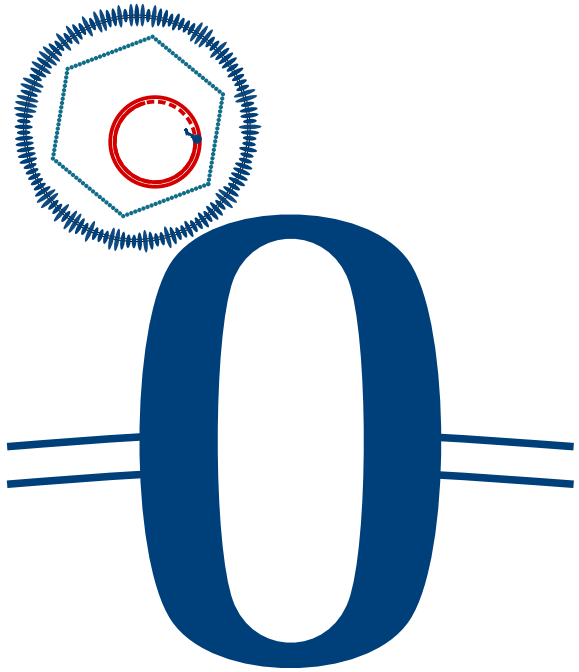
Optimization of an *in vitro* assay for identification of NTCP inhibitors



Pieter Van Brantegem
KU Leuven

Meet the Experts Transporter Conference Budapest
26 April 2018

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Affects 250 million people world wide

Is a common cause of cirrhosis and hepatocellular carcinoma

Cannot be eradicated yet

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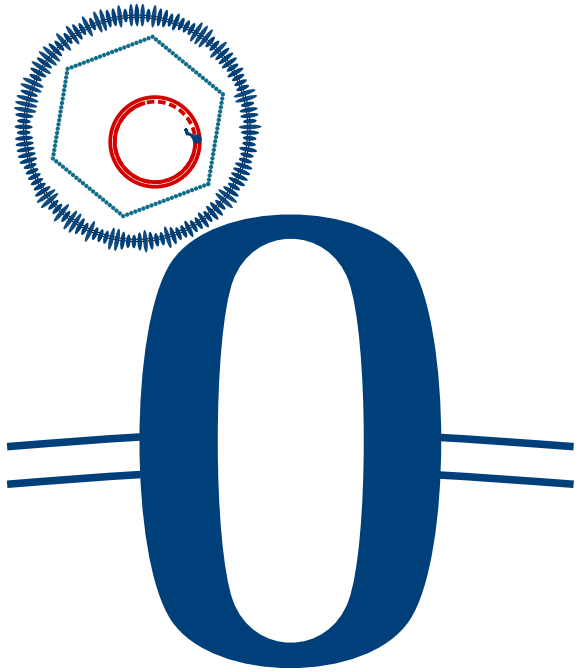

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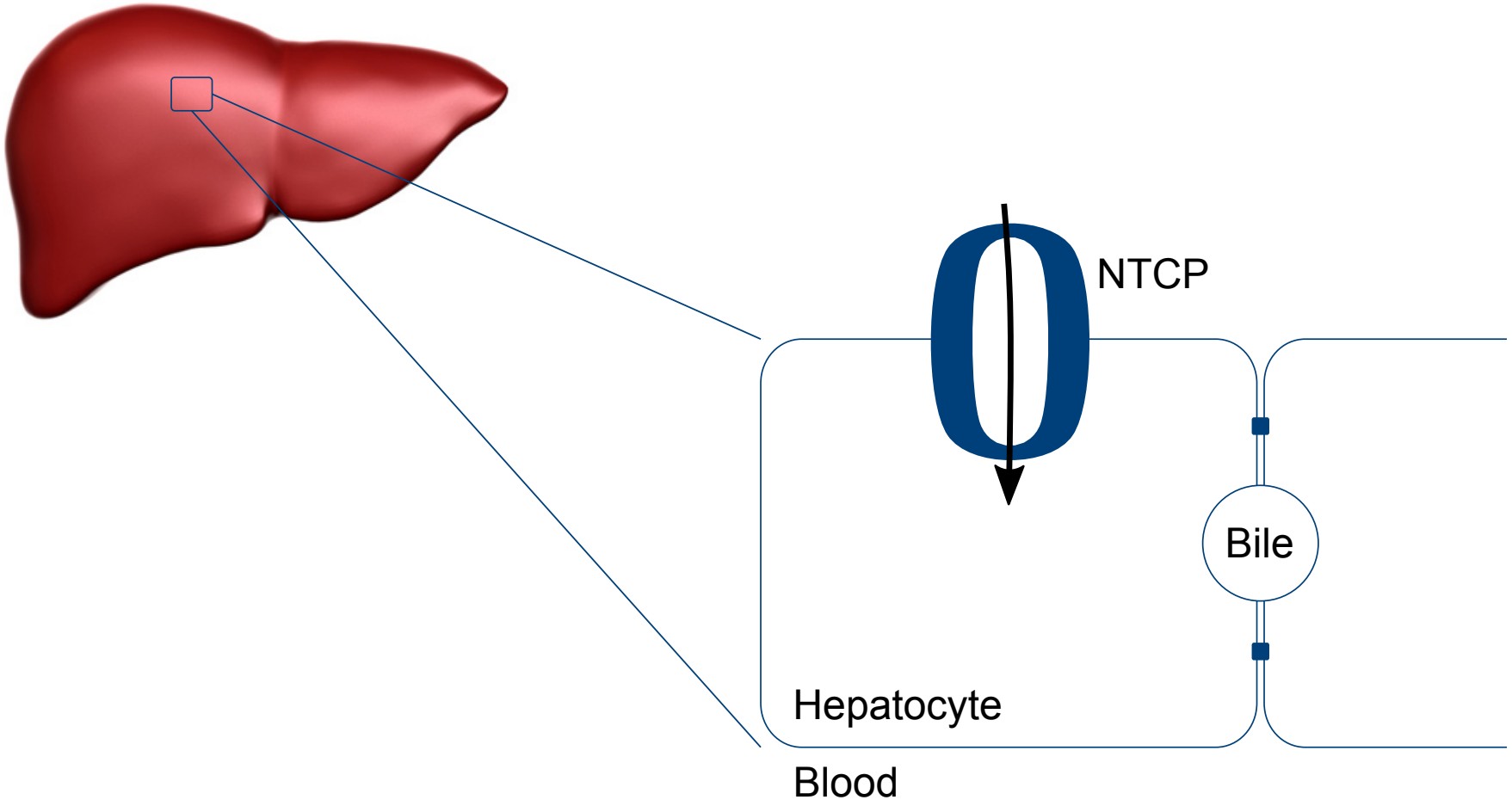
NTCP inhibition prevents HBV entry into hepatocytes



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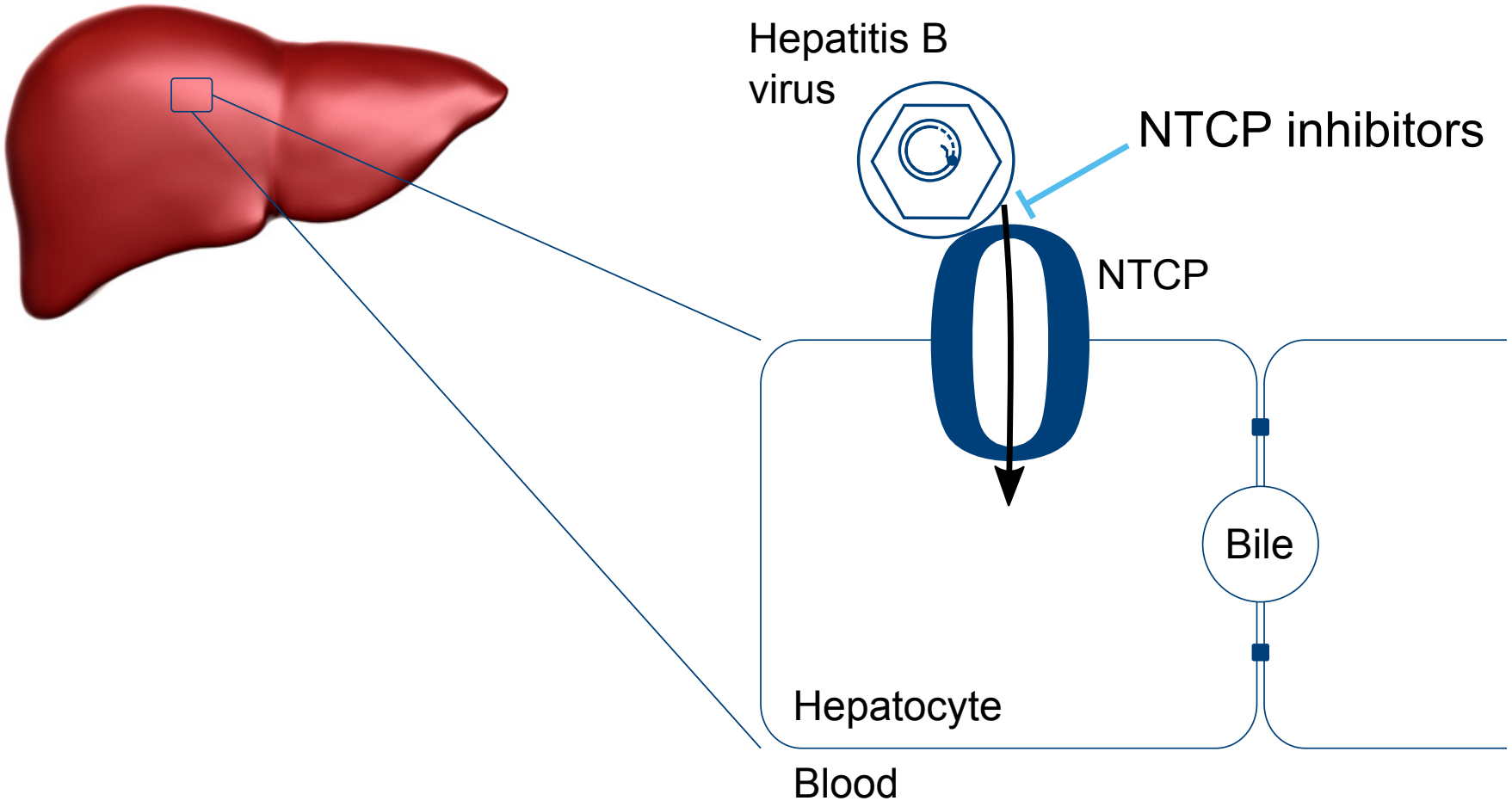

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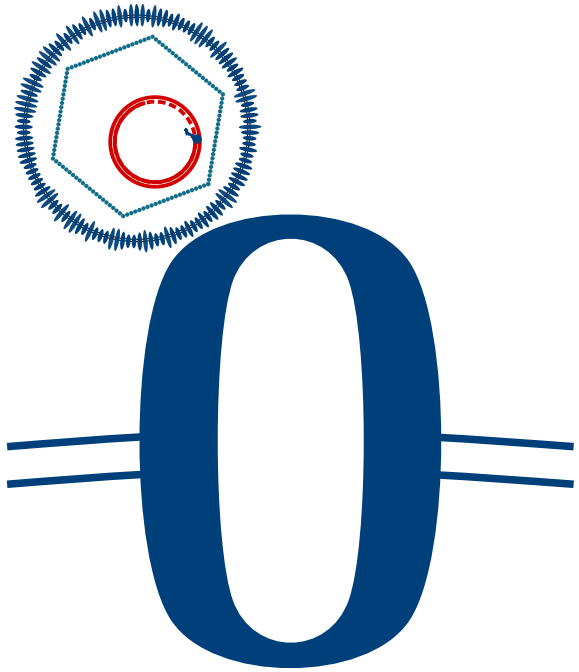

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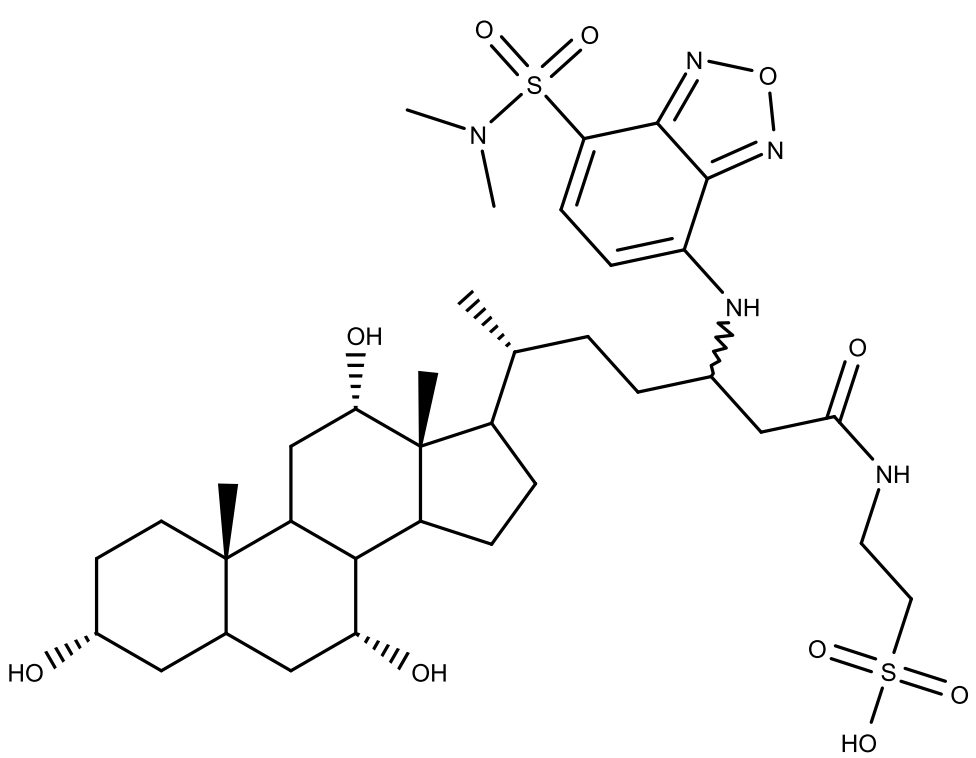

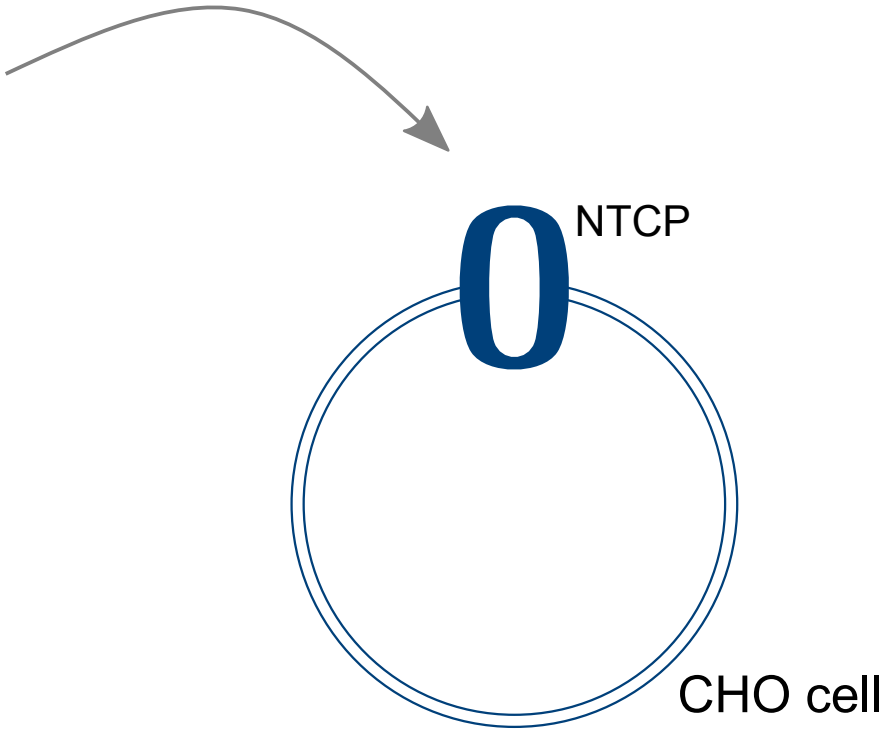

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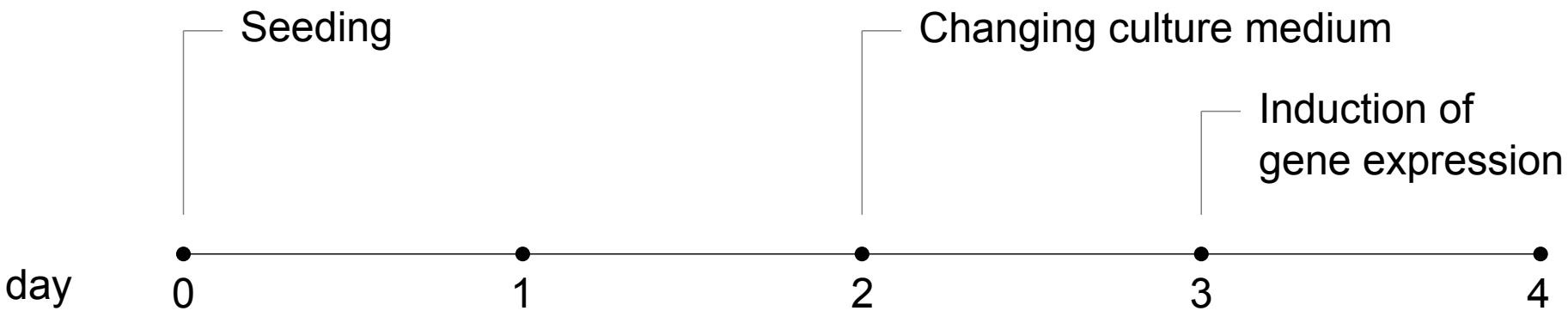

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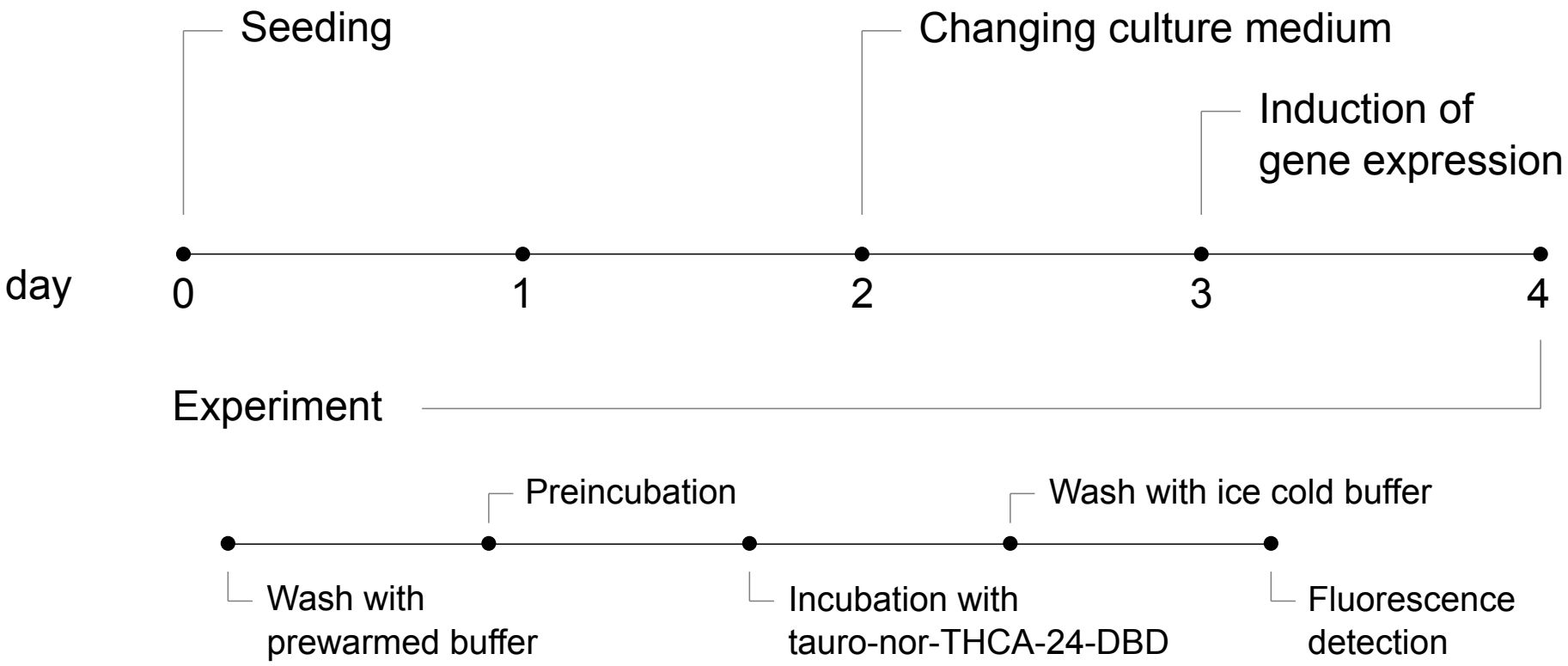

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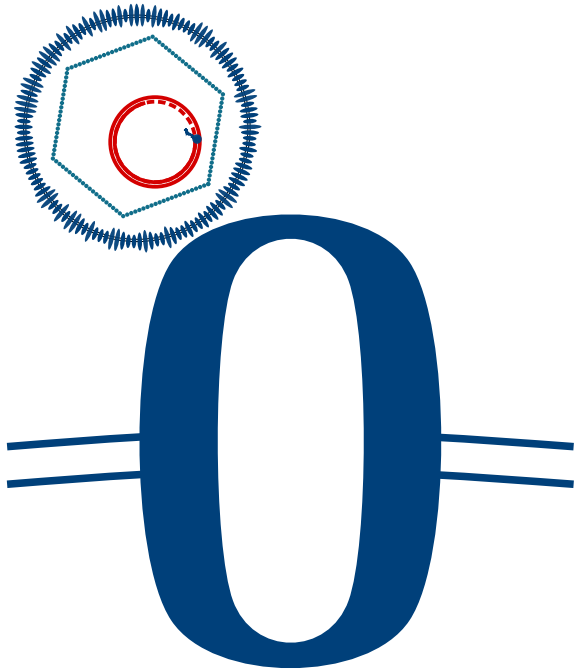

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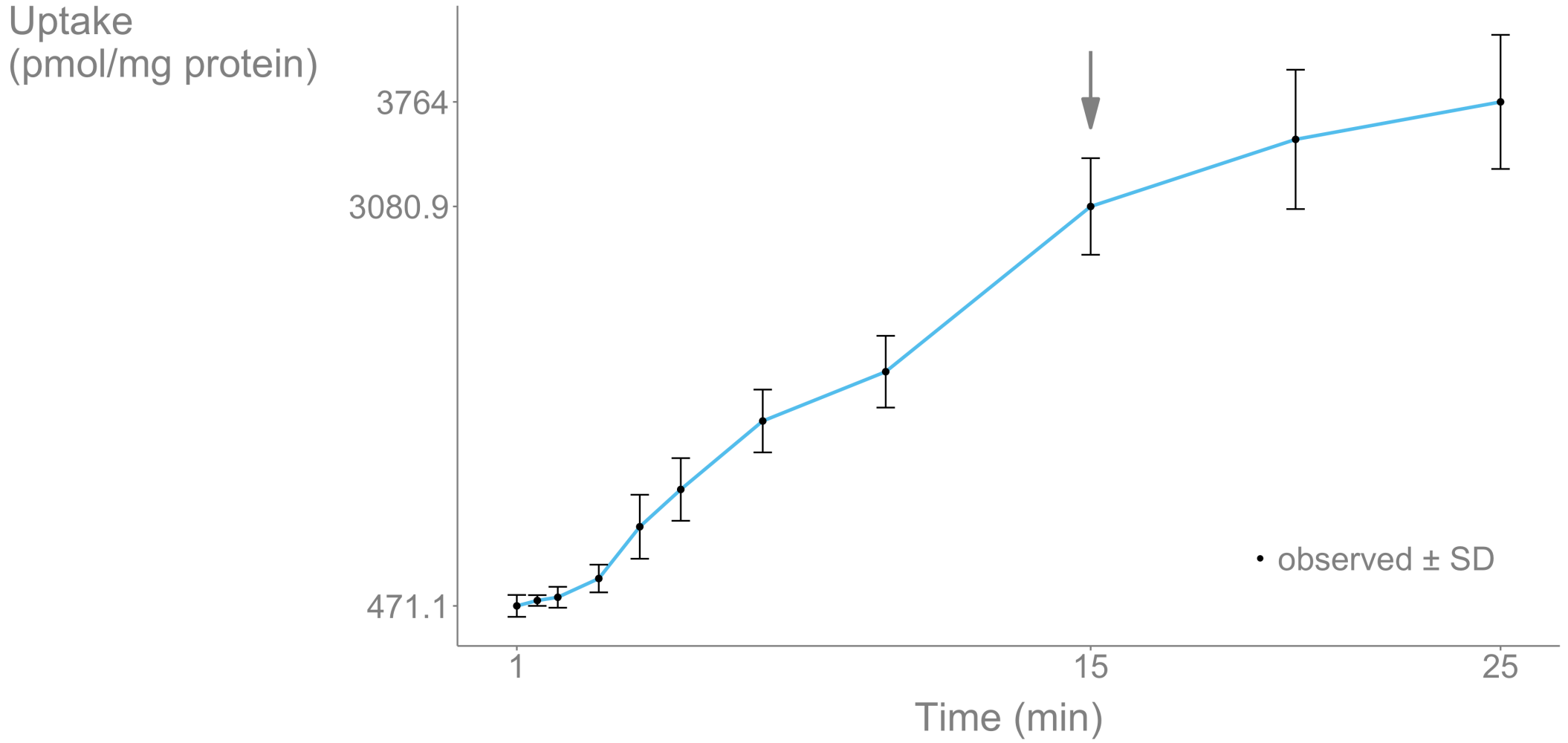
Uptake of 10 µM tauro-nor-THCA-24-DBD increased up to 20 minutes incubation



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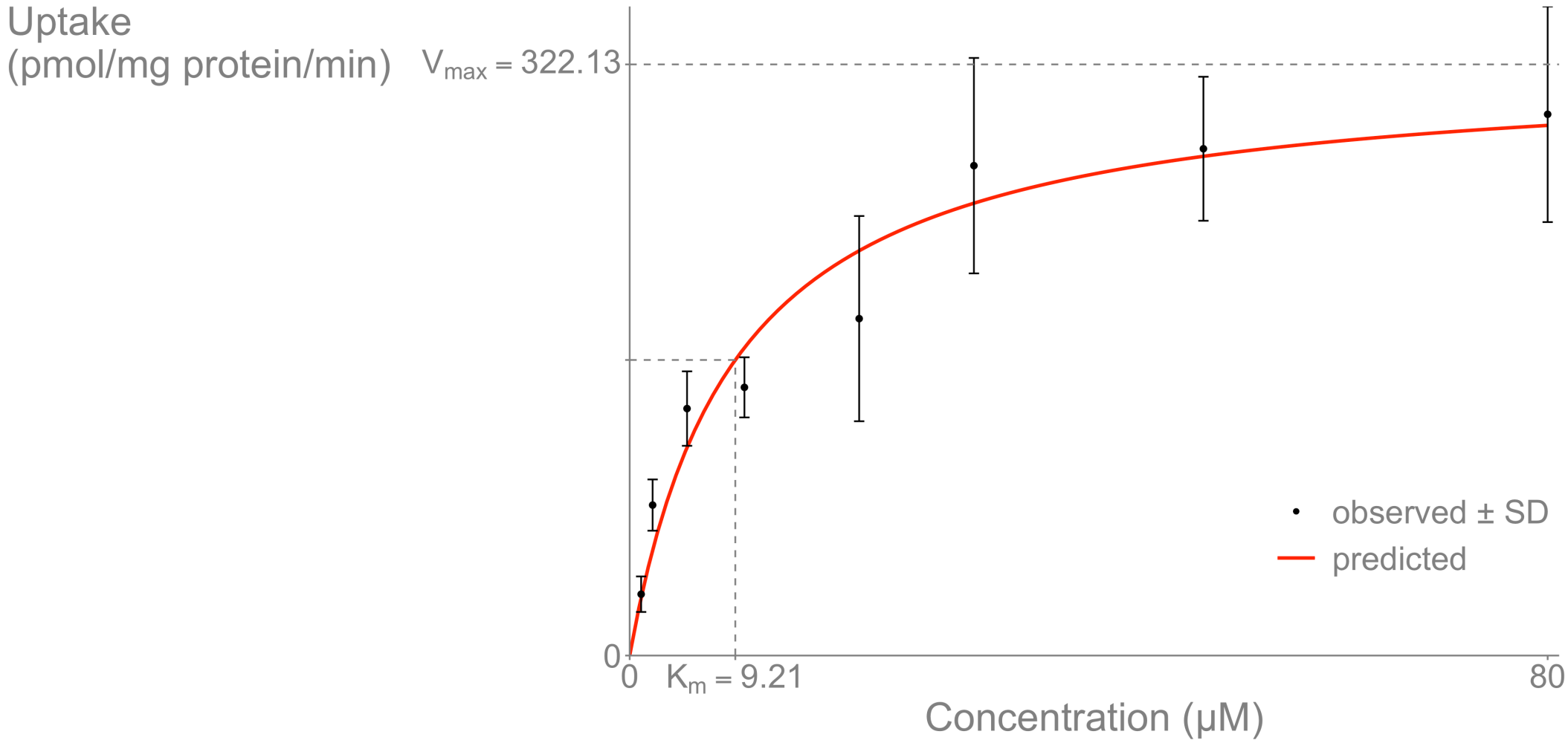

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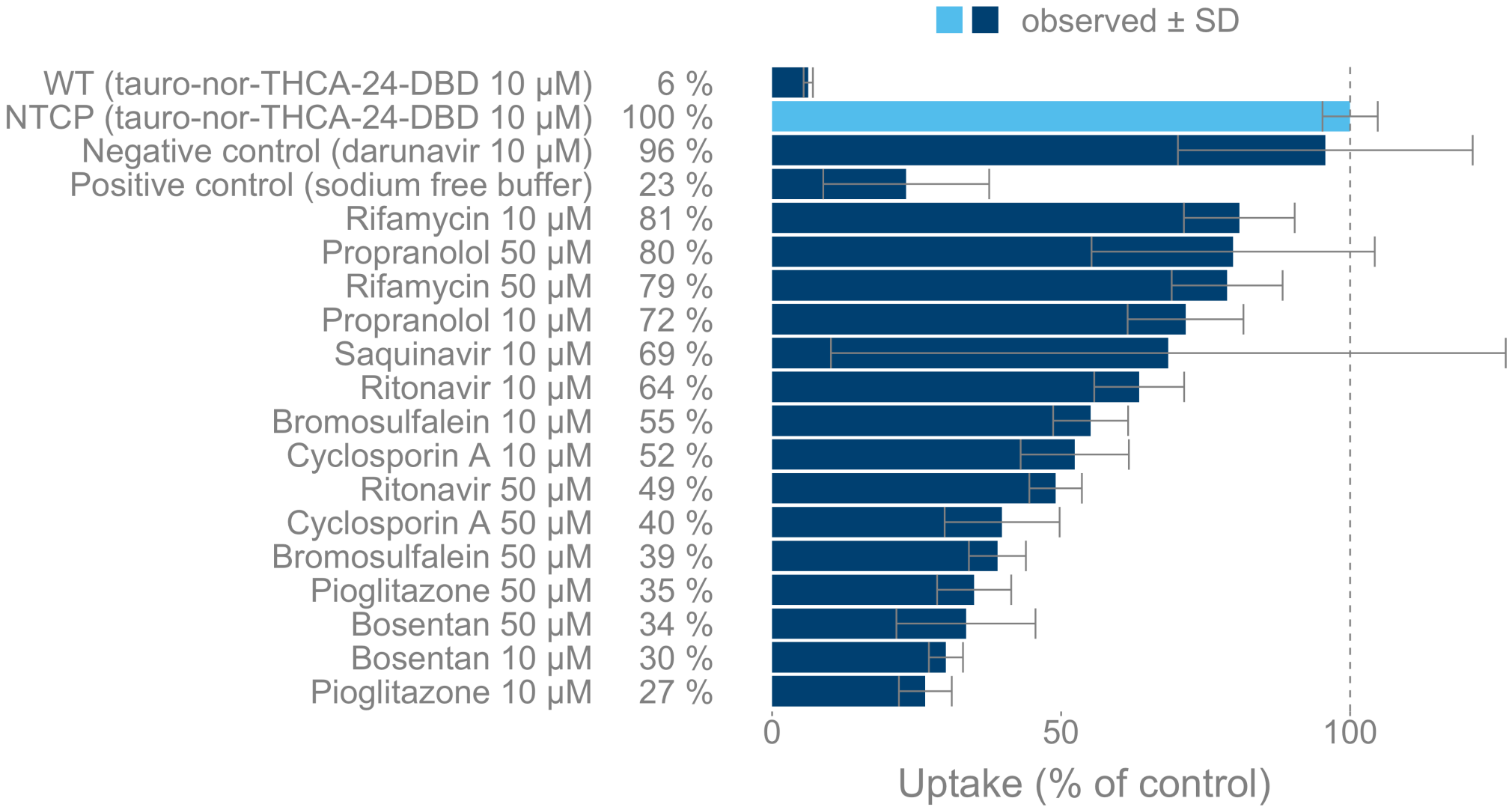

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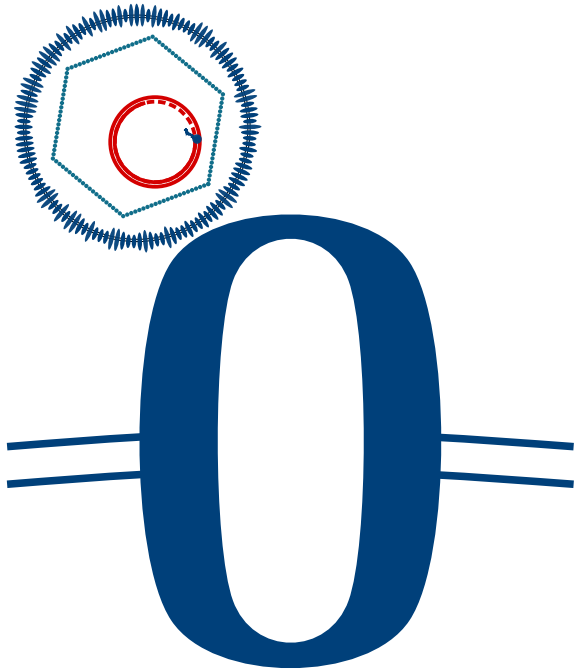

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The assay was optimized and will be used for identification of novel NTCP inhibitors

Uptake in wild-type cells remained below the LOQ at all time points

Inhibitory potential of 2000 compounds will be explored

Uptake will expressed to the control of each batch

A (proteo)chemometric model will be developed

Acknowledgement

KU LEUVEN

LLID
Laboratory of Liver Infectious Diseases

fwo Research Foundation Flanders
Opening new horizons

Prof. Bruno Stieger
University Hospital Zürich

Optimization of an *in vitro* assay for identification of NTCP inhibitors

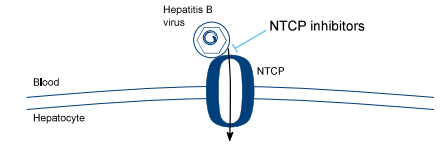
Pieter Van Brantegem, Philip Meuleman and Pieter Annaert

Chronic hepatitis B virus (HBV) infection

- affects up to 250 million people world wide
- is a common cause of cirrhosis and hepatocellular carcinoma
- cannot be eradicated yet

Sodium taurocholate co-transporting polypeptide (NTCP)

- is a hepatic transport protein
- is located at the sinusoidal membrane
- transports bile acids from the blood into hepatocytes
- has been identified as receptor for entry of the HBV into hepatocytes



NTCP inhibition to prevent HBV entry has been shown to be successful → We need to find small molecules that inhibit NTCP

Therefore, we developed an *in vitro* assay that allows high throughput screening

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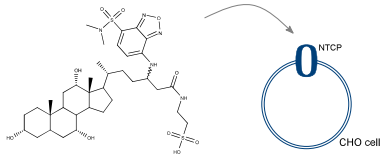

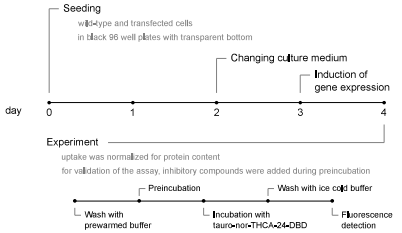

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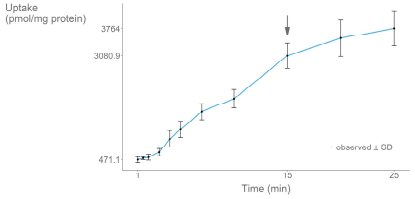

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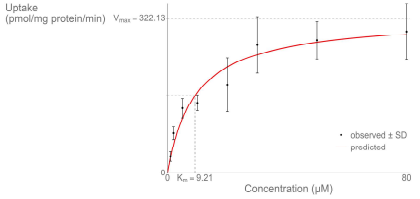

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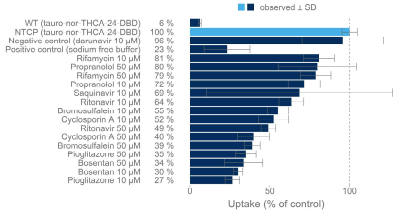
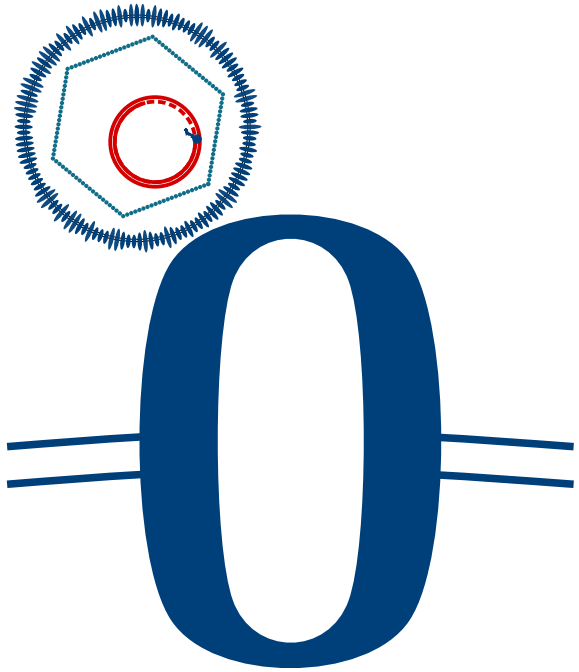


The assay was optimized and will be used for identification of novel NTCP inhibitors

- Uptake of tauro-nor-THCA-24-DBD in wild-type cells remained below the limit of quantification at all time points
  - as a consequence, no correction for uptake in wild-type cells will be made
- The inhibitory potential of 2000 compounds will be explored at equimolar concentrations of 10 µM and 15 minutes incubation time
- Uptake will be expressed relative to the control for each batch
- Compounds will be considered strong inhibitors if at least 50% uptake inhibition can be obtained
- Cyclosporin A 50 µM will be used as positive control

Optimization of an *in vitro* assay for identification of NTCP inhibitors



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