2016 HFNMRC Workshop on

NMR Screening Approaches

Hands-On : NMR Experiments

(1) Ligands Screening
(2) Metabolites Screening

Chi-Fon Chang
Senior Research Specialist, Genomics Research Center
Manager, High Field NMR Center
Academia Sinica

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(1) Ligands screening

Which Library/compounds to start with?

-Fragment library
-Spectrum library

Which NMR Experiments ?

-Ligand observation

-Protein observation

How to set up screening condition?

-Experimental conditions

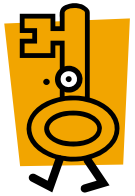
After screening.....

-Hits identifying & validating

-Follow up “chem-is-try”

-Structure……

Selection of Fragments? What Compounds to Start with?



"Rule of three (Ro3)" or "Rule of V"

(Method in Enzymology, 493,219, 2011)

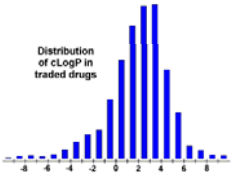
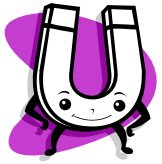


Table 9.1 Criteria used to define fragment libraries

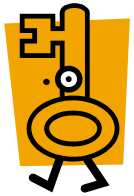
Property	Rule of three	Rule of V	Vertex avg[a]
MW (Da)	≤ 300	150–300	249.7 ± 49.4
clogP	≤ 3	≤ 3	1.5 ± 1.1
H-bond donors	≤ 3	≤ 3	1.0 ± 0.8
H-bond acceptors	≤ 3	≤ 6	3.1 ± 1.3
Rotatable bonds	≤ 3	≤ 6	3.1 ± 1.8
PSA ($Å^2$)	≤ 60	≤ 80	60.2 ± 21.5

[a] Average values $\pm$ standard deviation for 1100 compounds in the Vertex diverse fragment libraries.



HFNMRC library: MAYBRIDGE Ro3 1000

NMR Experiments to Observe Changes of NMR single



NMR methods for compound screening and hit validation (Nat.Rev Drug Discov, 7,738,2008)

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]

Flow Diagram for NMR Screening

From Ligands' point of view.....

HTS Compound Library → **Which compounds?**
Ex: MAYBRIDGE Ro3 1000

-Compound handling & storage !!

HTS Compound 1D NMR → **1D NMR Spectra for each Compound**

-Double confirm Compound quality & property
-1D 1H NMR spectra database

Compound "Grouping" ⇄ **1D NMR Spectra for each "Group"**

-5-10 compounds
-1D 1H NMR no overlap
-No interaction between compounds
-"Group spectra" database

HFNMRC: total 906 compounds in 195 groups!!

(Ready to look for binding!!)

Flow Diagram for NMR Screening

From Ligands' point of view…..

Target Proteins+ each Group (NMR screening Expts) → **Non-labeled protein**

- 1D 1H → Estimated protein needed

-1D STD → the ligand is in large excess (~30:1) to protein (ex: ~nM),
good for weak binding (mM), but missing tight binders (<1uM)

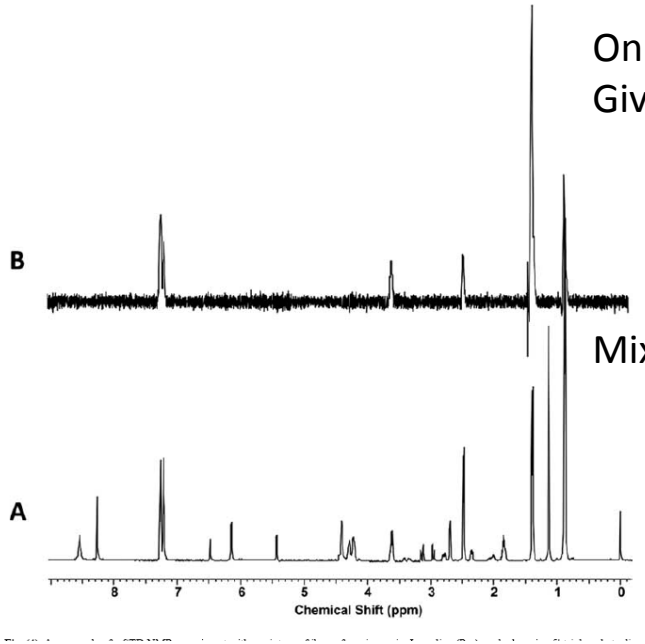
-1D Line-Broadening → Ex: The addition of 25uM of protein to 100uM
ligand with KD<nM will give 25% decrease in line width

-1D waterLOGSY → Solvent in H2O

Find the "Group "Hits

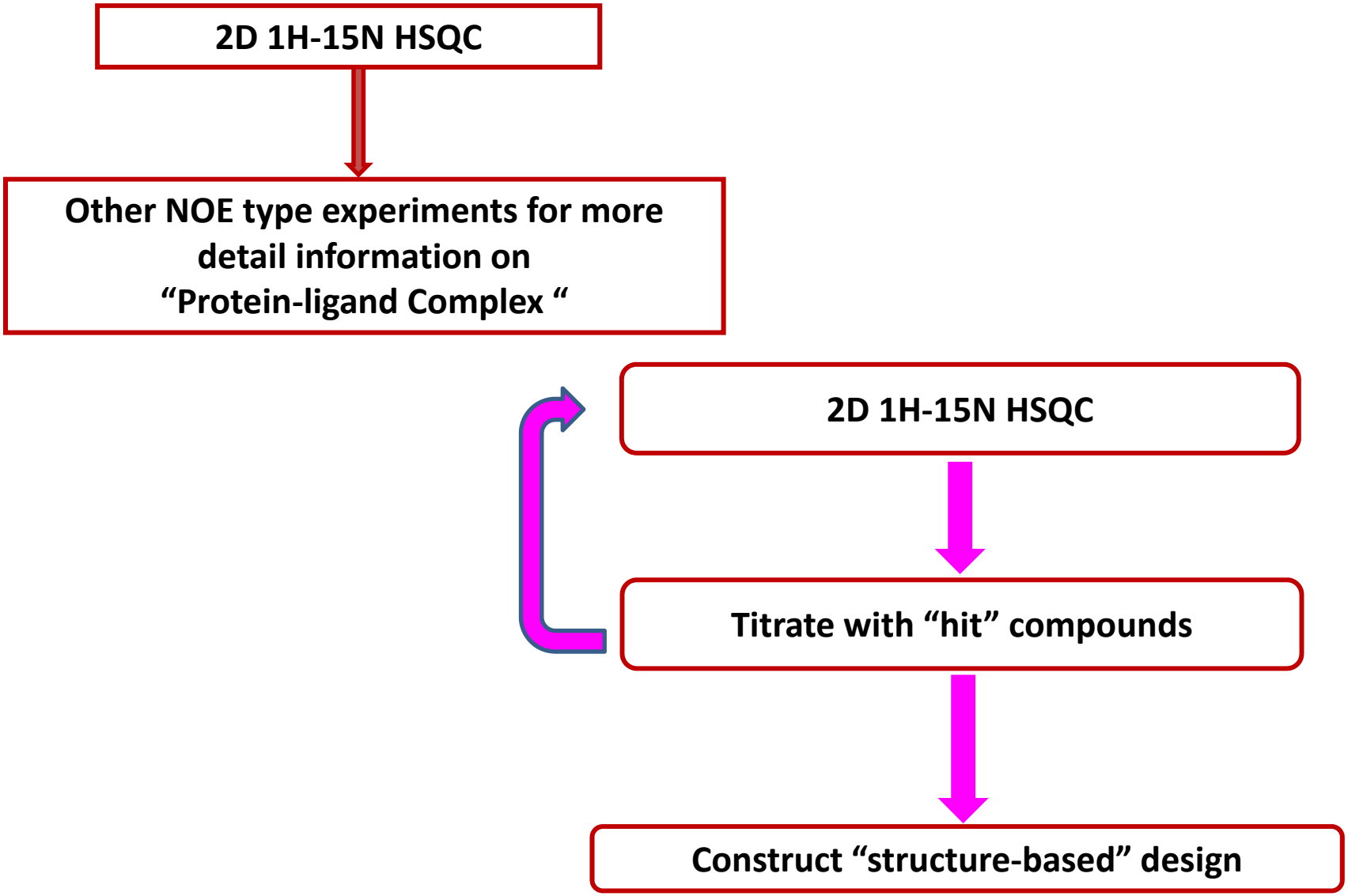
Narrow down to individual Hits

(Ready to look for stronger binder!!)

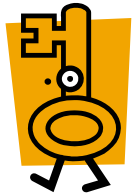


Flow Diagram for “hit” compounds

From Proteins’ point of view…..

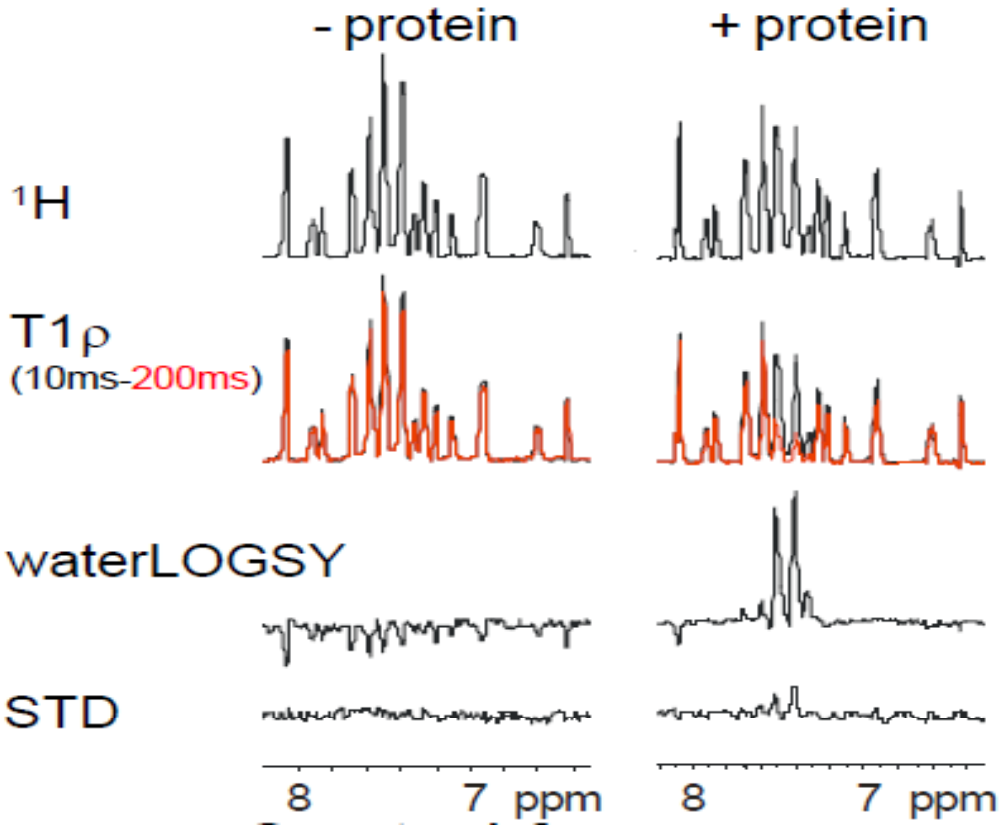


* Design and Characterization of a Functional Library for NMR screening Against Novel Protein Targets, Robert Powers et al>< Combinatorial Chemistry & High Throughput Screening, 2006,9,515-534



HFNMRC: Observe changes of ligands spectrum

- less protein
- no 15N needed

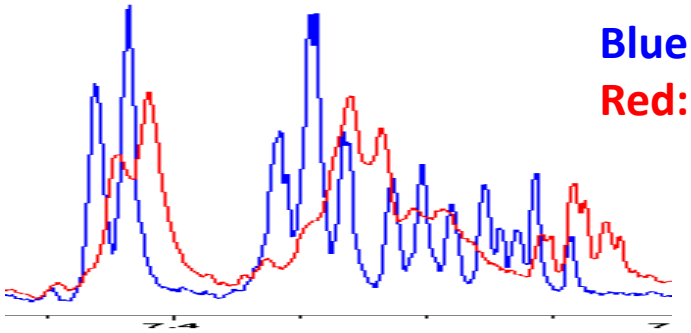


Example : 0.2mM compounds + ~0.005mM Protein , i.e. ~40:1

Total protein needed: 0.005mM*500ul*200 groups

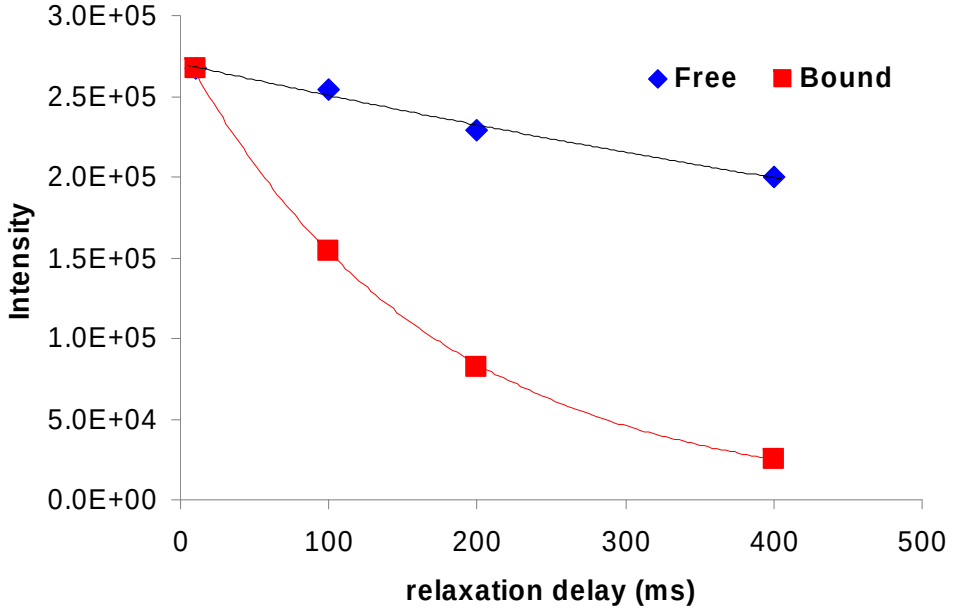
Example of NMR approach : line broadening & T1ρ experiments

<Example of 1D 1H Line Broadening simple 1D experiments with solvent suppression >



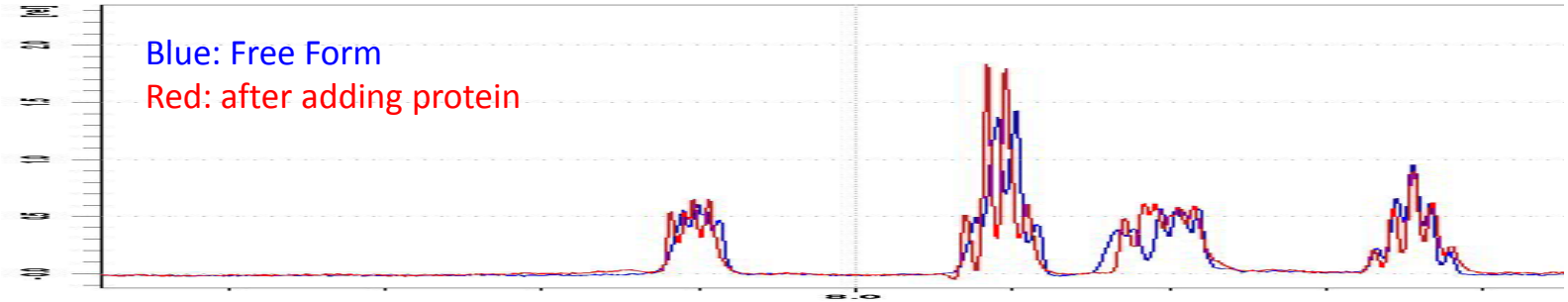
Blue: Free Form
Red: Bound From

<Example of 1D Relaxation Effect T1ρ experiments >

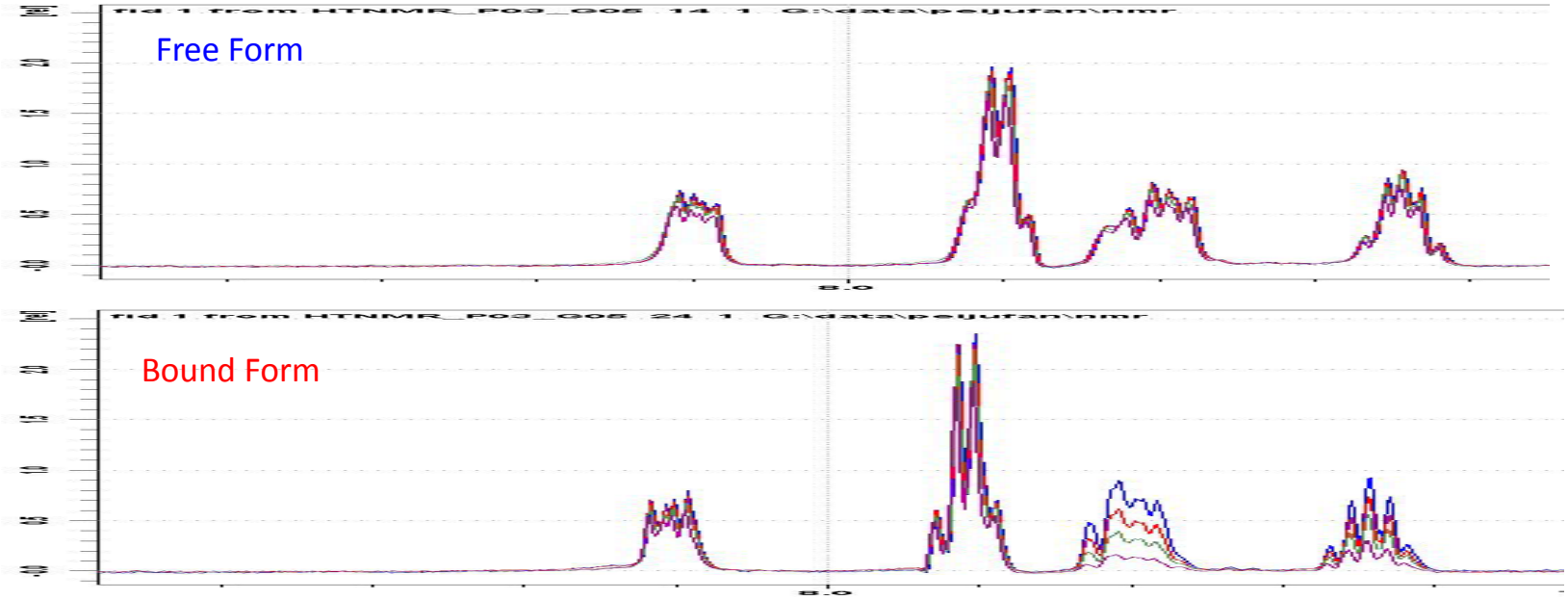


1D 1H Line Broadening /Chemical Shift perturbation

Data for NDM1

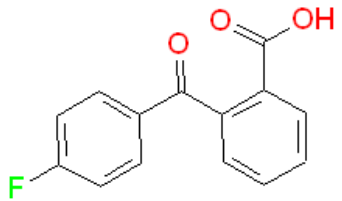


1D T1ρ experiment

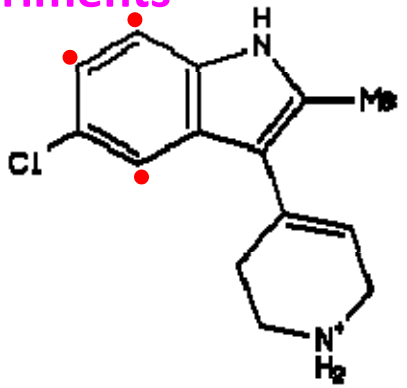


Picked Compound & Structure

Compound # P03- D3(DP00596)



Example of NMR approach : STD & waterLOGSY experiments

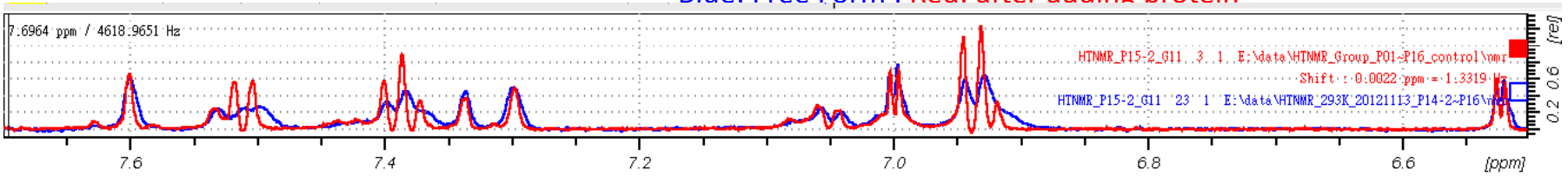


<Example of 1D STD >

Data for PDK

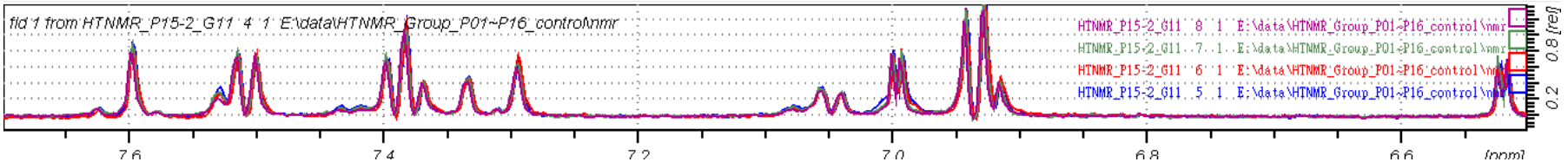
1D 1H Line Broadening /Chemical Shift perturbation

Blue: Free Form : Red: after adding protein

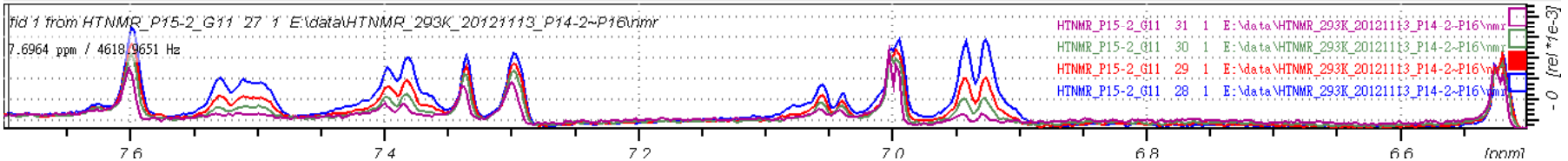


1D T1ρ experiment

Free Form → no significant decay

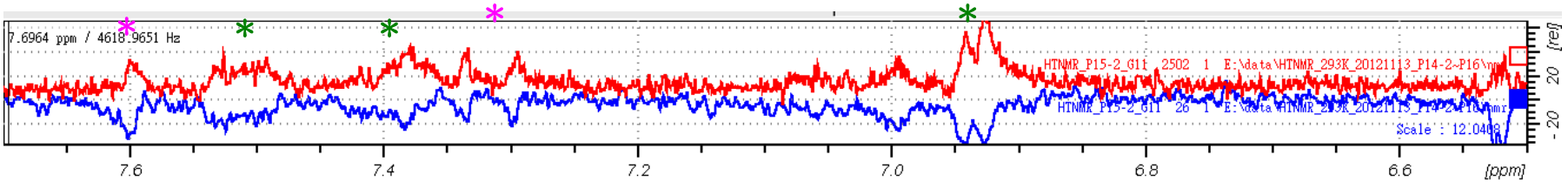


Bound Form → significant decay (behaved like large molecule)



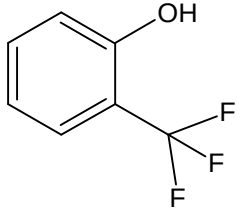
STD and waterLGOSY

Bound Form → Red:STD Blue: wLOGSY

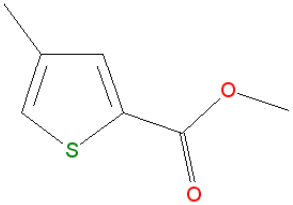


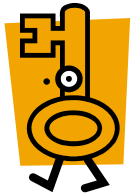
Picked Compound & Structure

Compound # P15-F11 (SB01911)



Compound # P15-F5 (GK01701)





HFNMRC: Observe changes of Protein spectrum
- 15N protein
- routine protein NMR experiments (ex: HSQC)

◆Questions to answer

How the protein interact with ligand?

◆NMR approaches

Step1: "Backbone assignment" for the protein

Step2: Follow "chemical shift" perturbation upon titration

Step3: Map the chemical shift perturbation result into protein structure (if available)

In HFNMRC (Compounds Screening)

Parameter Sets for compound-protein screening experiments :

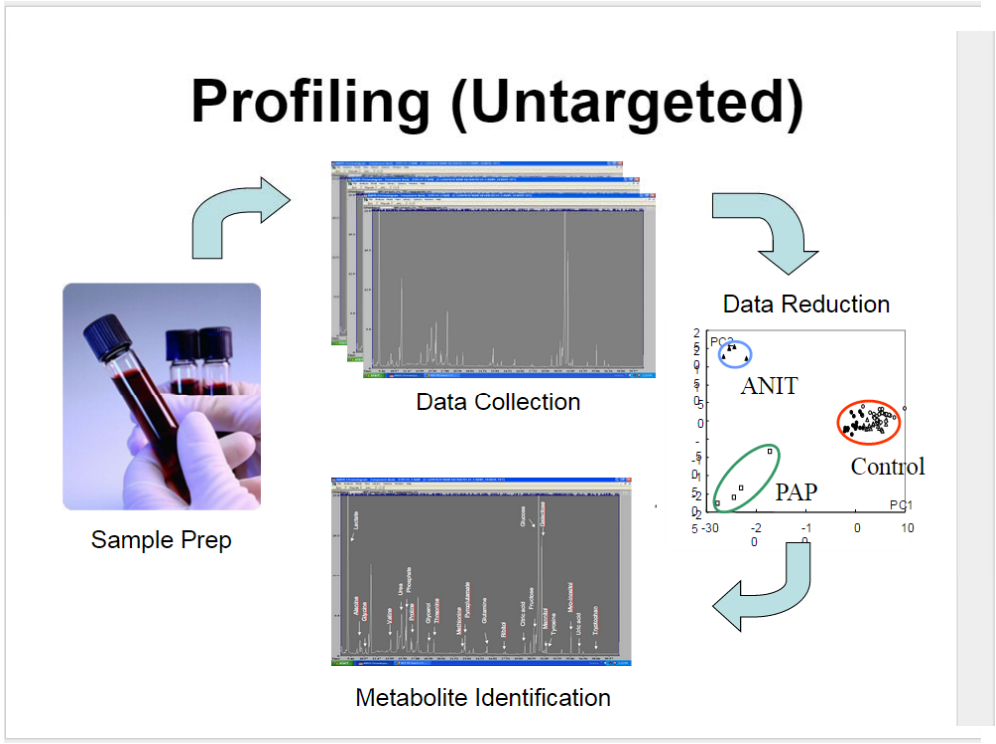
Standard Parameter Set	Pulse Program	Observed signals	Note
htnmr_ZGW5	zggpw5	Lignads	o1 @H2O
htnmr_STD	stddiffesgp3.cf	Lignads	fq2list
htnmr_T1roh	t1roh2des_2013.cf	Lignads	vdlist
htnmr_wLOGSY	epnogsygpno.cf	Lignads	

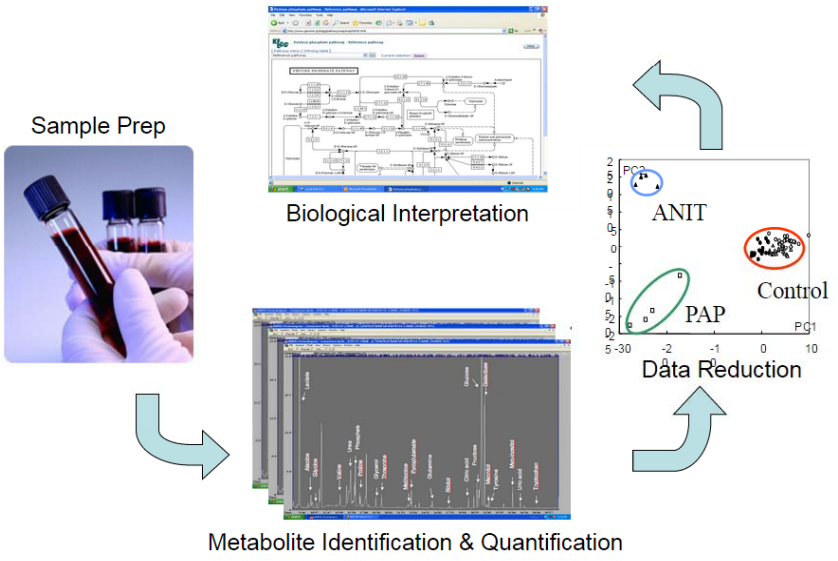
Parameter Sets for Protein NMR experiments :

Standard Parameter Set	Pulse Program	Observed signals	Note
std1_2D_15N-HSQC	fhsqcf3gpph	Protein	15N-labelled
std1_2D_15N-HSQC	hsqcetf3gpsi		
std1_2D_15N-HSQC	hsqcetfpf3gpsi		
std1_2D_15N-HSQC	hsqcfpf3gpphwg		

(2) Metabolites screening

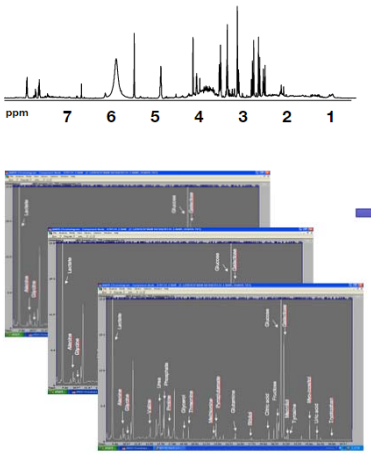
Approaches for Metabonomics Research

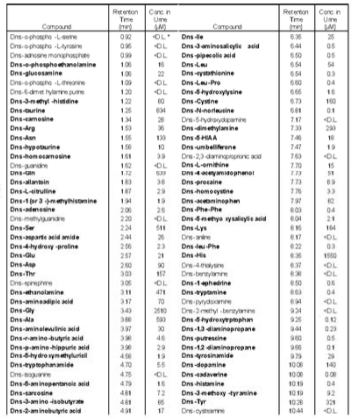




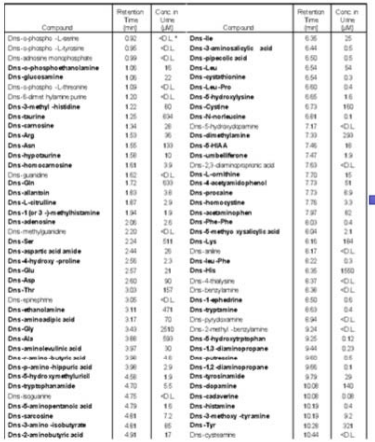
From David Wishart

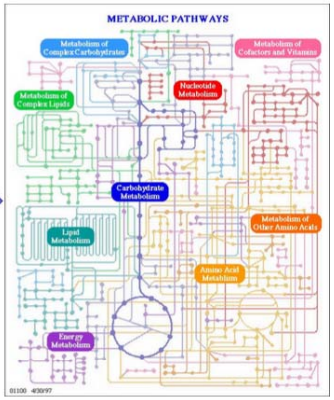
From Spectra to Lists



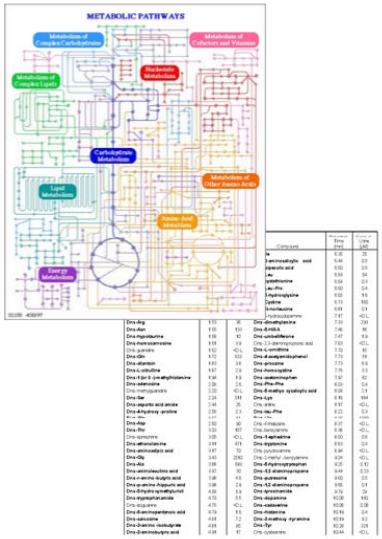


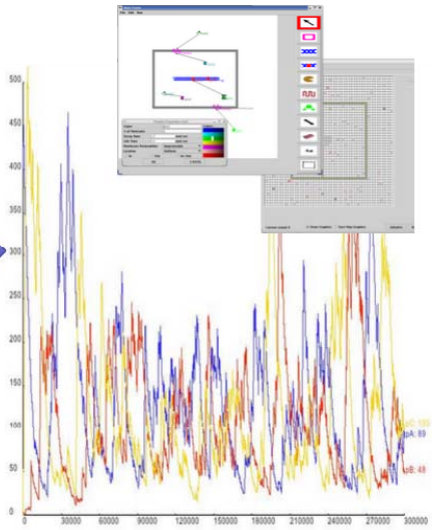
From Lists to Pathways





From Pathways & Lists to Models & Biomarkers

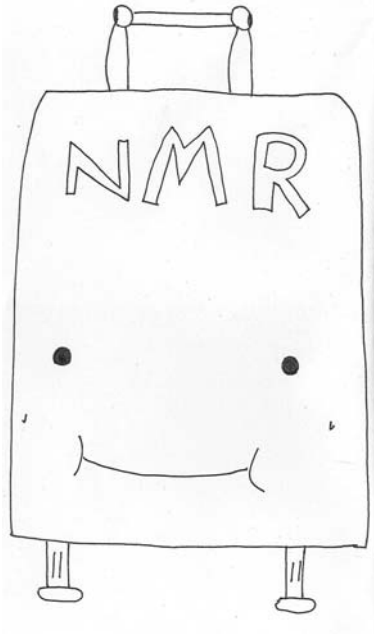




From David Wishart

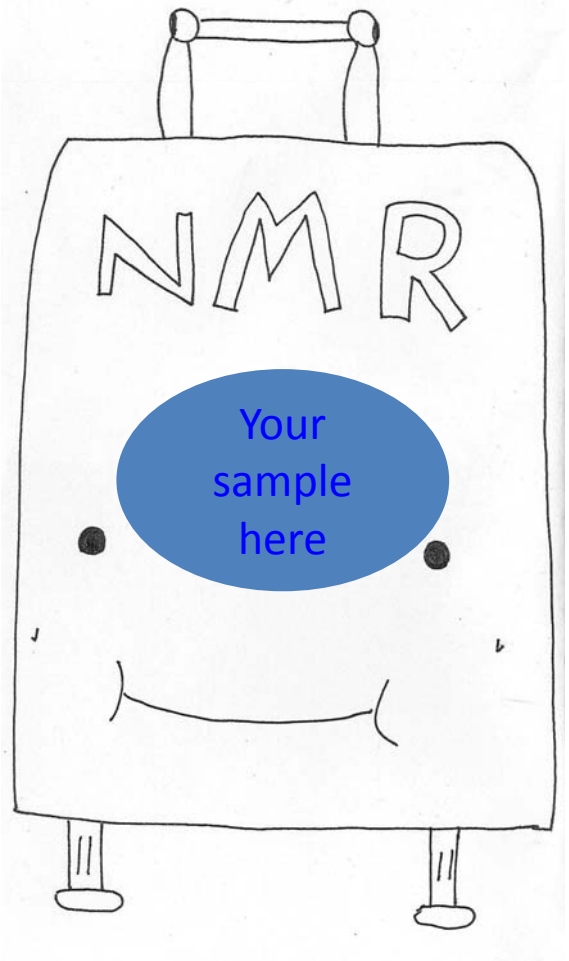
Analytical challenges for metabonomics

- **Identification**
  Assignment of the unknown compounds
- **Higher Resolution**
  Sample contains mixtures of compounds
- **Higher Sensitivity**
  Low concentration sample
- **Reproducibility**
  Reliable data for interpretation
- **High throughput**
  Large sample amount



Using NMR in Metabonomics Research

Technical support from High Field NMR Center



NMR measurement
Tell you whatever it can see

In HFNMRC (Metabonomics Profiling)

Parameter Sets for Metabonomics related experiments :

(with DIGMOD BASEOPT)

Standard Parameter Set	Pulse Program	Sample Type	Note
std3_Meta_URINE_O1	zgpr	URINE	URINE_5mm
std3_Meta_PROF_URINE_1H	noesygppr1d		
std3_Meta_PROF_URINE_JRES	jresgpprqf		

Standard Parameter Set	Pulse Program	Sample Type	Note
std3_Meta_PLASMA_O1	zgpr	PLASMA	PLASMA_5mm
std3_Meta_PROF_PLASMA_1H	noesygppr1d		
std3_Meta_PROF_PLASMA_CPMG	cpmgpr1d		
std3_Meta_PROF_PLASMA_DIFF	ledbpgppr2s1d		
std3_Meta_PROF_PLASMA_JRES	jresgpprqf		

(earlier set up std3_NTU_* based on Nature Protocals 2007, V2, No. 11, 2692)

In HFNMRC (Compounds /Metabolites identification)

2D 1H-1H Experiments

GRC Parameter Set	Experiment Details
std0_2D_COSY_cosygpppqf	1H-1H COSY
std0_2D_COSY-sol_cosygpprqf	1H-1H COSY with solvent supression
std0_2D_COSY-sol_cosyprqf	1H-1H COSY with solvent supression
std0_2D_COSY45_cosyqf45	1H-1H COSY
std0_2D_COSY-dec13C_cosydcgpqf	1H-1H COSY with 13C decoupled
std0_2D_COSY-DQF-sol_cosydfgpph19	1H-1H COSY with solvent supression
std0_2D_COSY-DQF_cosygpmfph	1H-1H COSY
std0_2D_TOCSY_dipsi2etgpsi	1H-1H TOCSY
std0_2D_TOCSY-sol_dipsi2etgpsi19	1H-1H TOCSY with solvent supression
std0_2D_TOCSY-sol_dipsi2phpr	1H-1H TOCSY with solvent supression

2D 1H-13C Experiments

GRC Parameter Set	Experiment Details
std0_2D_HSQC_hsqcetgpsisp2.2	1H-13C HSQC
std0_2D_HSQC-editing_hsqcedetgpsisp2.2	1H-13C edited HSQC
std0_2D_HSQC-nodec_hsqcetgpsisp.cf	Determine XH coupling
std0_2D_HSQC-sol-hsqcetgpprsisp2.2	1H-13C HSQC with solvent suppression
std0_2D_HMQC-hmqcetgpsi.2	1H-13C HMQC
std0_2D_HMQC-hmqcgpqf	1H-13C HMQC
std0_2D_HMBC_hmbcgplpndqf	1H-13C HMBC
std0_2D_HMBC_hmbcetgpl3nd	1H-13C HMBC J-filter to suppressed one-bond
std0_2D_HMBC-CIGAR_hmbcacgplpndqf.2	1H-13C HMBC optimized for long range signals

Research article

MRC

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Resolution-enhanced 2D NMR of complex mixtures by non-uniform sampling

Adrien Le Guennec,[a,b] Jean-Nicolas Dumez,[a] Patrick Giraudeau[b,c] and Stefano Caldarelli[a,d]*

NMR is a powerful tool for the analysis of complex mixtures and the identification of individual components. Two-dimensional (2D) NMR potentially offers a wealth of information, but resolution is often sacrificed in order to contain experimental times. We explore the use of non-uniform sampling (NUS) to increase substantially the resolution of 2D NMR spectra of complex mixtures of small molecules, with no increase in experimental time. Two common pulse sequences for metabolomics applications are analysed, HSQC and TOCSY. Specific attention is paid to sensitivity in resolution-enhanced NUS spectra, using the signal-to-maximum-noise ratio as a metric. With a careful choice of sampling schedule and reconstruction algorithm, resolution in the ^{13}C dimension for HSQC is increased by a factor of at least 32, with no loss in sensitivity and no spurious peaks. For TOCSY, multiplets can be resolved in the indirect dimension in a reasonable experimental time. These properties should increase the usefulness of 2D NMR for metabolomics applications by, for example, increasing the chances of metabolite identification.

In HFNMRC (general)

Easy steps for beginner :

1. Type "rpar" <enter> to load an appropriate parameter set
2. Type "getprosol" <enter> to load standard pulse values
3. Type "pulsecal" <enter> to do pulse calibration
4. Type "ns" <enter> to input number of scan
5. Type "rga" <enter> to find appropriate receiver gain
6. Type "zg" <enter> to collect spectrum
7. Type "ft" or "fp" <enter> to do Fourier Transfer
8. Click on phase to phase spectrum
9. Click on return, then save to save the spectrum
10. You data is done!!

In HFNMRC (practical)

Step 0: Set Temperature

Step 1: Load sample

Step 2: Match and Tune (wobb / atma)

Step 3: Lock and Shimming (lock solvent / topshim)

Step 4: Set up Experiment/Parameters

1. Type "rpar" <enter> to load an appropriate parameter set
2. Type "getprosol" <enter> to load standard pulse values
3. Type "pulsecal" <enter> to do pulse calibration
4. Type "gs" to confirmed "o1" for solvent suppression
5. Set "user's parameters" for "specific experiments"
6. Type "rga" <enter> to find appropriate receiver gain
7. Type "zg" <enter> to collect spectrum
8. Data processing for "specific experiments"

Let's try to set up the following experiments

Standard Parameter Set	Pulse Program	Observed signals	Note
htnmr_ZGW5	zggpw5	Ligands	o1 @H2O
htnmr_STD	stddiffesgp3.cf	Ligands	fq2list
htnmr_T1roh	t1roh2des_2013.cf	Ligands	vdlist

<Run 1>

Standard Parameter Set	Pulse Program	Observed signals	Note
htnmr_ZGW5	zggpw5	Ligands	o1 @H2O

- Confirmed H2O frequency for best solvent suppression result

<Run 2>

Standard Parameter Set	Pulse Program	Observed signals	Note
htnmr_STD	stddiffesgp3.cf	Ligands	fq2list

➢ "user's parameters" for "STD"

; ;d20: saturation time;d29: spinlock time [10 - 50 msec]
;d31: saturation time as executed
;l4: l4 = number of averages = (total number of scans) / NS
;l5: loop for saturation: p13 * l5 = saturation time
;NS: 8 * n
;DS: 4
;td1: number of experiments
;NBL: NBL = number of irradiation frequencies
;define FQ2LIST (irradiation frequencies); (list has to be stored in "/u/exp/stan/nmr/lists/f1")

Example: std_test ; units in Hz
o 600.13
10000
470

➢ "Processing scripts" for "STD" : std.cf

<Run 3>

Standard Parameter Set	Pulse Program	Observed signals	Note
htnmr_T1roh	t1roh2des_2013.cf	Ligands	vdlist

➢ "user's parameters" for "T1roh"

;l4: l4 = number of experiments = number of delays in vd-list
;NS: 8 * n
;DS: 4
;td1: number of experiments
;define VDLIST

Example: t1roh.cf , unit is "sec"
0.01
0.1
0.2
0.4

➢ "Processing scripts" for "T1roh" : t1roh.cf

We are here to help you!!