

Saturation Transfer Difference (STD) NMR
ref: Mayer and Meyer, JACS (2001) 123:6108

(1) Start with a regular ¹H spectrum

lock, shim, tuning, sample check, reference phase, reference intensity,
record any optimized parameters:

D1= o1p= SW=. TD =

(2) create a new experiment number ("edc" OR "ix" OR "wra" OR . . .)

rpar satxfr-bbo

Update your parameters: D1, o1p, SW, TD

(3) set the frequency of irradiation of gaussian pulse train:

eda --> sp07 --> offset of sp2 (NEED TO ALREADY KNOW THE OFFSET, can be
determined from the "zg" reference spectrum of step 1)

rga

zg

(NOTE saturation transfer of on vs. off resonance irradiation - either separately or
alternatively vis phase cycling)

STD

protein only:

1. selective saturation leads to efficient/rapid total saturation (in the spin-diffusion limit, i.e. high field and high MW, saturation times of 250ms were sufficient)
2. Tip filter (~30ms) removes broad protein peaks and leaves sharp lines due to small molecules (important for clarity where proteins and ligand signals overlap in 1D ¹H spectra)
3. 1mole protein (MW>10kDa)

protein-ligands:

1. on/off rates (Kd ~ exp-03(=mM) to exp-08(=nM))
2. optimal excess (>20-fold)
3. strongest signals = closest contact with the protein

STD Gaussian calibrations

86Hz (mayer+meyer) power = 4.3 nutations

if using a 50ms gaussian pulse - how much power do we need?

1. reference phase = zg (pulprog)
2. pulprog selzg (single gaussian and acquire)
gaussian = sp1 & p11
3. start with approx amplitude (sp1) equivalent to a 5.8ms 180
4. paropt on pulse duration (constant amplitude) to estimate the number of nutations at 50ms (86Hz = 4.3 nutations)(paropt: 2.5m, 2.5m, 21)(number 20 is 50ms)
5. to fine-tune sp1, paropt of sp1 holding 50m constant