Supporting information

for:

Spin-labeling Insights into How Chemical Fixation Impacts Glycan Organization on Cells

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I. Results from fitting with Easy Spin

Two component fits gave suitable results where incorporation of a third component did not decrease the RMSD within the SNR ratios of our data.[1] All theoretical fits were performed with Easy Spin using the following tensor values: g_{xx} = 2.0070, g_{yy} = 2.0062, g_{zz} = 2.0033, Axx = 6.7 G, Ayy = 6.7 G, Azz = 35G, as described in our earlier reports [1-3]. For these fittings, we allowed $S_{2,0}$ to vary but in all cases, similar to results obtained previously,[1] values of zero or near zero were obtained. For this report, only single samples of each cell batch were prepared. From our prior report [1] we ascertain the relative error in component populations arises from cell variability. Hence, we set the relative errors of the weights at 10% to account for the level of variability seen with EGE labeling.

We recognize that unique parameters for fits from single frequency EPR data have a high degree of uncertainty as the uniqueness of the parameters obtained from the fits are in question. For our data sets collected only at X-band frequency, we place errors on correlation times according to the range of correlation times broken into three different motional regimes with $\tau_R \leq 2$ ns as "fast", 2 ns $< \tau_R \leq 10$ ns as "intermediate", and 10 ns $< \tau_R <$ 100 ns as "slow". Variability in values of τ_R are observed to be ± 0.3 ns, ±1 ns, and ±10 ns within these regimes. These variabilities are the summations and averages over all simulations performed to date from our MGE and EGE glycan labeling schemes using the DBCO-SL[1-3] and the determined values of the g-tensor and A-tensor from high field measurements of a DBCO-SL azide-containing monosaccharide.[3]

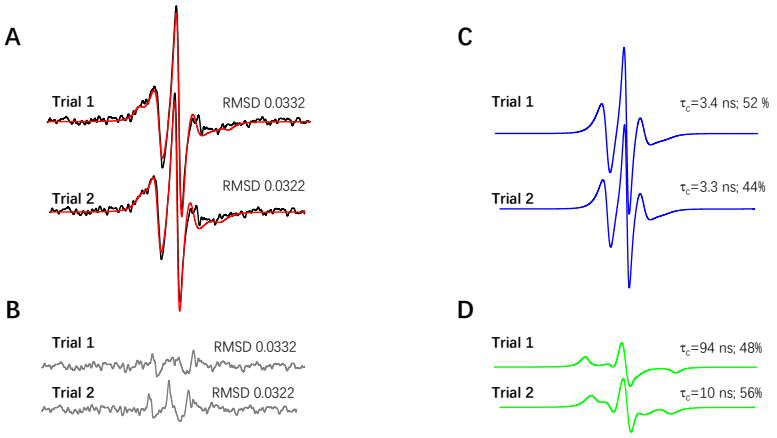

Figure S1a. Easy Spin fitting where (A) shows overlay of experimental (black) to a 2-component fit (red) with the residual shown in (B), component 1 in (C) and component 2 in (D). Example variability in spectral fitting with two-components for HeLa cells treated with ManNAz SL>F. Uncertainty for the intermediate-motion-regime and slow-motion-regime is illustrated by this example. This data set provided the most difficulty in finding uniqueness of fits, particularly with the (D) "slow" motional component, but it is well known that at X-band frequencies the lineshape becomes relatively insensitive to motions slower than ~ 10 ns without imposing an ordering potential.[4-6]

Table S1. Summary of results from fitting with easy spin.

	Component 1				Component 2				
Sample	Linewidth (mT) [a]	τ_R (ns)[b]	Weight (%)	$S_{2,0}$ [c]	Linewidth (mT)[a]	τ_R (ns)[b]	Weight	$S_{2,0}$ [c]	RMSD
ManNAz F > SL	0.13	0.6	69	0.001	0.04	13	31	0	0.03
ManNAz[d] SL > F	0.07 0.61	3.4 3.3	52 44	0 0	0.48 0.37	93 10.0	48 56	0 0	0.03 0.03
GlcNAz F > SL	0.10	2	48	0.0003	0.11	8	52	0	0.02
GlcNAz SL> F	0.21	2	60	0	0.12	15	40	0	0.03
sai9Az F>SL	0.15	0.7	20	0	0.06	5	80	0	0.03
sia9Az SL>F	0.13	1	18	0.0001	0.18	7	82	0.003	0.03

[a]Linewidth (mT): Lorentzian broadening (in mT) utilized in fitting algorithm. The relatively low numbers indicate the absence of any significant dipolar interactions.
[b]τ_R (ns): The rate of isotropic rotational diffusion. Relative error given by motional regime as ± 0.3 ns, ±1 ns , and ±10 ns for $\tau_R \leq 2$ ns as "fast", 2 ns < $\tau_R \leq$ 10 ns as "intermediate", and 10 ns < τ_R < 100 ns as "slow"
[c]$S_{2,0}$: Order parameter calculated from orientating potential $\lambda_{2,0.}$ The order parameter is essentially zero in these fits, but this parameter was left to change incase ordering was needed.
[d]Results shown for two separate fitting runs demonstrating the variability in parameters that give acceptable fits based upon the RMSD.

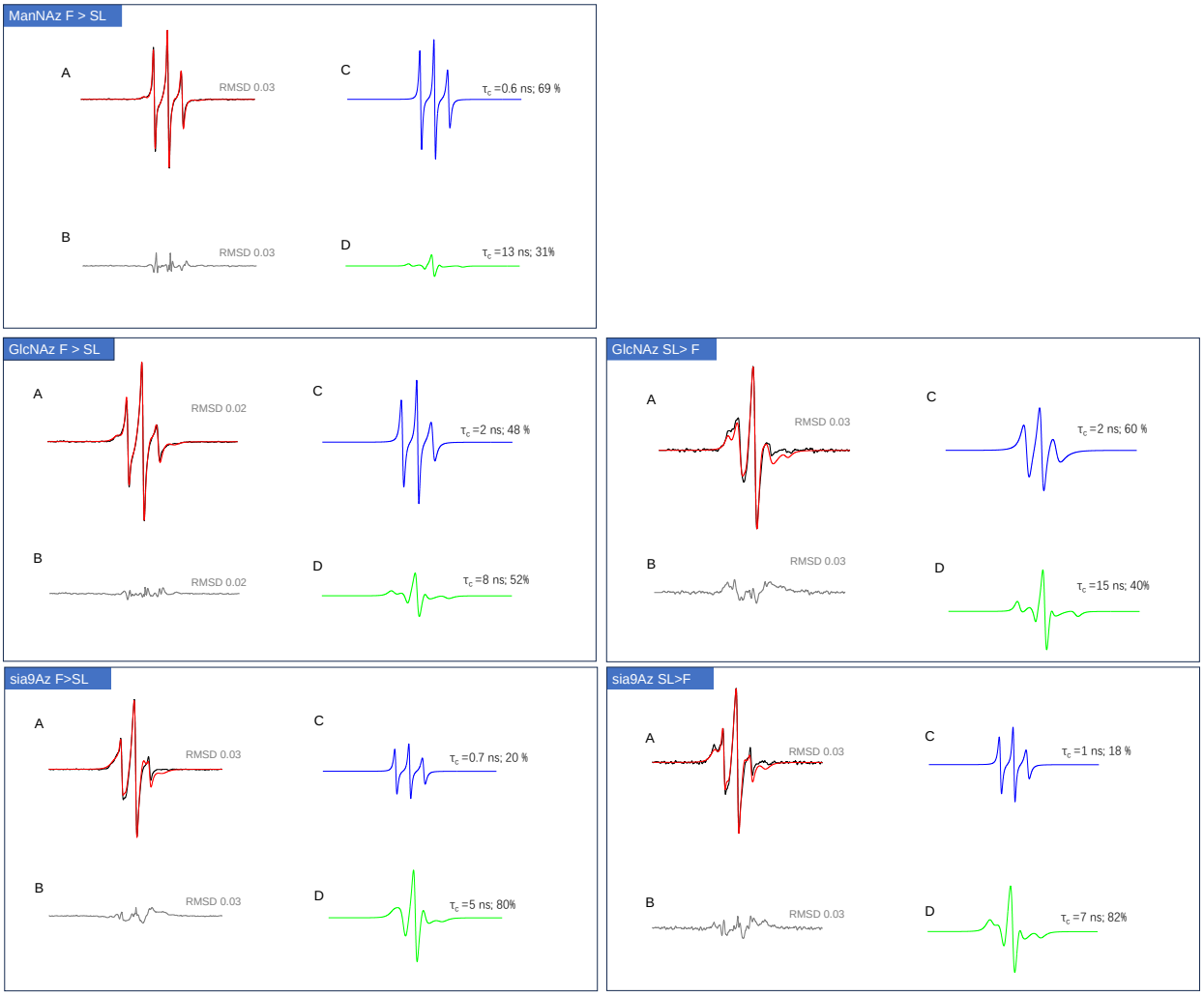

Figure S1b. Easy Spin fitting for X-band EPR spectra from HeLa cells treated with either ManNAz (top), GlcNAz (middle), or sia9Az (bottom) where (A) shows overlay of experimental (black) to a 2-component fit (red) with the residual shown in grey in (B), component 1 rendered in blue in (C) and component 2 plotted in green in (D).

II. EPR spectra obtained with ascorbic acid

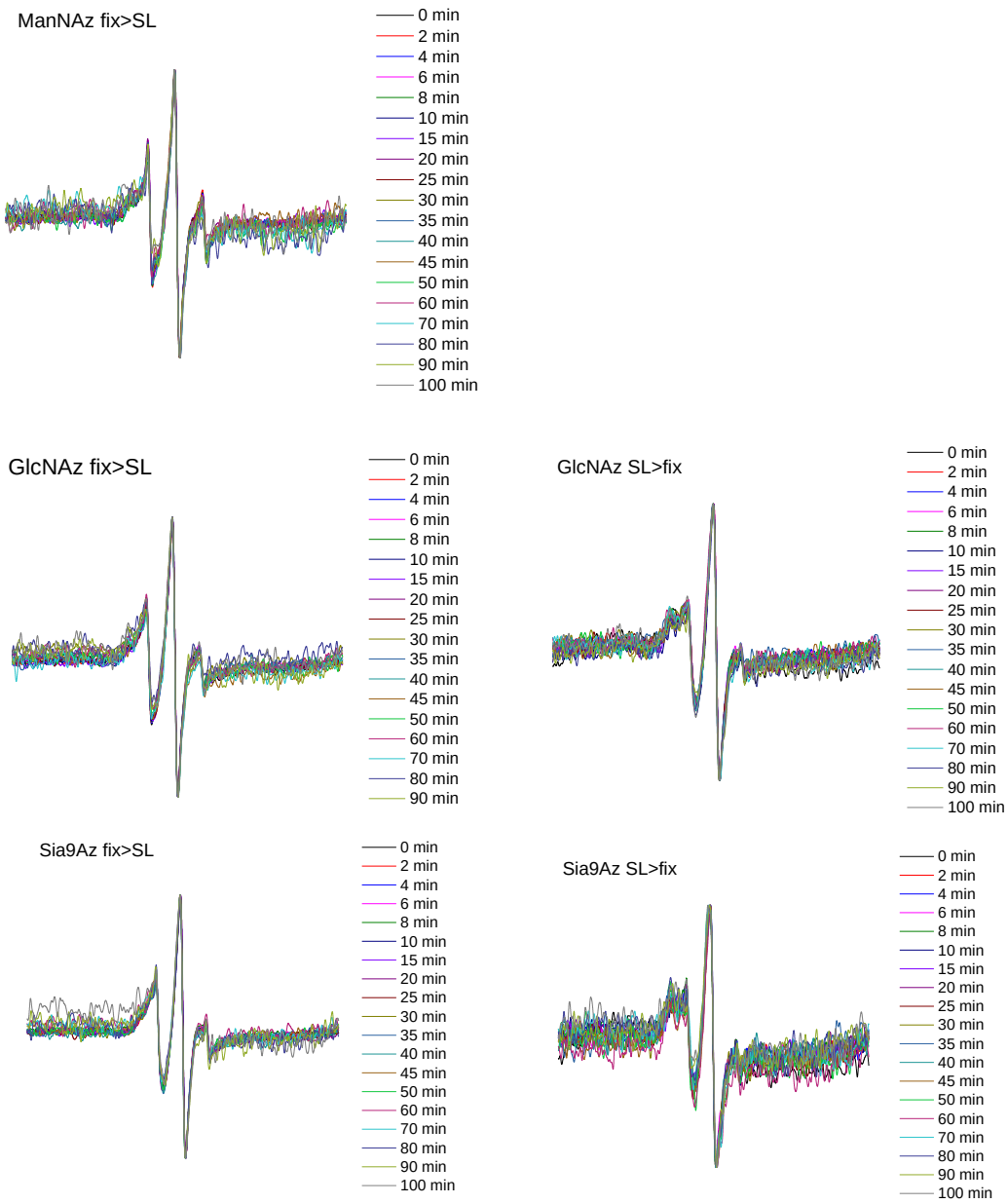

Figure S2. Overlay of time course EPR spectra for HeLa cells treated with **1** (top), **2** (middle), or **3** (bottom) with either (left) chemical fixation prior to spin labeling (F>SL) or (right) spin-labeling prior to chemical fixation (SL>F). Spectra shown are scaled with equal intensity of the central transition and overlain to demonstrate that there is no change in the relative shape (i.e., percentage of the 2-components) over the 100 min time course of exposure to ascorbic acid. For analysis of signal decay as a function of time, spectra were NOT scaled for evaluation of the intensity of the spectral lines (i.e., same level of SNR as obtained with instrumental gain/detector settings).

III. References:

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