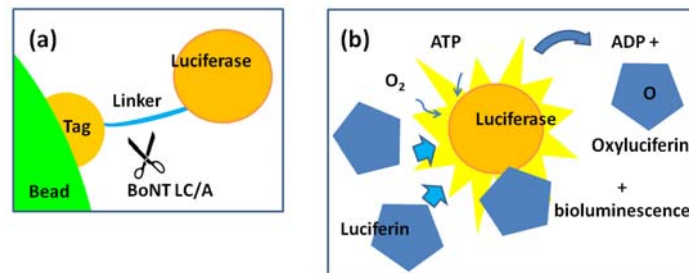


## OPTIMIZING THE LUCIFERASE RELEASE ASSAY FOR THE AUTOMATED DETECTION OF BOTULINUM NEUROTOXIN TYPE A

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**Background.** We compared the effect of length and number of truncated SNAP-25 polypeptide sequences in a linker for the luciferase release assay to determine the proteolytic activity of Botulinum Neurotoxin type A (BoNT/A) (as shown in the schematic). The purpose of this study was the optimization of the automated detection of BoNT/A in a centrifugal microfluidic device.

**Methods.** The DNA encoding truncated SNAP-25 polypeptides was inserted between that of firefly luciferase and HaloTag (Promega) using recombinant DNA techniques. Protein constructs were expressed in *E. coli*, and purified by size-exclusion chromatography, or immobilized onto HaloLink Resin. After incubation with BoNT/A light chain (LC/A), the cleavage products were analyzed by SDS-PAGE and Western Blot. Alternatively, after incubating with LC/A, the released luciferase was detected by the generated bioluminescence signal in a micro-plate reader or centrifugal microfluidic device.

**Results.** The SDS-PAGE and Western Blot analysis of the protein construct, containing two consecutive sequences of SNAP-25 amino acids 146-206 in the linker, showed that the Gln(197)-Arg(198) bond of SNAP-25 closest to the C-terminus was more susceptible to hydrolysis by LC/A than the one closest to the N-terminus. A similar result for a construct without the HaloTag further indicated that the difference in reactivity at the two sites was not due to the HaloTag but due to differences in the overall length of the polypeptide linker. These results were supported by experiments on the release of bound luciferase after LC/A incubation. Here the half-maximal effective concentration ( $EC_{50}$ ), derived from the four-parameter logistic fit of bioluminescence, after 20 minutes incubation with LC/A were  $849 \pm 63$  pM and  $10,971 \pm 476$  pM for the two- and one- SNAP-25 sequence constructs in the linker, respectively. The  $EC_{50}$  of a construct with an almost full-length SNAP-25 polypeptide, amino acids 16-206, in the linker was  $187 \pm 9$  pM. Therefore, the greater sensitivity to LC/A hydrolysis was due to the longer polypeptide linker.

**Conclusions.** These results strongly suggest that the  $EC_{50}$  of a luciferase release assay can be further reduced by using two or more of the longer sequences of SNAP-25 (amino acids 16-206) to tether the luciferase.

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