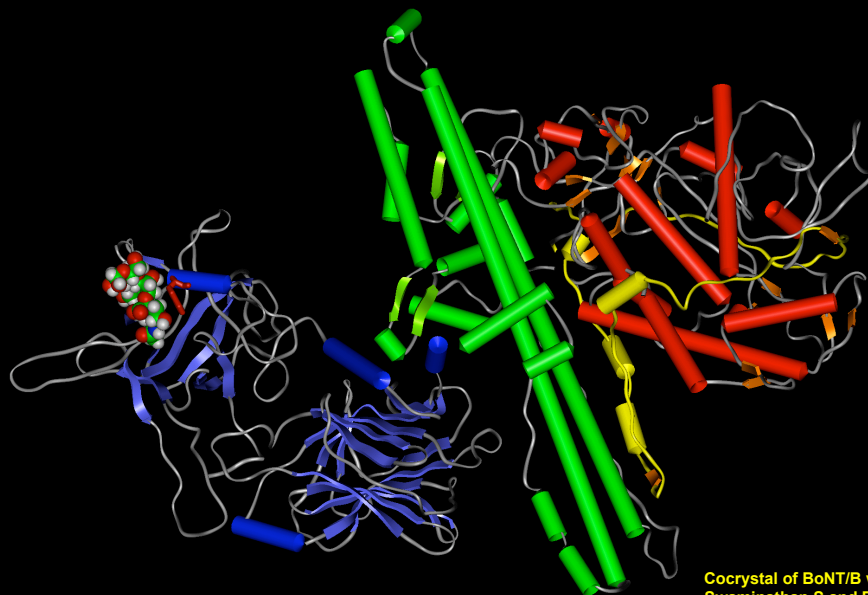
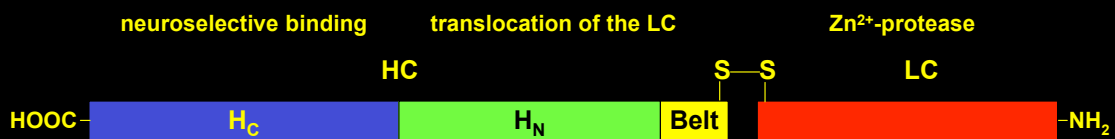


Botulinum Neurotoxin: Research activities on alternative activity tests in Europe

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Structure of the Clostridial Neurotoxins



Cocrystal of BoNT/B with sialyllactose.
Swaminathan S and Eswaramoorthy S,
Nat Struct Biol 2000, 7 (8), 693-9.

Three groups of assays to detect/determine BoNT

1st group

Assays appropriate to detect the protein BoNT or the encoding substances

- irrespective whether it possesses proteolytic activity or is inactivated by denaturation.

2nd group

Assays appropriate to detect the proteolytic activity of LC-BoNT

- irrespective whether the enzymatic LC is isolated, connected to the heavy chain or the heavy chain is denaturated.

3rd group

Assays appropriate to determine the full biological activity

- i.e. to test the suitable function of all three domains of BoNT: the binding/uptake, translocation/release of LC, and proteolytic activity of LC.

1st Group

Immunochemical detection of BoNT protein

ELISA

Widespread use in BoNT research

- Biotecon GmbH, Potsdam, Dr. Frevert
- Merz Pharmaceuticals GmbH, Frankfurt/M, Dr. Taylor
- Pasteur Institute, Paris, Dr. Popoff (DRET)

Immunoaffinity chromatography

- Applied Biotechn. in the Tropics, Göttingen, Dr. Gessler (BMBF)

Detection of DNA encoding BoNT (indirect)

PCR

- Dep. Hygiene, Univ. Helsinki, Dr. Lindstrøm, Fin. RP, Nat. Tech. Ag.
- Wehrwissenschaftl. Institut, Bundeswehr, Munster, Dr. Dierstein
- Pasteur Institute, Paris, Dr. Popoff (DRET)

DNA-Microarray

- Friedrich-Löffler-Institut, Jena, Dr. Seyboldt (BMBF)

2nd Group

Assays appropriate to detect the proteolytic activity.

Detection of cleaved substrates

Fluorescence detection using

FRET (SNAPtide™)

ELISA

- Biotecon GmbH, Potsdam, Dr. Frevert
- Merz Pharmaceuticals GmbH, Frankfurt/M, Dr. Eisele
- NIBSC, London, Dr. Sesardic

Endopep-MS

- Health Protection Agency, Salisbury, Dr. Shone

3rd Group

Assays determining the full biological activity

Binding and catalytic activity can be determined separately, this gives, however, no information whether the LC is translocated.

All steps can only be tested in intact cells:

- in cell cultures (primary cultures, cell lines, stem cells)
- *in situ* preparations
- *ex vivo* systems
 - mice phrenic nerve hemidiaphragm assay
NIBSC, London, Dr. Sesardic
Inst. Neurology, University of Rostock, Dr. Benecke
Med. Hochschule Hannover, Dr. Rummel/Dr. Bigalke (SET)

The mice phrenic nerve hemidiaphragm assay will be presented in detail in session 4A (Tue 8.30 am).