Sample Notes

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Important Notes on suitable NMR samples

- *For high resolution NMR studies:* Suitable compounds must be soluble (> 0.1 mM) and stable (> 2 day), and may include small synthetic or natural products, metabolites, carbohydrates, peptides, proteins (monomer < 40 kDa, oligomer < 120 kDa), DNA/RNA (< 30 bp) etc. The larger biopolymers (proteins, DNA/RNA, polysaccharides) require *isotope labeling* (^{13}C, ^{15}N, ^{2}H). Our NMR laboratories may be consulted to produce proteins with various stable isotopes and labeling patterns.
- *Solvent, organic buffer components*: must be *deuterated* to minimise their strong ^{1}H background signals! Exceptions:
    - ^{19}F NMR: no need for deuteration!
    - Biomolecules: use H_2O (not D_2O) if the observed ^{1}H are *labile* (N-H, O-H etc.)!
- *Total Salt/ion concentration*: should be minimised for maximal NMR sensitivity, and must not exceed 250 mM in general. However, for special cases our NMR platform disposes of an adapted high salt TXI probehead (600 MHz) that can tolerate up to 5 M salt – please consult with us!

Important Notes on Binding Studies:

- *For ligand detected binding studies by conventional ^{1}H NMR*: Only the observed ligands need to comply with the requirements cited above, while the unobserved target protein may even be insoluble or immobilised. These studies yield information (i.e. identity and binding epitope) only for the ligand, but not for the unobserved target protein. Mixtures of compounds (≤ 10) may be used for faster screening as they remain distinguishable via their observed ^{1}H spectra. Yet, the solvent (typically DMSO for ligand stock solutions, and water for the screening sample) and buffer components must be *deuterated* to minimise detrimental ^{1}H background signals. This method is *particularly suited to detect weakly binding ligands* with a K_D between 10^{-5} M and up to 10^{-2} M, but typically *fails to detect strongly binding ligands* with a $K_D \leq 10^{-6}$ M!
- *For ligand detected binding studies by **^{19}F** NMR*: The extreme spectral dispersion of ^{19}F allows to distinguish and employ mixtures of up to 10^2 *fluorinated* compounds by ^{19}F NMR. Alternatively, a single *fluorinated spy ligand* may be used to indirectly screen (via *competitive displacement*) also *un*fluorinated compounds, but at reduced sampling rates since these can not be mixed and spectrally distinguished anymore. Key advantages of ligand screening by ^{19}F NMR are: no need for solvent and buffer deuteration, fastest

sampling rates (when using large mixtures of fluorinated compounds), and widest detectable affinity range from very weak ($K_D \leq 10^{-2}$ M) to irreversible binding ($K_D = 0$).

- *For target (protein) detected binding studies by NMR*: Here, the target protein must be accessible to direct NMR observation and comply with the requirements cited above, implying much larger solubility and amounts of proteins. Moreover, these must typically be ^{15}N labeled to record their 2D $^{1}H,^{15}N$ correlation spectrum, where indicative changes then indicate ligand binding. While sampling rates are far lower than in complementary ligand detected screening, the more demanding and expensive protein detected NMR screening requires no solvent or buffer deuteration, directly indicates and distinguishes binding sites, and has a reduced lower, but *no upper affinity limit* (K_D range: 0 to 10^{-3} M).
- At CiC bioGUNE, sample changers are installed on both 600 MHz spectrometers to enable automated screening on larger sample numbers.