# Results from a comparison of 3mm and 5mm NMR sample tubes with high salt concentrations in a cryoprobe.

by Murthy Karra

It is well known that the presence of salt in NMR samples is highly detrimental to the S/N gain in using cryogenically cooled probes[1]. A electrically conductive sample due to the presence of small conductive ions will add resistance to the NMR coil and as a result reduce the S/N ratio significantly. A recent paper [2] by Kelly and co-workers examined this issue and concluded that this reduction was a result of a combination of concentration and mobility of ions in solution. They suggest that by switching to buffers of lower mobility ions, the S/N can be recovered.

Another way to reduce the overall salt amounts is to reduce the sample volume and this was demonstrated in a presentation recently given by Dr. Detlef Moskau at Vanderbilt University. It was shown that the S/N loss can be recovered if the sample NMR tube is switched from a 5mm tube to a 3mm. This is due to a reduction in volume (hence a reduction of total salt amount) and a concomitant increase in S/N.

Dr. Moskau demonstrated this effect for a glucose solution at different salt concentrations and different sample tube sizes. As a follow-up, $^{1}H/^{15}N$ HSQC experiments were performed on a sample of Ubiquitin ($^{15}N/^{2}H$ 70%, kindly provided by Susan Meyn) with different concentrations and NMR tube sizes.

Shown below are the results of Signal-to-Noise comparisons between 3mm and 5mm NMR sample tubes. The first histogram shows that even though the total amt of protein is reduced by a factor of 3, the S/N is fairly similar. The second experiment keeps the total amount of protein a constant and results in an average increase of 2.3 in S/N ratio.

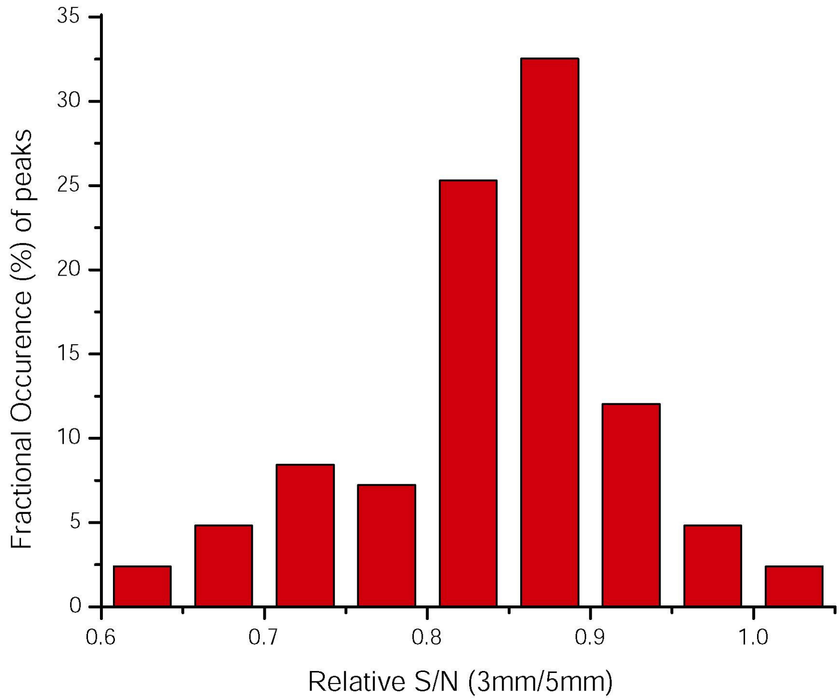
Some comments:

1. The 3mm sample tube was placed in a 5mm tube with the outer annulus filled with $D_2O$. No effort was made to center the 3mm tube but no adverse effects on shimming (as monitored by a reference line) or line shapes were observed.
2. NMR samples usually contain 5-10% D2O for lock purposes which can now be eliminated with a corresponding increase in S/N of exchangeable amide protons.
3. The $^{1}H$ pulse widths show great improvements (~40%) which is very useful to avoid excessive sample heating, better decoupling and spin-lock pulses.
4. The total amount of water is also reduced (by a factor of 3) and this helps to reduce radiation damping and better water suppression.
5. This results are valid only if you have a high salt concentration and a cryoprobe.
6. An increase in S/N ~ 2.3 corresponds to a time reduction of ~ 5.3 which would be very useful for all 3D and 4D NMR experiments (which use proton excitation and detection).

Spectometer = 800MHz with TCI Cyroprobe

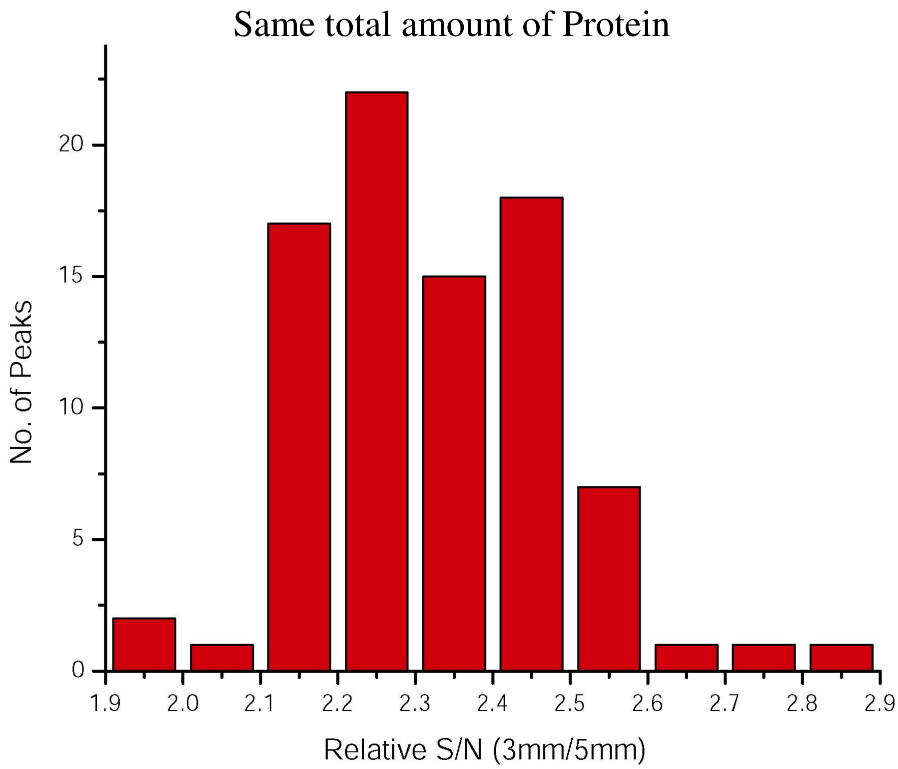
$^{1}H/^{15}N$ HSQC

Same concentration of Protein: Reduced Amount




| NMR Tube Diameter(OD) | **5mm** | **3mm** |
|---|---|---|
| Sample | Ubiquitin | Ubiquitin |
| Isotope Labels | $^{15}N/^{2}H$ (70%) | $^{15}N/^{2}H$ (70%) |
| Concentration | 0.2mM | 0.2mM |
| Salt Concentration | 240mM Phosphate | 240mM Phosphate |
| Sample Height | 3.6cm | 3.6cm |
| $D_2O$ conc. | 4% | 4% |
| Sample Volume | 500µL | 165µL |
| pH | 6.5 | 6.5 |
| $^{1}H$ $\pi/2$ length | 17.1µs | 10.3µs |
| Expt. time | 43mins | 43mins |
| Sample Amt(total) | 100 nanomoles | 33 nanomoles |

Spectometer = 800MHz with TCI Cyroprobe
$^{1}H/^{15}N$ HSQC




| NMR Tube Diameter(OD) | **5mm** | **3mm** |
|---|---|---|
| Sample | Ubiquitin | Ubiquitin |
| Isotope Labels | $^{15}N/^{2}H$ (70%) | $^{15}N/^{2}H$ (70%) |
| Concentration | 0.15mM | 0.45mM |
| Salt Concentration | 325mM Phosphate | 325mM Phosphate |
| Sample Height | 3.6cm | 3.6cm |
| $D_2O$ conc. | 10% | 10% |
| Sample Volume | 500μL | 165μL |
| pH | 6.5 | 6.5 |
| $^{1}H$ $\pi/2$ length | 17.2μs | 10.3μs |
| Expt. time | 22 mins | 22 mins |
| Sample Amt(total) | 75 nanomoles | 75 nanomoles |

**Average S/N gain per unit weight protein ~ 2.3**

1. Triebe, R.; Nast, R.; Marek, D.; Withers, R.; Baselgia, L.; Haberli, M.; Gerfin, T.; Calderon, P. In *40th Experimental Nuclear Magnetic Resonance Conference*; Orlando, FL, 1999; p 198.
2. Low-Conductivity Buffers for High-Sensitivity NMR Measurements, Kelly, A. E.; Ou, H. D.; Withers, R.; Dotsch, V.; J. Am. Chem. Soc.; 2002; 124(40); 12013-12019.