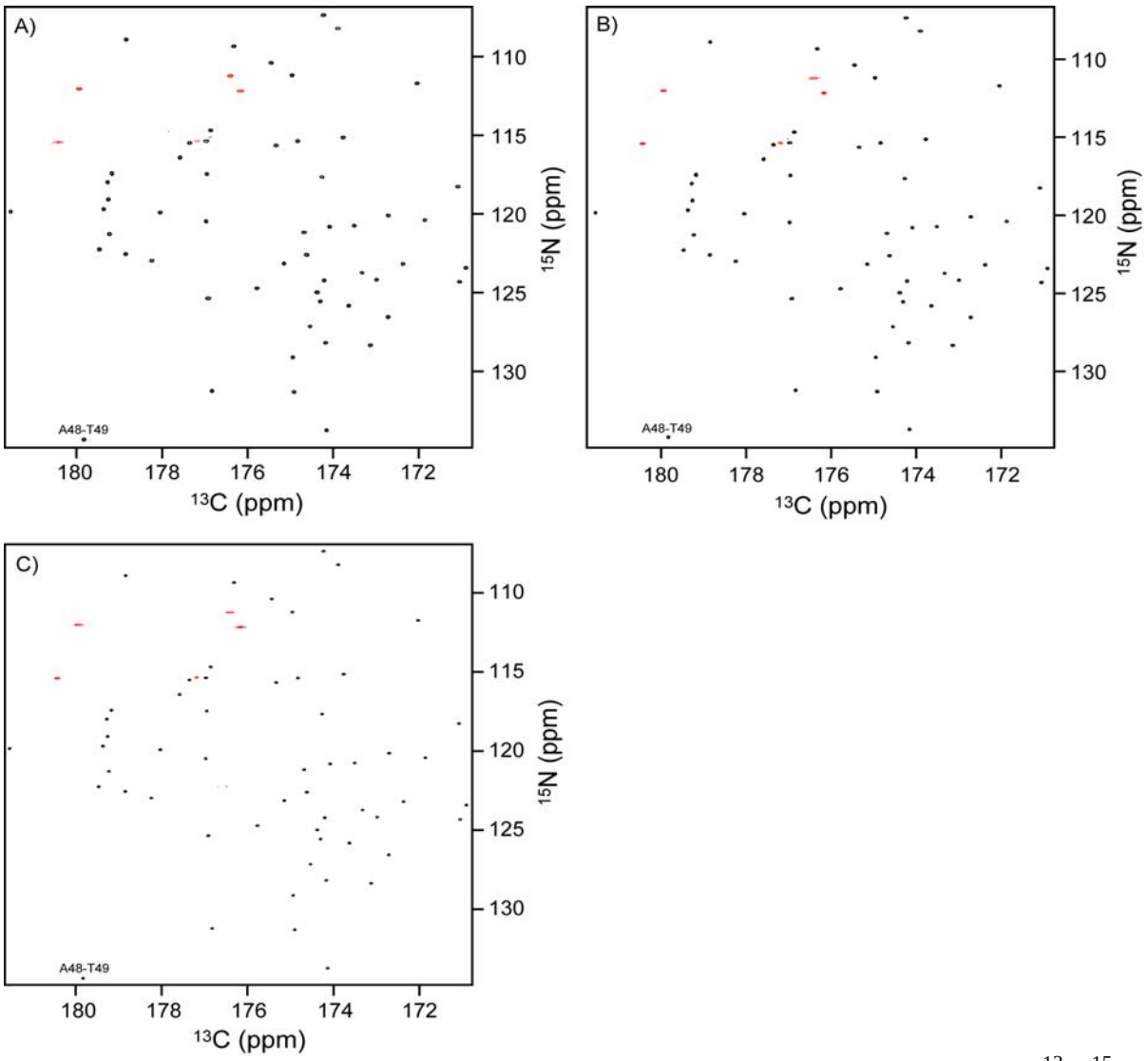
$^{13}C^{\alpha}$ decoupling during direct observation of carbonyl resonances in solution NMR of isotopically enriched proteins

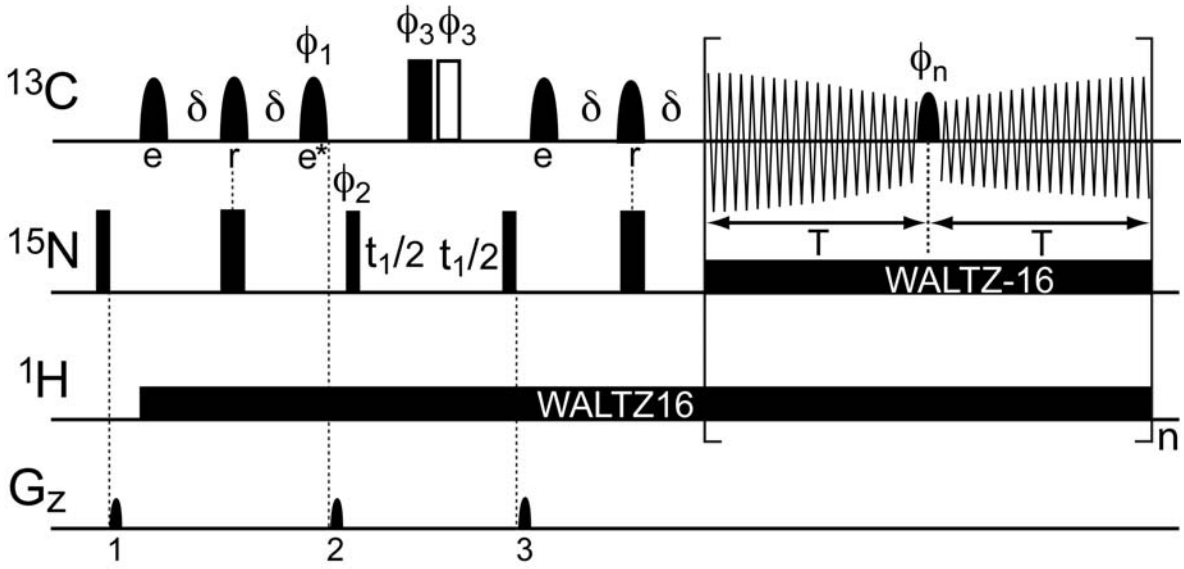
Jinfa Ying, Fang Li, Jung Ho Lee, and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, U.S.A.

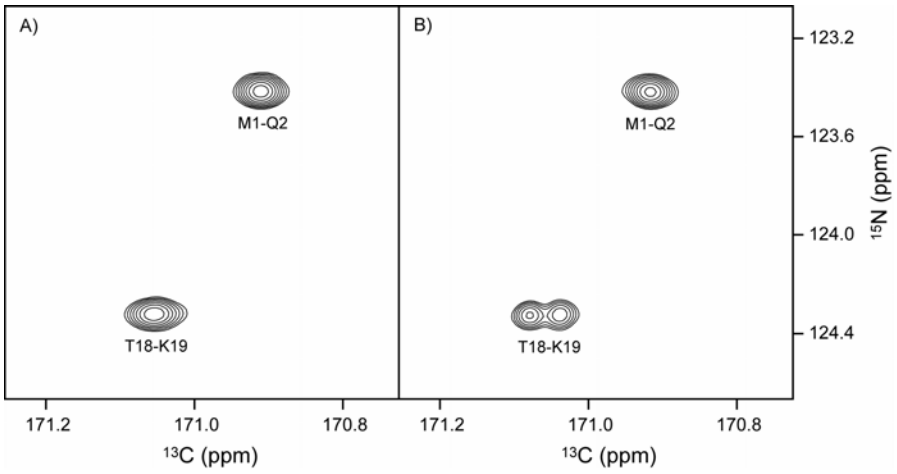
Supplementary Material



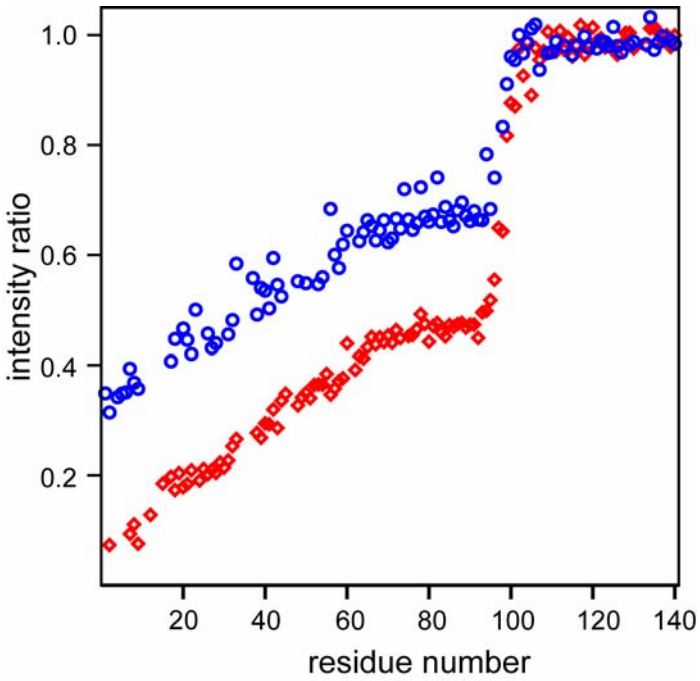
Supplementary Fig. S1. LOW-BASHD decoupled 2D NCO spectra of uniformly $^{13}C/^{15}N$-enriched GB3 recorded with the pulse scheme of Fig. S2, at (A) 125.7 MHz ^{13}C frequency, (B) 151 MHz ^{13}C frequency, and (C) 201.2 MHz ^{13}C frequency. The ^{13}C carrier was set to 177 ppm, and the LOW-BASHD decoupling was achieved using 180, 150, and 113 μs central lobe $^{13}C^{\alpha}$ selective sinc pulse, cosine-modulated at 14.8, 17.8, and 23.7 kHz for A), B), and C) respectively. The time domain matrix consisted of (A) 220 × 512 complex data points, or acquisition times of 139.5 ms (^{15}N) and 204.8 ms ($^{13}C'$), and with 8 scans and 1.5 s interscan delay the total measurement time was approximately 2 hours; (B) 256 × 512 complex data points, or acquisition times of 135.7 ms (^{15}N) and 204.8 ms ($^{13}C'$), and approximately 2 hours of total measurement time with 8 scans and 1.5 s interscan delay; (C) 350 × 640 complex data points, or acquisition times of 138.6 ms (^{15}N) and 204.8 ms ($^{13}C'$), and approximately 6 hours of total measurement time with 16 scans and 1.5 s interscan delay. Correlations for the sidechains of Asn and Gln resonances (colored red) are incompletely decoupled from the $^{13}C^{\beta}$ and $^{13}C^{\gamma}$ resonances because these resonate outside the LOW-BASHD decoupled region. The labeled A48-T49 correlation is folded in the ^{15}N dimension.



Supplementary Fig. S2. Pulse sequence used for recording the 2D LOW-BASHD NCO spectra. Narrow and wide bars represent 90° and 180° pulses, respectively. Pulse durations should scale inversely with the magnetic field strength, and at 201 MHz ^{13}C frequency the following values were used: The ^{13}C' selective EBURP2, ReBURP, and time-reversed EBURP2 pulses are marked with e, r, and e*, respectively, and have a duration of 375 μs (centered at 177 ppm). The LOW-BASHD ϕ_n $^{13}C^{\alpha}$ decoupling pulses have durations of 113 μs and shapes of the center lobe of a sinc function, with their amplitude cosine-modulated at a frequency of 23.7 kHz (at 201 MHz ^{13}C frequency, generating sidebands at ±118 ppm from the ^{13}C' carrier). Phase cycling: ϕ_1 = x, -x, ϕ_2 = x, x, -x, -x, ϕ_3 = x, x, x, x, -x, -x, -x, -x, ϕ_{rec} = x, -x, -x, x. The phase of ϕ_n follows the MLEV16 pattern as defined for Main Text Fig 1. States-TPPI was used for the quadrature detection of the indirect ^{15}N dimension by incrementing the phase of ϕ_2. Gradient pulses $G_{1,2,3}$ (z-axis) have durations of 1.1, 0.7 and 1.0 ms, and strengths of 33.6, 25.9, and 28.7 G/cm.



Supplementary Fig. S3. Comparison of a small region of the LOW-BASHD decoupled 2D NCO spectra of uniformly $^{13}C/^{15}N$-enriched GB3 recorded with the pulse scheme of Fig. S2 and (A) parameters of Fig. S2, and (B) the width of the $^{13}C^{\alpha}$ pulse reduced from 113 to 100 μs, and the interpulse delay reduced from 5 to 3.52 ms. Region (A) is taken from the same spectrum as Fig. 3, main text. The residual ~12 Hz splitting for the T18-K19 correlation results from the 3-spin effect involving T18-$^{13}C^{\beta}$.



Supplementary Fig. S4. The α-synuclein NCO volume ratio as a function of residue of spectra recorded in the presence and absence of SUVs (red diamonds), together with the corresponding volume ratios observed in the ^{1}H-^{15}N HSQC spectrum (blue circles). Ratios are extracted from the same spectra as used for Fig. 4, main text, but volumes have somewhat larger statistical uncertainties than the peak heights used for that figure, resulting in increased scatter.

Bruker pulse sequence code (Topspin 2.1 and Topsin 3.1) for the LOW-BASHD NCO experiment

```
#include <Avance.incl >
#include <Grad.incl>

define pulse PG1
define pulse PG2
define pulse PG3

#define BASH

;******definitions for BASH decoupling*******
define loopcounter tdCount

"d28=d29*0.5-p6*0.5-6u"   ;d29=delay between two 180 pulses

#ifdef BASH
"tdCount = aq/d29+1"
#else
"tdCount = aq/(d28*2)+1"  ;use d28*2 ensure enough points when BASH is off
#endif

dwellmode explicit   ;for DQD/digital

;l6 to be incremented in each block of the BASH acquisition
"l6=0"
;**** end of definitions for BASH decoupling****

"d11=30m"
"d12=200u"

;Increments
"in0=inf1/2"

;Gradient Pulses
"PG1=1.1m"
"PG2=0.7m"
"PG3=1.0m"

;Delays
"d2=12.5m"
"d3=d2-20u-de"

"d0=in0*0.5-p4*2-2u-p7*0.635"
```

```
;13C frequency offsets. Set o1p=176ppm from command line.
"cnst1=177"
"cnst2=56"

"spoff4=bf1*((cnst2-cnst1)/1000000)"  ; Cab 180


1 ze
  1m
  10u reset:f1
2 10u do:f2 do:f3
  10u LOCKH_OFF
  d11
  d12
3 d12
4 d12
    d1
    1m UNBLKGRAD
    10u pl1:f1  ; C
    10u pl2:f2  ; H
    10u pl3:f3  ; N

   (p7 ph0):f3
    3u
    PG1:gp1
    100u

    3u pl31:f2
    3u cpds1:f2 ph0   ; 1H WALTZ16

   (p10:sp10 ph0):f1
    d2
   (center (p12:sp12 ph0):f1 (p7*2 ph0):f3)
    d2
   (p10:sp11 ph5):f1
    3u
    PG2:gp2
    100u pl4:f1

   (p7 ph7):f3
    d0
   (p4*2 ph4):f1
    4u
   (p4*2:sp4 ph4):f1
    d0
   (p7 ph0):f3
```

```
    3u
    PG3:gp3
    90u
    10u BLKGRAMP

   (p10:sp10 ph0):f1
    d2
   (center (p12:sp12 ph0):f1 (p7*2 ph0):f3)
    d3
    10u pl30:f3
    10u cpds3:f3 ;cpds2:f2

  ACQ_START(ph30,ph31) ;total delay here=de

   0.05u DWL_CLK_ON
   0.1u REC_UNBLK

44  d28

#ifdef BASH
  ;for mlev16 decoupling phase cycling
  if  "l6%16==0  ||  l6%16==1  ||  l6%16==5  ||  l6%16==6  ||  l6%16==10  ||  l6%16==11  ||
l6%16==12 || l6%16==15"
    {
      "cnst6=0"
    }

  if "l6%16==2 || l6%16==3 || l6%16==4 || l6%16==7 || l6%16==8 || l6%16==9 || l6%16==13
|| l6%16==14"
    {
      "cnst6=180"
    }

    4u
    1u REC_BLK
    1u ip6+cnst6
   (p6:sp6 ph6):f1
    5u iu6
    1u REC_UNBLK
#endif

    d28

lo to 44 times tdCount

   0.1u REC_BLK
```

```
  0.05u DWL_CLK_OFF

   3u ru6
 100u rcyc = 2
   10u do:f2 do:f3
   10u LOCKH_OFF
   d11 wr #0  if #0 zd
   d12 ip7
 lo to 3 times 2
   d12*0.5 id0
   d12*0.5 ip31*2
 lo to 4 times l3
   1m do:f3
   1m do:f2

   1m BLKGRAD
exit

;Phases
ph0=0
ph1=1
ph2=2
ph3=3
ph4=0 0 0 0 2 2 2 2
ph5=1 3
ph6=0
ph7=0 0 2 2
ph8=0
ph30=0
ph31=0 2 2 0
```