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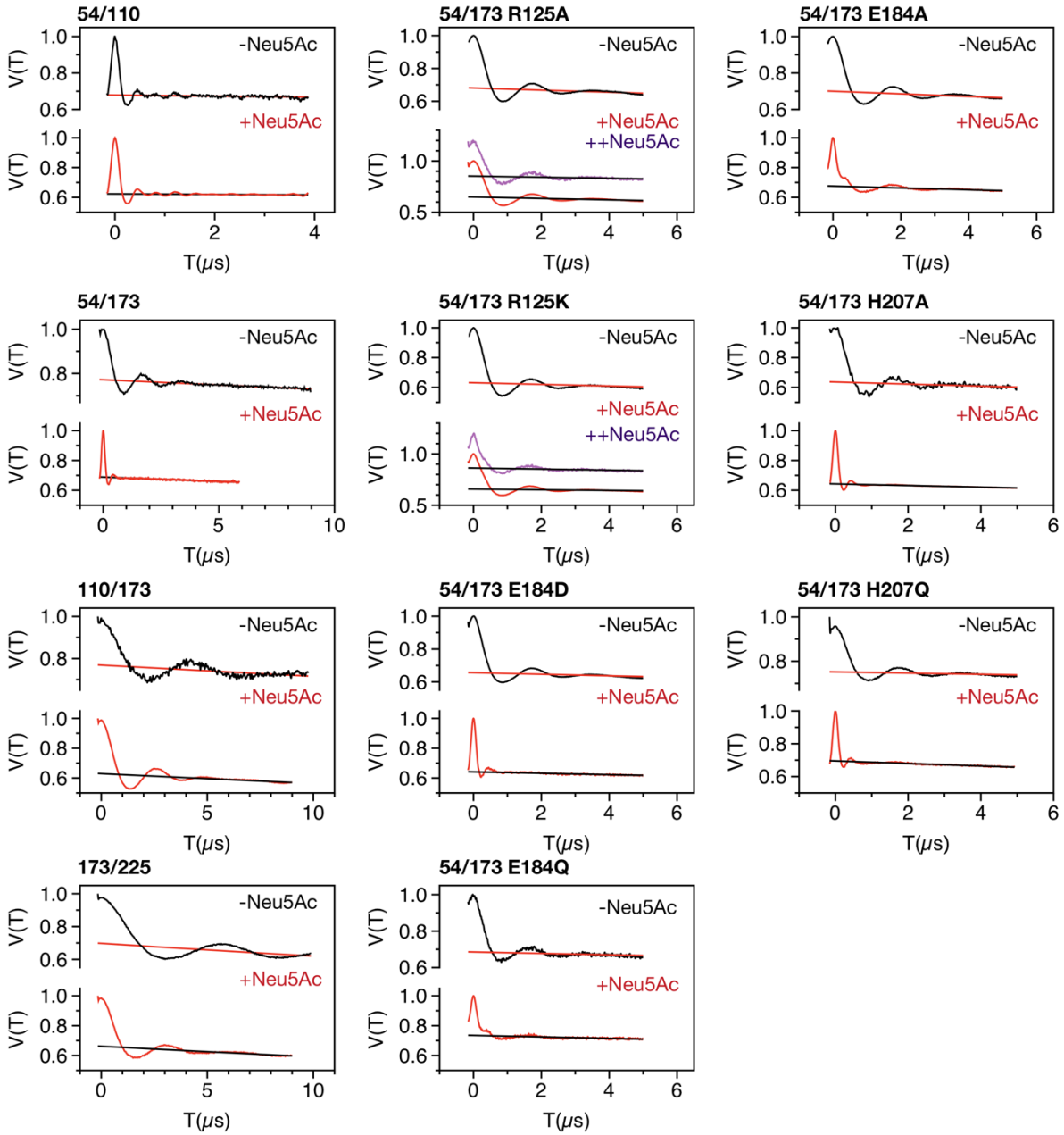
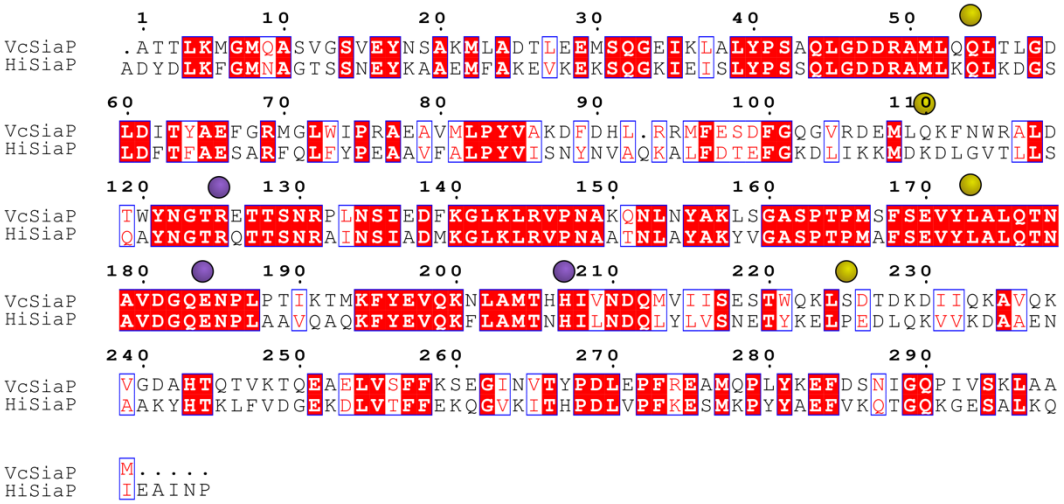
Supplemental Information

PELDOR Spectroscopy Reveals Two Defined States of a Sialic Acid TRAP Transporter SBP in Solution

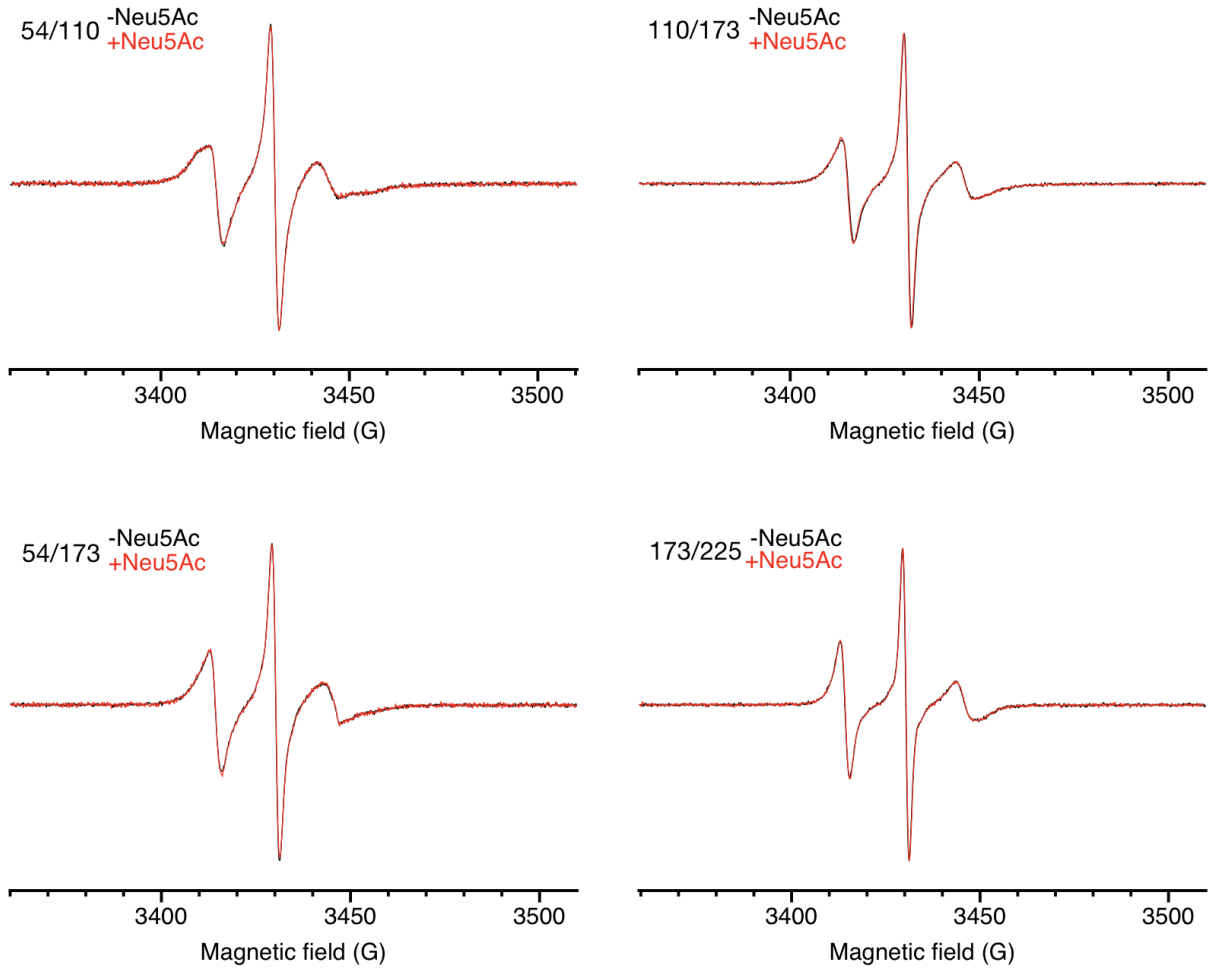
Janin Glaenzer, Martin F. Peter, Gavin H. Thomas, and Gregor Hagelueken



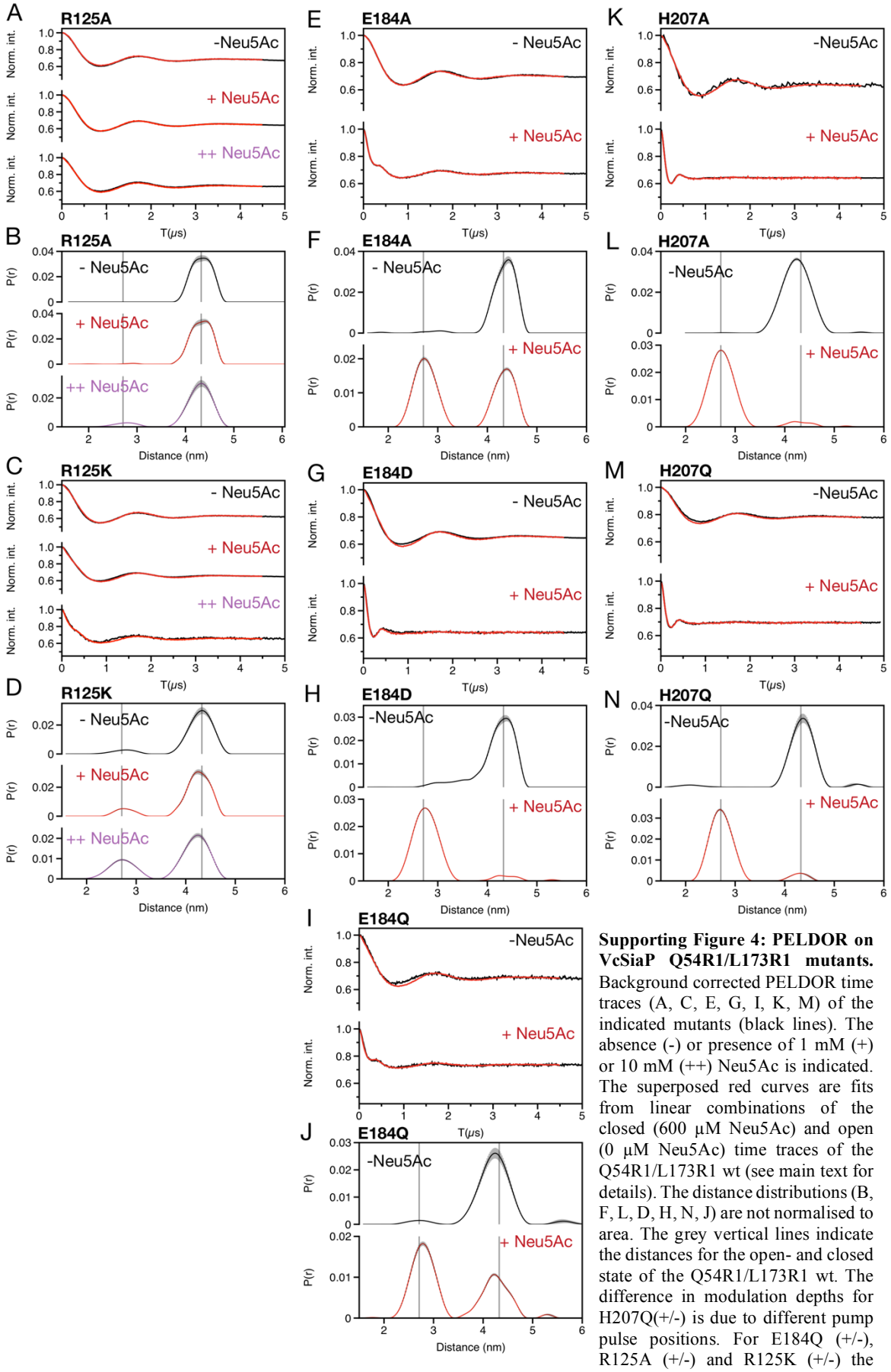
Supporting Figure 1: Uncorrected PELDOR time traces for the indicated mutants in the absence (-) or presence of 1 mM (+) or 10 mM (++) Neu5Ac. The intermolecular background that was used for the background correction is indicated. The difference in modulation depths for H207Q(+/-) is due to different pump pulse positions. For E184Q (+/-), R125A (+/-) and R125K (+/-) the small differences in modulation depths are due to slightly different pump pulse lengths (14 vs 16 ns).



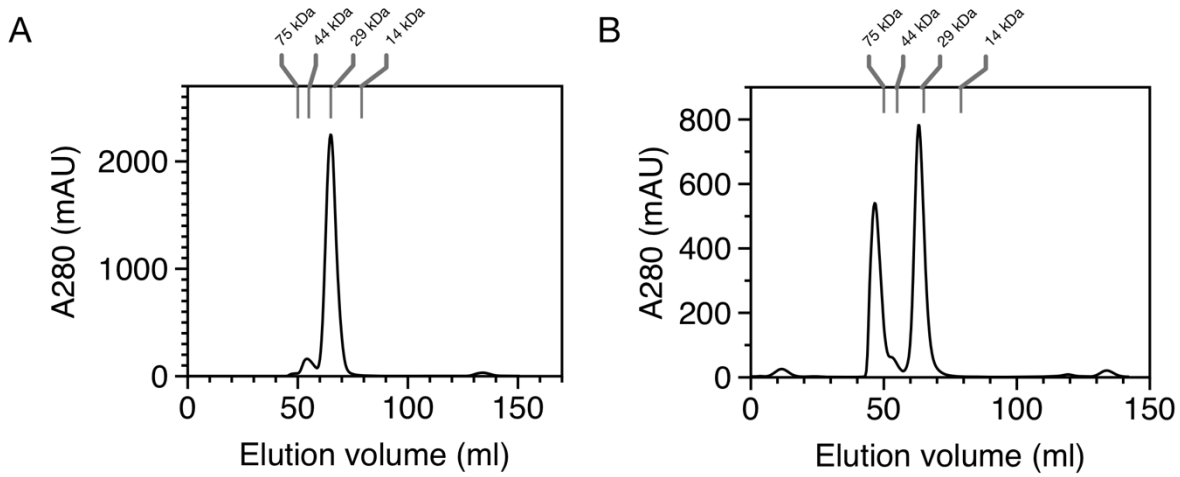
Supporting Figure 2: Sequence alignment of HiSiaP from *Haemophilus influenzae* and VcSiaP from *Vibrio cholerae.* Spin label positions are indicated by yellow spheres. Mutated residues of the conserved triad (R125, E184, H207) are marked by purple spheres.



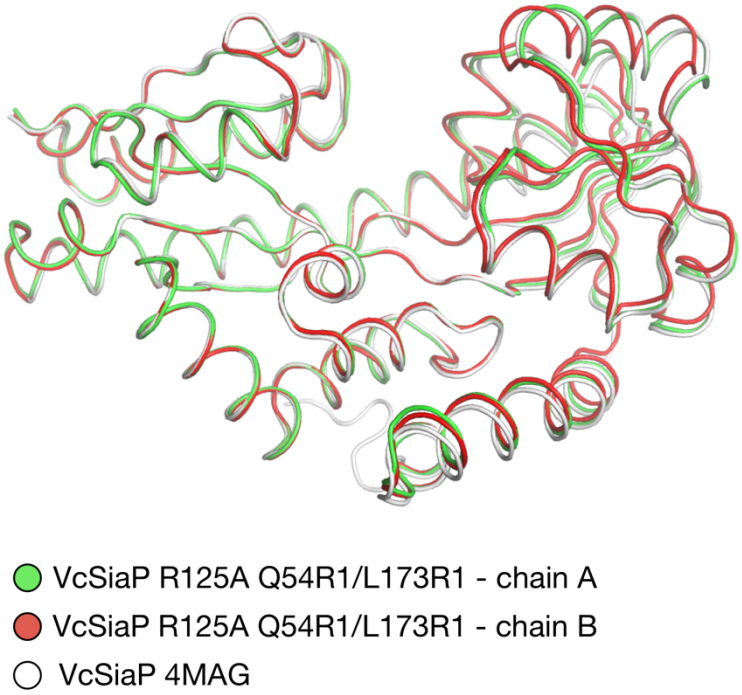
Supporting Figure 3: Room temperature *cw*-X-band EPR spectra of the indicated VcSiaP double mutants in the absence (-) and presence (+) of 1 mM Neu5Ac.



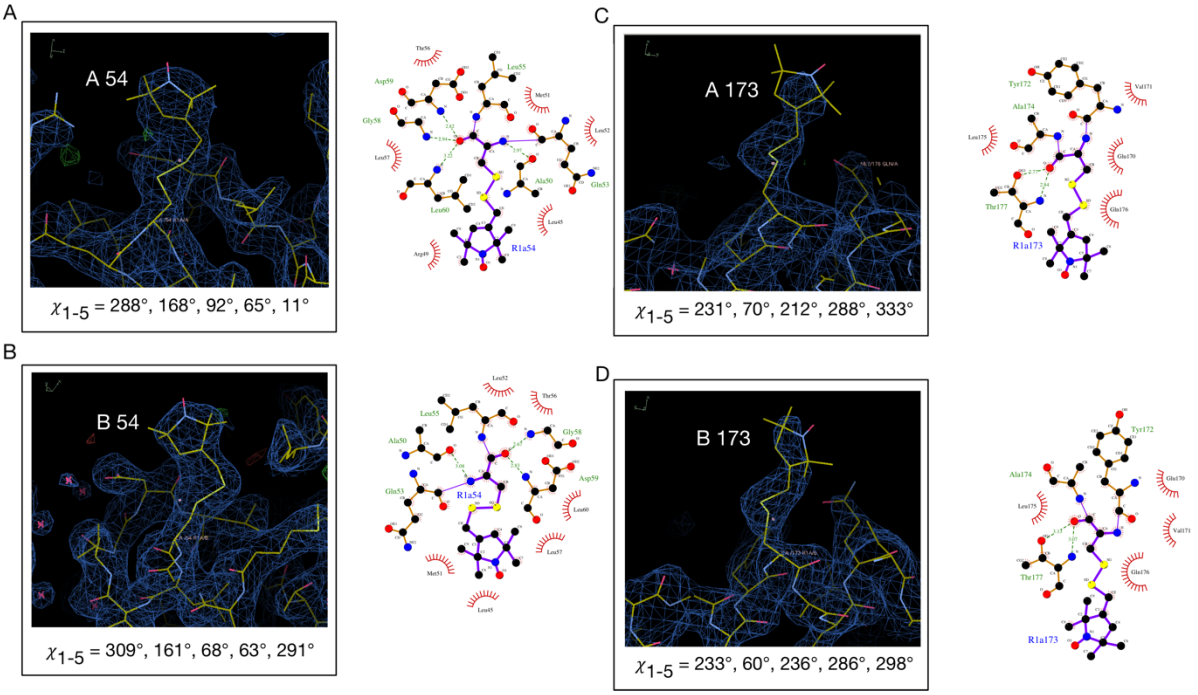
Supporting Figure 4: PELDOR on VcSiaP Q54R1/L173R1 mutants. Background corrected PELDOR time traces (A, C, E, G, I, K, M) of the indicated mutants (black lines). The absence (-) or presence of 1 mM (+) or 10 mM (++) Neu5Ac is indicated. The superposed red curves are fits from linear combinations of the closed (600 µM Neu5Ac) and open (0 µM Neu5Ac) time traces of the Q54R1/L173R1 wt (see main text for details). The distance distributions (B, F, L, D, H, N, J) are not normalised to area. The grey vertical lines indicate the distances for the open- and closed state of the Q54R1/L173R1 wt. The difference in modulation depths for H207Q(+/-) is due to different pump pulse positions. For E184Q (+/-), R125A (+/-) and R125K (+/-) the small differences in modulation depths are due to slightly different pump pulse lengths (14 vs 16 ns).



Supporting Figure 5: Gelfiltration of VcSiaP Q54R1/L173R1 mutants. A) VcSiaP Q54R1/L173R1 "wt". A Superdex 75 16/60 column was used. Molecular weight markers are indicated. The protein runs as a monomer, no aggregates were observed. **B)** VcSiaP Q54R1/L173R1 H207Q. A Superdex 75 16/60 column was used. In contrast to all other mutants, aggregates where observed for the H207 mutants. The monomer peak at ~65 ml was isolated and used for the PELDOR measurements.



Supporting Figure 6: Superposition of spin labelled VcSiaP R125A Q54R1/L173R1 with the VcSiaP wild-type. Cartoon models of VcSiaP R125A Q54R1/L173R1 chain A (green) and B (red), superimposed onto residues 1-100 of the VcSiaP wt structure (PDB-ID: 4MAG). Chain A is almost identical to the wt structure, whereas chain B is in a slightly more closed conformation.



Supporting Figure 7: Conformation of the R1 side chains in the VcSiaP R125A Q54R1/L173R1 structure. A) Left: Electron density (blue mesh, 2Fo-Fc contoured at 1.0 σ) observed at the Q54R1 site (chain A). The protein is shown as yellow stick model. The dihedral angles of the R1 side chain are given. Right: Ligplus scheme depicting the interactions of the R1 side chain (purple) with its molecular environment. Covalent bonds are shown as solid lines, polar interactions as dashed lines and nonpolar interactions as red arcs. Distances are given in Å. **C-D)** same as A) but for the indicated R1 sidechains.

Supporting Table 1

[Neu5Ac$_{tot}$] (µM)	VcSiaP open/close (%)		[closed] (µM)			[open] (µM)		
	Linear combination	Integration	Linear combination	Integration	Average	Linear combination	Integration	Average
0	100/0 (n.d.)	100/0	0.0 (n.d.)	0.0	0.0	25.0 (n.d.)	25.0	25.0
1	96/4 (±0.9)	96/4	1.0 (±0.2)	1.0	1.0	24.0 (±0.2)	24.0	24.0
10	58/42 (±0.6)	59/41	10.6 (±0.2)	10.4	10.5	14.4 (±0.2)	14.6	14.5
24	20/80 (±1.8)	22/78	20.0 (±0.5)	19.5	19.7	5.0 (±0.5)	5.5	5.3
120	8/92 (±1.2)	10/90	23.0 (±0.3)	22.5	22.8	2.0 (±0.3)	2.5	2.3
600	0/100 (n.d.)	3/97	25.0 (n.d.)	24.3	24.6	0.0 (n.d.)	0.7	0.4

[#]Values in parentheses represent the estimated error of the linear combination fitting procedure (3*σ).

Supporting Table 2

Mutant	Neu5Ac (mM)	VcSiaP Open/close (%)	
		Linear combination[#]	Integration
R125A	0	100/0 (±1.2)	100/0
	1	100/0 (±0.9)	99/1
	10	94/6 (±1.8)	96/4
R125K	0	97/3 (±0.9)	99/1
	1	85/15 (±2.4)	88/12
	10	64/36 (±3.0)	69/31
E184D	0	90/10 (±2.1)	93/7
	1	8/92 (±1.5)	9/91
E184Q	0	89/11 (±3.3)	96/4
	1	37/63 (±2.1)	39/61
E184A	0	100/0 (±1.2)	98/2
	1	45/55 (±0.9)	45/55
H207A	0	94/6 (±3.0)	99/1
	1	4/96 (±0.9)	7/93
H207Q	0	99/1 (±3.0)	97/3
	1	5/95 (±1.2)	7/93

[#]Values in parentheses represent the estimated error of the linear combination fitting procedure (3*σ)