corresponding site of the other molecule. The structural superposition of BoNT/A – GT1b complex showed that the electron density overlapped onto the GT1b in the structure of BoNT/A, suggesting that the observed electron density corresponds to bound 3'-siallyllactose. However, we could not construct a model of 3'-sialyllactose, as the observed electron density was too small and ambiguous.

To confirm that the observed electron density was derived from 3'-sialyllactose, crystals of apo form OFD05HC was prepared under similar conditions in which MES buffer and magnesium sulfate were used, and the structure was determined at a resolution of 3.1 Å [2]. No electron density was observed around the GBS, although the structures of OFD05HC were quite similar (RMSD 0.29 and 0.62 Å over 418 Cα atoms for two molecules in an asymmetric unit, respectively). These results confirmed that the electron density observed in the complex structure was derived from 3'-sialyllactose. The side chains as well as the main chains around GBS of the apo and complex forms were superposed well, suggesting that no major structural changes occurred after binding with 3'-sialyllactose.

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References

[1] N. Nuemket *et al*., Biochem. Biophys. Res. Commun. **41** (2011) 433.
[2] N. Nuemket *et al*., Acta Crystal. Sect. F Struct. Biol. Cryst. Commun. **66** (2010) 608.

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