SUPPLEMENTARY INFORMATION

Sugar-decorated Carbon Dots: A novel tool for targeting Immunomodulatory Receptors

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Interactions between sialic acid (Sia) and sialic acid-binding immunoglobulin-like lectins (siglecs) regulate the immune system, with aberrations contributing to pathologies such as autoimmunity, infectious disease and cancer. Over the last decade, several multivalent Sia ligands have been synthesized to modulate the Sia-binding affinity of proteins/lectins. Here, we report a novel class of multivalent siglec probes through the decoration of α(2,6)-sialyllactose ligands on inherently fluorescent carbon dots (CD). We show that the preference of α(2,3)-linked Sia for Siglec-1 can be altered by increasing the multivalence of Sia ligands present on the CD, and that a locally high glycan concentration can have a direct effect on linkage specificity. Additionally, micromolar (IC50 ~ 70 μM) interaction of α(2,6)-sialyllactose-CD (6-CD) with Siglec-2 (CD22) revealed it was capable of generating a significant cytotoxic effect on Burkitt's lymphoma (BL) Daudi B cells. Finally, we show that 6-CD is capable of forming trans interactions with CD22 on masked BL Daudi cells as a direct result of clustering of the Sia moiety on the CD surface. Overall, our glycoengineered carbon dots represent a novel high affinity molecular probe with multiple applications in sialoglycoscience and medicine.

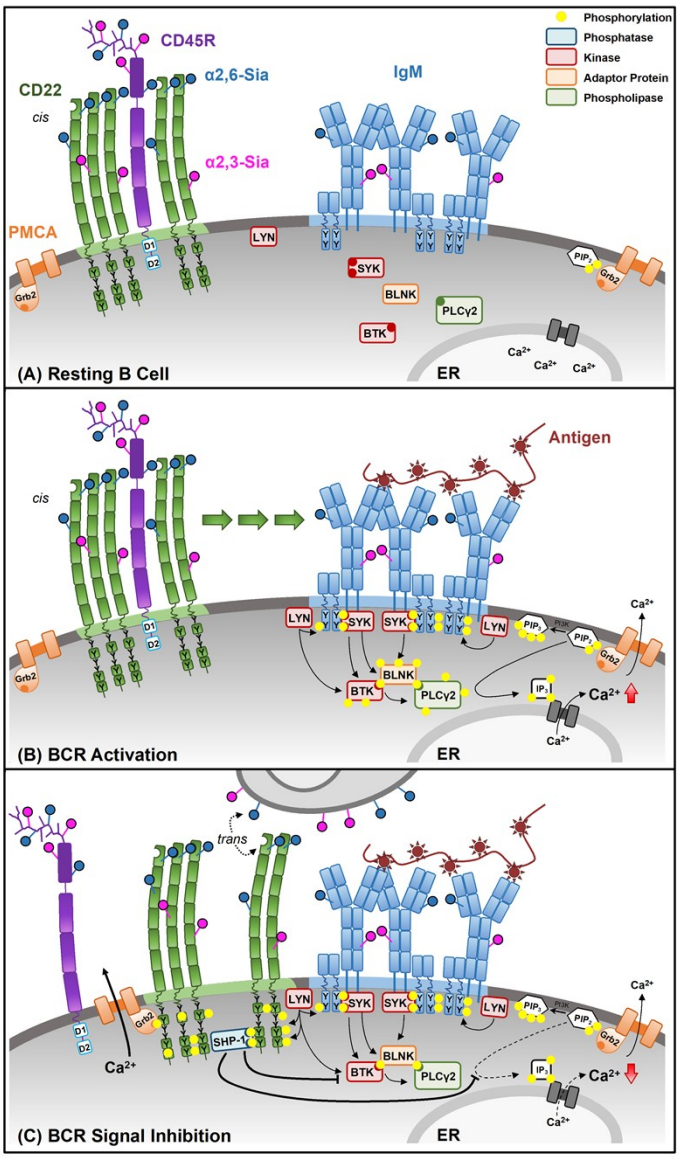

Figure S1 CD22 regulation of the B cell receptor (BCR) signal, adapted from Meyer et al., (2018).[1] A) The BCR is closed in resting B cells and CD22 forms homooligomers through cis-interactions. B) Binding to the BCR by specific antigens induces a conformation opening, this leads to activation and phosphorylation of immunoreceptor tyrosine-based inhibition motifs (ITIMs). Subsequent signalling cascades through SYK recruitment leads to Ca^{2+} release out of the endoplasmic reticulum. Finally, CD22 clusters are recruited to the BCR. C) BCR signal inhibition relies on recruitment of CD22. Additionally, recruitment of CD22 can occur through binding ligands of other cells via trans-interactions. Here, the proximity of CD22 to BCR leads to phosphorylation of the ITIMs of CD22 by LYN.[2] Subsequent signalling cascades through SHP-1 dephosphorylation inhibit further Ca^{2+} release and negatively regulate the BCR signal.[3]

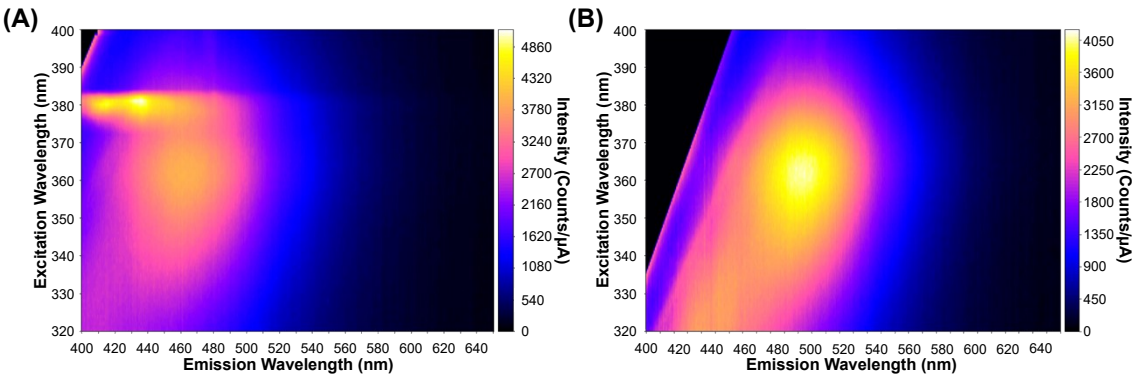

Figure S2 Photoluminescent spectra of (A) 3-CD; (B) 6-CD

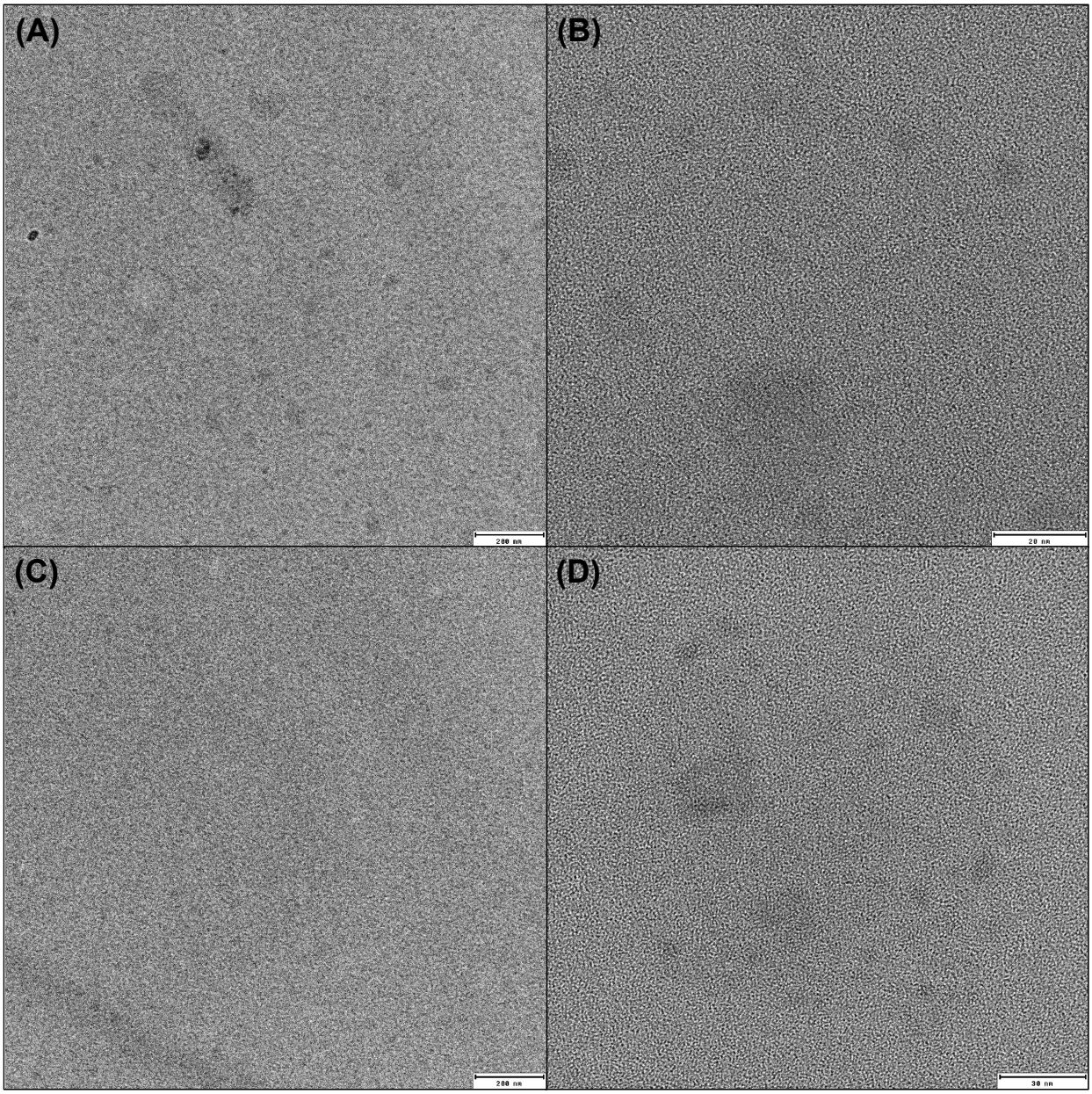

Figure S3 Transmission Electron Microscopy images of (A-B) 3-CD; (C-D) 6-CD.

Table S1 Dynamic Light Scattering measurements of hydrodynamic diameter and zeta potential measurements.

	Diameter (nm)	Average (nm)	Polydispersity Index	Average
	77.17		0.38	
6-CD	84.51	84.67 ± 7.58	0.34	0.36 ± 0.02
	92.32		0.37	
	75.42		0.31	
3-CD	80.41	76.27 ± 3.79	0.38	0.34 ± 0.04
	72.98		0.34	

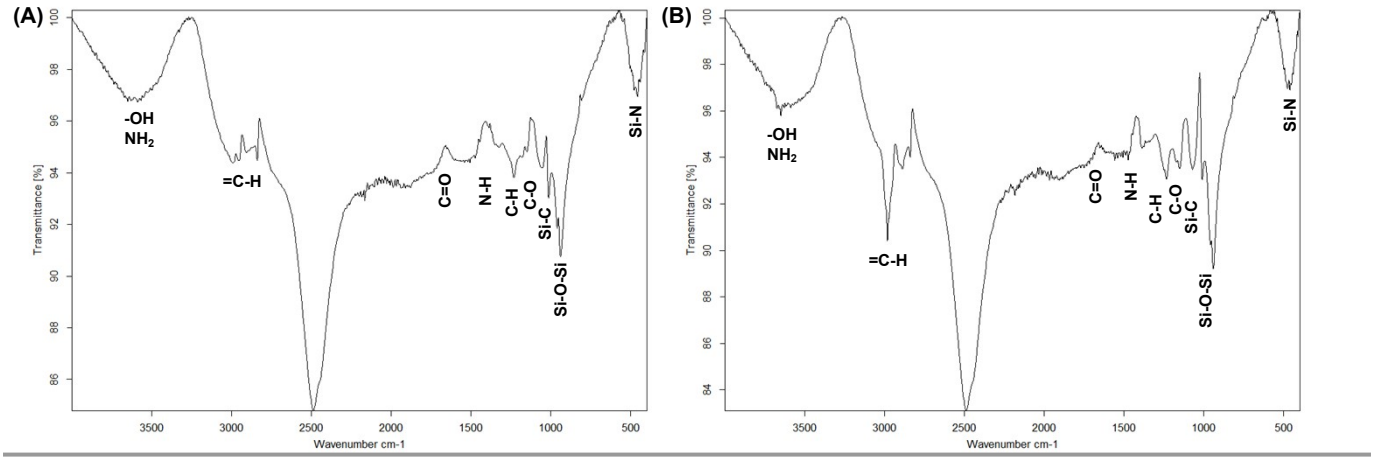

Figure S4 Fourier Transform Infrared Spectroscopy of (A) 3-CD; (B) 6-CD

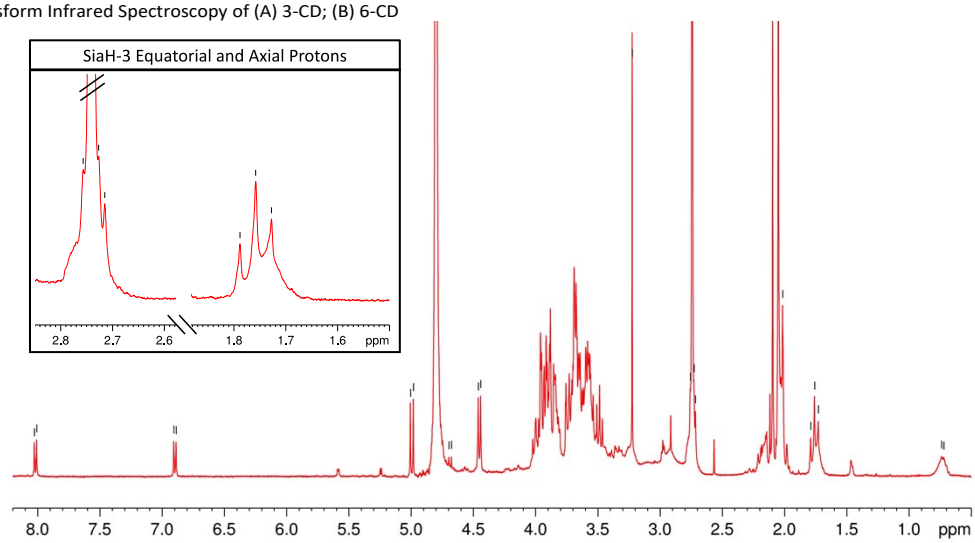

Figure S5 ^{1}H NMR spectrum of 6-CD at 400 MHz and 298 K in D_2O, where it is clear specific assignments are provided. Chemical shifts: 4.99 (d, J = 9.3 Hz, GlcH-1, ~90% α anomer), 4.80 (s, D_2O), 4.69 (d, J = 8.1 Hz GlcH-1, ~10% β anomer), 4.45 (d, J = 7.8 Hz, GalH-1), 4.00–3.40 (m, Gal, Glc and Sia ring protons), 2.75 (dd, J = 11.9, 4.2 Hz, SiaH-3eq), 2.10 – 2.00 (s, $(CH_3O)_3Si$) and (s, CH_3CONH-), 1.76, (t, J = 12.2 hz, SiaH-3ax), 0.73 (d, J = 6.9, CH_2Si). Impurities: 8.02 (d, J = 7.8 hz, DMAP*), 6.90 (d, J = 7.7 Hz, DMAP*), 3.22, (s, MeOD), 2.74 (brs, DMSO).*DMAP: 4-Dimethylaminopyridine

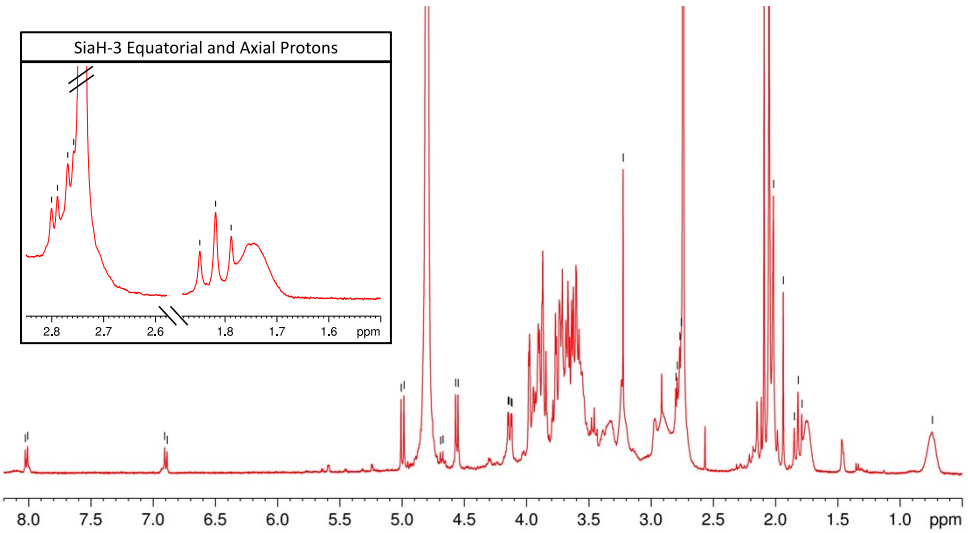

Figure S6 ^{1}H NMR spectrum of 3-CD at 400 MHz and 298 K in D_2O, where it is clear specific assignments are provided. Chemical shifts: 5.00 (d, J = 9.2 Hz, GlcH-1, ~90% α anomer), 4.80 (s, D_2O), 4.68 (d, J = 7.8 Hz ~10% Glc-1 β anomer), 4.56 (d, J = 7.8 Hz**,** GalH-1), 4.133 (dd, J = 9.8, 3.1 Hz GalH-3), 4.2–3.40 (m, Gal, Glc and Sia ring protons), 2.78 (dd, J = 12.5, 4.7 Hz, SiaH-3eq), 2.10 − 2.0 (s, $(CH_3O)_3Si$), 1.94 (s, NHAc-Sia), 1.82, (t, J = 12 hz, SiaH-3ax), 0.74 (s, CH_2Si). Impurities: 8.02 (d, J = 7.7 hz, DMAP*), 6.90 (d, J = 7.7 Hz, DMAP*), 3.22, (s, MeOD), 2.74 (brs, DMSO). *DMAP: 4-Dimethylaminopyridine

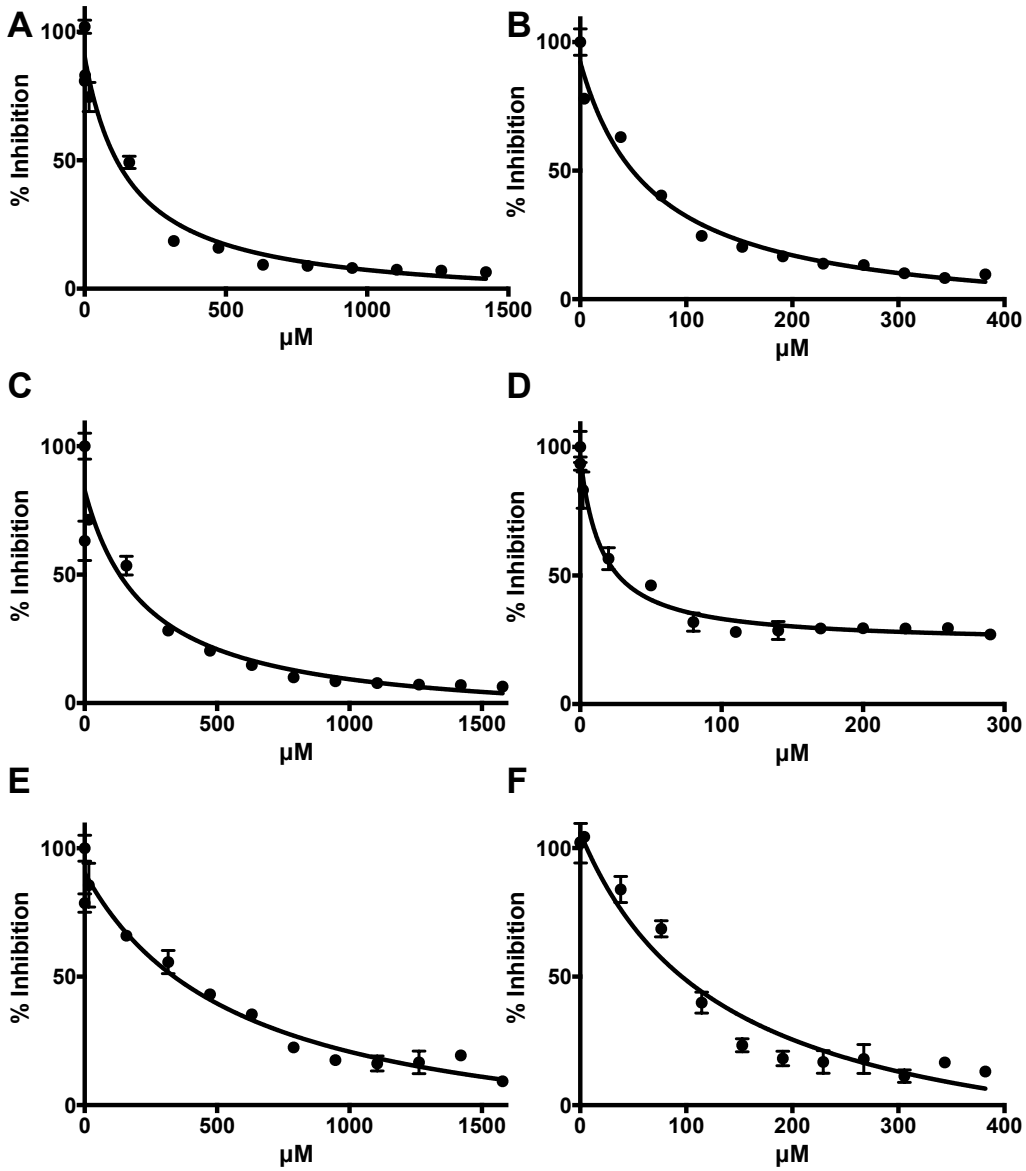

Figure S7 IC_{50} curves of Hapten inhibition assays against different Carbon Dots using least three titrations, standard deviations were within 15 %. (A) CD22 inhibition with 2,6-SL; (B) CD22 inhibition with 6-CD; (C) Siglec-1 inhibition with 2,3-SL; (D) Siglec-1 inhibition with 3-CD; (E) Siglec-1 inhibition with 2,6-SL; (F) Siglec-1 inhibition with 6-CD.

REFERENCES

1. S. J. Meyer, A. T. Linder, C. Brandl and L. Nitschke, Front Immunol, 2018, 9, 1371.
2. R. J. Cornall, J. G. Cyster, M. L. Hibbs, A. R. Dunn, K. L. Otipoby, E. A. Clark and C. C. Goodnow, Immunity, 1998, 8, 497-508.
3. G. M. Doody, L. B. Justement, C. C. Delibrias, R. J. Matthews, J. Lin, M. L. Thomas and D. T. Fearon, Science, 1995, 269, 242-244.

