# Different Biophysical Properties of Cell Surface α2,3- and α2,6-Sialoglycans Revealed by Electron Paramagnetic Resonance Spectroscopic Studies

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# I. Flow Cytometry Results of Pd2,6ST-Mediated Engineering and Fluorescence Labeling of Cell Surface Sialoglycans

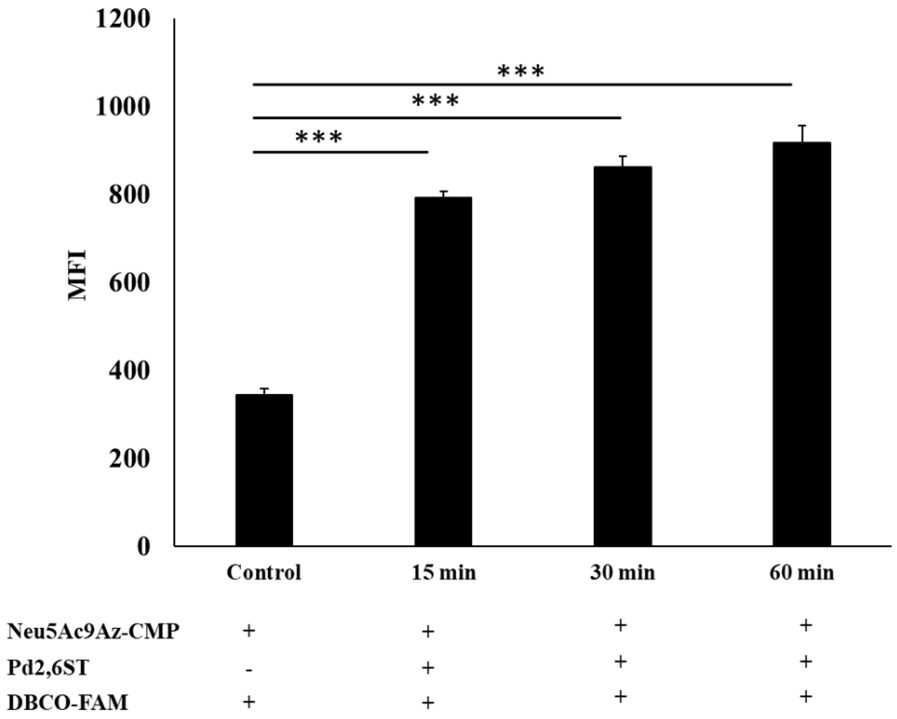


**Figure S-1:** FACS results of engineering and fluorescence labeling of cell-surface glycans with Pd2,6ST derived from bacterium *Photobacterium damsel*. After treatment with sialidase A (0.5 U/μL) at 37 °C for 1 h, HeLa cells were incubated with Neu5Ac9Az-CMP (0.1 μM) without Pd2,6ST for the control or with Neu5Ac9Az-CMP (0.1 μM) and Pd2,6ST (4 U/mL) for 15, 30, and 60 min, respectively. Thereafter, the cells were incubated with DBCO-FAM (50 μM) at rt for 1 h to attach the fluorescent tag. Finally, the cells were analyzed with a flow cytometer. *** The difference between two compared groups is statistically significant ($P < 0.01$).

## II. EPR Spectra and Theoretical Fitting Results

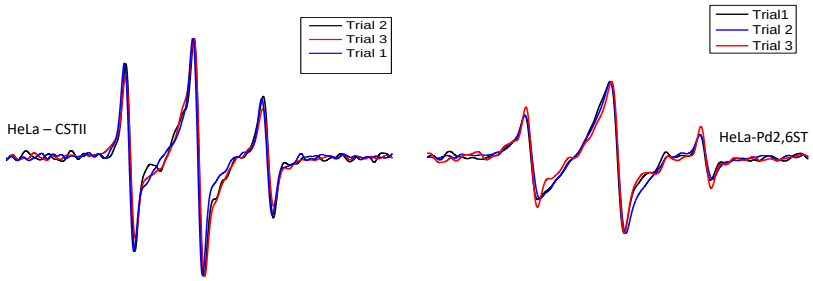


**Figure S-2**. Overlay of experimental EPR spectra showing reproducibility of enzymatic spin-labeling of HeLa cells using CSTII (left) and Pd2,6ST (right). The spectra are normalized to cell count and are control-subtracted.

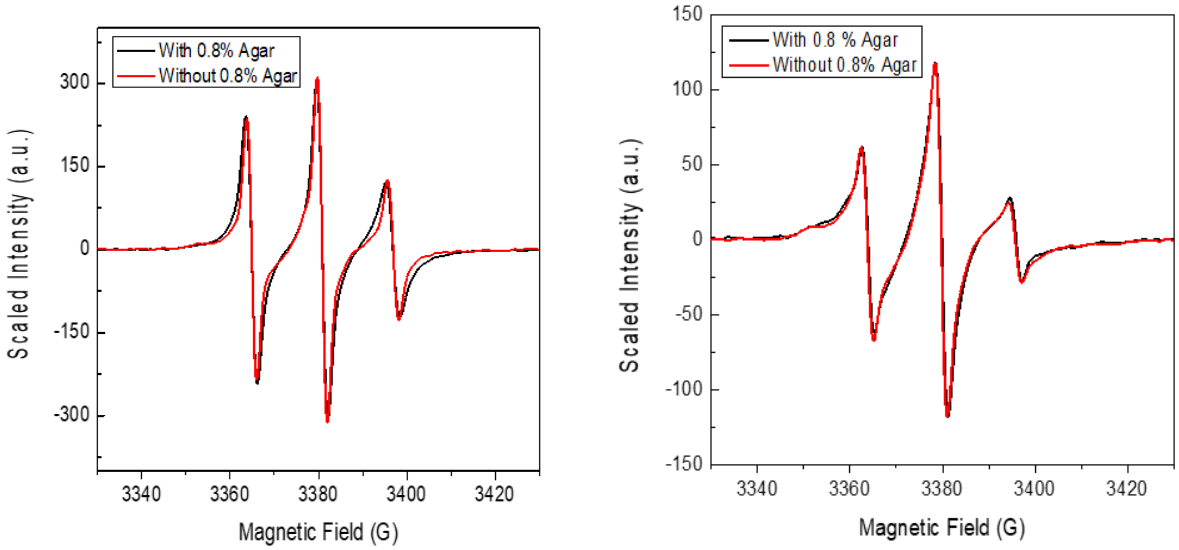


**Figure S-3**. Overlay of the EPR spectra of spin-labeled of HeLa cells treated with CSTII (left) and Pd2,6ST (right) in the presence (black) and absence (red) of agar.

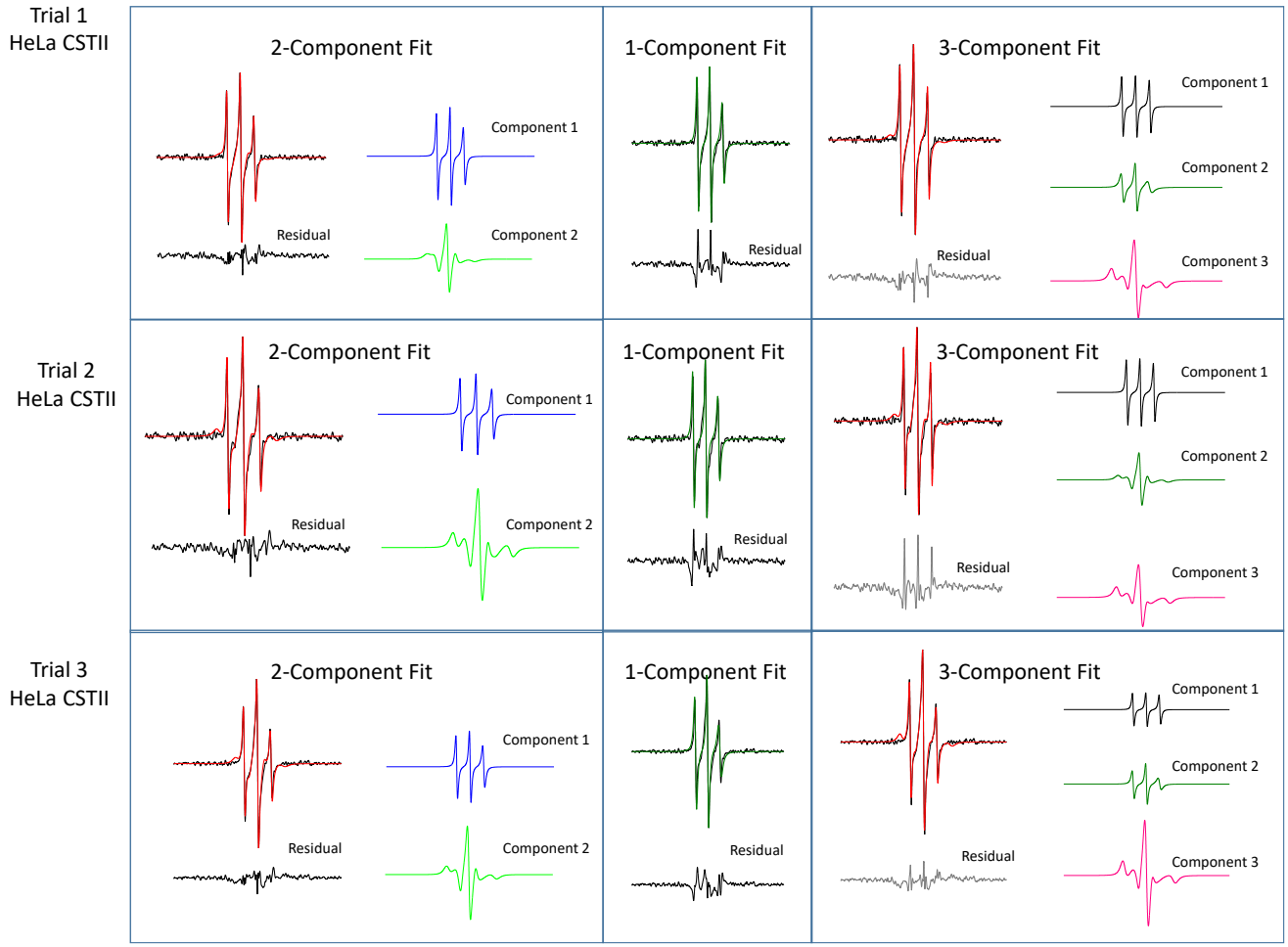


**Figure S-4**. Overlay of experimental X-band EPR spectra (black) and 2- (left column), 1- (middle column), and 3-component (right column) theoretical fit EPR spectra (red), as well as the components of theoretical fit spectra and the residuals (dark grey) between the experimental and theoretical fit spectra, for three separate experiments using CSTII for HeLa cell glycan engineering. All simulations were performed as described with Easy Spin using the following tensor values: gxx = 2.0070, gyy = 2.0062, gzz = 2.0033, Axx = 6.7 G, Ayy = 6.7 G, Azz = 35G. In all cases, the 2-component theoretical fit gave the best results.

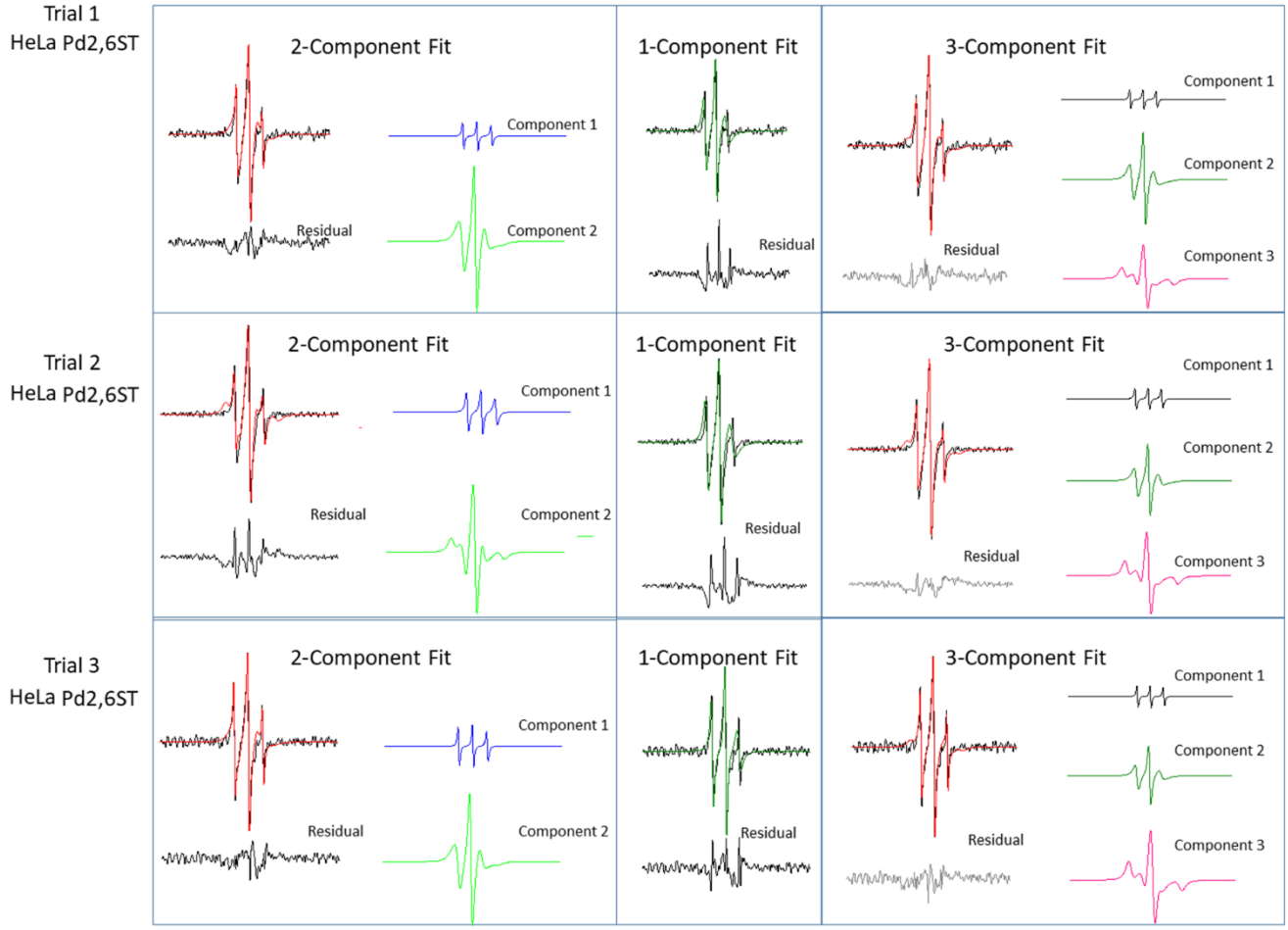


**Figure S-5**. Overlay of experimental X-band EPR spectra (black) and 2- (left column), 1- (middle column), and 3-component (right column) theoretical fit EPR spectra (red), as well as the components of theoretical fit spectra and the residuals (dark grey) between the experimental and theoretical fit spectra, for three separate experiments using Pd2,6ST for HeLa cell surface glycan engineering. All theoretical fits were performed as described with Easy Spin using the following tensor values: gxx = 2.0070, gyy = 2.0062, gzz = 2.0033, Axx = 6.7 G, Ayy = 6.7 G, Azz = 35G. The 2-component simulations gave the best results, except for Trial 2, where the 3-component results gave slightly better result than the 2-component results but the difference of 2- and 3-component simulation results was nearly negligible.

**Theoretical Fits to EPR spectra.**

Fits to spectra were performed as described previously using EasySpin. Parameters of line broadening and order parameter were included to demonstrate that when allowed to vary there is no need to include a restraining order potential or line broadening parameter indicative of dipolar interactions. Values are tabulated below. We note that they are essentially zero in each case.

**Table S-1**. EPR spectral parameters with 2-component theoretical fits for experimental spectra of spin-labeled HeLa cells engineered with CSTII.

| Trial | $h_1/h_0$[a] | Component 1 | | | | Component 2 | | | | RMSD |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Linewidth[b] (mT) | $\tau_c$ (ns)[c] | Weight (%) | $S_{2,0}$[d] | Linewidth[b] (mT) | $\tau_c$ (ns)[c] | Weight (%) | $S_{2,0}$[d] | |
| 1 | 0.79 | 0.137 | 0.371 | 58.1 | 0.001 | 0.030 | 5.54 | 41.9 | 0 | 0.031 |
| 2 | 0.78 | 0.140 | 0.323 | 40.7 | 0 | 0.124 | 9.11 | 59.3 | 0 | 0.031 |
| 3 | 0.68 | 0.146 | 0.399 | 42.4 | 0 | 0.024 | 7.49 | 57.6 | 0 | 0.030 |
| Average Values | 0.75± 0.06 | 0.14±0.01 | 0.36±0.04 | 47±10 | 0 | 0.06±0.06 | 7.4±1.8 | 53±10 | 0 | - |

[a]$h_1/h_0$: ratio of heights of the low field, central field lines.

[b]Linewidth (mT): Lorentzian broadening (in mT) utilized in fitting algorithm. The relatively low numbers indicate the absence of any significant dipolar interactions.

[c]$\tau_c$ (ns): The rate of isotropic rotational diffusion.

[d]$S_{2,0}$: Order parameter calculated from $\lambda_{2,0}$ potential. The order parameter is essentially zero in these fits.

**Table S-2**. EPR spectral parameters with 1-component theoretical fits for experimental spectra of spin-labeled HeLa cells engineered with CSTII.

| Trial | $h_1/h_0$ [a] | Component 1 | | | RMSD |
|---|---|---|---|---|---|
| | | Linewidth (mT) [b] | $\tau_c$ (ns) [c] | $S_{2,0}$ [d] | |
| 1 | 0.79 | 0.058 | 0.533 | 0.001 | 0.058 |
| 2 | 0.78 | 0.048 | 1.04 | 0 | 0.048 |
| 3 | 0.68 | 0.062 | 0.488 | 0 | 0.062 |
| Average Values | 0.75±0.06 | 0.06±0.01 | 0.7±0.3 | 0 | - |

[a]$h_1/h_0$: ratio of heights of the low field, central field lines.

[b]Linewidth (mT): Lorentzian broadening (in mT) utilized in fitting algorithm. The relatively low numbers indicate the absence of any significant dipolar interactions.

[c]$\tau_c$ (ns): The rate of isotropic rotational diffusion.

[d]$S_{2,0}$: Order parameter calculated from $\lambda_{2,0}$ potential. The order parameter is essentially zero in these fits.

**Table S-3**. EPR spectral parameters with 3-component theoretical fits for experimental spectra of spin-labeled HeLa cells engineered with CSTII.

| Trial | Component 1 | | | | Component 2 | | | | Component 3 | | | | RMSD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Linewidth[a] (mT) | $\tau_c$ [b] (ns) | Weight (%) | $S_{2,0}$ [c] | Linewidth [a] (mT) | $\tau_c$ [b] (ns) | Weight (%) | $S_{2,0}$ [c] | Linewidth [a] (mT) | $\tau_c$ [b] (ns) | Weight (%) | $S_{2,0}$ [c] | |
| 1 | 0.169 | 0.1 | 32.1 | 0 | 0.007 | 1.73 | 25.2 | 0 | 0.234 | 15.0 | 35.0 | 0 | 0.044 |
| 2 | 0.171 | 0.1 | 36.0 | 0 | 0.001 | 7.30 | 29.0 | 0 | 0.263 | 15.0 | 59.7 | 0 | 0.028 |
| 3 | 0.177 | 0.1 | 18.2 | 0 | 0.077 | 1.18 | 22.1 | 0 | 0.160 | 15.0 | 42.6 | 0 | 0.031 |
| Ave. | 0.17±0.07 | 0.1 | 29±11 | 0 | 0.03±0.05 | 3.4±3.7 | 25±4 | 0 | 0.22±0.06 | 15.0 | 46±13 | 0 | - |

[a]Linewidth (mT): Lorentzian broadening (in mT) utilized in fitting algorithm. The relatively low numbers indicate the absence of any significant dipolar interactions.

[b]$\tau_c$ (ns): The rate of isotropic rotational diffusion.

[c]$S_{2,0}$: Order parameter calculated from $\lambda_{2,0}$ potential. The order parameter is essentially zero in these fits.

**Table S-4**. EPR spectral parameters with 2-component theoretical fits for experimental spectra of spin-labeled HeLa cells engineered with Pd2,6ST.

| Trial | $h_1/h_0$[a] | Component 1 | | | | Component 2 | | | | RMSD |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Linewidth[b] (mT) | $\tau_c$ [c] (ns) | Weight (%) | $S_{2,0}$[d] | Linewidth[b] (mT) | $\tau_c$[c] (ns) | Weight (%) | $S_{2,0}$[d] | |
| 1 | 0.56 | 0.133 | 0.211 | 16.3 | 0.001 | 0.006 | 3.69 | 83.7 | 0.001 | 0.048 |
| 2 | 0.55 | 0.180 | 0.463 | 24.6 | 0 | 0.081 | 7.78 | 75.4 | 0 | 0.067 |
| 3 | 0.66 | 0.131 | 0.214 | 23.7 | 0.001 | 0.001 | 3.80 | 76.3 | 0 | 0.051 |
| Average Values | 0.59± 0.06 | 0.15±0.03 | 0.30±0.14 | 22±5 | 0 | 0.03±0.04 | 5±2 | 78±5 | 0 | - |

[a]$h_1/h_0$: ratio of heights of the low field, central field lines.

[b]Linewidth (mT): Lorentzian broadening (in mT) utilized in fitting algorithm. The relatively low numbers indicate the absence of any significant dipolar interactions.

[c]$\tau_c$ (ns): The rate of isotropic rotational diffusion.

[d]$S_{2,0}$: Order parameter calculated from $\lambda_{2,0}$ potential. The order parameter is essentially zero in these fits.

**Table S-5**. EPR spectral parameters with 1-component theoretical fits for experimental spectra of spin-labeled HeLa cells engineered with Pd2,6ST.

| Trial | $h_1/h_0$[a] | Component 1 | | | RMSD |
|---|---|---|---|---|---|
| | | Linewidth [b] (mT) | $\tau_c$ [c] (ns) | $S_{2,0}$[d] | |
| 1 | 0.56 | 0.050 | 1.97 | 0 | 0.074 |
| 2 | 0.55 | 0.059 | 2.28 | 0 | 0.084 |
| 3 | 0.66 | 0.070 | 1.51 | 0 | 0.072 |
| Average Values | 0.59±0.06 | 0.06±0.01 | 1.9±0.4 | 0 | - |

[a]$h_1/h_0$: ratio of heights of the low field, central field lines.

[b]Linewidth (mT): Lorentzian broadening (in mT) utilized in fitting algorithm. The relatively low numbers indicate the absence of any significant dipolar interactions.

[c]$\tau_c$ (ns): The rate of isotropic rotational diffusion.

[d]$S_{2,0}$: Order parameter calculated from $\lambda_{2,0}$ potential. The order parameter is essentially zero in these fits.

**Table S-6**. EPR spectral parameters with 3-component theoretical fits for experimental spectra of spin-labeled HeLa cells engineered with Pd2,6ST.

| Trial | Component 1 | | | | Component 2 | | | | Component 3 | | | | RMSD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Linewidth[a] (mT) | $\tau_c$ (ns) | Weight (%) | $S_{2,0}$ | Linewidth[a] (mT) | $\tau_c$ (ns) | Weight (%) | $S_{2,0}$ | Linewidth[a] (mT) | $\tau_c$ (ns) | Weight (%) | $S_{2,0}$ | |
| 1 | 0.149 | 0.1 | 10.5 | 0 | 0.001 | 3.12 | 49.5 | 0 | 0.300 | 20 | 40.0 | 0 | 0.046 |
| 2 | 0.182 | 0.1 | 12.0 | 0 | 0.011 | 3.02 | 40.0 | 0 | 0.305 | 20 | 48.0 | 0 | 0.041 |
| 3 | 0.139 | 0.1 | 12.2 | 0 | 0.001 | 2.69 | 32.9 | 0 | 0.349 | 20 | 54.9 | 0 | 0.047 |
| Ave. | 0.16±0.03 | 0.1 | 12±2 | 0 | 0.004±0.01 | 2.9±0.3 | 41±9 | 0 | 0.32±0.02 | 20 | 48±8 | 0 | - |

[a]Linewidth (mT): Lorentzian broadening (in mT) utilized in fitting algorithm. The relatively low numbers indicate the absence of any significant dipolar interactions.

[b]$\tau_c$ (ns): The rate of isotropic rotational diffusion.

[c]$S_{2,0}$: Order parameter calculated from $\lambda_{2,0}$ potential. The order parameter is essentially zero in these fits.

## III. Atomistic MD Simulations

All CGenFF parameters derived were subjected to the following checks:

| Parameter | Penalty check |
|---|---|
| All charges derived by analogy | ≤10 |
| All bonds and angle derived by analogy | ≤10 |

Video of the trajectories: The structure is aligned to the first dihedral angle, $\chi_1$, for clarity (see the separate file attached).

DBCO-SL-Dynamic-video.mp4