2023 NMR Training Course I - Fragment-based Screening using NMR

High-Field NMR Center
Academia Sinica

Mar. 10, 2023

2023 NMR Training Course (I)

Fragment-based Screening using NMR

High-Field NMR Center, Academia Sinica

Fragment-based drug discovery is a powerful method to identify drug-like candidates in the early stages of drug discovery. In recent years, NMR has been used extensively in FBDD. In this training course, we will briefly introduce how to apply NMR in fragment screening.

March 10, 2023

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Genomics Research Center

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Academia Sinica

Part I: Lectures ,GRC 1F Aditorium, Academia Sinica

09:40- Registration

10:00-10:30 Opening & Introductory Overview

10:30-11:00 Fragment-Base Screening Using NMR (I)

11:00-11:20 Break

11:20-12:00 Fragment-Base Screening Using NMR (II)

12:00-13:30 Lunch Break

13:30-14:00 Setting up Ligand-Observed NMR Experiments

Part II: Practical Hands-On, GRC NMR(1F,GRC) & HFNMRC(B2,IBMS)

14:00-14:10 Break

14:10-17:00 Hands-On

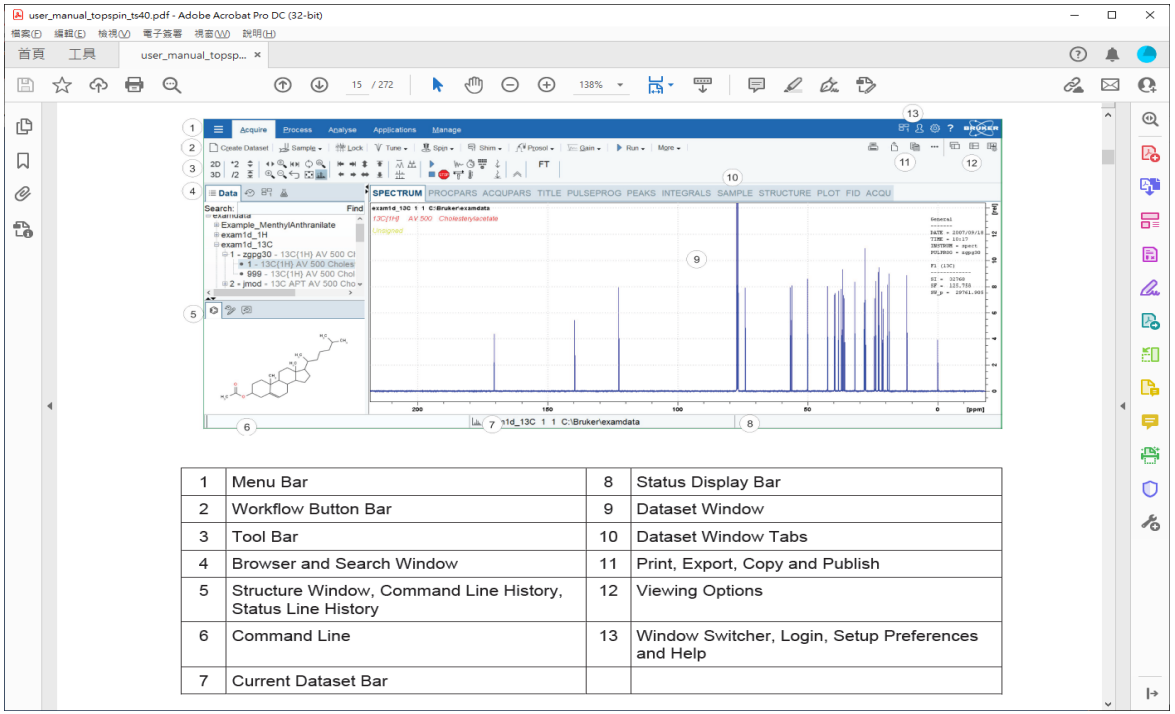
Opening & Introductory Overview

-News from High-Field NMR Center
-Brief Introduction on HFNMRC

HFNMRC News: AV500 is upgraded to AVANCE NEO 500

What's new for NEO ?

- NEO must use Topspin4.x or higher version
  NEO 必需使用Topspin4.x 以上版本操作



1	Menu Bar	8	Status Display Bar
2	Workflow Button Bar	9	Dataset Window
3	Tool Bar	10	Dataset Window Tabs
4	Browser and Search Window	11	Print, Export, Copy and Publish
5	Structure Window, Command Line History, Status Line History	12	Viewing Options
6	Command Line	13	Window Switcher, Login, Setup Preferences and Help
7	Current Dataset Bar		

- Routinely used commends are the same as Topspin2.x or Topspin3.x
  指令與Topspin2.x or Topspin3.x 相通

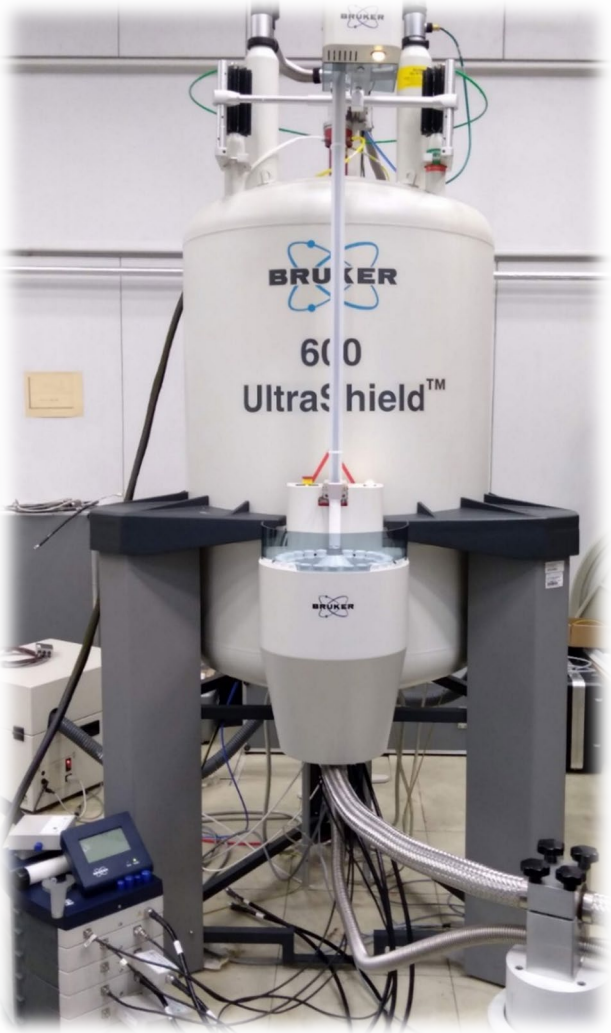
- Interface is different but not too difficult to follow
  介面稍有不同, 熟悉即可

- HFNMRC Standard Experiments and SOP are the same
  實驗設定方式與本核心其它NMR相同

Autosampler in HFNMRC : New and Old

AVIII600
(SampleCase-60 samples -2023)
With TCI Cryoprobe
(1H/13C/15N)

NEO 600
(SampleCase-24 samples -2022)
With TCI Cryoprobe
(1H/13C/15N)



Attention when using SampleCase:

(1)Pay attention on the length of your sample tube ! !

From the top of spinner to the top of tube <9.5cm

注意樣品管高度!! 樣品管不可突出spinner 9.5cm以上!!

(2) Manually switch sample command : sx #

→ your sample position , or empty position

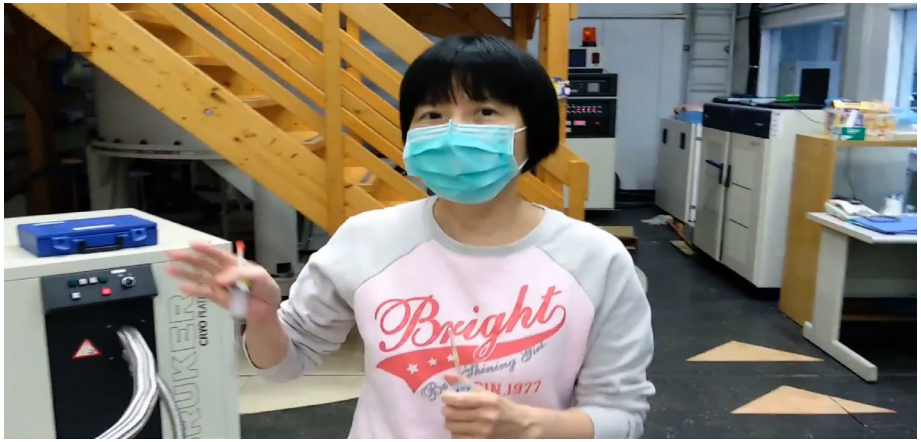
手動變更樣品指令: sx # (取出時可轉到沒有樣品的空號即可!!)

(3) To learn icon-NMR for automation, please contact us!

如欲學習設定icon-NMR, 請洽核心人員

How to use "SampleCase" & Topspin4.x

- SampleCase(24 on NEO600, Please see video), SampleCase(60 on AVIII600) 安裝自動送樣機 (影片介紹)



NEO500_IBMS
(SampleXpress-60 samples)
With QNP Cryoprobe
(1H/13C/19F/31P)

AV800_IBMS
(SampleJet-480 samples)
With TXI Cryoprobe
(1H/13C/15N)

•The SampleJet has five positions for well-plate sized NMR tube racks. This allows the handling of batches with up to 480 sample tubes.

•With Cooling capability

High Field NMR Center (www.nmr.sinica.edu.tw)

NMR	Install	Probe Head	Nuclei Detection	Remarks
AVIII850	2010	TCI_CRYO	1H/13C/15N	The highest NMR Field in Taiwan
		TXI_regular	1H/13C/15N	
AV800	2004	TXI_CRYO	1H/13C/15N	Sample Jet (480)
		TXI_regular	1H/13C/15N	
AVIII600	2008	TCI_CRYO	1H/13C/15N	Sample Case (60)
(AV600) NEO600	(2003) 2021	TXI_CRYO	1H/13C/15N	Sample Case (24)
AV600_CHEM	2002	QXI_regular	1H/13C/15N/31P	*Simultaneous decoupling on $^{1}H/^{19}F$
		*TBO_regular	1H/19F/BB	
(AV500) NEO500	(2009) 2022	*QNP_CRYO	1H/13C/31P/19F	Sample Xpress (60)
		TXI_CRYO	1H/13C/15N	

NMR Probes S/N ratio in HFNMRC

Regular Probe*	1H (EB)	Others
500MHz_TXI	450	
600MHz_TXI	1,218	
600MHz_BBO	465	465 (13C)
600MHz_QXI	1,193	85(31P)
600MHz_TBO	750	330(13C) 950(19F)
800MHz_TXI	2,077	

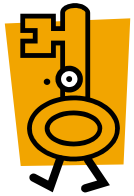
CRYO Probe*	1H (EB)	Others
500MHz_TXI	4,196	
500MHz_QNP	2,000	1,000 (13C) 1,197 (19F) 1,400 (31P)
600MHz_TCI_005	5,700	710(13C)
600MHz_TCI_121	6,530	950(13C)
800MHz_TXI	6,200	
850MHz_TCI	8,500	1,600(13C)

*Signal to Noise (S/N)value @ installed date

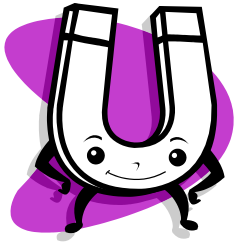
NMR & Probe	Topspin	1H (EB)	Others
NEO500_IBMS (Cryo QNP)	TP4.x	2,000	1,000 (13C) 988 (31P) 1,000 (19F)
AVIII600_IBMS (Cryo TCI_005)	TP2.x TP3.x	5,700	710(13C)
NEO600_IBMS (Cryo TCI_121)	TP4.x	6,530	950(13C)
AV600_CHEM (regular TBO)	TP2.x	406	377(13C) 241(31P) 342(19F)
AV800_IBMS (Cryo TXI)	TP2.x	6,200	N/A
AVIII850_IBMS (Cryo TCI)	TP2.x TP3.x	8,500	1,600(13C)

Currently installed system/Software

NMR Researches Across Diverse Fields



- ✓ Physics (fundamental)
- ✓ Chemistry
- ✓ Biology/ Biochemistry
- ✓ Material Science
- ✓ Food Science
- ✓ Pharmaceutical Research
- ✓ Medical Science
- ✓ Maybe many other more…..



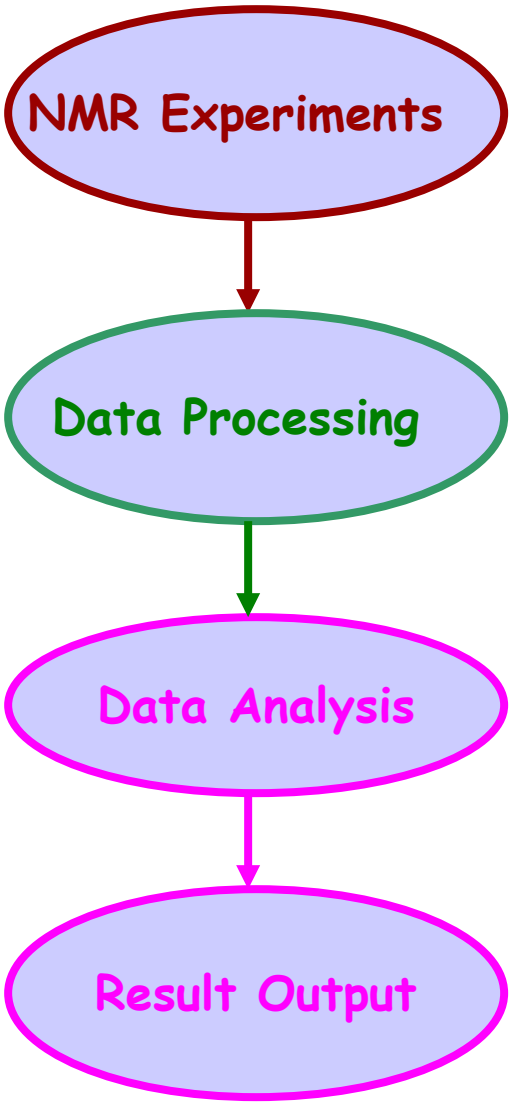
Service in GRC NMR Lab & HFNMRC, Academia Sinica

- Operate and maintain a state-of-the-art high field solution NMR core facility
- Provide high level technical supports to users
- Train users in applying advanced NMR techniques (Training Course, Workshops)
- Develop advanced NMR techniques
- Collaboration with researchers

Operation Mode

Type of Service	Description
Regular/Basic Service Mode	Data acquisition, experimental set up , technical consultation etc. but no data analysis
Collaborative Mode	facility members will work with collaborator on project design, planning, data analysis, structure determination and interpretation ……

User-friendly Platform



Information on web

homepage for user's information

Schedule on the web

spectrometer reservation

Simple Operation Manual

step by step instruction

Useful Experiments

1D, 2D, 3D, homo-, hetero- nuclear

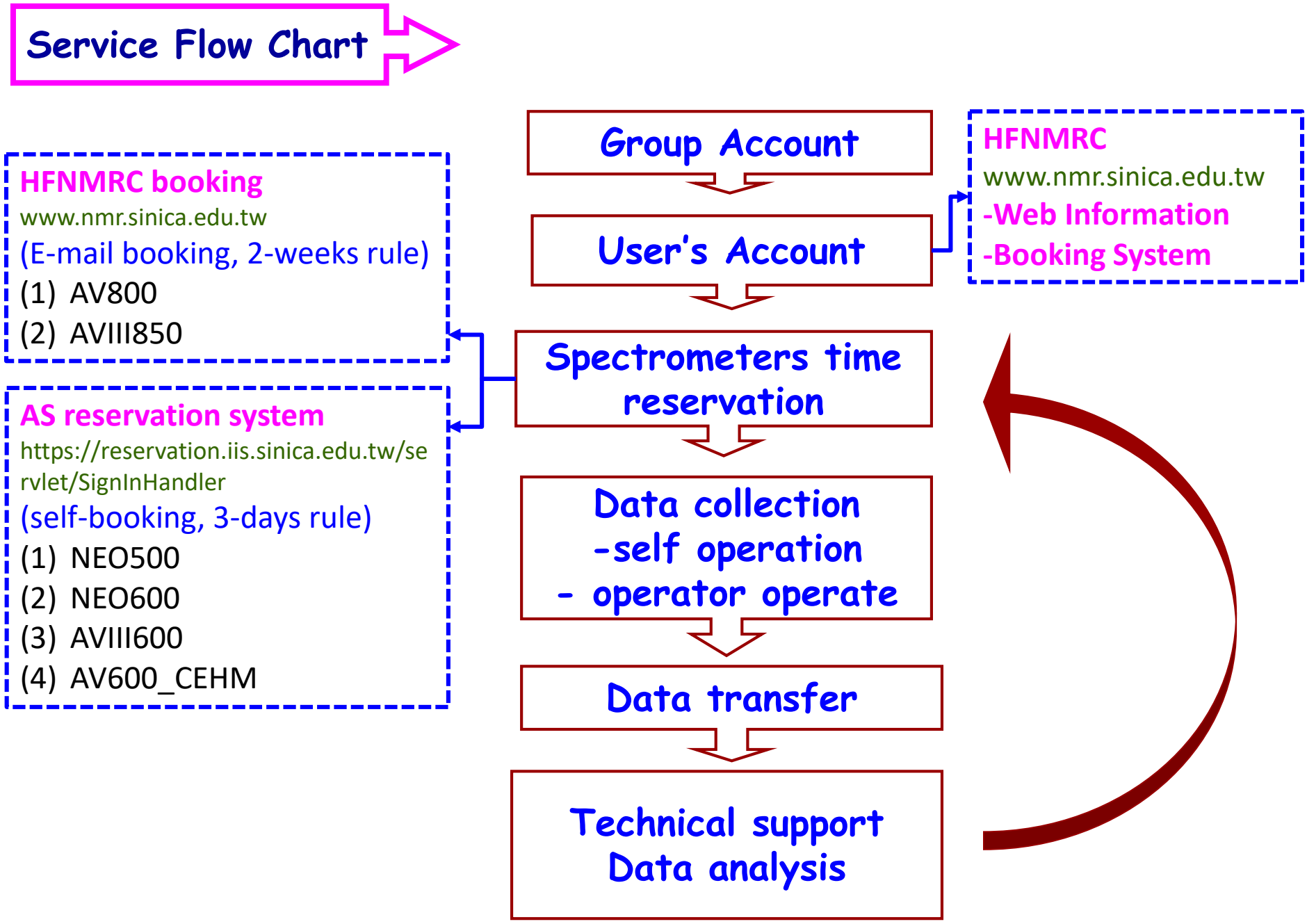
Parameters setting

expt/proc. parameter setting

NMR related Software

update and short instruction

Service Flow Chart

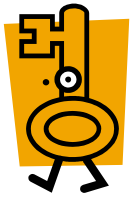


Applications of NMR

- What can NMR do?-

Experiences from Facility Users

Users Apply NMR mainly for the following…..



- Compound Identification / Structure Determination
- qNMR (Concentration/Purity of small molecules)
- Molecular Interactions (Protein-Protein, Protein-ligands……)
- Molecular Dynamics Studies
- Probing macromolecular folding and disordered states.
- Fragment Based Screening by NMR
- Metabonomics Researches (Human, E-coli, Perfused heart, Plant….)
- Food Analysis (oil, wine….)

Fragment-base Screening using NMR

-What is Fragment-base screening?
-Why Fragment-base screening?
-How HFNMRC can help ?

Fragment-Based Lead (Drug) Discovery

- **Fragment-based lead discovery** (**FBLD**) also known as **fragment-based drug discovery** (**FBDD**) is a method used for finding lead compounds as part of the drug discovery process.

- FBLD can be compared with high-throughput screening (HTS). In HTS, libraries with up to millions of compounds, with molecular weights of around 500 Da . are screened, and nanomolar binding affinities are sought. In contrast, in the early phase of FBLD, libraries with a few thousand compounds with molecular weights of around 200 Da may be screened, and **millimolar affinities** can be considered useful.

- Fragments are small organic molecules which are small in size and low in molecular weight. It is based on identifying small chemical fragments, which may bind only weakly to the biological target, and then growing them or combining them to produce a lead with a higher affinity.

From Wikipedia

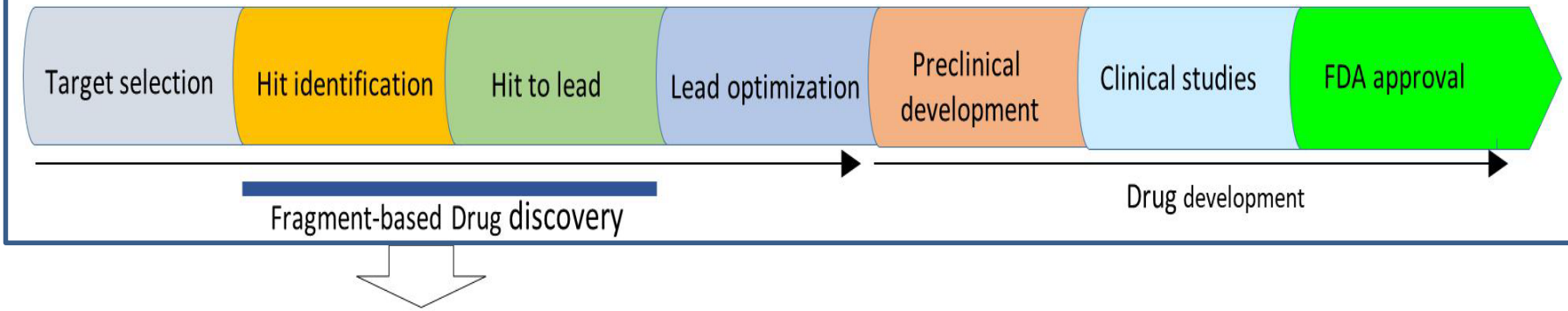
Advantages of Fragment-Based Screening

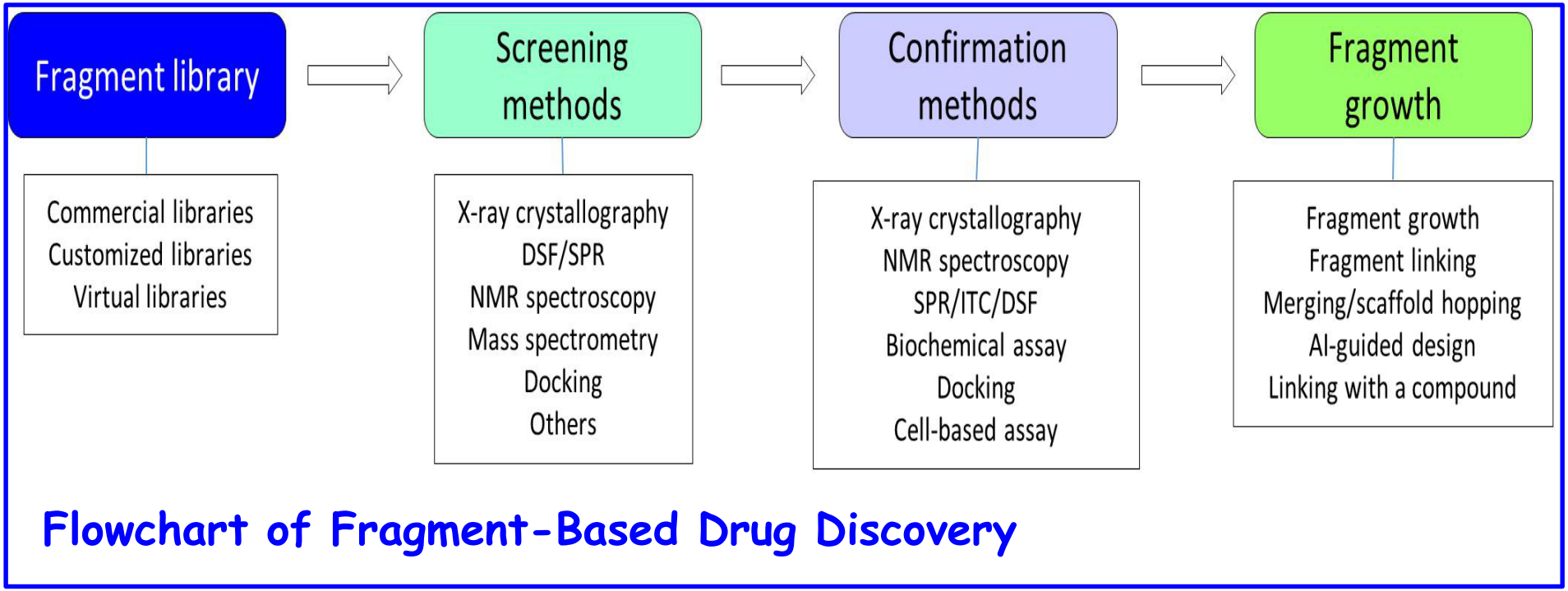
- Screening small fragments instead of large compounds
  - Increase the hit rate
  - Covers a greater portion of chemical diversity

- Screening with robust biophysical methods
  - Reduce false positive detection & increase confidence in weak hits
  - Do not require time-consuming assay development

- Leads to higher ligand efficiencies
  - Leading to smaller and more soluble compounds with higher chance for favorable pharmacokinetic properties

Drug Development Steps





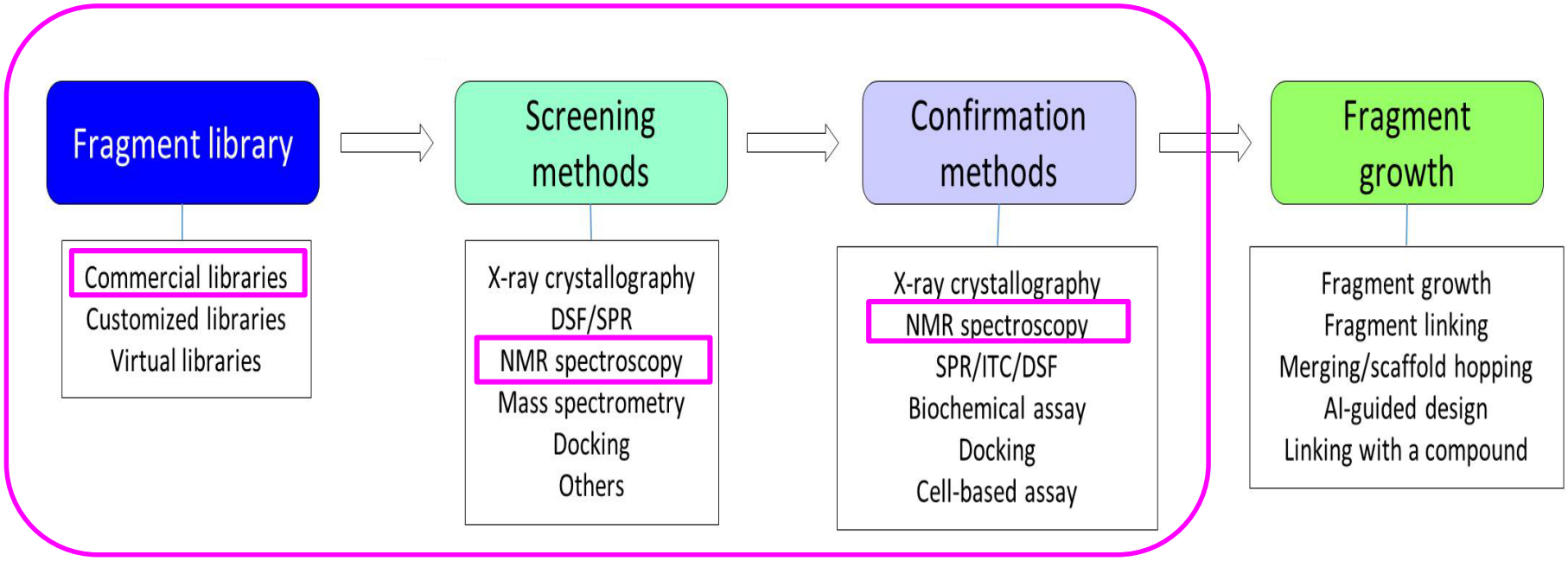
Flowchart of Fragment-Based Drug Discovery

Front. Mol. Biosci., 05 August 2020
Sec. Biophysics
Volume 7 - 2020 | https://doi.org/10.3389/fmolb.2020.00180

Fragment-base Screening using NMR (I)

-How HFNMRC can help?

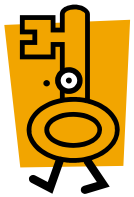
Fragment-baes Screening in HFNMRC



Advantages for NMR

(1) NMR studies are carried out in solution
(2) Unmodified biomolecule and ligand can be monitored directly
(3) Mixtures of compounds can be screened by NMR

Before Screening……Selection of Fragment Library

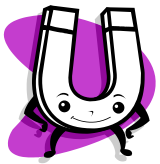


"Rule of three (Ro3)"

Type of compound	Fragment-like	Drug-like
Rule	Rule-of-Three*[34]	Rule-of-Five[21]
Thresholds		
MW	<300	≤500
cLogP	≤3	≤5
H-bond donors	≤3	≤5
H-bond acceptors	≤3	≤10
Typical values		
pIC_{50}	4.4**	8
HA	~15**	38
Ligand efficiencies		
LE	0.38	0.29
FQ	0.55	0.81

* The authors suggest number of rotatable bonds ≤3 and polar surface area ≤60 as additional useful criteria.** Median values taken from literature examples (see **Table S1**).

Schultes, Sabine et al. "Ligand efficiency as a guide in fragment hit selection and optimization." *Drug discovery today. Technologies* 7 3 (2010): e147-202 .



We use MAYBRIDGE Ro3 1000 in HFNMRC

How to set up "reliably" library?

-Fragment library

-NMR Spectrum library

How to set up screening condition?

-Experimental conditions

Which NMR Experiments for Screening ?

-Ligand observation NMR

-Hits identify

After screening.....

-Protein observation NMR

-Validate Hits / Binding Sites

-Follow up "chem-is-try"

-Structure……

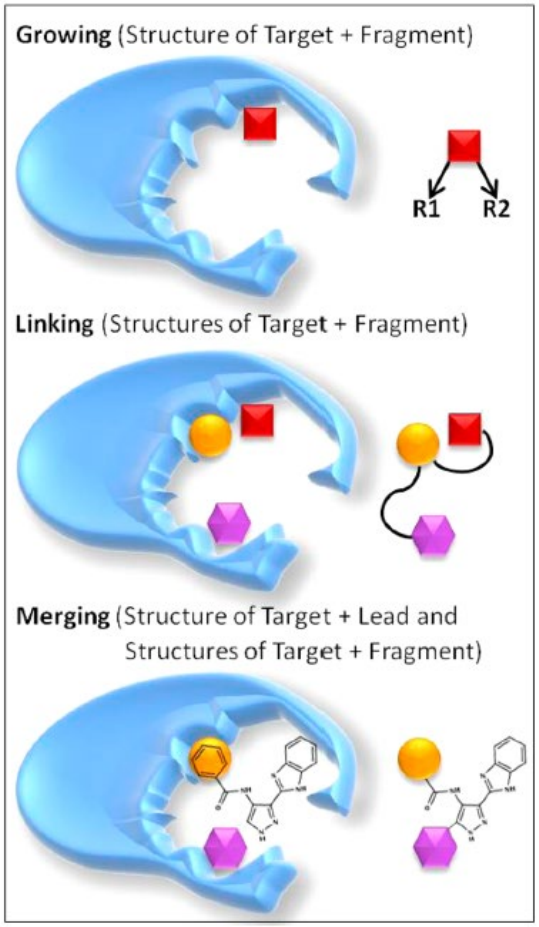


Figure 5. Strategies for using fragment positions in inhibitor design.

dx.doi.org/10.1021/ci400731w | J. Chem. Inf. Model. 2014, 54, 693−704

Step 1: Collect reference Spectrum for each compound

-1D 1H NMR to check compound purity and solubility

Step 2: Construct Group Mixtures (ex: 5 compounds/ group)

Mix ~5 compounds per Group

-Keep final concentration ~0.2mM
-Under buffer for later screening

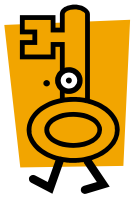
Collect 1D spectrum for each group

-1D 1H NMR no overlap
-No interaction between compounds

Construct final "Groups" for screening

HFNMRC Fragment vs. Spectra Library is ready!
We have total 906 compounds in 195 groups!!

Before start screening…. Anything else ?



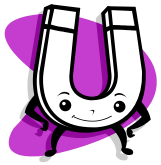
Setting sample condition!!

NMR screening prefer common biological buffers:

- **Phosphate (pH6.5-7.5)**
- **d18-HEPES (pH7-8)**
- **d11-TRIS (pH 7-9)**

Typical NMR sample for screening contain:

- **20-50mM Buffer**
- **0.2-0.5mM ligand**
- **Ligand : protein: 5-20 fold excess, depends on MW of the target protein**



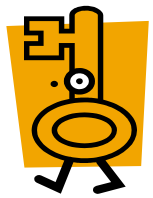
We choose PBS & d11-Tris @pH7 as our standard buffer, and keep [ligand]=0.2mM.

Step 3: Start screening using mutli-experiments

- 2-3 experiments to check for primary hits (exclude artifacts)

Analyze all data to identify Hits

Which NMR Experiments for Screening ?

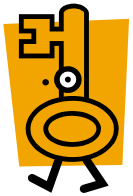


(Nat.Rev Drug Discov, 7,738,2008)

NMR methods for compound screening and hit validation

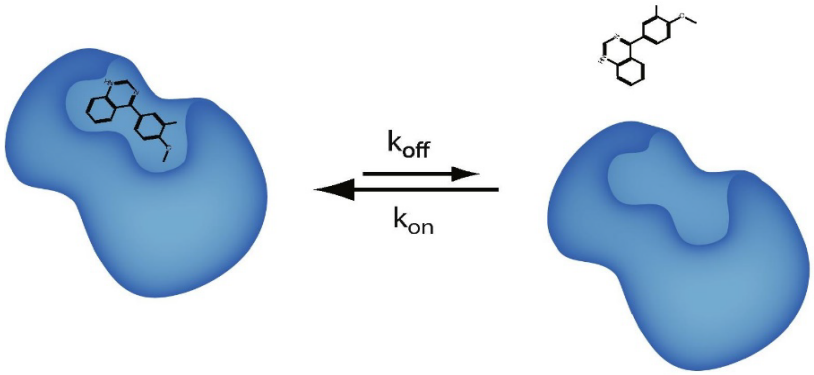
Approach	Observation	Use	Description and references to recent applications
Chemical-shift perturbation[1]	Target (protein or nucleic acid) resonances	Primary screening Hit validation Site of binding	Identifies compounds that bind by means of chemical-shift perturbation of resonances of the target[11,77,83–86]
STD NMR[2]	Ligand	Primary screening Hit validation	Identifies compounds that bind weakly; build-up curve identifies interacting functional groups[3,13,30,86–89]
WaterLOGSY[90]	Ligand	Primary screening	Identifies compounds that bind by using water-mediated NOEs[10,91]
SLAPSTIC (Using spin-labelled protein)[92]	Ligand	Primary screening	Highly sensitive detection of fragments that bind[5,92]
TINS[93]	Ligand	Primary screening Hit validation	Identifies compounds that bind by screening libraries against immobilized protein targets[93]
$T_{1\rho}$ and T_2 relaxation; line broadening[4]	Ligand	Primary screening Hit validation	Binding enhances relaxation; enables affinity estimates; build-up curve identifies interacting functional groups[94]
Transferred NOEs[95]	Ligand	Hit validation Conformation of flexible ligands	Gives information about the interaction of binders with the target[96,97]; determines bioactive conformation of flexible ligands such as peptides[14]
FABS[16,17]	Substrate or cofactor	Primary screening Hit validation	Uses reference substrates or cofactors to monitor enzymatic reactions[12,98–104]
FAXS[105,106]	Reference ligand	Primary screening Hit validation	Measures the displacement of a fluorinated 'spy' molecule[104,107]
Diffusion measurements[108,109]	Ligand	Primary screening Hit validation	Measures the difference in diffusion rates for ligands in the bound versus free state[110]

Which NMR properties to observe?



Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
fast	transverse relaxation	slow
strong positive	NOE cross-peaks	weak negative
slow	translational diffusion	fast



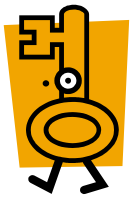
Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}

- Strong relaxation effects are imprinted on the ligand during its visit to the target protein, and read out on the free ligand after it dissociates from the target
- With ligands in large excess, most ligand molecules still visit the target several times and accumulated effects due to relaxation in the bound state. The "averaged effects" are measured.
- "Transferred" effects are terms to describe this process (ex: transferred NOEs, transferred cross-correlated relaxation….)

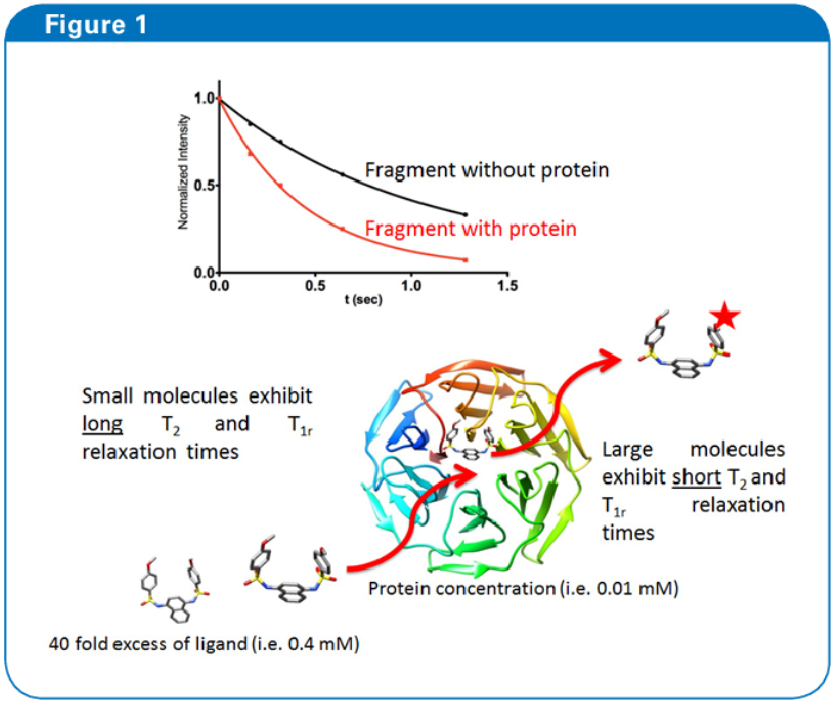
Alvar D. Gossert, Wolfgang Jahnke, NMR in drug discovery: A practical guide to identification and validation of ligands interacting with biological macromolecules, Progress in Nuclear Magnetic Resonance Spectroscopy,Volume 97,2016,Pages 82-125,

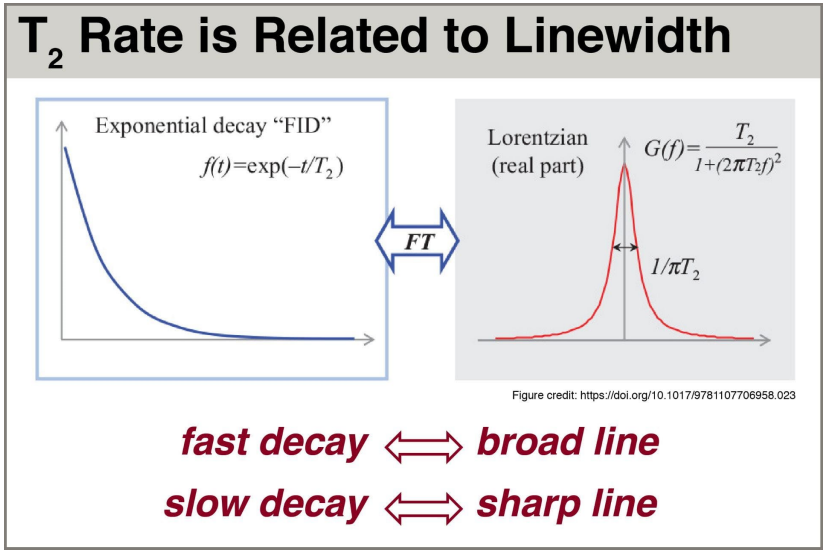
Ligand observed : The fast Koff rate enables detection of the ligands which ever bound to protein and still retain the NMR measureable properties of "bound-form".



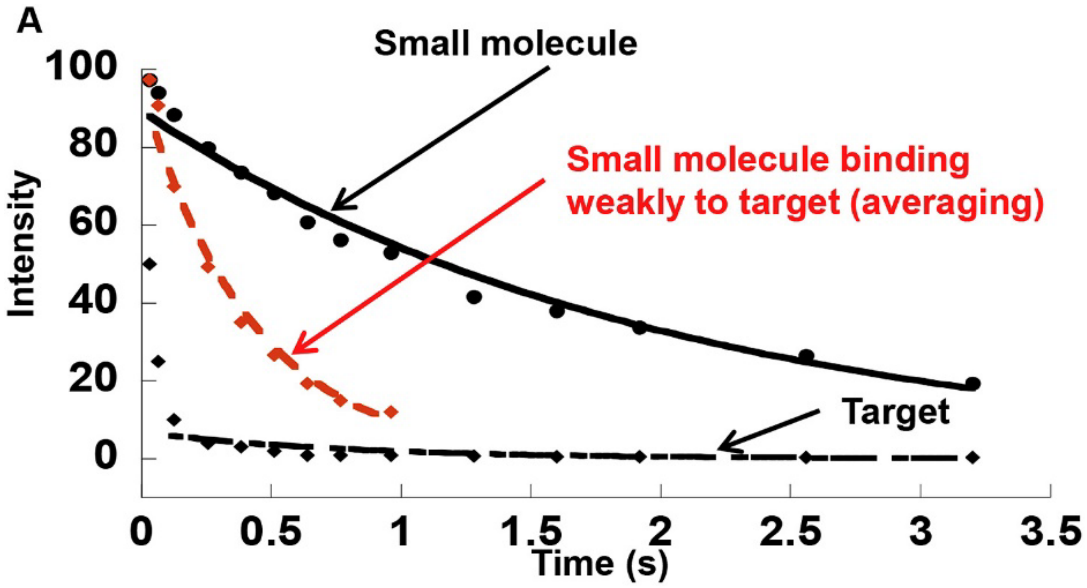
Relaxation-based approach (T_2 or $T_{1\rho}$)

- Transvers Relaxation effects (T2) is highly depend on molecular motions (rotational correlation time, τ_c : slower/longer cause shorter T2)
- NMR signal line width is proportional to transverse relaxation $1/T_2$ (or $T_{1\rho}$: mixed relaxation due to T1 & T2).
- Line broadening effect (intensity drop) could be observed for bound ligand

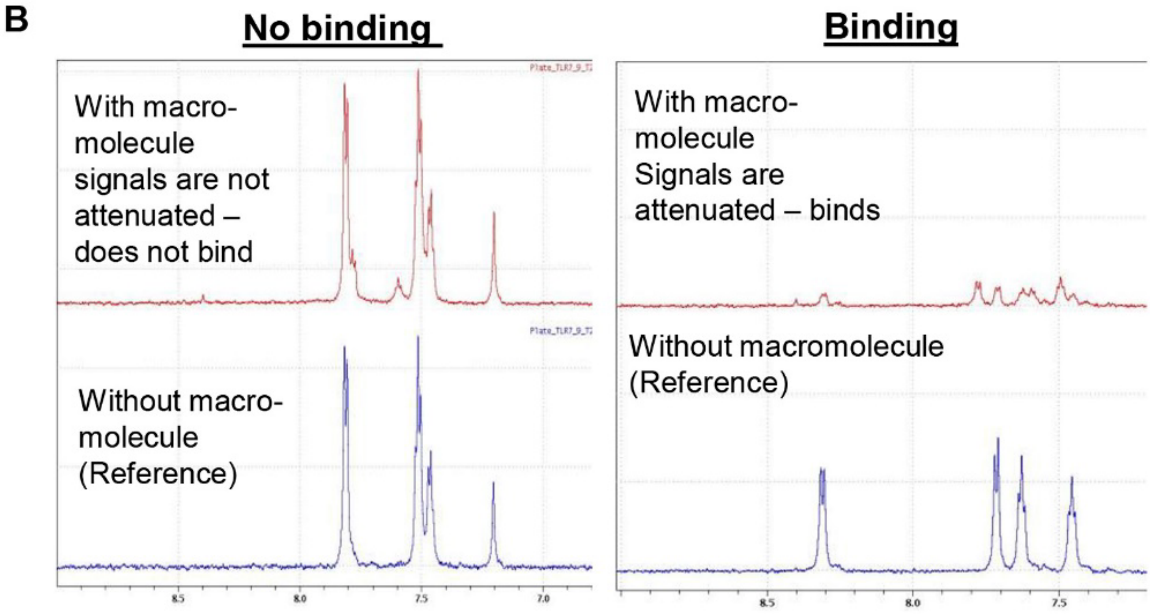




Practical Aspects of Fragment-Based Screening Experiments in TopSpin
D. Baldisseri, Bruker Biospin 2018 Chemistry, Biology



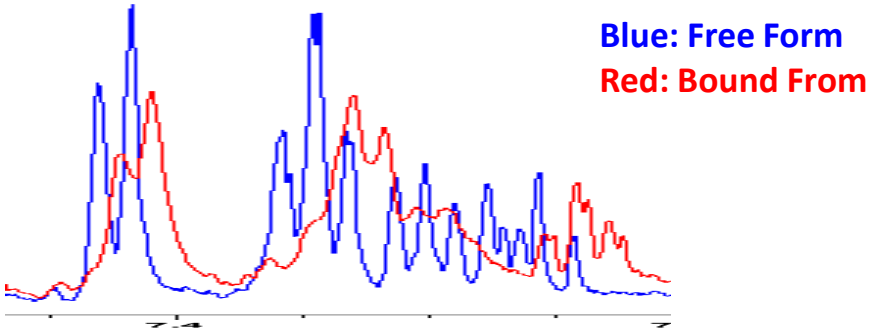
Note: due to the possible false-positive results , “ratio of ratios” (relaxation factor f, f<<1 for binding, the binding cutoff is generally factor f in the presence of protein is 0.2 units smaller than in the absence of protein)



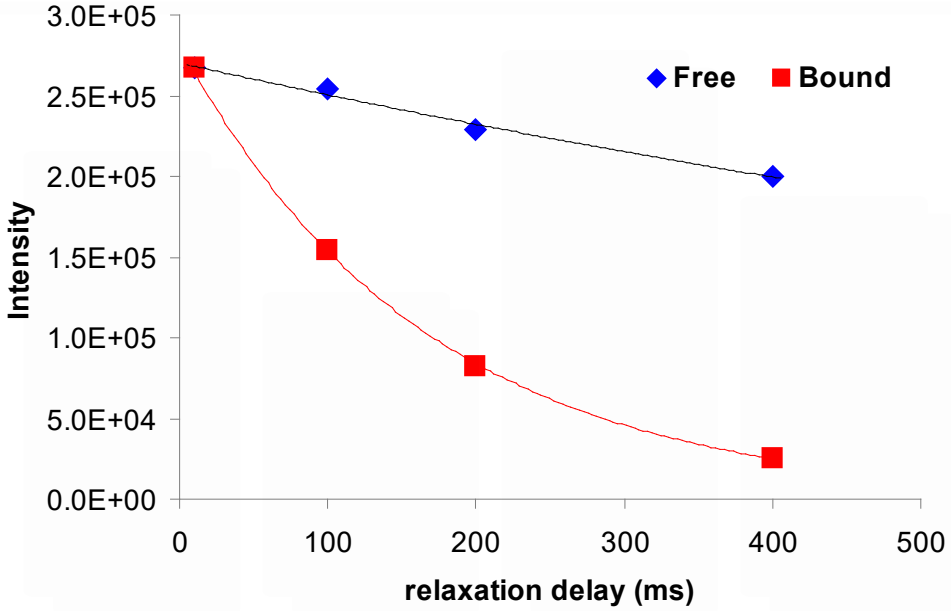
Mary J. Harner, Luciano Mueller, Kevin J. Robbins, Michael D. Reily,NMR in drug design,Archives of Biochemistry and Biophysics,Volume 628,2017,Pages 132-147,

Example from HFNMRC data: Line broadening & T1ρ

<Example of 1D 1H Line Broadening>



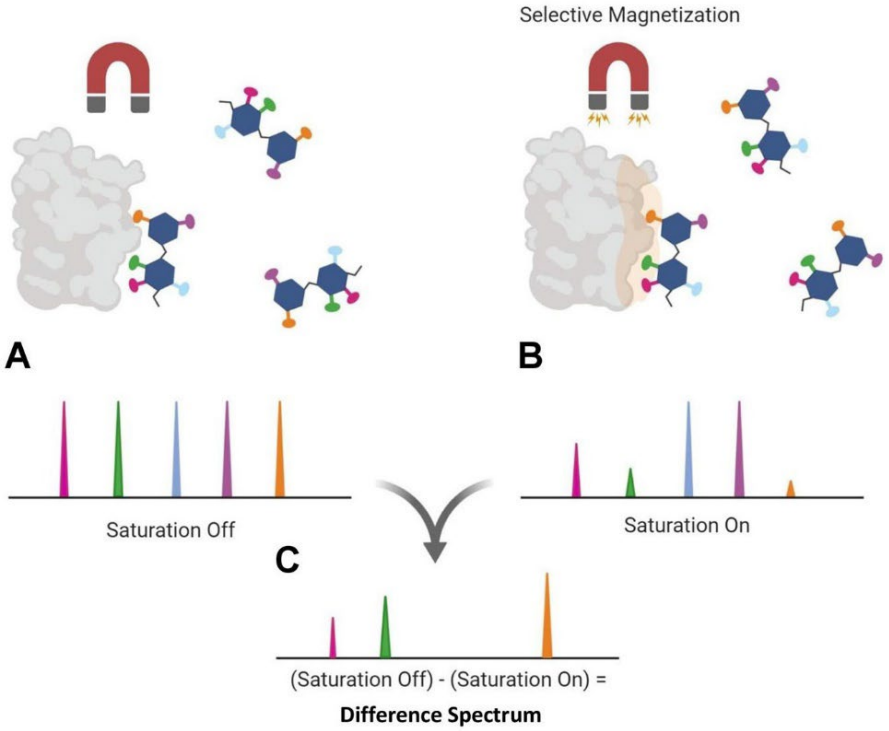
<Example of 1D Relaxation Effect >



NOE-based Approaches

(1) Saturation transfer difference (STD)

- Magnetization transfer between ligand and target protein, ie. NOE between bound ligand and protein
- Signal intensities were attenuated by saturation transfer for bound ligand
- Small positive signal (STD effect) only observed for bound ligand



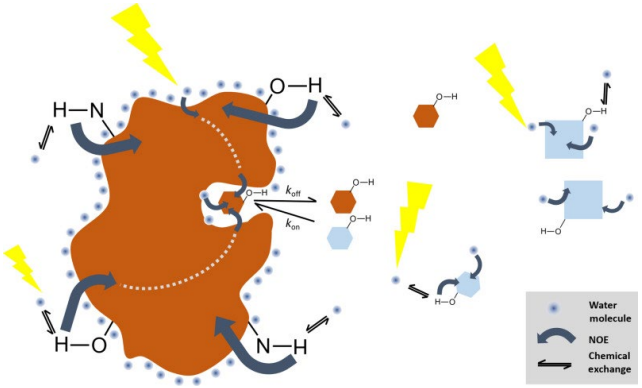
Note: aggregation leading to strong false positive STD effects (line broaden due to aggregation). Thus, a protein-observed follow-up is always necessary.

Vignovich WP, Pomin VH. Saturation Transfer Difference in Characterization of Glycosaminoglycan-Protein Interactions. SLAS TECHNOLOGY: Translating Life Sciences Innovation. 2020;25(4):307-319. doi:10.1177/2472630320921130

NOE-based Approaches

(2) Water Ligand-Observed by Gradient SpectroscopY (WaterLOGSY)

- What is measured is the NOE between H2O & Ligand
- Bulk water magnetization is transferred to
  (i) target bound ligand and exchangeable protons on target protein (slowly tumbling bound ligand/protein with long τ_c ,negative NOE)
  (ii) compounds free in solution (quickly tumbling with small τ_c ,positive NOE)
- Bound ligand can be distinguished from non-binders by opposite sign of signals
  (i) binder: positive signals (negative NOE are used to phased as positive peak), or negative but less intense than control, or not detectable (average effect)
  (ii) non-binder: negative signals (should have same intensity as control)



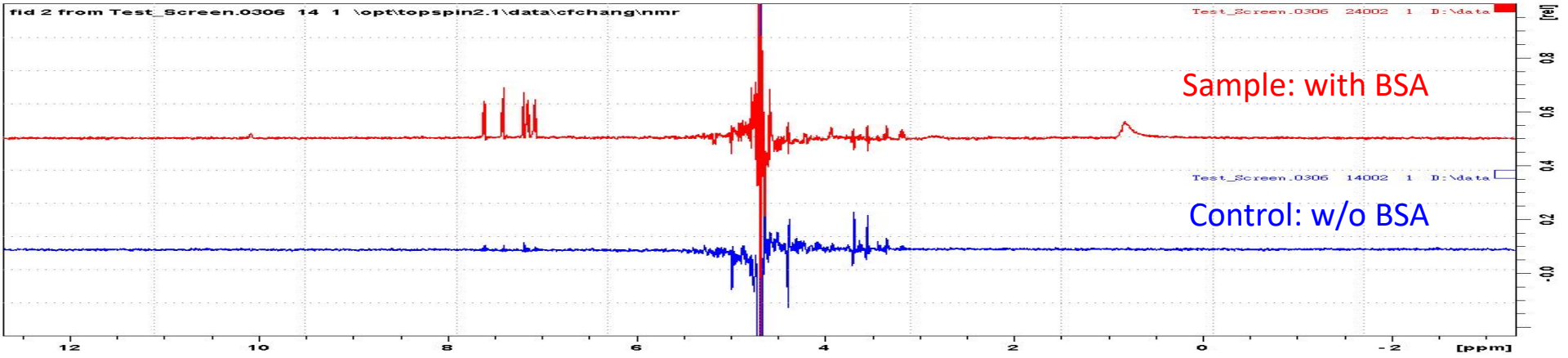
Note: Exchangeable protons will undergo chemical exchange with H2O, thus always positive, should be excluded from binding analysis

Renjie Huang, Ivanhoe K.H. Leung,Chapter Fourteen - Protein–Small Molecule Interactions by WaterLOGSY,Methods in Enzymology,Academic Press,Volume 615,2019,Pages 477-500,

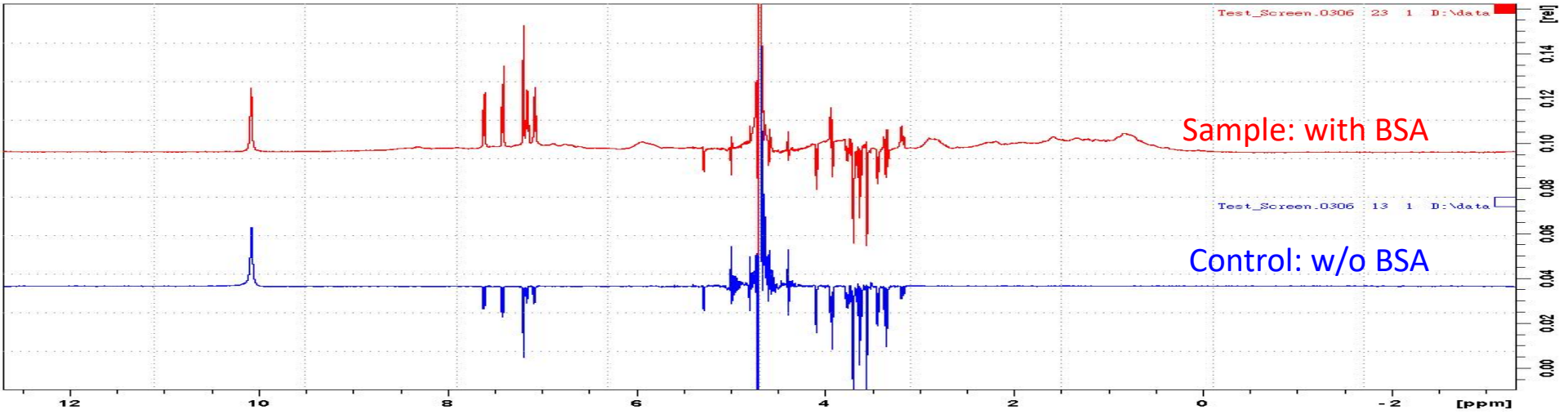
Example from HFNMRC data: STD & WaterLOGSY

(Testing Sample: BSA 0.1mM, **Trp 2mM**, sucrose 2mM, 10% D2O, 90%H2O)

<Example of 1D 1H STD>



<Example of 1D WaterLOGSY> (note on phase correction)



Which NMR Experiments for Screening (1H-NMR) ?

Water-LOGSY

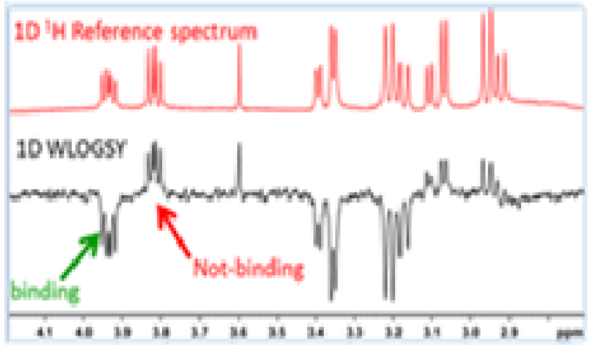
Binders have **opposite phase** to non-binders

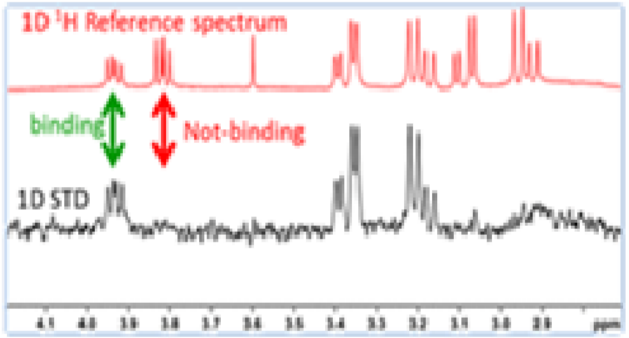
Saturation Transfer Difference (STD)

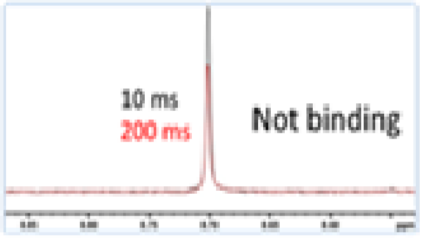
Binders **appear** in difference spectrum, non binders do not

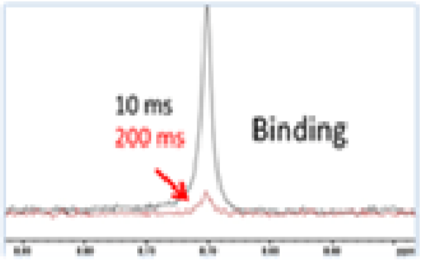
$T_2/T_{1\rho}$

Binders show **strong attenuation**









Practical Aspects of Fragment-Based Screening Experiments in TopSpin D. Baldisseri, Bruker Biospin 2018 Chemistry, Biology

For 1H experiment, we choose

(1) Solvent-suppressed 1H (chemical shift)

(2) waterLOGSY (NOE based saturation transfer experiments)

(3) STD (NOE based saturation transfer experiments)

(4) T1ρ (Relaxation based line broadening effects)

Which NMR Experiments for Screening (19F-NMR) ?

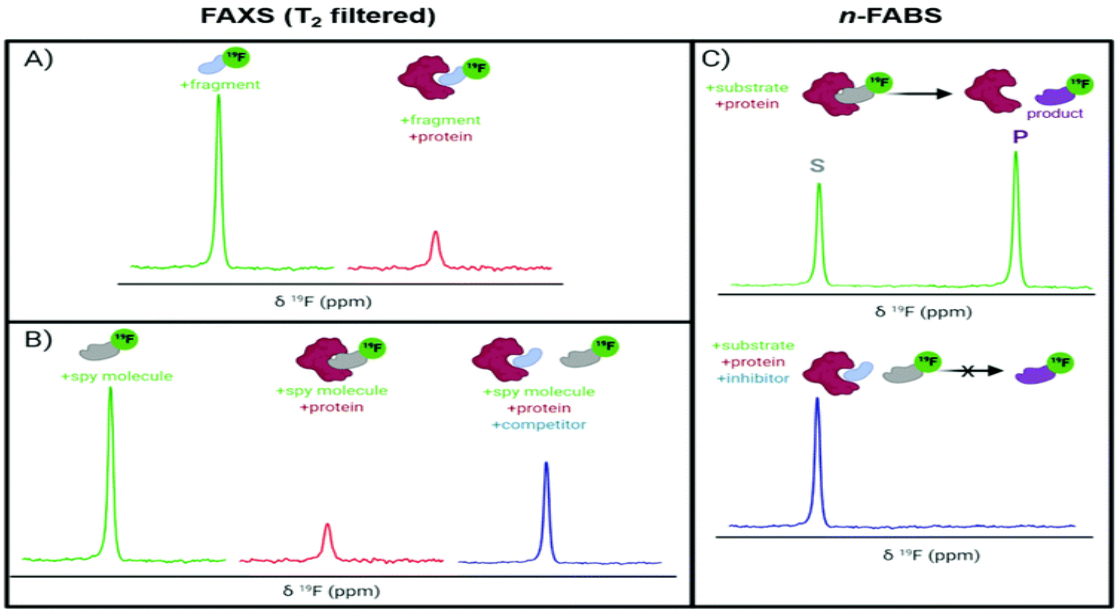
For compounds with 19F (19F library)

-few 19F signals for each compound, thus, >20 ligands can be mixed without overlap (higher throughput)

-no need for solvent suppression

-line broadening of 19F upon binding is significant than 1H detection

-We plan to do T2 experiment for 19F screening (relaxation based transfer)



DOI: 10.1039/D1CB00085C (Review Article) *RSC Chem. Biol.*, 2021, **2**, 1312-1330

Our approach for 1H NMR screening

"Free" Compound Groups → ·1D 1H spectrum ·T1ρ spectrum

- 0.2mM compounds
- PBD or d18-Tris Buffer @pH7.0

↓

Compound Groups + Target Protein → ·1D 1H spectrum ·T1ρ spectrum ·WaterLOGSY spectrum ·STD spectrum

- 0.2mM compounds + ~0.01mM Protein
- PBD or d18-Tris Buffer @pH7.0

↓

Find the " Hit Groups" → Analyze all spectrum Free vs. Complex

↓

Narrow down to "individual Hit"

Fragment-base Screening in HFNMRC

HFNMRC Service Charge

(Starting 2021.01.01)

	Item	Academia Sinica (NT$)	Academia (NT$)	Non-academia (NT$)
1	500MHz[1]	100/hr	110/hr	150/hr
2	600MHz[1]	120/hr	130/hr	180/hr
3	800MHz[1]	180/hr	190/hr	270/hr
4	850MHz[1]	200/hr	210/hr	300/hr
5	Small Molecule Data Collection[2]	1,000/expt	1,000/expt	1,500/expt
6	Experiment Setup Fee for Macromolecule[2]	1,500/expt	1,500/expt	2,000/expt
7	Fragment Based Screening[3] (data collection)	70,000/case	70,000/case	100,000/case
8	Fragment Based Screening[3] (data collection/processing)	100,000/case	100,000/case	150,000/case

* This fee might be adjusted according to the operational status.

1. In case of regular Room Temperature Probe, reduce N.T.80 per hr.

2. Data collection only, no data analysis nor interpretation is included.

3. Follow existing Fragment Library SOP in HFNMRC. Please find "Data collection Service" application form for details. Should you need other services, please contact us.

(Last updated: 01 Jan. 2021)

https://www.nmr.sinica.edu.tw/Doc/AS_HFNMRC_F008_FBSApForm.pdf

SOP from Bruker (Topspin3.5PL7 and TopSpin4.x)

Experiment	Recommended [ligand]/[protein]	
Target Size	~30KDa protein	~100KDa protein
T1ρ	20-fold excess	8-fold excess
STD/waterLOGSY	10-fold excess	5-fold excess

Table 1. Standard FBS screening experiments and parameter options in TopSpin.

Parameter set	Pulse program	nD	List	ZGOPTNS	AUNMA	AUNMP
SCREEN_STD	stddiffesgp.3	2	FQ2LIST (exam_std)	-DCALC_POWER	au_zg_std	proc_std (relax)
WLOGSY_PREP	ephogsygpno.2	1	n.a.	-DFLAG_FP -DFLAG_SL	au_zg_wlogsy	proc_wlogsy (prepare)
SCREEN_WLOGSY	ephogsygpno.2	1	n.a.	-DFLAG_FP -DFLAG_SL	au_zg_wlogsy	proc_wlogsy
SCREEN_T1R	t1rho_esgp2d	2	VPLIST (t1r_screening)	-DTEMPCOMP	au_zg_wlogsy	proc_std (relax)
SCREEN_T2	cpmg_esgp2d	2	VCLIST (t2_screening)	-DTEMPCOMP	au_zg_wlogsy	proc_std (relax)
SCREEN_19F_T2	19Fcpmg_screen	2	VCLIST (19F_simple_cpmg_screen)		au_zg_19F_wvm	proc_T2_19F (relax)

Practical Aspects of Fragment-Based Screening Experiments in TopSpin D. Baldisseri, Bruker Biospin 2018
Chemistry, Biology

SOP in HFNMRC (pp and au program depends on Topspin version)

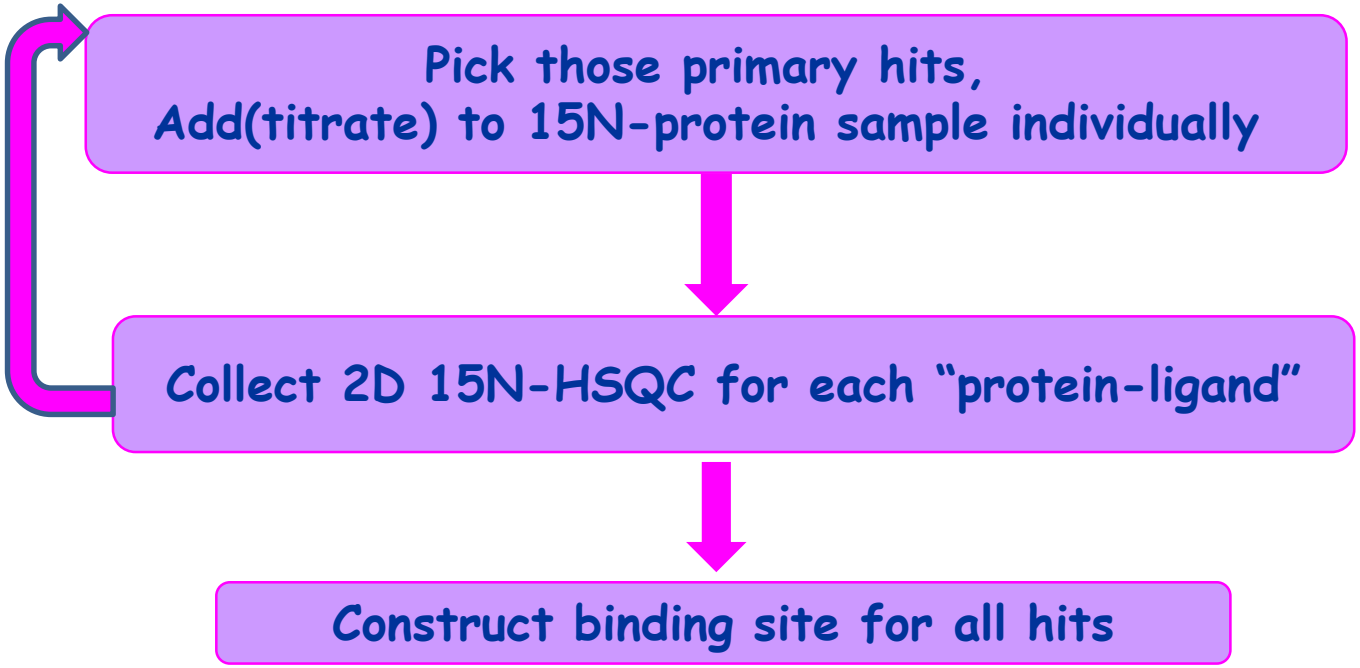
Parameter Set	Pulse Program	nD	List	Process AU
htnmr_ZGW5	zggpw5	1	n.a.	n.a.
htnmr_ZGESGP	zgesgp	1	n.a.	n.a.
htnmr_STD	stddiffesgp.3 stddiffesgp3.cf	2	STD.cf	stdsplit or std.cf
htnmr_T1rho	t1rho2des_2016.cf	2	T1rho.cf	t1rho.cf
htnmr_wLOGSY	ephogsgpno.2	1	n.a.	n.a.

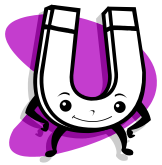
Step 3: Start screening using mutli-experiments

- 2-3 experiments to check for primary hits (exclude artifacts)

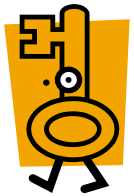
Analyze all data to identify Hits

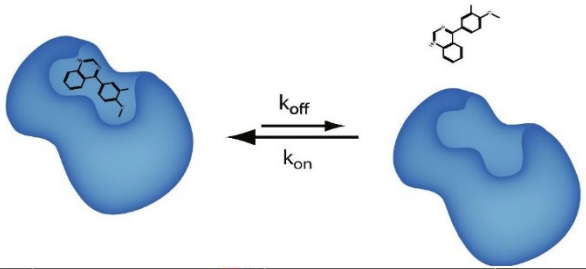
Step 4: Evaluating protein-ligand interactions (Validation)

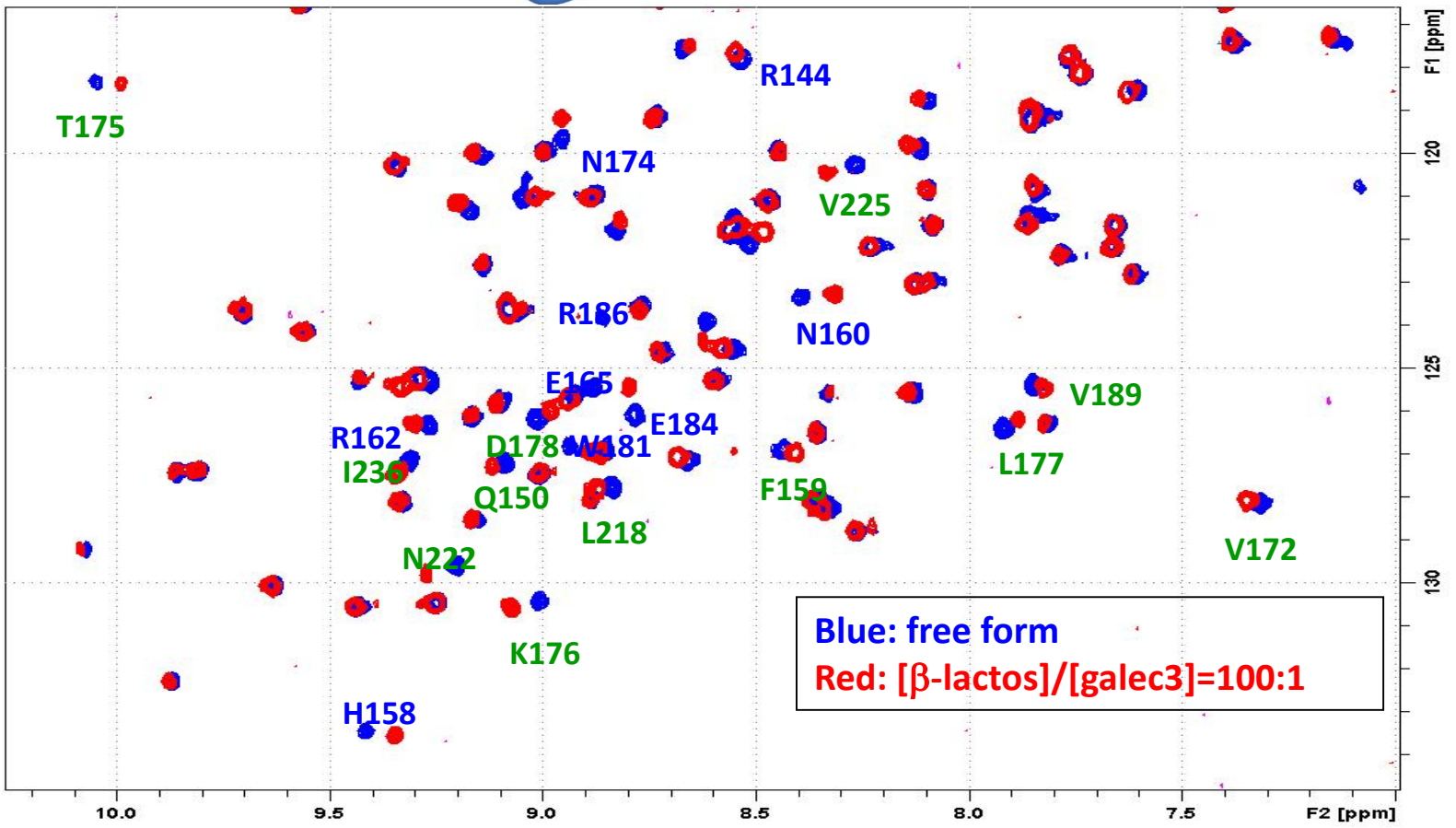




Protein observed : 15N-1H HSQC (finger print for protein) chemical shift perturbation upon titration with "hit" ligand







Our approach for NMR hit validation

"Free" Protein NMR → ·Backbone Assignments ·15N labelled protein

- 0.1mM 15N-labelled Protein
- PBD or d18-Tris Buffer @pH7.0

↓

Target Protein + Hit Compounds → ·1D 1H spectrum ·2D 15N-1H HSQC

- ~0.1mM Protein + compounds (titration)
- PBD or d18-Tris Buffer @pH7.0

↓

Titration to find " Active Site" → Analyze Chemical Shift perturbation Free vs. Complex

↓

SAR (structure-activity relationship) by NMR → Provide information for Compound Optimization

Fragment-base Screening in HFNMRC

Step 1: Collect reference Spectrum for each compound

-1D 1H NMR to check compound purity and solubility

Step 2: Construct Group Mixtures (ex: 5 compounds/ group)

Mix ~5 compounds per Group

-Keep final concentration ~0.2mM
-Under buffer for later screening

Collect 1D spectrum for each group

-1D 1H NMR no overlap
-No interaction between compounds

Construct final "Groups" for screening

HFNMRC Fragment vs. Spectra Library:
(1) MAYBRIDGE Ro3 1000 , 906 compounds in 195 groups!!
(2) OTAVA 19F Library (under construction….)

Step 3: Start screening using mutli-experiments

- 2-3 experiments to check for primary hits (exclude artifacts)

Analyze all data to identify Hits

Step 4: Evaluating protein-ligand interactions (Validation)

Pick those primary hits,
Add(titrate) to 15N-protein sample individually

Collect 2D 15N-HSQC for each "protein-ligand"

Construct binding site for all hits

Provide these information for user's further characterization

Examples

Acknowledgement

NMR Facility Members

Pei-Ju Fang

Yi-Ping Huang

Geok-Soon Lee

Tsun-Ai Yu

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Setting up Ligand-Observed NMR Experiments

NMR System Overview

Group	NMR	System	Probe	Location
1	AV600R	**Topspin2.1**	**Dual(1H/13C)**	**GRC, 1F**
2	AV600L	**Topspin3.2**	**TCI(1H/13C/15N)**	**GRC, 1F**
3	NEO500	**Topspin4.2.0**	**QNP(1H/13C/19F/31P)**	**IBMS, B2**
4	AVIII600	**Topspin2.1 &** Topspin3.2	**TCI(1H/13C/15N)**	**IBMS, B2**
5	NEO600	**Topspin4.1.3**	**TCI (1H/13C/15N)**	**IBMS, B2**
6	AV800	**Topspin2.1**	**TXI(1H/13C/15N)**	**IBMS, B2**
7	AVIII850	**Topspin2.1 &** Topspin3.2	**TCI(1H/13C/15N)**	**IBMS, B2**
8	AV400	**Topspin2.1**	**Dual (1H/13C)**	**IBMS, B2**

(Testing Sample: BSA 0.1mM, Trp 2mM, sucrose 2mM, 10% D2O, 90%H2O)

Parameter Set	Pulse Program	nD	List	Process AU
htnmr_ZGESGP	zgesgp	1	n.a.	n.a.
htnmr_STD	stddiffesgp3.cf stddiffesgp.3	2	STD.cf	stdsplit or std.cf
htnmr_T1rho	t1rho2des_2016.cf t1rho_esgp2d	2	T1rho.cf	t1rho.cf
htnmr_wLOGSY	ephogsgpno.2	1	n.a.	n.a.

Parameter Set	Parameters to optimized (d1=3sec, ns=8)
htnmr_ZGESGP	O1 & 90 deg pulse (optional), ns
htnmr_STD	O1, 90deg pulse, ns, d20 (saturation time, ex: 2sec) STD list (FQ2LIST, ex: -40ppm & 0.8ppm)
htnmr_T1rho	O1, 90 deg pulse, ns T1rho list (VDLIST or VPLIST, ex: 10ms, 200ms, 300ms)
htnmr_wLOGSY	O1, 90 deg pulse, ns, d8 (mixing time ex:0.8-1.2s. 1.7s…)

Simple Operation Guide for HFNMRC Users

by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

PART I: Preparation (Software & Hardware)

Step 1: Starting topspin

1. Login into computer
2. Double click on "**topspin**" icon (Fig.1)
   (Topspin window will pop out)

Step 2: Temperature control

1. Type "**edte**" <enter>
2. The temperature control sub-window will pop out, set up your target temperature (Fig. 2)

Step 3: Loading sample (no autosampler)

1. Put NMR tube into **Spinner** and adjust sample position using **Sample Position Gauge** (Fig.3)
2. Click "**Lift**" button on [BSMS] (Fig.4) , you should hear the air flow
3. Load your sample (make sure sample can "float" on the top of magnet before releasing the sample)
4. Click "**Lift**" button on [BSMS] again, the sample should go down to the probe

Step 3': Loading sample (with autosampler)

1. Put NMR tube into **Spinner** and adjust sample position using **Sample Position Gauge** (Fig.3)
2. Load your sample(s) to autosampler, and note your sample position(s)
3. Type "**sx #**" (# is your sample position number) to load sample into NMR probe

Note:

- Depends on hardware, it could be topspin2.x , topspin3.x or Topspin4.x
- Check temperature before loading sample is highly recommended.
- For regular 5mm NMR tube, fill in 450-500ul sample solution is recommended.
- For NEO 600 SampleCase, tube above spinner must less than 9.5cm
- In case autosampler couldn't load sample correctly, try to switch to other position. If error show up, ask facility members to help.

Step 4: Lock & Wobble (with ATM*)

1. Start a new data set :
   type "**edc**" <enter> , or "**new**" <enter> (Fig.5)
2. Select Standard Experiment (ex: 1GRC_1D_1H) ;
   Or, Type "**rpar 1GRC_1D_1H***"
3. Type "**getprosol**" <enter> to read in standard pulses
4. Type "**lock**" <enter> to choose your D-solvent
5. Type "**atma**"<enter> to auto adjust 1H frequency or "**atmm**"<enter> to manually tune/match

* ATM is the auto Tune and Match accessory installed on probe. For those probes without ATM accessory (ex: Dual probe on AV400 & TXI/QXI on AV600_CHEM) , if needed, type "**wobb**" to do wobble manually.

Step 5: Shimming

1. (optional) Type "**rsh** " (not necessary, but you can select previously saved shim file for specific probe/solvent)
2. Type "**topshim**" <enter>
   or "**topshim tunea**" <enter> (recommended)
   or "**topshim convcomp**"<enter> (for NEO only)
3. Manually adjust shim if necessary

Note:

- Use 1H experiment to optimize hardware setting
- Why we should getprosol ?
- What is lock doing?
- When and why should we do wobble(atma) ?
- What is shimming?
- More options are available if typing "topshim gui"

By now, Lock & Shim has been optimized. Unless you change temperature or sample, it's not necessary to lock/shim again during later experiment set up and data collection!

However, wobble (atma) might be needed depends on the experiments you set up.

PART II: Experiment Set up & Data Collection

● Steps for Setting up 1D Experiment:

1. Type "**edc**" or "new" <enter> to set up new EXPNO
2. Select Standard Experiment (1GRC*) ;
   Or, Type "**rpar 1GRC*** " <enter>
3. Type "**getprosol**" <enter> to read in calibrated pulses information from standard samples
4. Type "**atma**"<enter> or "**atmm**"<enter> to optimized nuclei frequencies (if no ATM probe, check it manually by typing "**wobb**")
5. (optional) For 1H experiment, type "**pulsecal** " <enter> to optimized 90 degree pulse for your own sample (recommend for sample in H20/D2O, or 2D experiments)
6. Type "**ns**" <enter> to fill in scan number you like
7. (optional)Type "**eda**" or "**ased**" <enter> to fill in other parameters if needed (ex: O1, SW, TD,....)
8. Type "**rga**" <enter> to auto adjust receiver gain
9. Type "**zg** " <enter> to collect 1D FID
10. Type " **efp**" <enter> to do Fourier Transform
11. Type "**apk**" <enter> to auto-phasing the spectrum
12. Type "**abs n**" <enter> to adjust baseline

(Spectrum is ready for further processing/analysis: calibration, peak picking, integration ....)

Note:

- Select most commonly used expts from our standard parameter sets
- May skip if already done for required nuclei
- Write down the corresponding power level (dB/dBW) and pulselength (us) for later usages
- ased only display parameter needed for specific experiment
- Can be combined as " efp, apk, abs n"

● Extra notes:

- If using optimized pulses from "pulsecal" for other experiments (Fig.6) Type "**getprosol Δ 1HΔ<pulse in us >Δ<power level in dB or dBW>**" (ex: getprosolΔ1HΔ**10.2**Δ**-3.5**) instead of "getprosol"

● Steps for Setting up 2D Experiment:

1. Type "**edc**" or "**new**" <enter> to set up new EXPNO
2. Select Standard Experiment (1GRC*) ;
   Or, Type "**rpar 1GRC*** " <enter>
3. Type "**getprosol**" <enter> to read in calibrated pulses information from standard samples
4. Type "**atma**"<enter> or "**atmm**"<enter> to optimized nuclei frequencies (if no ATM probe, check it manually by typing "**wobb**")
5. (recommended) Use 1H pulse calibrated by pulsecal "**getprosol Δ 1HΔ<pulse us >Δ<level in dB or dBW>**"
6. Type "**ns**" <enter> to fill in scan number you like
7. (recommended) Type "**eda**" or "**ased**" <enter> to double confirm all parameters
8. Type "**rga**" <enter> to auto adjust receiver gain
9. Type "**zg** " <enter> to collect 2D spectrum
10. After the first series data is done,
    Type " **rser 1**" <enter> to call out the 1st FID
11. Do "**efp, apk, abs n**" to check spectrum quality (if signal not strong enough, **stop** the data acquisition, increase scan number (step 6), and **rga, zg** again.)
12. Type "**xfb**" <enter> to Fourier Transform 2D

(Spectrum is ready for further processing/analysis)

Note:

- Select most commonly used expts from our standard experiment sets
- Important but may skip if already done for required nuclei
- For other nuclei, you may use default values
- ased would be easier

PART III: Remove Sample and Logout

1. Click "**Lock**" botton on [BSMS] to turn off lock (Fig.4)
2. With Autosampler: type "**sx #**" where # is an empty position
3. Without Autosampler:
   Click "**Lift**" botton on [BSMS] to turn on air and remove your sample
   Click "**Lift**" botton again to turn off air
4. **Exit Topspin** and **Logout Computer**

● Notes on Useful Commands

1. > edte → edit temperature
2. > lockdisp → to open lock sub-window
3. > lock → to lock the field for selected D-solvent field
4. > edc → copy current data set to a new one
5. > rpar → to read in available parameter set and overwrite current data
6. > getprosol→ read in default pulses and parameters (standard samples)
7. > atma → auto tune and match nuclei frequency
8. > atmm → manually tune and match nuclei frequency
9. > pulsecal → auto determine 90 degree pulse for current sample
10. > ased → display acquisition parameters needed for specific experiment
11. > expt→ estimate experiment time
12. > rga → auto estimate receiver gain
13. > gs → start acquisition but no data saving (useful for optimization)
14. > zg → zero memory and start data collection (overwrite existing data)
15. > tr → transfer collected FID after current scan (save existing FID)
16. > tr #→ transfer collected FID after # scan
17. > go → start data collection , add on NS to existing data
18. > stop → stop data acquisition immediately
19. > halt → similar to stop but after the current status
20. > qu ** → submit commands (**) to spooler
21. > qumulti → submit multiple commands to multiple experiments
22. > sx # → to switch sample position (#) on autosampler
23. > efp → em (window function), ft (Fourier transform), pk (pick phase)
24. > apk → to auto pick phase
25. > abs n → to auto baseline correction without integration
26. > rser # → read series file # (ie. available FIDs)
27. > xfb → to do Fourier transform on both dimensions
28. > dpa → to display acquisition parameters
29. > ii → to initialize hardware connection
30. > ii restart → to initialize hardware connection

Fig 1. Topspin Icon

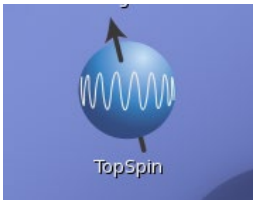
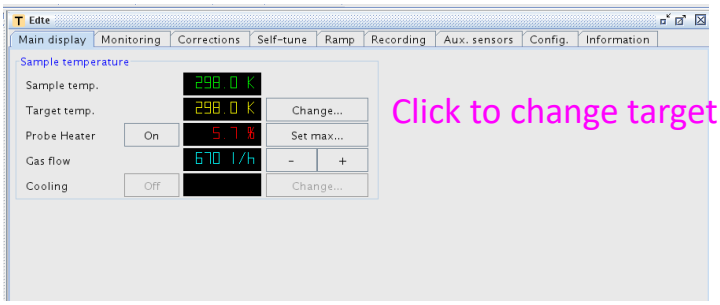


Fig 2. Temperature control window



Click to change target TEMP if needed

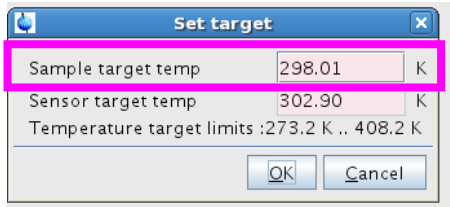


Fig 3. Sample and Spinner

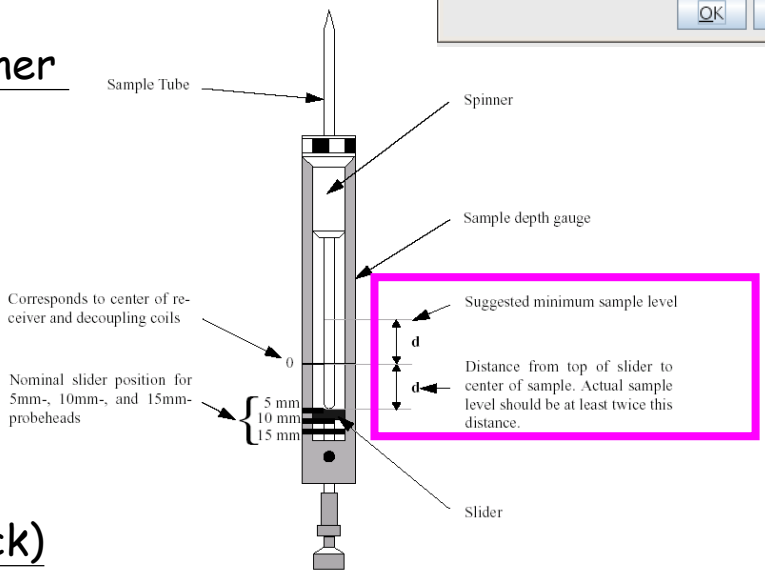


Fig 4. BSMS (Lift & Lock)

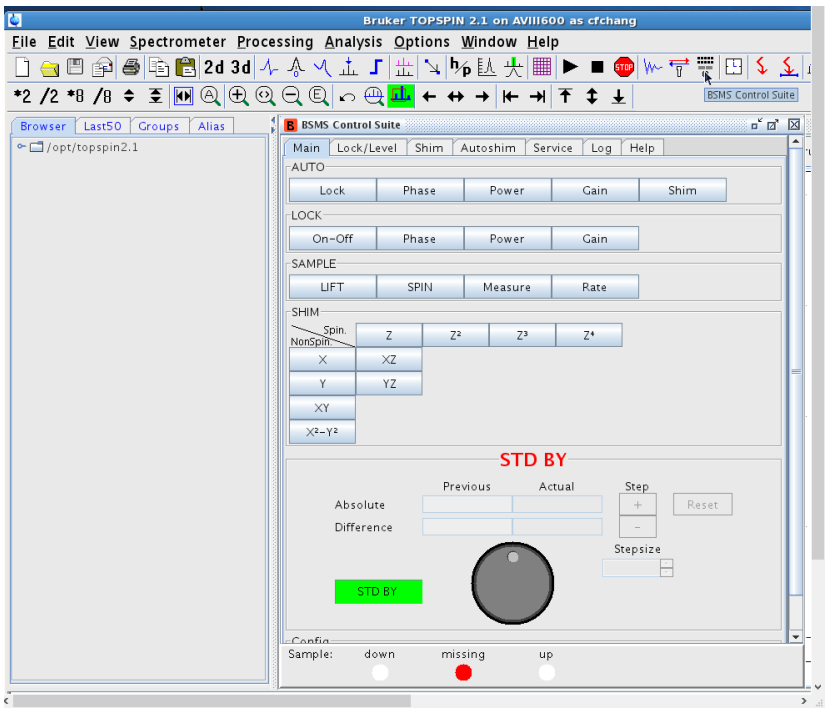
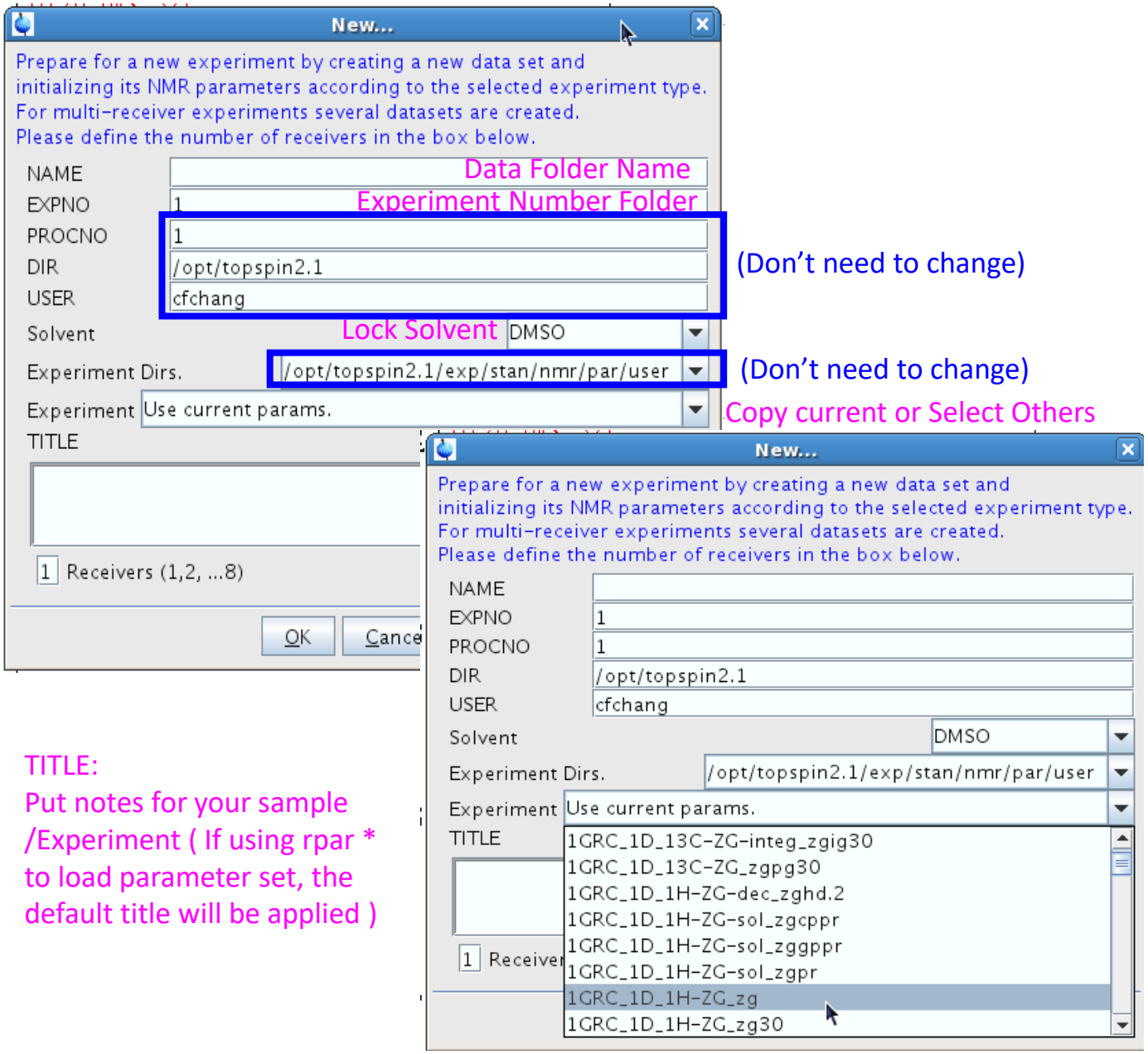
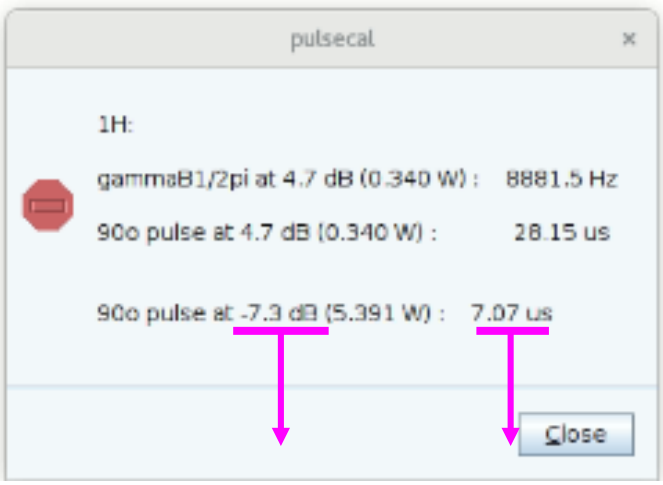


Fig 5. edc window



TITLE:
Put notes for your sample
/Experiment (If using rpar * to load parameter set, the default title will be applied)

Fig 6. "pulsecal" output window



power level In "dB"

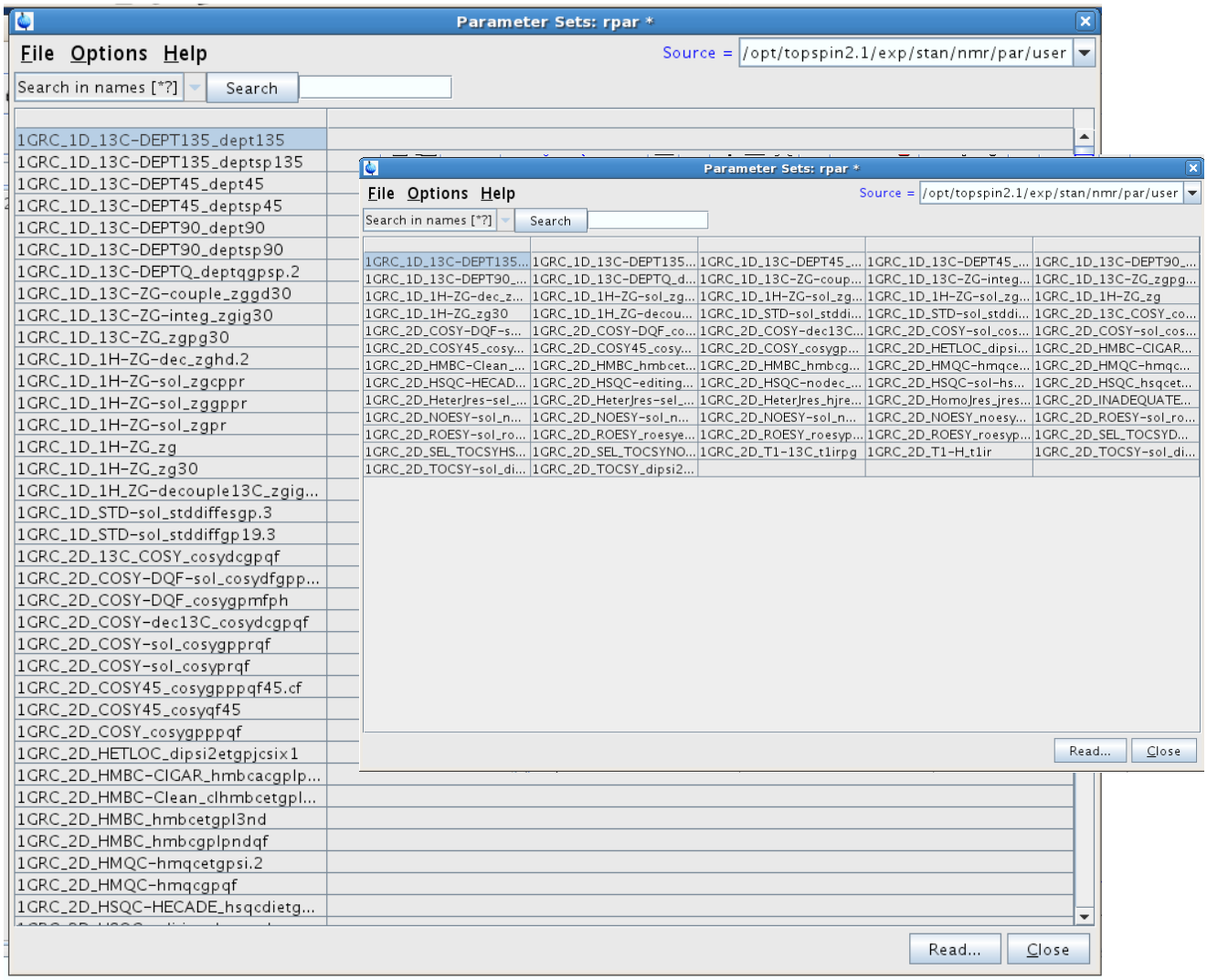
Pulse length In "us"

Pulse length In "us"

getprosol 1H 7.07 -7.3

power level In "dB"

Useful Parameter Sets for small molecules in HFNMRC



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