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Clickable Shiga Toxin tracer for drug delivery

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CLICKABLE SHIGA TOXIN TRACER FOR DRUG DELIVERY

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Directional click chemistry has a great potential for delivery applications without disturbing active binding site of cellular ligands. Commonly used antibody conjugate or N-Hydroxysuccinimid (NHS)-biotin coupling offers expensive and random probe attachments to a tracer molecule. One of natural cancer cell marker, Shiga Toxin B-subunit (STxB), is often produced heterologously in *Escherichia coli,* randomly labelled and used as molecular tool for Gb3 [Gb3; Gala(1-4)-Galb(1-4)-Glcb1-ceramide] tumour receptor detection, cell-specific vectorization, and imaging purposes. Complete process is excessively complex with inconsistence results that differs between each performed assay.

Here, we present the recombinant STxB production wt and with non-canonical amino acids (ncAAs). The products are manufactured using the proprietary technology of enGenes-X-press for growth decoupled production in *Escherichia coli (E. coli).* It delivers high quality and quantity of STxB analysed for yield, purity, receptor recognition and delivery of STxB into the cellular milieu. We evaluated the production at different condition in high-throughput µ-scale fed-batch-like cultivation and 1 L benchtop-bioreactor fed-batch cultivation. In our studies, we validated activity and selectiveness of the produced STxB (wt/ncAAs) to Gb3 receptor on tumour cells with the analytical methods to deduct the kinetics profiles of those interactomes. We have established manufacturing protocols for the soluble production of STxB (wt/ncAAs) that can be easily up-scaled. Furthermore, we showed that directional immobilization of dye increase specificity of STxB tracer inside the cell.