Supporting Information:

Double Lanthanide-Binding Tags: Design, Photophysical Properties and NMR Applications

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Solid phase peptide synthesis. The **dLBT1** peptide was synthesized on Fmoc-PAL-PEG-polystyrene resin (180 μmol/g, Applied Biosystems) on an ABI 431A Peptide Synthesizer (Applied Biosystems). Standard Fmoc (9-fluoenylmethoxycarbonyl) coupling procedures were used, including deprotection by 20% 4-methylpiperidine in NMP (1-methyl-2-pyrrolidinone, Sigma-Aldrich), double coupling, and acetic anhydride capping after each step. Peptide coupling steps used 4 eq. Fmoc-amino acid (GenScript or NovaBioChem) per eq. resin, N-Hydroxybenzotriazole (HOBt, GenScript) and 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, GenScript) activating agents, with diisopropylethylamine (DIPEA) in NMP, for one hour at room temperature. The full-length peptide N-terminus was used as a free amine. Side-chain deprotection and cleavage from the resin (to yield a C-terminal amide) was carried out using a cocktail of 94% trifluoroacetic acid (TFA), 2.5% 1,2 ethanedithiol (EDT),

2.5% H_2O, and 1% triisopropyl silane (TIS), shaking for 2 hours at room temperature. HPLC purification (*vide infra)* was unsuccessful at giving a purity greater than 60%.

Cloning of the GST-dLBT2 and GST-dLBT3 constructs. The gene for the double-LBTs were inserted into the pGEX-4T-2 plasmid (Amersham Biosciences), using a megaprimer strategy. A TEV (Tobacco Etch Virus) protease cleavage site was included to facilitate removal of the N-terminal GST (glutathione-S-transferase) fusion protein; the recognition sequence, ENLYFQG, is cleaved such that the C-terminal (dLBT) fragment is left with a terminal glycine. Two smaller primers (obtained from Invitrogen), "TEV-dLBT2_for_*BamHI*" (CGGGATCCGAAAACCTGTACTTCCAGGGUTACATCGACACCAACAACGATG GTTGGATTGAAGGCGACGAACTGTATAT) and "dLBT_rev_*XhoI*" (CCGCTCGAGTCACGCCAGCAGTTCATCGCCTTCGATCCAACCGTCGTTGTTG GTATCGATATACAGTTCGTCGCCTTC) were elongated by PCR using Platinum Taq polymerase (Invitrogen) to generate the double-stranded **dLBT2** insert. For **dLBT3**, the primer "TEV-dLBT3_for_*BamHI*" (CGGGATCCGAAAACCTGTACTTCCAGGGTTACATCGACACCAACAACGATG GTTGGATTGAAGGCGACGAACTGTATAT) was used with the same "dLBT_rev_*XhoI*" primer. The PCR products were digested and inserted into the pGEX-4T-2 vector.

Protein Sequence of GST-**dLBT2:**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPN

LPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRI

AYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVV

LYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHP

PKSDLVPRGS*ENLYFQ***GYIDTNNDGWIEGDELYIDTNNDGWIEGDELLA**

Protein Sequence of GST-**dLBT3:**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPN

LPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRI

AYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVV

LYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHP

PKSDLVPRGS*ENLYFQ***GPGYIDTNNDGWIEGDELYIDTNNDGWIEGDELLA**

Expression and purification of the GST-dLBT2 and –dLBT3 constructs. Starting from an overnight culture, BL21-(DE3)-Gold cells (Stratagene) expressing the desired GST-fusion were grown in 10 L of LB media containing carbenicillin antibiotic in a fermenter (BIOFLO 110, New Brunswick Scientific), at 37 °C. When the OD_{600} reached 0.45, the temperature was reduced to 30 °C, and protein production was induced with 0.2 mM isopropyl-β-D-1-thiogaltopyranoside (IPTG) at OD_{600} of about 0.65. After 5 hours, the cells were harvested by centrifugation and frozen at -80 °C until needed.

All purification was performed at 4 °C unless otherwise noted. The cell pellet from the 10 L growth was thawed and resuspended in a lysis buffer (400 mL PBS, 1

mg/mL lysozyme, 400 μL Protease Inhibitor Cocktail Set III (Calbiochem), 1 mM DTT), and incubated at 4 °C for about 20 minutes. 50 mL of a 5% NP40 solution (in PBS) was then added, followed by 10 minutes of rocking. Cells were lysed by sonication, and cellular debris was pelleted by centrifugation. Supernatant was incubated for 45 minutes with Glutathione-sepharose resin (Amersham Biosciences) at room temperature, washed extensively with PBS, and the GST-construct was then eluted using a 10 mM glutathione solution in 50 mM Tris (pH 8.0) buffer containing the same protease inhibitor cocktail. Elution fractions were analyzed by 15% SDS-PAGE and quantified using the Biorad BCA/BSA protein assay. Purified protein was stored at 4 °C until cleavage by mTEV protease.

Cleavage by mTEV protease and purification of dLBT2 and dLBT3 peptides. mTEV protease (mutant Tobacco Etch Virus protease) was expressed on site from expression vector pRK793, which was obtained from Dr. David S. Waugh; NCI, CCR, Macromolecular Crystallography Laboratory, Frederick, MD. The cleavage reaction was conducted in 50 mM Tris, 5 mM EDTA, 5 mM βME at 30 °C for 4 hours, and analyzed by 15% SDS-PAGE (*vide infra*) for completeness.

The solution was then prepared for HPLC. DMF (dimethylformamide, Sigma-Aldrich) was added to a concentration of up to 20%, and the mixture was acidified to pH < 5.0 using acetic acid. Precipitated protein was pelleted by centrifugation, and the supernatant was filtered and then purified by reverse phase HPLC on a Waters 600 automated control module with a YMC C_{18} preparative column using an acetonitrile/water gradient (5 minutes of 5% acetonitrile followed by a 30-minute linear

gradient to 95% acetonitrile) with 0.1% TFA. A Waters 2487 dual wavelength absorbance detector recorded at 228 nm and 280 nm. Peptide identification and purity was confirmed by MALDI mass spectroscopy on a PerSeptive Biosystems Voyager MALDI-TOF instrument using a 2,5-dihydroxybenzoic acid matrix.

Protein Sequence of His_6**-dLBT3-*Ubiquitin*:**

MKHHHHHH**GPGYIDTNNDGWIEGDELYIDTNNDGWIEGDELLA*MQIFVKTL TGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKE STLHLVLRLRGG***

Cloning, expression and purification of dLBT3-Ubiquitin. The construct for the expression of **dLBT3-**Ubiquitin was generated by inserting the appropriate **dLBT3**-encoding gene into the pET11a plasmid (Novagen). The His_6**-dLBT3-**Ubiquitin construct was expressed in *E. coli* BL21(DE3) using M9 minimal medium for isotopic labeling or LB medium for unlabeled samples. At an OD_{600} of 0.6 to 0.8, protein expression was induced by addition of 500 mg isopropyl ß-D-1-thiogalactopyranoside (IPTG) per liter of culture medium. After reaching an OD_{600} of 1.2 to 1.4, cells were harvested by centrifugation at 6,000 g. Cell pellets were solved in buffer 1, containing 100 mM NaCl and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7. After sonification and removal of cellular debris by centrifugation at 10,000 g, the His-tagged protein was extracted of the lysate by IMAC (immobilized metal affinity chromatography) on a Quiagen Ni-NTA-Superflow column. The protein was eluted with buffer 2, containing 100 mM NaCl, 20 mM HEPES and 150 mM imidazole at pH 7,

which was subsequently removed by dialysis against buffer 1. The His-tag was removed following the Quiagen TAGZyme protocol. The protein solution was incubated with 0.05 U of activated dipeptidyl aminopeptidase I (DAPase) per mg of protein for 2 hours at 37 °C. The DAPase I cleaves N-terminal dipeptides until it reaches the GPG stop point. Uncleaved protein and the C-terminal His-tagged DAPase I were removed using an inverse IMAC. For removal of fragments, the protein solution was again dialysed against buffer 1.

Native Ubiquitin was expressed in *E. coli* BL21(DE3) using M9 minimal medium for isotopic labeling or LB medium for unlabeled samples. At an OD_{600} of 0.6 to 0.8, protein expression was induced by addition of 500 mg IPTG per liter of culture medium. After reaching a OD_{600} of 1.2 to 1.4, cells were harvested by centrifugation at 6,000 g. Cell pellets were solved in buffer 3, containing 50 mM ammoniumacetate at pH 5. After sonification and removal of cellular debris by centrifugation at 10,000 g, the solution was heated to 90 °C to precipitate other proteins. Impurities were removed on a sepharose cation exchange column, followed by dialysis against buffer 3 and lyophilization.

The construct GPG-**sLBT1**-Ubiquitin was expressed and purified in a similar fashion, as described in the literature (J. Wöhnert et al, *J. Am. Chem. Soc.* **2003**, *125*, 13338-13339).

PAGE and Western Blot Analysis. Proteins were loaded onto 15% SDS-polyacrylamide gels in denaturing buffer, and subjected to electrophoresis at 120 V for about two hours. The gel was then washed twice for 15 minutes (each wash) with 100 mM NaCl, 10 mM HEPES pH 7.0 buffer, followed by incubation for 20 minutes in the same buffer containing 4 μM Tb^{3+}. Luminescent bands were visualized and processed as

described previously (K. J. Franz et al, *ChemBioChem* **2003**, *4*, 265-271) (Figure S1a). The same gel was then stained with Gel Code Blue (Pierce) to visualize all bands (Figure S1b).

Alternatively, an "anti-LBT" Western blot could be taken of the gel. The primary (monoclonal) antibody that recognized the antigen sequence Ile-Glu-Gly-Asp-Glu-Leu-Leu was generated in rabbits (Quality Controlled Biochemicals, Hopkinton, MA), and a goat-anti-rabbit alkaline phosphatase stain was used for visualization (Figure S1c).

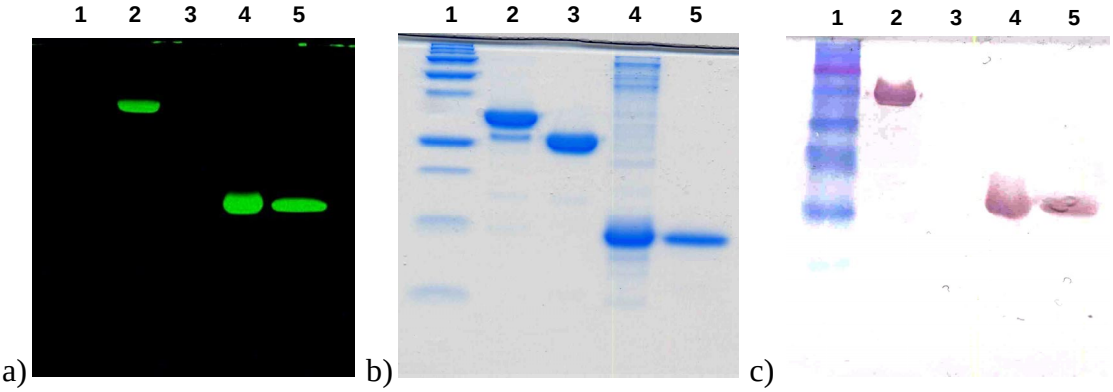

Figure S1. Purification and visualization of the dLBT constructs. For all panels, lane 1: protein mass ladder; lane 2: GST-ENLYFQ-**dLBT2**; lane 3: GST-ENLYFQ; lane 4: His_6-**dLBT3**-ubiquitin; lane 5: **dLBT3**-ubiquitin. (a) 15% PAGE treated with 4 μM Tb^{3+}, visualized on a UV-transilluminator, with enhancement for color and contrast. (b) The same gel, stained with Coomassie brilliant blue. (c) Western blot analysis using a primary LBT-antibody.

NMR ^{15}N Relaxation Data.

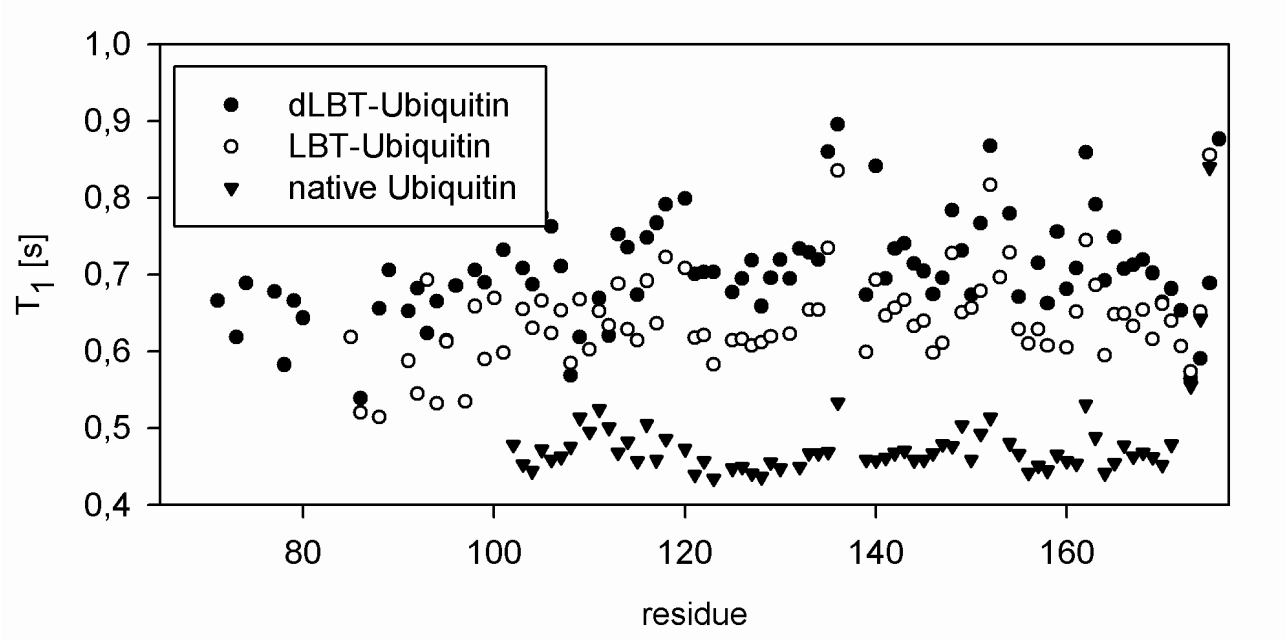

Figure S2a. ^{15}N relaxation data for **dLBT3**-ubiquitin, GPG-**sLBT1**-ubiquitin and native ubiquitin: (a) Spin-lattice relaxation time T_1; (Residues are numbered as in Figure 7, with the first methionine of ubiquitin set as M101.)

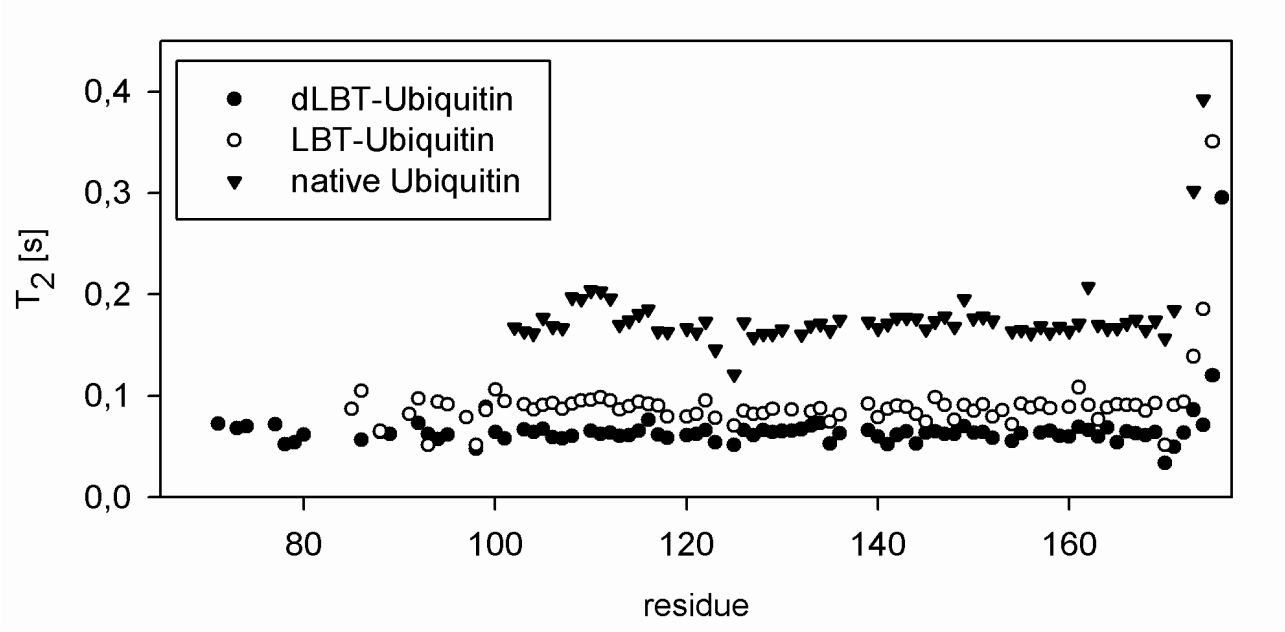

Figure S2b. ^{15}N relaxation data for **dLBT3**-ubiquitin, GPG-**sLBT1**-ubiquitin and native ubiquitin: (b) Spin-spin relaxation time T_2

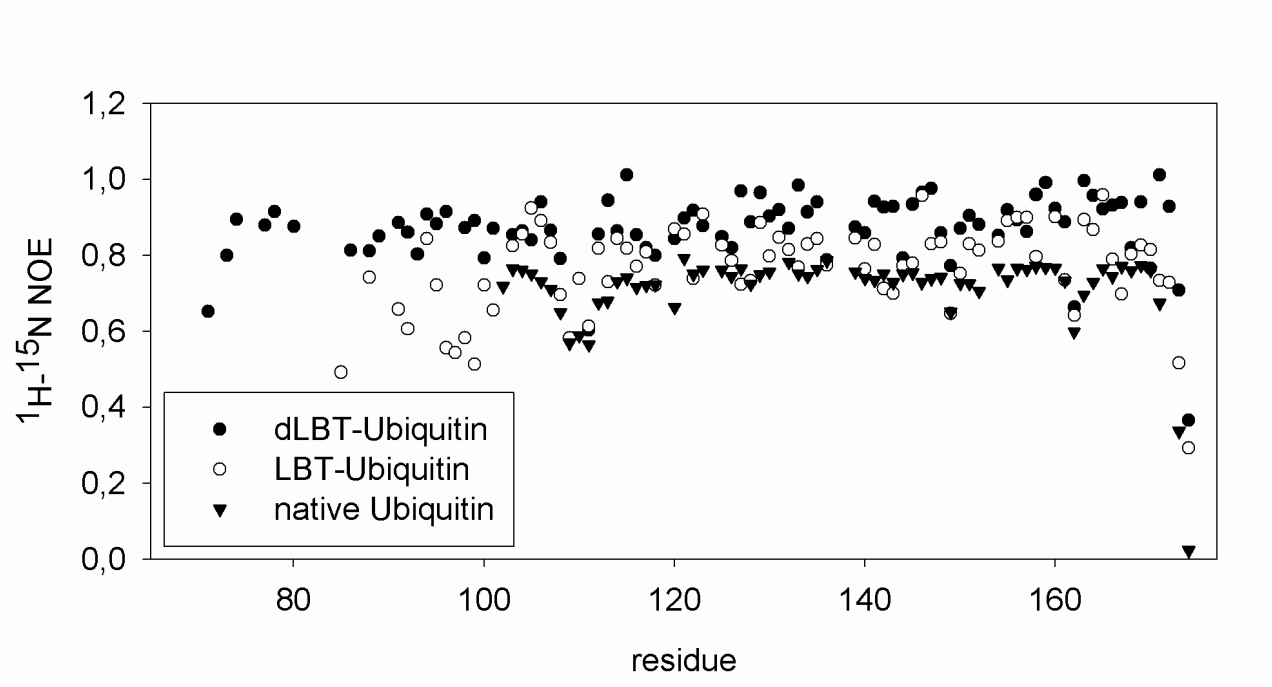

Figure S2c. ^{15}N relaxation data for **dLBT3**-ubiquitin, GPG-**sLBT1**-ubiquitin and native ubiquitin: (c) ^{1}H ^{15}N heteronuclear NOE.

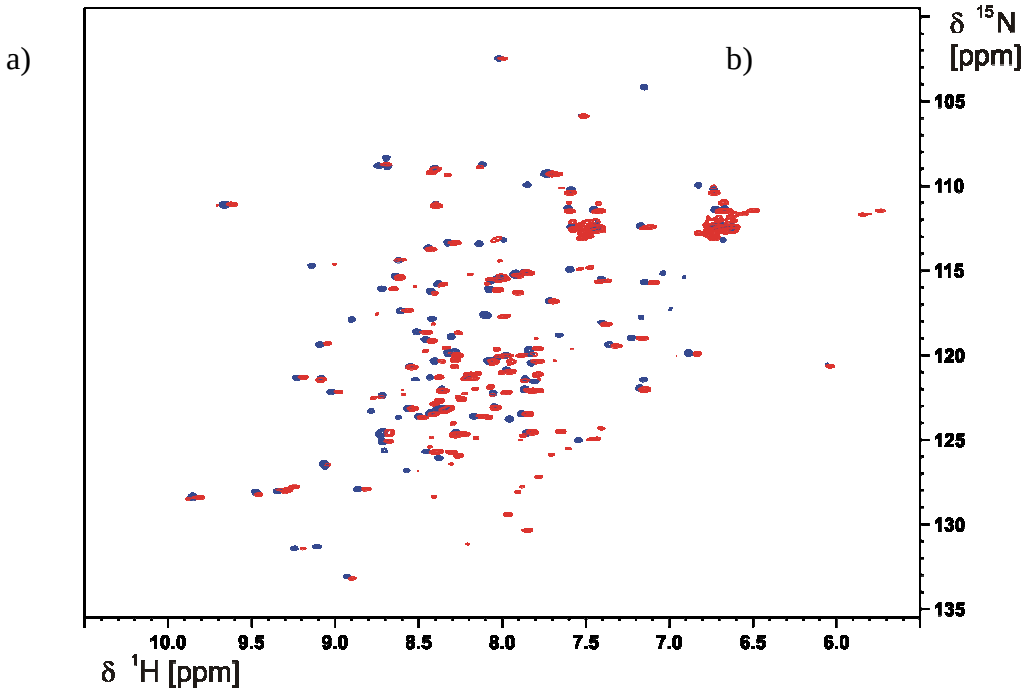

Figure S3a. ^{15}N HSQC spectrum comparing **dLBT3**-ubiquitin (2 eq. Lu^{3+}, diamagnetic) shown in blue with wildtype ubiquitin shown in red.

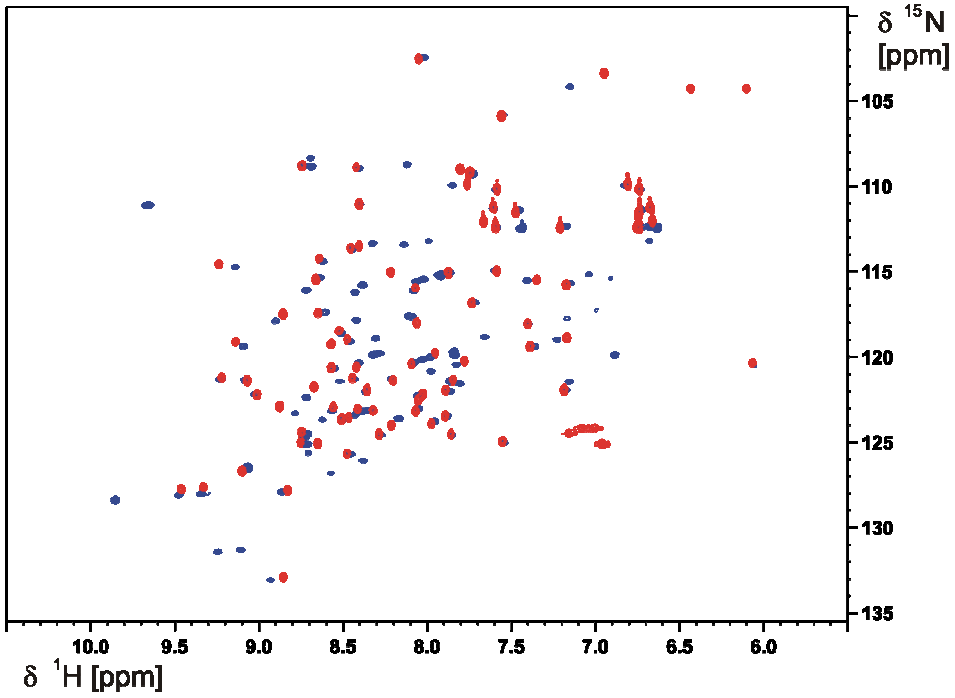

Figure S3b.
^{15}N HSQC spectrum comparing **dLBT3**-ubiquitin (2 eq. Lu^{3+}, diamagnetic) shown in blue with **dLBT3**-ubiquitin (2 eq. Eu^{3+}, paramagnetic) shown in red.

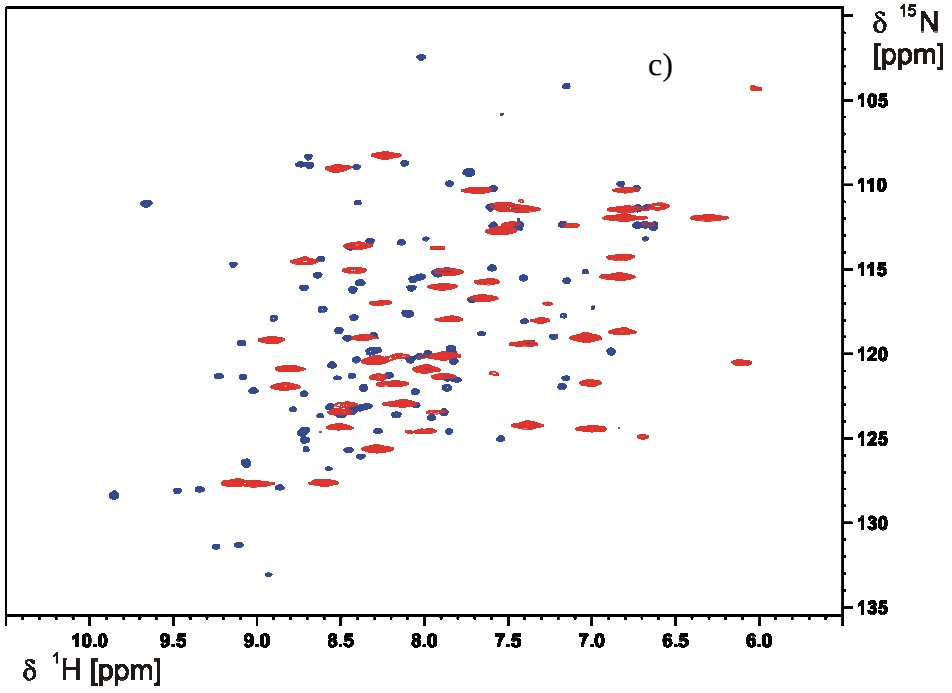

Figure S3b.
^{15}N HSQC spectrum comparing **dLBT3**-ubiquitin (2 eq. Lu^{3+}, diamagnetic) shown in blue with **dLBT3**-ubiquitin (2 eq. Tm^{3+}, paramagnetic) shown in red.

Table S1. Relaxation data and order parameters for **dLBT3**-ubiquitin.

Residue	HetNOE	ΔNOE	T1	ΔT1	T2	ΔT2	S^2	ΔS^2
71	0.6511	0.0092	0.6659	0.0181	0.0723	0.0059	0.7296	0.0276
73	0.7999	0.0113	0.6185	0.0108	0.0681	0.0013	0.6291	0.0703
74	0.8943	0.0126	0.6885	0.0133	0.0701	0.0010	0.8945	0.0414
77	0.8780	0.0124	0.6774	0.0216	0.0716	0.0025	0.7623	0.0679
80	0.8760	0.0124	0.6434	0.0163	0.0615	0.0021	0.6594	0.0672
88	0.8104	0.0115	0.6560	0.0102	0.0642	0.0009	0.6863	0.0549
89	0.8499	0.0120	0.7059	0.0058	0.0619	0.0012	0.7636	0.0359
92	0.8603	0.0122	0.6823	0.0149	0.0730	0.0015	0.7471	0.0514
93	0.8019	0.0113	0.6233	0.0146	0.0620	0.0034	0.6236	0.0717
94	0.9070	0.0128	0.6654	0.0106	0.0569	0.0014	0.6517	0.0555
95	0.8829	0.0125	0.6146	0.0249	0.0619	0.0015	0.8038	0.0397
98	0.8715	0.0123	0.7057	0.0284	0.0476	0.0025	0.8451	0.0566
99	0.8913	0.0126	0.6897	0.0302	0.0889	0.0395	0.8562	0.217
100	0.7919	0.0112	0.8190	0.0130	0.0640	0.0019	0.9573	0.012
101	0.8707	0.0123	0.7323	0.0178	0.0581	0.0032	0.8607	0.0365
103	0.8536	0.0121	0.7079	0.0102	0.0666	0.0014	0.8028	0.037
104	0.8637	0.0122	0.6868	0.0196	0.0640	0.0016	0.7622	0.0556
105	0.8406	0.0119	0.7774	0.0204	0.0675	0.0019	0.9207	0.039
106	0.9407	0.0133	0.7626	0.0225	0.0590	0.0024	0.9751	0.0322
107	0.8647	0.0122	0.7108	0.0232	0.0578	0.0007	0.7942	0.057
108	0.7907	0.0112	0.5683	0.0134	0.0601	0.0042	0.5434	0.0764
111	0.6022	0.0085	0.6688	0.0160	0.0622	0.0016	0.7109	0.0233
112	0.8560	0.0121	0.6207	0.0194	0.0637	0.0011	0.5908	0.0843
113	0.9443	0.0134	0.7527	0.0213	0.0603	0.0047	0.9335	0.037
114	0.8634	0.0122	0.7358	0.0237	0.0608	0.0010	0.8791	0.0504
115	1.0110	0.0143	0.6734	0.0126	0.0654	0.0017	0.7343	0.0513
116	0.8537	0.0121	0.7482	0.0136	0.0760	0.0114	0.9246	0.0685
118	0.7999	0.0113	0.7916	0.0208	0.0587	0.0009	0.9473	0.0228
120	0.8438	0.0119	0.7992	0.0192	0.0611	0.0028	0.9180	0.0307
121	0.8976	0.0127	0.7010	0.0130	0.0623	0.0013	0.7949	0.0463
122	0.9178	0.0130	0.7033	0.0173	0.0659	0.0018	0.8003	0.0461
123	0.8765	0.0124	0.7033	0.0335	0.0543	0.0023	0.8667	0.0650
125	0.8476	0.0120	0.6767	0.0156	0.0513	0.0009	0.7256	0.0546
126	0.8193	0.0116	0.6949	0.0216	0.0657	0.0016	0.7596	0.0643
127	0.9679	0.0137	0.7183	0.0104	0.0607	0.0011	0.8338	0.0318
128	0.8879	0.0126	0.6587	0.0053	0.0662	0.0012	0.6663	0.0477
129	0.9647	0.0136	0.6952	0.0243	0.0640	0.0025	0.8830	0.0537
130	0.9023	0.0128	0.7195	0.0143	0.0651	0.0008	0.8390	0.0391
131	0.9199	0.0130	0.6951	0.0174	0.0654	0.0010	0.8110	0.0481
132	0.8705	0.0123	0.7340	0.0264	0.0670	0.0011	0.8517	0.0530
133	0.9839	0.0139	0.7283	0.0116	0.0704	0.0034	0.9270	0.0391
134	0.9140	0.0129	0.7192	0.0205	0.0729	0.0007	0.8768	0.0473
135	0.9407	0.0133	0.8598	0.0146	0.0525	0.0011	0.9741	0
136	0.7866	0.0111	0.8951	0.0169	0.0628	0.0011	0.9081	0.0165

139	0.8748	0.0124	0.6733	0.0090	0.0660	0.0009	0.7158	0.0485
140	0.8587	0.0121	0.8413	0.0382	0.0600	0.0031	0.9597	0.0409
141	0.9422	0.0133	0.6945	0.0089	0.0521	0.0021	0.7517	0.0450
142	0.9270	0.0131	0.7334	0.0127	0.0613	0.0013	0.8744	0.0295
143	0.9275	0.0131	0.7403	0.0132	0.0646	0.0010	0.8921	0.0318
144	0.7923	0.0112	0.7144	0.0301	0.0529	0.0019	0.8723	0.0424
145	0.9339	0.0132	0.7044	0.0164	0.0633	0.0020	0.8170	0.0465
146	0.9650	0.0136	0.6745	0.0374	0.0646	0.0065	0	0.0443
147	0.9756	0.0138	0.6953	0.0099	0.0621	0.0031	0.7633	0.0480
148	0.8585	0.0121	0.7836	0.0164	0.0625	0.0004	0.9111	0.0327
149	0.7718	0.0109	0.7311	0.0119	0.0699	0.0013	0.8630	0.0139
150	0.8701	0.0123	0.6735	0.0109	0.0637	0.0020	0.7147	0.0497
151	0.9043	0.0128	0.7663	0.0226	0.0643	0.0019	0.9788	0.0258
152	0.8812	0.0125	0.8677	0.0261	0.0587	0.0011	0.9735	0
154	0.8517	0.0120	0.7794	0.0124	0.0551	0.0028	0.9509	0.0266
155	0.9187	0.0130	0.6711	0.0274	0.0628	0.0006	0.8244	0.0682
157	0.8625	0.0122	0.7153	0.0134	0.0633	0.0010	0.8213	0.0410
158	0.9592	0.0136	0.6623	0.0219	0.0656	0.0007	0.7886	0.0662
159	0.9911	0.0140	0.7555	0.0184	0.0606	0.0013	0.9603	0.0300
160	0.9233	0.0131	0.6810	0.0122	0.0595	0.0029	0.6875	0.0532
161	0.8870	0.0125	0.7080	0.0081	0.0694	0.0013	0.8061	0.0341
162	0.6628	0.0094	0.8593	0.0110	0.0660	0.0017	0.9253	0.0093
163	0.9967	0.0141	0.7916	0.0153	0.0599	0.0008	0.9783	0.0185
164	0.9563	0.0135	0.6919	0.0069	0.0683	0.0012	0.7634	0.0424
165	0.9207	0.0130	0.7491	0.0190	0.0542	0.0088	0.8944	0.0378
166	0.9316	0.0132	0.7077	0.0105	0.0648	0.0014	0.7858	0.0389
167	0.9381	0.0133	0.7129	0.0105	0.0629	0.0023	0.8138	0.0382
168	0.8190	0.0116	0.7195	0.0249	0.0610	0.0010	0.7890	0.0642
169	0.9407	0.0133	0.7019	0.0171	0.0644	0.0021	0.8228	0.045
170	0.7657	0.0108	0.6642	0.0212	0.0336	0.0086	0.7895	0.0322
171	1.0112	0.0143	0.6817	0.0738	0.0493	0.0090		0
172	0.9285	0.0131	0.6532	0.0160	0.0634	0.0041	0.7819	0.0549
173	0.7068	0.0100	0.5642	0.0195	0.0860	0.0022	0.5884	0.0415
174	0.3659	0.0052	0.5910	0.0205	0.0713	0.0023	0.4280	0.0400
175	-1.2838	0.0182	0.6889	0.0504	0.1203	0.0111	0.3409	0.1358

Table S2. Relaxation data and order parameters for GPG-**sLBT1**-ubiquitin

Residue	HetNOE	ΔNOE	T1	ΔT1	T2	ΔT2	S^2	ΔS^2
85	0.4918	0.0070	0.6185	0.0313	0.0872	0.0036	0.6754	0.0745
88	0.7412	0.0105	0.5141	0.0109	0.0650	0.0024	0.5350	0.0395
91	0.6574	0.0093	0.5875	0.0260	0.0818	0.0038	0.7479	0.0689
92	0.6051	0.0086	0.5448	0.0209	0.0968	0.0036	0.5763	0.0609
94	0.8435	0.0119	0.5322	0.0189	0.0938	0.0041	0.6380	0.0619
95	0.7213	0.0102	0.6122	0.0247	0.0913	0.0034	0.8332	0.0539
97	0.5431	0.0077	0.5344	0.0143	0.0790	0.0028	0.4773	0.0446
98	0.5825	0.0082	0.6586	0.0254	0.0511	0.0120	0.8463	0.0453
99	0.5126	0.0072	0.5898	0.0245	0.0859	0.0048	0.6464	0.0639
100	0.7207	0.0102	0.6696	0.0185	0.1058	0.0017	0.8799	0.0226
101	0.6552	0.0093	0.5982	0.0140	0.0944	0.0030	0.7386	0.0325
104	0.8556	0.0121	0.6305	0.0048	0.0864	0.0006	0.9044	0.0165
105	0.9241	0.0131	0.6656	0.0085	0.0907	0.0013	0.9970	0
106	0.8903	0.0126	0.6239	0.0128	0.0925	0.0014	0.9442	0.0240
108	0.6962	0.0098	0.5848	0.0542	0.0921	0.0009	0.7810	0.1060
109	0.5813	0.0082	0.6676	0.0642	0.0955	0.0181	0.8918	0.0668
110	0.7381	0.0104	0.6020	0.0371	0.0960	0.0010	0.9508	0.0128
111	0.6124	0.0087	0.6526	0.0593	0.0982	0.0031	0.9069	0.0223
113	0.7295	0.0103	0.6876	0.0115	0.0867	0.0036	0.9454	0.0131
114	0.8443	0.0119	0.6291	0.0260	0.0896	0.0017	0.9634	0.0471
115	0.8184	0.0116	0.6145	0.0119	0.0940	0.0029	0.8952	0.0356
116	0.7703	0.0109	0.6917	0.0155	0.0918	0.0027	0.9733	0.0107
118	0.7213	0.0102	0.7227	0.0219	0.0795	0.0018	0.9287	0.0253
120	0.8694	0.0123	0.7081	0.0191	0.0792	0.0020	0.9608	0
121	0.8557	0.0121	0.6173	0.0080	0.0820	0.0017	0.8848	0.0269
122	0.7378	0.0104	0.6211	0.0509	0.0951	0.0028	0.9624	0.0239
123	0.9076	0.0128	0.5834	0.0117	0.0783	0.0040	0.8123	0.0460
125	0.8261	0.0117	0.6145	0.0112	0.0704	0.0007	0.8294	0.0385
126	0.7853	0.0111	0.6162	0.0094	0.0850	0.0016	0.8666	0.0220
127	0.7236	0.0102	0.6076	0.0097	0.0817	0.0020	0.8175	0.0202
128	0.7325	0.0104	0.6115	0.0078	0.0828	0.0019	0.8072	0.0162
129	0.8865	0.0125	0.6193	0.0124	0.0868	0.0038	0.9240	0.0252
131	0.8460	0.0120	0.6226	0.0048	0.0864	0.0018	0.9151	0.0161
133	0.7682	0.0109	0.6538	0.0243	0.0843	0.0020	0.9012	0.0399
135	0.8438	0.0119	0.7344	0.0106	0.0744	0.0007	0.9629	0.0145
136	0.7741	0.0109	0.8356	0.0201	0.0814	0.0018	0.8373	0.0177
139	0.8457	0.0120	0.5993	0.0138	0.0924	0.0018	0.7863	0.0439
140	0.7635	0.0108	0.6933	0.0138	0.0786	0.0018	0.9451	0.0166
142	0.7118	0.0101	0.6565	0.0114	0.0902	0.0010	0.9203	0.0220
143	0.6999	0.0099	0.6671	0.0319	0.0891	0.0022	0.9427	0.0353
144	0.7720	0.0109	0.6328	0.0133	0.0818	0.0015	0.9120	0.0259
145	0.7793	0.0110	0.6393	0.0223	0.0740	0.0020	0.9345	0.0345
147	0.8294	0.0117	0.6107	0.0228	0.0911	0.0008	0.8799	0.0618
148	0.8356	0.0118	0.7279	0.0093	0.0760	0.0016	0.9478	0.0112
149	0.6461	0.0091	0.6506	0.0140	0.0909	0.0012	0.8510	0.0291
150	0.7510	0.0106	0.6564	0.0172	0.0853	0.0031	0.9186	0.0318
151	0.8292	0.0117	0.6786	0.0318	0.0914	0.0009	0.9788	0.0094

152	0.8119	0.0115	0.8167	0.0343	0.0793	0.0007	0.8484	0.0357
154	0.8361	0.0118	0.7287	0.0177	0.0718	0.0021	0.9515	0.0262
155	0.8901	0.0126	0.6291	0.0188	0.0919	0.0025	0.9727	0.0278
156	0.8994	0.0127	0.6099	0.0143	0.0884	0.0024	0.9638	0.0228
157	0.8994	0.0127	0.6289	0.0102	0.0918	0.0018	0.9841	0.0223
158	0.7957	0.0113	0.6077	0.0171	0.0878	0.0015	0.8579	0.0519
161	0.7353	0.0104	0.6513	0.0164	0.1083	0.0015	0.8937	0.0186
162	0.6418	0.0091	0.7450	0.0174	0.0907	0.0015	0.8852	0.0201
164	0.8663	0.0123	0.5948	0.0169	0.0881	0.0023	0.8984	0.0552
165	0.9591	0.0136	0.6483	0.0069	0.0916	0.0034	0.9431	0.0270
166	0.7894	0.0112	0.6489	0.0174	0.0911	0.0016	0.9581	0.0285
167	0.6977	0.0099	0.6329	0.0153	0.0908	0.0013	0.8496	0.0291
169	0.8268	0.0117	0.6158	0.0116	0.0927	0.0019	0.8772	0.0358
171	0.7340	0.0104	0.6395	0.0052	0.0907	0.0012	0.9003	0.0124
172	0.7287	0.0103	0.6070	0.0097	0.0938	0.0017	0.8195	0.0210
173	0.5157	0.0073	0.5737	0.0243	0.1388	0.0033	0.5617	0.0230
174	0.2929	0.0041	0.6510	0.0419	0.1853	0.0059	0.4102	0.0331
175	-1.2531	0.0177	0.8558	0.1600	0.3505	0.0636	0.1656	0.0662
176	-7.7651	0.1098						