



astex<sup>TM</sup>  
therapeutics

**Less is More – Sample Size, Cryoprobes and  
Screening**

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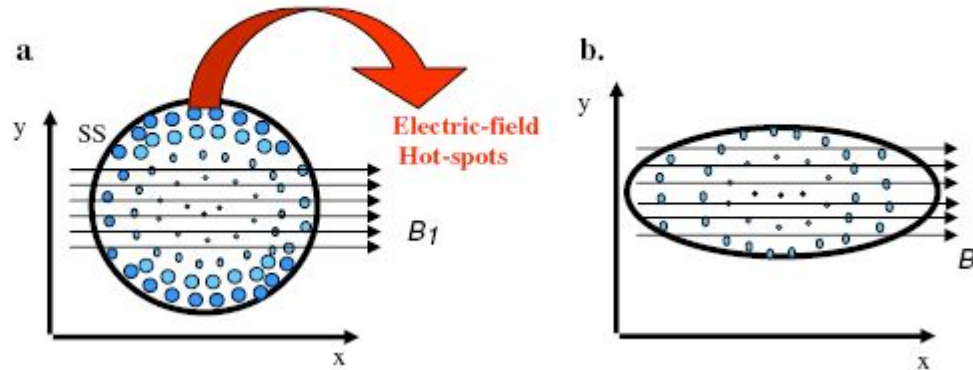
PSDI NMR Workshop  
29<sup>th</sup> October 2007

# Outline

- DRX500U + 5mm TXI Cryoprobe + BACS-60
- Sample formats & Automation
  - 5mm tube (40mm/550ul samples)
  - 2.5mm capillary (40mm/150ul samples)
  - 2mm capillary (40mm/40ul samples)
- Lock and Acquisition Parameters
- Lineshape, Baseline, Water Suppression
- Sensitivity ( $D_2O$ ,  $H_2O$ , salt)
- B1 Homogeneity
- Quality control

# Sample Geometries

Fig. 3 Schematic diagram showing where the electric-fields are generated in a cross-section of a cylindrical NMR tube (a). Changing the geometry of the NMR tube to a squashed or S-tube (b) helps avoid the “hot-spot” regions of the RF field and places more sample in the lower electric-field-generating regions of the NMR tube



$$R_s \propto \sigma \int E^2 dV$$

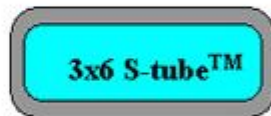
E-field in sample

conductivity of sample

- de Swiet, JMR (2005) **174**, 331 [Varian]
- Voehler et al., JMR (2006) **183**, 102 [Georgia State Uni./Bruker]
- Robosky et al., Anal. Bioanal. Chem. (2007) **387**, 529 [Pfizer/Varian]

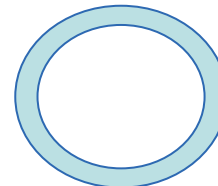


cross section: 14mm<sup>2</sup>  
volume : 600μL

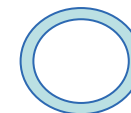


cross section: 10.7mm<sup>2</sup>  
volume : 460μL

$R_s$  (5mm tube) >  $R_s$  (3x6 S-tube)



5mm OD  
550ul



2.5mm OD  
150ul



2.0mm OD  
40ul

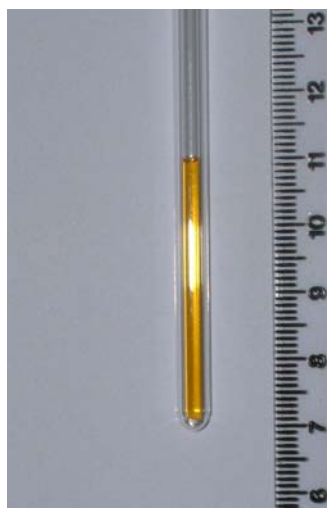
# Title

## Fragment screening with Ligand-detection

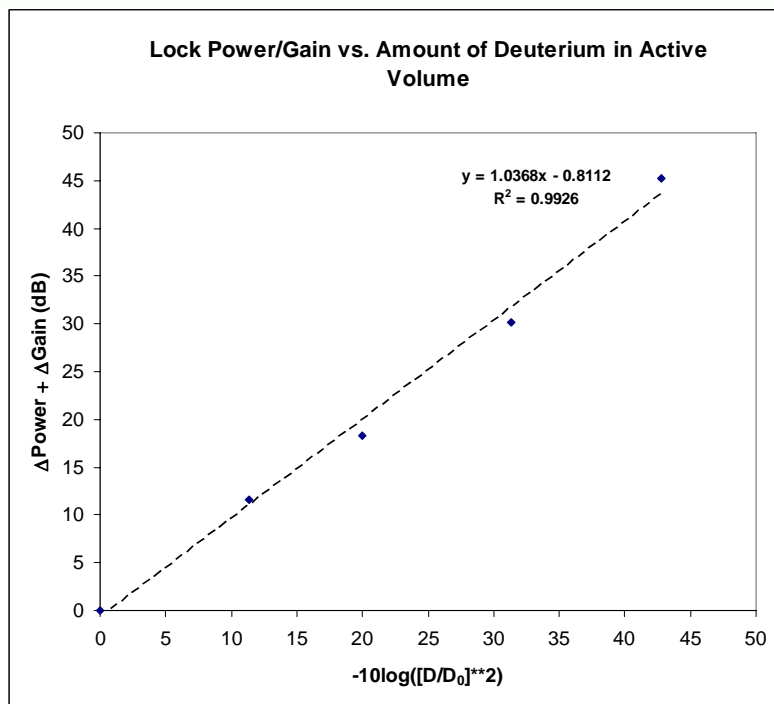
- Weak interactions  $\Rightarrow$  [Ligand] = 1mM
- Ligand:Protein ratio is fixed (100:1)  $\Rightarrow$  [Protein] = 10uM
- Usually good sensitivity [10mins/experiment in 5mm tubes with cryoprobe]
- Would prefer to save protein at expense of longer acquisitions [20-30mins]
  - but concentrations are fixed  $\Rightarrow$  reduce sample volume and increase NS

NMR tube	Description	Volume (40mm depth)	Relative Volume	Wall thickness (mm)
NE-UL5	Standard 5mm tube	550ul	1.0	0.41
NE-H5/3	5mm tube with 3mm extension	200ul	0.36	0.24
WD 520-1A	5mm Tube with 2.5mm extension	150ul	0.27	0.16
NE-262-2.5	2.5mm capillary	150ul	0.27	0.16
NE-262-2	2.0mm capillary	40ul	0.07	0.44

# Tubes, Capillaries and Automation



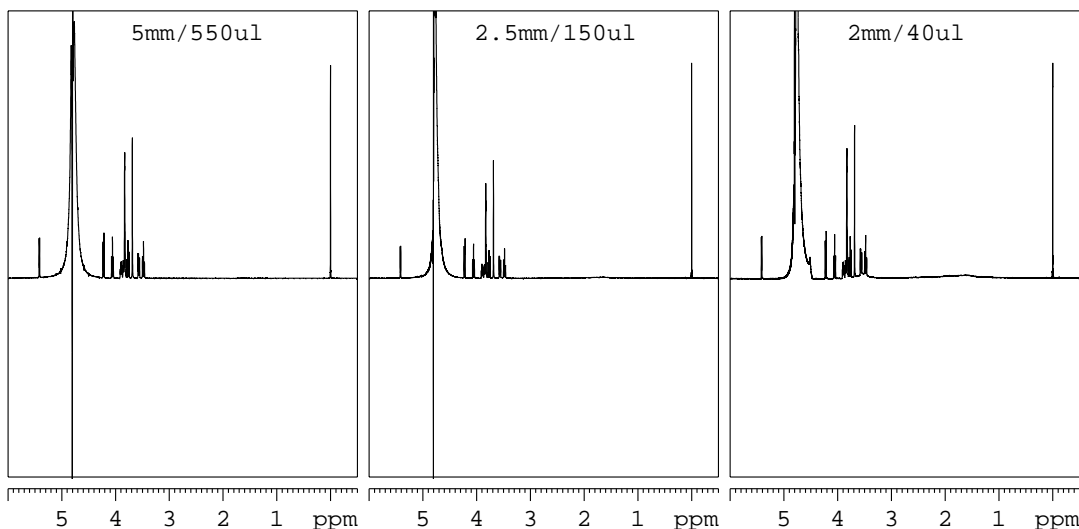
# Lock Parameters



- $(\text{Lock Power} + \text{Lock Gain})^{1/2} \propto [D]$

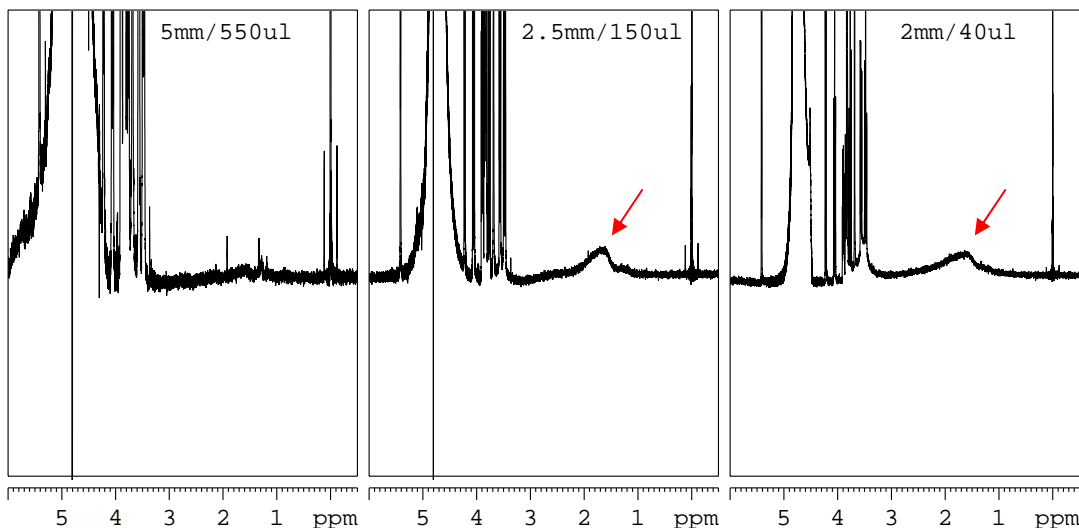
Solvent	Volume (ul)	total[D]	$-10\log[D]**2$	POWER (dB)	GAIN (dB)	$\Delta\text{Power} + \Delta\text{Gain (dB)}$
100% D2O	550	1	0.0	-20	104.4	0
100% D2O	150	0.27	11.4	-20	116.0	11.6
10% D2O	550	0.1	20.0	-15	117.7	18.3
10% D2O	150	0.027	31.4	-10	124.6	30.2
10% D2O	40	0.0073	42.7	-10	139.6	45.2

# Lineshape/Solvent Suppression



2mM sucrose, 0.5mM TSP, 10% D2O

Sample	$\Delta v_{1/2}(1)$	$\Delta v_{1/2}(2)$	Anomeric Splitting
5mm/550ul	109Hz	38Hz	75%
2.5mm/150ul	89Hz	27Hz	83%
2mm/40ul	100Hz	40Hz	79%



Sample	Relative Volume	Relative S/N (TSP)
5mm/550ul	1	1
2.5mm/150ul	0.27	0.45
2mm/40ul	0.073	0.13

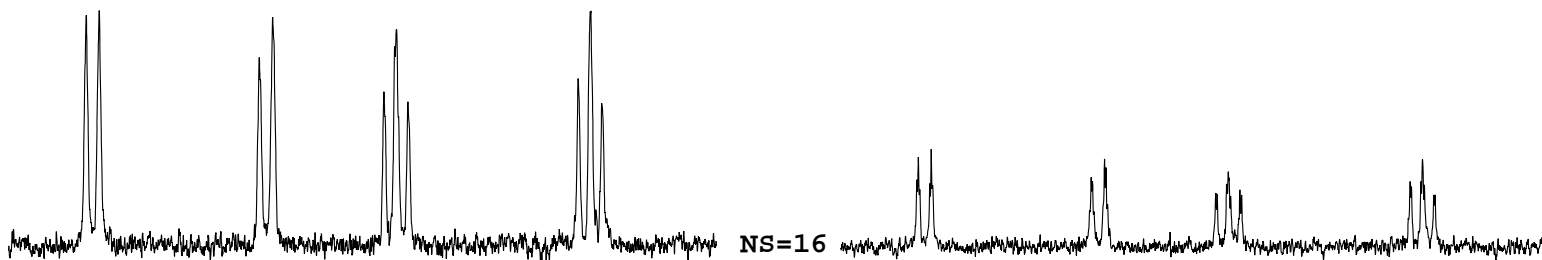
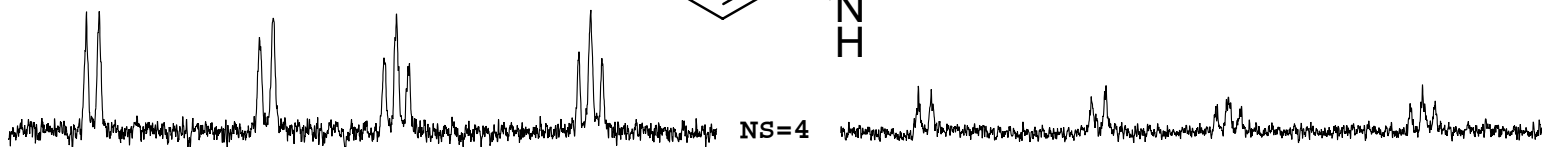
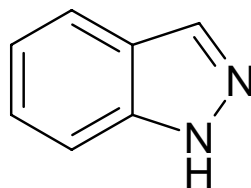
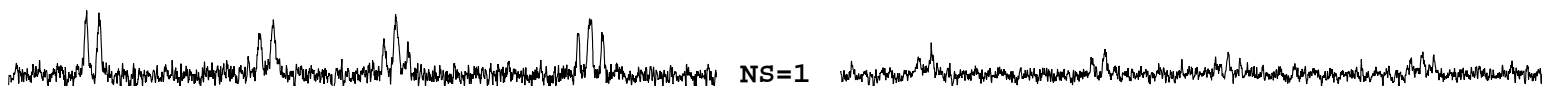
↙ susceptibility artefact

# Typical Spectra

100uM Indazole  
0.1M Phosphate Buffer pH6.8  
T=27°C, D<sub>2</sub>O

550ul in 5mm tube  
40mm sample height  
PW90=10.2us

150ul in 2.5mm capillary  
40mm sample height  
PW90=9.2us



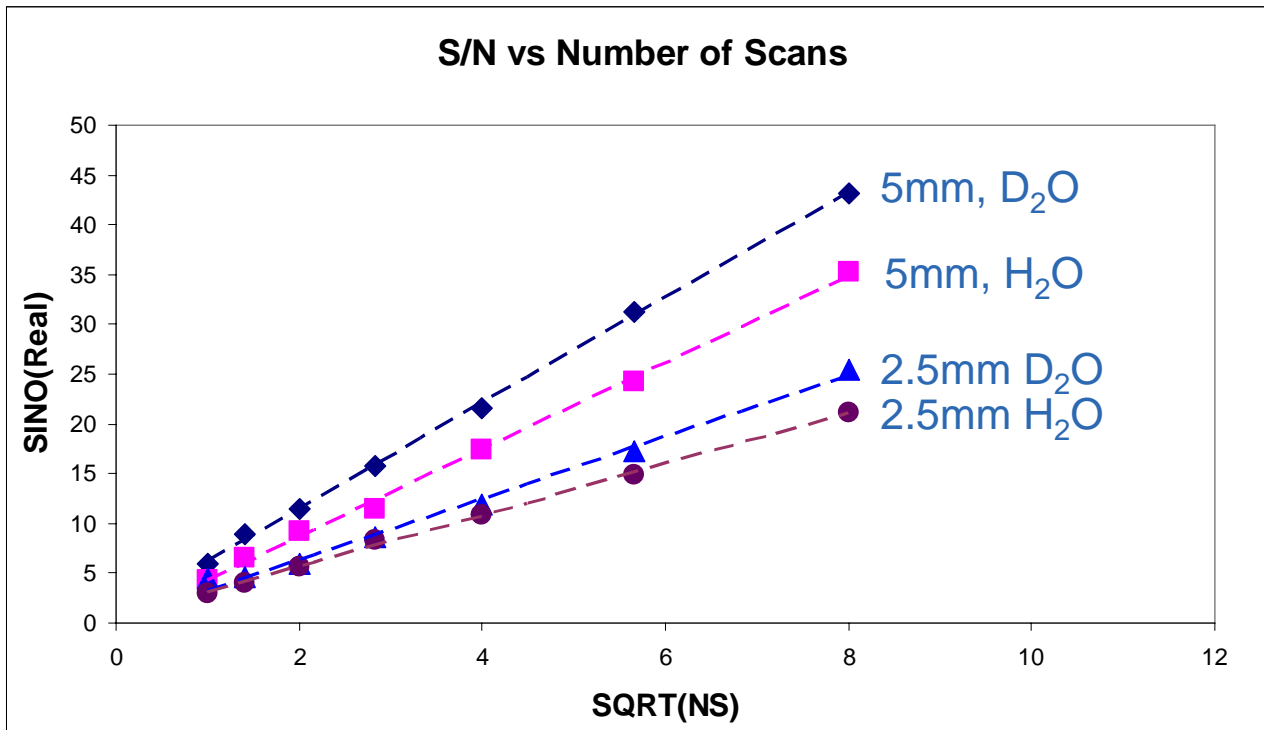


# Solvent Effects

Sample: 100uM indazole, 0.1M phosphate, D<sub>2</sub>O or H<sub>2</sub>O

D<sub>2</sub>O: 1-pulse with 1.6s presat

H<sub>2</sub>O: 1-pulse with WATERGATE



Sample	Relative S/N (TSP)
5mm/D <sub>2</sub> O	1
5mM/H <sub>2</sub> O	0.82

Sample	Relative S/N (TSP)
2.5mm/D <sub>2</sub> O	1
2.5mM/H <sub>2</sub> O	0.84

Sample	Relative S/N (TSP)
5mm/H <sub>2</sub> O	1
2.5mM/H <sub>2</sub> O	0.59

# Salt Tolerance

2mM sucrose, 0.5mM TSP, 10% D<sub>2</sub>O  
0, 0.1, 0.5M NaCl

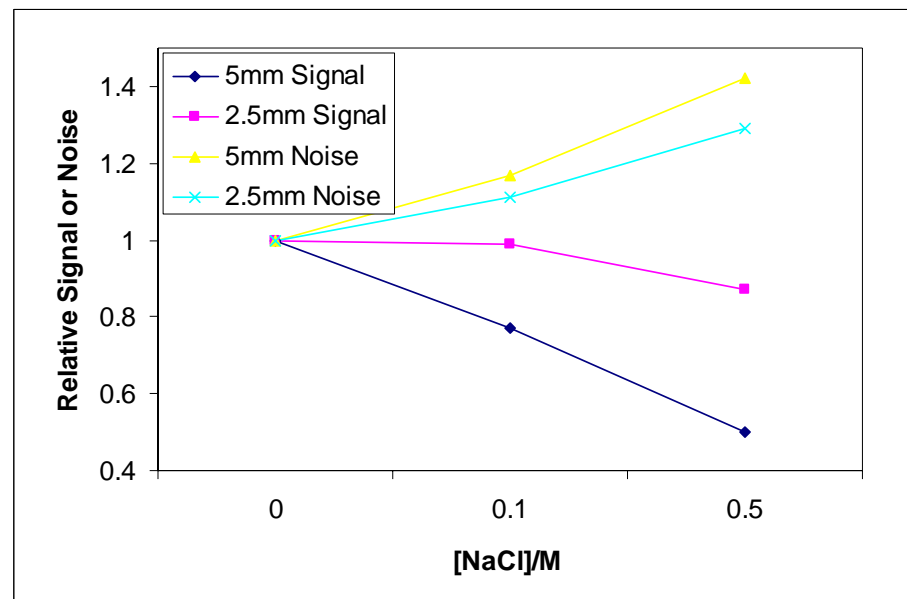
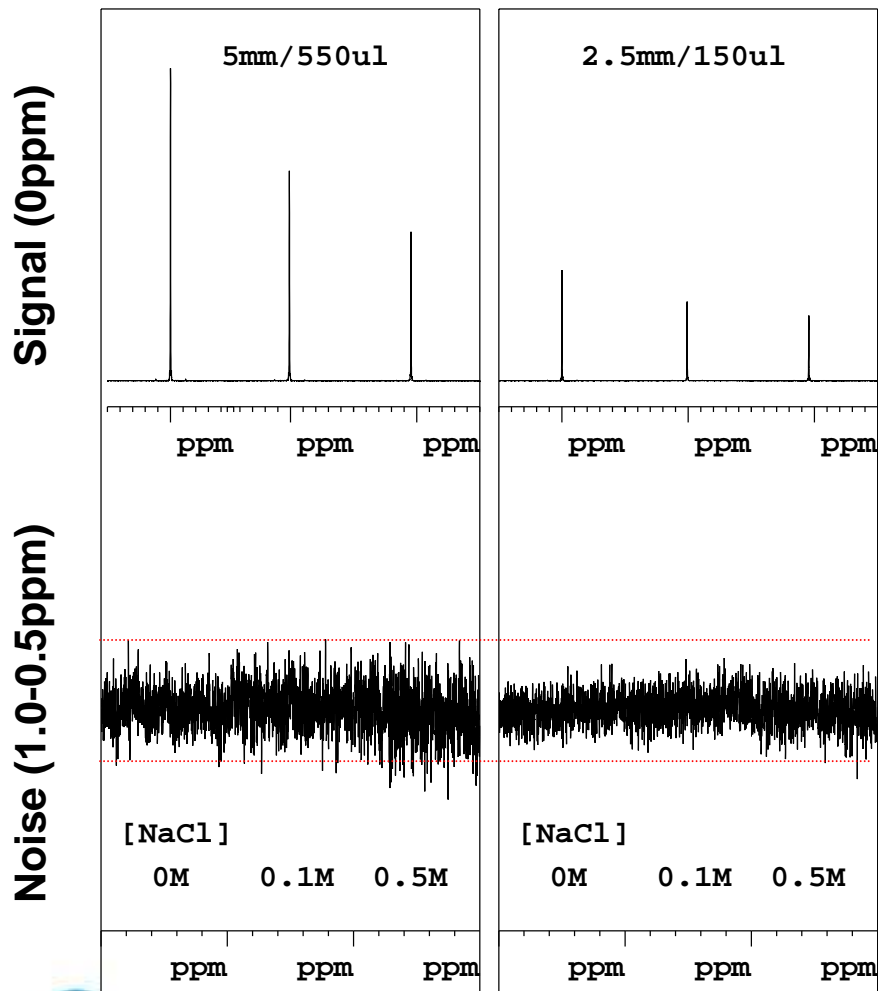
	Tube OD		
NaCl conc. (M)	5mm	2.5mm	2mm
0	9.1	8.6	8.6
0.1	11.2	8.6	8.7
0.5	16.8	9.6	8.7
	PW90 (us)		

	Tube OD		
NaCl conc. (M)	5mm	2.5mm	2mm
0	1.0	0.47	0.16
0.1	0.57	0.24	0.15
0.5	0.34	0.21	0.17
	Relative S/N		

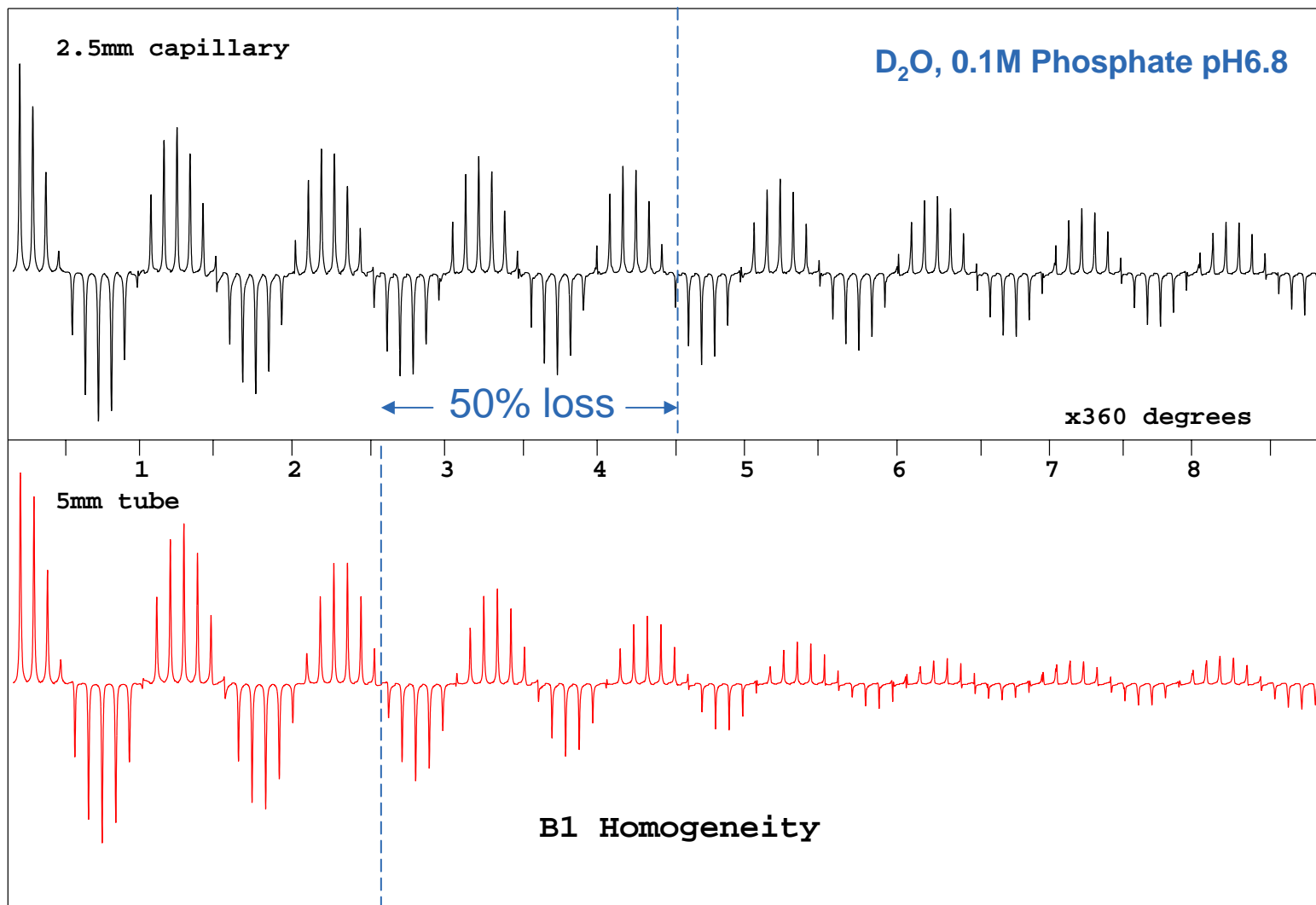
	Tube OD		
NaCl conc. (M)	5mm	2.5mm	2mm
0	1.0	1.7	2.2
0.1	0.57	0.88	2.1
0.5	0.34	0.77	2.3
	Relative S/N per unit volume		

# Loss of Signal or Increase in Noise?

2mM sucrose, 0.5mM TSP, 10% D2O  
0-0.5M NaCl



# B1 Homogeneity



# Quality Control

New Era (2.5mm OD/ 150ul)			
Capillary Number	Linewidth	Intensity	Sample Height
1	3.12	110825	38
2	3.12	108121	38
3	3.30	104623	38
4	3.30	106907	38
5	3.48	103487	39
6	3.30	106123	38
7	3.30	107161	37
<b>AVERAGE</b>	3.27	106750	38
<b>STD DEV</b>	0.12	2389	1
<b>% VARIATION</b>	4	2	2

Brand X (2.5mm OD/150ul)			
Capillary Number	Linewidth	Intensity	Sample Height
1	3.85	89555	40
2	5.50	72553	40
3	5.32	73884	38
4	3.85	87930	41
5	4.03	81729	41
6	3.67	95975	39
7	3.48	92631	40
<b>AVERAGE</b>	4.24	84894	40
<b>STD DEV</b>	0.82	9102	1
<b>% VARIATION</b>	19	11	2

Other considerations:

Capillary closures (2-5% failure rate)

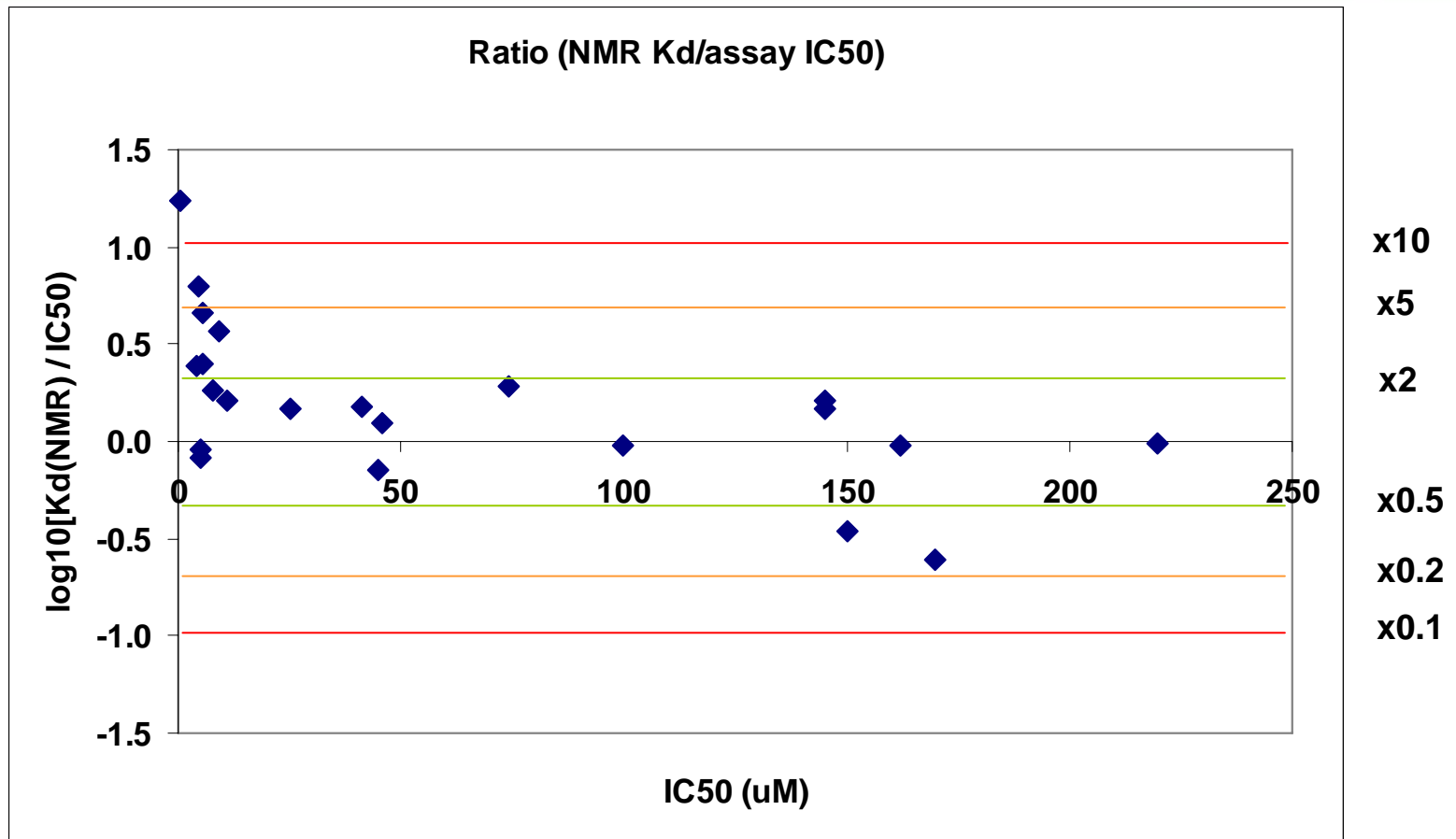
Capillary length (+/- 2mm for automated filling)

Breakages

# Summary: Low-volume NMR

- Reduced protein consumption (typ. 3-4x)
- Disposable capillaries
- Cheaper automation (BACS + Hydra)
- Improved salt tolerance
  - Up to 1M NaCl is routine
  - shorter pulses (less sample heating)
  - better sensitivity per unit sample, particularly at high salt
- Better pre-saturation (less radiation damping)
- Improved B1 Homogeneity

# NMR Screening vs Assay data



Competition format NMR – displacement of 'spy' {Kd=85uM, [spy]=250uM}

# Materials and Methods



- Capillaries & Collars – New Era, New Jersey USA
- Hydra Liquid handling – Matrix Technologies, Manchester UK
- Custom-made 96-well blocks – Radleys Scientific, Saffron Walden UK



