

# Tag der Forschung

1. Dez 2023

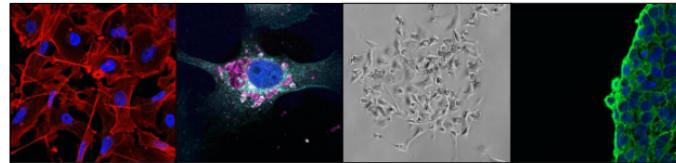


# Programm

<b>UHRZEIT</b>	<b>FACHBEREICH</b>	<b>LEHRSTUHL/ NACHWUCHSGRUPPE</b>	<b>REFERENT*IN</b>	<b>PROGRAMMPUNKT/ VORTRAGSTHEMA</b>
9:00 – 9:10	<b>Begrüßung</b>			
9:10 – 9:30	<b>Pharmakologie</b>	Prof Biel	Prof Biel PD Dr. Fenske Dr. Mehlfeld	Pharmakologie
9:30 – 9:40		Prof Koch	Prof Koch	Molekulare Pharmakologie
9:40 – 9:45	<b>Pharm. Technologie</b>	Prof Friess	Prof Friess	Pharmaeugetische Technologie
9:45 – 10:05		Prof Merkel	Dr. Winkeljann & alle Senior Scientists	Pharmazeutische Technologie
10:15 – 10:25	<b>Pharm. Biologie</b>	Prof. Wagner	Dr. Berger	Pharmazeutische Biotechnologie
10:25 – 10:35		Prof. Fürst	Prof. Zahler	Pharmazeutische Biologie
10:35 – 10:45	<b>Pharm. Chemie</b>	Prof. Bracher	Prof. Bracher	Pharmazeutische und Medizinische Chemie
10:45 – 10:50		Dr. Müller	Dr. Müller	Pharmazeutische und Medizinische Chemie
10:50 – 11:00		Prof. Merk	Fr. Stiller	Pharmazeutische und Medizinische Chemie
11:00 – 11:10		Prof Huc	Prof Huc	Pharmazeutische und Medizinische Chemie
11:10 – 11:15		Dr. Konrad	Dr. Konrad	Pharmazeutische und Medizinische Chemie
11:15 – 11:20		Dr. Thorn-Seshold	Dr. Thorn-Seshold	Pharmazeutische und Medizinische Chemie
11:20 – 11:30	<b>Klinische Pharmazie</b>	Prof. Scherf-Clavel	Prof. Scherf-Clavel	Klinische Pharmazie
11:30	<b>Zeit zum Austausch (in kleiner Runde)</b>			

## Vorstellung einer neuen Seminarreihe

### CANCER BIOLOGY SEMINAR SERIES



Monday 15.01.2024

17:00 st

Wieland-HS

Lina Ouologuem

„Targeting the lysosome in cancer  
immunity”

Anna Milton

“Characterization of cancer progression in  
Non-Small Cell Lung Cancer (NSCLC) cell  
lines”

## CHE HOCHSCHULRANKING 2024

### STUDIERENDENBEFRAGUNG

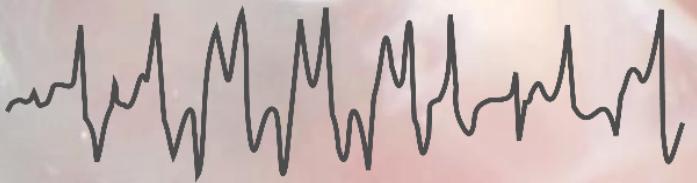
- Ab 4. Dezember bis 4. Februar 2024
- Einladung via E-Mail an ...@campus-lmu.de
- **anonym**
- Veröffentlichung der Ergebnisse im ZEIT Studienführer



**Ihre zahlreiche Teilnahme ist sehr wichtig!**



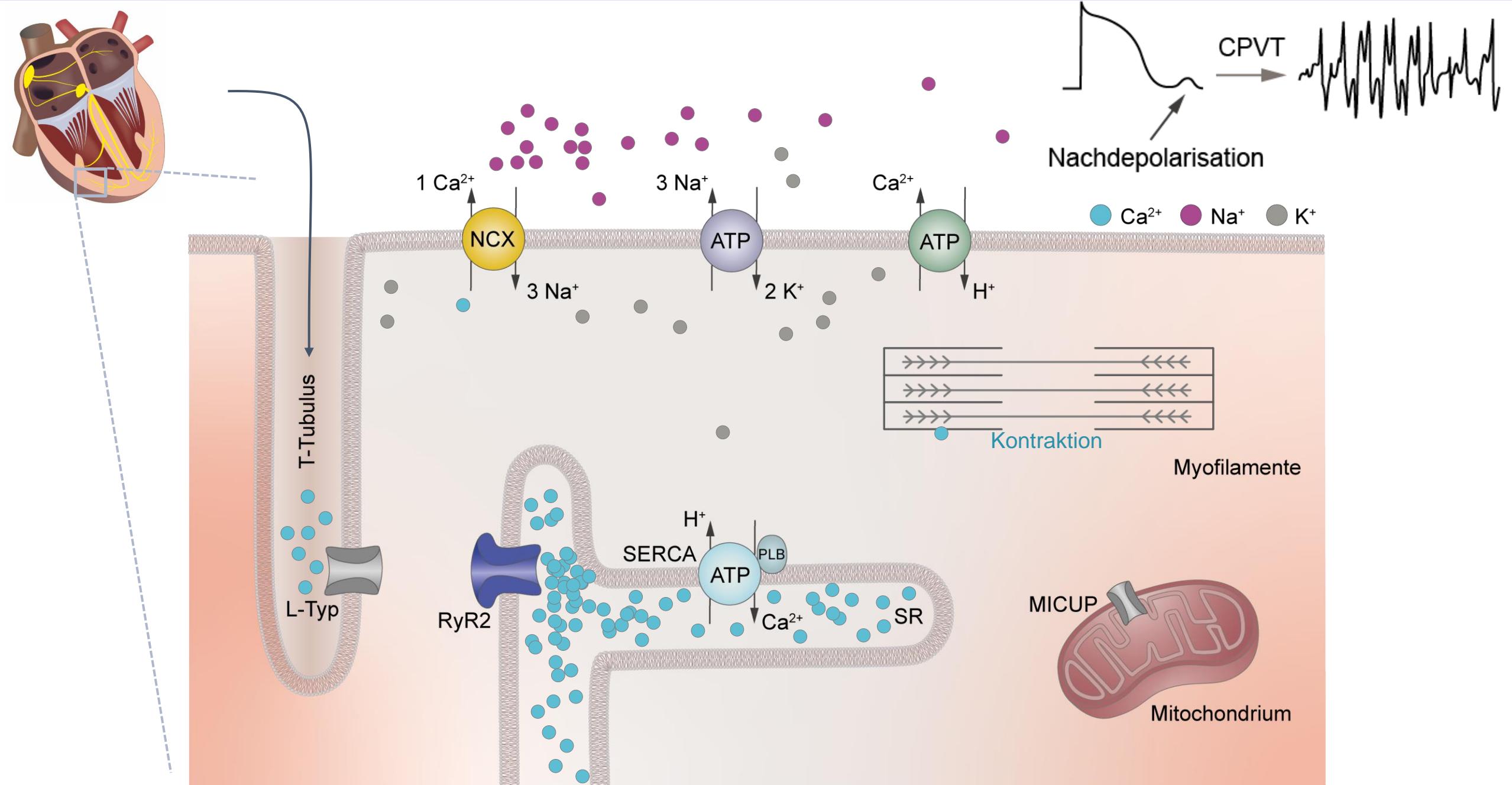
# Gentherapeutische Ansätze zur Behandlung der catecholaminergen polymorphen ventrikulären Tachykardie



PD Dr. Stefanie Fenske

Arbeitsgruppe molekulare kardiovaskuläre Pharmakologie

# Elektromechanische Kopplung und katecholaminerge polymorphe ventrikuläre Tachykardie (CPVT)



# Therapie der CPVT / Projektziel

## Therapie

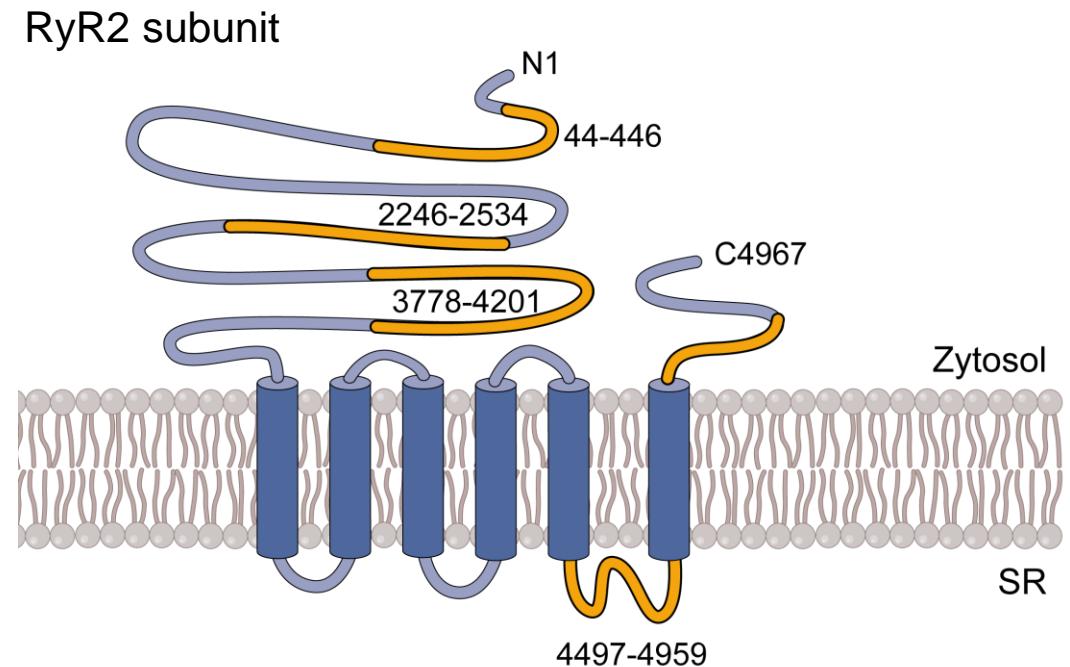
- $\beta$ -Adrenozeptor-Blocker
- Implantation eines Defibrillators

## Herausforderungen der Gentherapie

- gain-of-function Mutationen
- sehr großes Gen (ca. 15 000 bp coding sequence)
- > 160 Mutationen

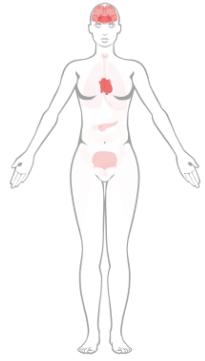
## Projektziel

- Entwicklung eines universellen Gentherapie-Ansatzes zur Behandlung der CPVT

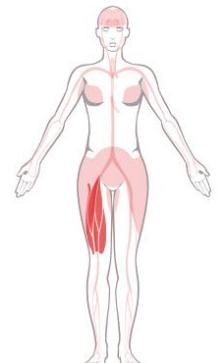


# Projektziel

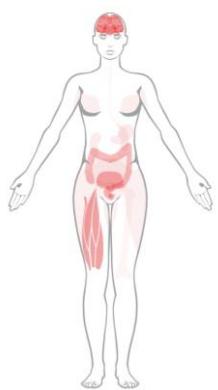
expression pattern



RyR2  
heart muscle  
isoform



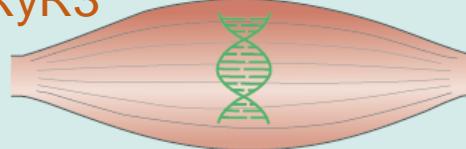
RyR1  
skeletal muscle  
isoform



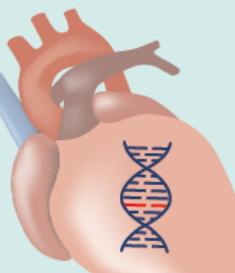
RyR3  
brain  
isoform

## Katecholaminerge polymorphe ventrikuläre Tachykardie

RyR1  
RyR3



RyR2



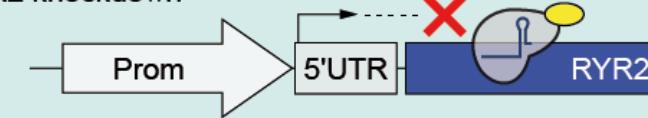
RYR2 Gen mit  
CPVT mutation



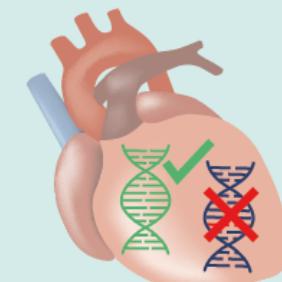
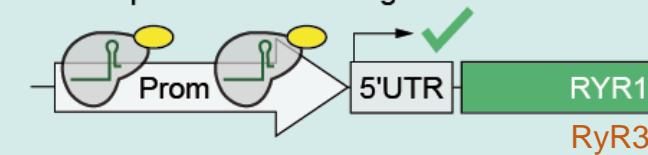
Gesundes  
RYR1 Gen

## Gentherapie Ansatz

RYR2 knockdown



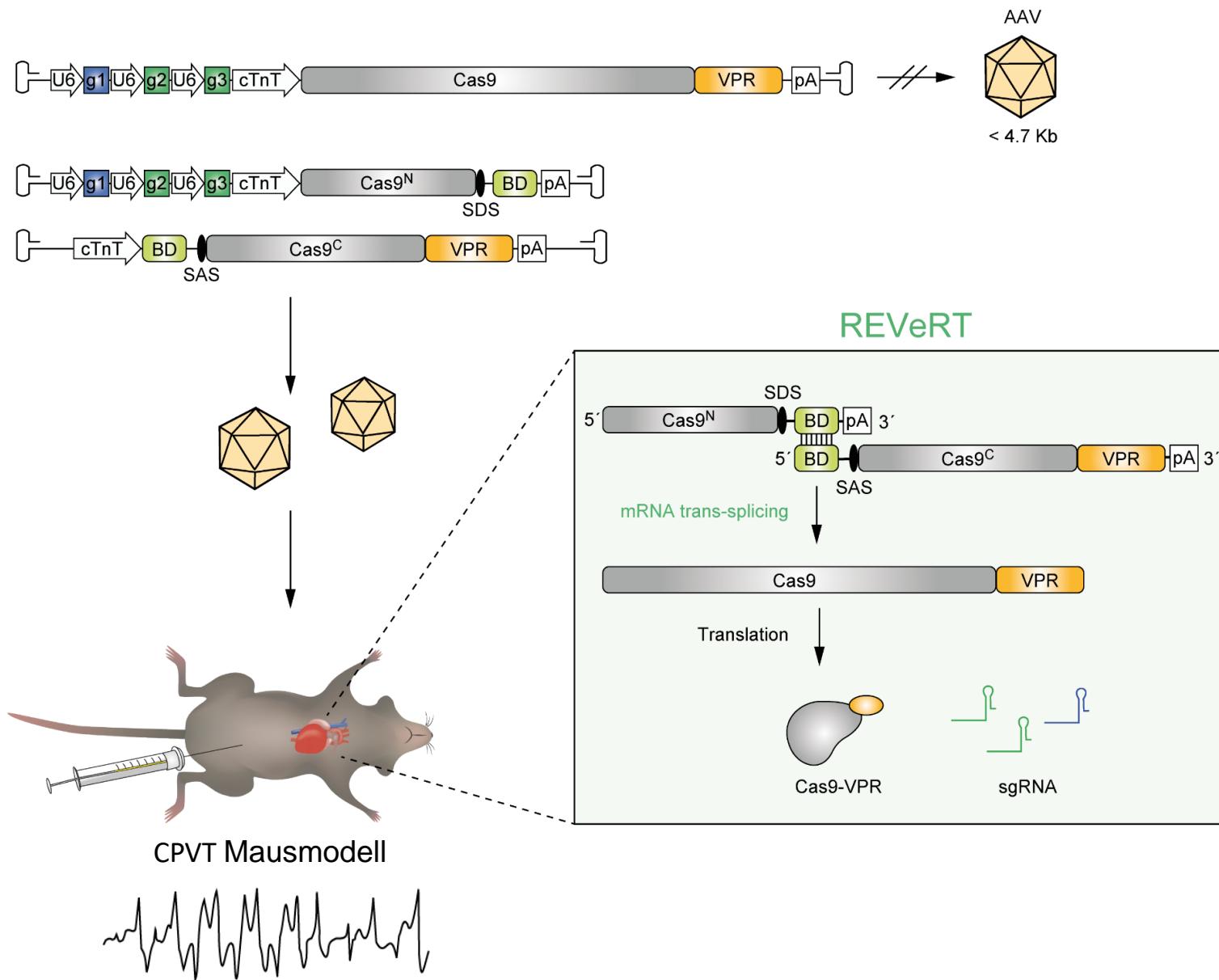
RYR1 transkriptionelle Aktivierung



guide  
RNAs

✓ Aktivierung  
✗ knockdown

# Duale rekombinante Adeno-assoziierte Viren (AAVs) als Genfährten

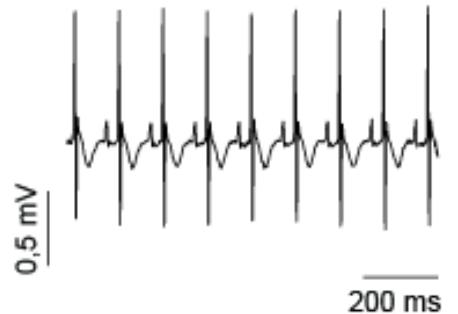


# Monitoring der Gentherapie

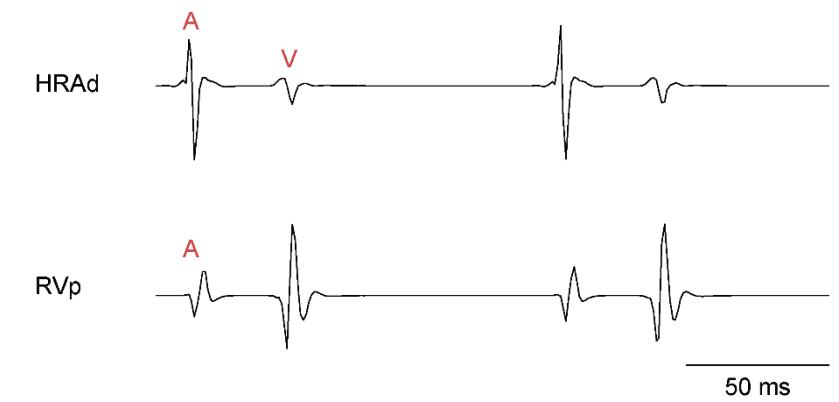
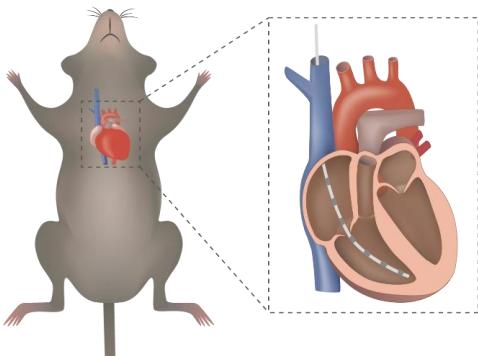
## Telemetrische EKG Messungen



RYR1TA & RYR2KD post

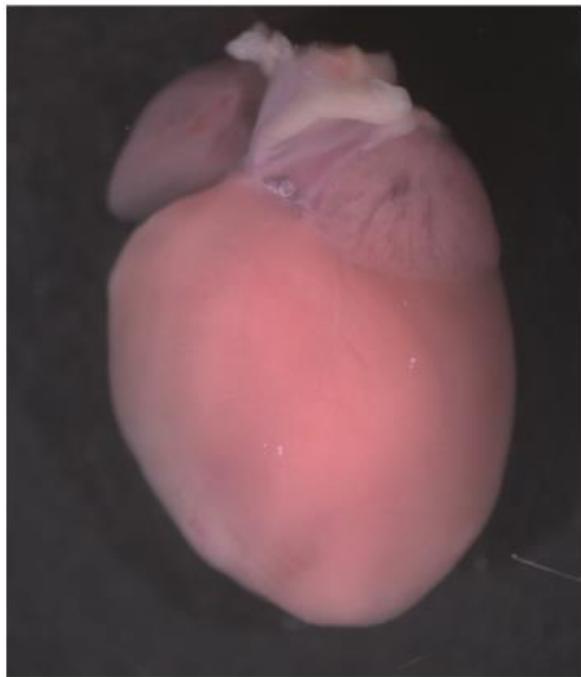


## Herzkatheter-Untersuchung



# Monitoring der Gentherapie

Isoliertes Mausherz

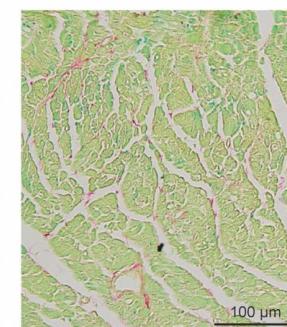
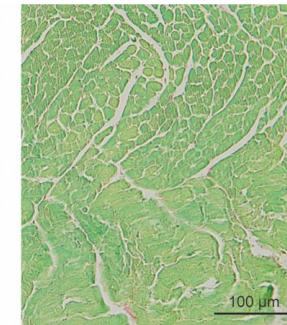


Fibrose-Färbung

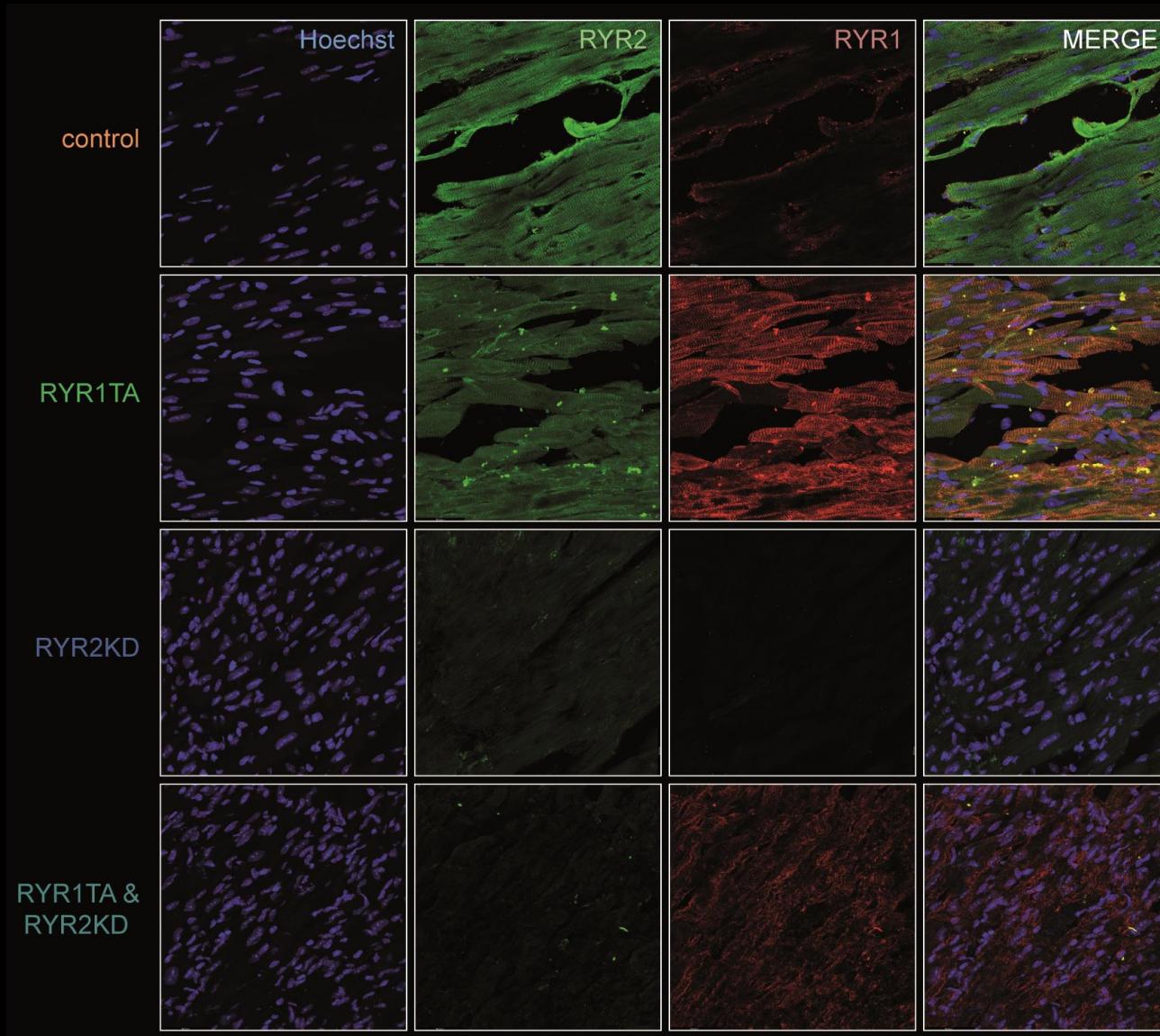
untreated



treated



# Expression der RyR Isoformen nach der Behandlung



# AK Biel - AG molekulare kardiovaskuläre Pharmakologie



Funding



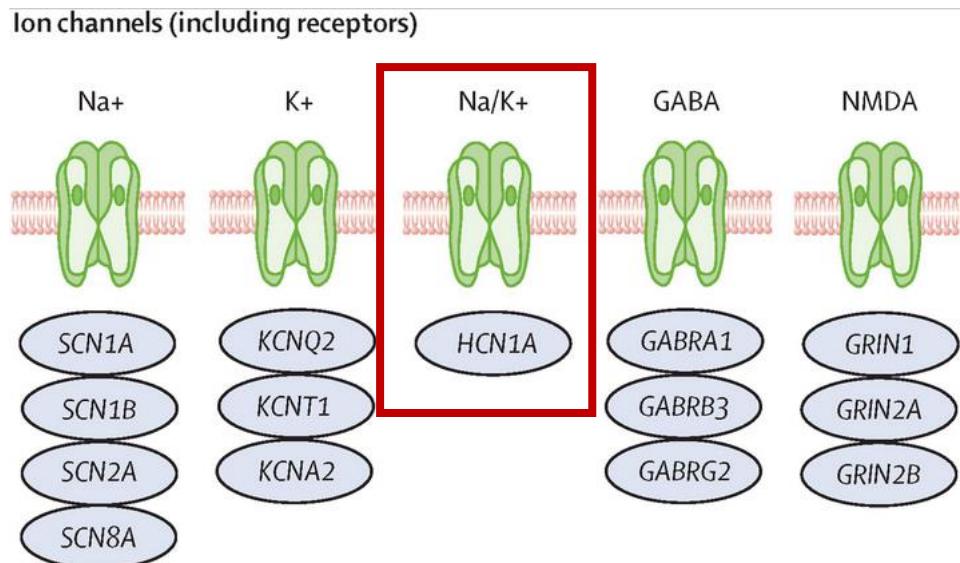
# Gentherapeutische Ansätze zur Behandlung neuronaler, degenerativen Erkrankungen

Dr. Verena Mehlfeld  
Tag der Forschung  
01. Dezember 2023

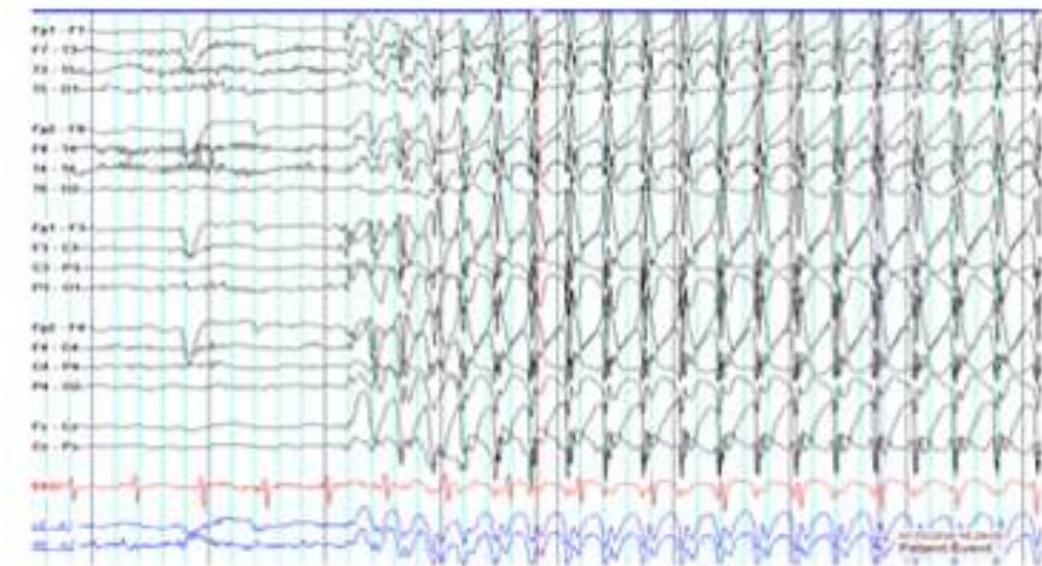


# Channelopathies

Identifizierte Gene, bei denen Mutationen zu genetisch bedingten Epilepsien führen



Absence Seizure

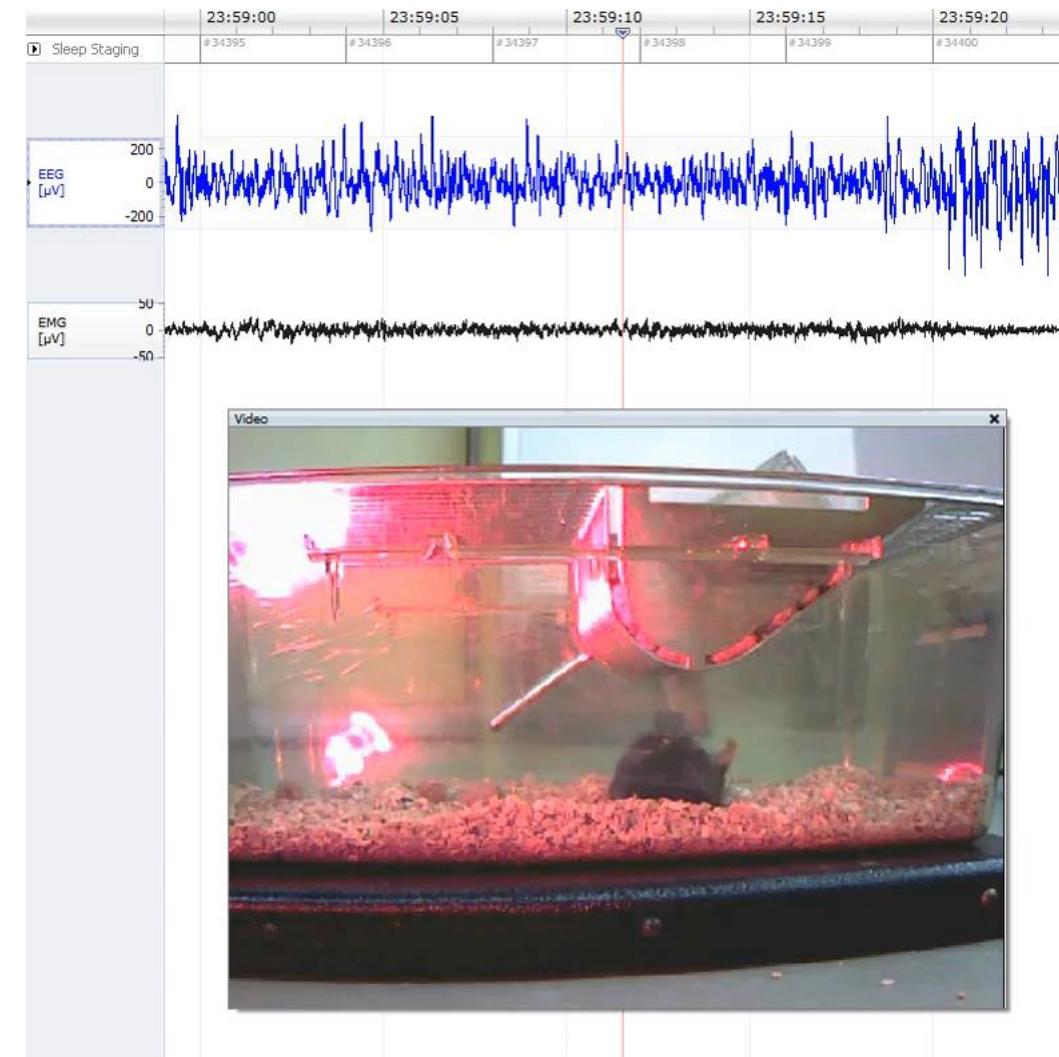
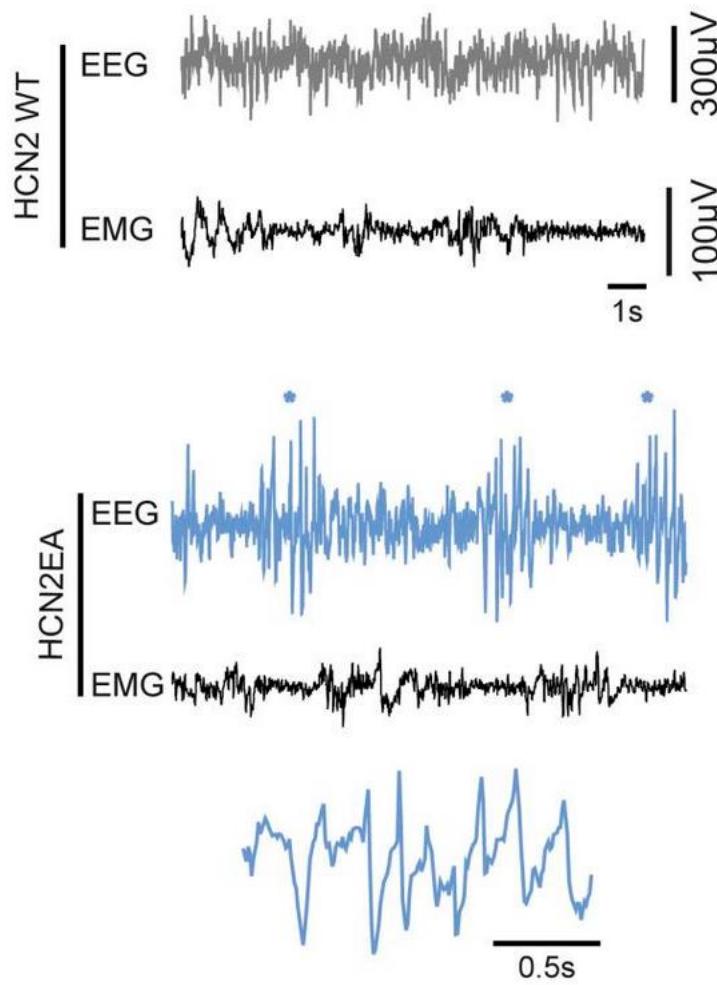


[https://www.thelancet.com/cms/attachment/af2fbfc3-cc25-48a6-9303-6f8cc590ac63/gr1\\_lrg.jpg](https://www.thelancet.com/cms/attachment/af2fbfc3-cc25-48a6-9303-6f8cc590ac63/gr1_lrg.jpg)

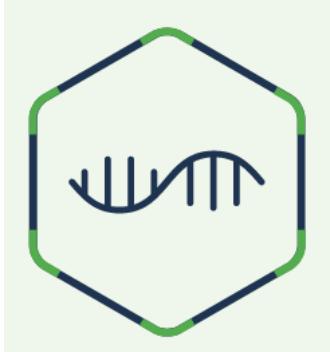
# Absence Epilepsie



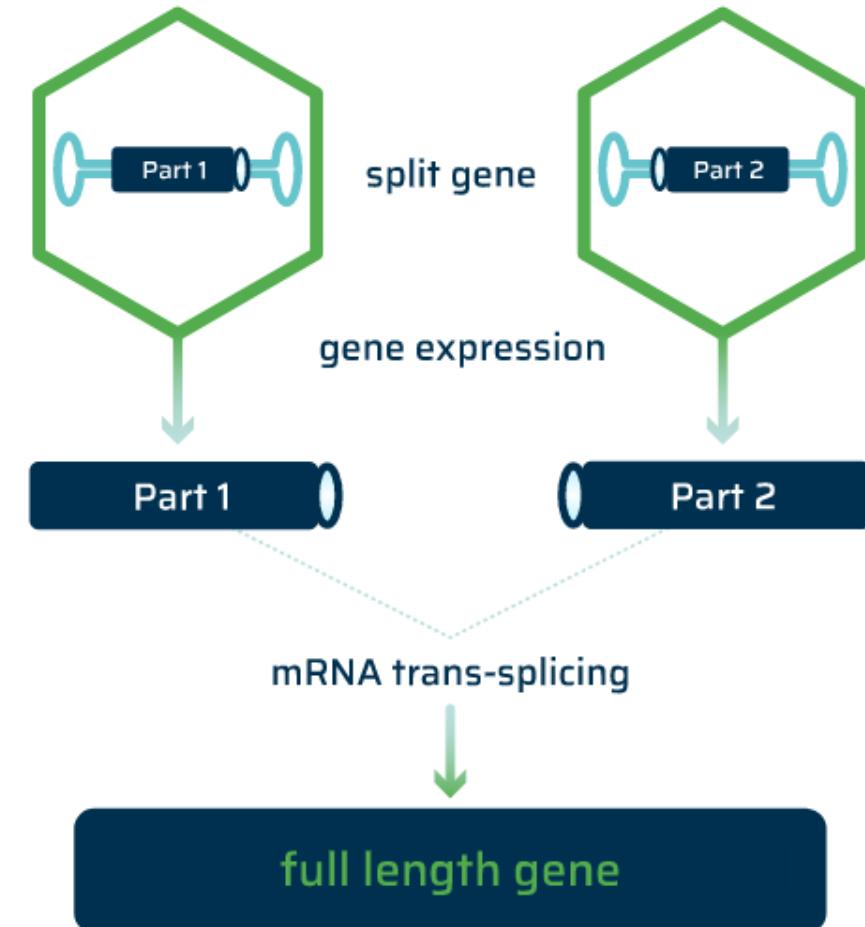
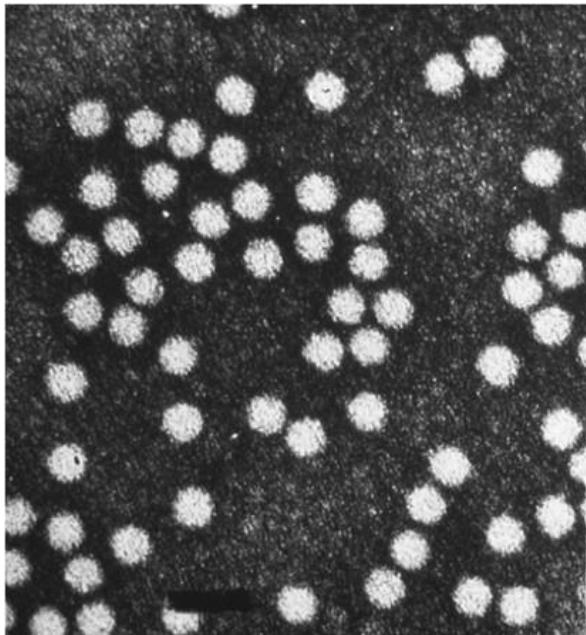
# HCN2EA – Absence Epilepsie



# Gen- (Supplementation)- Therapie



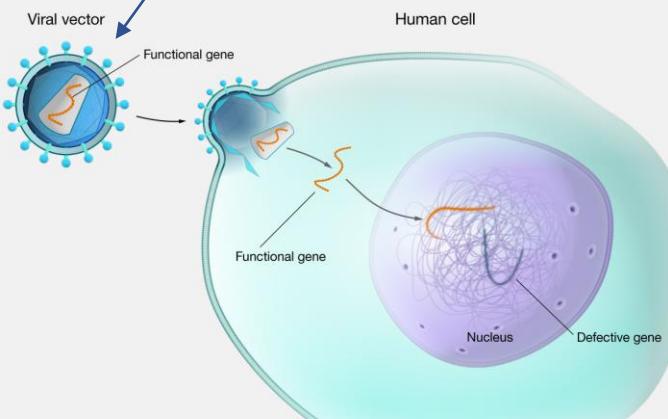
- recombinanter Adeno-assozierter Virus (rAAV)



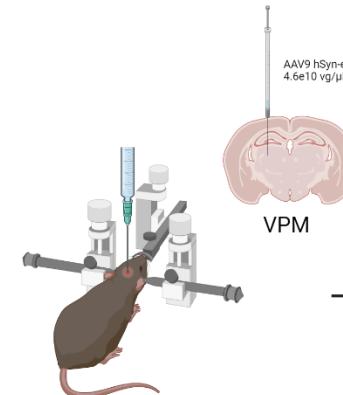
# rAAVs als Genfährten

HCN2

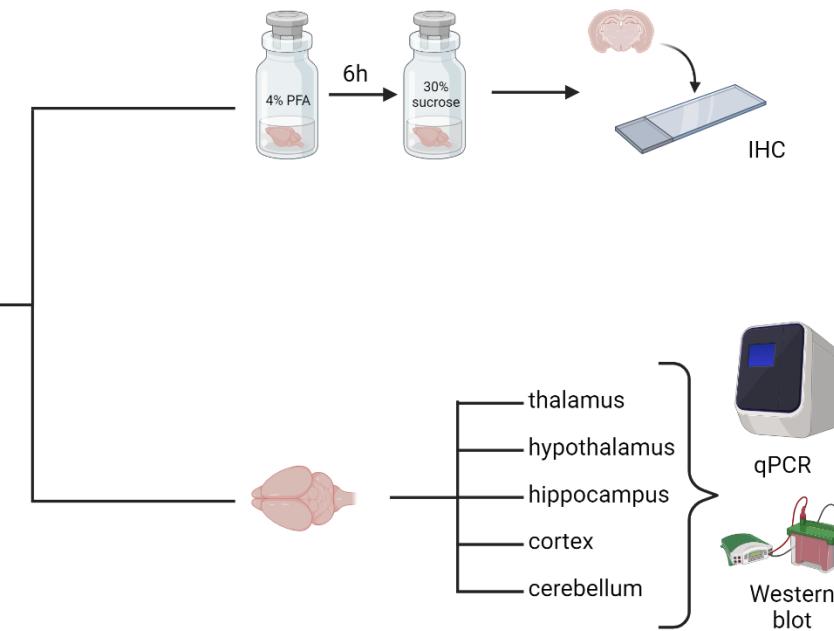
Doktorarbeit Eda Arslantas



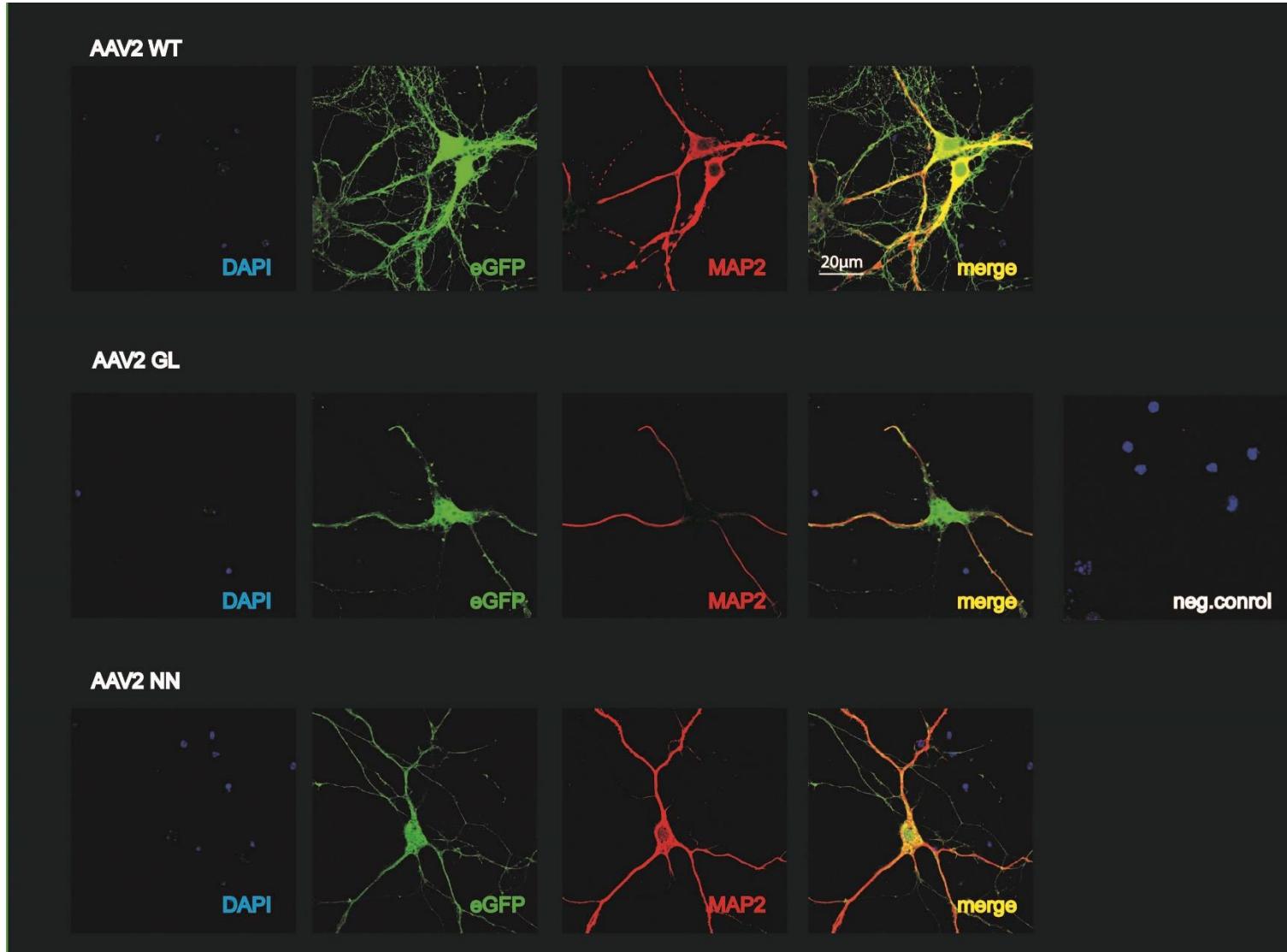
<https://www.genome.gov/genetics-glossary/Gene-Therapy>



3 weeks



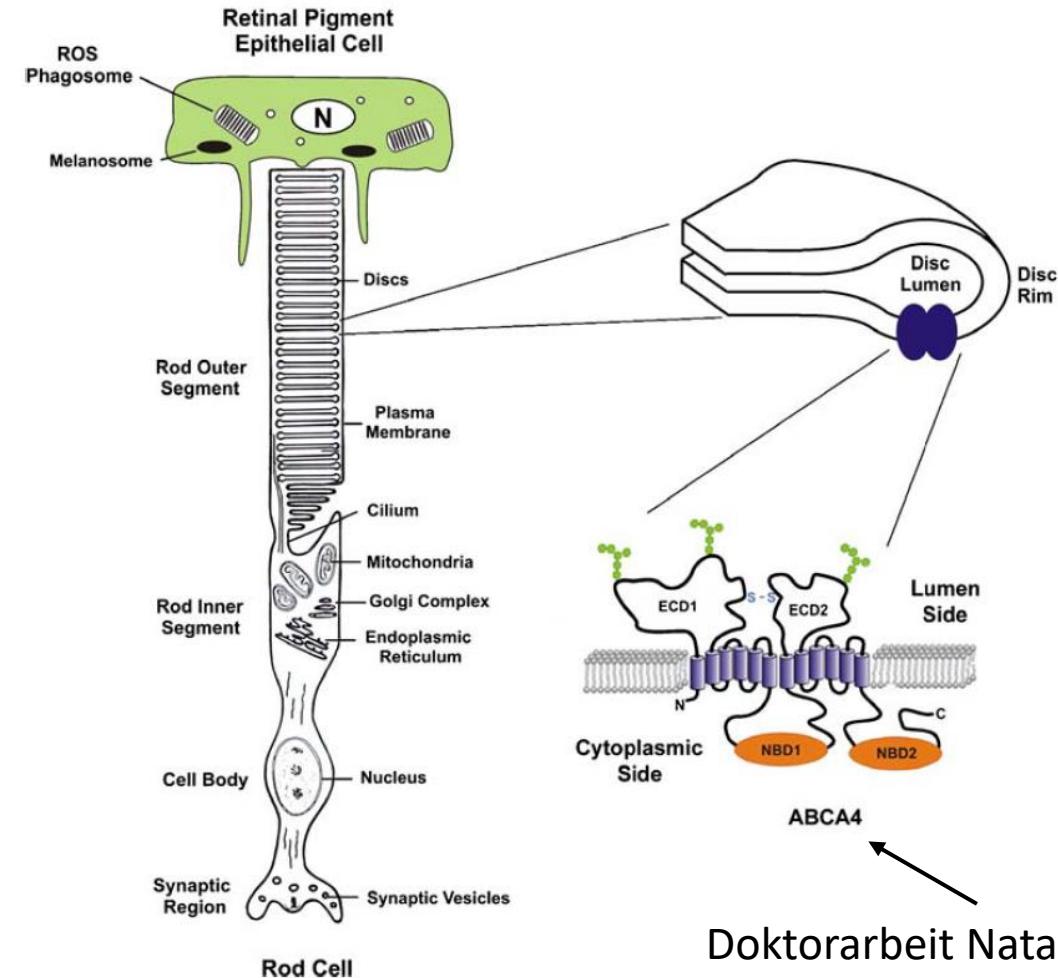
# Genföhren-Charkterisierung in der Zellkultur



Primäre Neuronenkultur / Zellkultur  
Doktorarbeit Eda

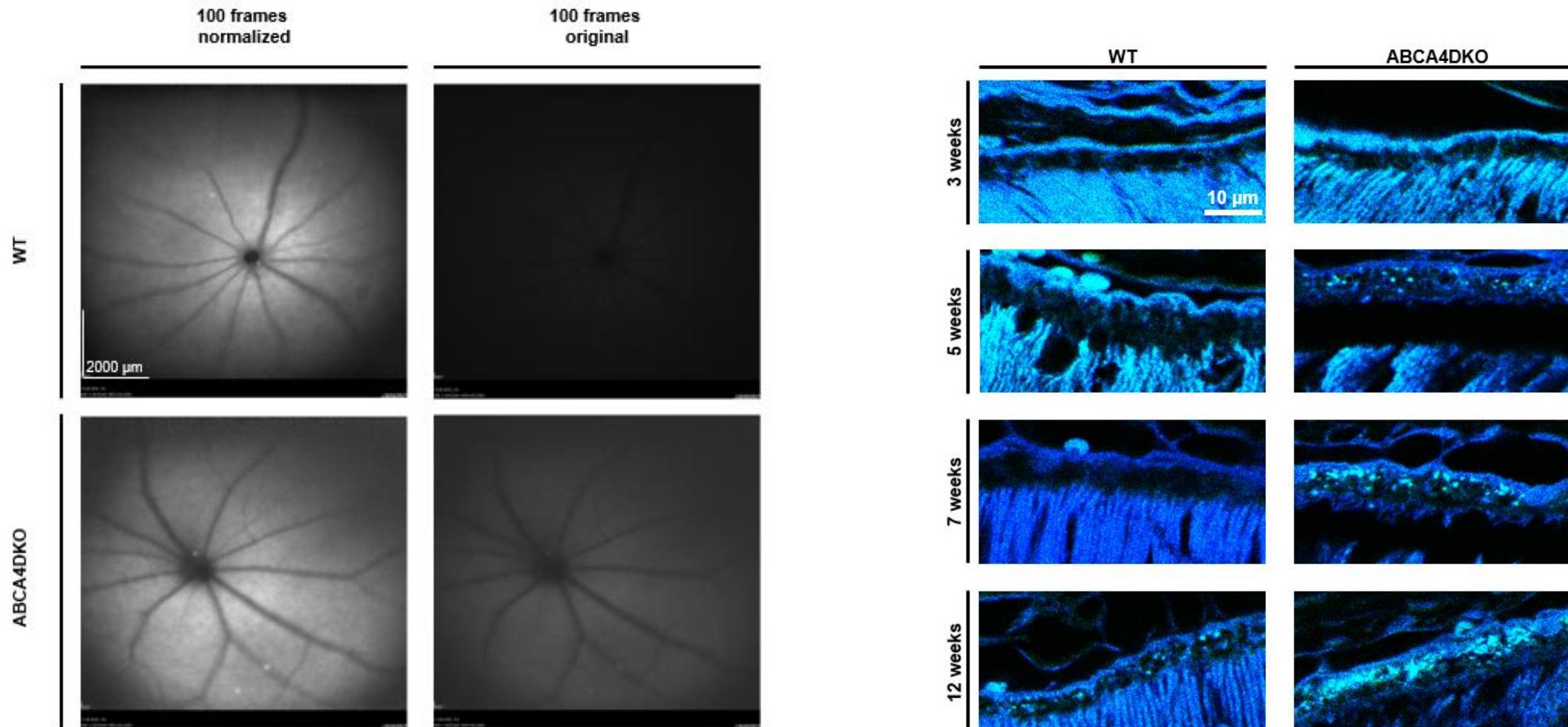


# Morbus Stargardt



Doktorarbeit Natalie Klippe

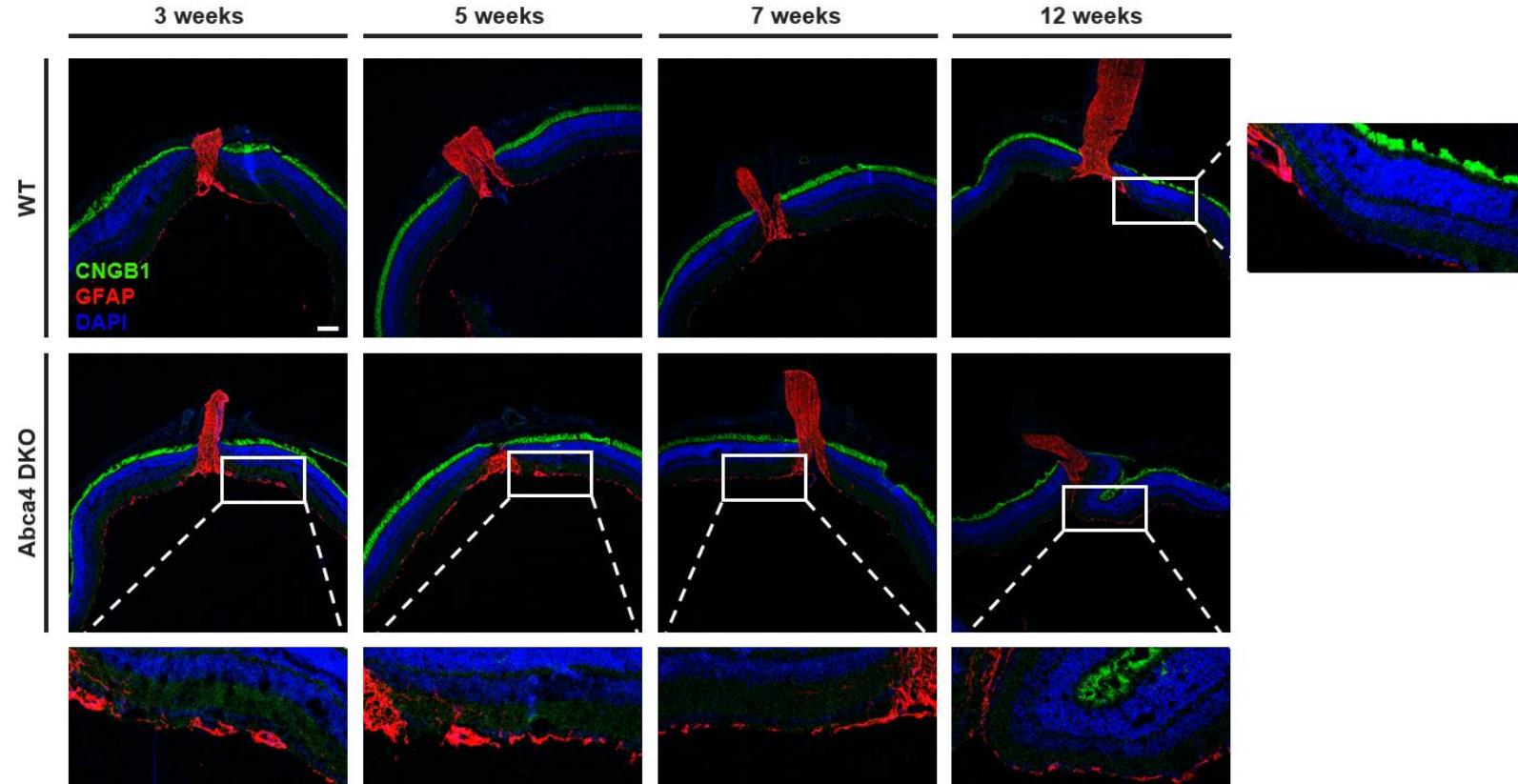
# Pathogenese des Morbus Stargardt



Blaue Autofluoreszenz des Fundus

Autofluoreszenz in Schnitten der Retina  
Masterarbeit Franz Kynast

# Pathogenese des Morbus Stargardt



Infiltration von Gliazellen in die Retina

Doktorarbeit Natalie





# Gentherapie von Augenerkrankungen

Tag der Forschung – 1.12.2023

Susanne Koch

## Retinitis pigmentosa (RP)

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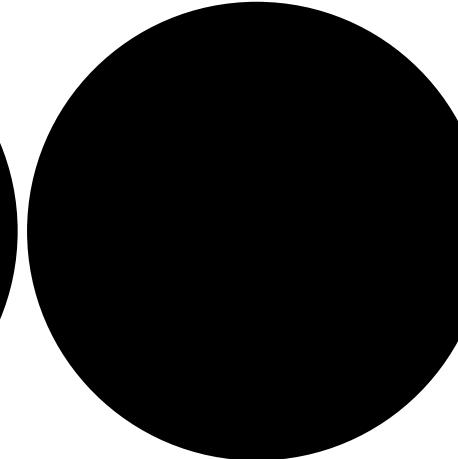
Normal Vision



Decreased Vision



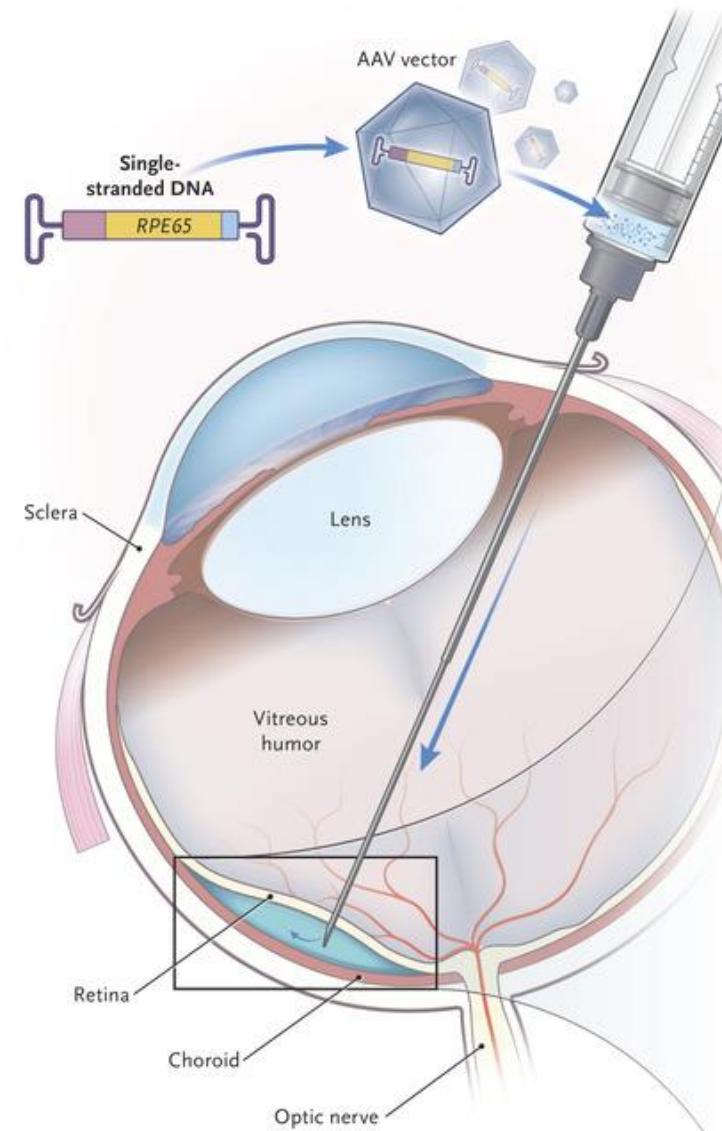
Tunnel Vision



Blindness

- disease progression: night blindness > tunnel vision > blindness
- most common cause of inherited blindness
- prevalence 1:4000
- > 70 disease-causing genes, eg *PDE6b*
- no treatment

# Luxturna: First gene therapy for inherited retinal dystrophy (LCA)



N Engl J Med 2019; 381:455-464

Hannah



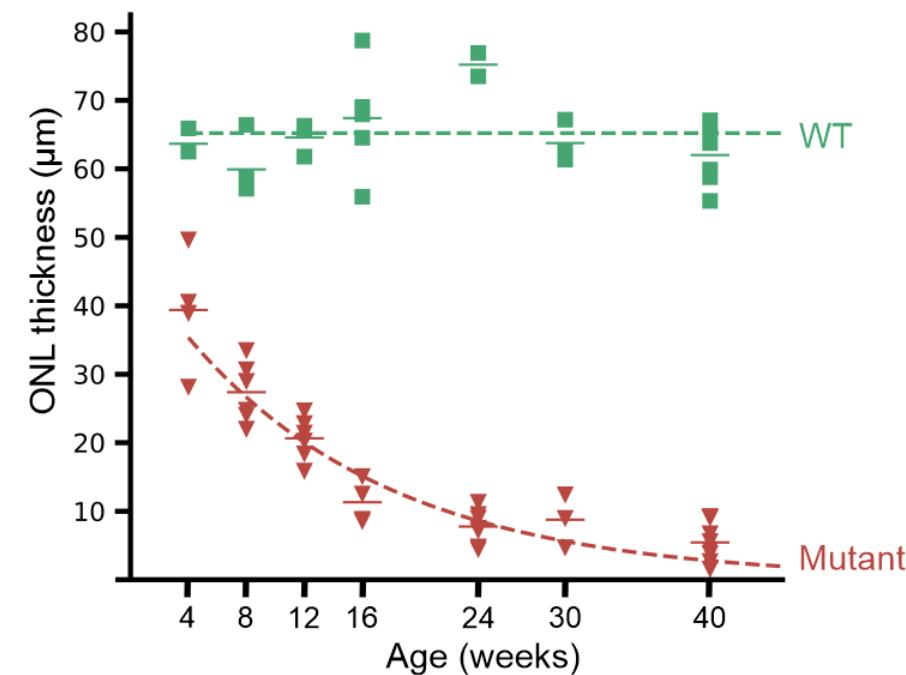
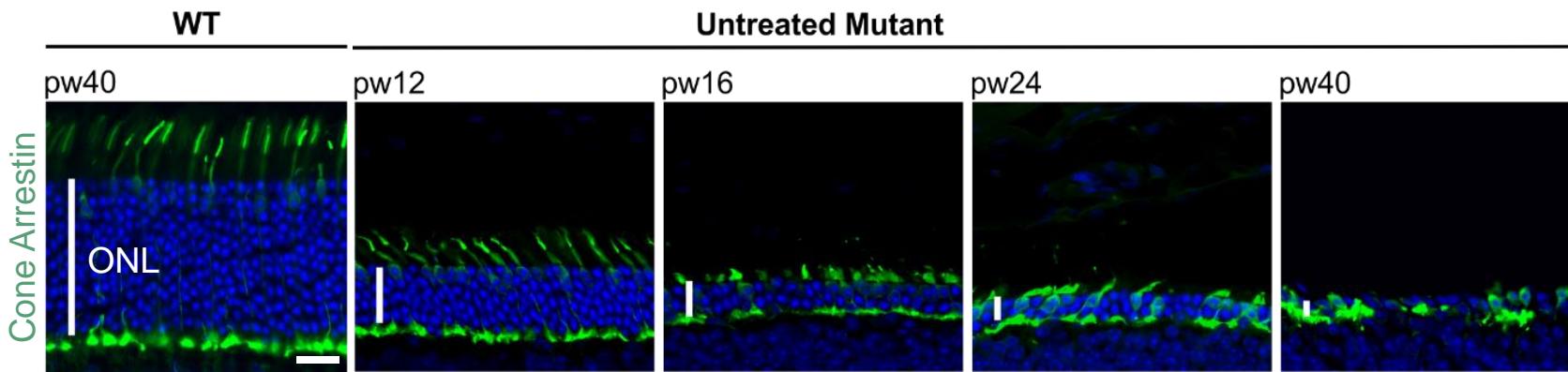
<https://www.chop.edu/stories/gene-therapy-inherited-blindness-hannah-s-leber-congenital-amaurosis-story>

„I can see the stars!  
They are not star-shaped“

„When can I have my second eye injected?“

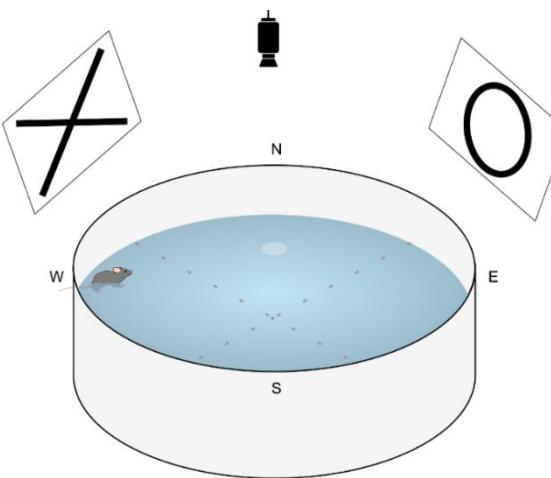
- 1. Therapeutic time window  
(„Point of no return?“)**
  
- 2. Development of  
„gene-independent“ treatments  
Identification of disease mechanisms**

## *Pde6b*-deficient disease progression



## Vision-guided behavior – water maze

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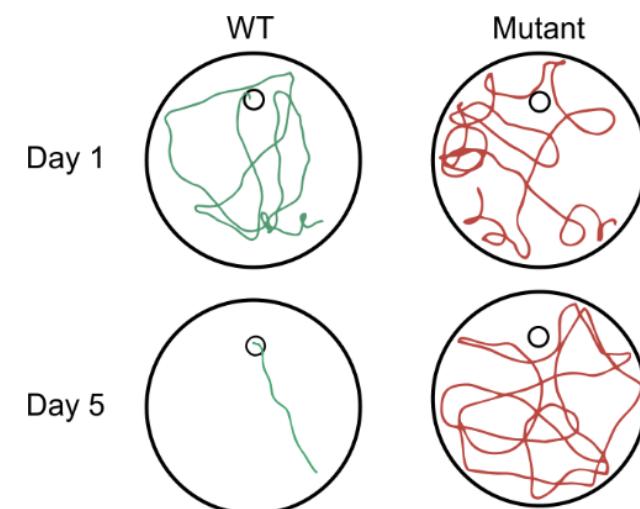
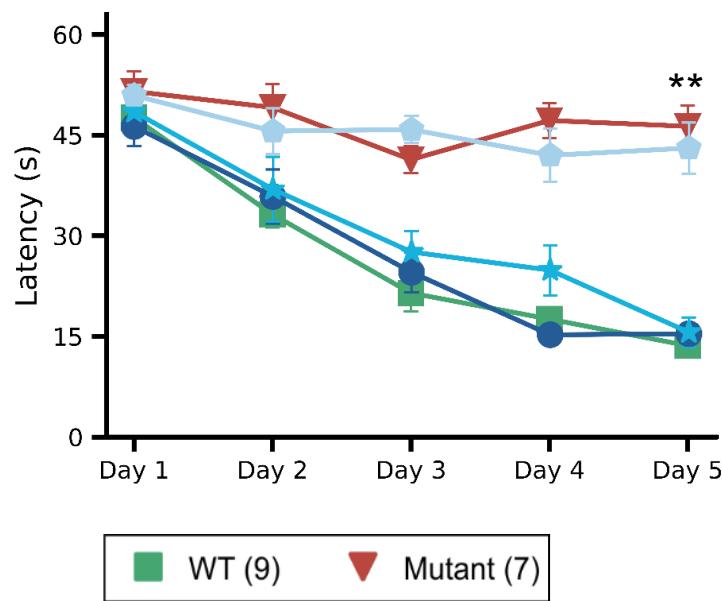
Day1



Day5

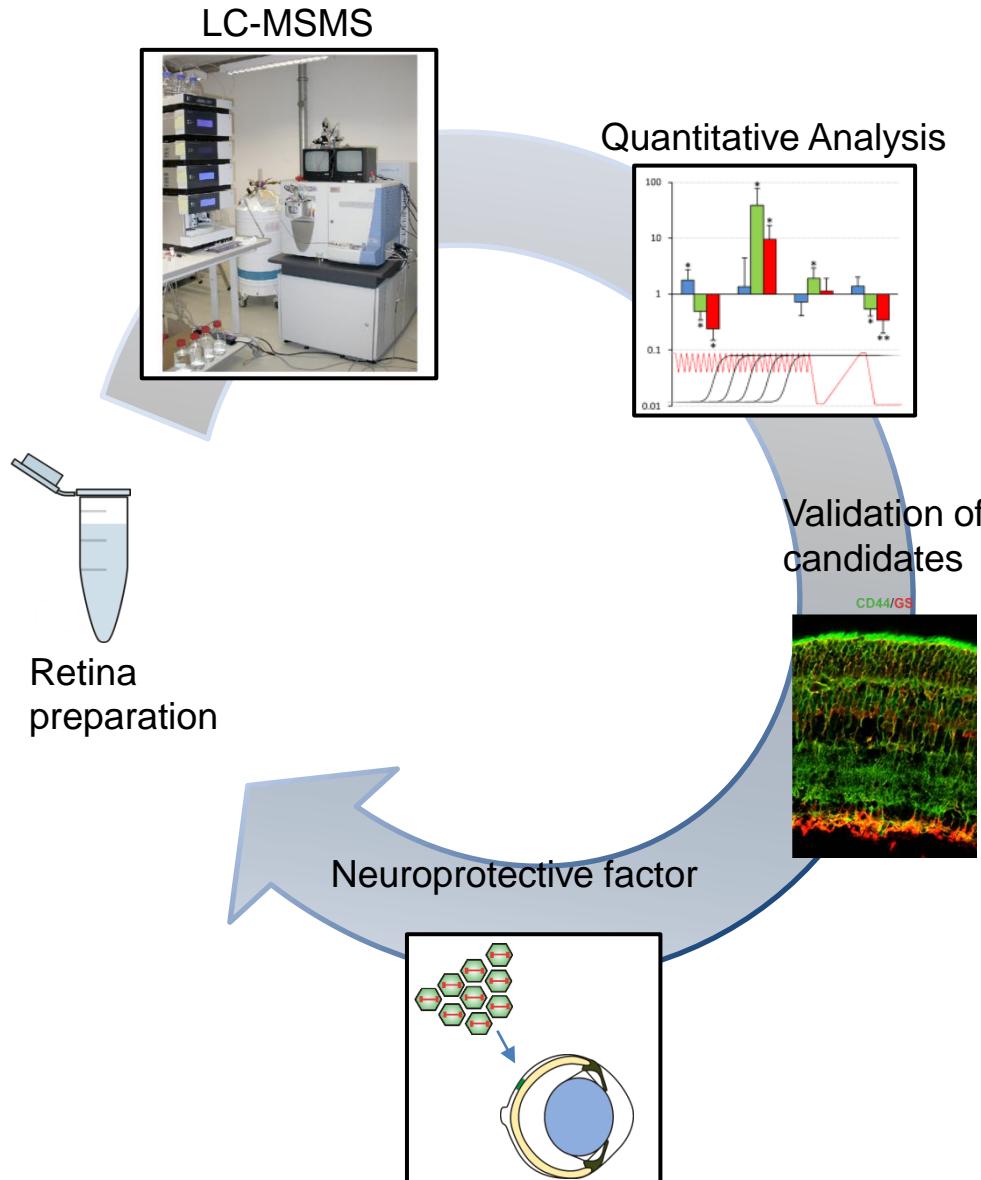
## Rescue of vision-guided behavior by treatment at pw16 or earlier

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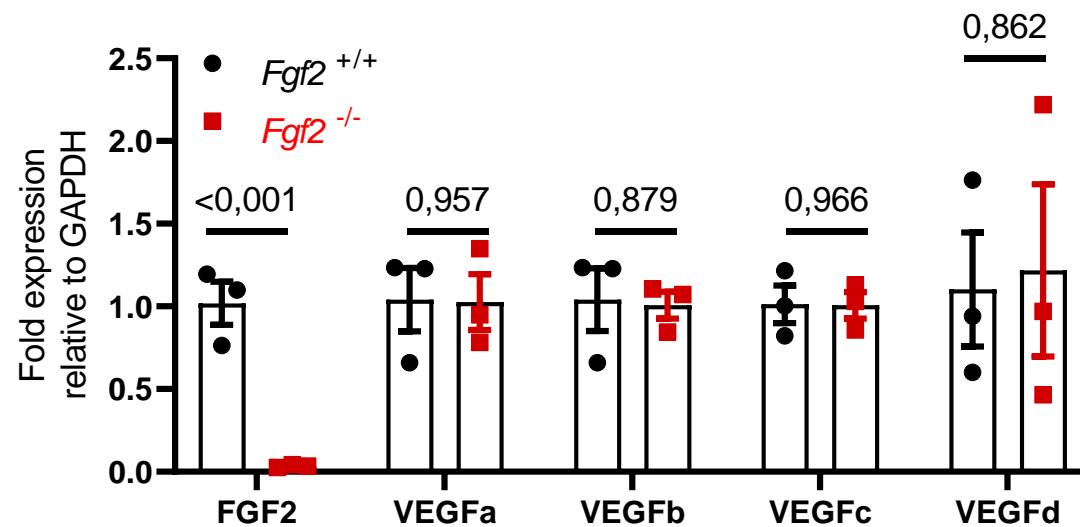
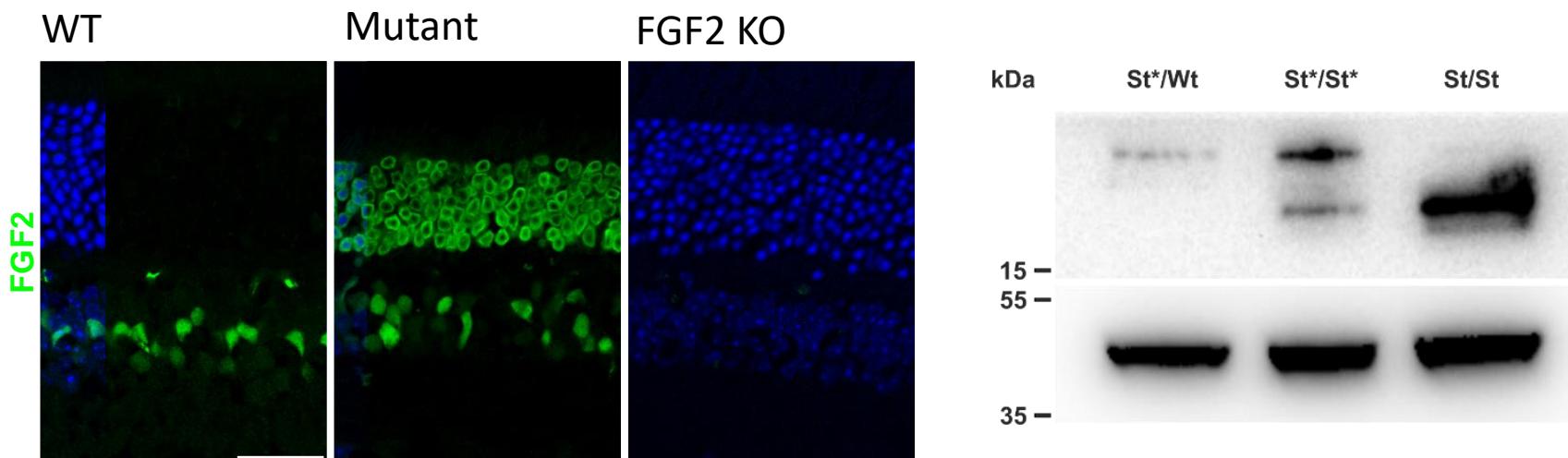


1. Therapeutic time window  
(„Point of no return?“)
  
2. **Development of**  
**„gene-independent“ treatments**  
**Identification of disease mechanisms**

## Identification of disease mechanisms



## FGF2 upregulation





Danke für Ihre Aufmerksamkeit



Monika Ayten  
Hanaa Ghanawi  
Nundehui Diaz Lezama  
Felia Haffelder



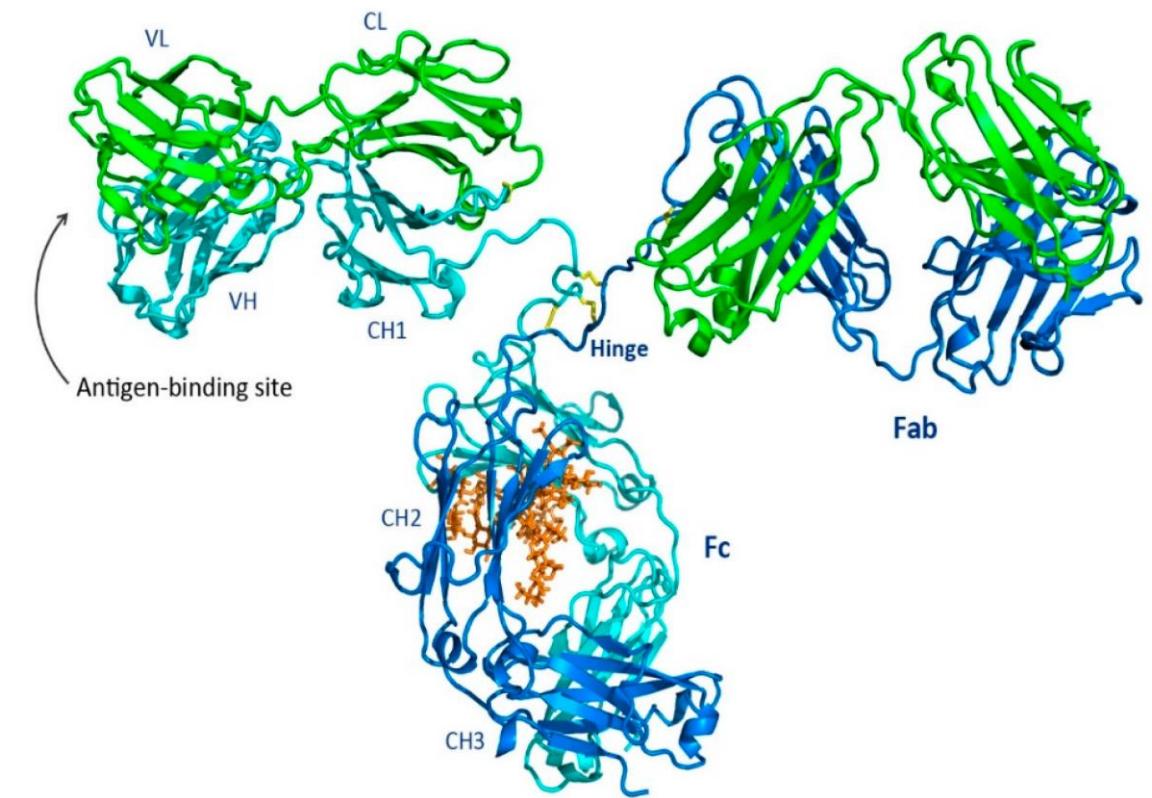
**Research groups: Prof. Merkel, Prof. Winter, Prof. Friess ~ 40 people**

### **General Infrastructure – Highlights:**

- Sterile facility with filling machine, lyophilizer in class A, isolator etc.
- Cell culture labs, flow cytometry
- Chemistry labs for polymer synthesis
- Nanoparticles preparation, e.g. Assembler, Microfluidics
- Parenteral formulation handling and processing equipment e.g. TFF, filling and capping equipment, LFs, lyophilizers, spray-dryers, cryo-mill
- Equipment for preparing and testing inhalative dosage forms
- Protein / NP analytical equipment e.g. DLS plate reader, Protein-DSCs, nano-DSF, Bio-FT-IR, CD, Fluorimeters, LCs, FPLCs, AF4s
- Further analytical equipment e.g. DSC, TG, DVS, Karl-Fischer, Zetasizer, NTA, SVP, Injection Force Measurement, Laser Diffraction, Cascade impactor, FACS, PCR
- Animal inhalation facility (BMC)



- #1 Comirnaty® SARS-CoV-2 vaccine (29.0 B\$)
- #2 Humira® Anti-TNF mAb (21.2 B\$)
- #3 Spikevax® SARS-CoV-2 vaccine(19.5 B\$)
- #3 Keytruda® Anti-PD1 mAb (19.5 B\$) LYOPHILIZATE
- #5 Eliquis® Factor Xa inhibitor (11.9 B\$)
- #6 Revlimid® Immunmodulator (11.3 B\$)
- #7 Stelara® Anti-IL-12/IL-23 mAb (10.1 B\$)
- #8 Biktarvy HIV INSTO/NRTI/NtRTI (9.7 B\$)
- #9 Opdivo® Anti-PD1 mAb (8.9 B\$)
- #10 Dupixent Anti-IL-4/IL-13 mAb (7.4 B\$)





## Protein Aggregation: Mechanisms and Analytics

**Protein-Protein-Interactions:**  
Analytics,  
Formulation Effects  
Mutation, Subspecies Effects

**Protein-Material-Interactions:**  
Adsorption,  
New Containers,  
Surfactants,  
Modeling



**Protein Formulation:**  
High Concentration,  
Suspensions,  
Modeling

**Lyophilization / Frozen Bulk:**  
Process Understanding,  
Scale Down of Bulk Freezing,  
Protein and NP Formulation,  
Understanding the FC

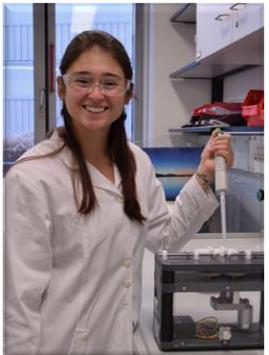
# Current Ph.D. Students @ LMU And Their Projects



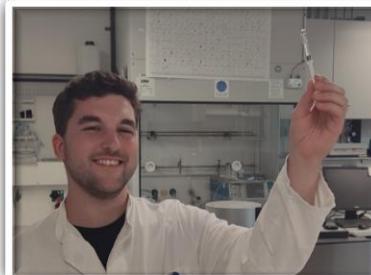
**KEN LO PRESTI**  
Protein Mobility  
And Accessability  
In Lyophilisates



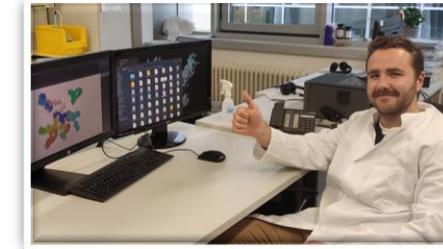
**RICARDA NAGEL**  
Protein Behaviour  
In The Freeze  
Concentrate



**ELENI PAPADOULOS**  
New Surfactants For  
Protein Stabilization



**DANIEL FUCHS**  
Universal Container  
For Biologics



**JONAS BINDER**  
Ion Cloud Effect On  
Proteins - Modeling



**TIM SARTER**  
Protein Behaviour At  
Interfaces - Modeling



**LASSE BEHRENS**  
Lyophilisation And  
Stabilization Of LNPs



**ELENA RICHERT**  
Shrinkage And  
Moisture Effects  
In Lyophilisation

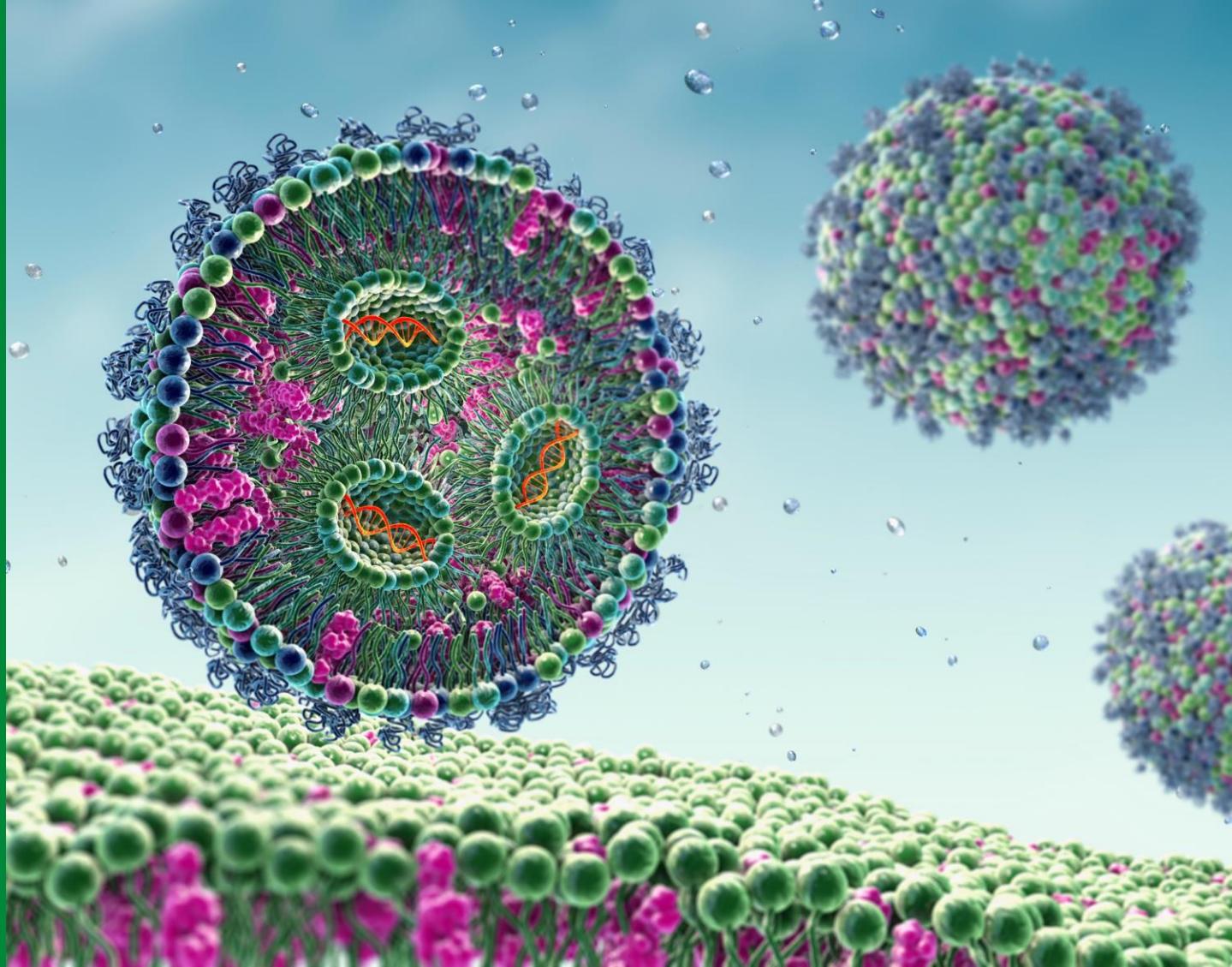
## DO YOUR OWN RESEARCH PROJECT!

- Wahlpflichtfach
- Studentische Hilfskraft
- Bachelorprojekt
- Masterprojekt



## Internships in Pharmaceutical Technology and Drug Delivery

AK Merkel and all sub-groups



# Research Focus



Dr. Simone Carneiro

## Pulmonary Delivery

Formulation of Inhalable Dosage Forms  
Nebulization  
Spray- and Freeze-Drying  
Powder Analytics



## Cancer

Lung Cancer  
Chemosensitivity, Mutations, Cancer Bio  
Therapeutic CRISPR/Cas Delivery  
Combination Therapy  
Ovarian and Breast Cancer



Dr. Karin Bartel



## Inflammatory Diseases

Asthma  
COVID-19  
T Cell Targeting  
Macrophage Targeting  
Cancer Immunology  
Rheumatoid Arthritis



Dr. Benjamin Winkeljann

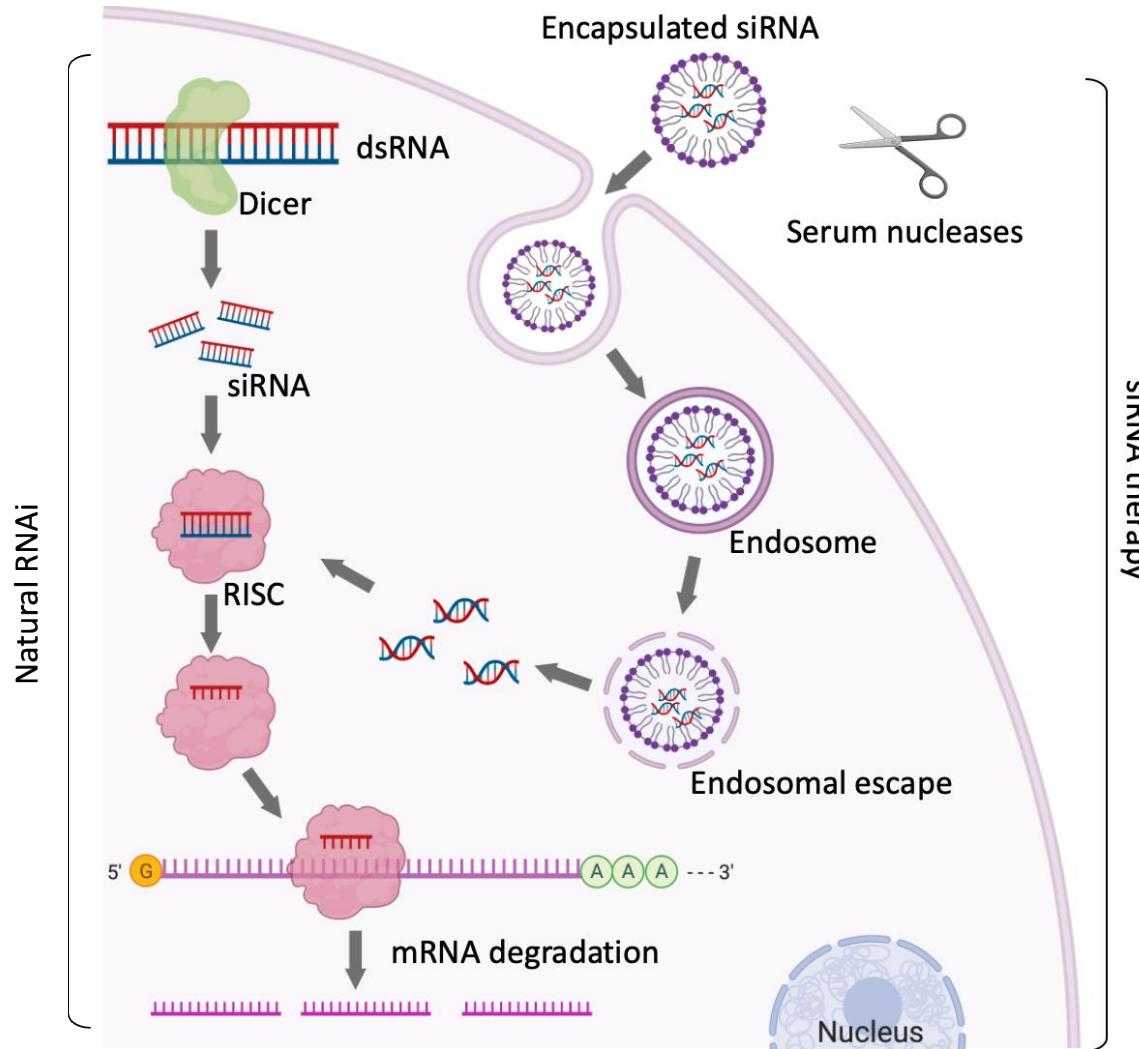
## Material Science

Polymer Synthesis  
Nucleic Acid Formulation  
Molecular Dynamics Simulations  
In situ forming hydrogels  
Rheology  
Material Characterization and  
Degradation  
Microfluidics  
Machine Learning



Dr. Valentin Fell

# RNA Delivery



# Methods

Polymer Synthesis and Characterization

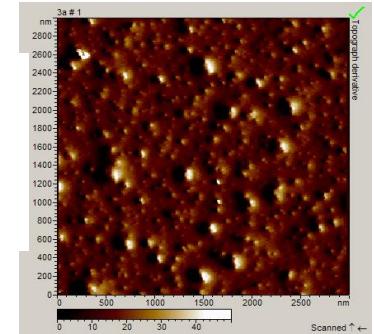
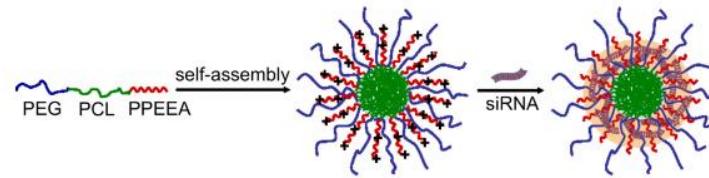
Nanoparticle Preparation (Precipitation,  
Evaporation, Microfluidics)

Particle Characterization (DLS, LDA,  
AFM, SEM)

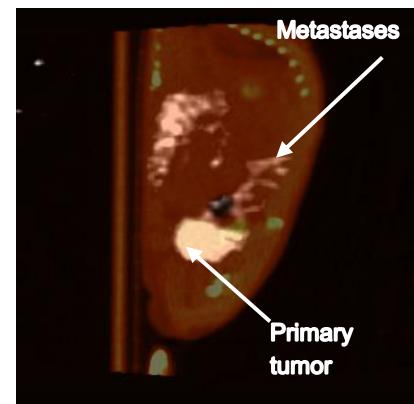
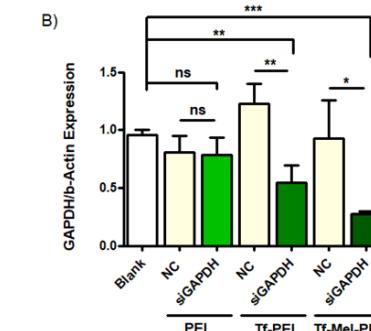
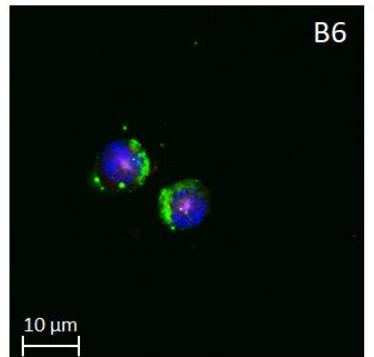
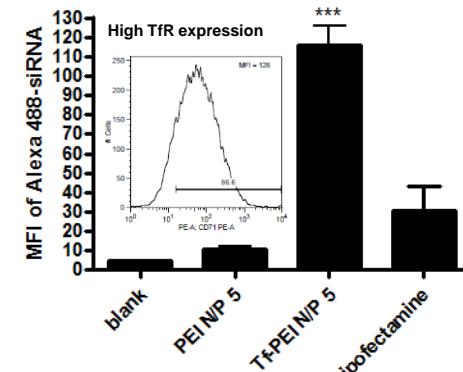
Cellular Uptake (Flow Cytometry,  
Confocal Microscopy, HPLC)

Gene Silencing (Flow Cytometry, qRT-  
PCR, Western Blot, ELISA)

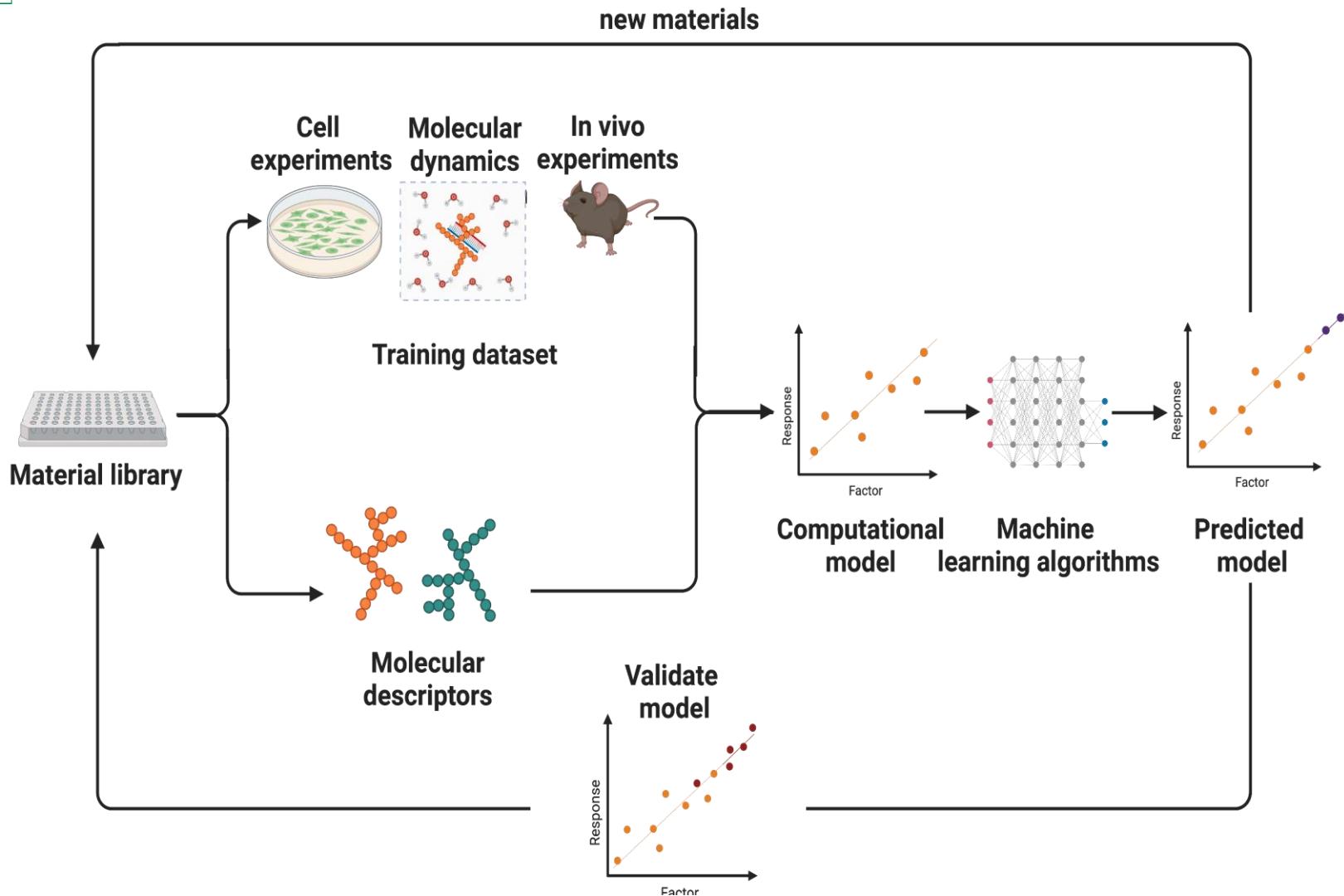
Biodistribution *in vivo*



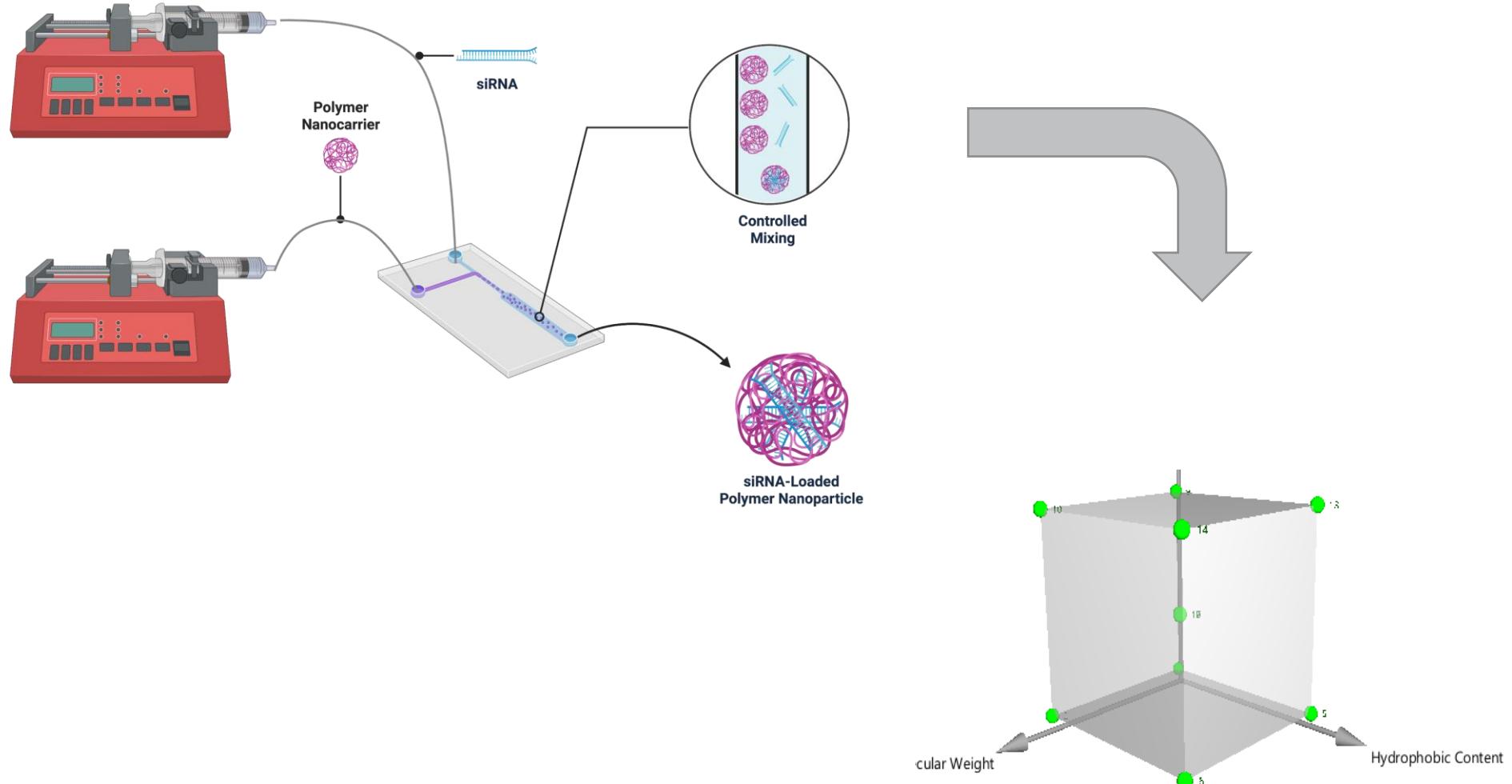
siRNA uptake in activated T cells (high TfR)



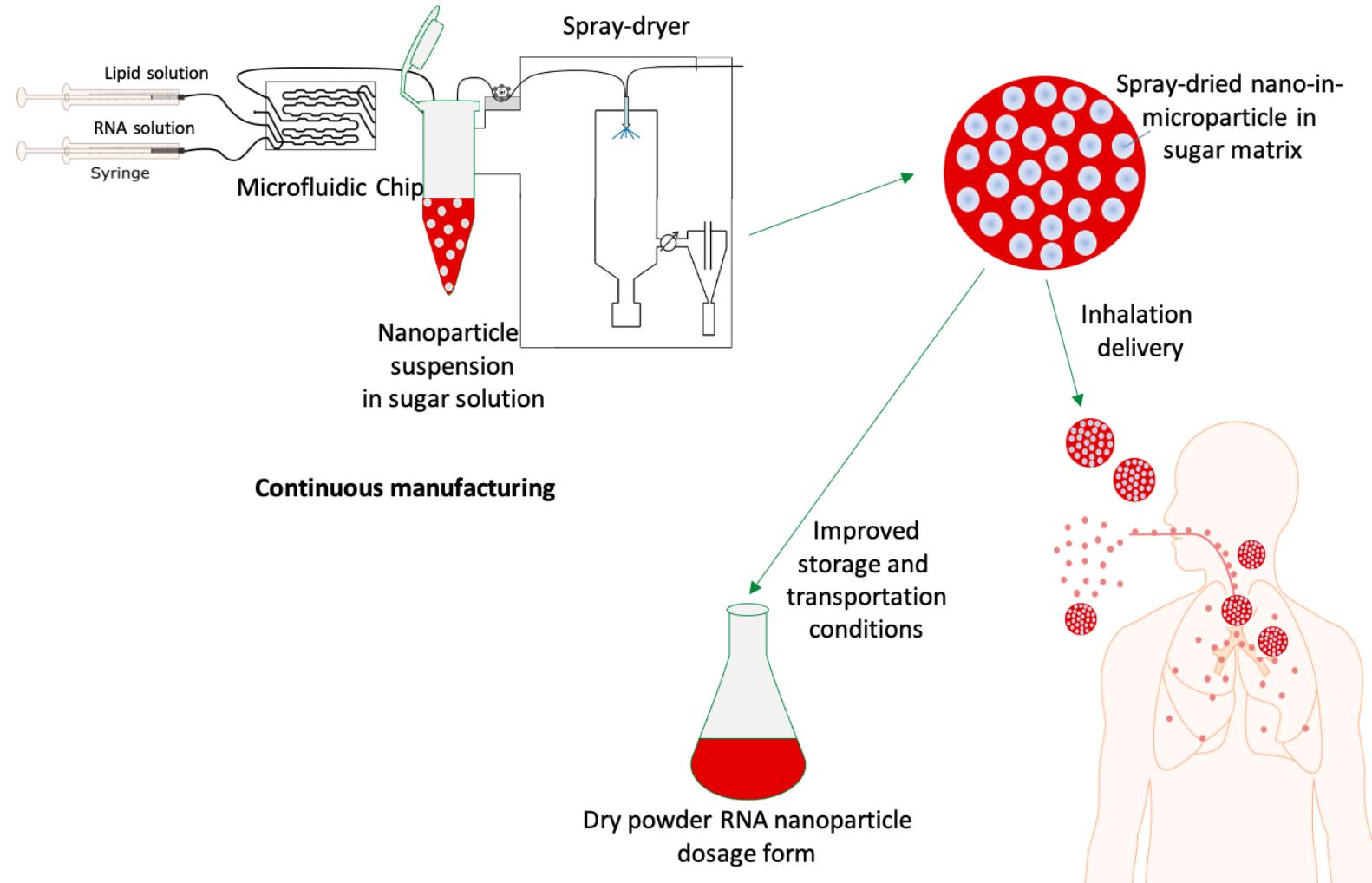
# AI in RNA Delivery



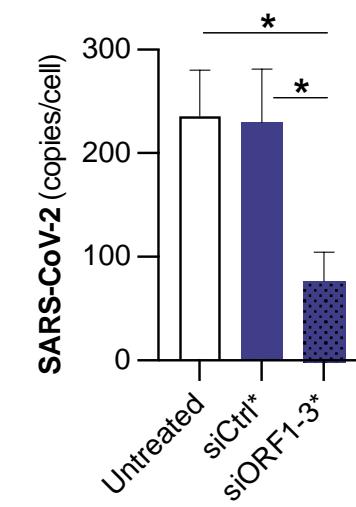
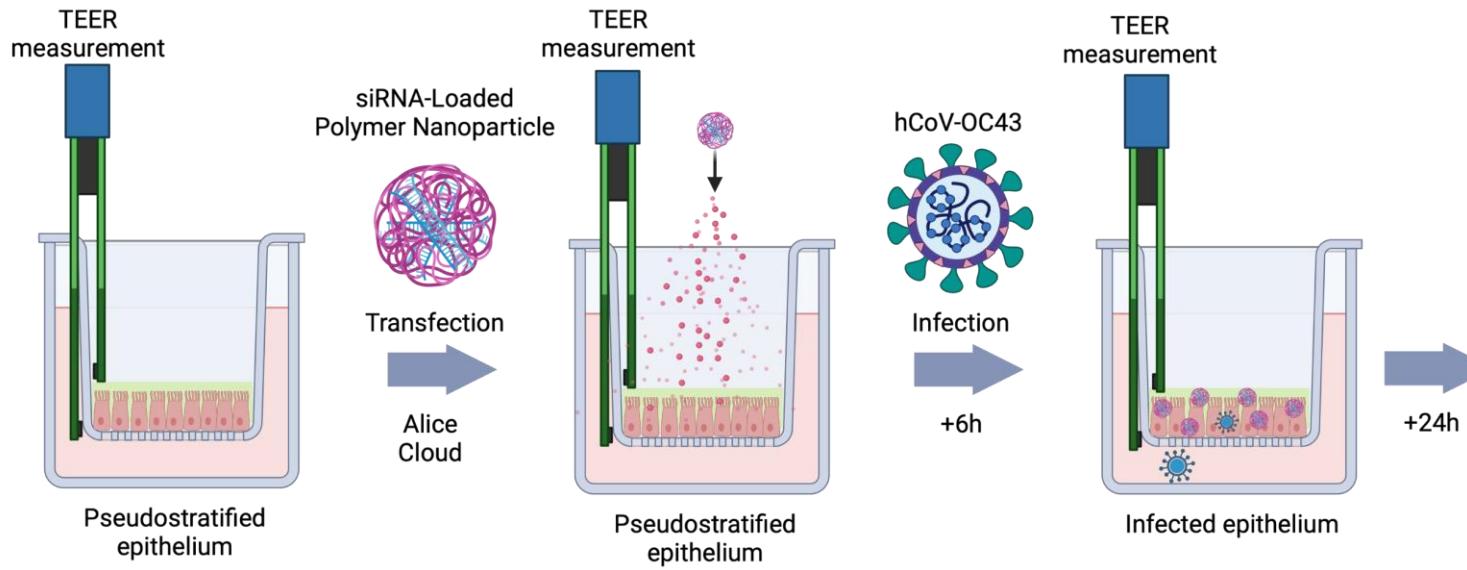
# Microfluidic Mixing and DoE



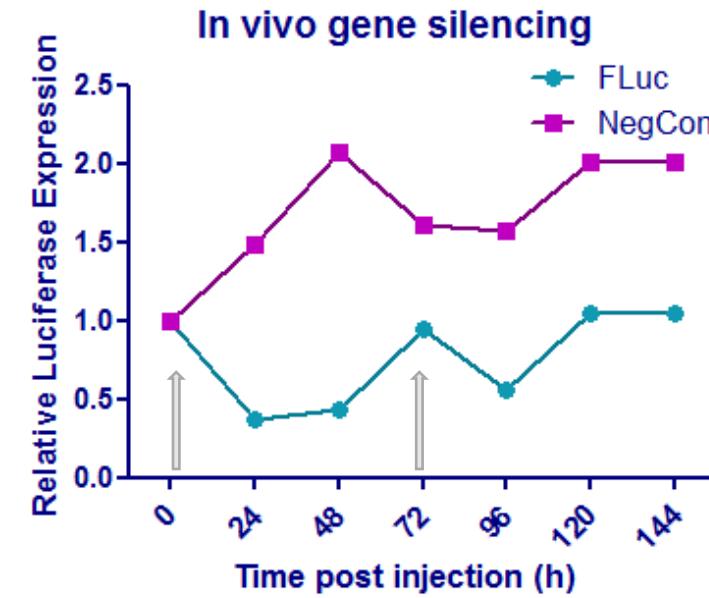
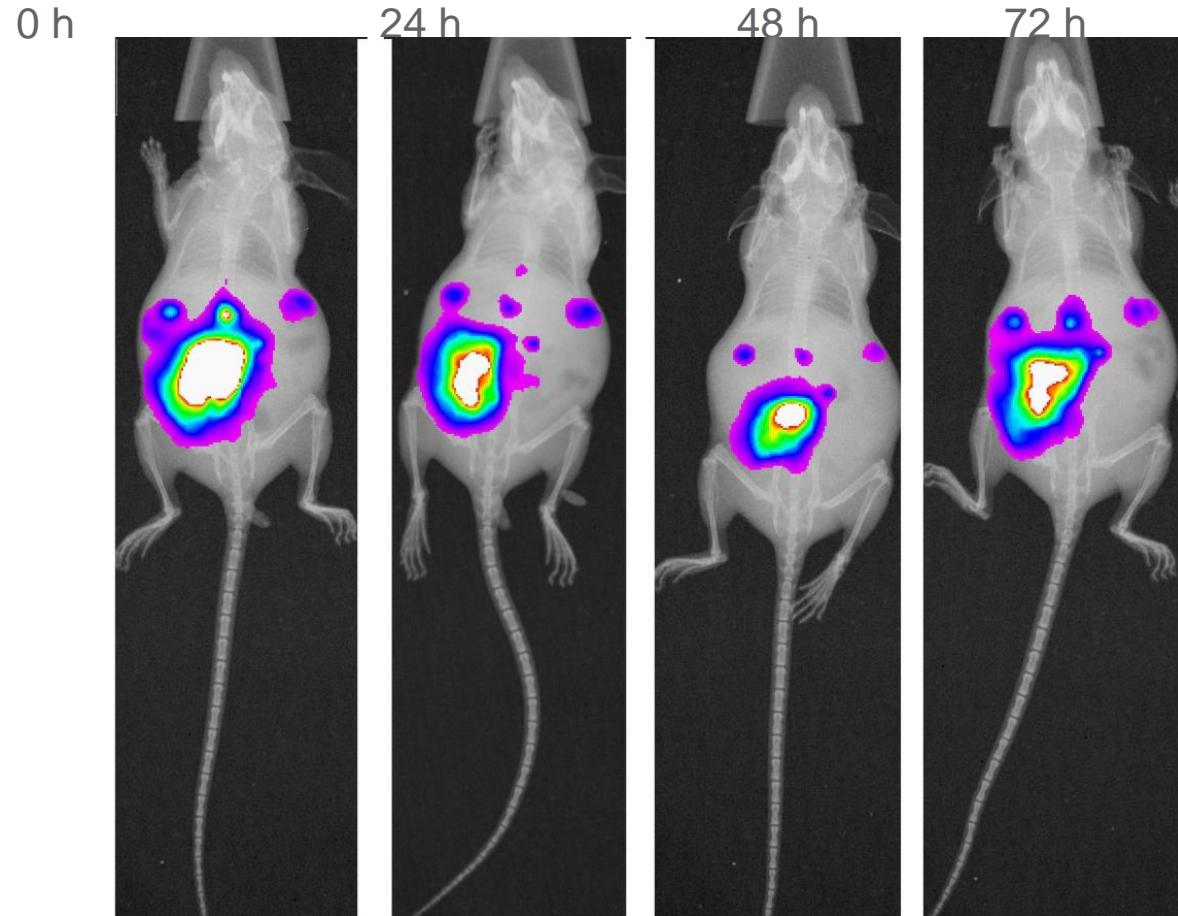
# Spray Drying



# Cell Culture Models



# In vivo gene silencing

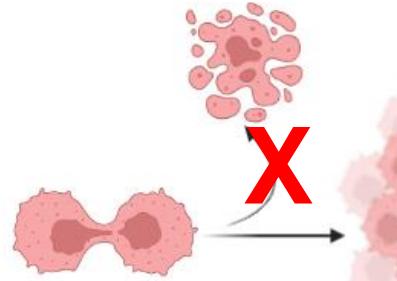


# The Endolysosomal System in Cancer



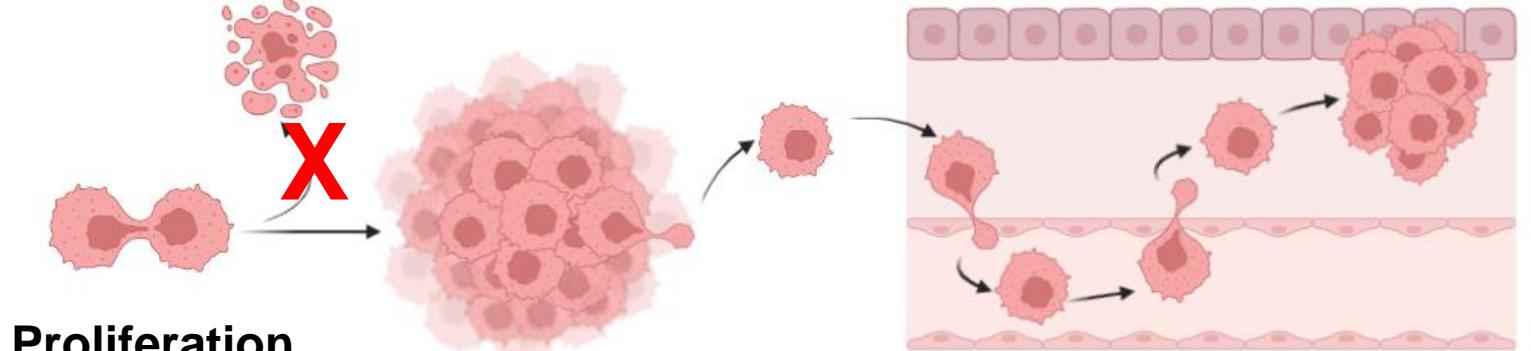
Dr. Karin Bartel

## Apoptosis



## Proliferation

Primary Tumor



Migration & Invasion

Metastasis

## Research Aims

- 1) Characterizing ES function in **cancer hallmarks**
- 2) Develop **novel modulation strategies of the activity of ES proteins**
- 3) Develop **novel treatment strategies**

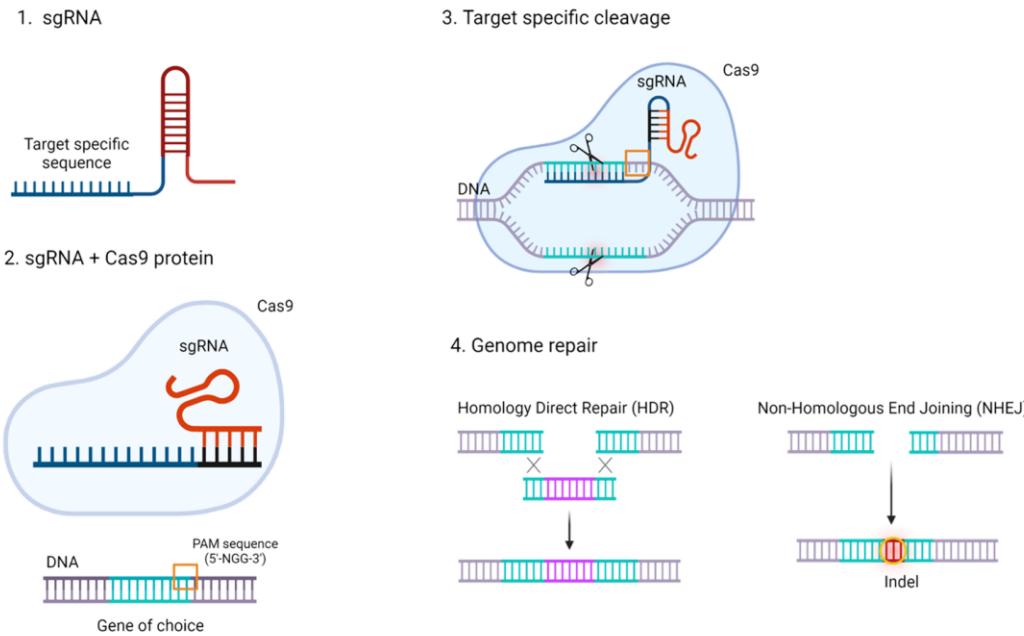
## What could you do in the lab?

- 2D and 3D cell culture of different cancer cells
- Treatment with cytostatics and novel drugs/RNA
- Functional studies on cancer cells (e.g. flow cytometry, metabolic activity, boyden chamber)
- Cell biological assays (e.g. qPCR, Western Blot, Confocal microscopy, gene editing)
- Molecular Biological methods (e.g. cloning, PCR, sequencing)

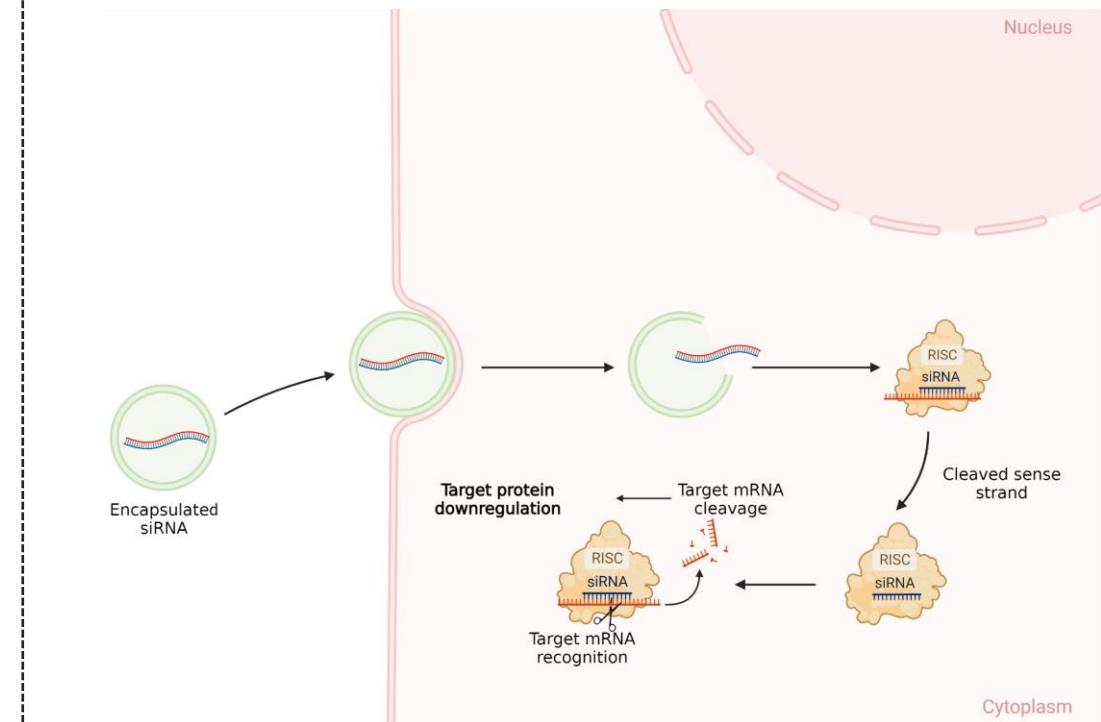
# Lipid nanoparticles for delivery of various RNA-cargoes to the lungs



## Delivery of CRISPR/Cas9 to enhance gene editing in lung cancer



## Delivery of siRNA to enhance transfection and gene silencing in pulmonary cells

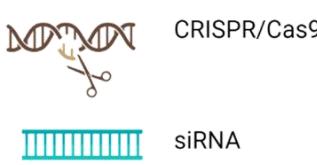
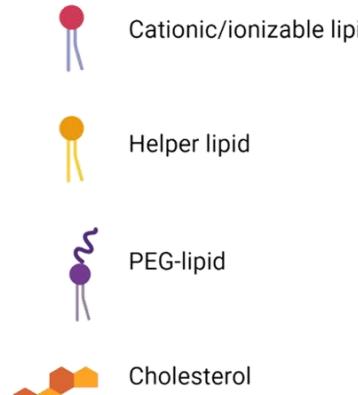
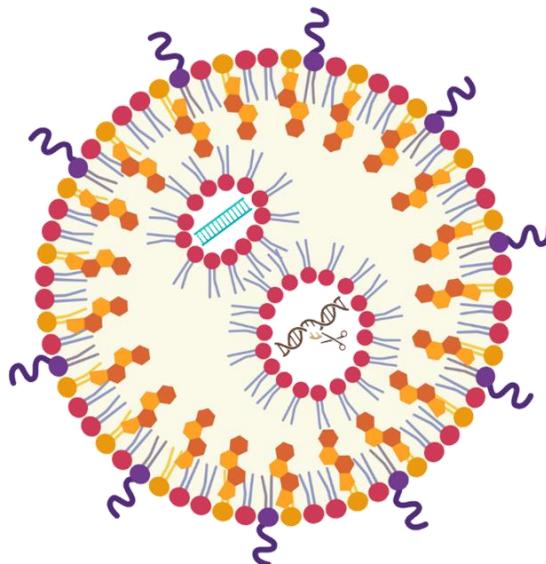


Dr. Simone Carneiro

# LNP as a remarkable non-viral delivery system

Direct delivery of RNA  
cargoes to the lungs

Instability in biological  
media



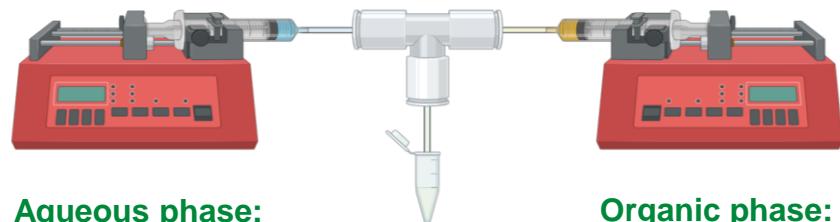
- Monodisperse nanoparticle formation: increased stability
- Efficient encapsulation of nucleic acids
- Protect the cargo from degradation
- Low toxicity profile
- Facilitate cellular uptake
- Enable endosomal escape and cargo release into the cytosol

**Extensive validation on the market:**

- 2 mRNA-COVID vaccines
- Onpattro®

# Methodology

## RNA-based LNP preparation and characterization

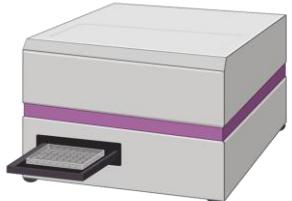


Aqueous phase:  
RNA dissolved in an  
acidic buffer

Organic phase:  
lipids

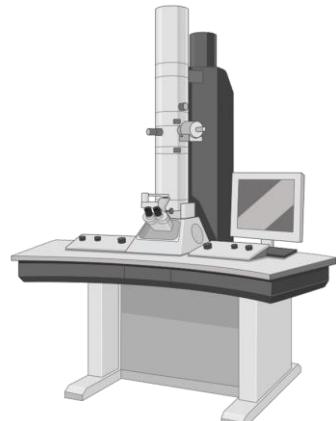


Particle size  
Polydispersity  
Zeta Potential



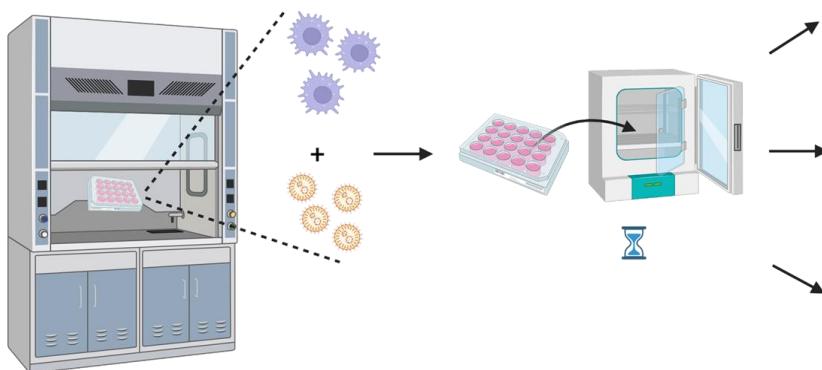
Encapsulation efficiency

## Particle morphology



## Cell culture studies and biological activity

### Submerged cell culture studies:



Gene knockdown

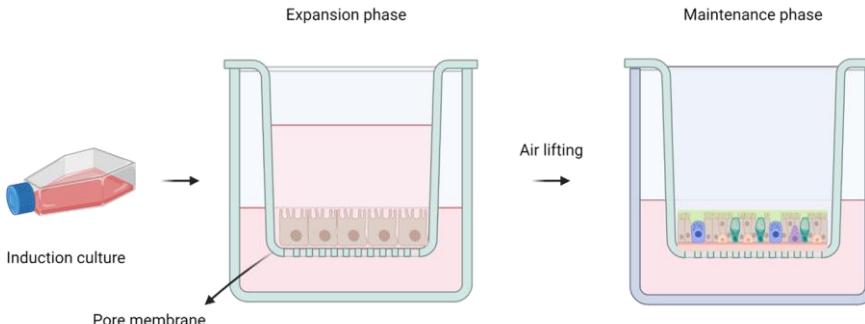


Cell uptake



Gene editing

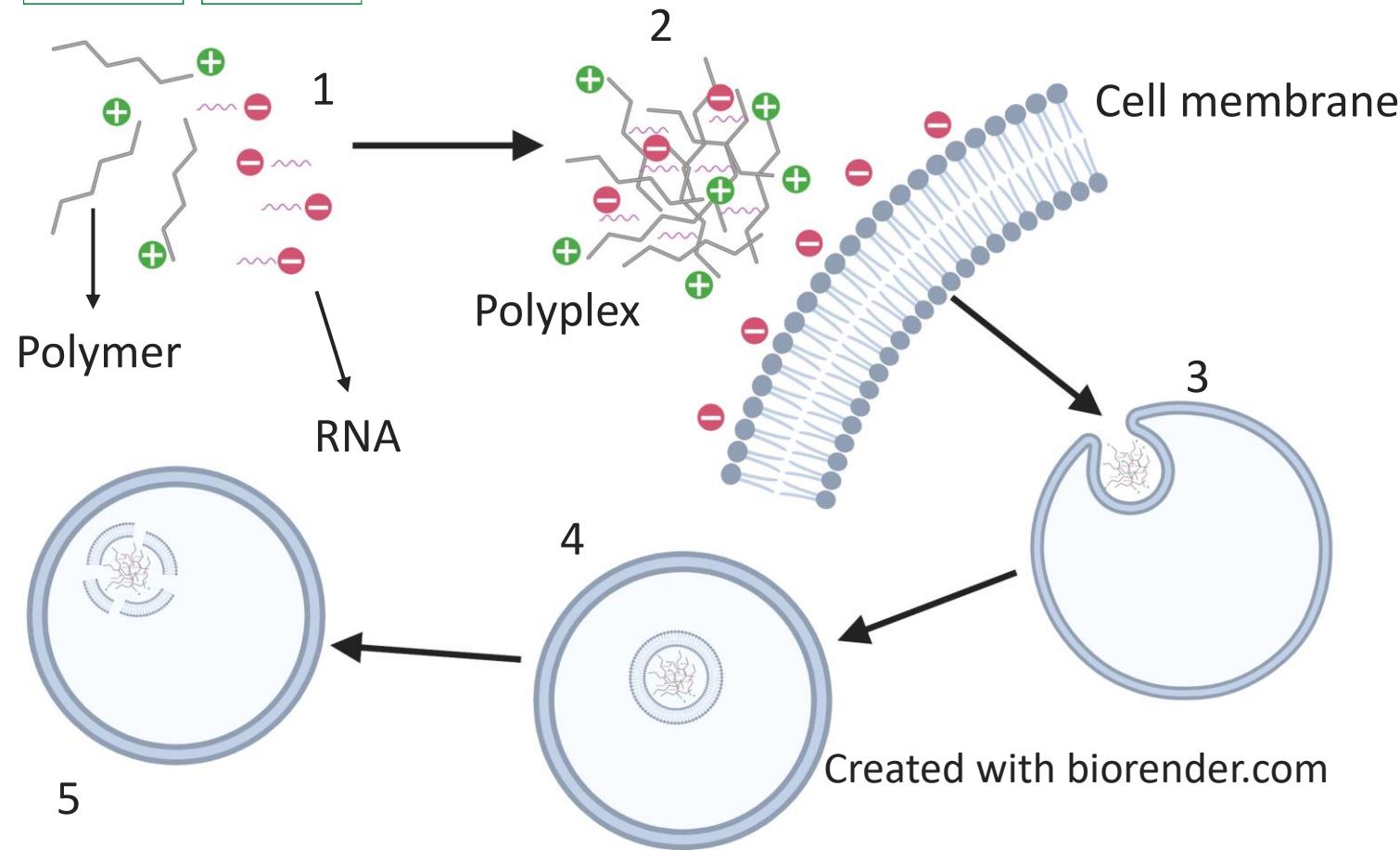
### Cells cultured at the air-liquid interface:



Differentiation  
towards a  
mucociliary type

Tight junctions

# What is the mechanism of encapsulation/release?



Dr. Valentin Fell



-  
Polymers for  
RNA delivery

## Simplified mechanism<sup>[1-5]</sup>

- 1) Polyplex formation by charge recognition
- 2) Polyplex-cell membrane interaction
- 3) Internalisation by vesicle formation
- 4) Endosomal trafficking
- 5) Endosomal escape & release of RNA cargo → bottleneck

[1] *Biomacromolecules*, 2016, **17**, 76-87.

[2] *WIREs Nanomedicine and Nanobiotechnology*, 2013, **5**, 449-468.

[3] *Biomaterials*, 2014, **35**, 2066-2078.

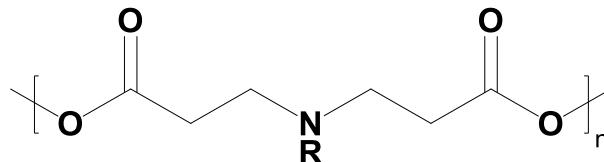
[4] *J. Am. Chem. Soc.*, 2012, **134**, 1902-1905.

[5] *Nano Lett.*, 2010, **10**, 3684-3691.

# Why are polymers interesting for RNA delivery?

- Polymers can be designed so that they are biocompatible<sup>[1]</sup> and/or biodegradable<sup>[6]</sup>
- If polymers are moderately positively charged, they can bind to the negative RNA<sup>[7]</sup> and negative cell membrane<sup>[1]</sup>
- However, a too high positive charge of the polyplex leads to cytotoxicity<sup>[1]</sup>

## Prominent example: Poly( $\beta$ -amino ester)s (PBAE)



- Easy to synthesise<sup>[8]</sup>
- No by-products
- Many variations by choice of monomers from large pool of amines & diacrylates
- Biodegradable

## What are the problems?

- Cytotoxicity<sup>[1]</sup>
- Poor encapsulation efficiency<sup>[7]</sup>
- Problematic solubility<sup>[9]</sup>
- Bioaccumulation<sup>[10]</sup>
- Uncontrolled release of RNA outside cell<sup>[1]</sup>
- No internalisation into the cell<sup>[11]</sup>
- No endosomal escape of the cargo<sup>[12]</sup>
- If biodegradable: release of harmful molecules
- Too large (>150 nm) and too broad size distribution<sup>[13]</sup>



More research necessary!

[1] *Biomacromolecules*, 2016, **17**, 76-87.

[6] *Molecular Pharmaceutics*, 2016, **13**, 134-143.

[7] *J. Controlled Release*, 2016, **229**, 120-129.

[8] *Advanced Healthcare Materials*, 2019, **8**, 1801359

[9] *Polymer*, 2009, **50**, 3895-3904.

[10] *Pharm. Res.*, 2008, **25**, 2216-2230.

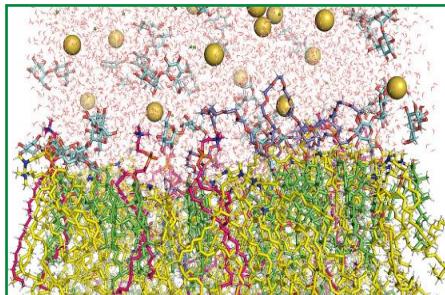
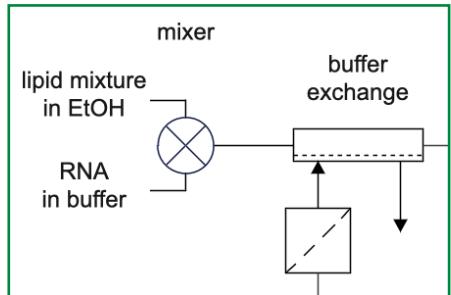
[11] *Biomacromolecules*, 2014, **15**, 1299-1310.

[12] *Advanced Therapeutics*, 2019, **2**, 1900047.

[13] *Nanotechnology*, 2017, **28**, 224001.

# Engineering Unit Operations in Processing of Nanocarriers for RNA delivery.

We combine engineering fundamentals, the use of simulation tools, and experimental techniques to advance pharmaceutical processes. Specifically, we focus on the processing of lipid nanoparticle systems for RNA delivery to the lung.



**Dr. Ben  
Winkeljann**

Apply fundamentals in process engineering, incorporating heat and mass transfer aspects and materials science.

Utilize modern data science and simulation approaches to accelerate process understanding and optimization.

Validate the formulations physicochemical properties and biological performance in multiple experimental settings.





Merkel Lab: Adrian Kromer, Alexandra Mößlang, Alice Hirschmann, Anny Nguyen, Benjamin Winkeljann, Carolina Fuchs, David Jürgens, Fabian Link, Felix Sieber-Schäfer, Gabriele Brandstätter, Gabriele Loiudice, Joschka Müller, Katharina Prüßmann, Katharina Steinegger, Katrin Wiebe, Lasse Hagedorn, Leon Reger, Leonie Deßloch, Min Jiang, Simone Carneiro, Nora Martini, Regine Bahr, Sabine Kohler, Siyu Chen, Stina Rademacker, Susanne Petzel, Valentin Fell

# Pharmazeutische Biotechnologie

Lehrstuhl Prof. E. Wagner

Dr. Simone Berger  
01.12.2023



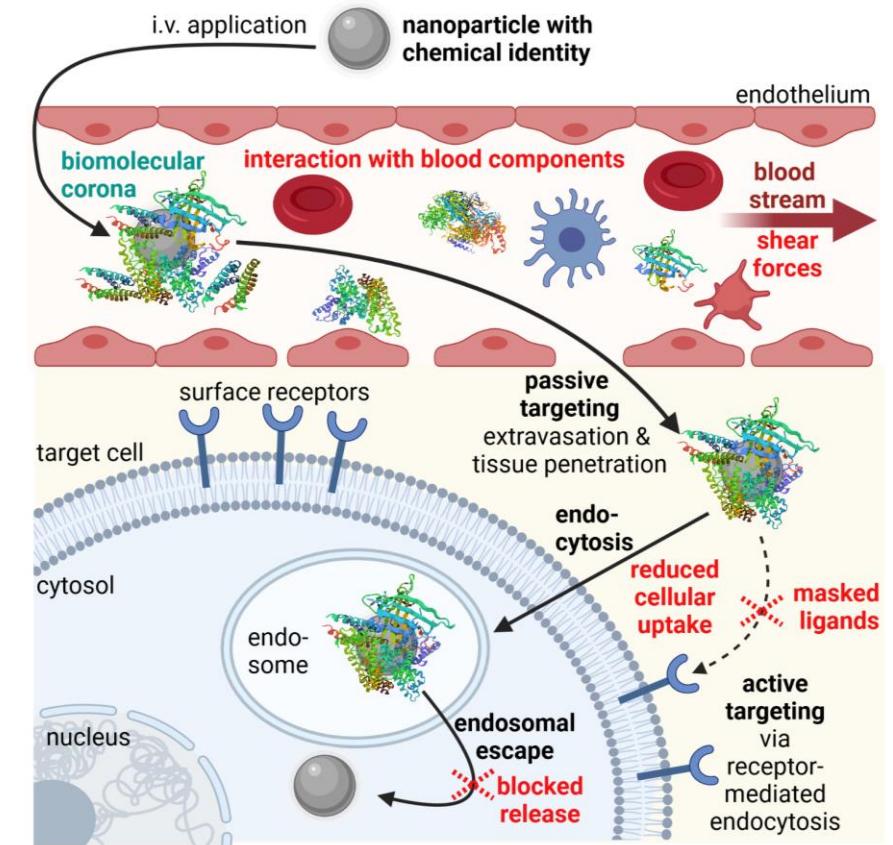
# Sequence-defined carriers for dynamic nucleic acid delivery



## Therapeutic nucleic acids

- Classical gene therapy (e.g., pDNA, mRNA)
- Modulation of gene expression at various levels of natural mRNA biosynthesis and function (e.g., siRNA)
- Genome editing systems (e.g., CRISPR/Cas9 mRNA or protein + sgRNA)
- Protein-interacting therapeutic nucleic acids (e.g., aptamers, poly(I:C))

Lächelt & Wagner (2015), Chem Rev.



S. Berger et al. (2022) Biophysics Reviews.

Requirements on the delivery system:

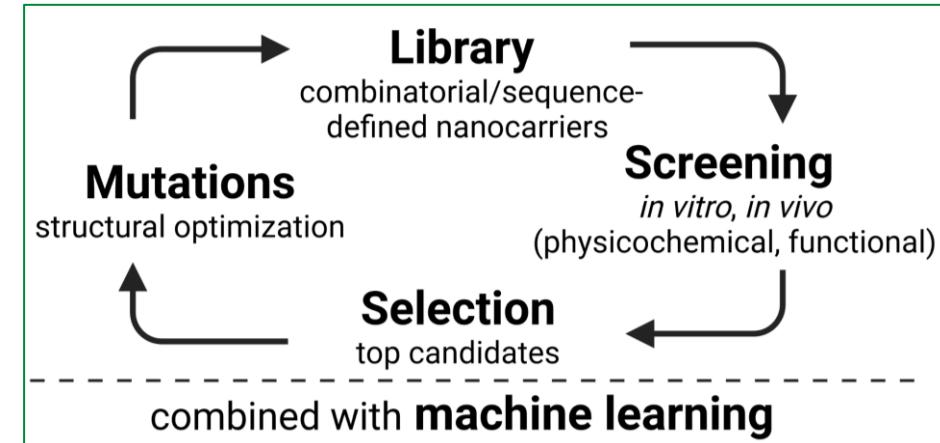
- Extracellular stability
- Efficient cellular uptake
- Endosomal escape
- Intracellular disassembly & cargo release

# Optimization of sequence-defined nanocarriers

## → Chemical evolution strategy

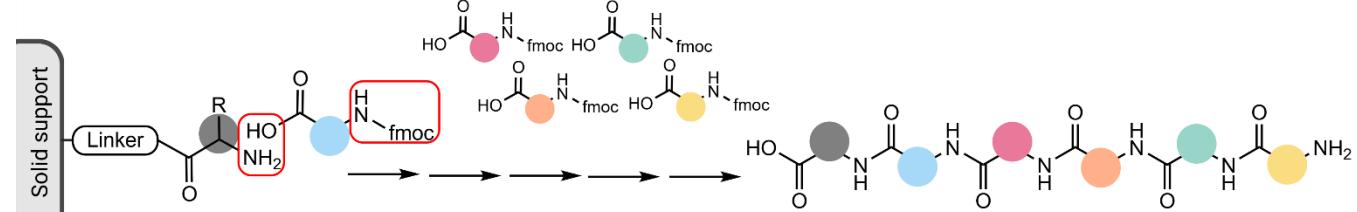
### → Learn from viruses & natural evolution

- Identification of **chemical motifs** for delivery (*natural and artificial amino acids, building blocks*)

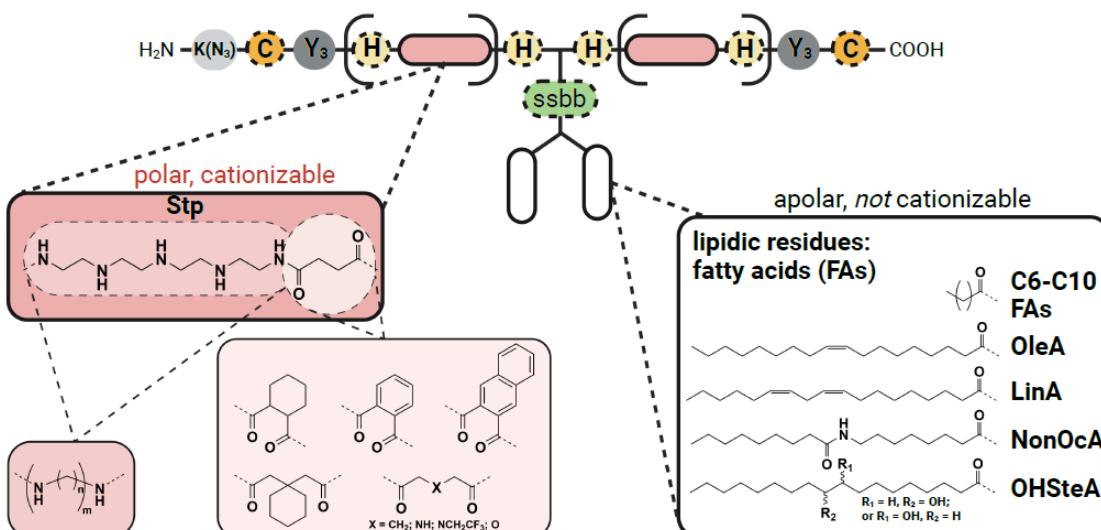


### → Solid phase-assisted peptide synthesis

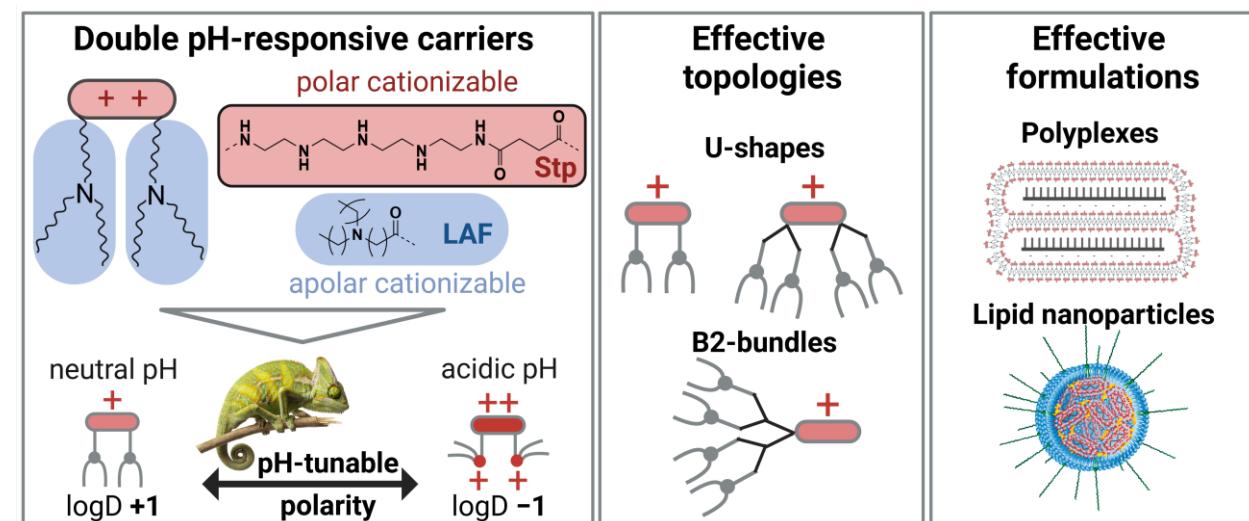
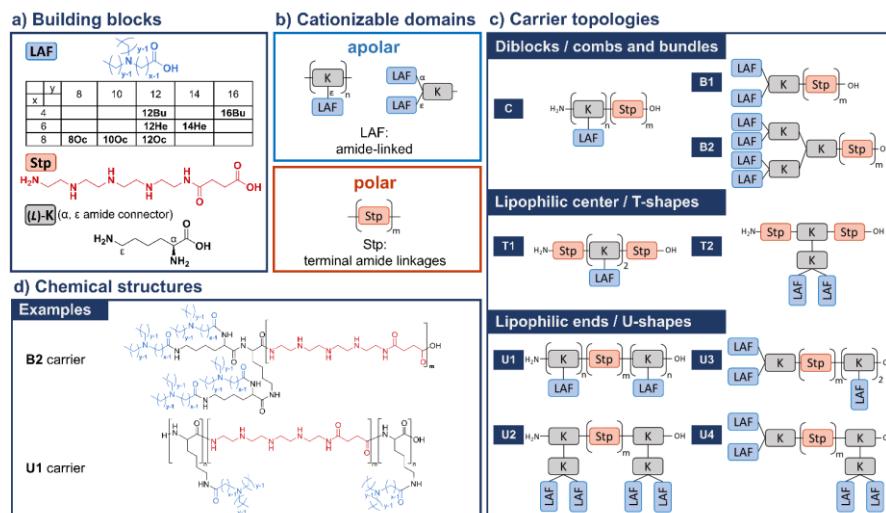
- Creation of **xenopeptide** carrier libraries



# Different modifications within a sequence-defined oligoaminoamide (OAA): 2D and 3D structure variations



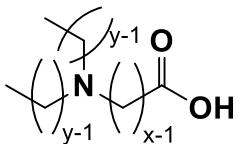
Berger, Lächelt, Wagner (2023), PNAS. In press



## Synthesis & Analytics

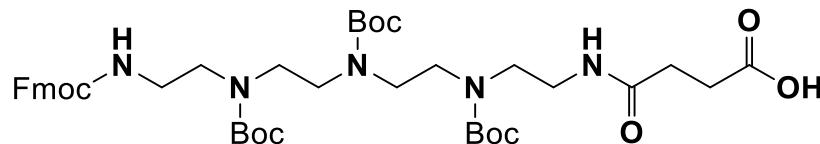
of building blocks and carrier structures for nucleic acid delivery

LAF = lipo amino fatty acids



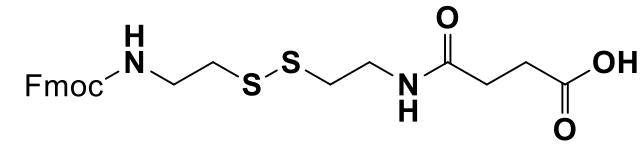
Thalmayr, Grau et al. (2023), *Adv Mater.*

Stp = succinoyl tetraethylene pentamine



Schaffert et al. (2011), *Org Lett.*

ssbb = cystamine disulfide building block

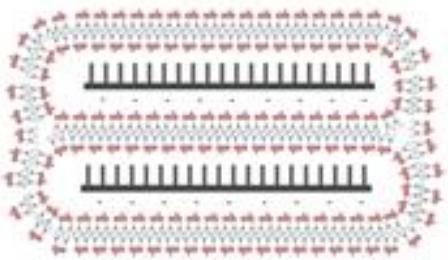


Klein, Reinhard et al. (2016), *Nanoscale*

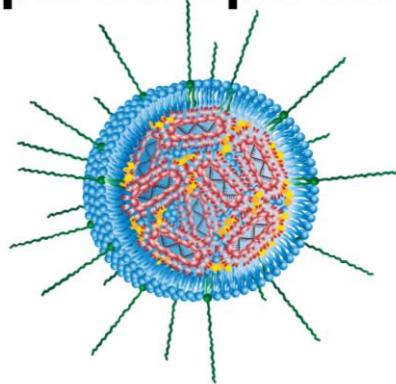
- **In-process-controls:** TLC; analytical HPLC, LC-MS; MS – ESI, EI, MALDI-TOF; <sup>1</sup>H-NMR; (GC) ...
- **Purification:** Extraction, dry/flash column chromatography, size exclusion chromatography, dialysis, preparative HPLC, lyophilization ...
- **Analytics:** MS – ESI, EI, MALDI-TOF; <sup>1</sup>H-NMR, <sup>13</sup>C-NMR; HPLC, LC-MS; (GC) ...

# Formation and characterization of nanoparticles

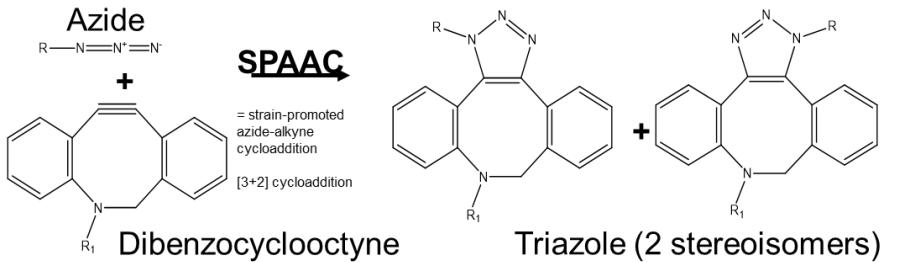
## Polyplexes



## Lipid nanoparticles



## Functionalization with shielding and targeting units: strain-promoted azide-alkyne cycloaddition



### ➤ Physicochemical characterization

- Particle shape, size, polydispersity, and surface charge
- Nucleic acid binding/compaction efficiency; encapsulation
- ...

### ➤ Mechanistic studies

- Hydrophilic/hydrophobic balance, logD
- Uptake studies, targeting efficiency
- Transfection kinetics
- Stability and deficiency in serum
- Lytic potential, endosomal escape
- Biodegradability
- ...

### ➤ Biological evaluation *in vitro*

- (cell culture, different cell lines)
- Transfection efficiency (reporter assays)
  - Toxicity
  - ...

### ➤ Transfection efficiency and toxicity *in vivo*

- (different tumor models in different mouse strains)
- Biodistribution
  - Transfection efficiency
  - Biocompatibility
  - ...

## ➤ Bachelor- & Masterarbeiten

### ➤ WPF BSc

- Labor (Begleitung von Doktoranden), Datenauswertung, Literaturrecherche
- Report, Präsentation

### ➤ WPF StEx

- 2 Labor-Schnuppertage: Einblick in Festphasen-Synthese, Zellkultur, Durchflusszytometrie und CellWatcher
- Literaturrecherche
- Erarbeitung einer Präsentation zu einer der 4 Stationen im Kontext einer wissenschaftlichen Fragestellung und Vorstellung im Arbeitskreis

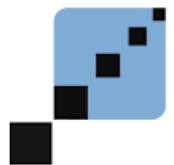
# Thank you for your attention!

Prof. E. Wagner

All team members of  
AK Wagner

## Questions ???

[simone.berger @cup.uni-muenchen.de](mailto:simone.berger@cup.uni-muenchen.de)



**CeNS**  
Center for NanoScience | LMU





Prof. Dr.  
Robert Fürst

# Naturstoffe in der Vaskulären Biologie



Prof. Dr.  
Stefan Zahler



Dr. Matthias Völkl



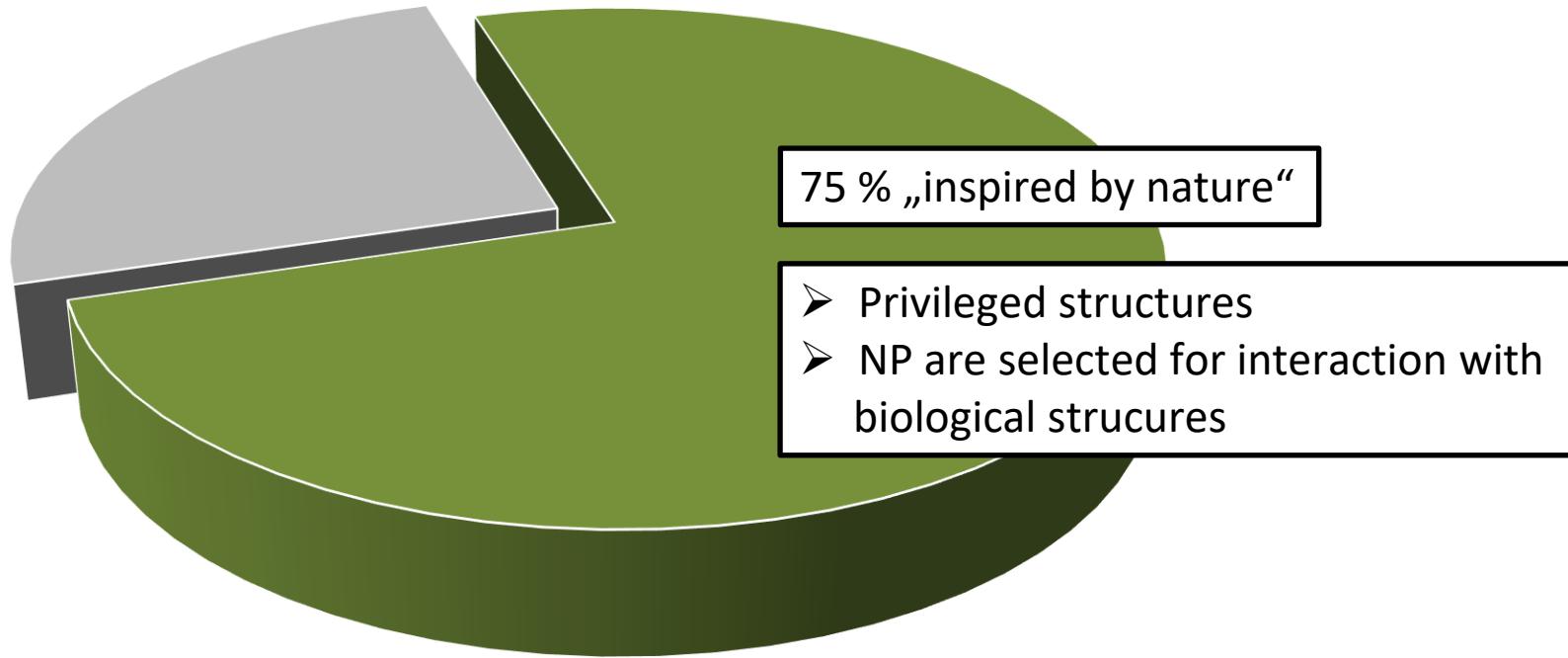
Dr. Benjamin Kirchweger



Dr. Thomas Zech

# Background

## Natural products (NPs) – an invaluable source for **therapeutic leads**



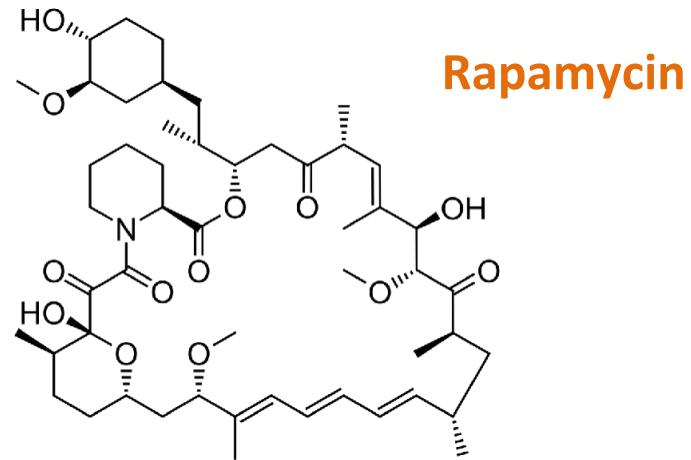
All newly approved drugs from **1981 to 2019** ( $n = 1,881$ )

*Modified from Newmann and Cragg, 2020*

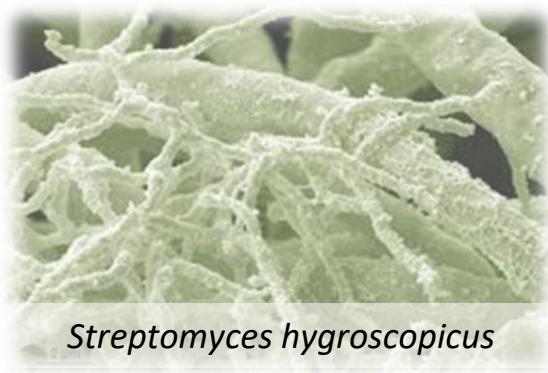
# Background



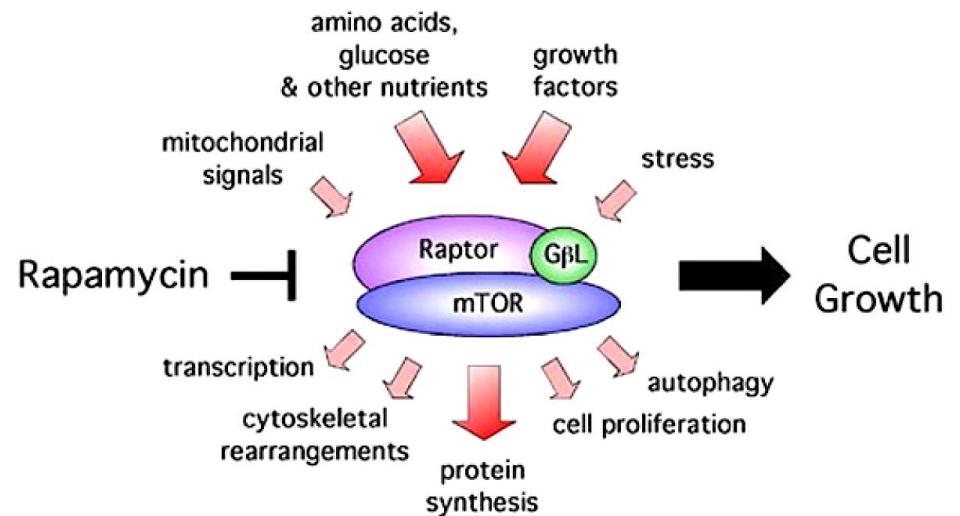
Natural compounds – indispensable **chemical tools** to understand biological/pharmacological systems



Rapamycin

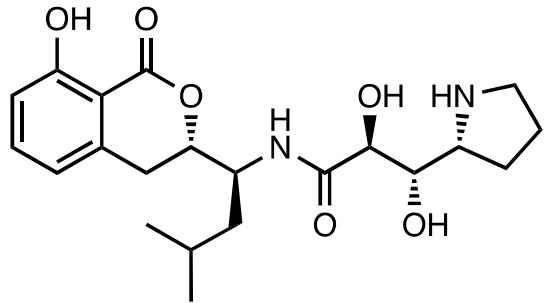
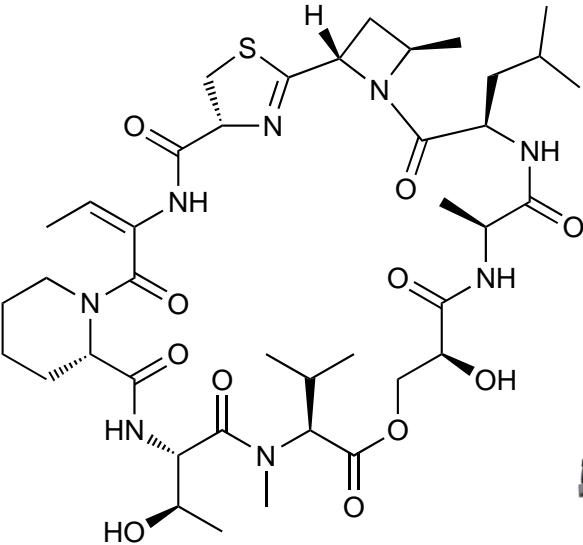


*Streptomyces hygroscopicus*

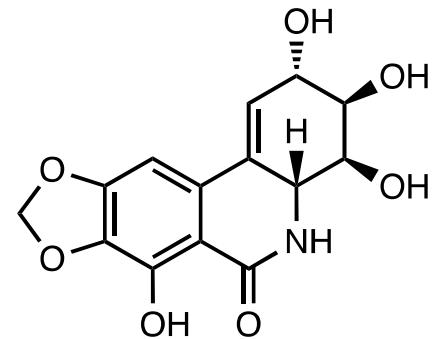


→ **mTOR** =  
mammalian/mechanistic target of rapamycin

# Background



Nature's  
toolbox

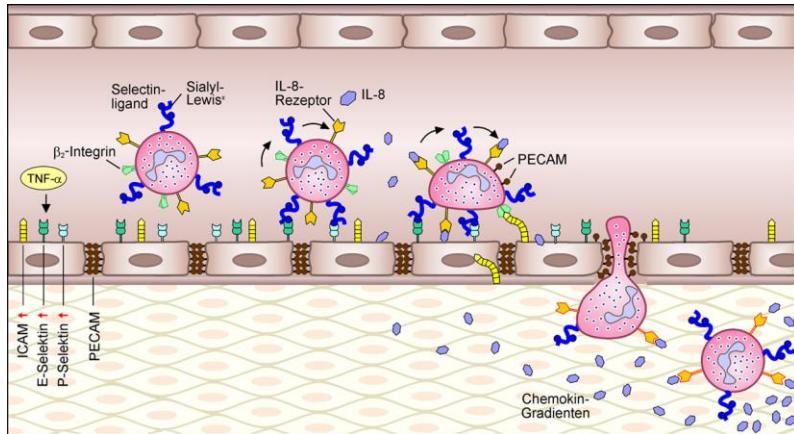


- To discover innovative **anti-inflammatory** and **anti-cancer** compounds
- To identify new drug **targets** for therapy

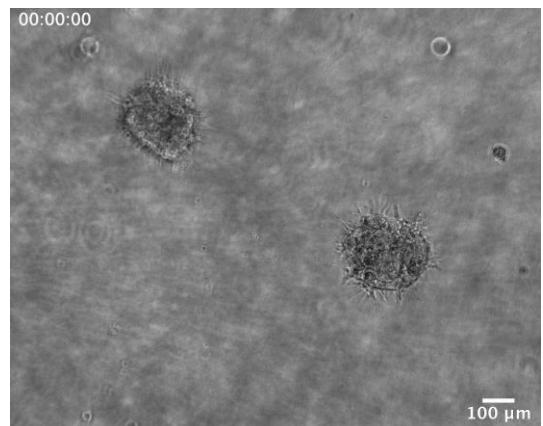
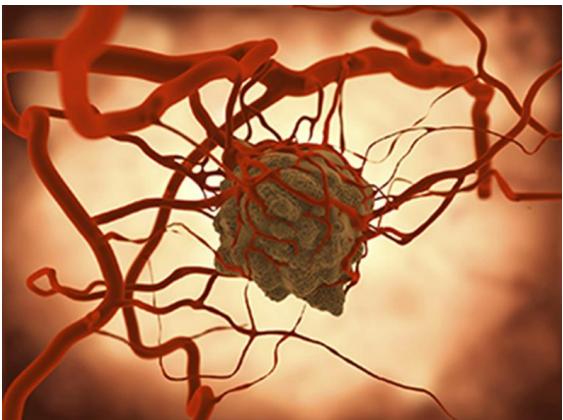




- Inflammation (interaction of leukocytes and endothelial cells)



- Angiogenesis (generation of new blood vessels)



# Methods in the lab

- Cell culture of vascular cells, isolation of primary leukocytes
- Functional assays (leukocyte adhesion, migration, proliferation, sprouting, ...)
- Fluorescence microscopy (fixed samples and living cells)
- qPCR
- Flow cytometry
- Gene silencing and overexpression
- Western blot

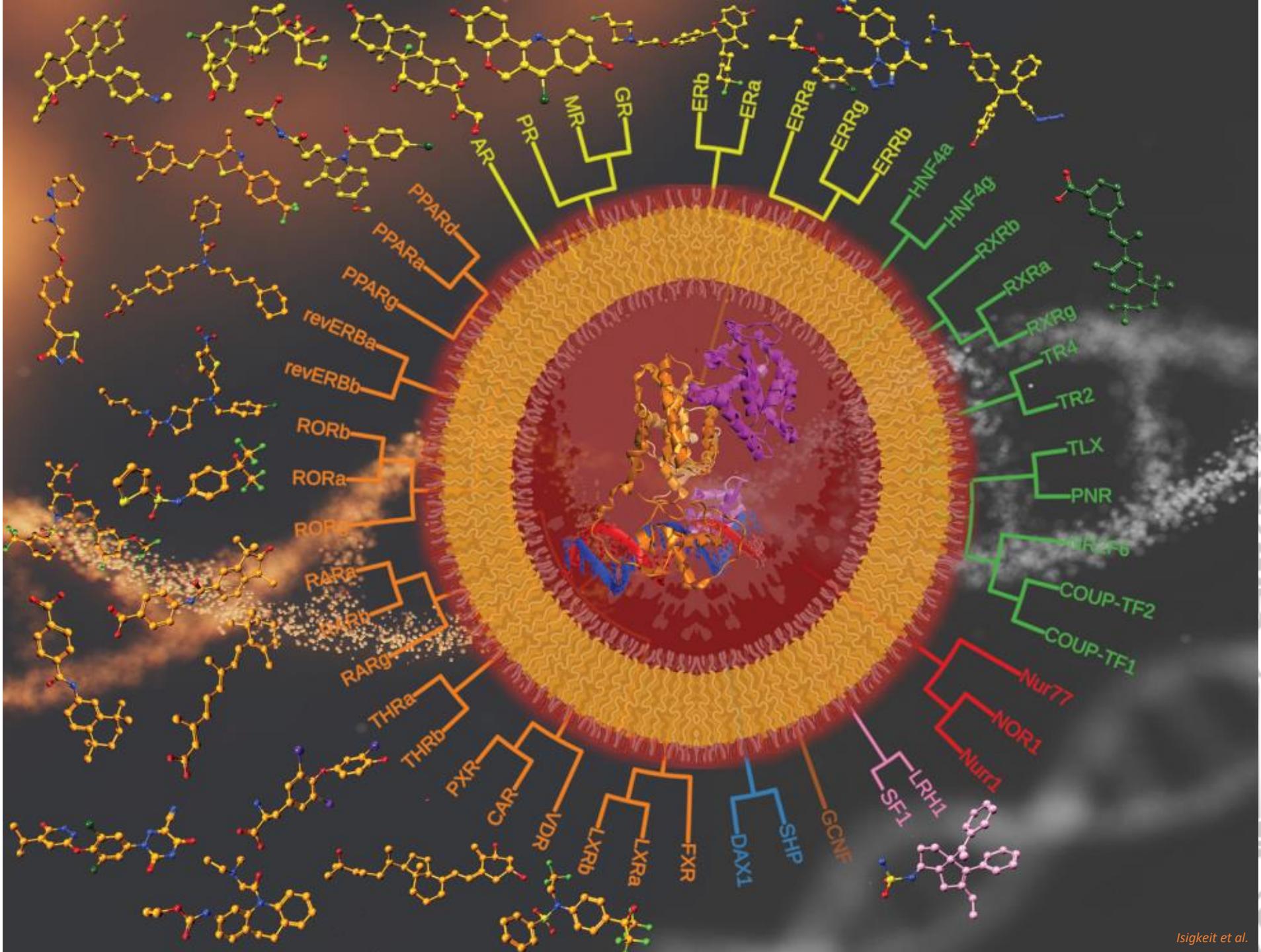
## Weitere Informationen



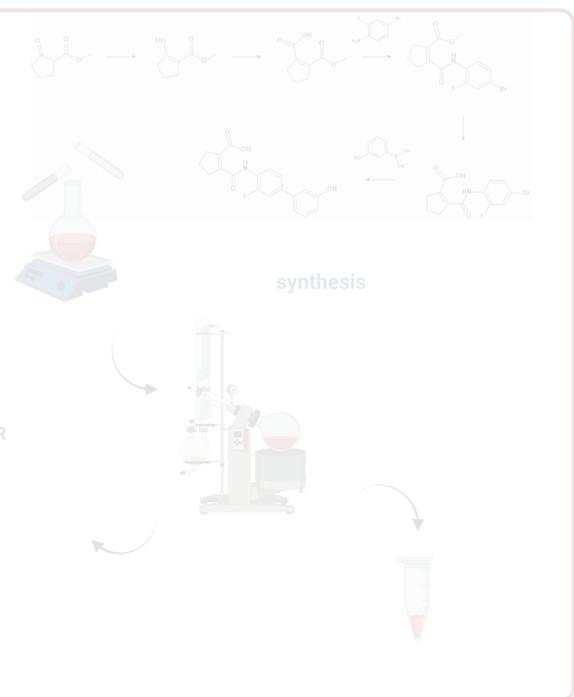
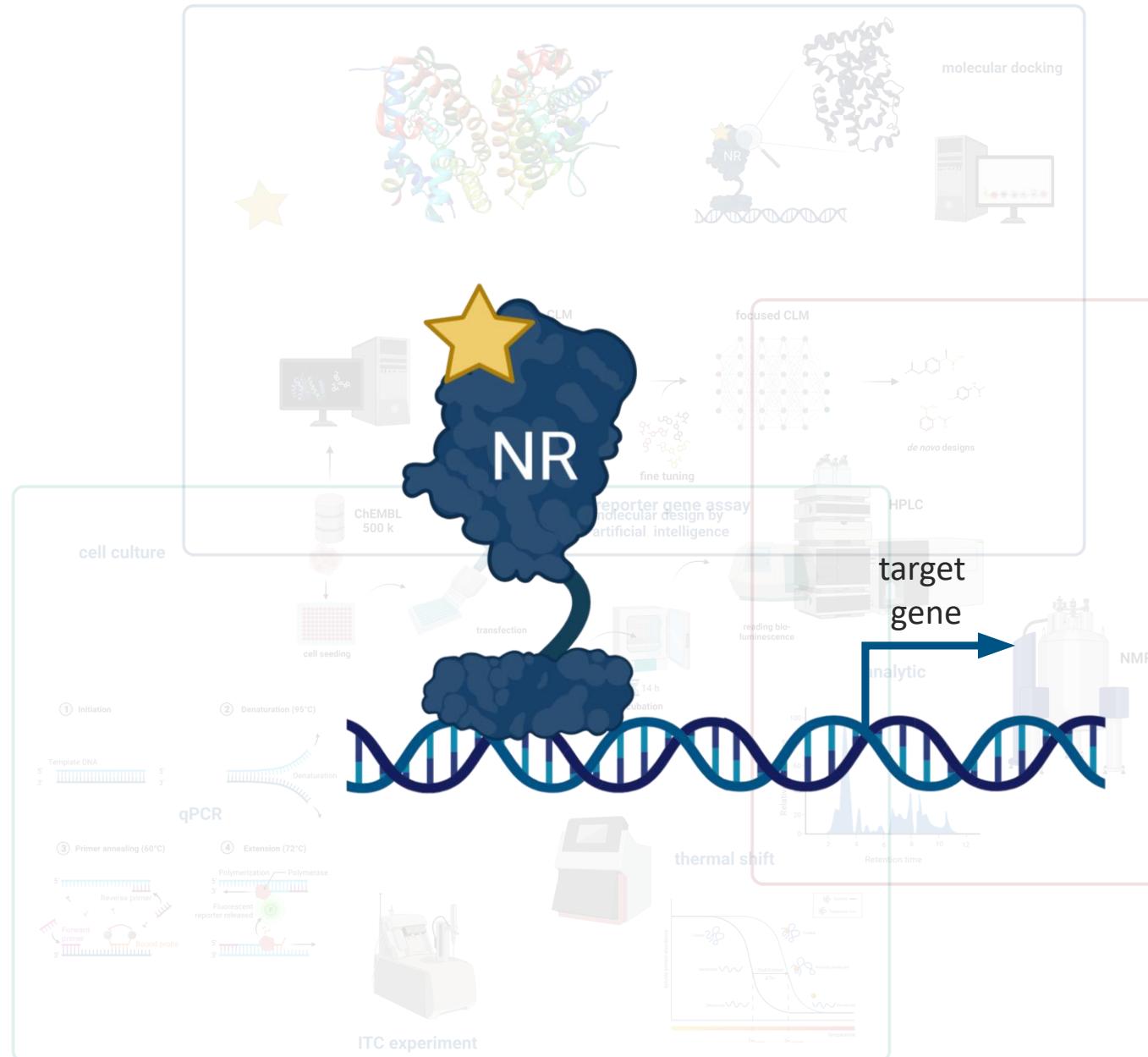
- Im Anschluss an diese Veranstaltung
- Weitere Fragen zu Forschungspraktika, Bachelor- und Masterarbeiten am AK Fürst per E-Mail an Prof. Zahler ([stefan.zahler@lmu.de](mailto:stefan.zahler@lmu.de))

# Tag der Forschung AK Merk

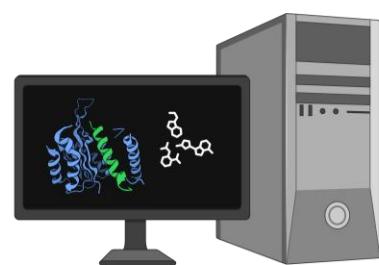
Lehrstuhl für pharmazeutische &  
medizinische Chemie



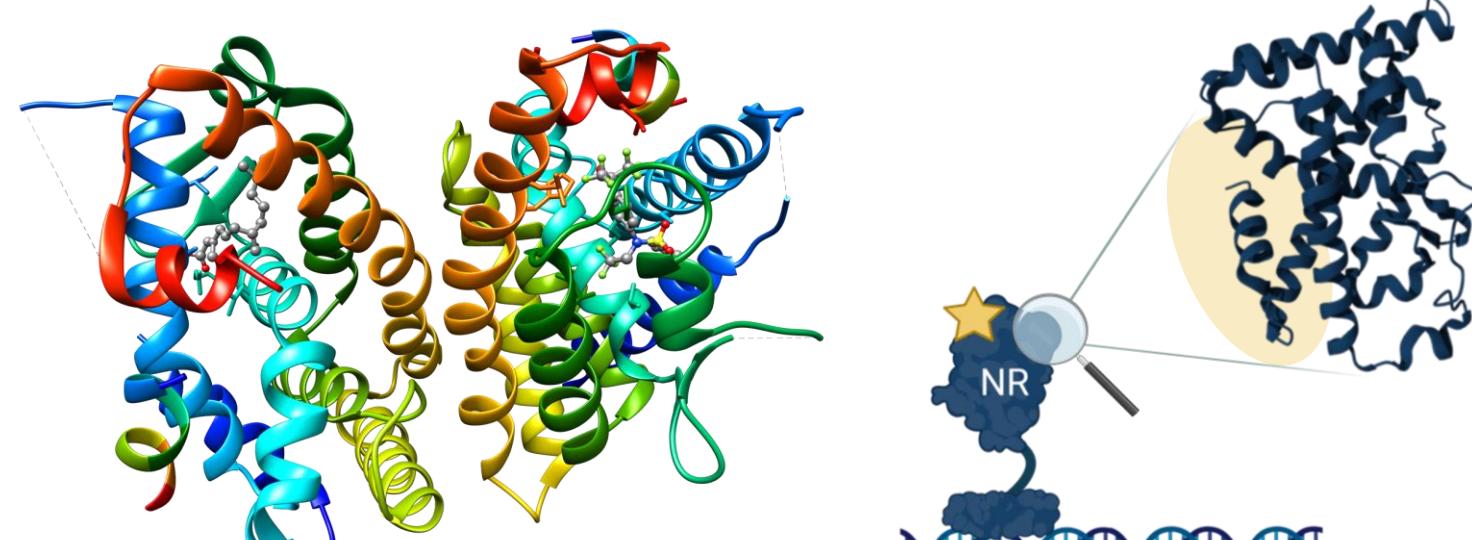
## Targeting nuclear receptors in neurodegeneration and beyond



computational  
chemistry



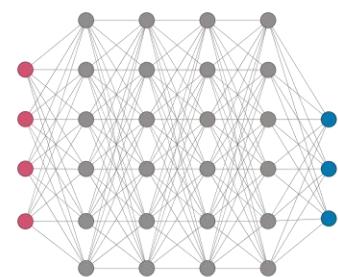
ChEMBL  
500 k



molecular docking

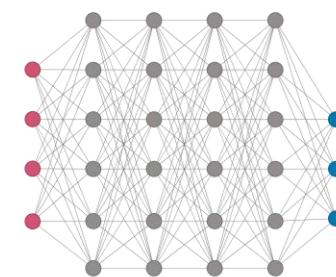


CLM

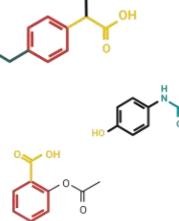


fine tuning

focused CLM

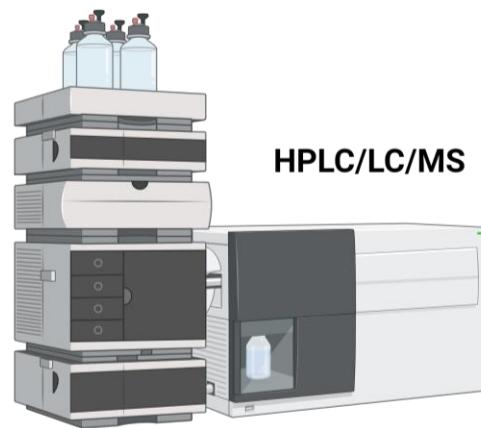


*de novo* designs

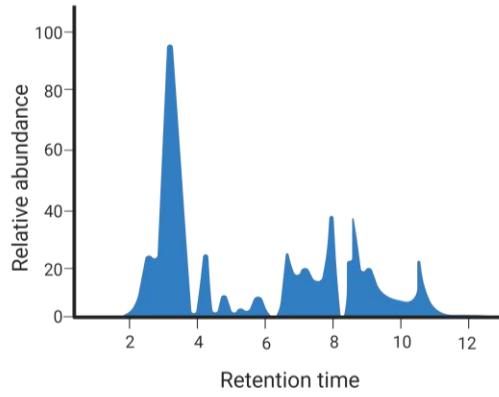


molecular design by  
artificial intelligence

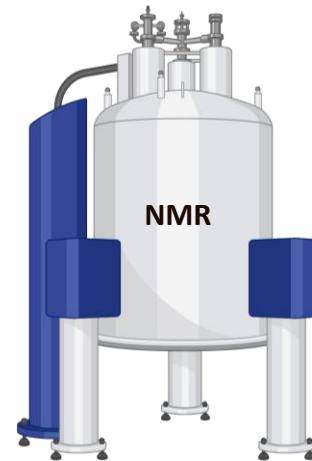
# chemistry



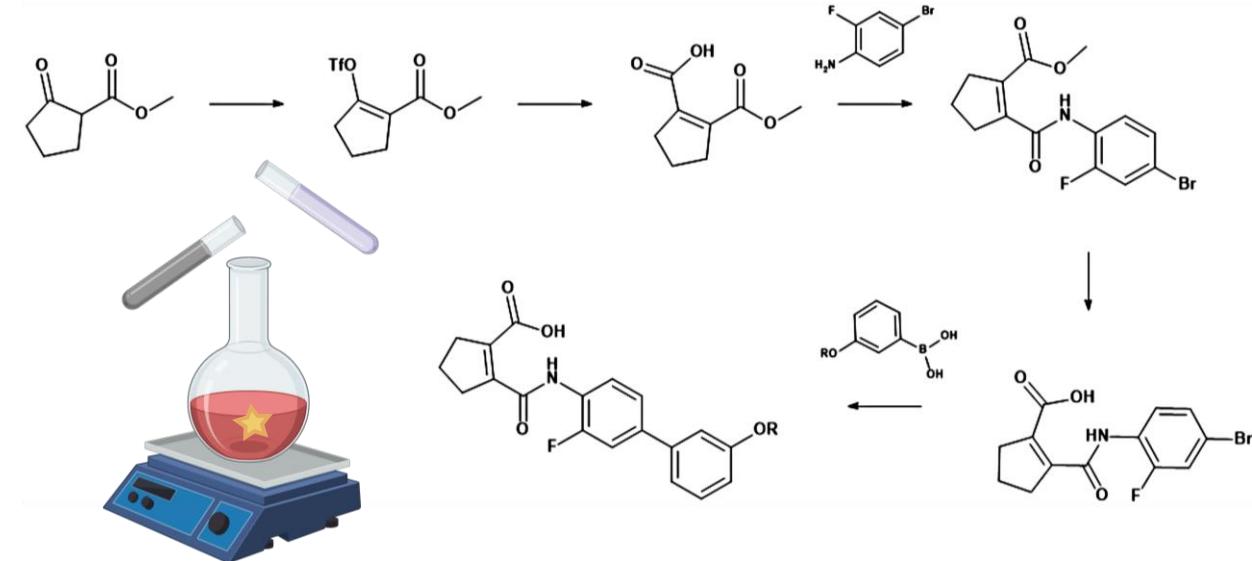
HPLC/LC/MS



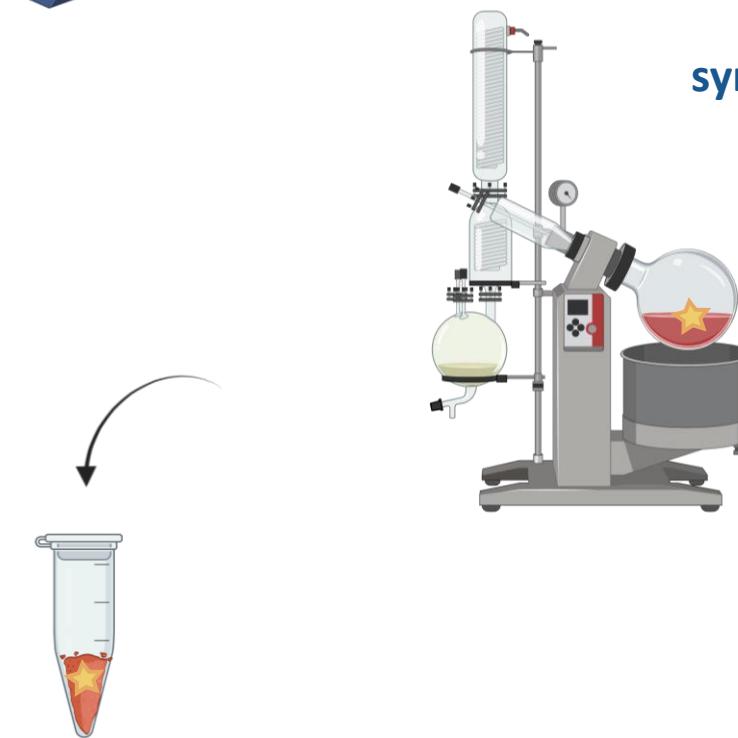
analytics



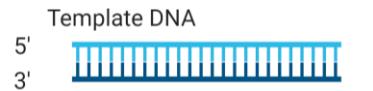
NMR



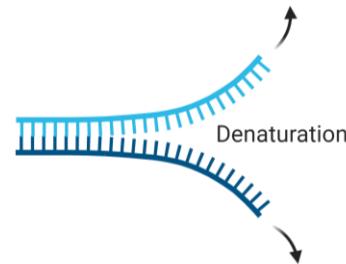
synthesis



### ① Initiation

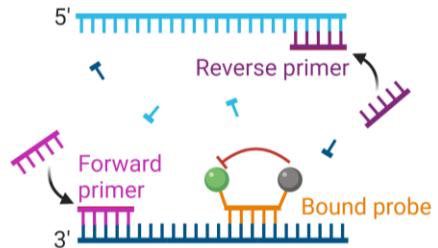


### ② Denaturation ( $95^{\circ}\text{C}$ )

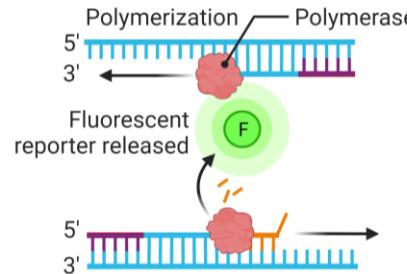


★ qPCR

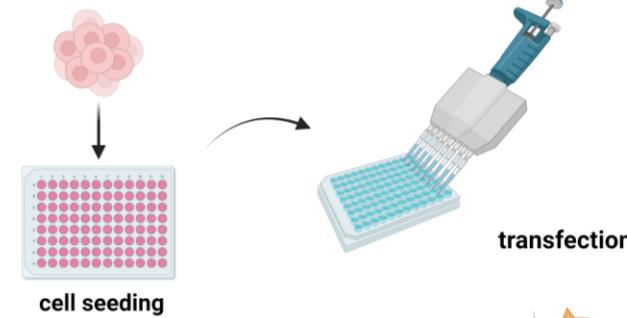
### ③ Primer annealing ( $60^{\circ}\text{C}$ )



### ④ Extension ( $72^{\circ}\text{C}$ )



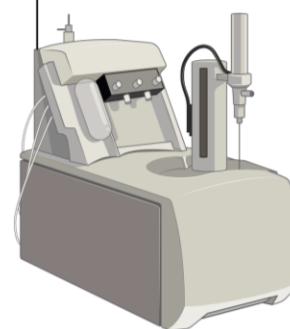
## cell culture



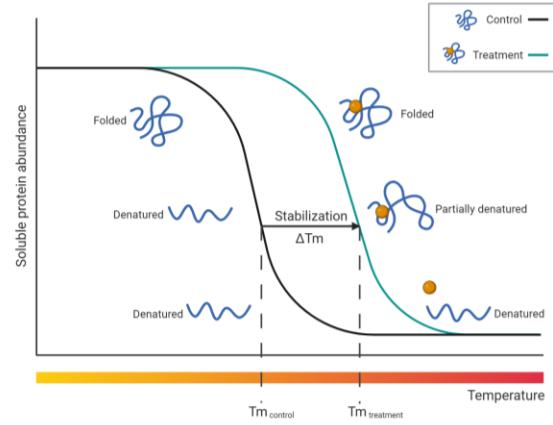
## reporter gene assay



## isothermal titration calorimetry



★ thermal shift assay



# **potent Nurr1 agonists**

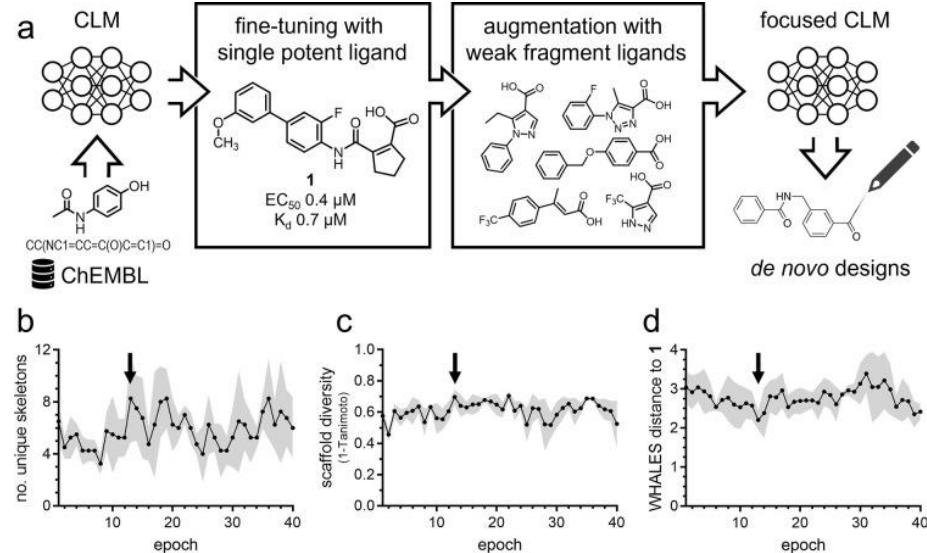
published in 2023



# De Novo Design of Nurrl Agonists via Fragment-Augmented Generative Deep Learning in Low-Data Regime

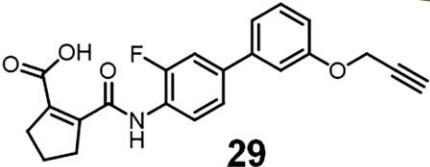
M. Ballarotto, Sabine Willems, Tanja Stiller, Felix Nawa, J. Marschner, F. Grisoni, D. Merk less

Published in Journal of Medicinal... 31 May 2023 • Biology

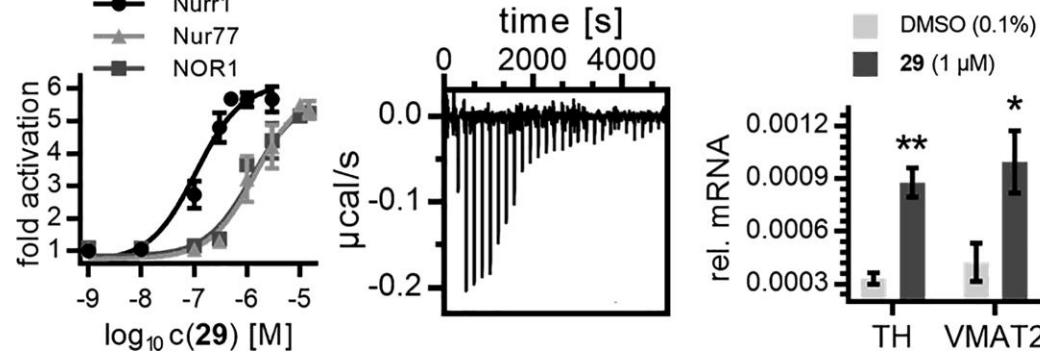


## Development of a Potent Nurr1 Agonist Tool for In Vivo Applications

Jan Vietor, Christian Gege, Tanja Stiller, Romy Busch, Espen Schallmayer, Hella Kohlhof, Georg Höfner, Jörg Pabel, Julian A. Marschner, and Daniel Merk\*

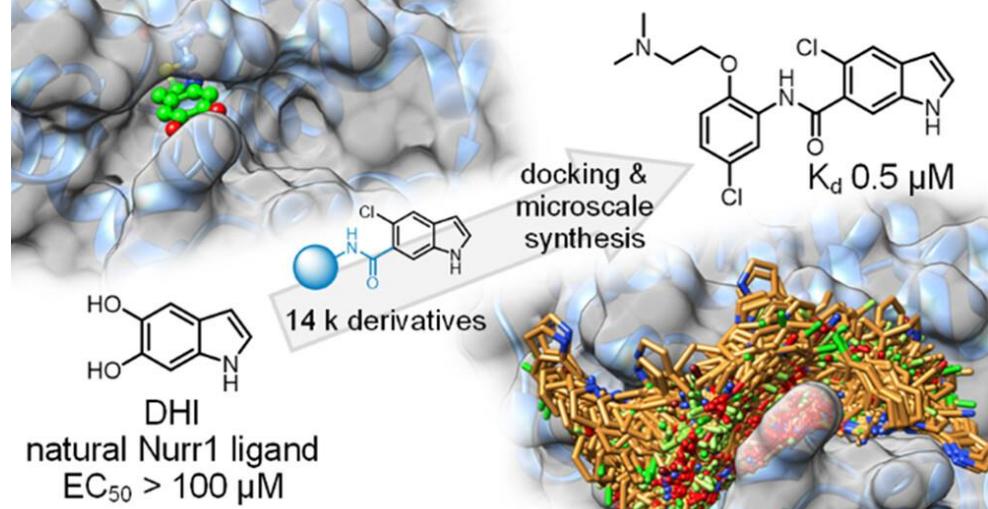


$EC_{50}(\text{Nurr1}) = 0.11 \mu\text{M}$   
 $K_d(\text{Nurr1}) = 0.3 \mu\text{M}$   
>10-fold selective vs. Nur77, NOR1  
PK (rat, 5 mpk p.o.):  $t_{1/2} = 4.4 \text{ h}$ ,  $c_{\max} = 56 \mu\text{M}$



## Structure-Guided Design of Nurr1 Agonists Derived from the Natural Ligand Dihydroxyindole

Minh Sai,<sup>‡</sup> Jan Vietor,<sup>‡</sup> Moritz Kornmayer, Markus Egner, Úrsula López-García, Georg Höfner, Jörg Pabel, Julian A. Marschner, Thomas Wein, and Daniel Merk<sup>\*</sup>



# AK Merk

Prof. Dr. Daniel Merk  
Senior Scientists/Postdocs

Dr. Georg Höfner

Dr. Julian Marschner

Dr. Jörg Pabel

Dr. Thomas Wein

## PhD Students

Romy Busch

Markus Egner

Xiu Ge

Emily Hank

Tim Hörmann

Till Kasch

Max Lewandowski

Úrsula López

Felix Nawa

Minh Sai

Katharina Scholz

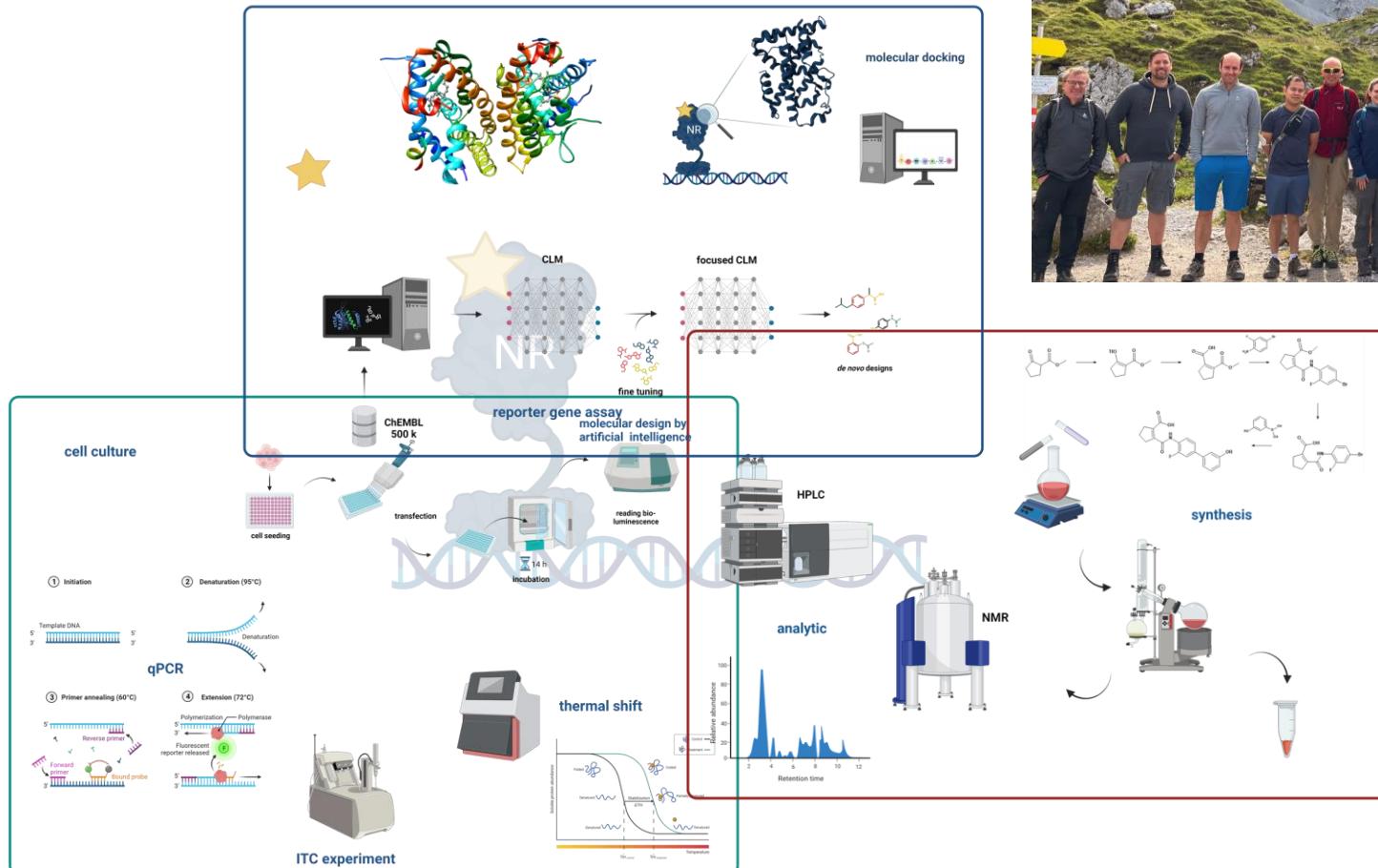
Tanja Stiller

Jan Vietor

## Technicians

Silke Duensing-Kropp

Tanja Franz



Wahl- & Forschungspraktika  
Bachelor- & Masterarbeiten  
PJ & PhD

# Uncovering and targeting vulnerabilities in drug-resistant and metastatic lung cancer cell lines with new small molecule drug candidates

Ludwig Maximilian University Munich  
David B. Konrad  
1<sup>st</sup> of December 2023



david.konrad@cup.lmu.de

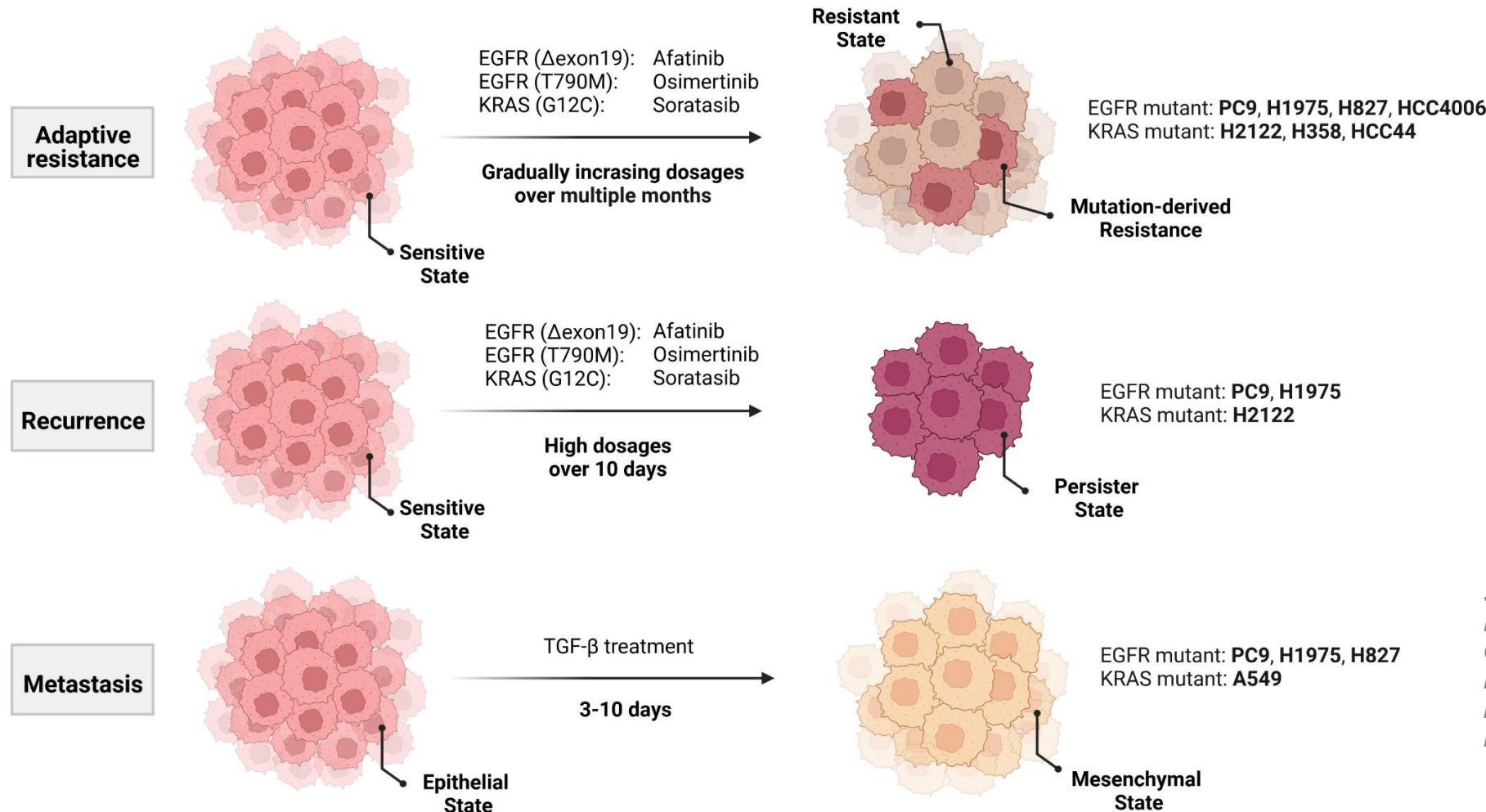


konrad.cup.uni-muenchen.de



@DavidBKonrad

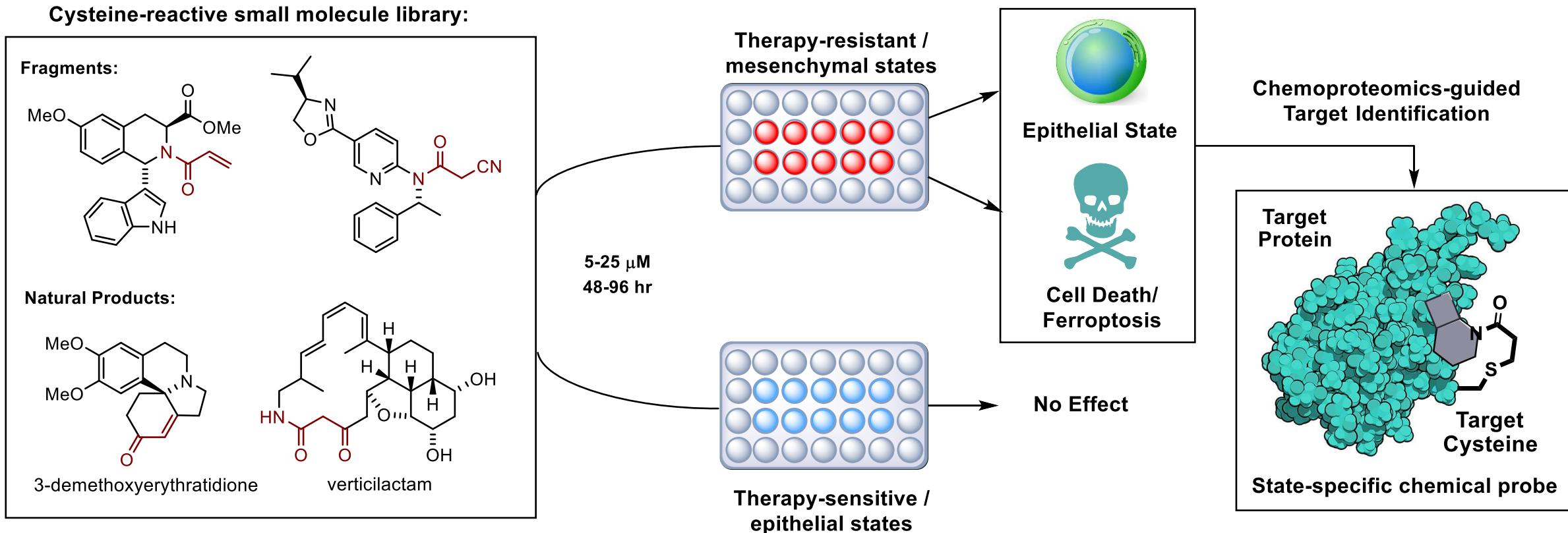
# Advanced NSCLC Model Cell Lines



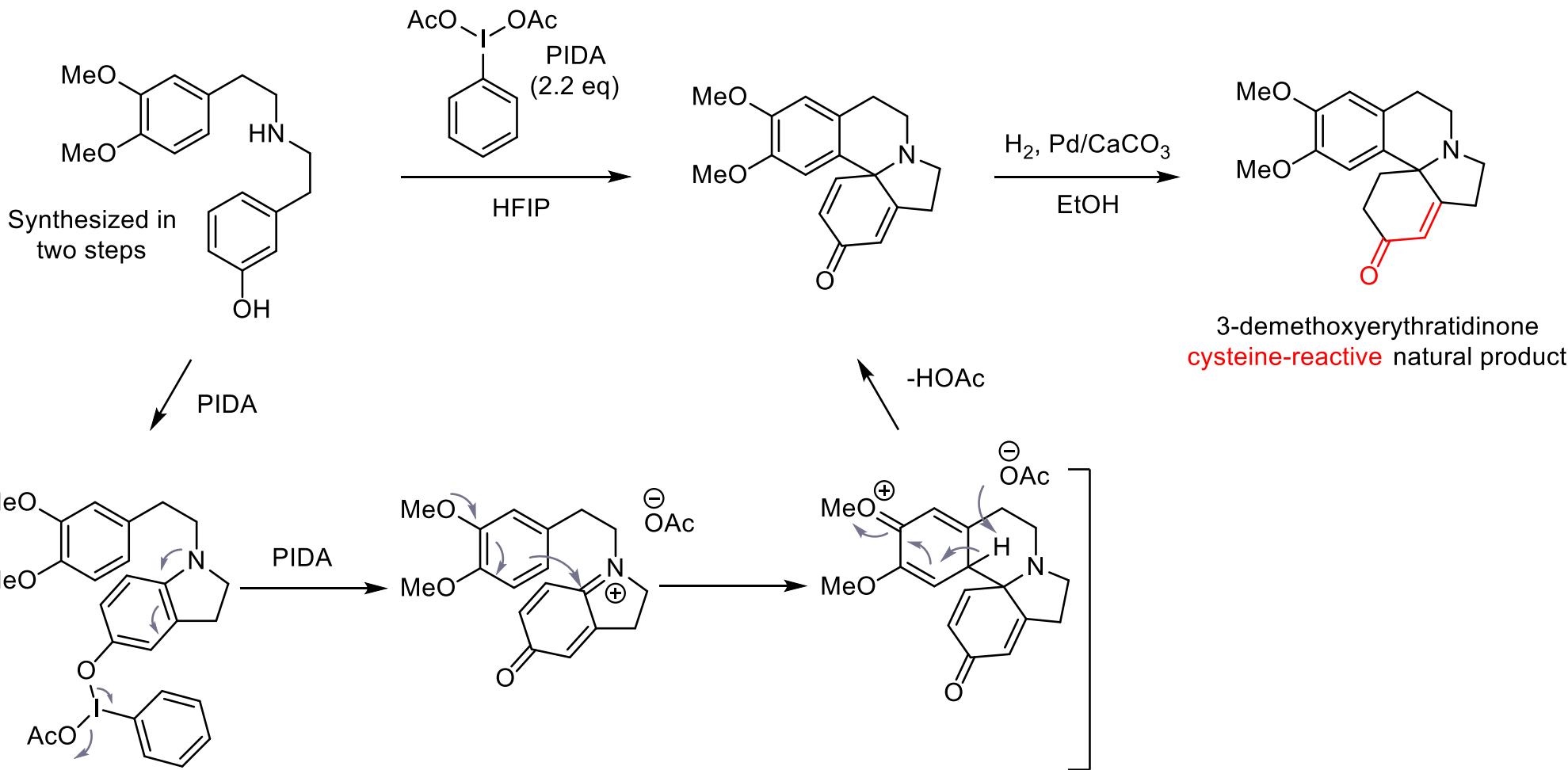
Sci. Rep. 2020, 10, 10597.  
Nature 2017, 551, 247-250.  
Cancer Res. 2021, 81, 3051-3066.  
Nat. Rev. Cancer 2014, 14, 535-546.  
Mol. Cancer 2018, 17, 29.  
Nature 2019, 575, 217–223.

→ Availability of the same cell lines in early and advanced states enables to pinpoint and characterize pathways that underlie therapy resistance, metastasis and recurrence.

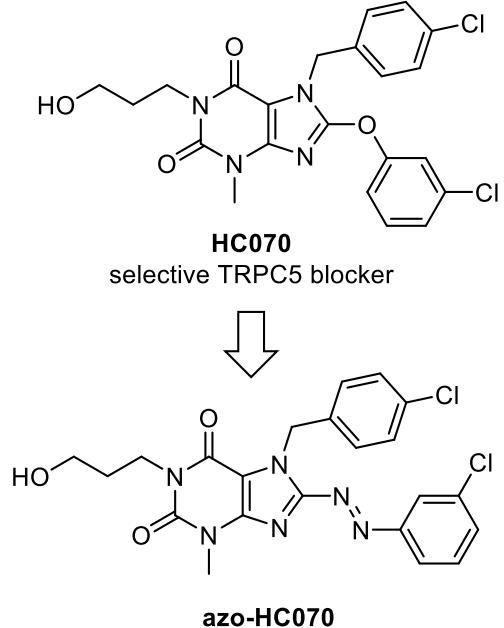
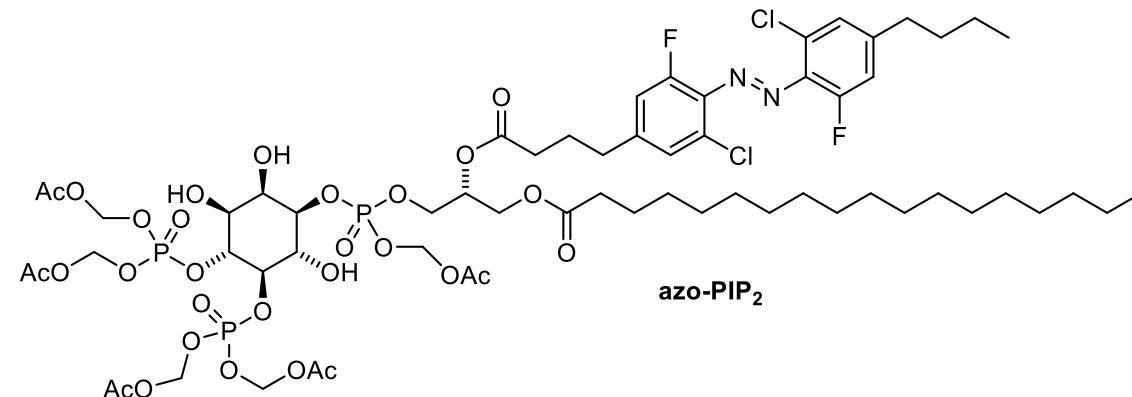
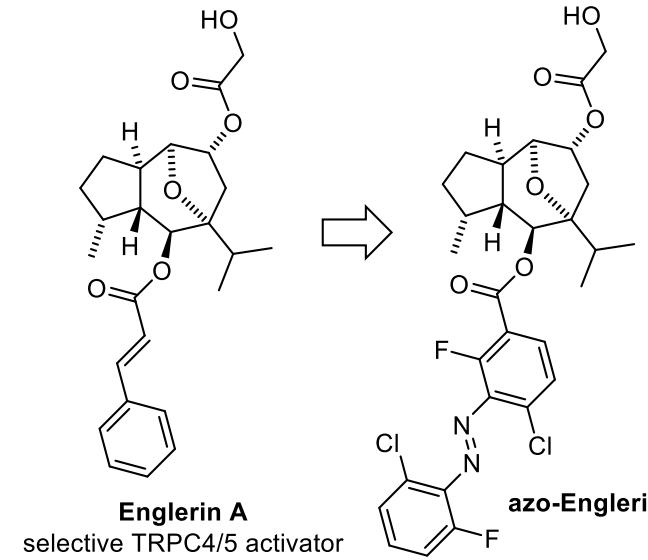
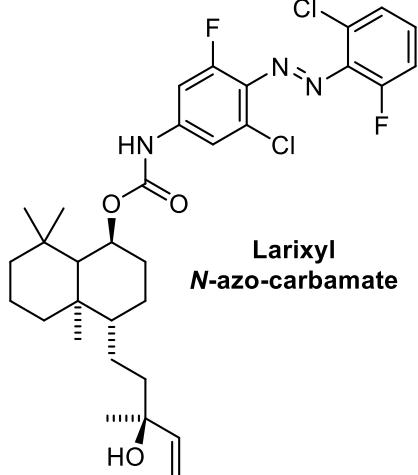
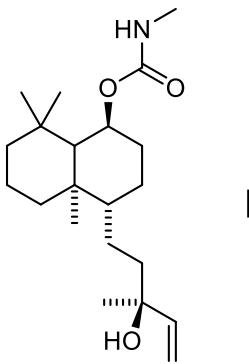
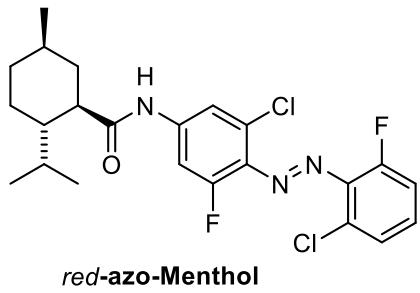
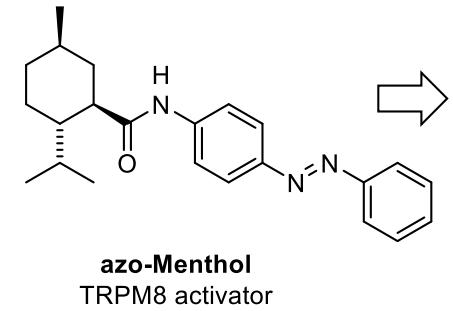
# Small Molecules that Specifically Target Advanced NSCLC



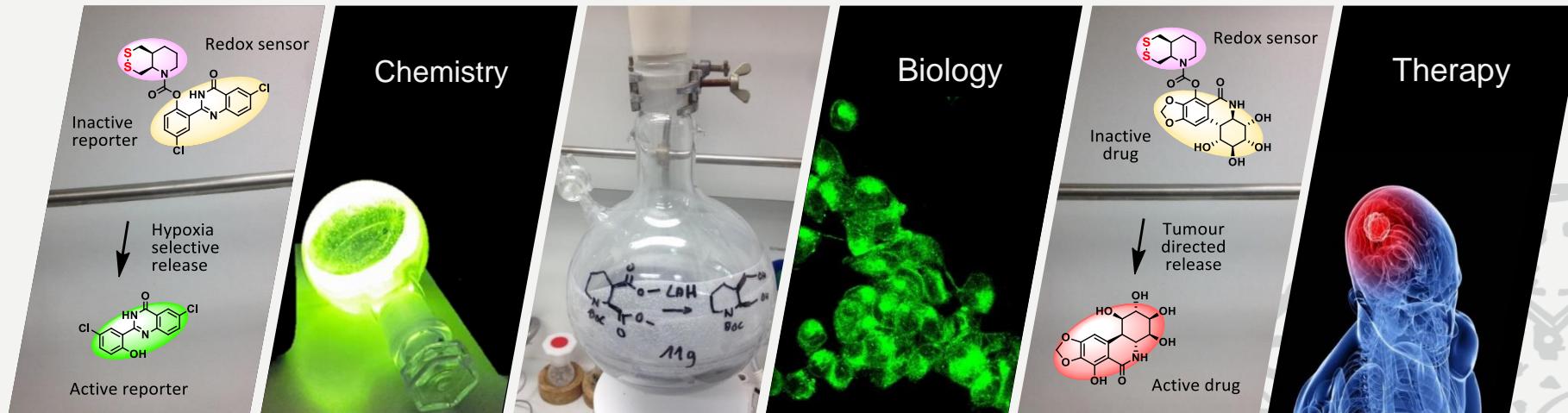
# Expedient Synthesis of Complex Molecules



# Photoswitchable TRPC Channel Probes



# interfacing organic chemistry with biochemistry and biology

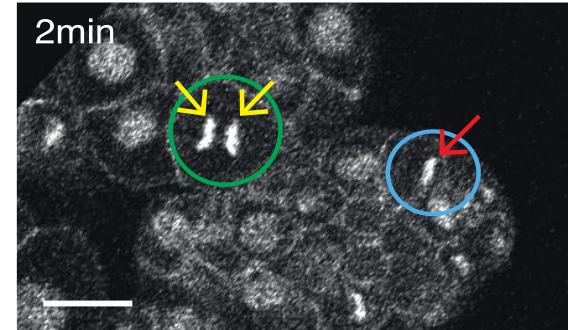
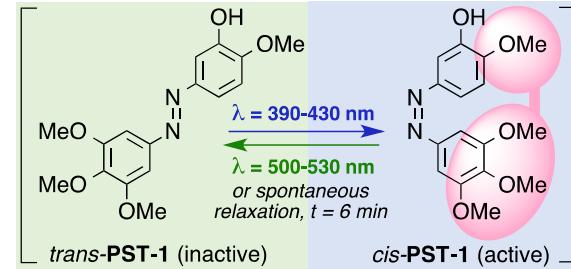
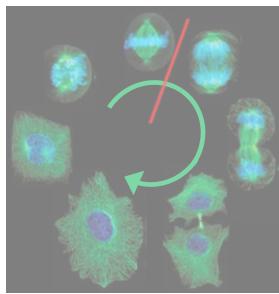
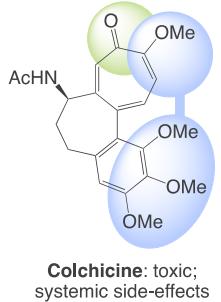


Thorn-Seshold Research Group  
Pharmaceutical Chemistry

# 1. using chemistry & light to control biology

## *med chem + photochemistry for cell biology*

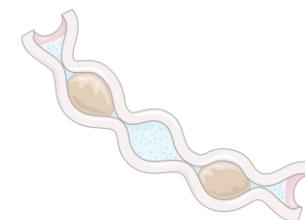
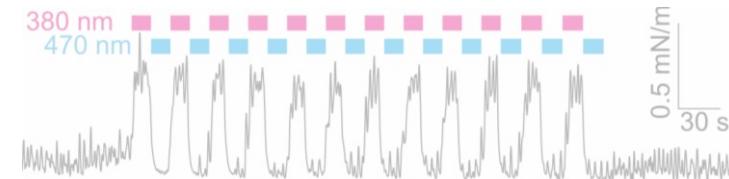
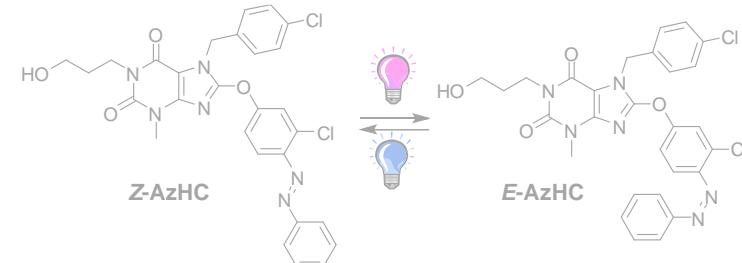
cell division & migration (microtubules)



***light-controlled antimitotics***

*Cell* 2015, [doi.org/f7kcp](https://doi.org/f7kcp); *Science* 2017, [doi.org/gbwmgk](https://doi.org/gbwmgk);  
*Angewandte* 2021, [doi.org/g94z](https://doi.org/g94z)

peristalsis (TRPC4 ion channel)



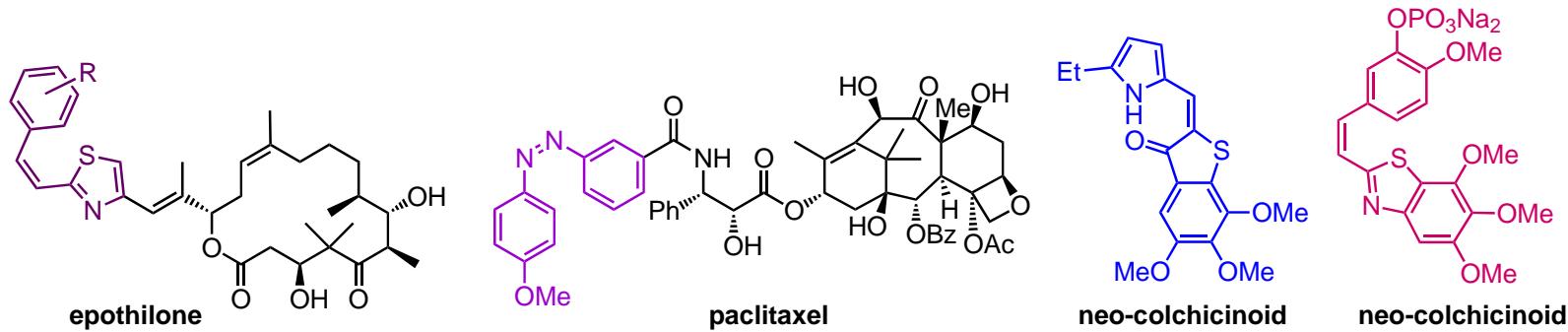
light-controlled intestine contractility:  
Michael Schaefer, Leipzig; *Angewandte* 2022  
([doi.org/h2wb](https://doi.org/h2wb)) & new studies in prep (2024)

organic chemistry Praktika & PhD available

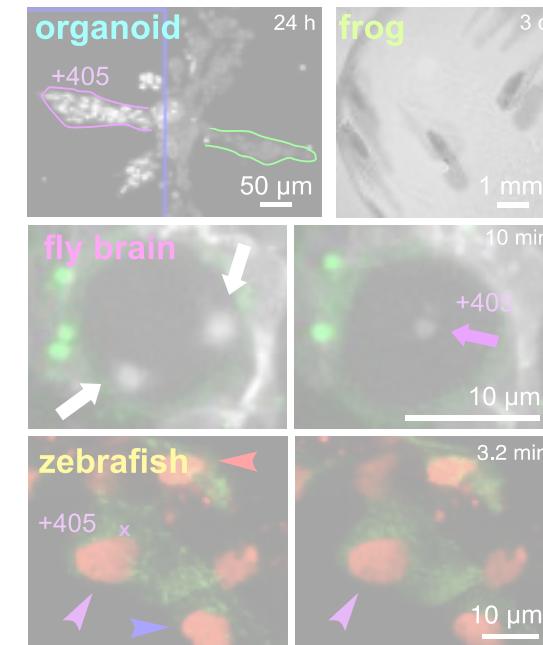
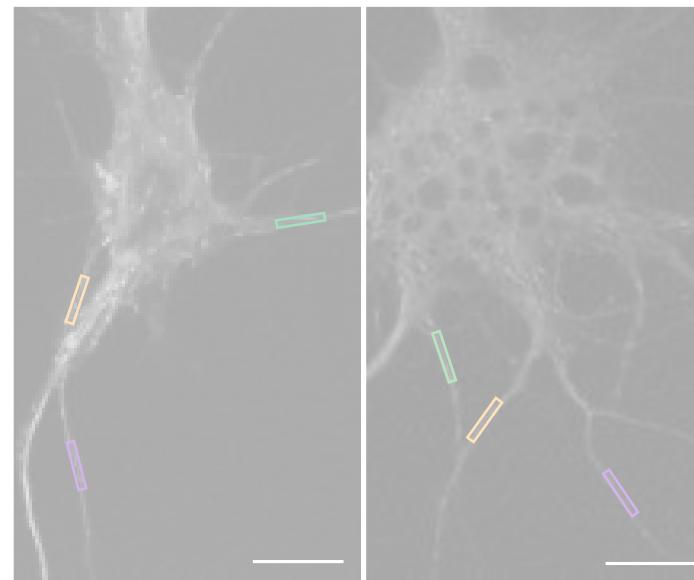
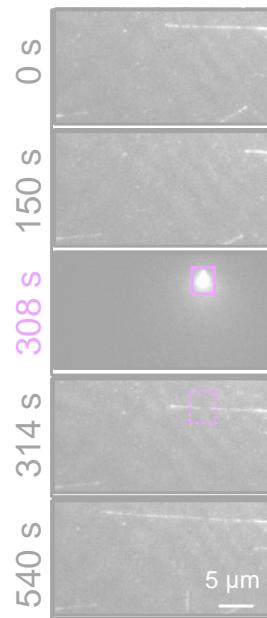
<https://thorneshold.cup.uni-muenchen.de/research/tubulin/>

# 1. using chemistry & light to control biology

*med chem + photochemistry for cell biology*



each with  
its own  
biology

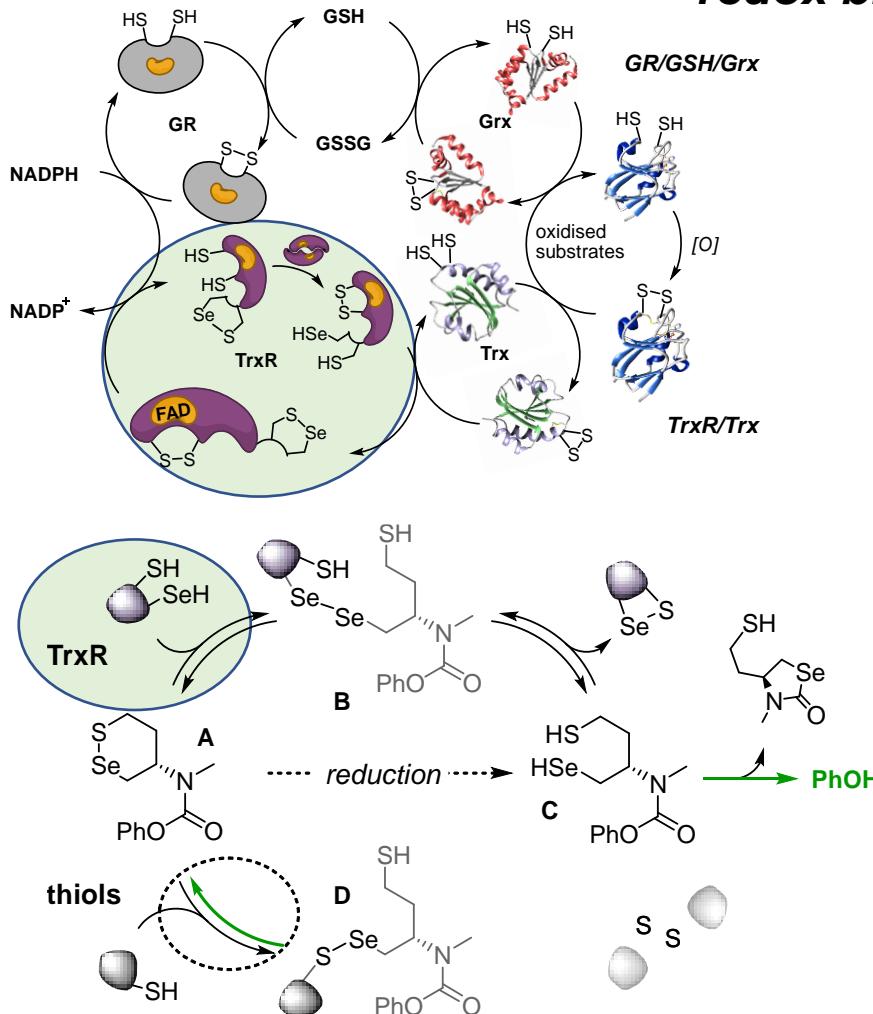


organic chemistry Praktika & PhD available

<https://thorneshold.cup.uni-muenchen.de/research/tubulin/>

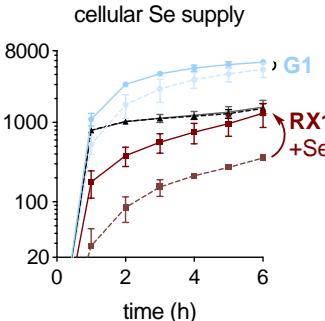
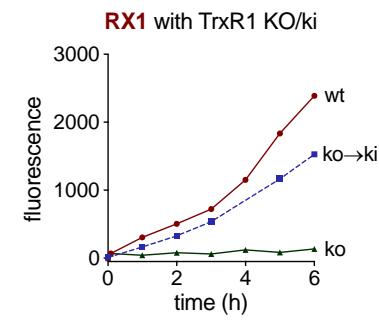
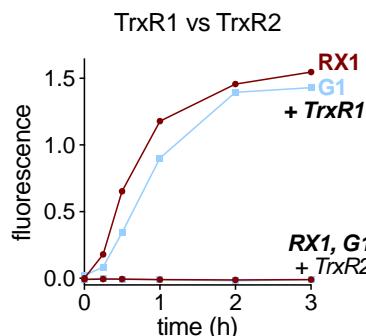
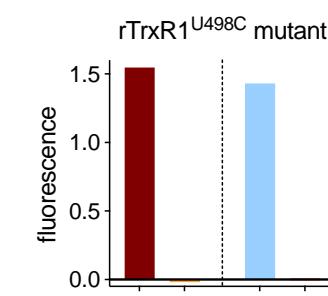
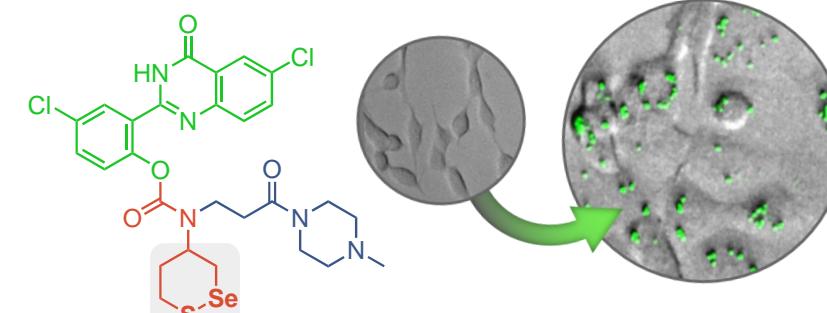
## 2. chemical probes to light up biology

### redox biochemistry



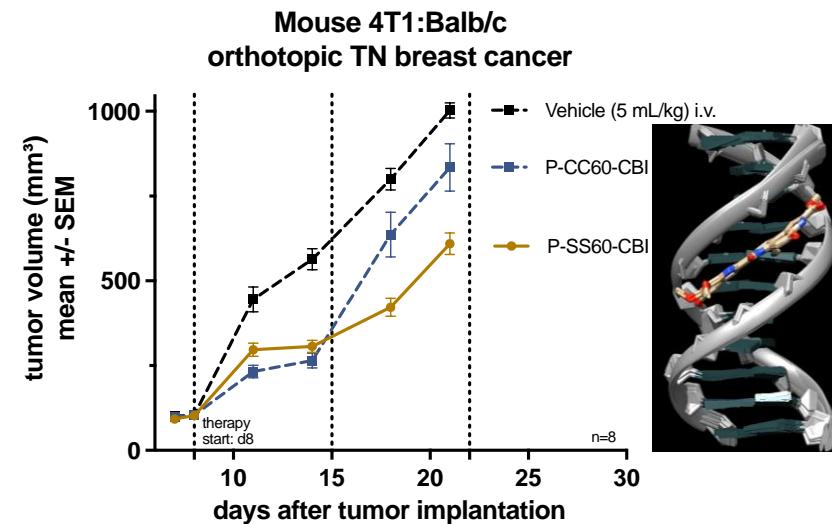
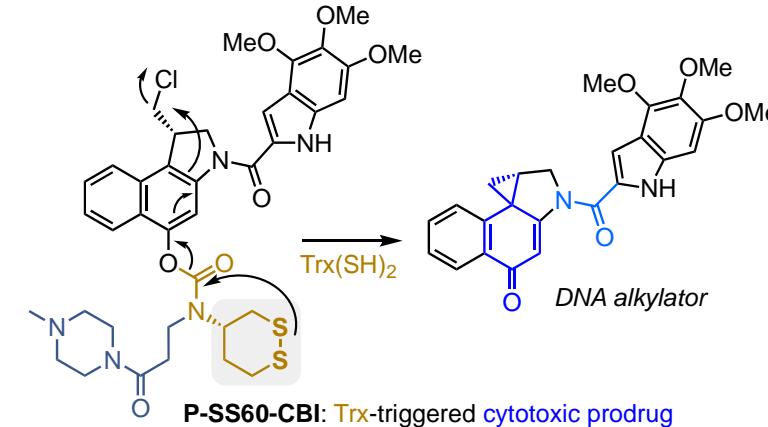
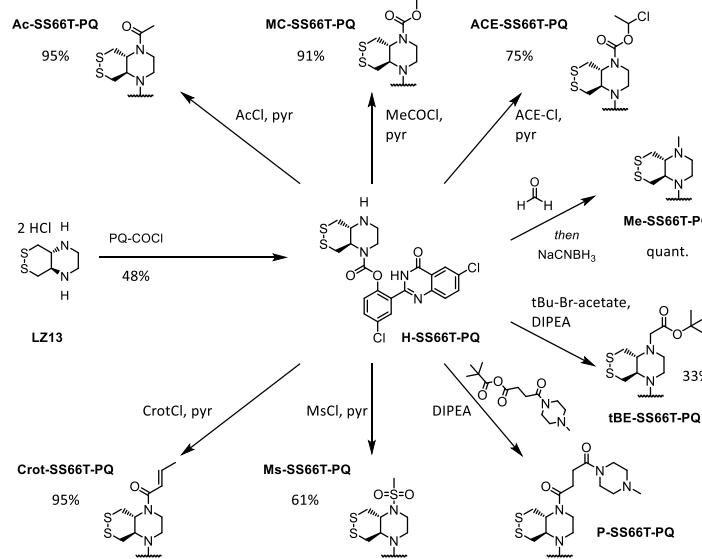
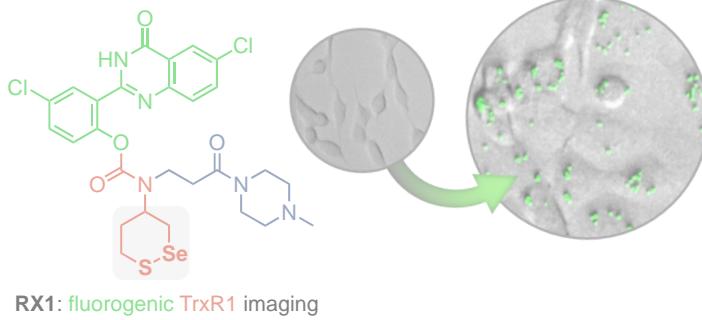
biochemistry Praktika & PhD available

<https://thorneshold.cup.uni-muenchen.de/research/redox/>



## 2. chemical prodrugs to treat pathology

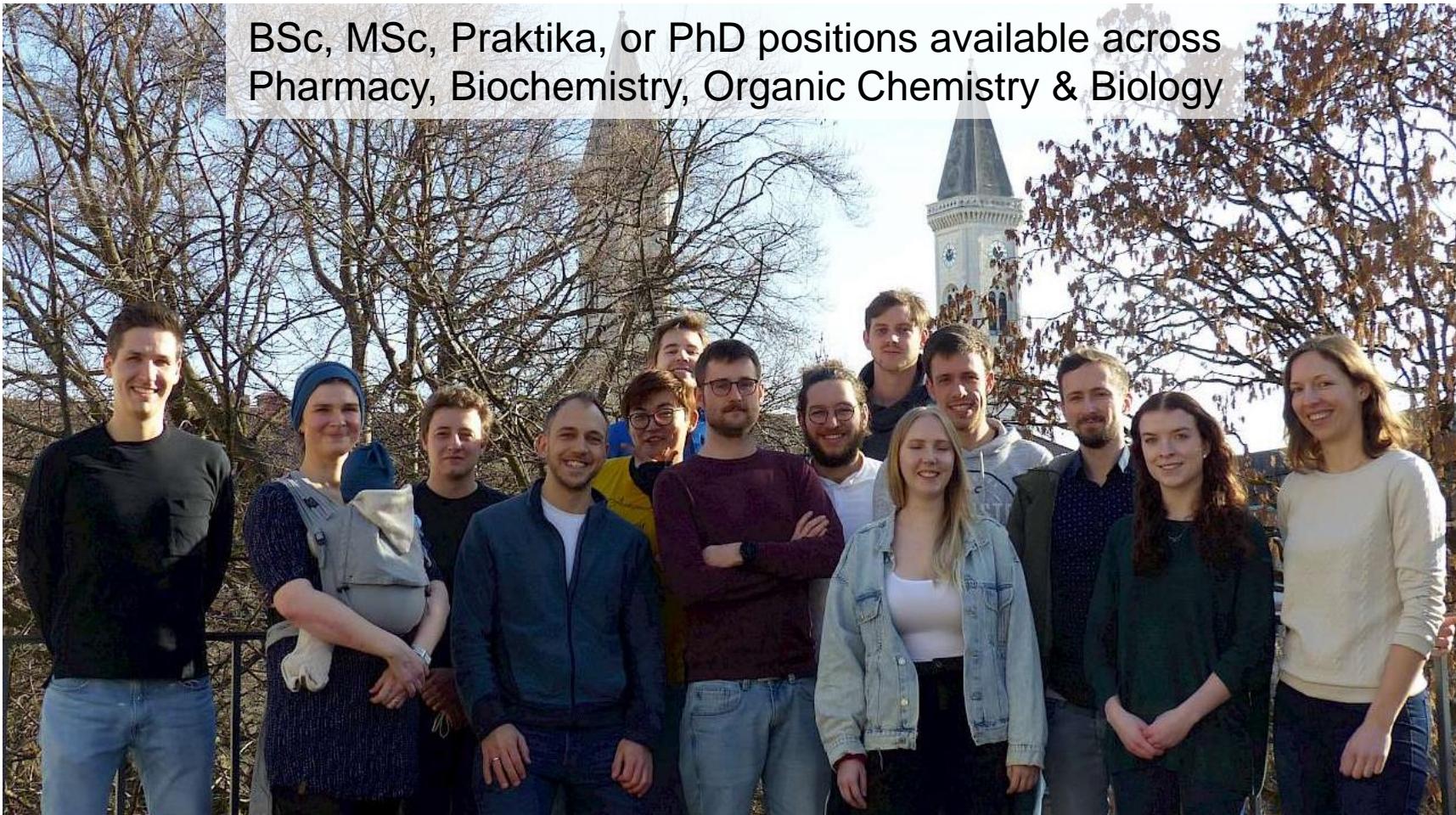
### *redox biology*



biochemistry Praktika & PhD available

<https://thorneshold.cup.uni-muenchen.de/research/redox/>

# join us @ the Thorn-Seshold group!



BSc, MSc, Praktika, or PhD positions available across  
Pharmacy, Biochemistry, Organic Chemistry & Biology



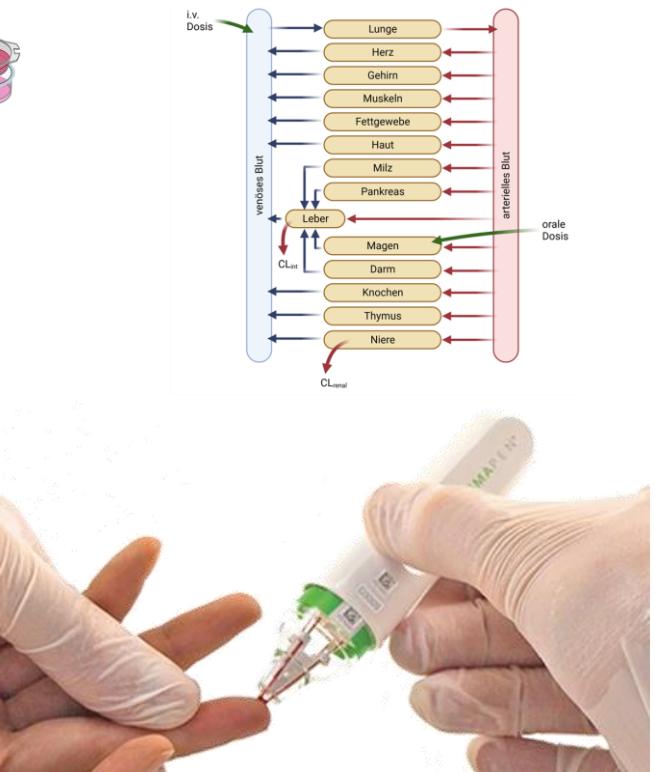
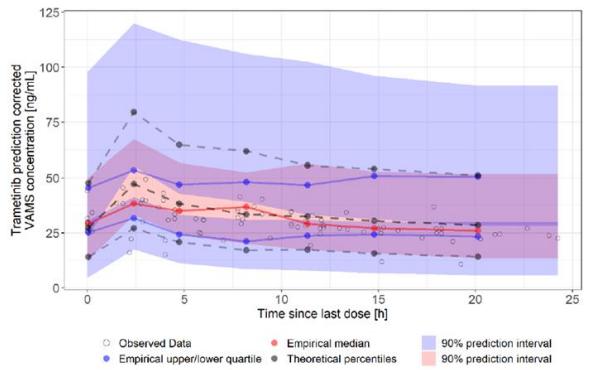
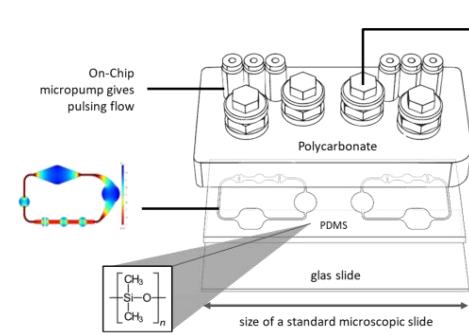
group meets Tuesdays 0915-1045

<https://thornseshold.cup.uni-muenchen.de/research>

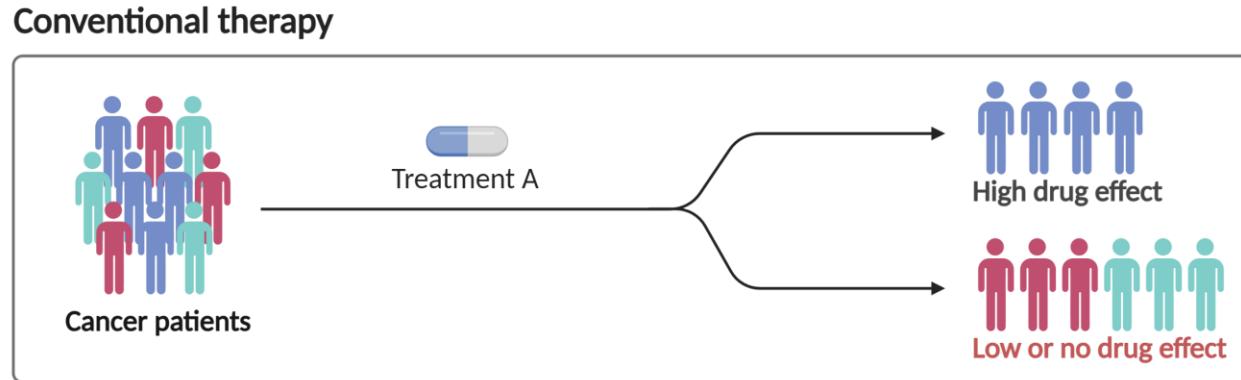
@ThornSesholdLab

# 1. One-Dose-fits-all?

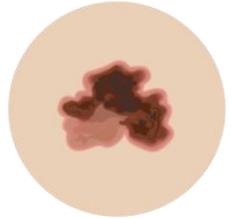
## Microsampling and Models for the win!



# The setting: Oral targeted anti-cancer therapy is a case of precision medicine



Example



Melanoma

Non mutated

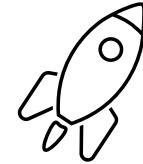
vs.

BRAF<sup>V600E</sup> mutated

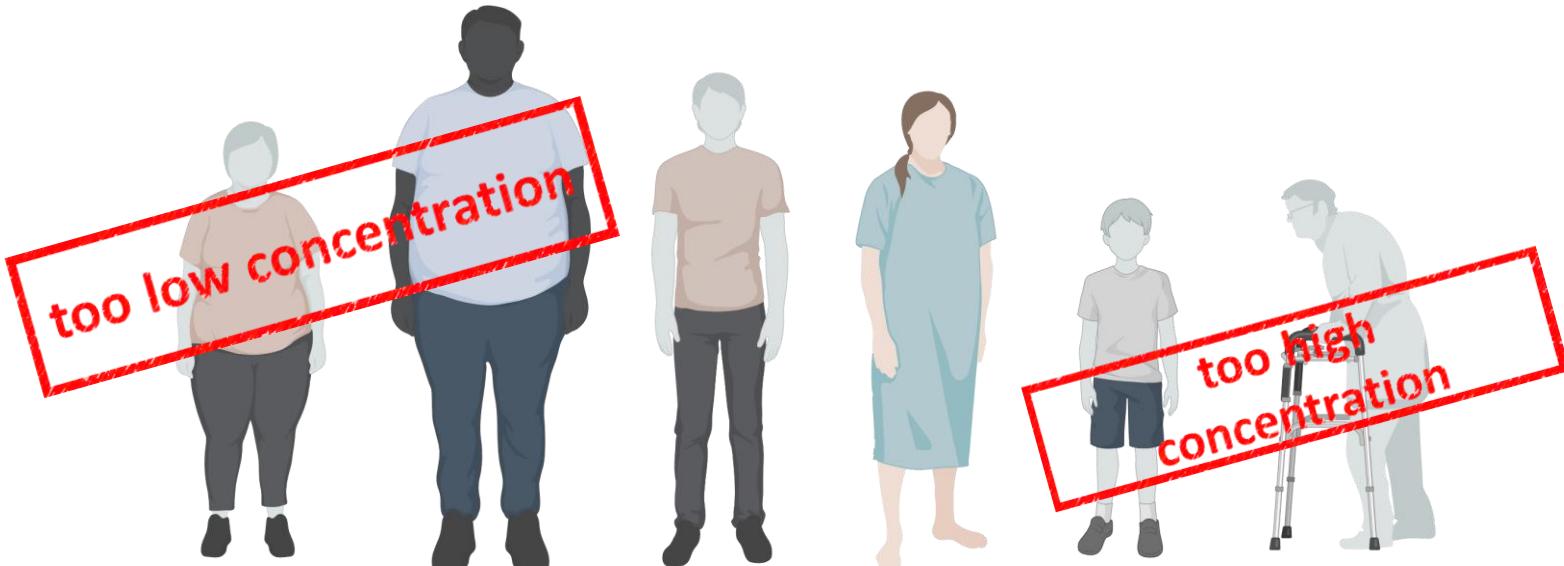
**The setting:**

**All oral targeted antineoplastic drugs are dosed by the one-size-fits-all approach**

But it's no rocket science ...

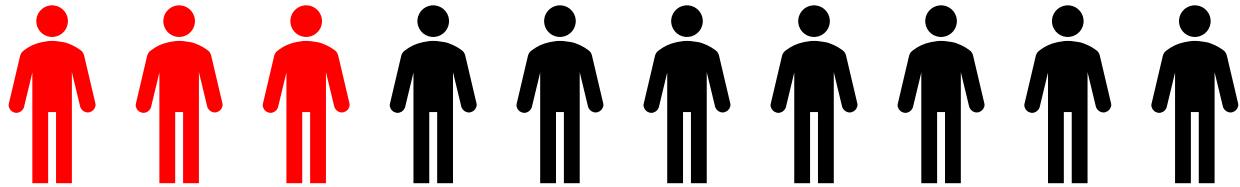


... what happens if these patients receive the same dose of a given drug?



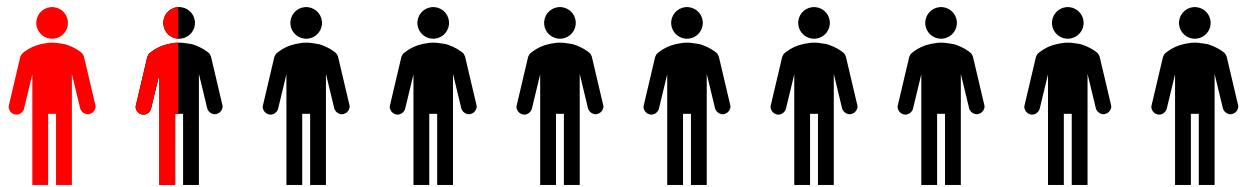
## The Setting:

All oral targeted antineoplastic drugs are dosed by the one-size-fits-all approach



30 % of Patients receive a too low dose

→ risk of suboptimal effectiveness



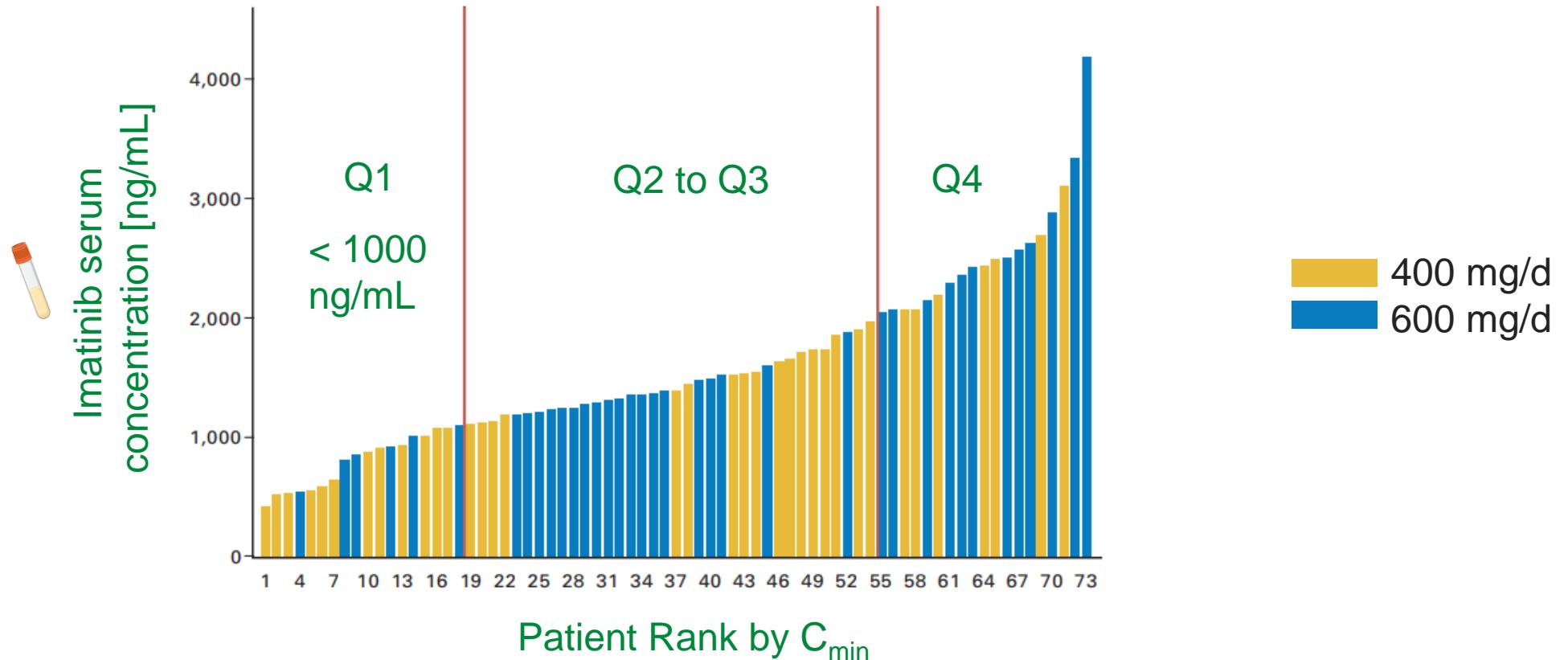
15 % of Patients receive a too high dose

→ risk of increased toxicity

## The Problem:

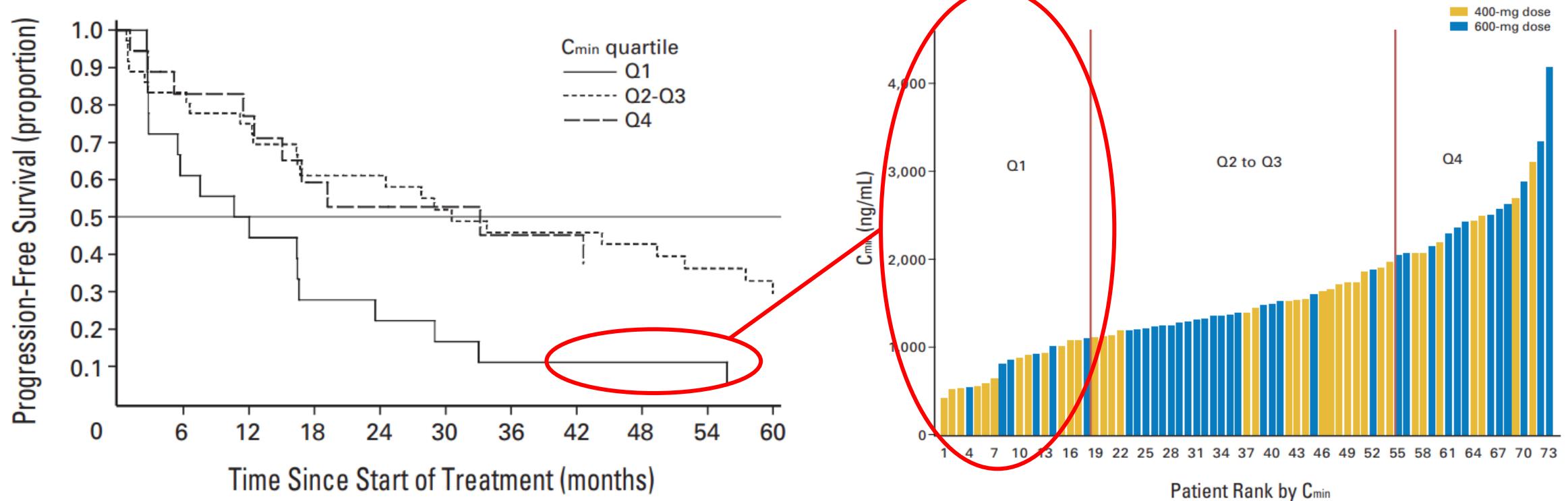
### OAD Example: Imatinib in chronic myelogenous leukemia (CML) PK/PD variability

one-dose-fits-all?



## The Problem:

Imatinib concentration below 1000 ng/mL is a risk factor for disease progression

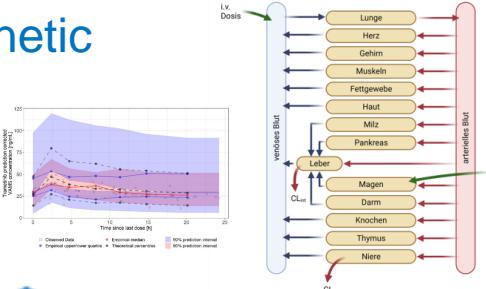


# The solution:

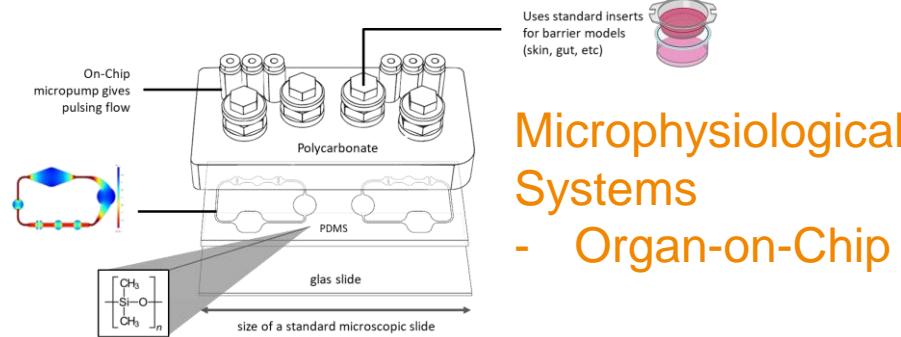
## Precision dosing für orale Tumortherapien / Methoden und Ziele unserer Forschung

### Pharmacokinetic Models

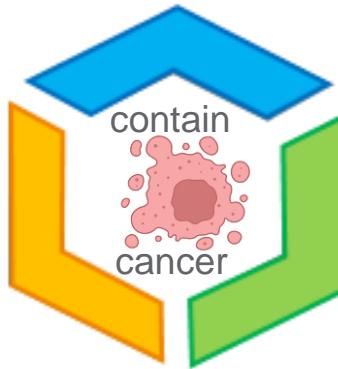
- PBPK
- popPK



Interpretation of measured values  
Optimising the dose  
Recognising risk factors



### Microphysiological Systems - Organ-on-Chip



Understanding PK/PD  
Target concentrations ?  
Relationship PK/Tox?

### Bioanalytical Methods

- Microsampling
- LC-MS/MS

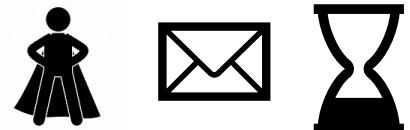


Care for outpatients  
Increase sample stability  
Generate more data

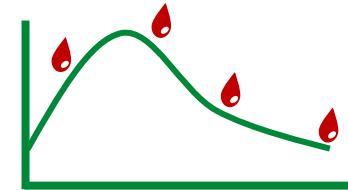
## The solution:

### Microsampling offers many advantages for monitoring outpatient therapies

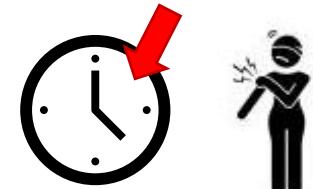
Patients can collect samples themselves at-home and sent it to the lab by mail



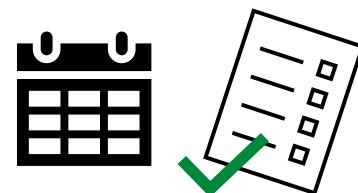
Patients can collect multiple samples



Samples can be collected on an ad hoc basis (e.g. occurrence of adverse events)

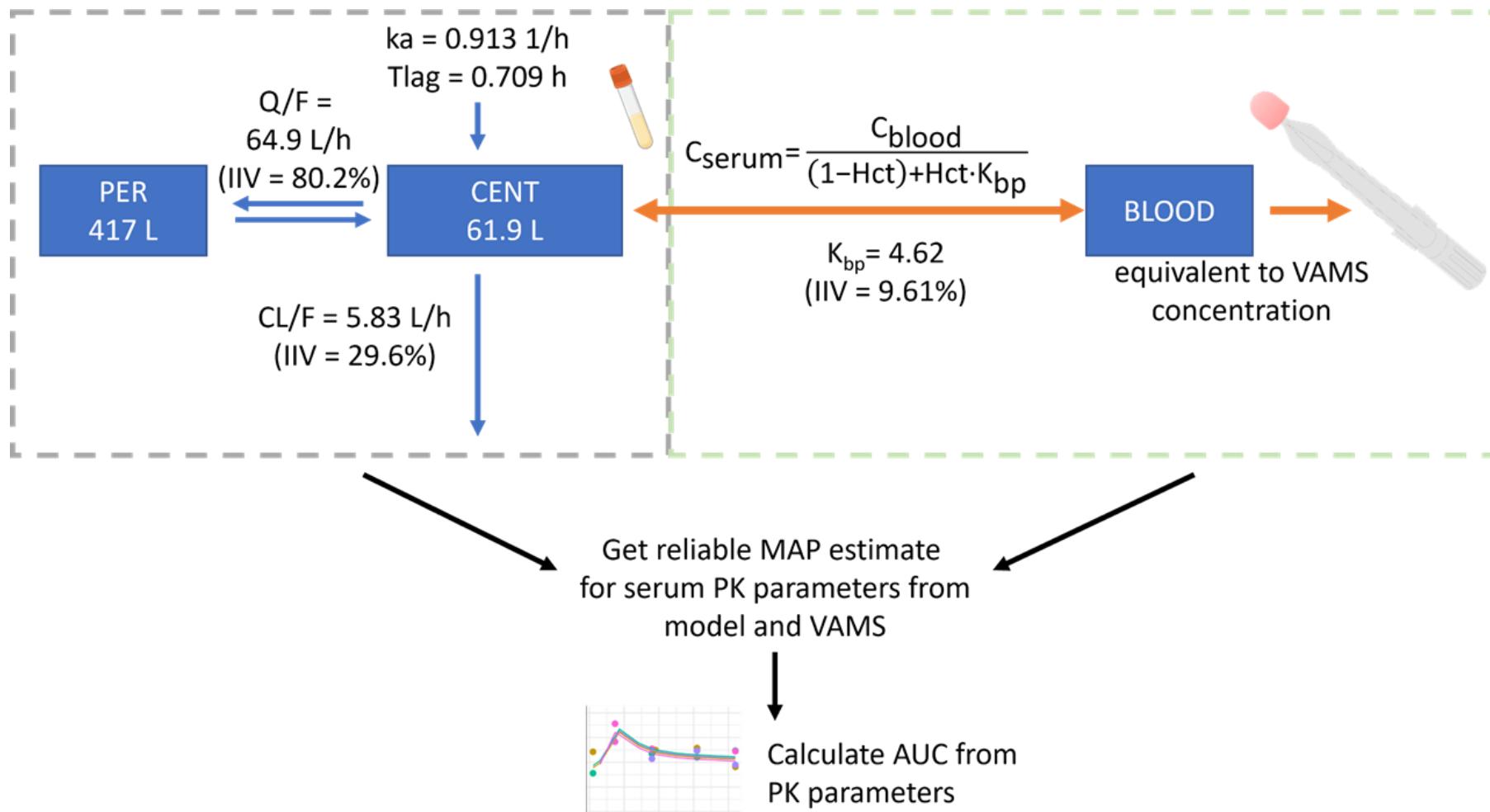


Results will be available until next doctors appointment

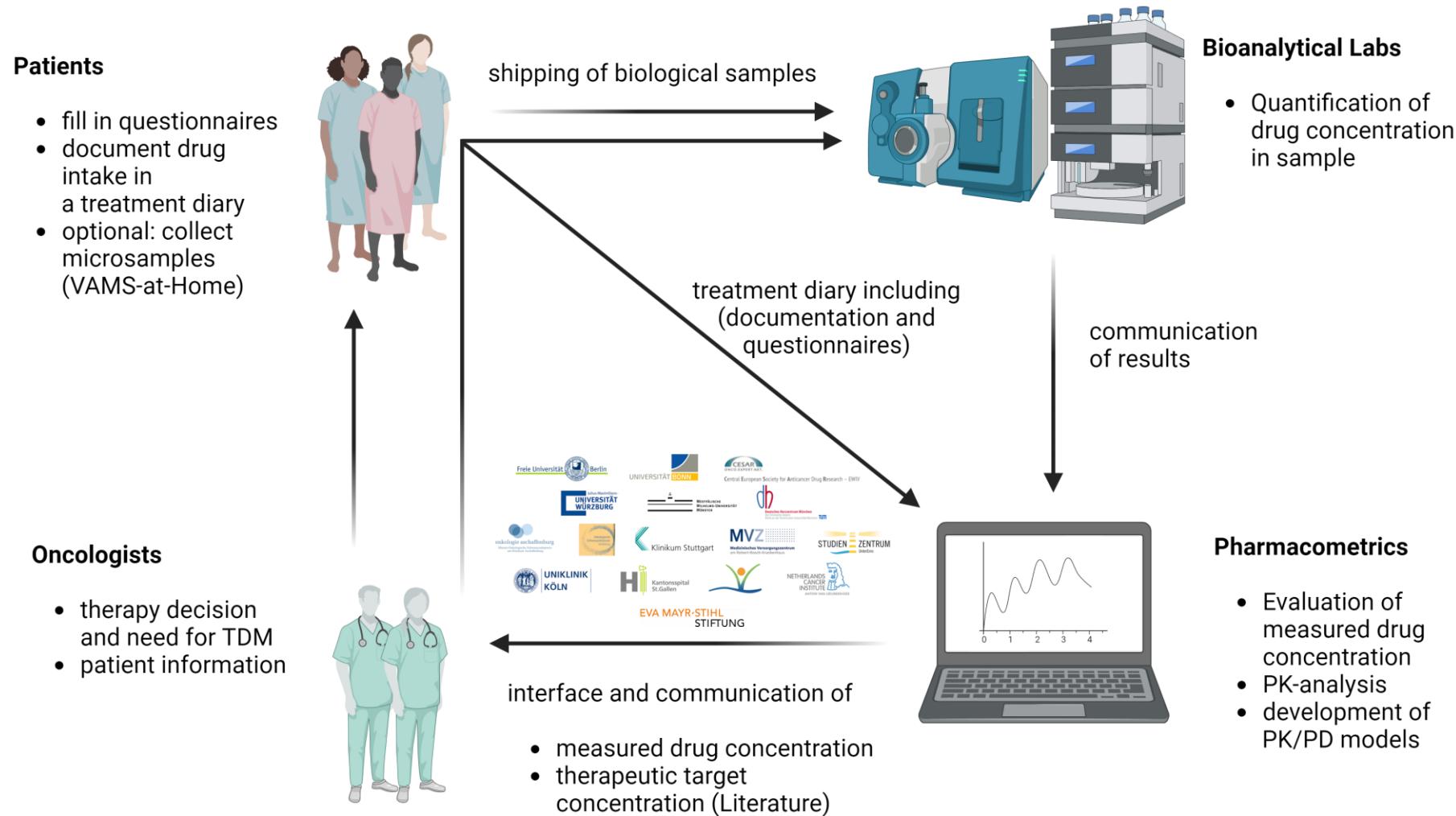


## The solution:

### Model informed precision dosing supported by microsampling



# Bigger goals: build a framework to foster TDM of OAD





**Vielen Dank für Ihre Aufmerksamkeit!**

**Zeit zum Austausch mit den Lehrenden und  
Forschenden**

