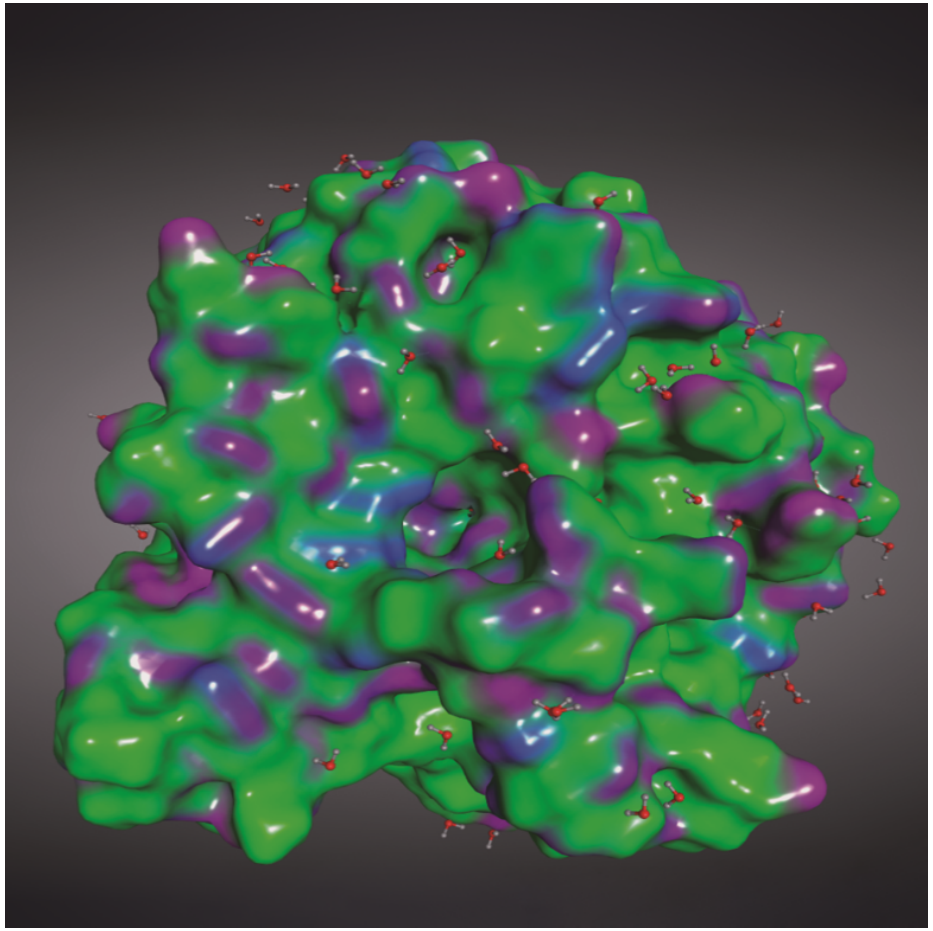
**Meeting KDDF – IZI-MWT**
**July 9th 2019**

# Fraunhofer
## IZI

**Department of Drug Design and Target Validation**

# Department of Drug Design and Target Validation (IZI-MWT)

- Discovery and development of small molecules and biologics for **neurodegenerative (AD) and metabolic disorders**
- Target validation (*in vitro* and *in vivo*), **cell and animal models**
- **Drug Design** (*in silico*) and **Computational Chemistry**
- **Assay development** (biomarkers)
- (GLP-) Analytics for pre-clinical and clinical studies of small molecules



# Who We Are

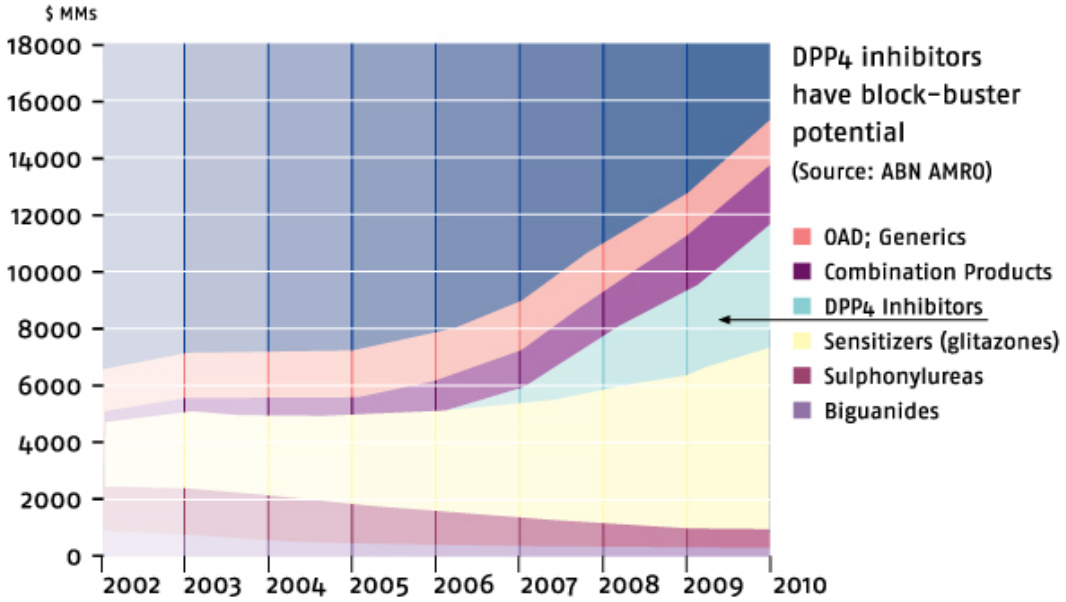
- Deciphering new treatment paradigms, delineation therapeutic molecules and validation of therapeutic strategies in preclinical studies
- Main field of activity: Pathologic post-translational modifications, misfolding of proteins and formation of pathological aggregates, target validation
- Development and testing of small molecules as well as biological agents (biologicals)
- Molecule design for diagnostic and therapeutic applications
- Generation of pharmacologically relevant *in vitro* and *in vivo* models

- **Department = 3 UNITS**

1. Unit **Drug Design and Analytical Chemistry** – Medicinal chemistry, pre-clinical and clinical analytics
2. Unit **Protein and Drug Biochemistry** – Enzyme characterization and protein drugs
3. Unit **Molecular Biotechnology** – *in vitro* / *in vivo* pharmacology

# Where we are coming from:
## A new Mechanism of Action – Fighting Type 2 Diabetes

- Improving glucose control by inhibition of dipeptidyl peptidase 4 (DP4)
- Since 2007 on the market
- DP4-inhibitors: 9.5 billion US-$ turnover in 2012

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# Where we are coming from:
## A new Mechanism of Action – Fighting Alzheimer's Disease

- Pivotal toxicity of pyroglutamate (pGlu)-modified Abeta peptides in Alzheimer's disease
- Concept currently evaluated in phase II clinical studies
- Enabled IPO of Probiodrug AG at EURONEXT on October 27, 2014

LETTERS

nature medicine

Glutaminyl cyclase inhibition attenuates pyroglutamate Aβ and Alzheimer's disease–like pathology

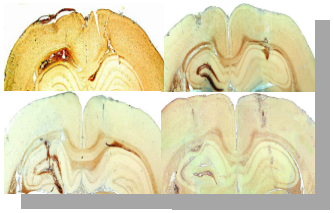
Stephan Schilling[1], Ulrike Zeitschel[2], Torsten Hoffmann[1], Ulrich Heiser[1], Mike Francke[2], Astrid Kehlen[1], Max Holzer[2], Birgit Hutter-Paier[3], Manuela Prokesch[3], Manfred Windisch[3], Wolfgang Jagla[4], Dagmar Schlenzig[1], Christiane Lindner[5], Thomas Rudolph[5], Gunter Reuter[5], Holger Cynis[1], Dirk Montag[6], Hans-Ulrich Demuth[1,4] & Steffen Rossner[2]

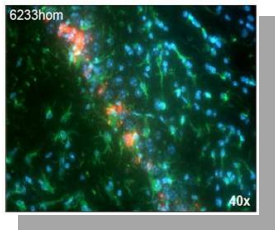
LETTER

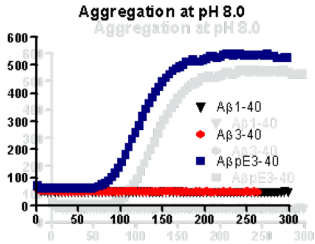
doi:10.1038/nature11060

Prion-like behaviour and tau-dependent cytotoxicity of pyroglutamylated amyloid-β

Justin M. Nussbaum[1]*, Stephan Schilling[2]*, Holger Cynis[2], Antonia Silva[1], Eric Swanson[1], Tanaporn Wangsanut[1], Kaycie Tayler[3], Brian Wiltgen[3], Asa Hatami[4], Raik Rönicke[5], Klaus Reymann[5], Birgit Hutter-Paier[6], Anca Alexandru[7], Wolfgang Jagla[7], Sigrid Graubner[7], Charles G. Glabe[4], Hans-Ulrich Demuth[2,7] & George S. Bloom[1,8]





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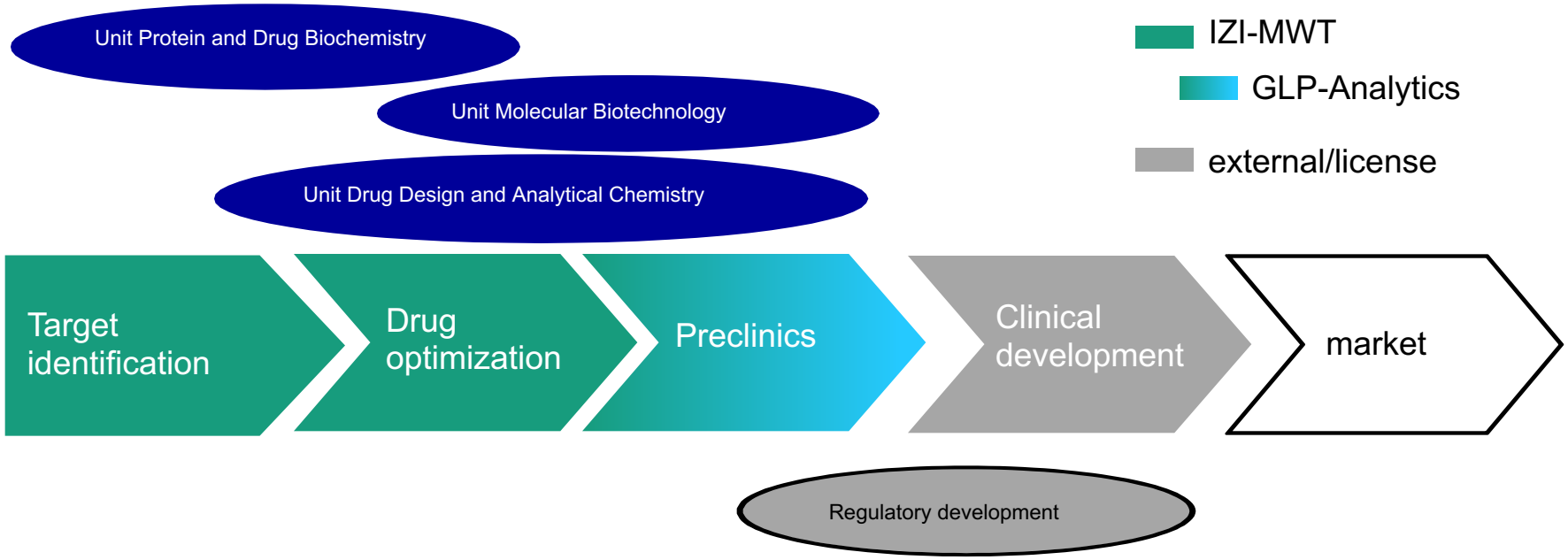
# Unit Allocation within the Drug Discovery Process




- medical use patents
- composition of matter patents

# Drug Design and Analytical Chemistry Unit

**Computer chemistry and bioinformatics**

- Exact and rapid semi-empirical and quantum chemical methods
- MD simulations
- X-ray crystallography → quality assurance and refinement of models
- Molecular docking, incl. HTvS campaigns
- Department-wide central knowledge management system (ELN und DMS)

**Medicinal and peptide chemistry**

- Synthesis, purification and analysis of small molecules (without restrictions, multicomponent reactions, enantioselective synthesis)
- Synthesis, purification and analysis of peptides (fully automated, wide range of labels)
- Aided by peptide synthesizers, microwaves, »on-site« mass spectrometry

## Small Molecules

**Bioanalytics**

- Binding analysis of ligand-protein and protein-protein interaction by biophysical methods (SPR, ITC)
- ELISA-based assay development for biomarker analysis
- Qualification of methods for preclinical and clinical analytics

**Analytical Development**

- Development of methodology for preclinical and clinical trials (LC-UV/VIS and LC-MS coupling)
- Metabolism studies using LC-MS (highly sensitive MS)
- MALDI-TOF/TOF for peptide analytics in biological and non-biological matrices
- Space-resolved MALDI-TOF/TOF for analysis of tissue slices (MALDI-Imaging)

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# Protein and Drug Biochemistry Unit

**Isolation and characterization of proteins as drugs or drug targets**

## Areas of competence

- Isolation and characterization of proteins for in vitro and in vivo analysis
- Development and application of enzyme assays for drug characterization *in vitro*
- Isolation, characterization and humanization of antibodies
- Protein drugs

## Methods

- Molecular cloning of target gene sequences
- Heterologous expression of proteins in *E. coli*, yeast, insect and mammalian cells
- Column chromatographic purification of proteins
- Analysis of enzyme structure and function *in vitro* (spectroscopy and X-ray structure analysis, enzyme assays)
- Structure-based optimization of antibodies (protein engineering)

## Scientific Focus

- Characterization of small molecule inhibitors for application as novel drugs in fibrosis and kidney protection
- Scientific projects with industrial partners (assays and inhibitor characterization, e.g. probiodrug AG)
- Development of antibodies against modified target proteins, main focus Alzheimer's Disease

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# Molecular Biotechnology Unit

**Target identification and target validation for human pathologies**

**In vitro pharmacology**

- Characterization of drugs with regard to toxicity and transport and/or efficacy in cellular model

**In vivo pharmacology**

- Establishing and phenotyping of animal models for pharmacological drug testing
- ADME screening in vivo

## Methods

- Mammalian cell culture (S2)
- Primary cell culture
- Organotypic slice cultures
- Cell-biological analyses (FACS, RT-PCR, WB)

- Development and phenotyping of transgenic animal models
- Animal pharmacology
- Stereotactic CNS injections
- Immunocytochemistry and histochemistry

## Technologies

**Cell culture**

- > 100 permanent cell lines and primary cells
- In vitro-assays for substance characterization
- S2 laboratory

**Animal facility**

- Microsurgery and macrosurgery
- Comprehensive phenotyping of animal models, incl. a wide range of cognitive tests (Y maze, water maze, contextual fear conditioning, open field, rota rod, tail suspension etc.)

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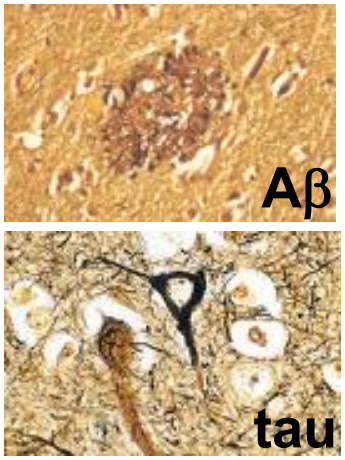
# Development of post-translational modification-specific antibodies against Alzheimer‘s disease

# Alzheimer‘s Disease (AD)

- Amyloidoses : deposition of amyloid peptides Aβ and tau
  - Aβ - main component of plaques
  - Tau - intracellular neurofibrillary tangles (from P-tau)




- Signs: progressive cognitive decline, disorientation
- Diagnosis: MRT, PET, cognitive tests (MMSE, ADAS-Cog)

- Affected population

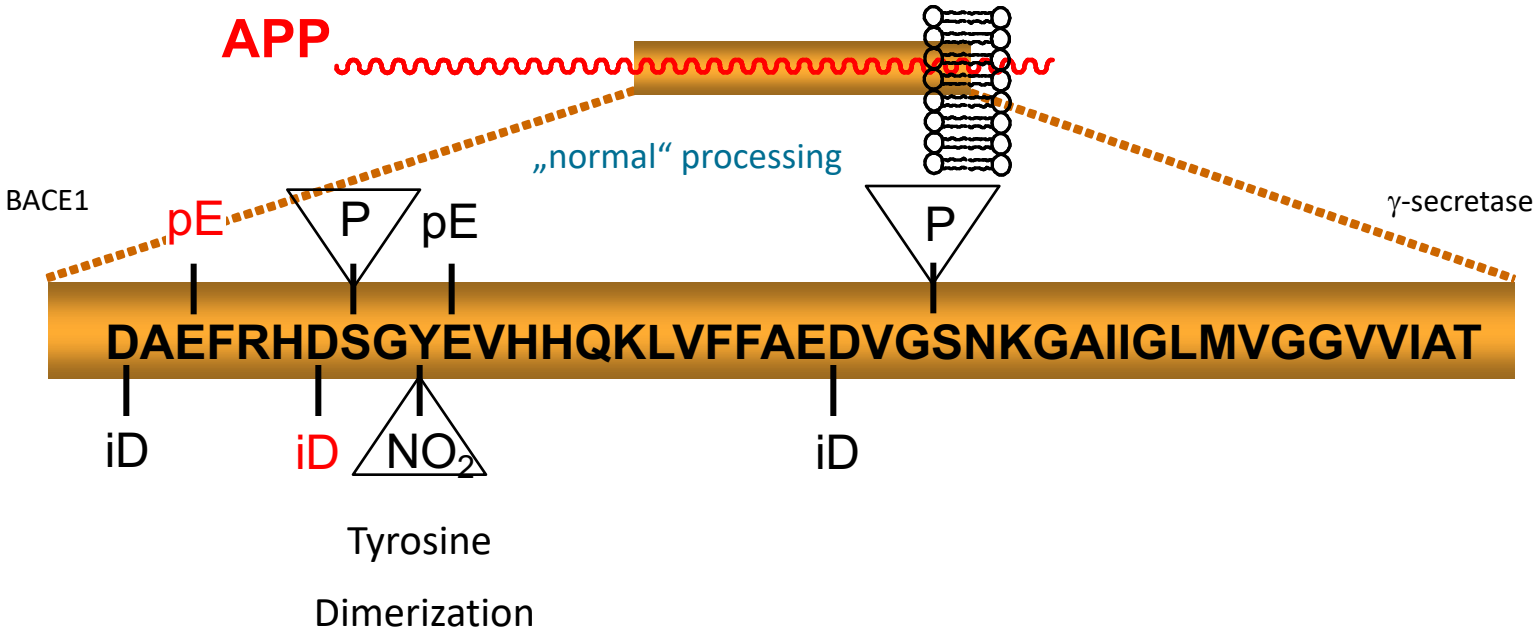
**Worldwide**

| 2010 | 2013 | 2050 (estimated) |
|---|---|---|
| 36 million | 44 million (+22%) | 132 million (+300%) |

Source: Alzheimer´s Disease International

# Protein Structure Modifications of Aβ: Attractive Anchor points for Drug Development

- specific for the disease state
- no physiological function of „abnormal“ products
- generation of neoepitopes – specific targeting with low risk of side effects



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# Why targeting a modified form of Aβ?

- Modified (aged) Aβ only prominent in brain, therefore
  - No capture of antibody in periphery
  - No increase of Aβ concentration in plasma upon treatment

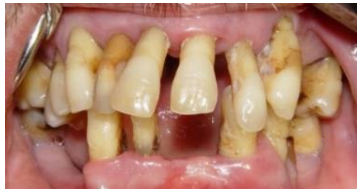
- Lower epitope density of particularly modified species
  - Better distribution of the antibody within the brain tissue
  - Lower reactivity within CAA, thus lower risk of ARIAs

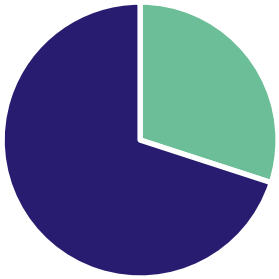
**Antibodies targeting modified Aβ display features for: lower dosing and lower risk of side effects**

# Development of selective and local acting anti-bacterials for the curation of Periodontitis

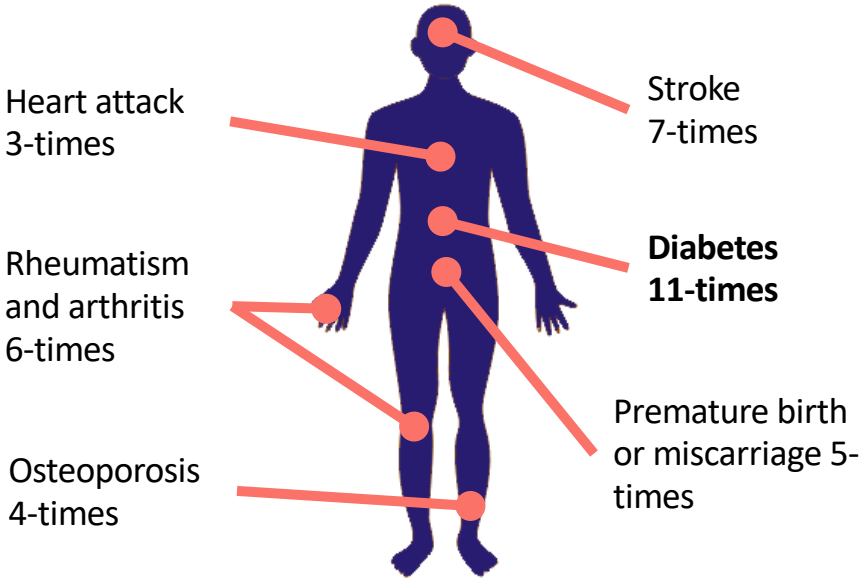
# **Periodontitis –**
# The Most Abundant and Neglected Infectious Disease Worldwide

Inflammatory process caused by
**specific bacteria**





Affects nearly **30%** of the population worldwide



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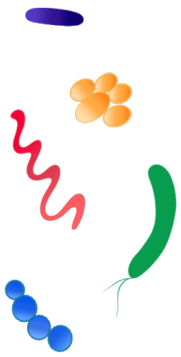
# Pathogenesis

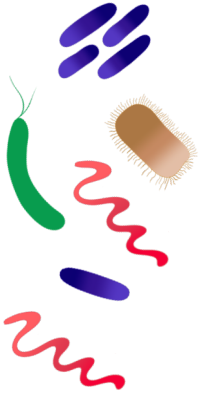


Symbiotic microbiota

- Immunodeficiency
- Obesity
- Smoking
- Aging

➢ Key stone pathogens

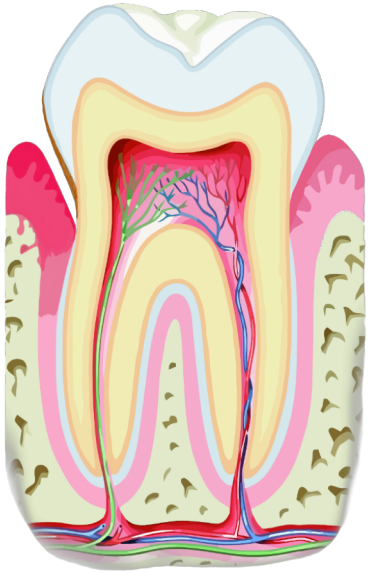
Homeostasis breakdown



Dysbiotic microbiota

Nutrients

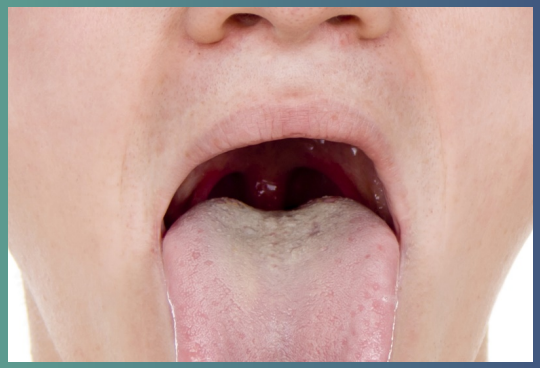
Inflammatory tissue breakdown



Driven by *Porphyromonas gingivalis* –
The „Key-Stone-Pathogen“ in Periodontitis

# Current therapy

- Manual debridement of the biofilm and daily disinfection (SRP)
- Systemic application of broad spectra antibiotics

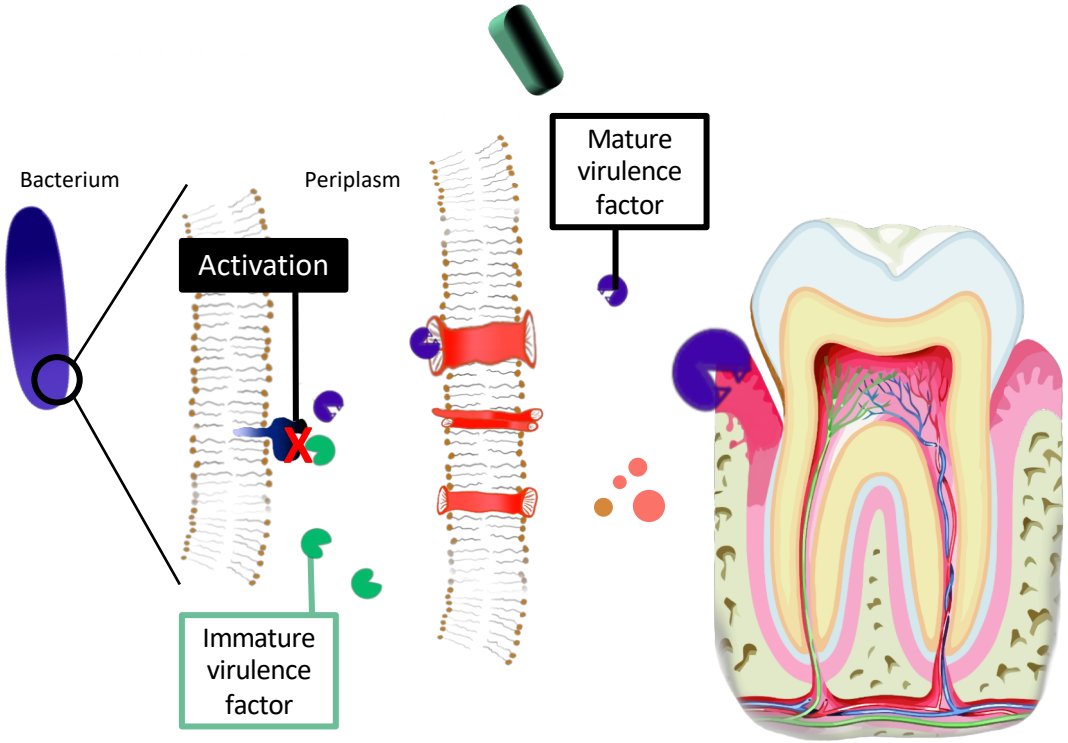


- Development of resistence and disruption of intestinal microbiom and oral flora
- ➢ Loss of metabolic and immunologic support

# Our Approach
## Target

Novel and Local **Inhibitors** for **Selective Targeting** of Periodontitis Causing **Pathogens**

- Inactivation of Gingipain Maturation Process
- **2 patent applications filed**




Physiological biofilm stays intact



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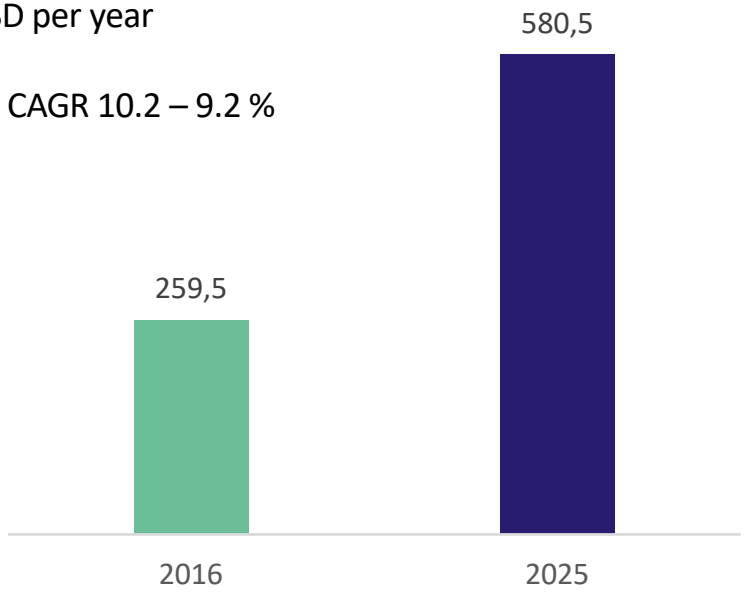
# Market
## Volume and Drivers

Current Periodontitis drug market valued 347 million USD per year

- CAGR 10.2 – 9.2 %




- Predisponed Diabetes Patients **2X**
- Local therapeutic solutions on the rise
- Expanding Asia-Pacific market due to better access to health care infrastructure
  - High patient numbers
  - Rising expenditures
- Main Driver: Increase of **antibiotic resistance** and **aging population**



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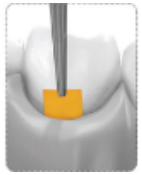
# **Market**
## Competitors

### Local Applications





- **Resistance and biofilm disruption still occurring**

### New Target

- Development of a vaccine against periodontitis – by targeting *P.gingivalis* as a the key pathogen
- Molecular Target: gingipains
- Were able to show positive results in mice

➢ **External Validation of P. gingivalis**

Expected Superiority in Clinical Benefit



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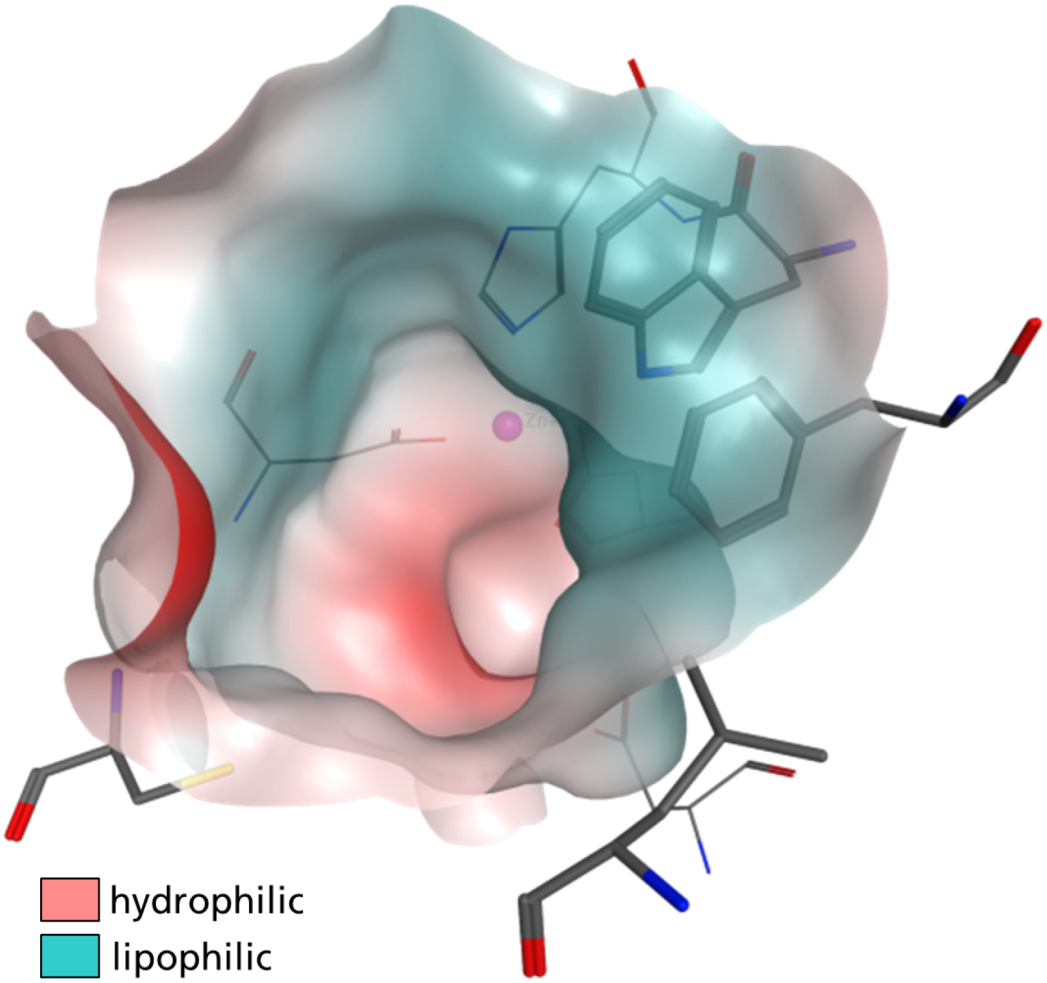
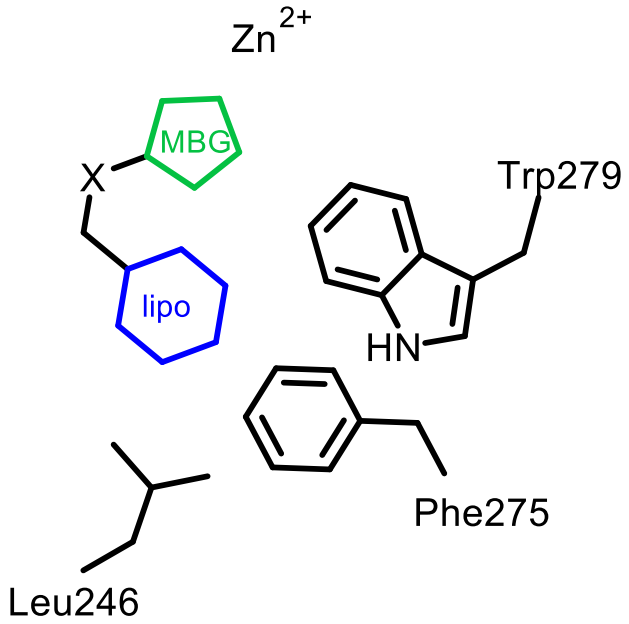
# Inhibitor Design Strategy



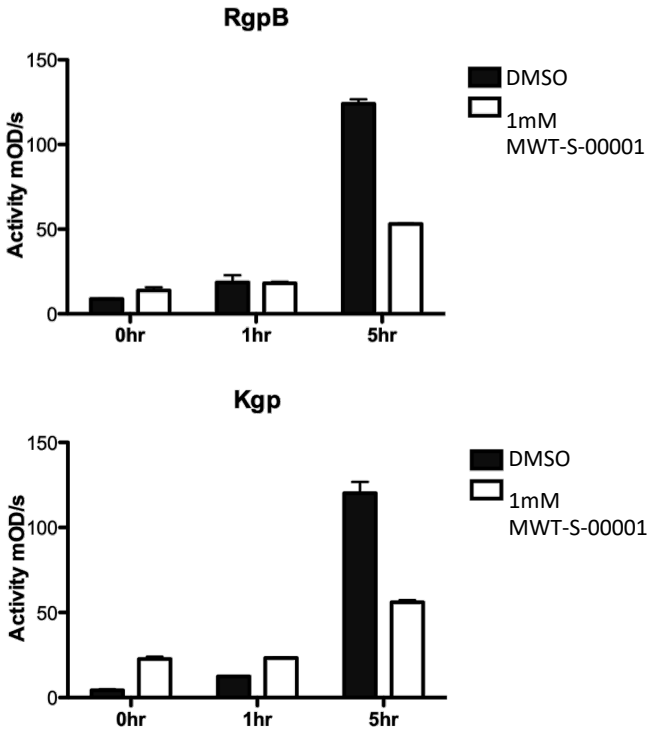




X-Ray unpublished data

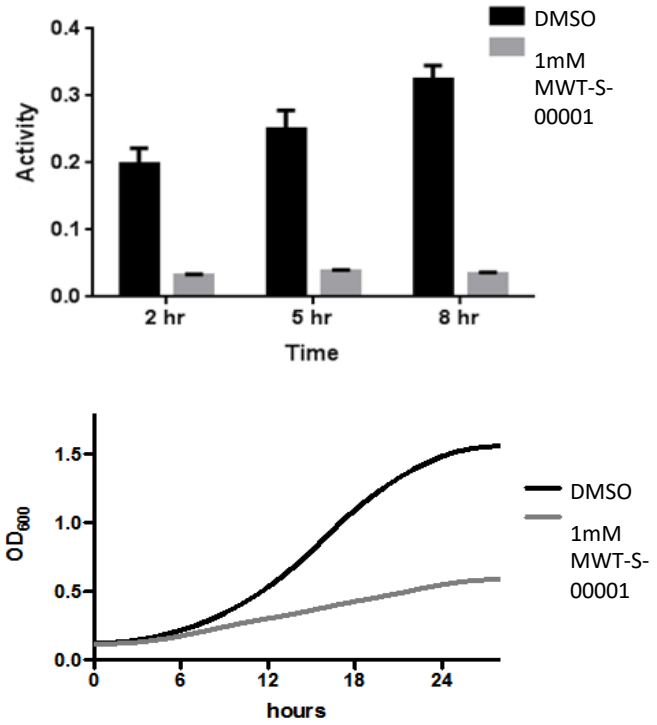
# 1st »Proof of Principle« in Cell Culture

MALOPOLSKA CENTRE OF BIOTECHNOLOGY

»MWT-S-00001«: secretion of gingipains




»MWT-S-00001«: growth inhibition




**Enzyme inhibition leads to diminished secretion of virulence factors and growth inhibition of pathogens → compound reaches bacterial site of action**



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# Hijacking Uptake Mechanisms

zmk bern
Zahnmedizinische Kliniken der Universität Bern

| | | Red Complex | | | | Control | |
|---|---|---|---|---|---|---|---|
| Inhibitor (µM) | $K_i$ (nM) | P.g. ATCC 33277 MIC (µM) | P.g. M5-1-2 MIC (µM) | T.f. ATCC 43037 MIC (µM) | P.i. ATCC 25611 MIC (µM) | S. g. ATCC 10558 MIC(µM) | A.a. ATCC 33384 MIC (µM) |
| CHX (%) | - | ≤0.002 | ≤0.002 | ≤0.002 | ≤0.002 | ≤0.002 | ≤0.002 |
| Doxy (mg/ml) | - | ≤3.13 | ≤3.13 | ≤3.13 | ≤3.13 | ≤3.13 | ≤3.13 |
| Bim-YYY-XXX | 358 | 0.98 | 0.98 | 0.98 | ≤0.49 | >2000 | 1000 |
| Mtz-YYY-XXX | | 10 – 20[Lit] | | | | | |

**Improved activity and selectivity by modifying the uptake with conjugation!**

MIC...minimal inhibitory concentration
CHX...chlorhexidine (antiseptic)
Doxy...doxycycline (antibiotic)
Mtz...metronidazol (conjugated antibiotic)
Bim...benzimidazole (Inhibitor conjugated)



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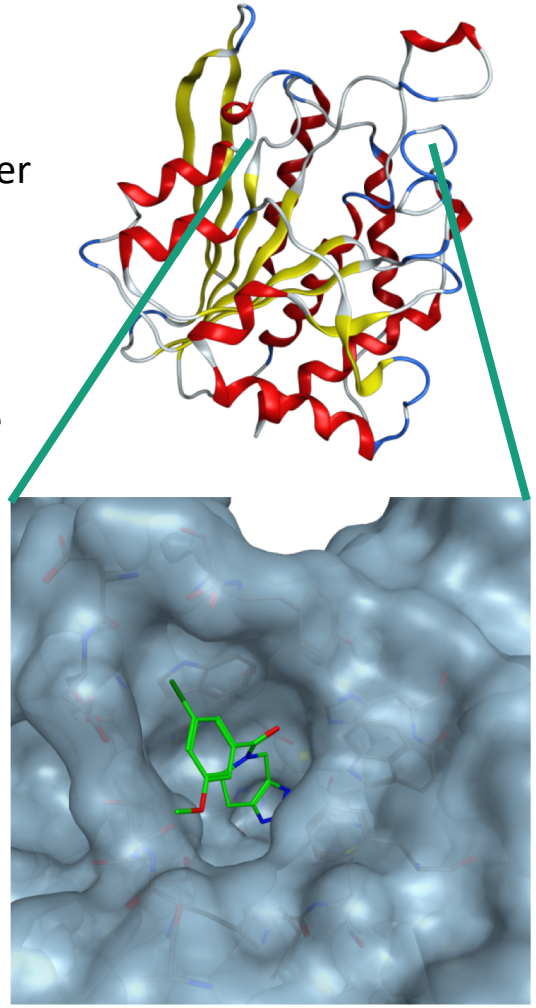
# Summary & Outlook

- Medicinal Chemistry
  - SAR of different novel compound classes → IP generation
  - Porphyrin conjugates exhibit improved *in vivo* activity → further exploration of transport mechanisms necessary
- Crystallization efforts
  - Co-crystallization with Tetrahydroimidazopyridine-inhibitor successful → structure based compound optimization possible
- *In vivo* characterization of selected compounds
  - Preliminary PK-data → Acceptable PK-profile and oral bioavailability of selected compound classes
- Development of an controlled release and locally applied formulation (in progress)



# Preclinical development platform *in vitro* AND *in vivo*

# Molecular Biotechnology Unit

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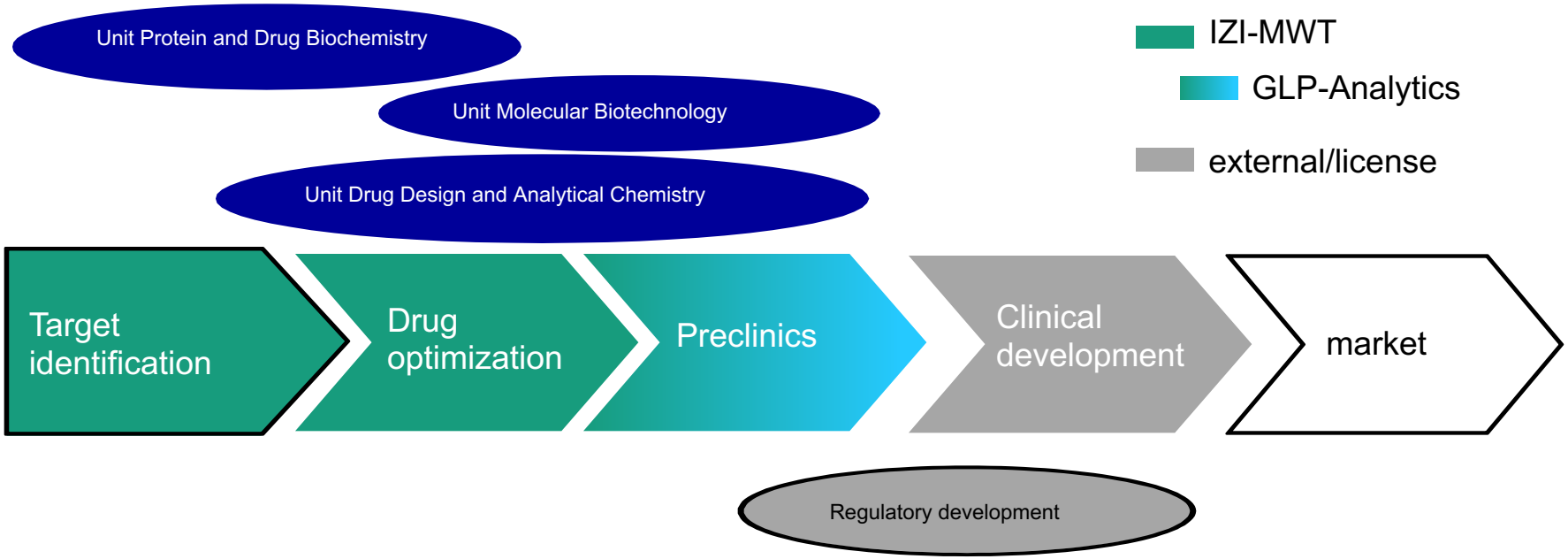
# Unit Allocation within the Drug Discovery Process




IP

- medical use patents
- composition of matter patents

Validation of proprietary targets by contribution of all units –
From target isolation to proof of principle



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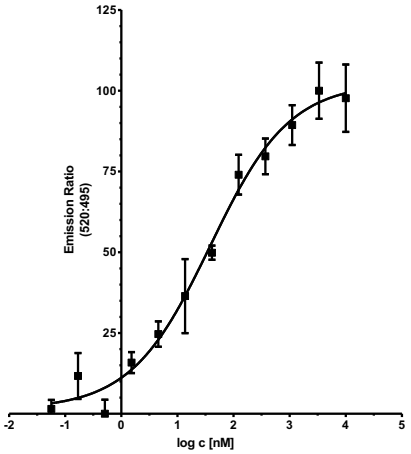
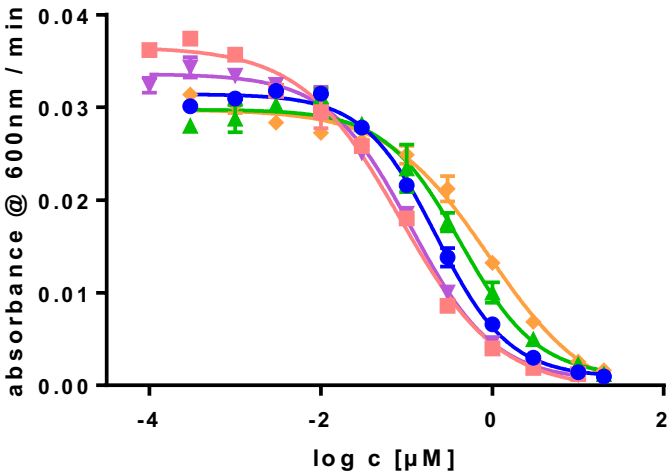
# Example: Developement of RORγt-Modulators

- Preclinical development of RORgt-modulators for autoimmune diseases
- Industry cooperation with Immunic AG (Munich)
  - Spin-off from 4SC (Munich)

Compound characterization *in vitro*




- Assay transfer
- Novel Assays
- Validation



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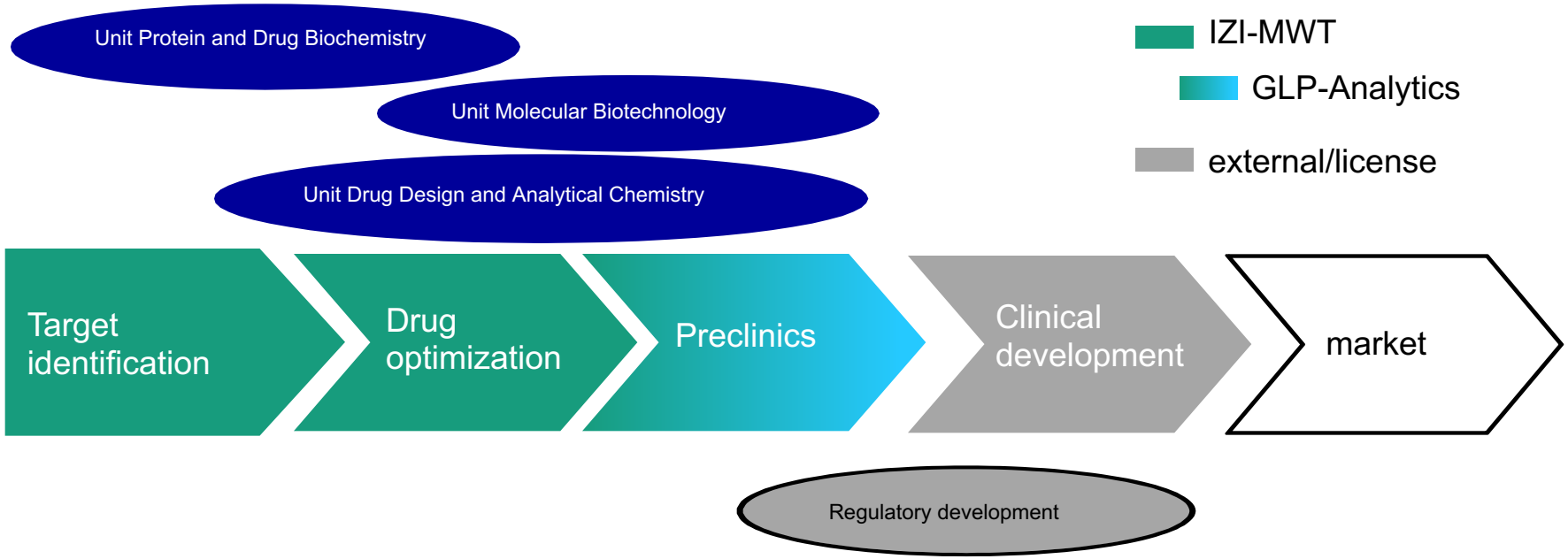
# *In vivo* Platform




Safety

Selectivity

Efficacy

PK/PD/BD

IND studies

GLP - analytics

# Preclinical Development Platform

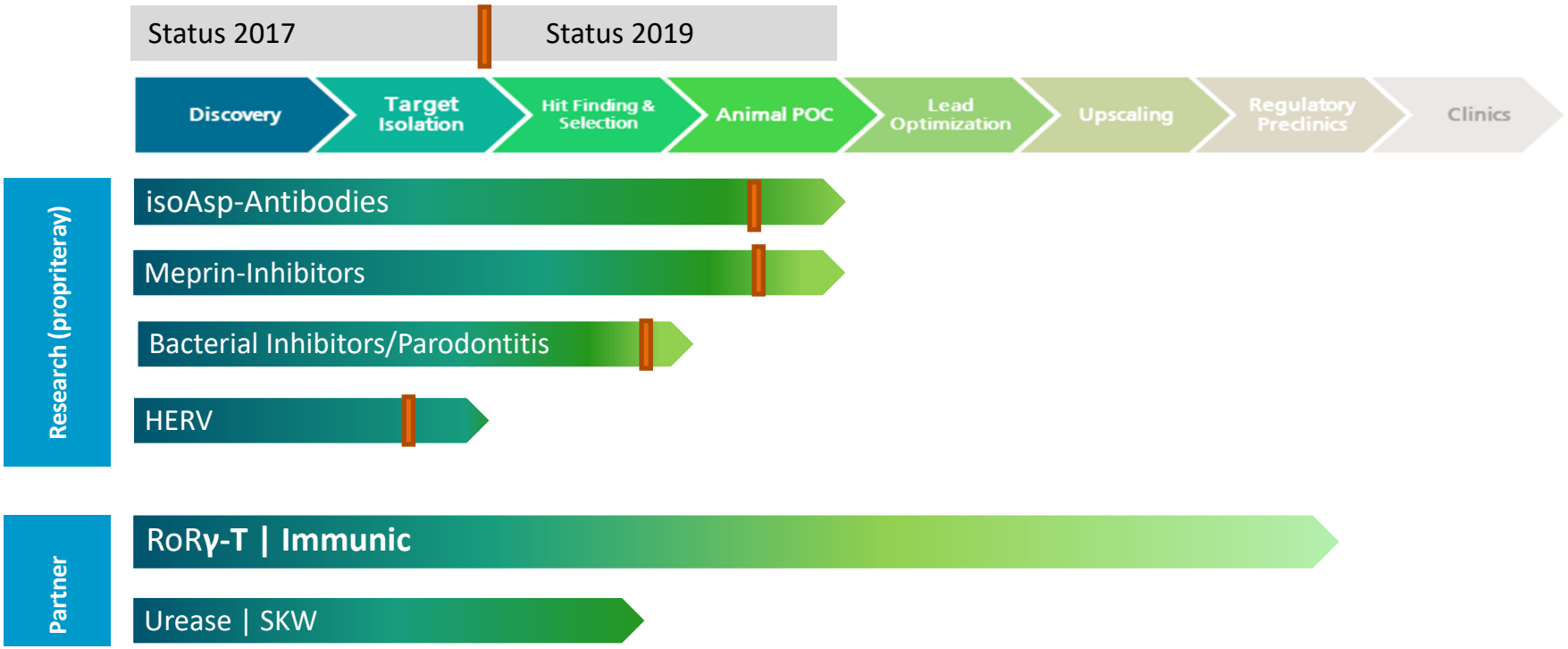
- **Tailored solutions for industrial and academic partners and internal projects**
  - Internal Assays
  - Scouting and Management for **non-GLP studies**
    - Genotoxicity (AMES, micronucleus)
    - Stability (microsomal stability)
    - pKa – determination
    - CYP – inhibition
    - Extended PK studies
    - Safety Screen
    - Off – target screening
    - Exploratory toxicity studies *in vivo*

# Preclinical Development Platform

- **Internal assays for IND enabling and clinical studies under GLP**
  - GLP-Analytics

- **Scouting and Management for IND enabling studies under GLP**
  - GLP-Genotoxicity
  - GLP-Metabolism
  - GLP-Pharmacology
  - GLP- General Toxicology (rat and dog)

# Summary and Outlook




**Extension of capabilities to clinics under way**



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