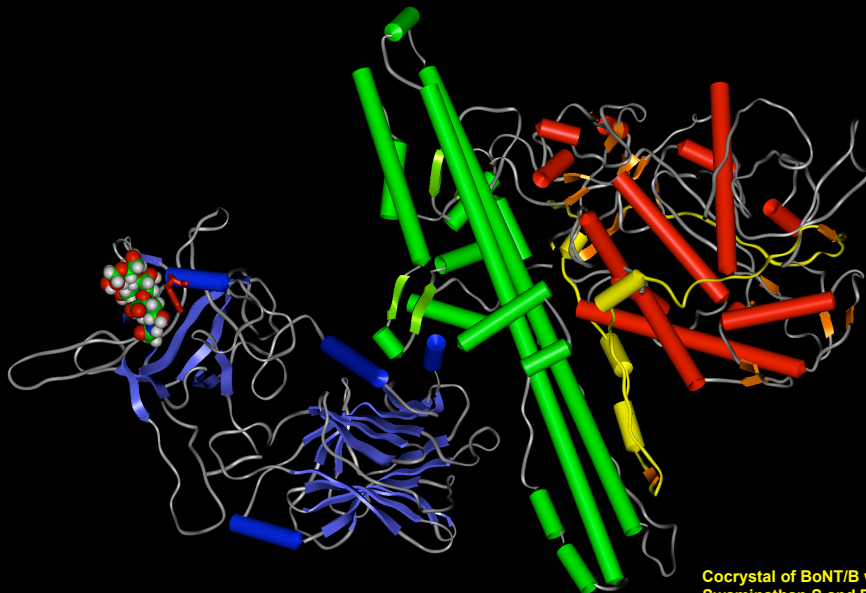
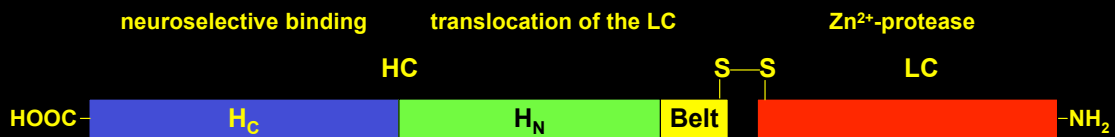


## Botulinum Neurotoxin: Determination of activity using a mice *ex vivo* test

Andreas Rummel

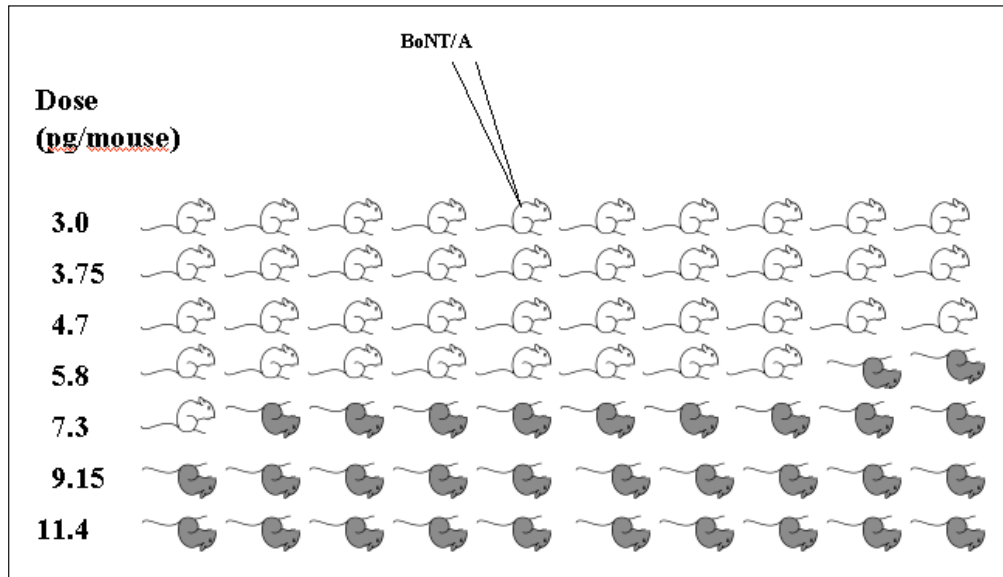
**MHH** Institut für  
Toxikologie  
Medizinische Hochschule Hannover  
Germany

## Structure of the Clostridial Neurotoxins

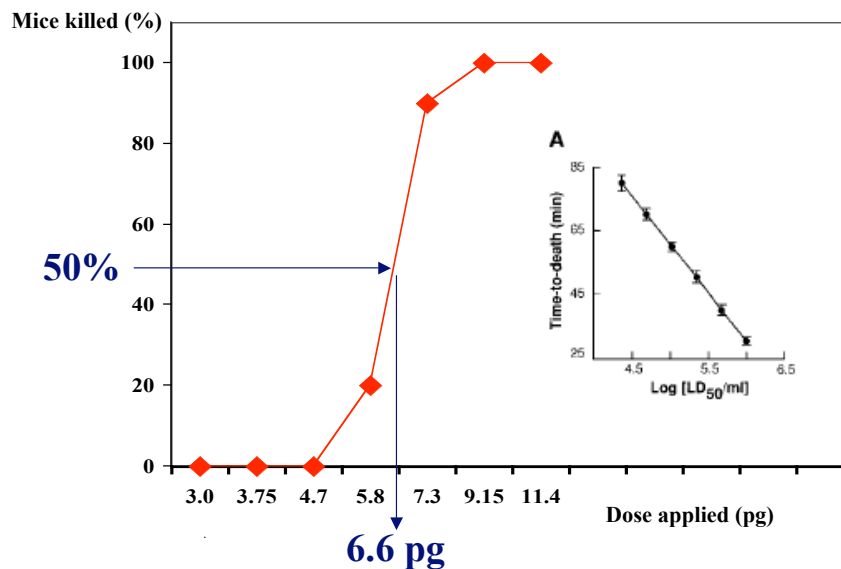


Cocrystal of BoNT/B with sialyllactose.  
Swaminathan S and Eswaramoorthy S,  
*Nat Struct Biol* 2000, 7 (8), 693-9.

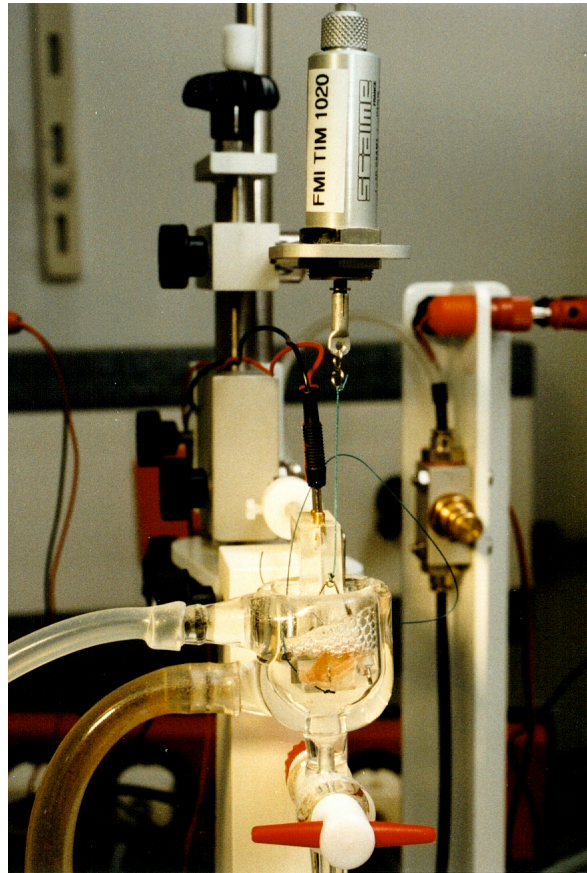
## Determination of activity of clostridial neurotoxins by the mice bioassay



## Determination of activity of clostridial neurotoxins by the mice bioassay



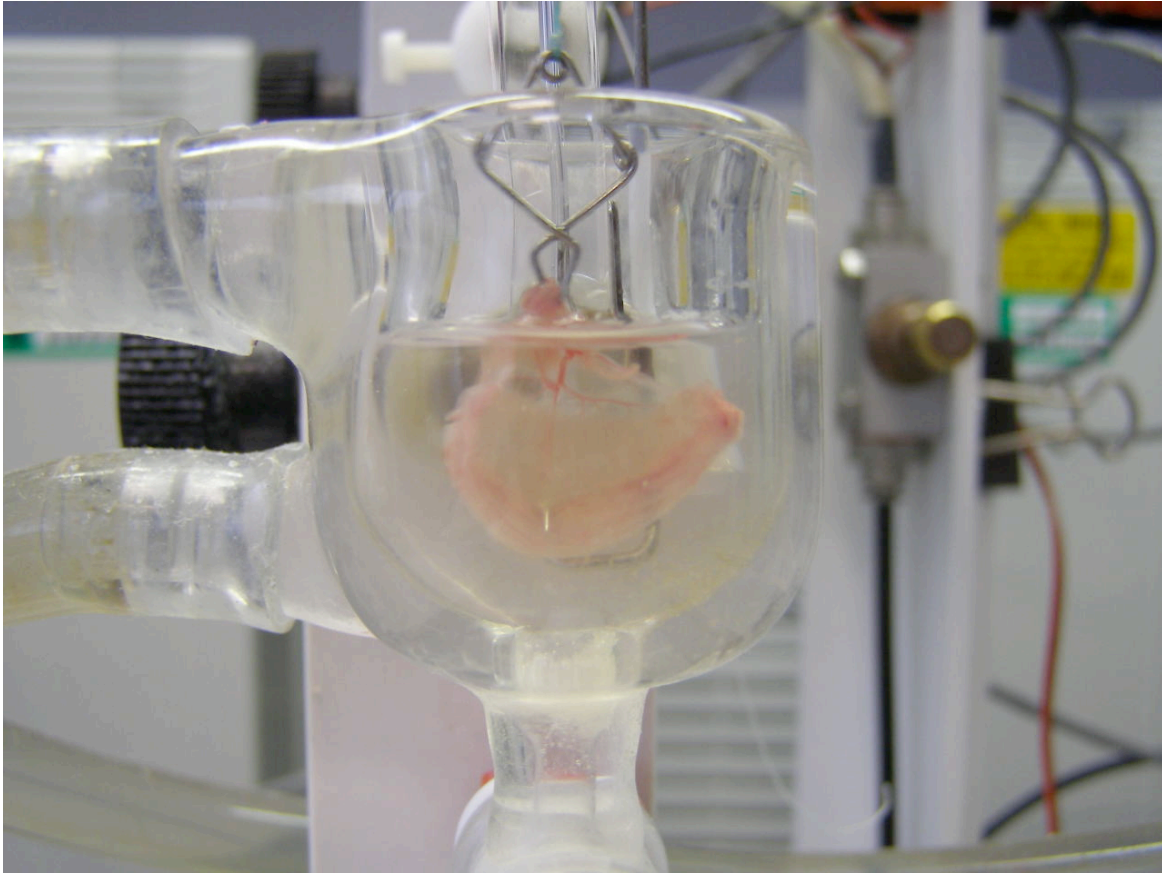
Determination of activity of clostridial neurotoxins by an *ex vivo* assay (MPN, mice phrenic nerve hemidiaphragm)



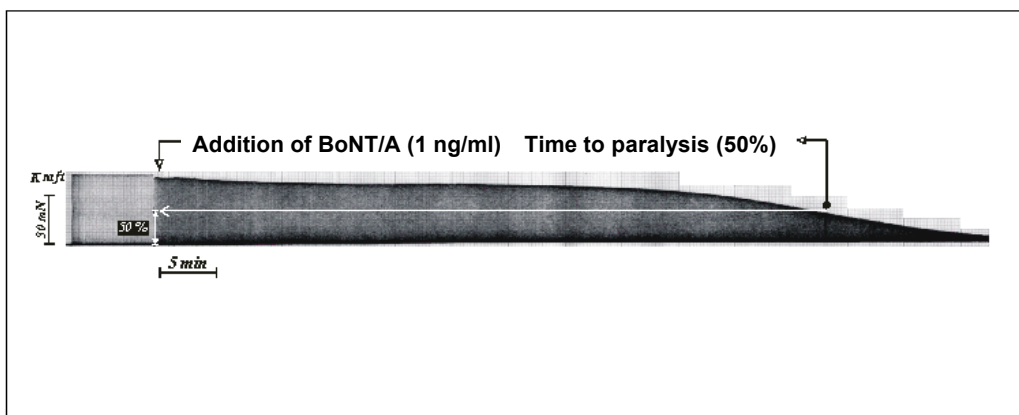




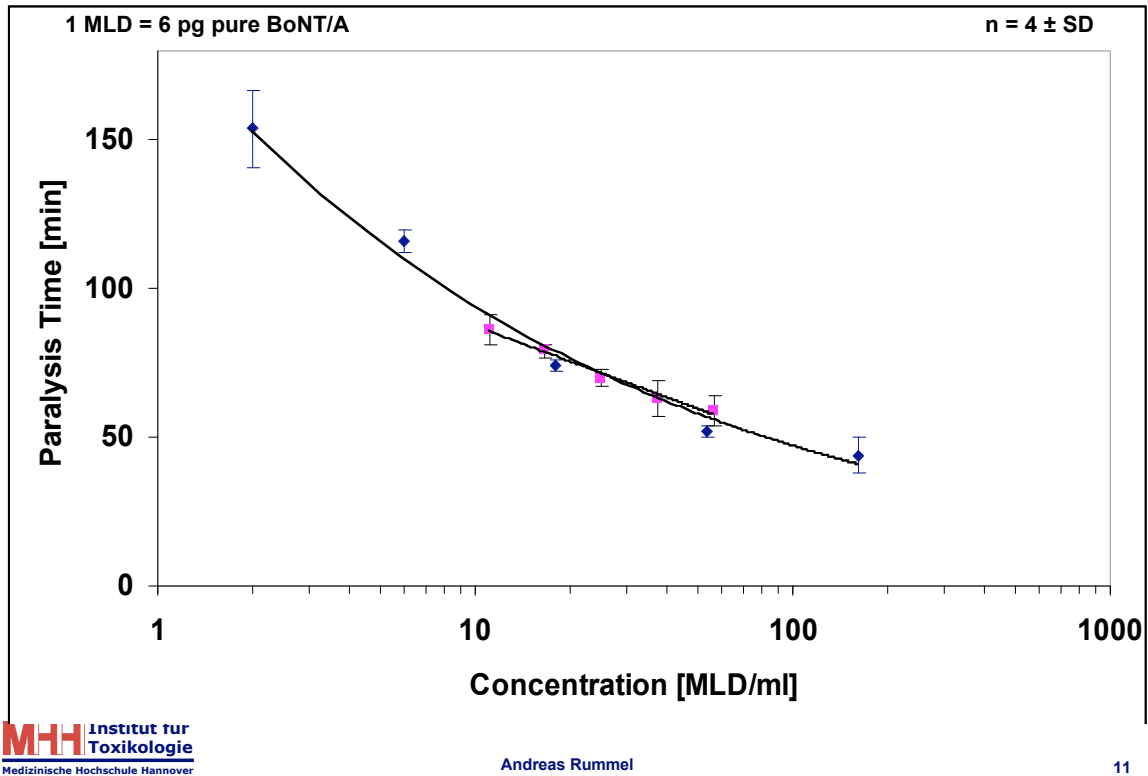




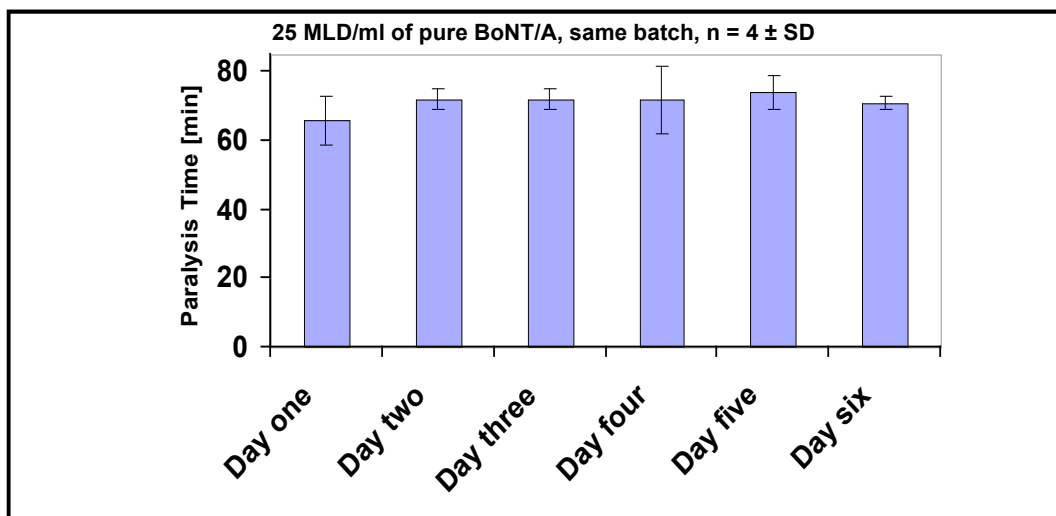
## Read out of the MPN assay



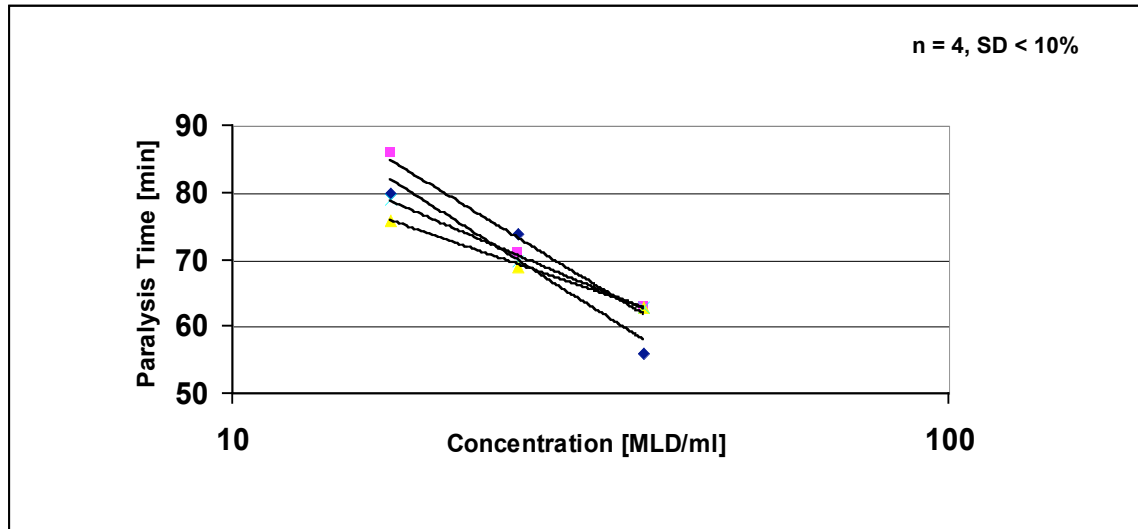
## Concentration-response-curves of BoNT/A in MPN assay



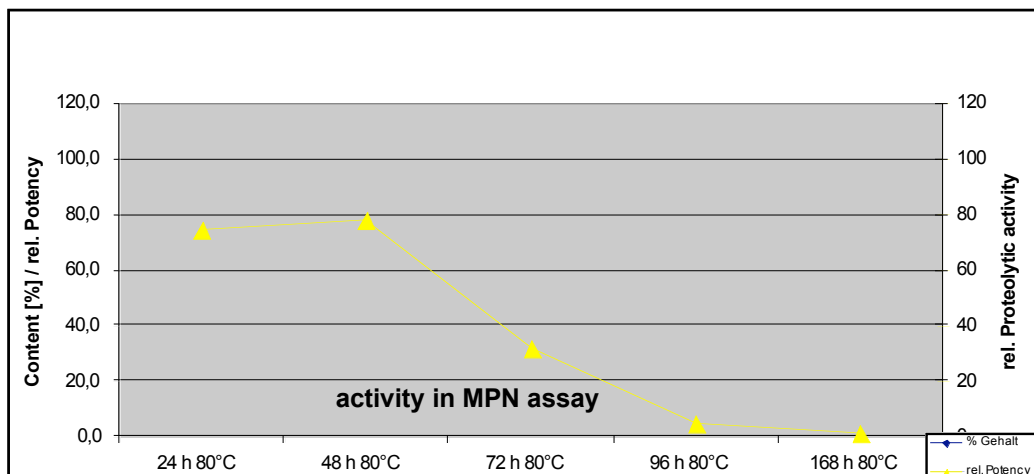
## Day to day variability of BoNT/A in MPN assay



## Comparison of 4 batches of pure BoNT/A in MPN assay

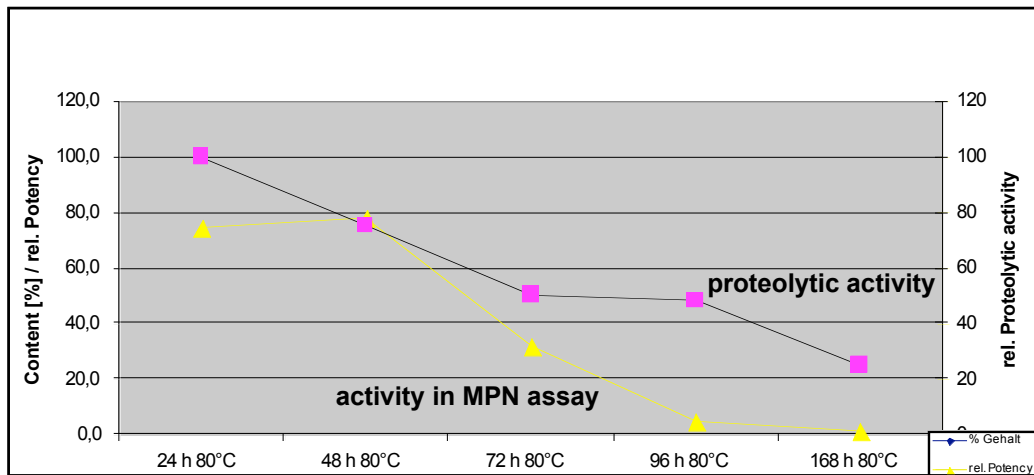


## Heat stress stability of BoNT/A in MPN assay

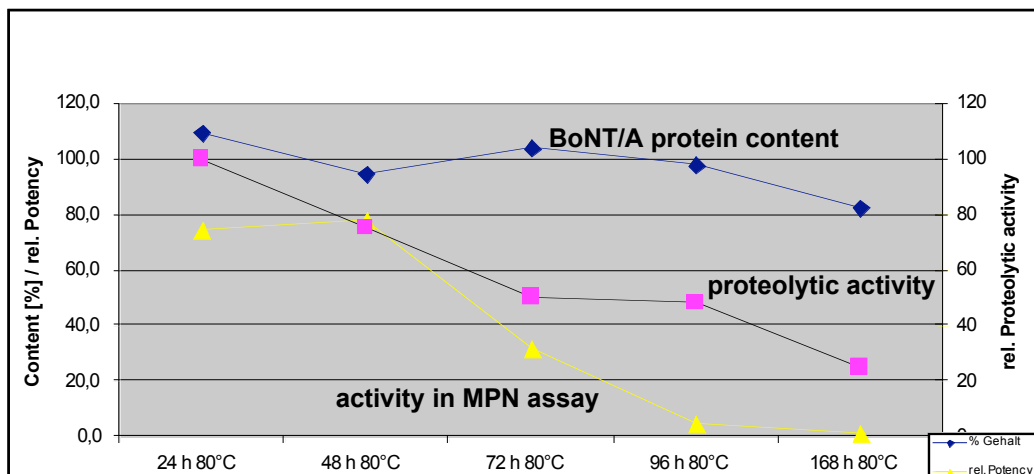




## Heat stress stability of BoNT/A in MPN assay



## Heat stress stability of BoNT/A in MPN assay



## Advantages of MPN assay

- No animal experiment but resembles mice bioassay
- Results within 2 hours
- Experimental conditions can easily be varied
- Can also be used to quantify neutralizing antibodies  
=> service offered by [www.toxogen.de](http://www.toxogen.de)

## Disadvantages of MPN assay

- Animals are still required
- Requires experimental skills
- Sophisticated and expensive equipment (\$ 100,000)
- Lavishes much time and money on validation under GMP regulations
- Only 4 tests can be performed per day under GMP (for screening up to 12 tests per person/facility/day)
- Test is costly under GMP regulation

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Personnel:

Hans Bigalke  
Kirstin Häfner  
Christina Knorr  
Beate Laske  
Kristina Reincke  
Andreas Rummel