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Supporting Information of
Improving Dipolar Recoupling for Site-Specific Structure and Dynamics Studies in Biosolids NMR: Windowed RN-Symmetry Sequences

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Tables:

Table S1. Summary of acquisition and processing parameters for the 2D/3D wPARS dipolar coupling measurements.

Acquisition	ω3	ω2	ω1	Processing	ω3	ω2	ω1
U-^{15}N-labeled N-acetyl-valine (NAV)							
^{1}H-^{15}N wPARS 2D	-----	1k complex; sw=20 kHz;	16 real; sw=7 kHz;	NMRPipe	-----	90-degree sinebell; FFT;	FFT;
U-^{13}C,^{15}N-Met-Leu-Phe (MLF) tripeptide							
^{1}H-^{15}N wPARS 2D	-----	1k complex; sw=20 kHz;	16 real; sw=7 kHz;	NMRPipe	-----	90-degree sinebell; FFT;	FFT;
^{1}H-^{13}C wPARS 2D	-----	1k complex; sw=50 kHz;	16 real; sw=16.7 kHz;	NMRPipe	-----	90-degree sinebell; FFT;	FFT;
^{1}H-^{13}C wPARS 3D	1k complex; sw=50 kHz;	200 real; sw = 30 kHz;	16 real; sw=16.7 kHz;	NMRPipe	45-degree sinebell; FFT;	45-degree sinebell; forward linear prediction of 200 points; FFT;	FFT;
U-^{13}C,^{15}N-dynein light chain (LC8) protein							
^{1}H-^{13}C wPARS 3D	1k complex; sw=50 kHz;	200 real; sw = 30 kHz;	16 real; sw=16.7 kHz;	NMRPipe	45-degree sinebell; FFT;	45-degree sinebell; forward linear prediction of 200 points; FFT;	FFT;

Table S2. Dipolar couplings for aromatic sidechains in U-^{13}C,^{15}N-LC8 prepared with and without Cu(II)-EDTA doping: (H) Histidine, (W) Tryptophan, (F) Phenylalanine, and (Y) Tyrosine. The dipolar lineshapes were recorded by 3D R10$_1^3$-based ^{1}H-^{13}C(^{13}C) wPARS.

Residue	Dipolar Coupling (kHz)		η_D	
	Without	With Cu^{2+}	Without	With Cu^{2+}
Carbons on rigid residues				
H55 (Cδ)	21.8	22.9	0.02	0.34
W54 (Cδ2)	19.6	21.0	0.39	0.17
W54 (Cε2)	19.9	19.7	0.20	0.24
W54 (Cε3)	21.2	19.6	0.25	0.05
W54 (Cζ2)	21.5	21.6	0.22	0.26
W54 (Cζ3)	22.8	21.3	0.42	0.26
W54 (Cη2)	-----	21.8	-----	0.19
Carbons on mobile residues				
F62 (Cδ)	19.2	20.2	0.22	0.06
F76 (Cδ)	12.2	12.6	0.52	0.24
Y41 (Cδ)	13.2	12.8	0.98	0.86
Y41 (Cε)	13.4	10.8	0.99	0.96
Y75 (Cδ)	11.3	11.4	0.86	0.87
Y75 (Cε)	11.5	11.4	0.88	0.95

Figures:

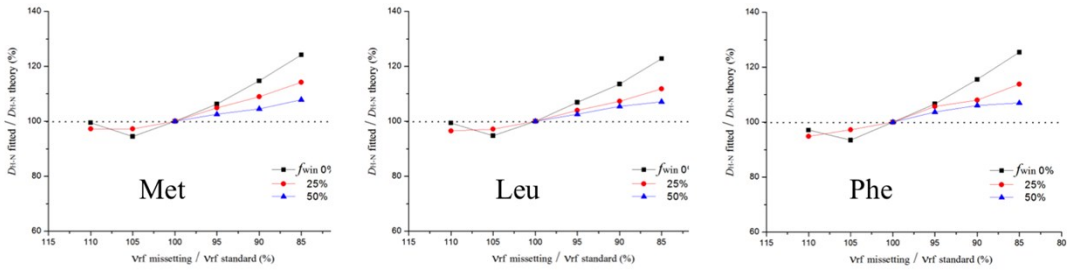

Figure S1. Experimental performance of windowed-DIPSHIFT 2D in MLF. Errors (expressed in %) in ^{1}H-^{15}N dipolar couplings plotted as a function of the ^{1}H rf field mismatch, shown in % of the correct theoretical value. Different window fractions of 0%, 25%, 50% were tested and shown as filled squares, triangles and circles, respectively. Experiments were performed with MAS frequency of 14 kHz.

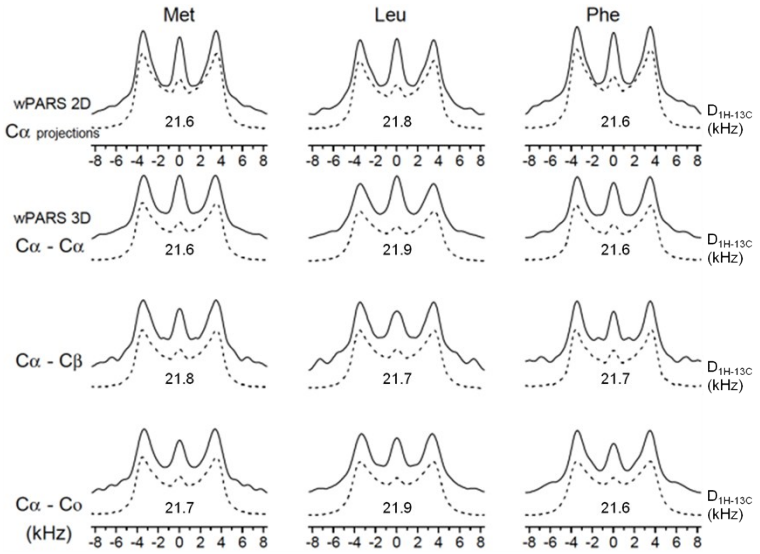

Figure S2. ^{1}H-^{13}C$_{\alpha}$ dipolar line shapes of MLF extracted from 2D wPARS (top row) and from 3D R10$_1^3$-based ^{1}H-^{13}C(^{13}C) wPARS (three bottom rows). Experimental and fitted lineshapes are shown as solid and dashed lines, respectively. Experiments were performed with MAS frequency of 20 kHz.

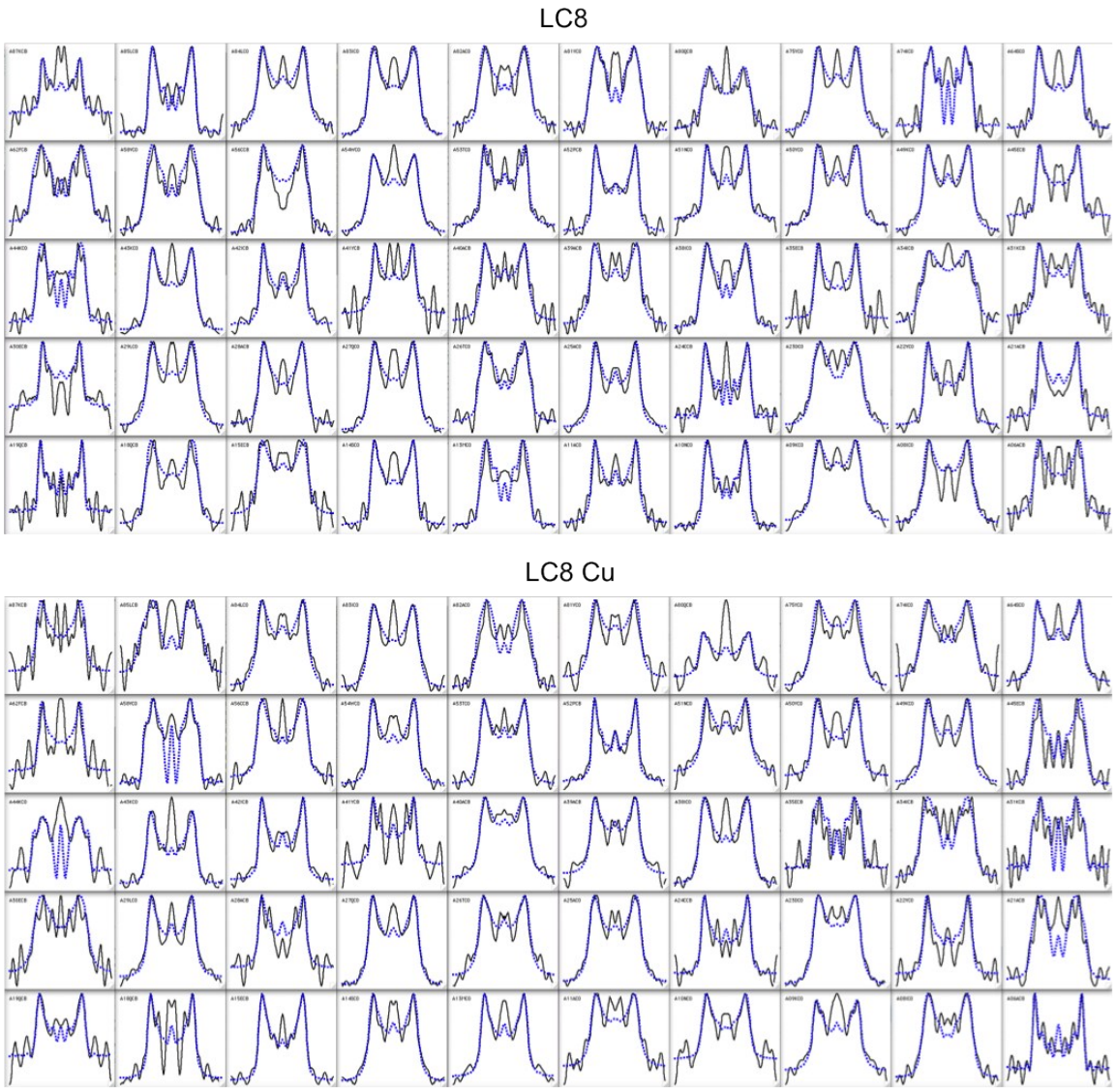

Figure S3. ^{1}H-$^{13}C\alpha$ dipolar line shapes for 50 residues possessing resolved cross peaks in U-^{13}C,^{15}N-LC8 3D wPARS spectra. The experimental and the best-fit line shapes are shown as black solid lines and blue dashed lines, respectively. The samples were prepared without (top) and with (bottom) Cu(II)-EDTA doping.

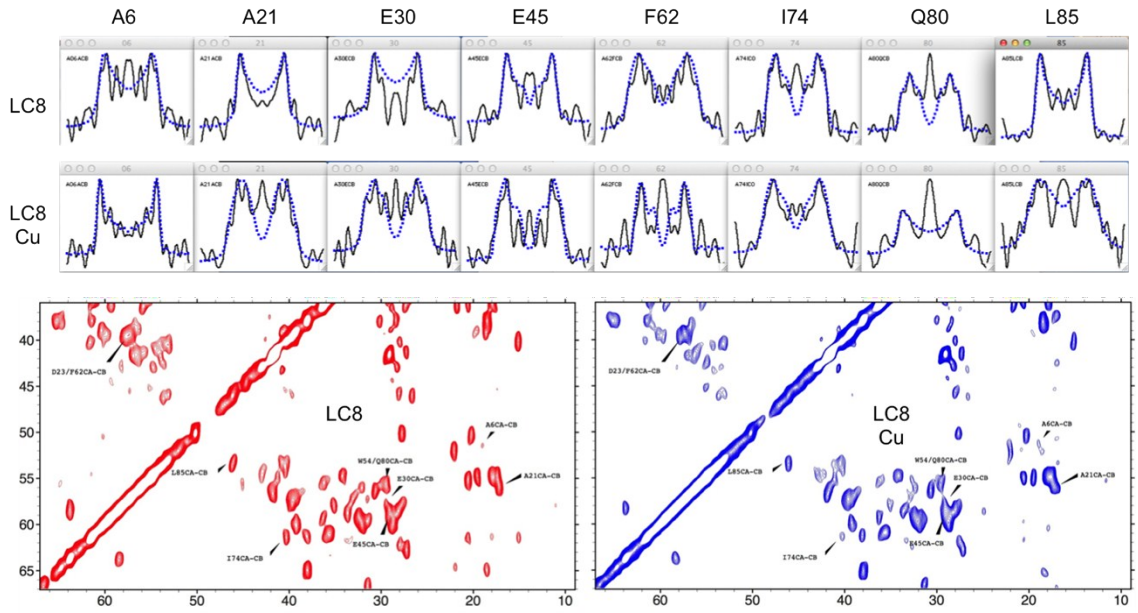

Figure S4. Top: Dipolar line shapes for U-^{13}C,^{15}N-LC8 residues exhibiting differences in dipolar couplings of more than 1.5 kHz in samples prepared with and without Cu(II)-EDTA doping. Experimental and best-fit line shapes are shown as black solid lines and blue dashed lines, respectively. Bottom: 2D planes of the 3D wPARS spectra containing the corresponding cross peaks.

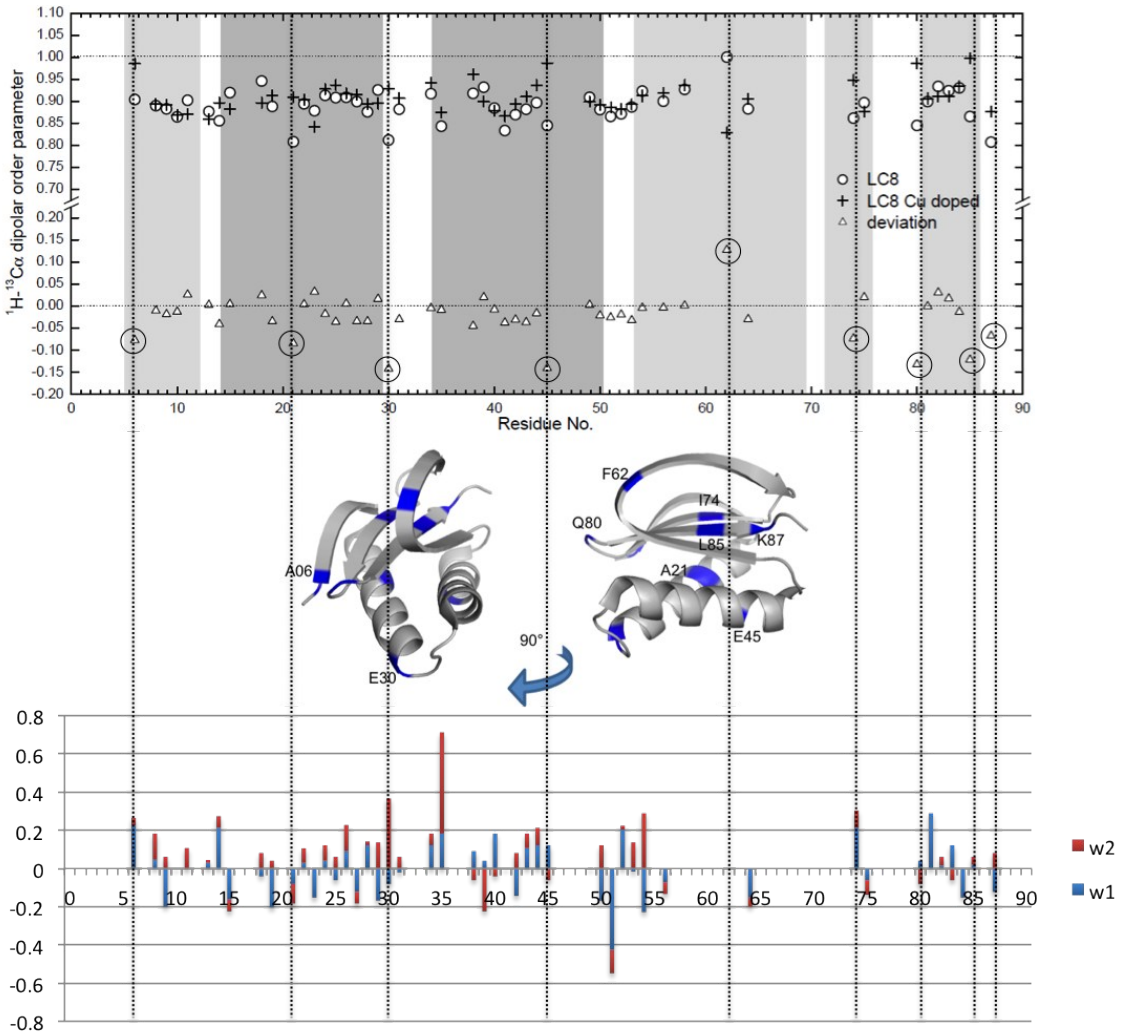

Figure S5. Chemical shift perturbations observed in nanocrystalline U-^{13}C,^{15}N-LC8 with and without Cu(II)-EDTA doping, compared to the dipolar coupling deviations as shown in Figure 5.

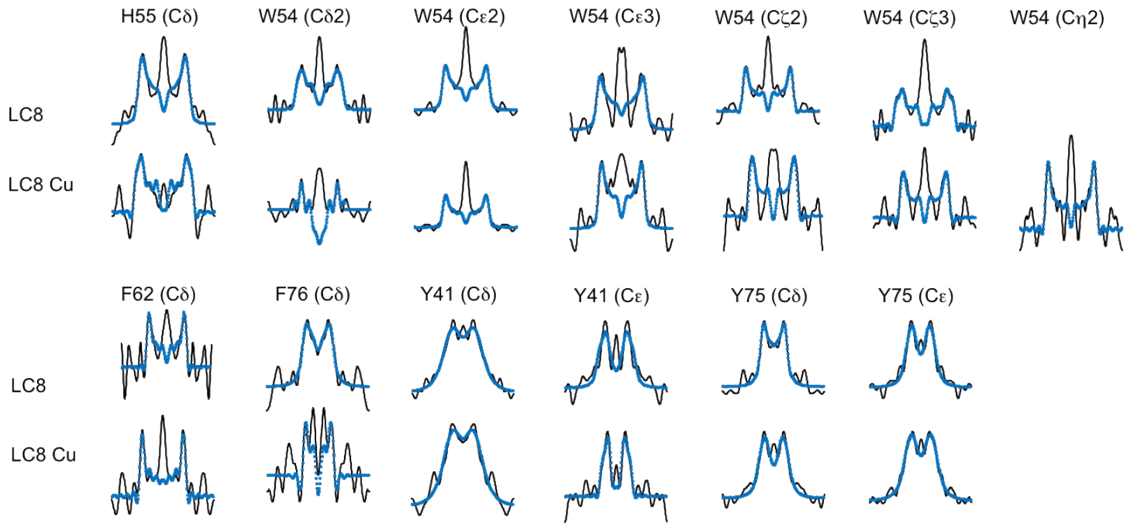

Figure S6. ^{1}H-^{13}C(δ,ε,ζ) dipolar line shapes for sidechains of 6 representative residues possessing resolved cross peaks in U-^{13}C,^{15}N-LC8 3D wPARS spectra. The experimental and the best-fit line shapes are shown as black solid lines and blue dashed lines, respectively. The samples were prepared without (top) and with (bottom) Cu(II)-EDTA doping.

Simpson data fitting scripts:

```
spinsys {
        channels 1H 13C
        nuclei 1H 13C
#       Euler: any  theta   phi
        dipole 1 2 22800 0 0 0
#       Euler angles: phi  theta  any
        shift 1 0p 0p 0 0 0 0
        shift 2 0p 0p 0 0 0 0
}

par {
  proton_frequency 599.8e6
  spin_rate        14000
  np               16
  crystal_file     rep168
  gamma_angles     13
  verbose          1101
  start_operator   I2x
  detect_operator  I2p
  variable N       10
  variable n       1
  variable v       3
  variable Hdec    125000
  variable rf      70000
  sw               spin_rate/2/n

# ntail is number of tail points in FID for baseline correction (0: no correction)
        variable ntail 0
# DCCinit is starting value of DCC fit
        variable DCCinit 10000.0
# LBinit is starting value of line broadening fit
        variable LBinit 50.0
# fcent is half width (Hz) of central region ignored by fit
        variable fcent 500
# fedge is half width (Hz) of total region included in fit
        variable fedge 3500
}

proc pulseq {} {
  global par
  maxdt 1.0

  set tr  [expr 1.0e6/$par(spin_rate)]
  set tRn [expr $par(n)*$tr/$par(N)]
        set delay 0
#       set delay [expr $tRn/4.0]
#       set delay [expr $tRn/2.0]
  set t90 [expr ($tRn-$delay)/2.0]
  set ph  [expr 180.0*$par(v)/$par(N)]

  reset
  for {set i 0} {$i < [expr $par(N)/2]} {incr i} {
    pulse $t90 $par(rf) $ph 0 0
```

```
   delay $delay
   pulse $t90 $par(rf) $ph 0 0
   pulse $t90 $par(rf) [expr -$ph] 0 0
   delay $delay
   pulse $t90 $par(rf) [expr -$ph] 0 0
  }
  pulseid 1 0 0 500000 0
  for {set i 0} {$i < [expr $par(N)/2]} {incr i} {
   pulse $t90 $par(rf) [expr $ph+180.0] 0 0
   delay $delay
   pulse $t90 $par(rf) [expr $ph+180.0] 0 0
   pulse $t90 $par(rf) [expr -$ph+180.0] 0 0
   delay $delay
   pulse $t90 $par(rf) [expr -$ph+180.0] 0 0
  }
  pulseid 1 0 0 500000 0
  store 1

  reset
  acq
  for {set i 1} {$i < $par(np)} {incr i} {
   reset
   prop 1 $i
   acq
  }
}

proc minuit {} {
        global mn g par

  set tr  [expr 1.0e6/$par(spin_rate)]
  set tRn [expr $par(n)*$tr/$par(N)]
        set delay 0
#       set delay [expr $tRn/4.0]
#       set delay [expr $tRn/2.0]
  set t90 [expr ($tRn-$delay)/2.0]

        set f [fsimpson [list [list dipole_1_2_aniso $mn(DCC)] ] ]
        faddlb $f $mn(LB) 0

#  subtract tail
        if {$par(ntail) > 0}  {
           set tail 0.
           for {set m 1} {$m <= $par(ntail)} {incr m}  {
                set n [expr $par(np) + 1 - $m]
                set fn [findex $f $n -re]
                set tail [expr $tail + $fn/$par(ntail)]
           }

           for {set n 1} {$n <= $par(np)} {incr n} {
                set fn [findex $f $n -re]
                set fn [expr $fn - $tail]
                fsetindex $f $n $fn 0
           }
        }
        fzerofill $f 256
```

```
	fft $f

# scaling and base-line adjustment
	set jcent [expr 128*($par(sw) - 2*$par(fcent))/$par(sw)]
	set jedge [expr 128*($par(sw) - 2*$par(fedge))/$par(sw)]
	set j1 0.
	set f1 0.
	set f2 0.
	set g1 0.
	set fg 0.

# in the summation, do not use {set j $jedge} because jedge is not an integer
	for {set j 1} {$j <= $jcent} {incr j} {
	if {$j > $jedge} {
		set fj [findex $f $j -re]
		set gj [findex $g $j -re]
		set j1 [expr $j1 + 1]
		set f1 [expr $f1 + $fj]
		set f2 [expr $f2 + $fj*$fj]
		set g1 [expr $g1 + $gj]
		set fg [expr $fg + $fj*$gj]
	}
	}
	set c [expr $f2*$j1 - $f1*$f1]
	set a [expr ($fg*$j1 - $f1*$g1)/$c]
	set b [expr ($f2*$g1 - $f1*$fg)/$c]
	for {set j 1} {$j <= 256} {incr j} {
		set fj [findex $f $j -re]
		set gj [findex $g $j -re]
		if {$par(output) == 2} {fsetindex $g $j $gj 0}
		set fj [expr $a*$fj + $b]
		if {$par(output) == 2} {set gj 0}
		fsetindex $f $j $fj $gj
	}

	set chi2 0.
	set rms [frms $f $g -re {{-3500 -500} {500 3500}}]
	puts "$mn(DCC)	$mn(LB)	$rms	$chi2"
	if {$par(dev) == 2} {set rms $chi2}
	if {$rms < $par(bestrms)} {
		set par(bestrms) $rms
		fsave $f $par(name).fit
	}
	funload $f
	return $rms
}

proc main {} {
	global par mn g
	if {$par(exptype) == 1} {
	  set g [fload "PARSexp.fid"]

#  subtract tail
	  if {$par(ntail) > 0}  {
	    set tail 0.
	    for {set m 1} {$m <= $par(ntail)} {incr m}  {
```

```
            set n [expr $par(np) + 1 - $m]
            set gn [findex $g $n -re]
            set tail [expr $tail + $gn/$par(ntail)]
        }
        for {set n 1} {$n <= $par(np)} {incr n} {
            set gn [findex $g $n -re]
            set gn [expr $gn - $tail]
            fsetindex $g $n $gn 0
        }
      }
      fzerofill $g 256
      fft $g
    }  else  {

    set g [fload "example.spe"]
    }
    set par(bestrms) 1e6
    puts " DCC       LB       rms       chi^2"
    mnpar DCC $par(DCCinit)  100
    mnpar LB  $par(LBinit)   50
    mnminimize
    puts "DCC   $mn(DCC)"
    puts "LB    $mn(LB)"
    if {$par(dev) == 1} {puts "rms   $par(bestrms)"}
    if {$par(dev) == 2} {puts "chi^2 $par(bestrms)"}
    funload $g
}
```