Supplementary material to

The disorderly conduct of Hsc70: its interaction with the Alzheimer's related TAU protein as monitored by NMR.

Dedicated to the memory of Chad A. Dickey.

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Supplemental Table 1		
	Wild type	BETA (395-508)
	EC_{50} (μM)	EC_{50} (μM)
4R0N TAU	0.9 ± 0.2	3.4 ± 2.9
TAU1	17.0 ± 9.3	32.0 ± 22
NRLLLTG	6.3 ± 3.5	28.0 ± 9.8
C-PEPTIDE	0.2 ± 1.8	1.3 ± 0.6
TAU2	> 50	> 200
Huntingtin	> 200	> 200
KFERQ	> 200	> 200

Table S1: Binding of substrates to Hsp70 (HSPA1A)
and its truncation by fluorescence polarization (FP).
TAU1: GKVQIINKKG; Insulin C-peptide (C-PEP); TAU2: VQIVYK; huntingtin: MATLEKLMKAFESLKSF.
The Fam-HLA tracer was at 20 nM, wt-Hsp70 at 5 μM, Hsp70- BETA at 1 μM.

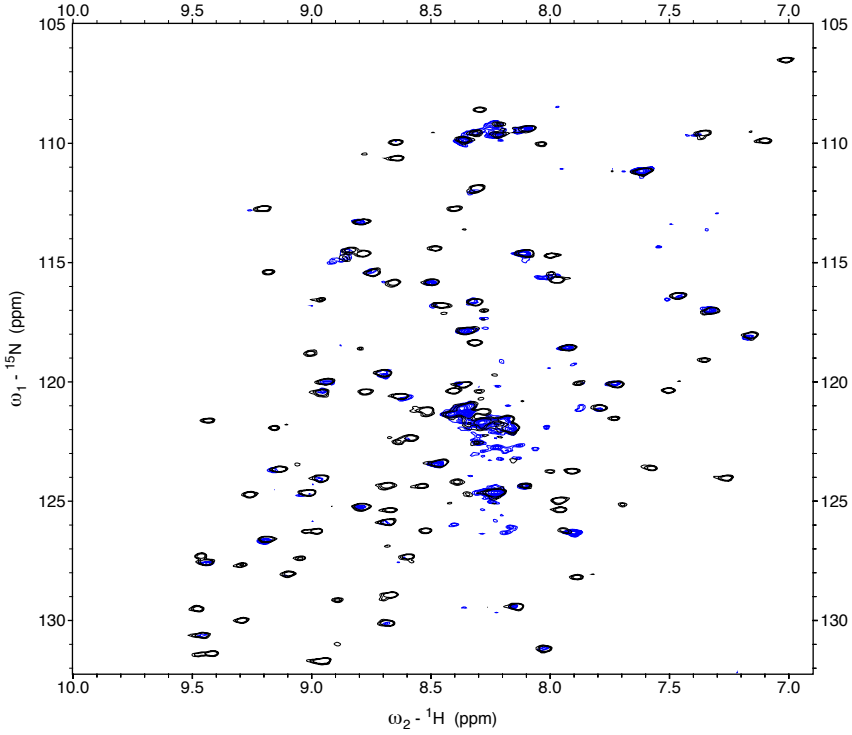

Fig S1 A Binding of Fam-HLA
Blue 50 uM BETA apo, Black, with 60 uM Fam-HLA

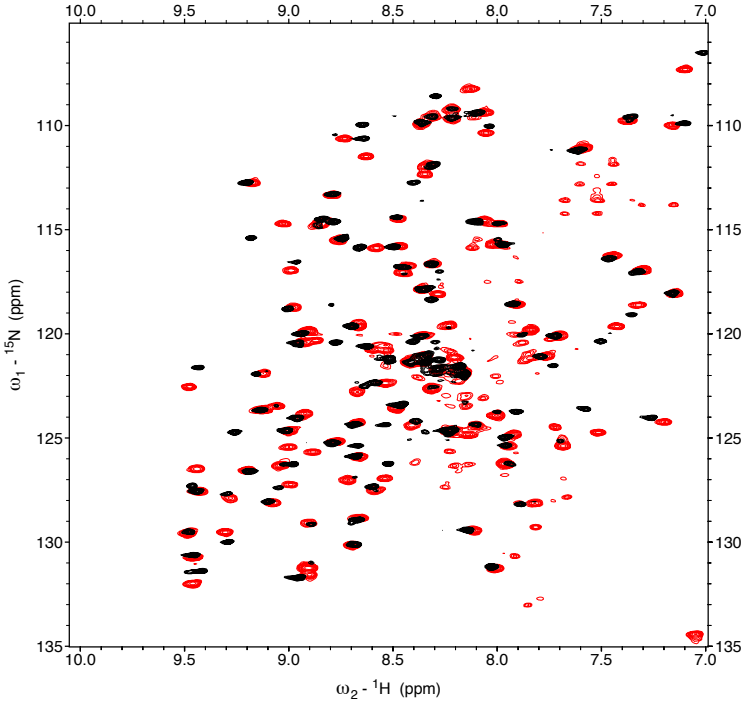

Fig S1 B Binding of Fam-HLA compared with TAU1
Black, 50 uM BETA with 60 uM Fam-HLA
Red, 35 μM BETA + 200 μM TAU1 (GKVQIINKKG)

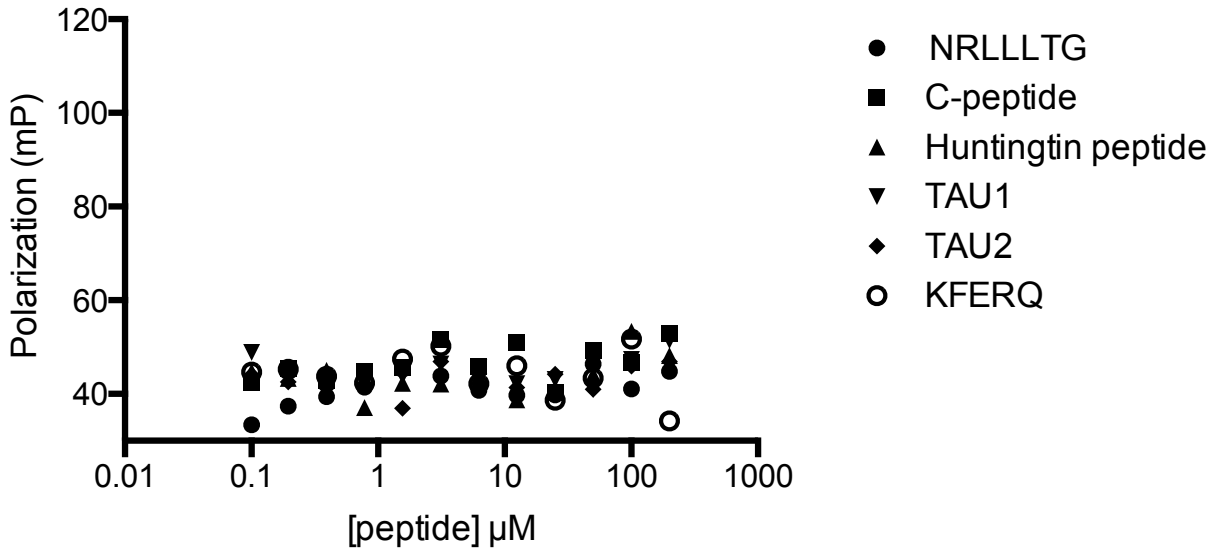

Figure S2.
Effect of the different peptides on the fluorescence polarization of the HLA tracer

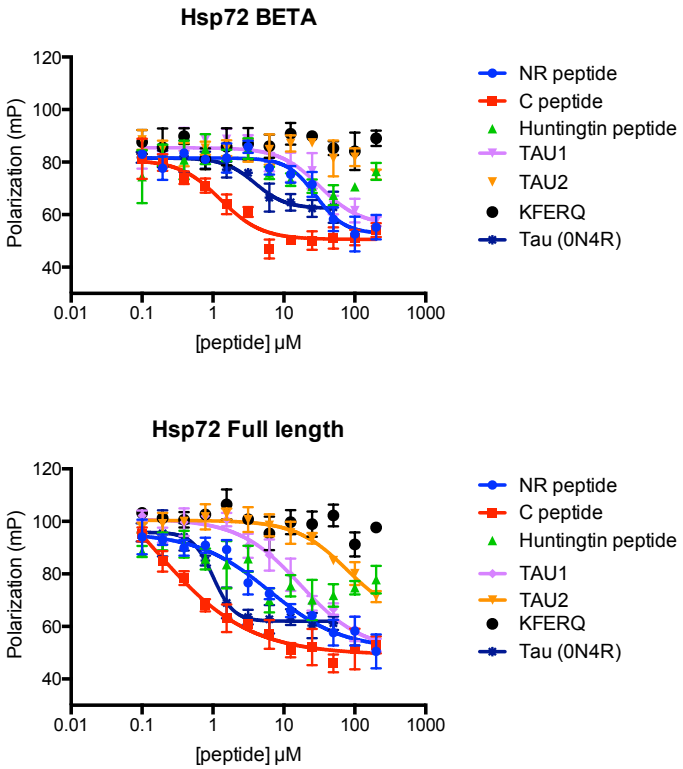

Figure S3. Interaction of peptides with inducible Hsp70 (HSPA1A) and its BETA construct.

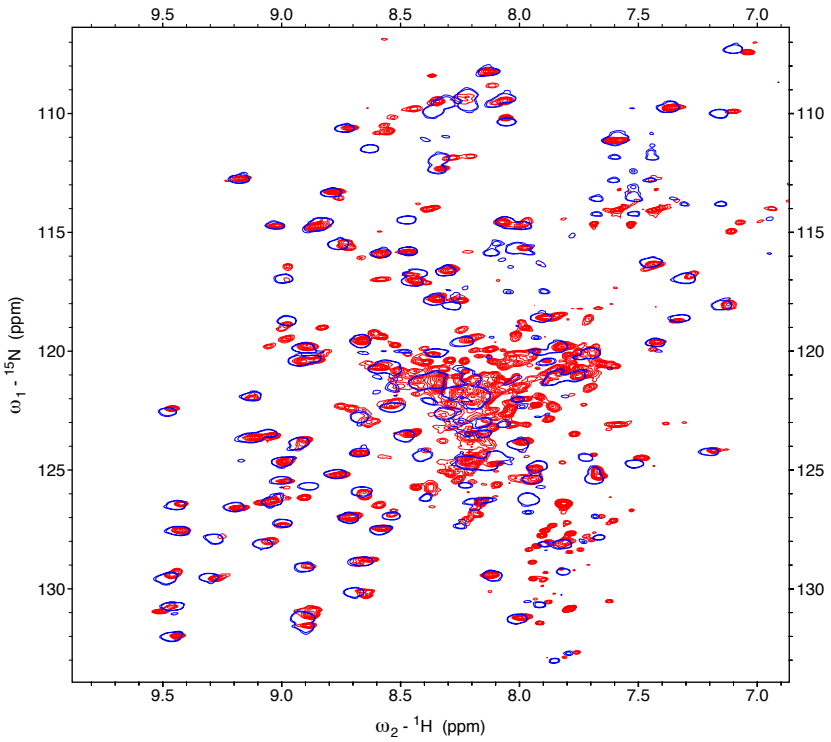

Figure S4.

^{15}N-^{1}H TROSY spectra of BETA with unlabeled TAU1 in trans (blue; 113 residues) and the BETA-TAU1-CIS construct (red, 165 residues)

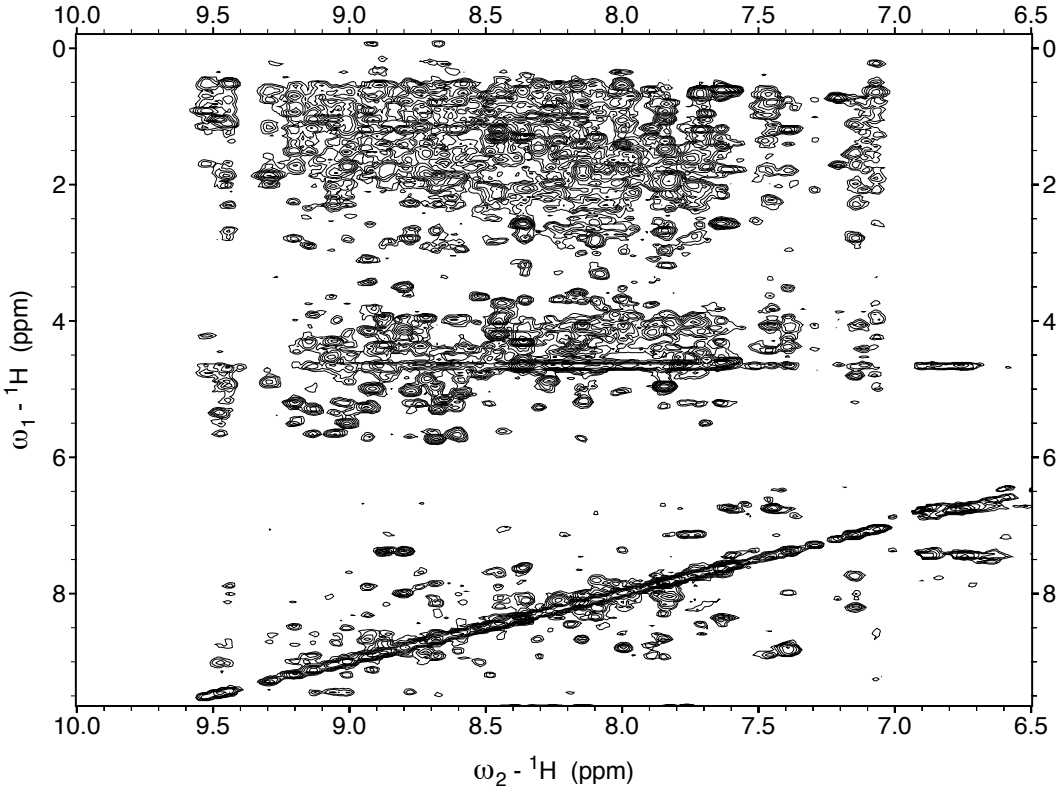

Figure S5. ^{1}H-^{1}HN Projection of the NUS 3D ^{1}H-τ_M-^{15}N-^{1}HN NOESY-TROSY spectrum of BETA-TAU1-CIS. The mixing time τ_M was 200 ms.

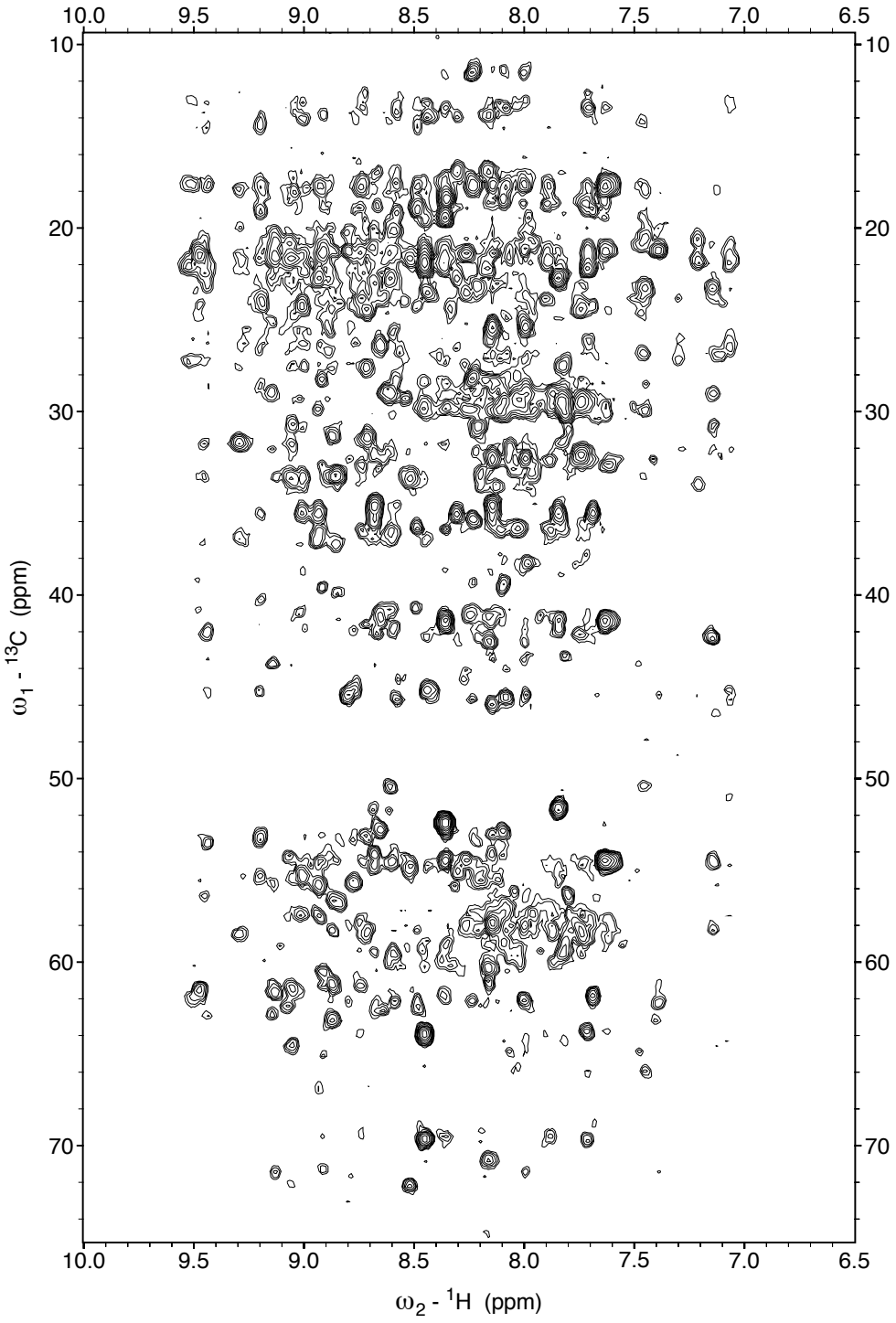

Figure S6. ^{13}C-^{1}HN Projection of the NUS 3D (^{1}H)-^{13}C-(^{1}H)-τ_M-^{15}N-^{1}HN HMQC-NOESY-TROSY spectrum of BETA-TAU1-CIS. The mixing time τ_M was 200 ms.

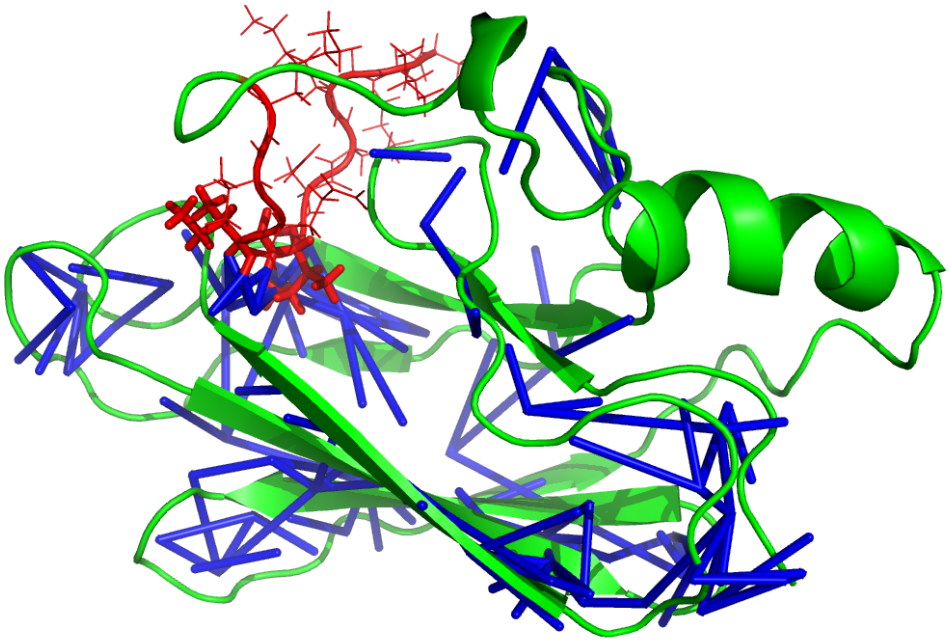

Figure S7
The molecular replacement structure for Hsc70 SBD with the sequence GKVQIINKKG in cis (red). In blue are non-sequential NOEs identified in the set of 3D NOE spectra confirming the structure of the BETA domain, and establishing details of the binding of GKVQIINKKG.

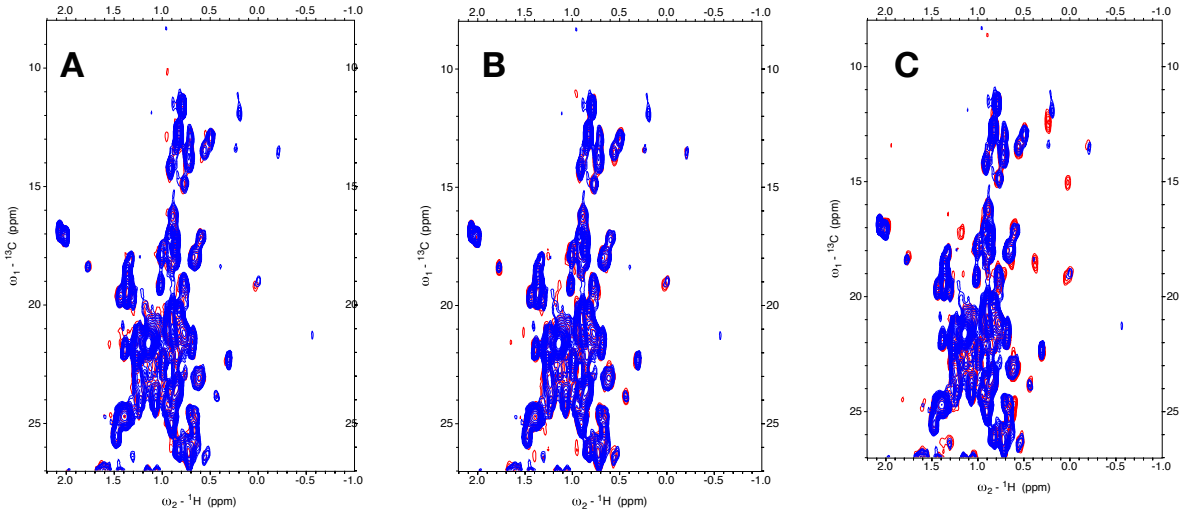

Figure S8.

Interaction tests with other peptides, methyl region of ^{13}C-^{1}H HSQC spectra

Panel A, 11 µM BETA in blue superposed on 11 µM BETA + 19 µM KFERQ (CMA recognition sequence)

Panel B, 11 µM BETA in blue superposed on 11 µM BETA + 12 µM MATLEKLMKAFESLKSF (Huntintin)

Panel C, 11 µM BETA in blue superposed on 11 µM BETA + 12 µM insulin C-peptide (red)

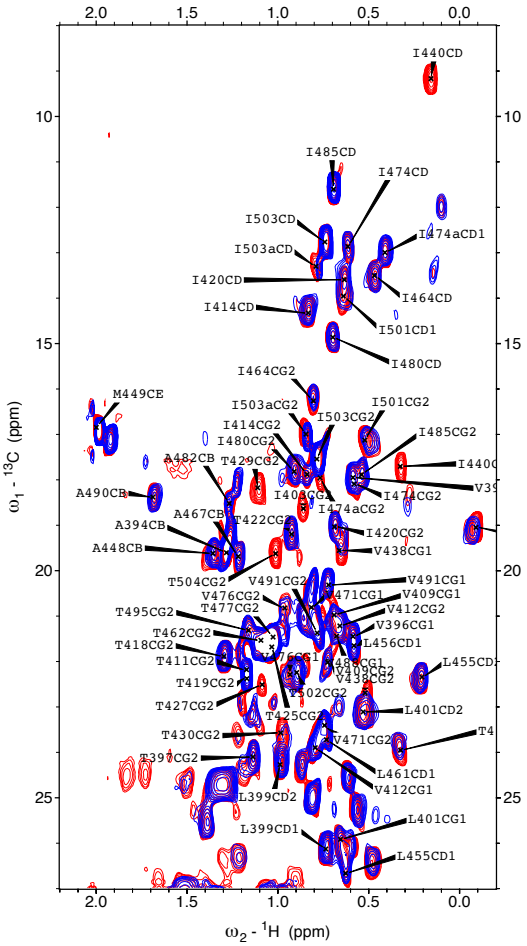

Figure 9.

Overlay of the ^{1}H-^{13}C constant-time HSQC of 35 µM Hsc70 BETA (blue) and 35 µM Hsc70-BETA with 200 µM GKVQIINKKG (red). The assignments are indicated (BMRB accession code 27395)