Supplementary Material

Accurate determination of rates from non-uniformly sampled relaxation data

Matthew A. Stetz and A. Joshua Wand*

Johnson Research Foundation and Department of Biochemistry & Biophysics,
University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104 USA

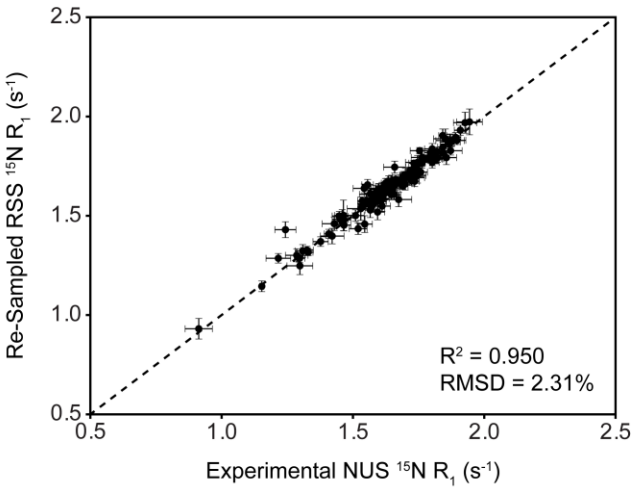

Fig. S1 Comparison of ^{15}N R_1 relaxation rates obtained from re-sampling of RSS data and from experimental NUS data for a sample of ^{15}N CaM.

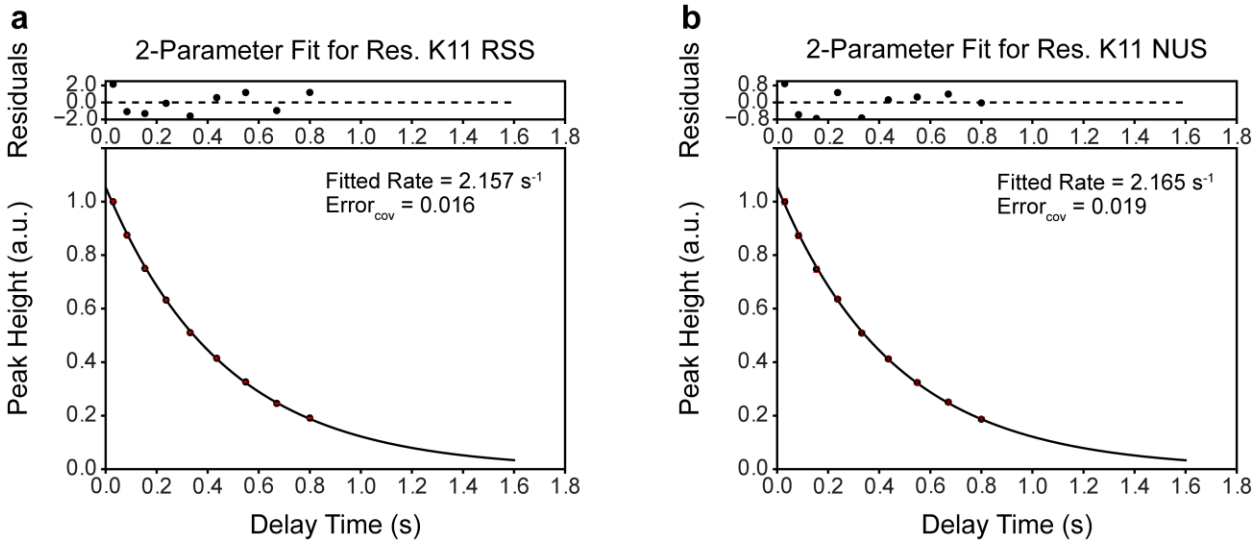

Fig. S2 (a) 2-parameter fit of ^{15}N R_1 decay for residue K11 of ubiquitin for RSS data collected with 120* RSS points points (28 ppm spectral width). (b) 2-parameter fit of ^{15}N R_1 decay for resiude K11 of ubiquitin for data collected with 25% NUS, 30* NUS points (28 ppm spectral width). Error bars representing the uncertainty in peak height are shown in red and are smaller than the symbols used. The fitted rate and error in the fitted rate determined by the covariance matrix of the fit are shown.

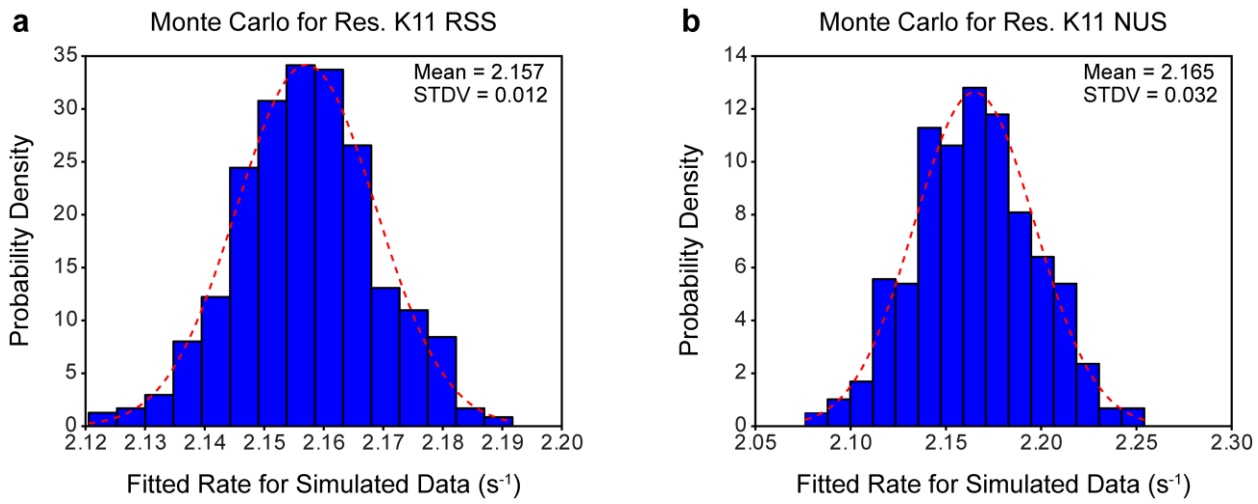

Fig. S3 (a) Histogram of fitted R_1 rates from 500 Monte Carlo simulations of the data shown in Fig. S1a. (b) Histogram of fitted R_1 rates from 500 Monte Carlo simulations of the data shown in Fig. S1b. In these simulations, peak heights measured at each delay were varied randomly within the boundaries of their uncertainties then the points were fit with a 2-parameter single exponential decay. The red dashed line is a fit to a normalized probability density function for a normal distribution. The normalization is such that the integral of the fitted function is 1. The standard deviation is an estimate of the error in the fitted rate. The error for the NUS data is ~3-fold larger than the error for the RSS data. This reflects an inherently lower reproducibility in peak height for NUS data relative to RSS data.

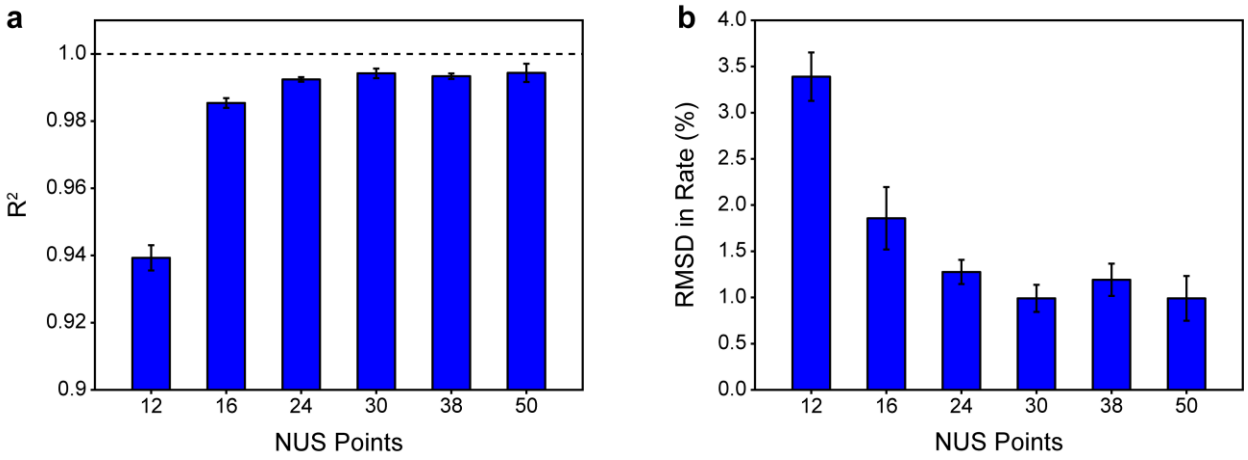

Fig. S4 The accuracy of NUS-derived relaxation rates depends strongly on the number of sampled NUS points at a fixed sampling density of 25%. Each data set has been sized to a uniform size of 512* points in the indirect dimension via zerofilling. RSS-derived and NUS-derived ^{15}N R_1 rates for ubiquitin are compared quantitatively for spectra collected with a variable number of points at a constant spectral width. (a) Dependence of the R^2 on the number of NUS points. (b) Dependence of the RMSD on the number of NUS points. Error bars are one standard deviation from the mean of three independent replicate data sets.

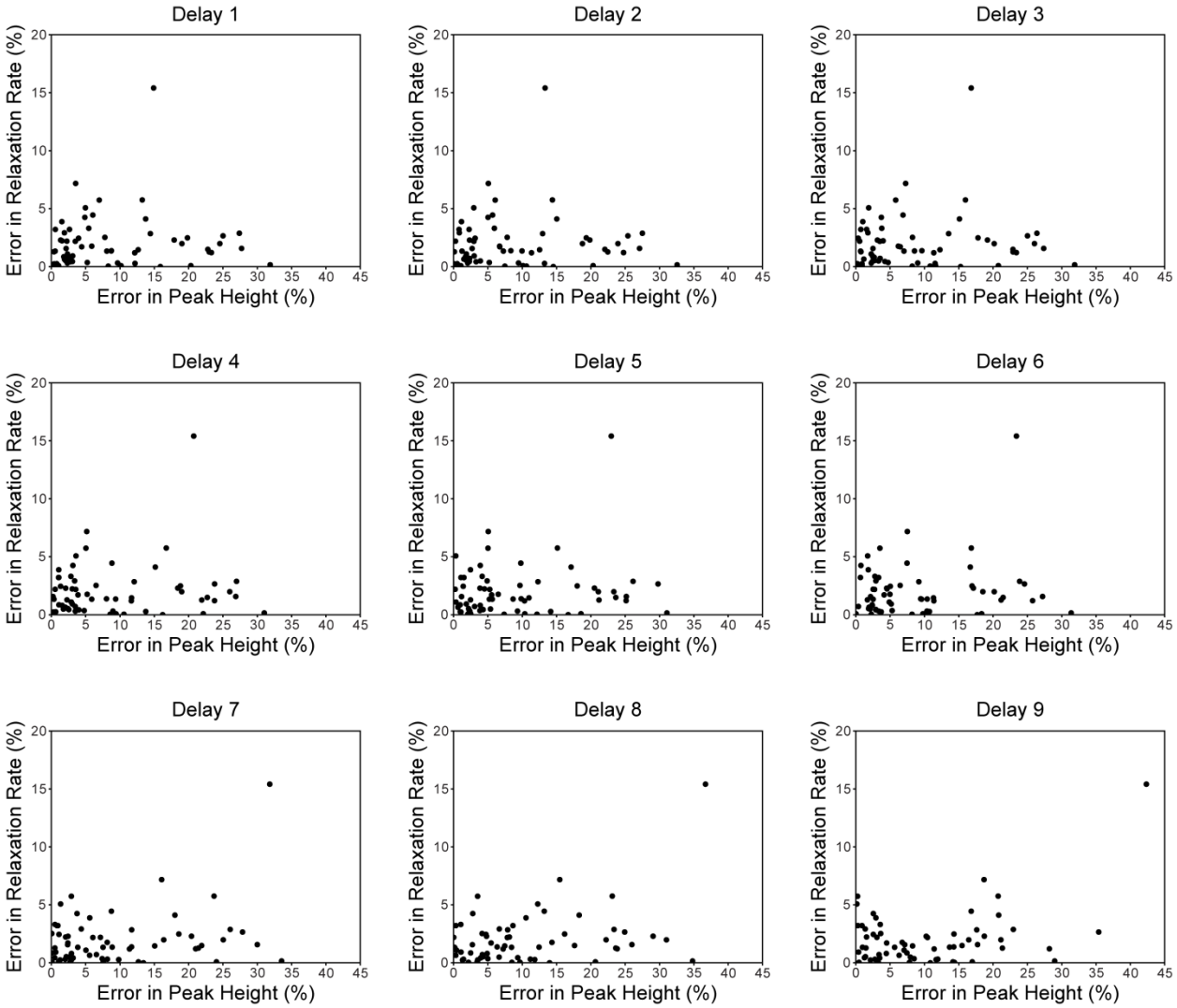

Fig. S5 The error in NUS-derived peak height is uncorrelated with the error in NUS-derived relaxation rate. NUS-derived peak heights were compared to RSS-derived references for each plane of a ^{15}N T_1 experiment collected on ubiquitin with 48* RSS points, 12* NUS points, and 28 ppm spectral width.

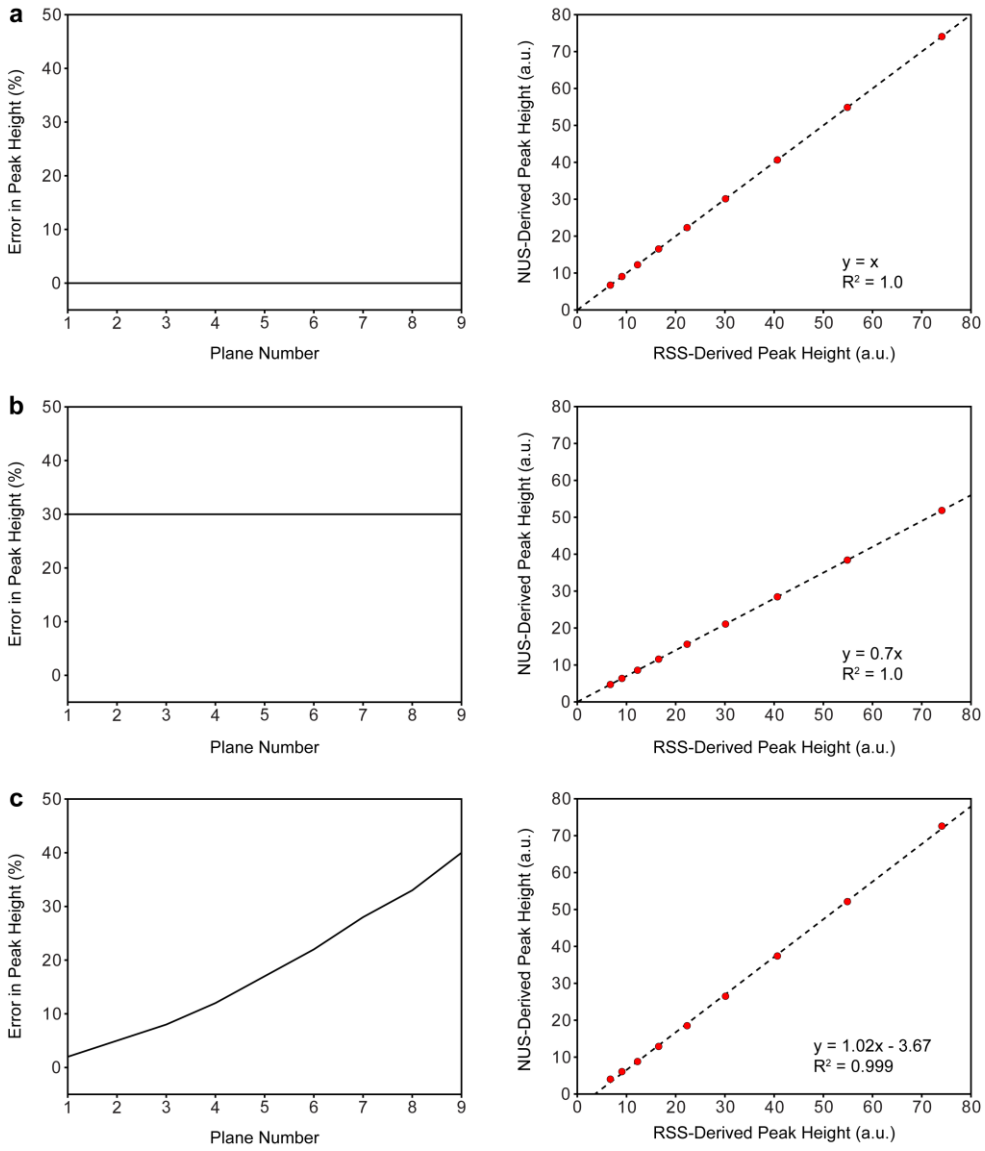

Fig. S6. Illustration of the derivation of the non-linearity factor. Left panels show the error in NUS-derived peak height for a single cross peak as a function of delay. Right panels show the correlation between RSS-derived and NUS-derived peak heights of the same cross peak where each data point is taken from a single plane in the relaxation series. (a) If peak heights are reconstructed exactly across all planes in the seires then reconstruction is accurate and consistent. Correlations of RSS-derived and NUS-derived peak heights over all planes in the series are straight lines with a slope of 1 and no intercept. (b) If peak heights are reconstructed with a 30% error across all planes in the series then reconstruction is inaccurate but consistent. Correlations of RSS-derived and NUS-derived peak heights over all planes in the series are straight lines with a scaled slope and no intercept. (c) If peak heights are reconstructed with variable errors across all planes in the series then reconstruction is neither accurate nor consistent. Correlations of RSS-derived and NUS-derived peak heights are slightly non-linear resulting in a non-zero intercept. All cross peaks are subject to this analysis and the absolute value of the intercept is defined as the non-linearity factor.

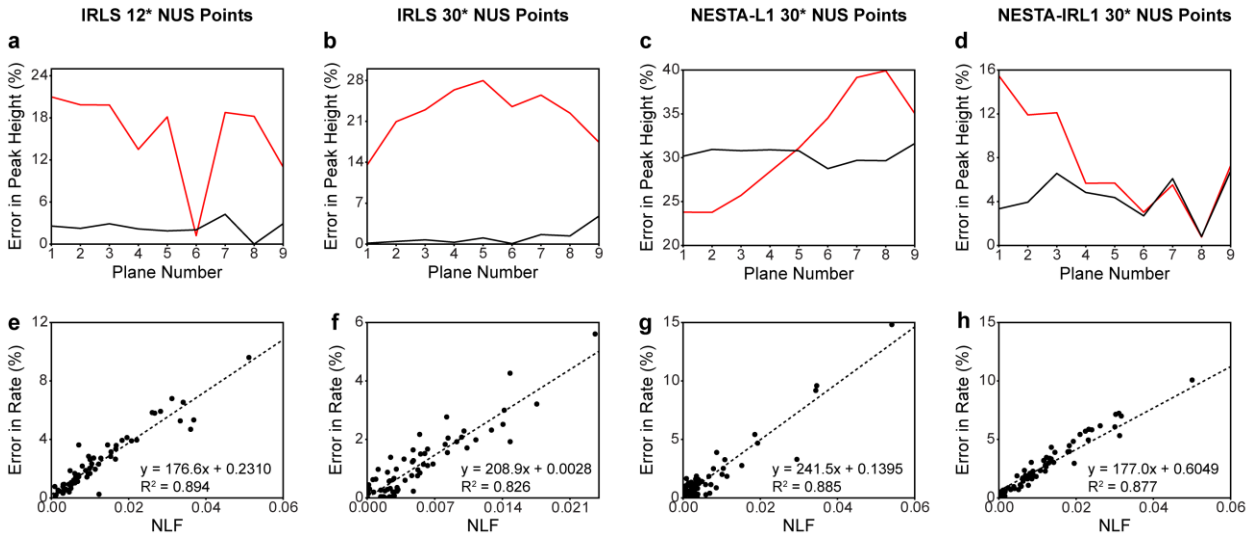

Fig. S7 The error in NUS-derived peak height as a function of relaxation delay for different reconstruction algorithms (a-d). Lines in red correposond to cross peaks which exhibited the largest error in relaxation rate. Line in black correspond to cross peaks which exhibited the smallest error in relaxation rate. The correlation between errors in relaxation rate and non-linearity factors for different reconstruction algorithms (e-f).

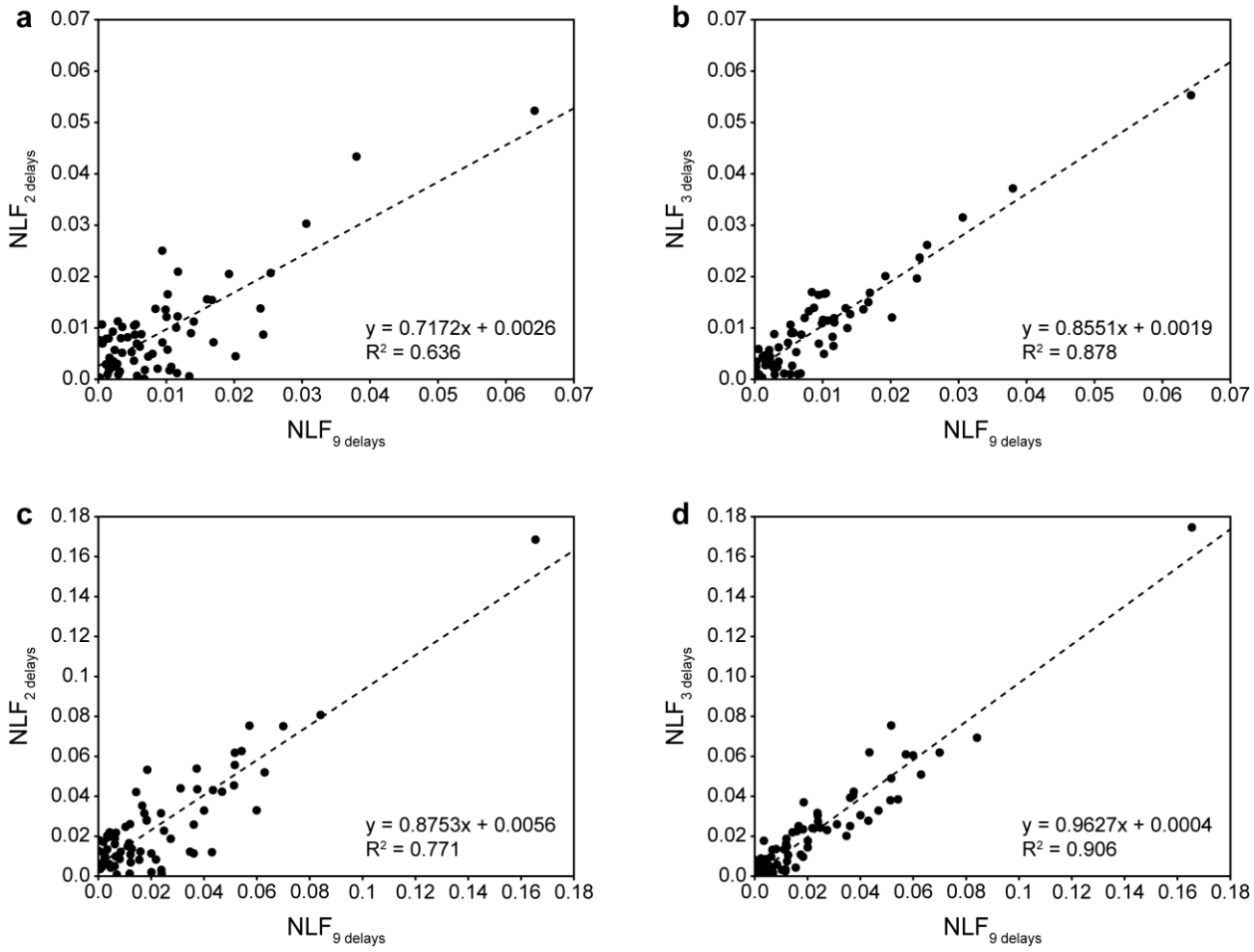

Fig. S8 Estimation of non-linearity factors from minimal reference data. Correlation between estimated and observed non-linearity factors for ubiquitin ^{15}N T_1 collected with 12* NUS points using (a) 2 RSS reference planes (shortest and longest delays) and (b) 3 RSS reference planes (shortest and two longest delays). Correlation between estimated and observed non-linearity factors for AK ^{13}N $T_{1\rho}$ collected with 26* NUS points using (a) 2 RSS reference planes and (b) 3 RSS reference planes.

Table S1 Summary of RSS-NUS Comparisons Using Alternative Reconstruction Algorithms

Algorithm	**Protein**	**NUS Points**	**R^2**	**RMSD**	**<NLF>**
IRLS	Ubiquitin	12*	0.950	3.08%	0.01187
IRLS	Ubiquitin	30*	0.983	1.59%	0.00538
NESTA-L1	Ubiquitin	30*	0.958	2.98%	0.00616
NESTA-IRL1	Ubiquitin	30*	0.922	3.57%	0.01284

Table S2 Summary of Additional RSS-NUS Comparisons

Experiment	Protein	Cross Peaks[1]	NUS Points	R^2	RMSD	<NLF>
$^{2}H\ R^{Q}(D_z)$	Ubiquitin	49	16*	0.993	3.10%	0.01499
$^{2}H\ R^{Q}(D_+)$	Ubiquitin	49	16*	0.992	3.37%	0.01722
$^{13}C\ T_1$	AK	106	26*	0.990	5.43%	0.02224
$^{13}C\ T_{1\rho}$	AK	106	26*	0.978	3.90%	0.02082
$^{15}N\ T_1$ TROSY	Ubiquitin 30% glycerol	72	32*	0.967	1.66%	0.00699
$^{15}N\ T_1$ TROSY	AK	312	16*	0.881	6.27%	0.02563
$^{15}N\ T_1$ TROSY	MBP	318	50*	0.885	3.57%	0.01577
$^{15}N\ T_{1\rho}$ TROSY	Ubiquitin 30% glycerol	72	32*	0.985	2.30%	0.00943
$^{15}N\ T_{1\rho}$ TROSY	AK	312	16*	0.915	5.94%	0.02923
$^{15}N\ T_{1\rho}$ TROSY	MBP	318	50*	0.942	3.18%	0.01494
$^{15}N\ T_1$	CaM	156	32*	0.919	2.94%	0.01076
$^{15}N\ T_1$	CaM	156	46*	0.975	1.85%	0.00847
$^{15}N\ T_2$	Ubiquitin	72	12*	0.975	3.72%	0.02148
$^{15}N\ T_2$	Ubiquitin	72	30*	0.990	2.82%	0.01109

[1] The number of cross peaks is taken to be the number observed cross peaks identified by automated peak picking