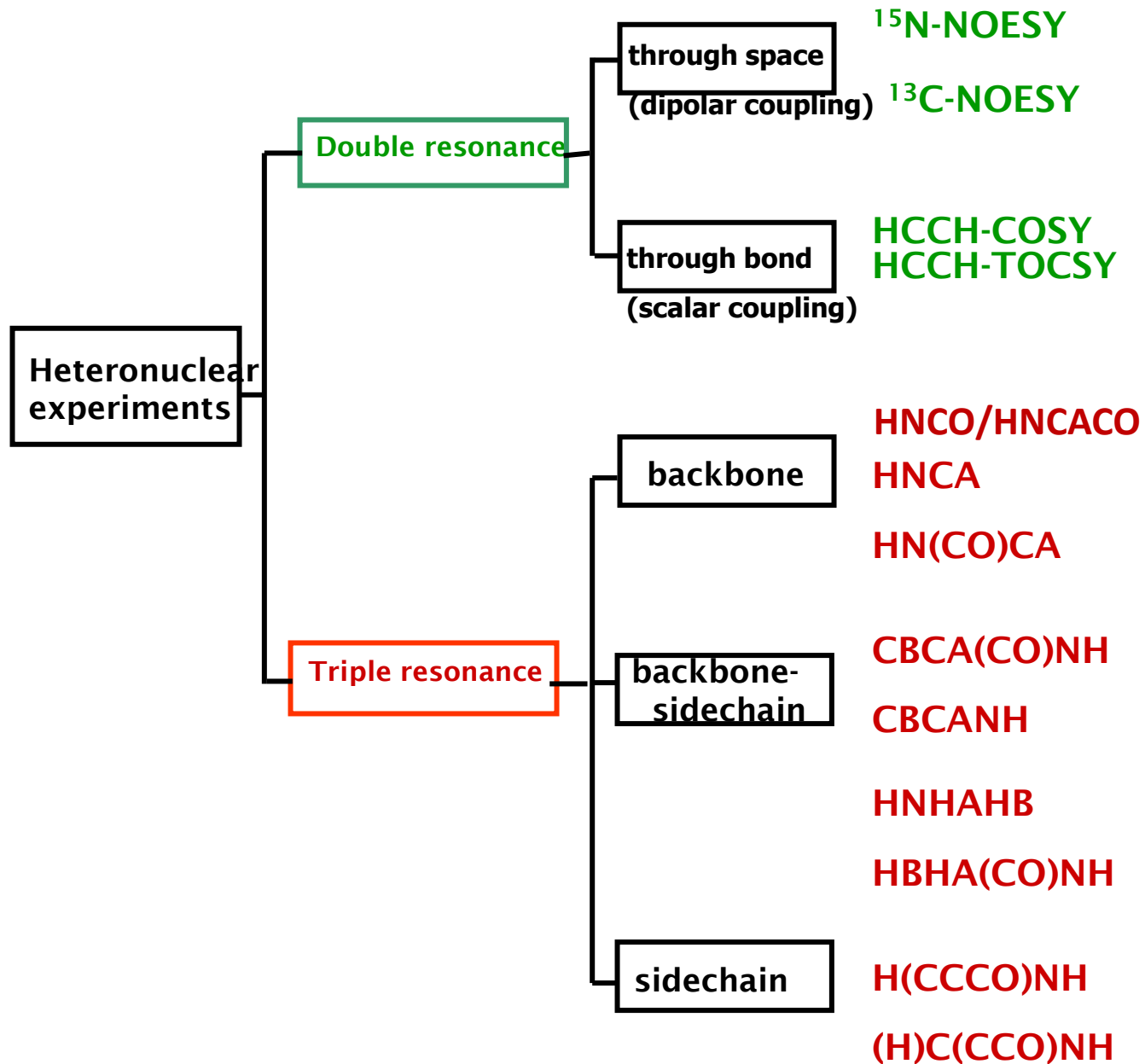


Triple resonance

How to select the right pulse program?

Choice of experiment



What type of experiment yields the best S/N?

protein mw. approximate cutoff in kDa	2D test exp. 1H-15N-correlation	triple resonance test exp. example HNCA	15N-decoupling program	comments
< 10kDa <i>small</i>	hsqcetf3gpsi2	HNCAGP3D	garp4	PEP, Rance S/N enhancement
> 10< 20kDa <i>medium</i>	hsqcf3gpph19	HNCAGPWG3D	bi_garp_pl2 (=bilevel decoupling)	with watergate shorter sequence
> 20kDa <i>large</i>	trosetf3gpsi.2	TRHNCAGP3D2	<i>not decoupled</i>	TROSY

Depending on the size of your protein, one of the three options gives the highest S/N (gradient enhanced, watergate, or, TROSY)

Knowing this, the rest of the triple resonance experiments should all be of that type.

1H-detected triple resonance - selection of experiment type depends on protein size

protein mw. approximate cutoff in kDa	2D test exp. 1H-15N-correlation	triple resonance test exp. example HNCA	15N-decoupling program	comments
< 10kDa <i>small</i>	hsqcetf3gpsi2	HNCAGP3D	garp4	PEP, Rance S/N enhancement
> 10< 20kDa <i>medium</i>	hsqcf3gppl19	HNCAGPWG3D	bi_garp_pl2 (=bilevel decoupling)	with watergate shorter sequence
> 20kDa <i>large</i>	trosetf3gpsi2	TRHNCAGP3D2	<i>not decoupled</i>	TROSY <i>ns=2 ok</i>
fast methods (alternative to the above conventional experiments at high S/N):				
< 20kDa <i>small medium</i>	b_hsqcetf3gpsi	B_HNCAGP3D	garp4.p62 = soft dec.	fast method: AQ<50ms, d1>50ms
> 20kDa <i>large</i>	b_trosetf3gp.2	B_TRHNCAGP3D	<i>not decoupled</i>	fast trosy method: d1 > 50ms, <i>ns=2 ok</i>

If the sample concentration is very high, you may use fast acquisition methods. You need to know whether HSQC or TROSY experiments give best S/N.

NOTE: Soft decoupling garp4.p62 should use $pcpd3=10*p21$ and $pl26=pl3+20dB$

Brutscher BEST triple res: new programming, example, b_hncacbgp3d

CNST54	8.3000002	H(N) chemical shift (offset, in ppm)
CNST55	5.0000000	H(N) bandwidth (in ppm)

Input: bandwidth and offset for 1H

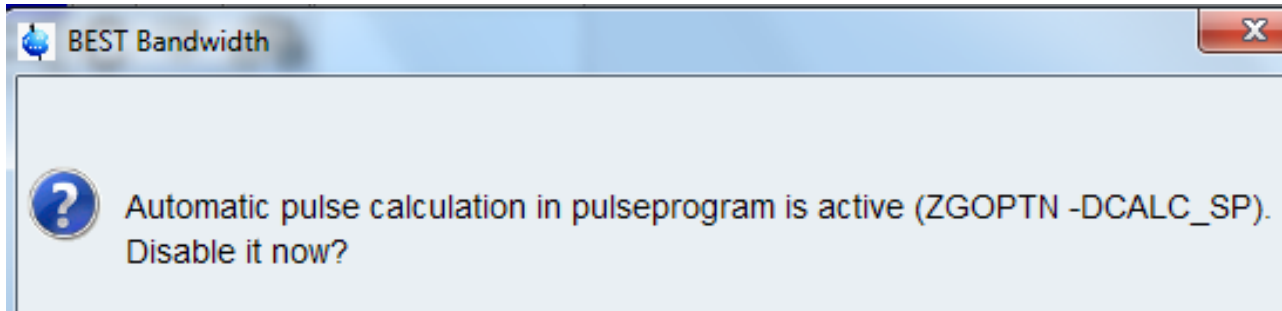
Selective pulse lengths and power levels are calculated automatically

```
# ifdef CALC_SP
```

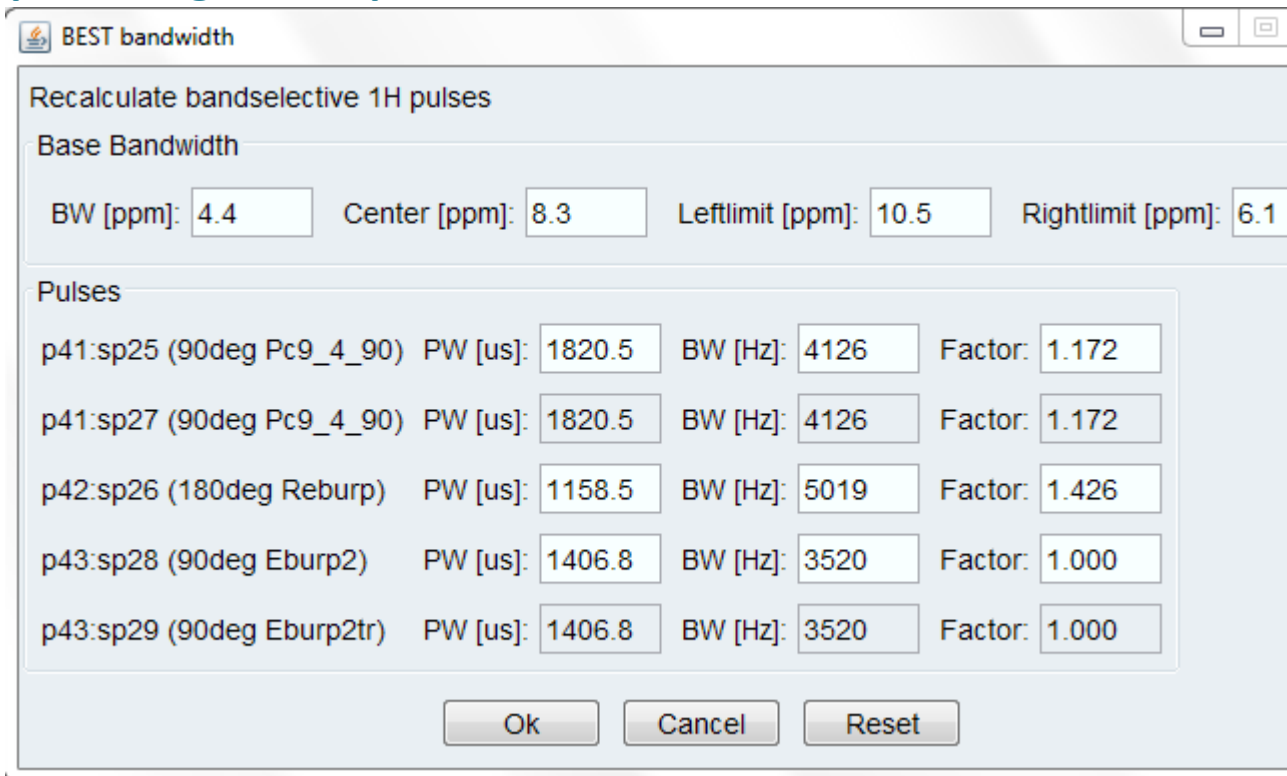
```
ZGOPTNS
```

```
-DCALC_SP
```

Brutscher BEST triple res: alternative BESTBW, example, b_hncacbgp3d



Bestbw command, input: bandwidth and offset for 1H
Selective pulse lengths and power levels are calculated and set automatically



Brutscher best triple res. 46 pulse programs (25 TROSY versions)

b_hncacbgp3d	b_hncacbgp3d.2	b_hncacbigp3d	b_hncacogp3d	b_hncacogp3d.2
b_hncacogp4d	b_hncagp3d	b_hncagp3d.2	b_hncaigp3d	b_hncocacbgp3d
b_hncocacbgp3d.2	b_hncocacbgp4d	b_hncocagp3d	b_hncocagp3d.2	b_hncocagp4d
b_hncogp3d	b_hncogp3d.2	b_hncoigp3d	b_hsqcetf3gpsi	b_hsqcetf3gpsi_wvm
b_hsqcetf3gpsi_wvm2	b_trhncacbgp3d	b_trhncacbgp3d.2	b_trhncacbigp3d	b_trhncacogp3d
b_trhncacogp3d.2	b_trhncagp3d	b_trhncagp3d.2	b_trhncagp3d	b_trhncocacbgp3d
b_trhncocacbgp3d.2	b_trhncocagp3d	b_trhncocagp3d.2	b_trhncocanconhetgp5d	b_trhncocanconhetgp5d.2
b_trhncocanhgp3d	b_trhncocanhgp3d.2	b_trhncocanhgp3d	b_trhncogp3d	b_trhncogp3d.2
b_trhncoigp3d	b_trosytf3gpsi	b_trosytf3gpsi.2	b_trosytf3gpsi.3	b_trosyf3gpqh
b_trosyf3gpqh.2				

TROSY versions 25 pulse programs

b_trhncacbgp3d	b_trhncacbgp3d.2	b_trhncacbigp3d	b_trhncacogp3d	b_trhncacogp3d.2
b_trhncagp3d	b_trhncagp3d.2	b_trhncagp3d	b_trhncocacbgp3d	b_trhncocacbgp3d.2
b_trhncocagp3d	b_trhncocagp3d.2	b_trhncocanconhetgp5d	b_trhncocanconhetgp5d.2	b_trhncocanhgp3d
b_trhncocanhgp3d.2	b_trhncocanhgp3d	b_trhncogp3d	b_trhncogp3d.2	b_trhncoigp3d
b_trosytf3gpsi	b_trosytf3gpsi.2	b_trosytf3gpsi.3	b_trosyf3gpqh	b_trosyf3gpqh.2

13C-detected triple resonance - selection of experiment type depends on protein size

protein mw. <i>approximate</i> cutoff in kDa	triple resonance exp. type Example CACO	Homo- decoupling	comments
< 10kDa <i>small</i>	c_hcaco_ia	ipap	virtual – make pulseprogram longer for small proteins only
> 10< 20kDa <i>medium (large)</i>	c_hcaco_s3	s3e	virtual – shorter, less relaxation losses
any <i>any</i>	c_caco_bshd	BASH- homodec method	bandselective homodecoupling, does not make pp longer, for bigger proteins
fast methods (alternative to the above conventional experiments at high S/N):			
< 10kDa <i>small (medium)</i>	c_hcaco_ct_iare.2 C_hcacon_iare.2	garp4.p62 = soft 15N- decoupling	13C-detected fast methods: AQ<50ms, d1>0.2-0.3sec

Notes- for sequential assignment: hcacon_iare3d.2 and hcanco_iare3d (PRO, GLY)

15N new 1st/2nd

#	Filename	RFF[Hz]	PuW[μs]	Pw[W]	
0	selective excitation				
1	select. inversion/refocussing				
2					
3					
4					
5					
6	T1rho, ramp down				
7	T1rho, ramp up				
8	na_: 180° adiabatic	Crp20,1,40.1	3989.42	1000.00	42.112
9	na_: 180°	Q3.1000	5501.28	600.00	80.079
10	180° selective	Q3.1000	2750.64	1200.00	20.020
11	180° (protein unselective ba	Reburp.1000	4569.56	1371.00	55.251
12	180° broadband	Bip720,50,20.1	4000.00	500.00	42.336
13					
14					
15					
16					

#	RFF[Hz]	PuW[μs]	Pw[W]	
0	cpd	1041.67	240.00	2.8711
1	TOCSY spinlock	1953.12	128.00	10.094
2	ROESY spinlock			
3	presat. (cw irradiation, RF fi			
4	2nd cpd (power gated)	816.99	306.00	1.7661
5	low power cpd	714.29	350.00	1.3500
6	bilev cpd (cw part)			
7				
8	TOCSY/hetero T. (high sele	3472.22	72.00	31.901
9	TOCSY/hetero T. (very high	1168.22	214.00	3.6111
10				
11				
12	low power presat. (cw irradi.,			
13				
14				
15	T1rho	1923.08	130.00	9.7855
16	Rexchange/T2	3125.00	80.00	25.840

15N-detection 10 pulse programs

n_can	n_cbcacon3d	n_cbcanc	n_con	n_dipsi2_hninept
n_hcan	n_hcan3d	n_hcccon3d	n_hninept_f2ig	n_noesy_hninept

4D 56 pulse programs

b_hncacogp4d	b_hncocacbgp4d	b_hncocagp4d	c_hbhacbacon_ia4d	c_hbhacbacon_ia4d
c_hcaconcano_ia4d	c_hcancaco_ia4d	c_hcccon_ia4d	c_hncacon_iare4d	c_hncanco_iare4d
cbcaonhgpwg4d	hacanhgpwg4d	hbcbcgcdcldgp4d	hbhacbaconhgpwg4d	hbhaconhgpwg4d
hccconhgpwg4d	hcchcogp4d	hcchdigp4d	hmqcnoesyhmqcnngp4d	hmqcnoesyhsqcgpsmwg4d
hmqcnoesyhsqcncgp4d	hncacbgpwg4d	hncacbgpwg4d.2	hncacogp2h4d	hncacogp4d
hncacogpwg2h4d	hncacogpwg4d	hncacogpwg4d_mq	hncannhgp4d	hncannhgpwg4d
hncocagp2h4d	hncocagp4d	hncocagpwg2h4d	hncocagpwg4d	hncocagpwg4d_mq
hncocagpwg4d.2	hncocagpwg4d.3	hsqcnoesyhmqccngp4d	hsqcnoesyhsqcccgp4d	hsqcnoesyhsqcncgp4d
hsqcnoesyhsqcncgp4d	hsqcnoesyhsqcnnngp4d	trhncacbgpwg4d	trhncacoetgp2h4d	trhncacoetgp4d
trhncacogp2h4d	trhncacogp4d	trhncacogpwg4d	trhncacogpwg4d_mq	trhncocagp2h4d
trhncocagp4d	trhncocagp2h4d	trhncocagp4d	trhncocagpwg4d	trhncocagpwg4d_mq
trhncocagp4d.2				

5D 25 pulse programs

b_trhncocanconhetgp5d	b_trhncocanconhetgp5d.2	c_hcaconcano_ia5d	c_hcaconcano_ia5d.2	c_hcaconcano_iare5d
c_hcaconcano_iare5d.2	c_hcaconcano_ia5d	c_hcaconcano_ia5d.2	c_hcancaco_ia5d	c_hbhacbaconcano5d
c_hncacon_iare5d	c_hncanco_iare5d	c_hncocanco_ia5d	c_hnconcano_ia5d	cbcaonhgpwg5d
hacaconcaconcoahagp5d	hacaconcaconcoahagp5d.2	hacaonhgpwg5d	hbhacbaconhgpwg5d	hccconhgpwg5d
hncocacbgpwg5d	hncocacbgpwg5d.2	trhncocacbgpwg5d	trhncocanconhetgp5d	trhncocanconhetgp5d.2

hncocanhgpwg6d

dr = dual receiver

dr_hsqcf3_con	dr_zg30	dr_zg30_igf3	dr_zg30_pgf3
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Homodecoupling options for small molecules

dipsi2gpphzs_psyche	noesygpphzs_psyche	reset_dipsi2gpphzs_psyche	reset_psyche_1d
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reset_dipsi2gpphzs_psyche	reset_hsqcetgppesp	reset_hsqcetgppsp	reset_psyche_1d	reset_ZS_1d
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dipsi2gpphzs_zshd	mlevphpp_zshd	noesygpphpp_zshd	noesygpphzs_zshd	zg_zshd
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c_caco_bshd	c_con_sq_bshd	fhsqcf3gpph_bshd	hsqctf3gp_bshd	hsqcetgpiajclrndsp_bshd
hsqcetgpsisp2.2_bshd	zg_bshd			

fhsqcf3gpph_bbhd	hsqcedetgpsisp2.3_bbhd	hsqcedetgppsp.3_bbhd	hsqctf3gp_bbhd	hsqcetgpsisp2.2_bbhd
hsqcetgppsp.2_bbhd	hsqcfpf3gpphwg_bbhd	zg_bbhd		