Compounds can be screened at **very low concentration** due to exquisite sensitivity of ^{19}F to binding and recent **advancements in hardware**
- **Large chemical shift range** allows screening of large mixtures
- **Fast acquisition** and **very clean NMR spectra**, enabling fast evaluation of screening data
- Fluorine is known to be involved in **favorable interactions** with proteins, e.g. carbonyl groups, Bissantz et. al (2010) *J. Med. Chem.*, 53
- Novel library design strategy based on "**local environment of fluorine**" concept, described by Vulpetti et. al (2009) *J. Am. Chem. Soc.*, 131



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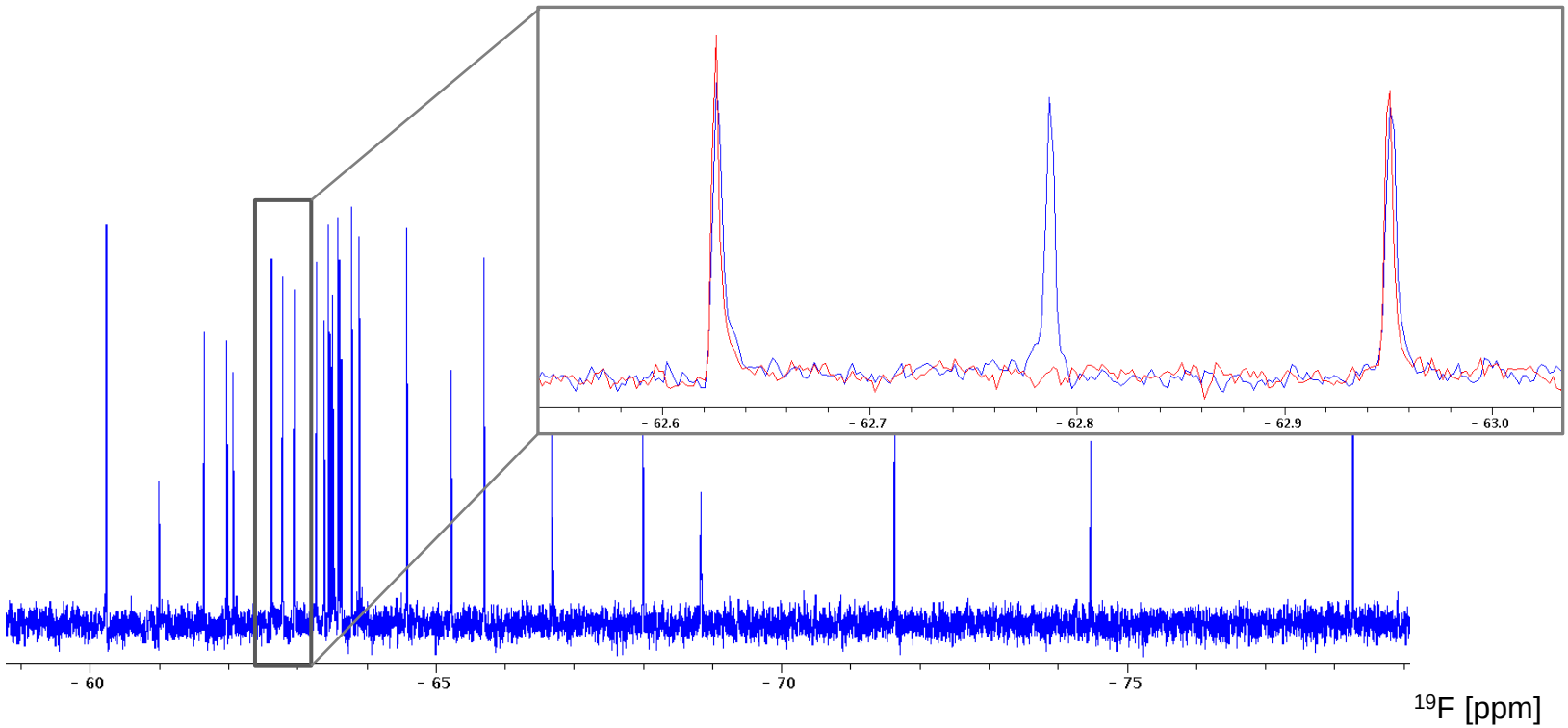
Simplification of data analysis

Signal decrease can be easily quantified and analyzed automatically

⟹ Addition of protein causes line broadening - binding



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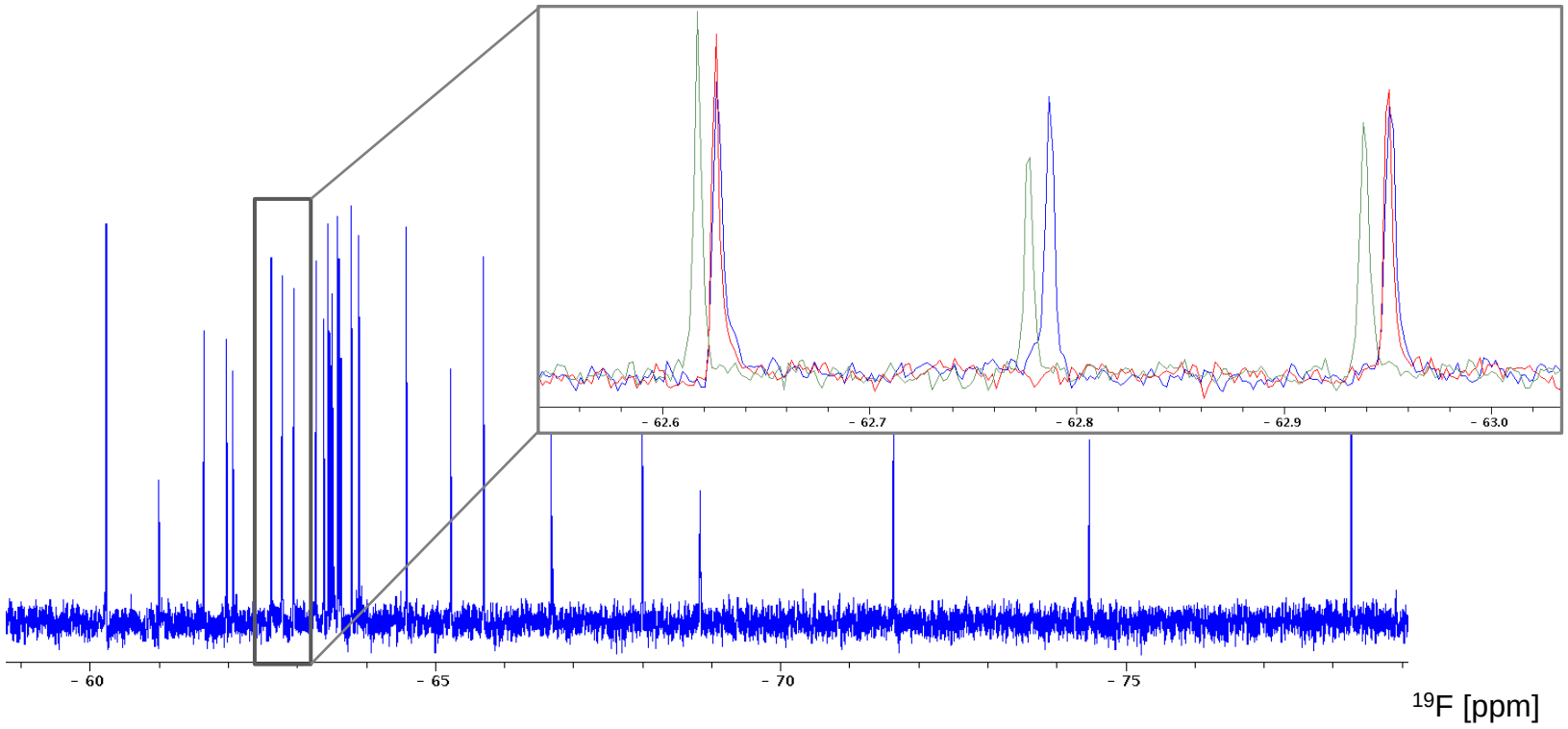
Different binding sites can be probed at screening stage with tool compounds

⟹ Addition of tool compound restores the compound signal



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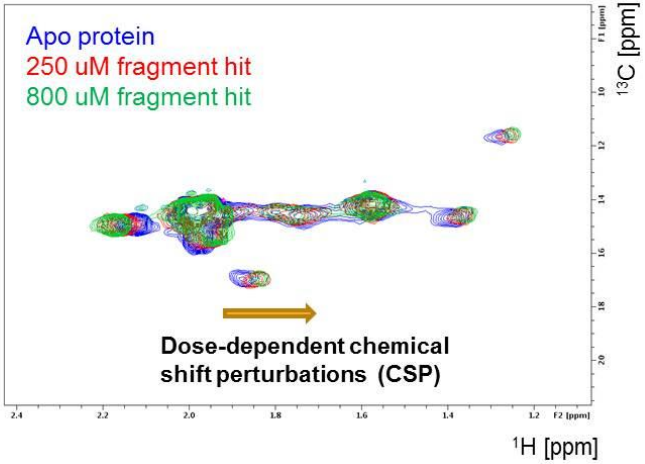
Primary screening hit could be validated and progressed into potent series

- ~ 100 analogs of fragment hit were tested in thermal shift & biochemical assays (orthogonal validation)
- Positive hits were further validated by 2D NMR chemical shift perturbation experiments

Compound	Stargazer thermal shift (≥3σ, 0.8 ° C)	Biochem IC_{50} [uM]	LE	2D NMR Chemical shift
Fragment hit	Yes	811	0.23	Yes
Analog 1	Yes	206	0.26	Yes
Analog 2	Yes	337	0.24	Yes
Analog 3	Yes	468	0.27	Yes



- Alternative chemotype identified by ^{19}F-based screening
- Analoging, 2D NMR and docking provided basis for chemistry
- Binding mode of more potent analogs could be confirmed by crystallography
- New fragment-based chemotype currently progressed into nM inhibitors with favorable ligand efficiency parameters being retained

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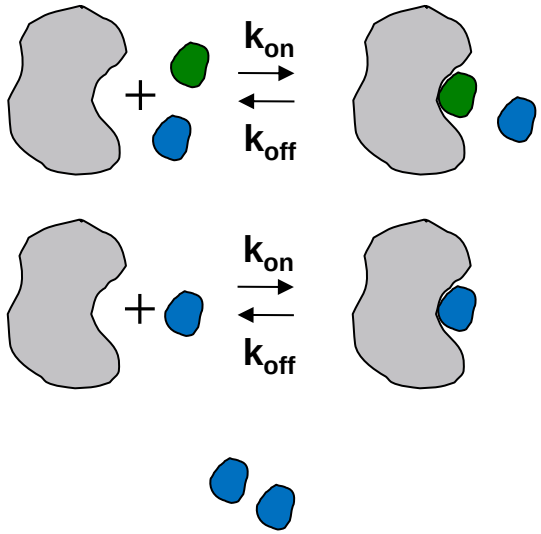
^{19}F-based NMR reporter assays

Quantitative and structural information from a single measurement

Reporter gets displaced by test compound

Reporter binds to protein

Reporter free in solution



Requirements

- Fluorinated reporter compound
- Sufficient solubility to avoid assay artifacts
- Specific binding, affinity in ~10-200 uM range
- Preferably crystal structure of bound reporter available

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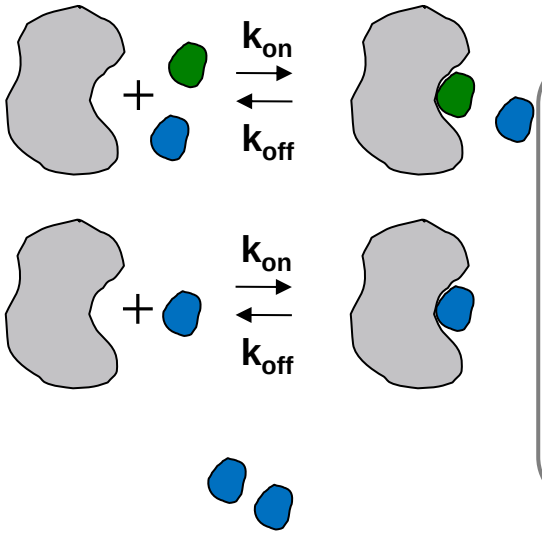


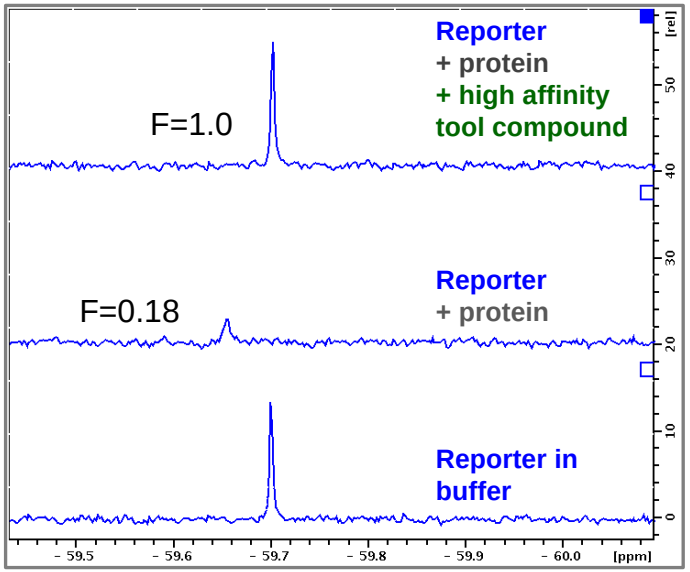
Example

CF3 group

Sol >600 uM

K_D (SPR): 150 uM
IC_{50}: 182 uM

X-ray available



^{19}F [ppm]

$$F(I)_{rep} \sim \frac{[ERep]}{[Rep]_{tot}} \sim K_D \text{ (test compound)}$$

Displacement of reporter by test compounds provides rank ordering/K_D and confirms localized binding event

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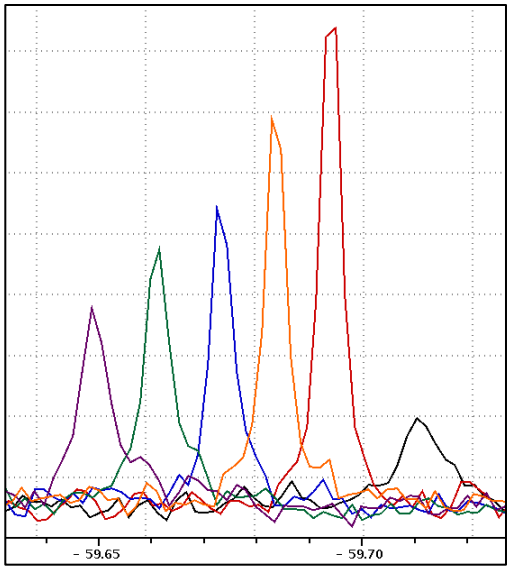
^{19}F-NMR reporter assay for diverse applications

Quantitative and structural information from a single measurement

Conditions

- 20 uM reporter
- 1-5 uM protein
- 50-500 uM test compound
- < 5 min acquisition



^{19}F [ppm] (individual spectra shifted for clarity)

Applications

- No restriction on test compounds (size, properties)
- Data acquisition and analysis is simple and fast
- Suitable for HTS and FBS hit validation
- Quantification enables prioritization and application in compound optimization

Protein + reporter
+ Compound 1 (IC_{50} = 33.7 nM)
+ Fragment 1 (SPR K_D = 60 µM)
+ Fragment 2 (SPR K_D = 240 µM)
+ Fragment 3 (SPR K_D = 190 µM)
+ Fragment 4 (SPR K_D = 320 µM)

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Summary

- Novel software tool development towards automated analysis of ligand-observed NMR binding data
  - Robustly identifies T1rho and STD hits single compound experiments in single compound experiments
  - Performance on mixtures good and being improved
  - WaterLOGSY module development started recently and being tested

- ^{19}F NMR provides fast and robust addition to compound screening repertoire
  - Very sensitive and less prone to observing unspecific binding
  - Libraries of ^{19}F-containing molecules enable primary screening (hit identification)
  - ^{19}F-based NMR reporter assay provides simple way to generate quantitative binding data and confirms binding in desired pocket

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