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Business Unit

Drugs

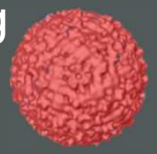
Drug Discovery Programs (Meprin, AD Ab)

Overview „Business Unit Drugs & Vaccines“

- Small organic molecules
- Biopharmaceuticals
  - Antibodies
  - Peptides
- Natural Products
- Cell Therapies
- Vaccines
  - Protein-/peptide-, DNA-based

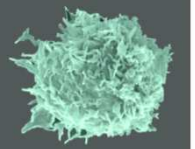
Infection

E.g. Emerging diseases Pathogen Control



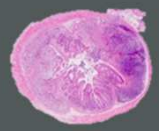
Oncology

E.g. Solid tumors, leukemias, CSC



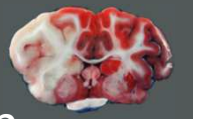
Inflammation

E.g. Auto-immune diseases (IBD, RA)



Neurology

E.g. Stroke and Neuro-degeneration



- **Drug discovery programs**
- Contract research, testing and manufacturing

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Proprietary Drug Candidates
Overview of selected partnering opportunities

- **New antibody against modified Aβ species for AD treatment ➡ preclinical stage (POC in mice demonstrated)**
- **Lead candidates for kidney failure and fibrosis (meprin β inhibitors) ➡ preclinical stage**
- RSV vaccine & therapeutics approaches
- Papilloma pseudoviruses for the delivery of genetic vaccines
- Clay minerals as toxin adsorber ➡ preclinical stage (POC in mice and rats demonstrated)
- GCSF for treatment of stroke-induced immunodepression ➡ preclinical stage (clinical stage in a different setting, PoC for neurogenic immunodepression in rodents demonstrated)
- Extracellular vesicles as cell-free tools for therapies

Drug design and target validation services

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Department Drug Design and Target Validation

Track Record*

Discovery of a new treatment against type 2 Diabetes

- Improved glucose control by inhibition of Dipeptidyl Peptidase 4 (DP4)
- Approved since 2007
- DP4-Inhibitors: 9.5 Billion US-$ in 2012

Discovery of a new treatment against Alzheimer's disease

- Discovery of pyroglutamate (pGlu)-modifzied Aβ-Peptide toxicity in neurodegenerative disorders.
- Currently in clinical Phase II
- Enabled IPO of Probiodrug AG at EURONEXT on Oktober 27th, 2014

LETTERS

nature medicine

Glutaminyl cyclase inhibition attenuates pyroglutamate Aβ and Alzheimer's disease–like pathology — Nature 2008

LETTER — Nature 2012

doi:10.1038/nature11060

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

* In 2015, Fraunhofer took over the entire preclinical development team of Probiodrug AG including the former CEO/CSO of the company

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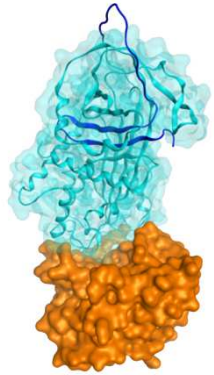
Department Drug Design and Target Validation
Dual Business Strategy



Drug Development Expertise

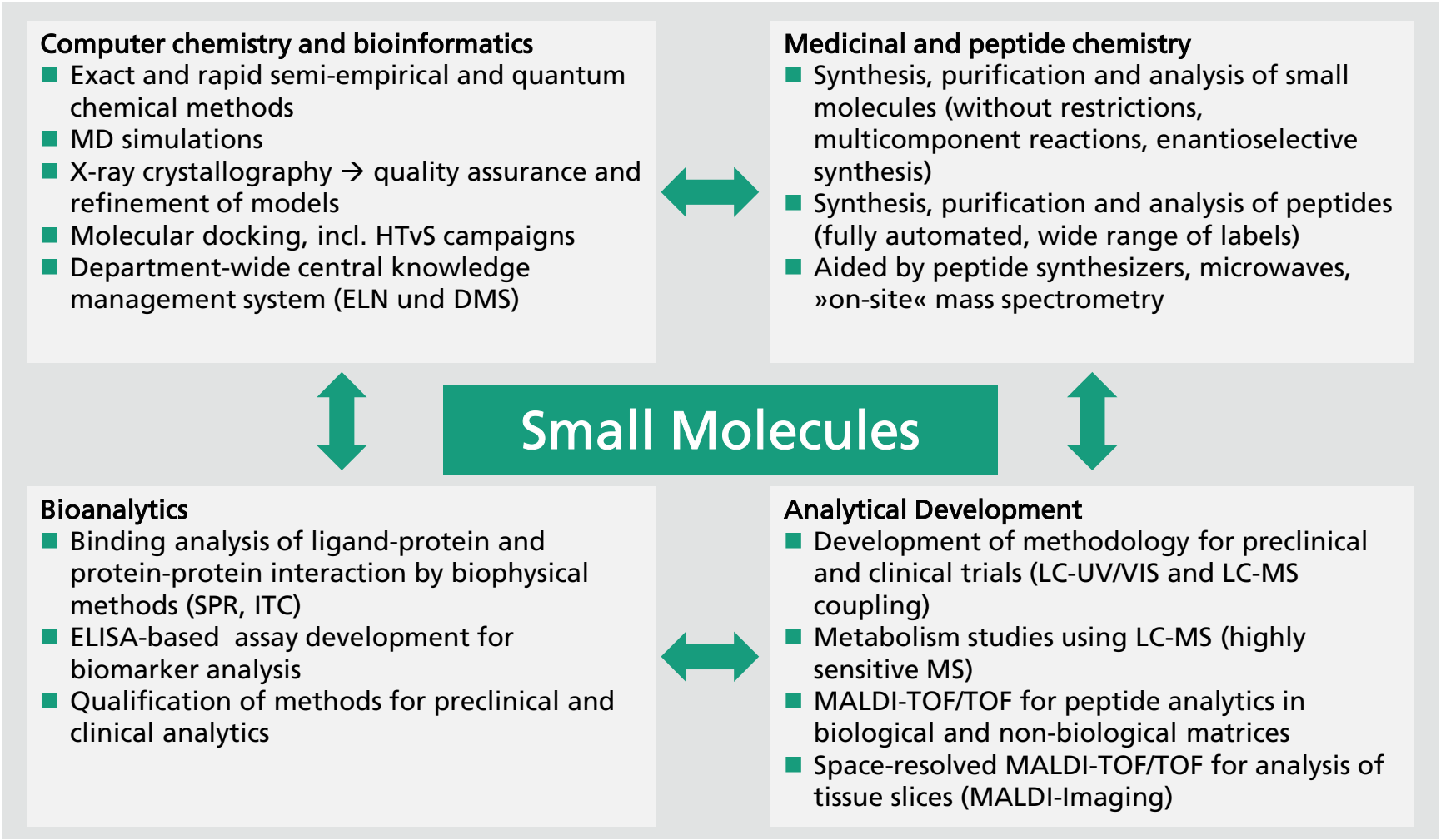
Development of a pipeline of proprietary drug candidates

- Antibody for AD treatement → POC in vivo
- Small molecules for treatment of fibrosis and kidney diseases → Lead cpds
- Small molecules as new antibiotics → Lead cpds

Provide external partners with drug R&D services on a fee for service base

- MoA exploration (neurodegenerative und inflammatory disorders)
- Target identification and validation
- (GLP-) Analytics for preclinical and clinical studies of small molecules
- Biomarker identification for disease monitoring and therapy monitoring
- Conduct and (if required) generation of relevant *in vitro* und *in vivo* models
- Drug development including in silico screening, comprehensive medicinal chenistry capabilities

Drug Design and Analytical Chemistry Unit



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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 & humanization of antibodies
- Development of other 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

- Small molecules as novel drugs in fibrosis and kidney protection
- Scientific projects with industrial partners (e.g. assays and inhibitor characterization)
- 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

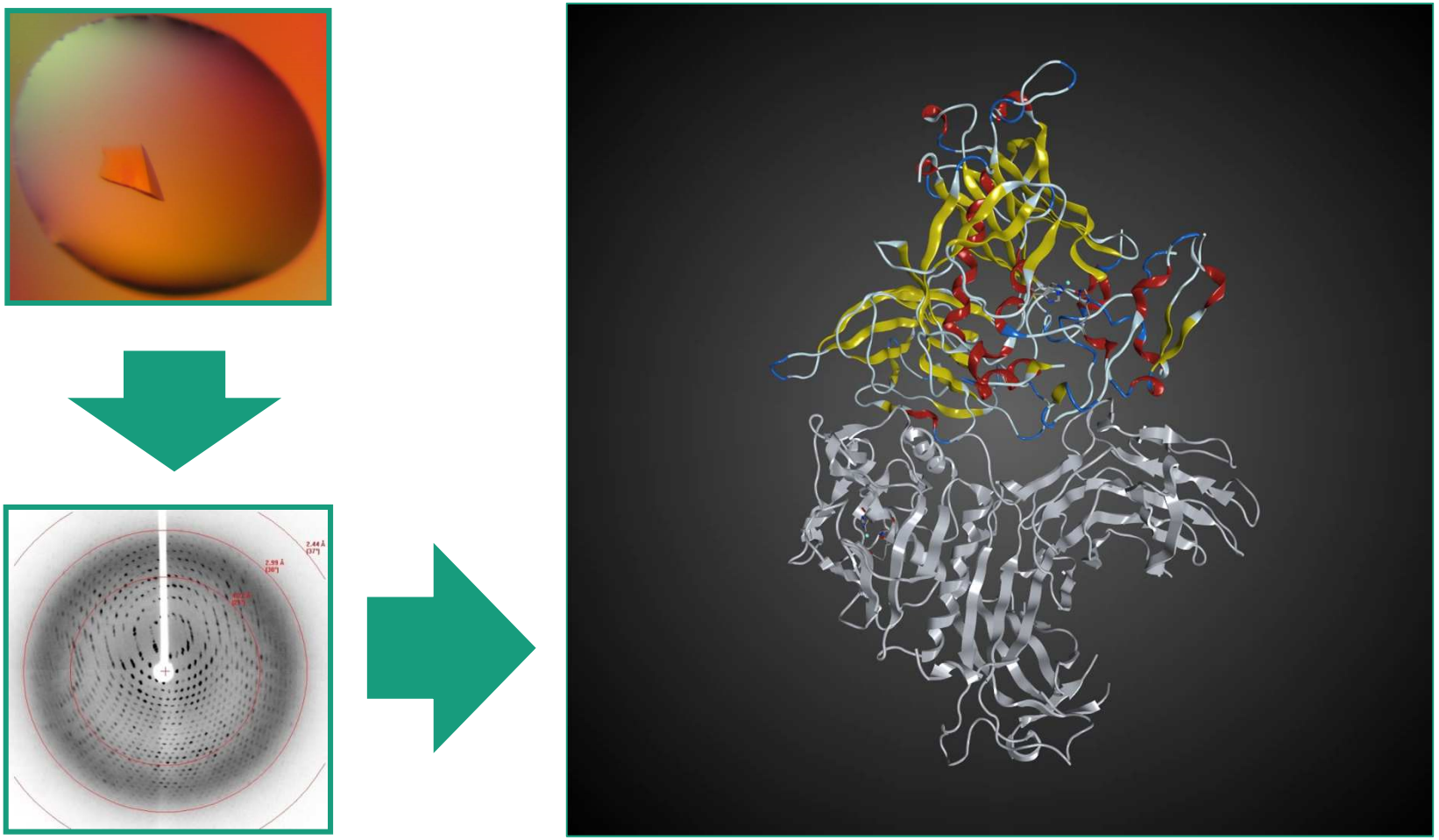
Cell culture

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

Animal facility

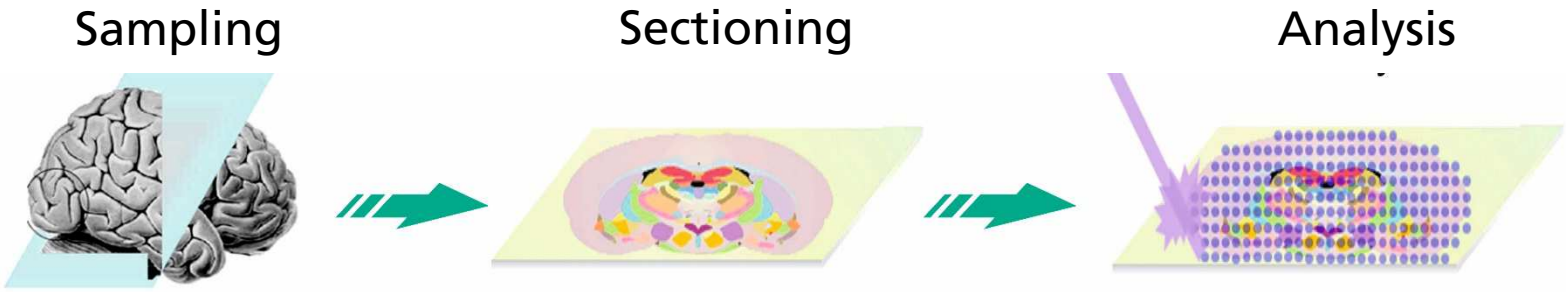
- Microsurgery and macrosurgery
- Comprehensive phenotyping of animal models, incl. a wide range of cognitive tests (Y maze, water maze, contextual fear conditioning, open field, rota rod, tail suspension etc.)

Selected Methods and Models - I
Structure-Based Design for Small Molecules and Biologics

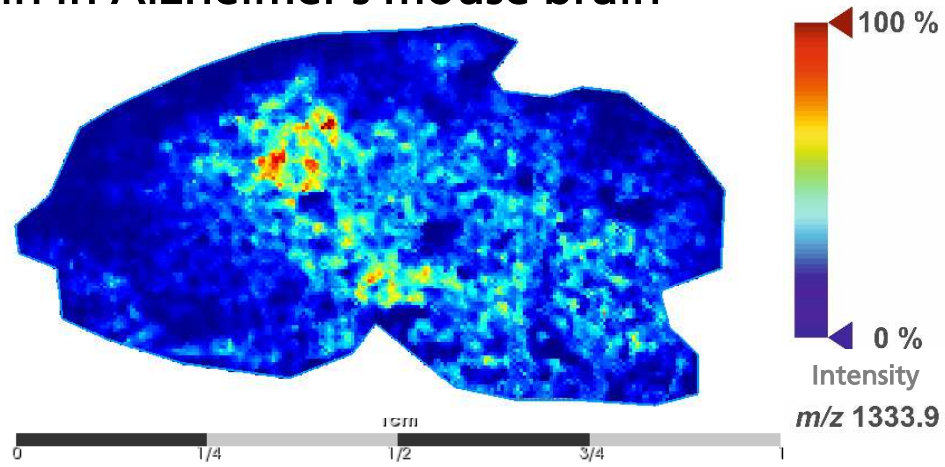


Selected Methods and Models - II
MALDI-Imaging

- Investigations on regional distribution of molecules in tissue sections



- **Localisation of basic myelin protein in Alzheimer's mouse brain**
  - 10 µm Sections
  - 50 µm lateral resulution
  - 17.800 mass spectras
  - Mass *m/z* 800-5000
  - 7,5 h for analysis



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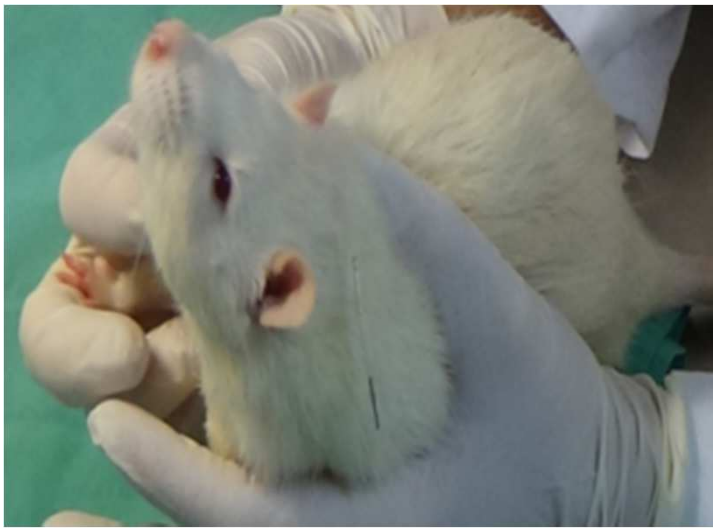
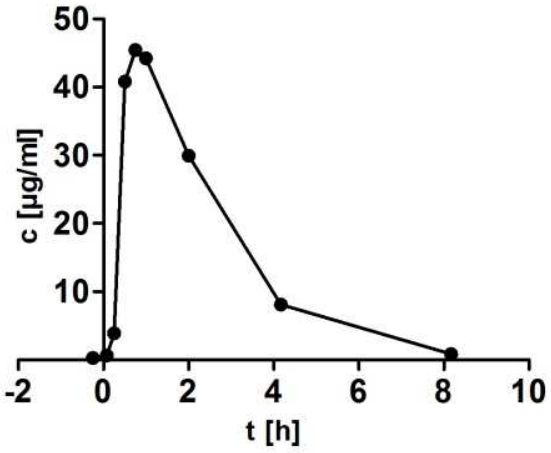
Selected Methods and Models - III
Animal Pharmacology

- Rat PK model (Catheterized SD rats)
- Combined with LC-MS/MS or LC-UV/VIS detection of small molecules





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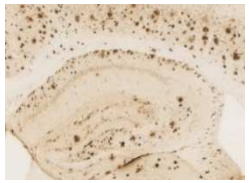
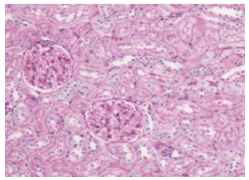
Selected Methods and Models - IV
Animal models IZI-MWT

Pharmacokinetics (PK) model (rat)

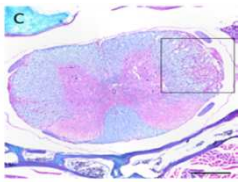


Alzheimer's disease mouse models (mouse)



Kidney models (mouse, rat)

In-stent restenosis model (rabbit)



Multiple Sclerosis model (mouse)

Reference Project (Industry)

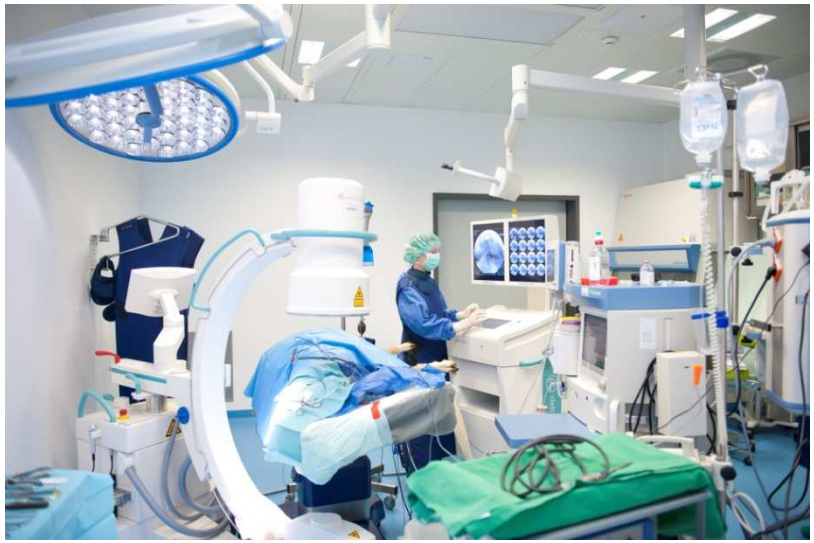
- **Preclinial development of RORγt-modulators for autoimmune diseases**
  - Synthesis of lead candidate and back-ups
    - Route scouting for upscaling
  - Non-GLP and GLP analytics
  - Generation of target proteins
  - Compound characterization *in vitro*
  - Compound pharmacology *in vivo*
  - Scouting for and management of preclinical development
    - Compound characterization (chemistry)
    - Toxicology
    - Metabolism
    - Efficacy
    - Pharmacology

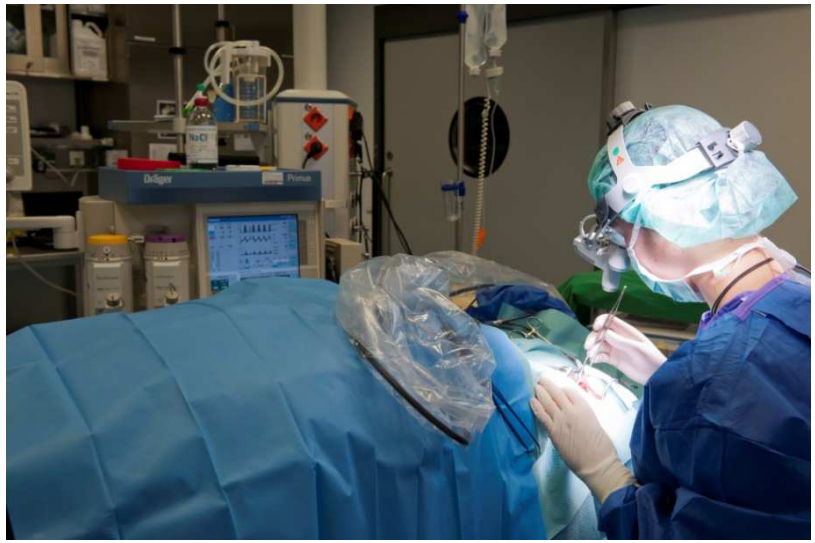
Animal Facilities and Capabilities

Animal Facilities and Capabilities

- Up to BSL-3 laboratories for immunological assays and animal testing:
  - Completely equipped with isolated ventilated cages for mice and rats
  - 3 different hygiene levels (quaratine, experimental, breeding)
  - GLP certificate for small and large animal facility
  - License for biosafety level 1-3, permission to work with animal epidemic pathogens
  - Surgery facility for large animals
  - Imaging area with NMR, CT, X-ray and bioluminescence

Surgery Facility for Large Animals





- Operation faciltiy with 2 fully equiped operation rooms including intensive care and C-arm X-Ray
  - Aseptic surgery with inhalation anesthesia and artificial respiration
  - Online measurement of vital parameter (e.g ECG, BP, Temp., SPO2, CO2)
  - Intraoperative measurement of blood gases and metabolism
  - Intraoperative C-arm X-ray with DSA

Imaging Core Unit – Magnetic Resonance Imaging

MRI specifications:

7T (300MHz) with ParaVision 5.1

gradient: B-GA09

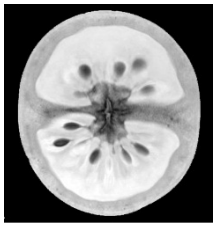
high performance shim upgrade

gradient strength up to 375 mT/m

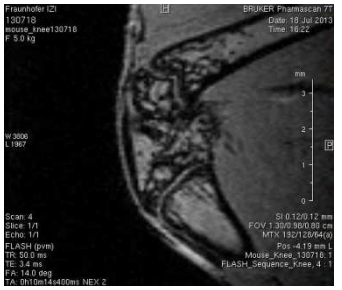
4 receiver channels

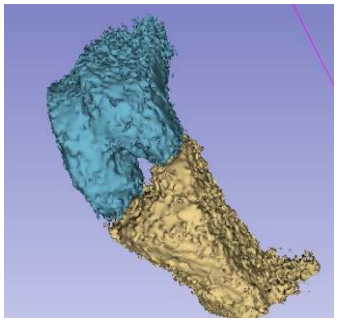
application-specific coils kits

advanced physiological monitoring system

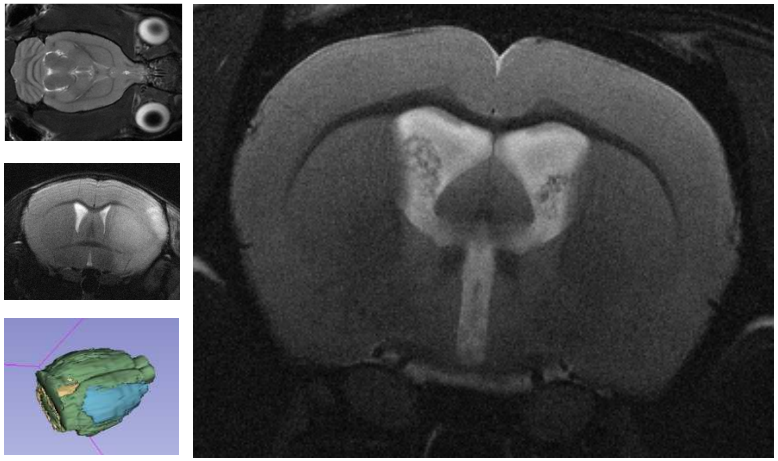


tomatoe

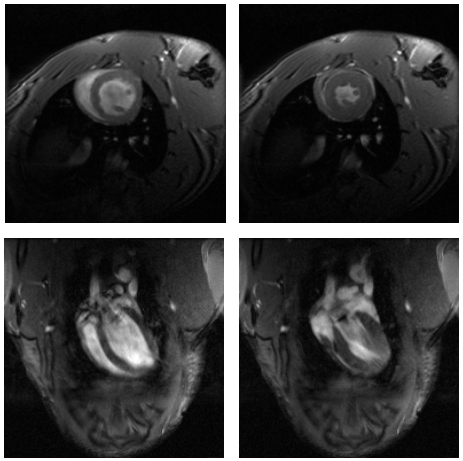




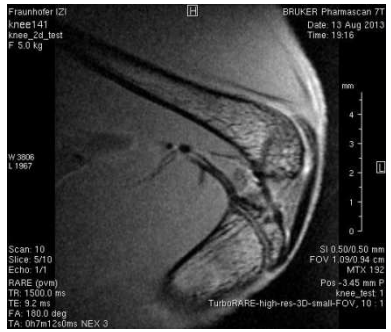
3D mouse knee model



rat (right) and mouse brain (left) anatomy + stroke



heart function in a rat



2D mouse knee

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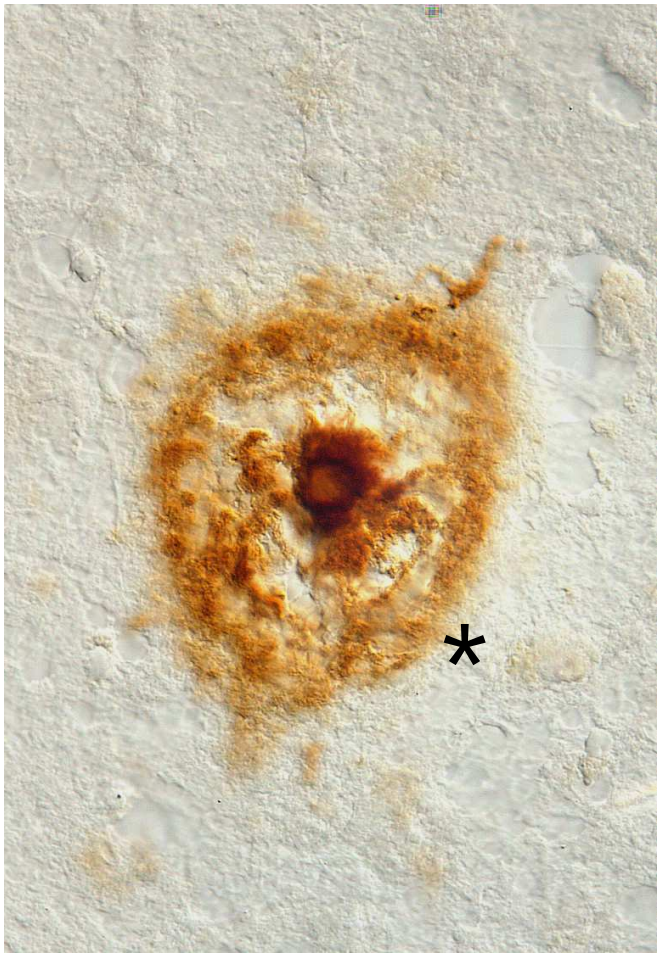
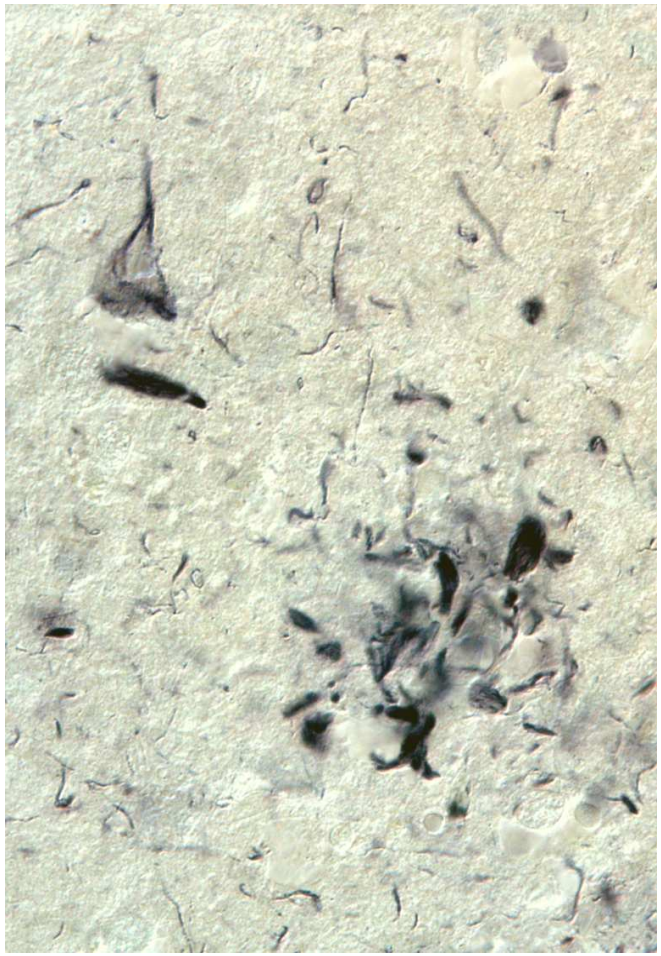
New approaches for AD treatment

Hallmarks of Alzheimer Pathology

β-amyloid plaques

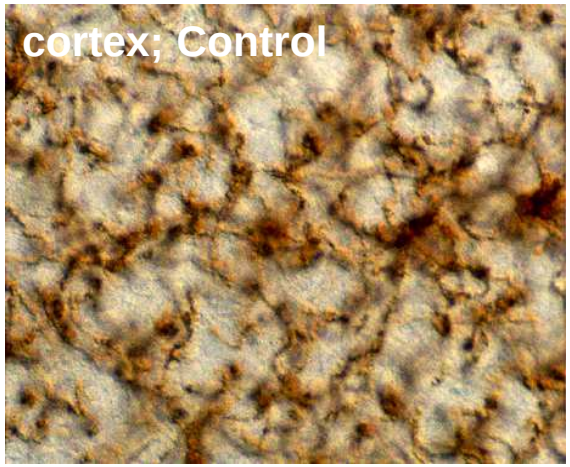


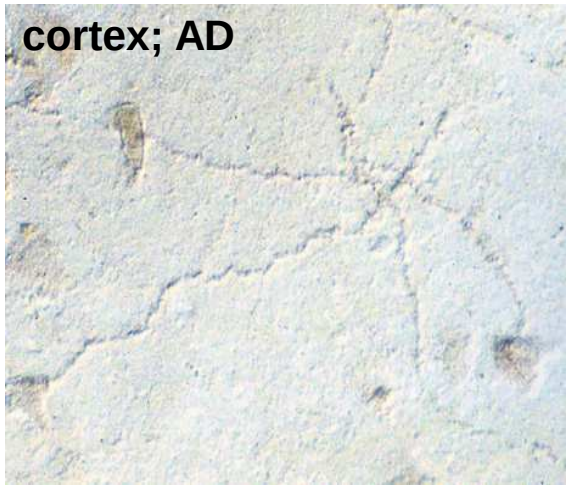
neurofibrillary tangles



cholinergic degeneration

inflammation





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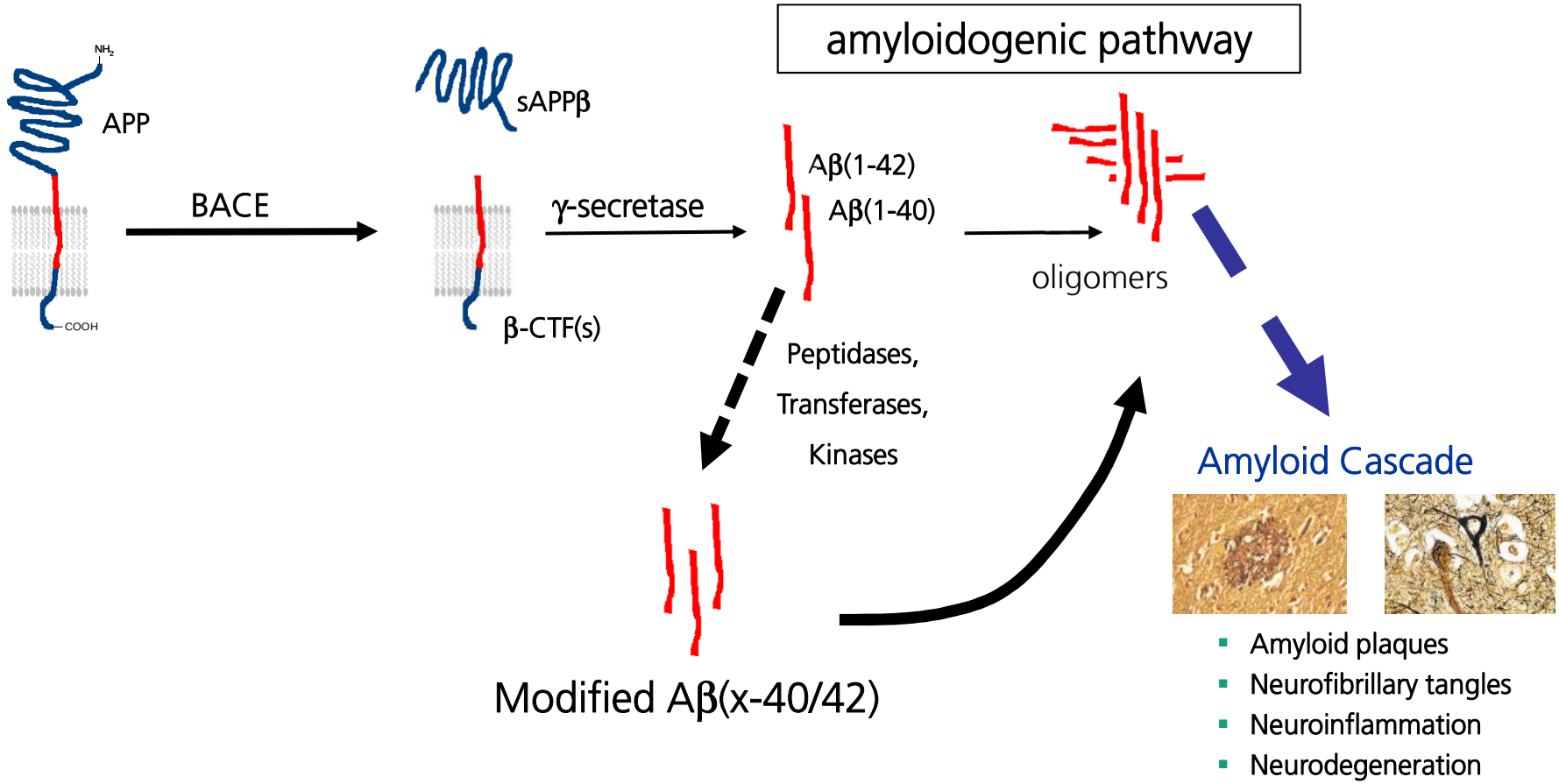
Processing of Amyloid Precursor Protein (APP)



~80% of Aβ in AD is N-terminally truncated and modified

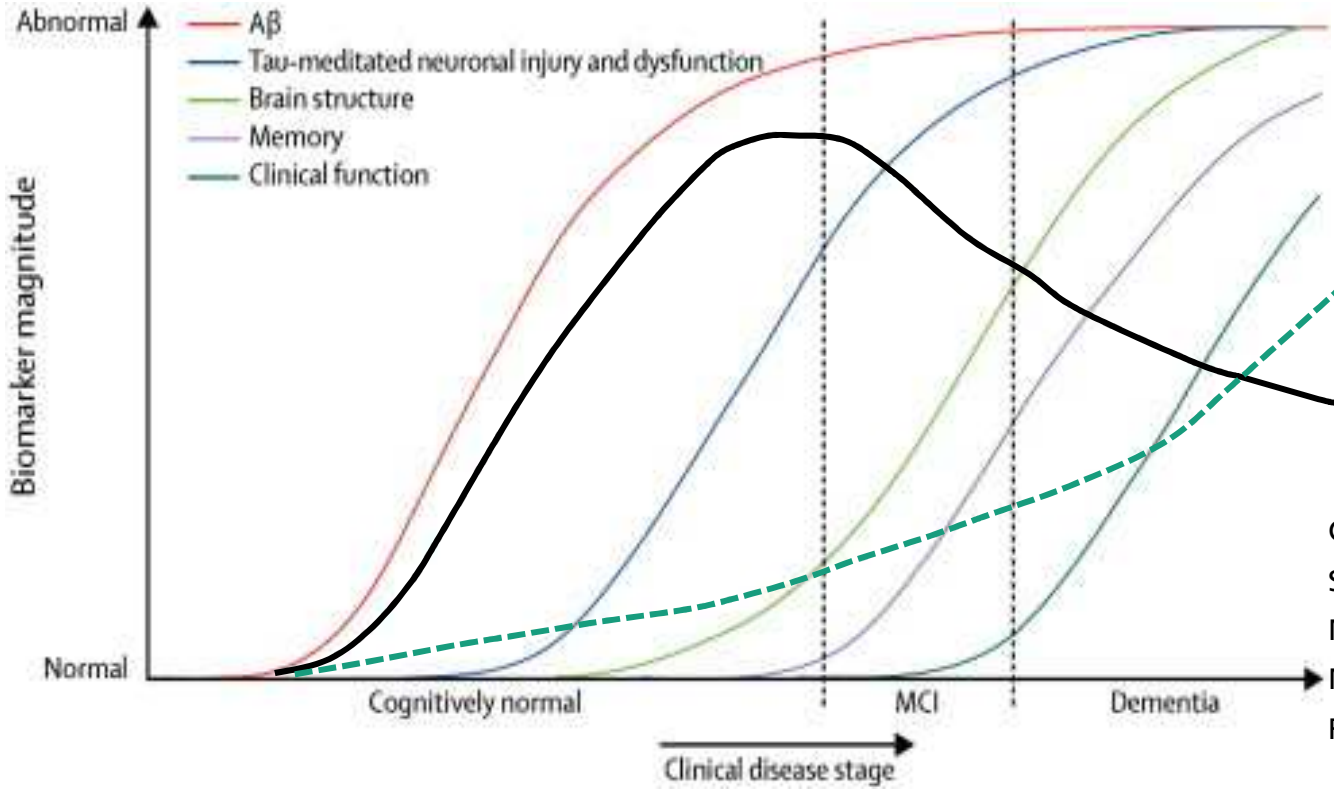
Aβ: The right target, addressed in the wrong way

- Mutations in APP predispose for familial AD
- ApoE4 genotype: strongest risk factor – associated with Aβ transport
- Other risk factors (e.g. CD33) – associated with Aβ phagocytosis
- Tau mutations not associated with AD

The „Amyloid Cascade“: Correct model for Disease initiation, but addressed in the wrong way by pharmaceutics

Search for the right target addressed in the right way

AD: About Loaded Gun and Trigger



Aβ(x-40/42), posttranslational modifications

Aβ(1-40/42)

Güntert et al., *Neuroscience*, 2006
Schilling et al., *Nat Med*, 2008
Morawski et al., *JAD*, 2014
Mandler et al., *Acta Neuropathol*, 2014
Rijal Upadhaya et al., *Brain*, 2014

Accumualtion of N-terminally truncated and modified Aβ correlates with disease progression

Scheme modified from Villemagne et al., Lancet Neurol, 2013

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The Fraunhofer isoAsp-A**β** Antibody: Unique Characteristics

- 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**

The Fraunhofer antibody to target modified Aβ: lower dosing, low risk of side effects, high efficacy

* Cerebral amyloid angiopathy ** Amyloid Related Imaging Abnormalities

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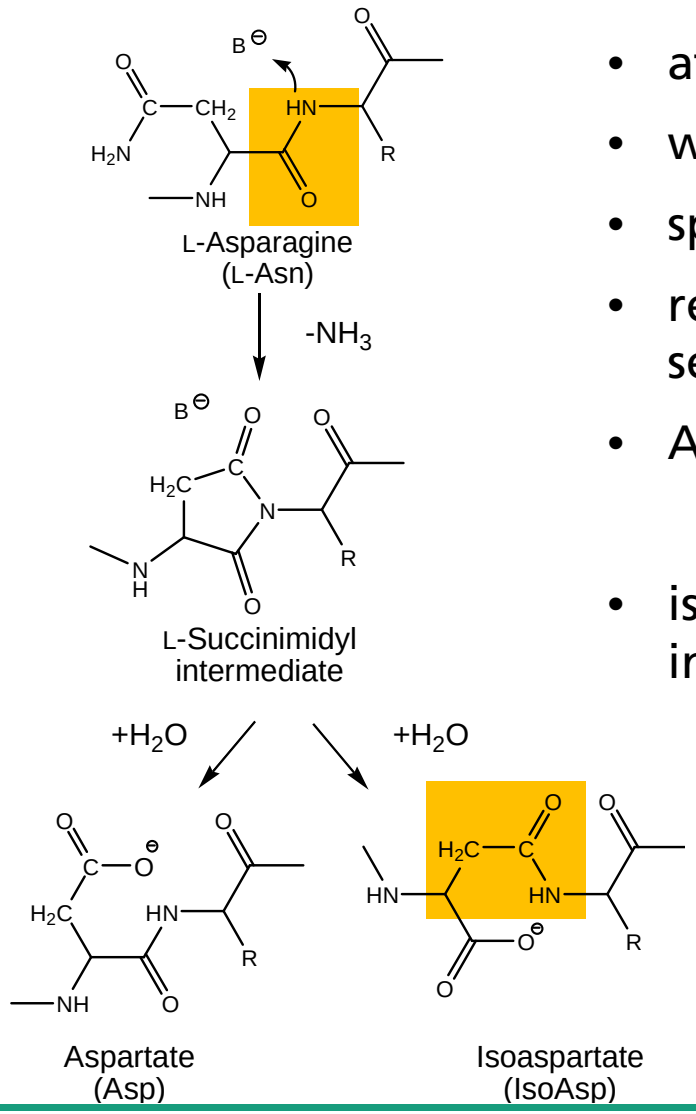
IsoAspartate (iD) formation – a molecular switch



- at Asp or Asn residues (at Asn ~2-3 times faster)
- within peptides and proteins
- spontaneous reaction
- reaction velocity dependent on primary and secondary structure
- Aβ Tottori mutation (D7N) – link to familial AD

- isoAsp produces „kink“ in peptide chain and may introduce charge

Isoapartate-modified Aβ: Effective anchor for antibody-mediated clearance?

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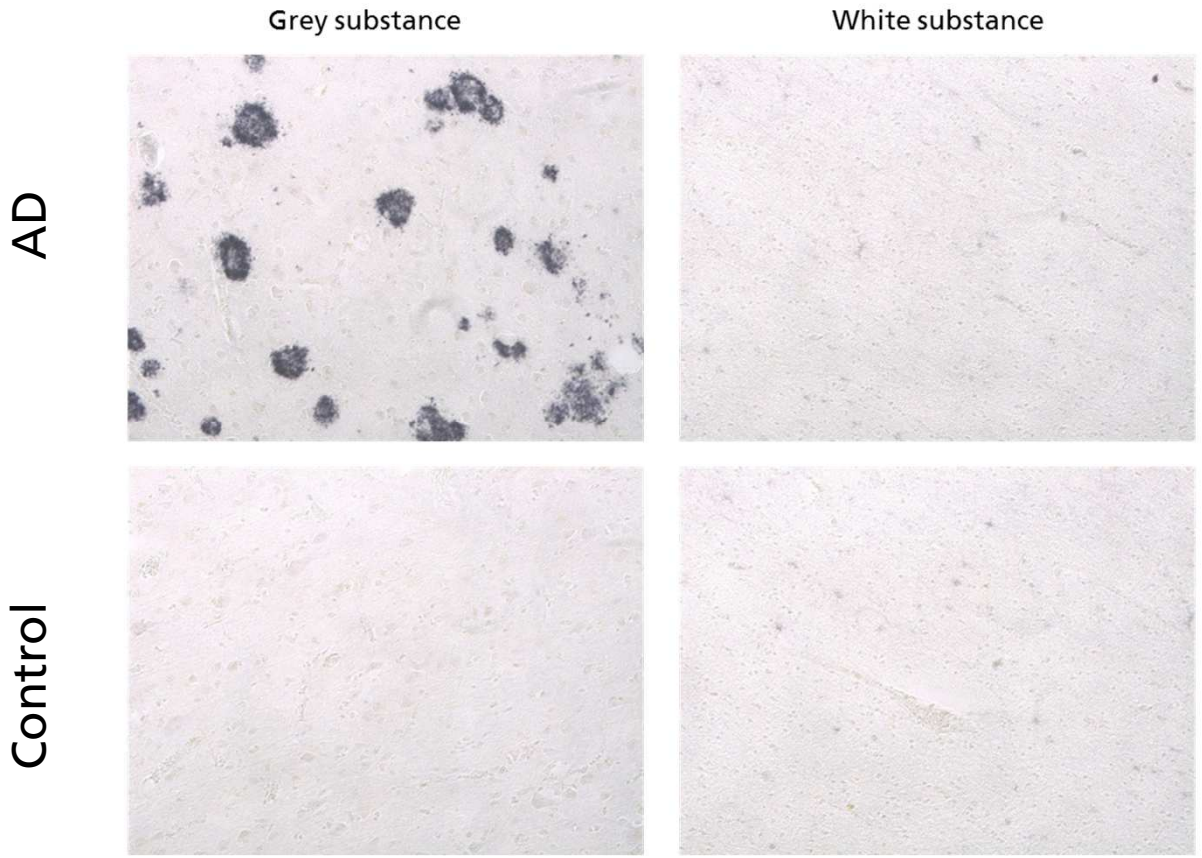
IsoAsp7 antibody affinity and specificity (SPR)

#clone	isoAsp7(L) Aβ	Aβ1-18	selectivity factor
K29	136 nM	9009 nM	~70
K23	5 nM	378 nM	~80
K16	55 nM	3990 nM	~70
K11	**6 nM**	**2700 nM**	**~400**

K11 seleceted for further characterization

Antibody K11: Human AD Brain



New antibody: Reactivity with deposits in AD brain, no tissue cross-reactivity in control brain observed

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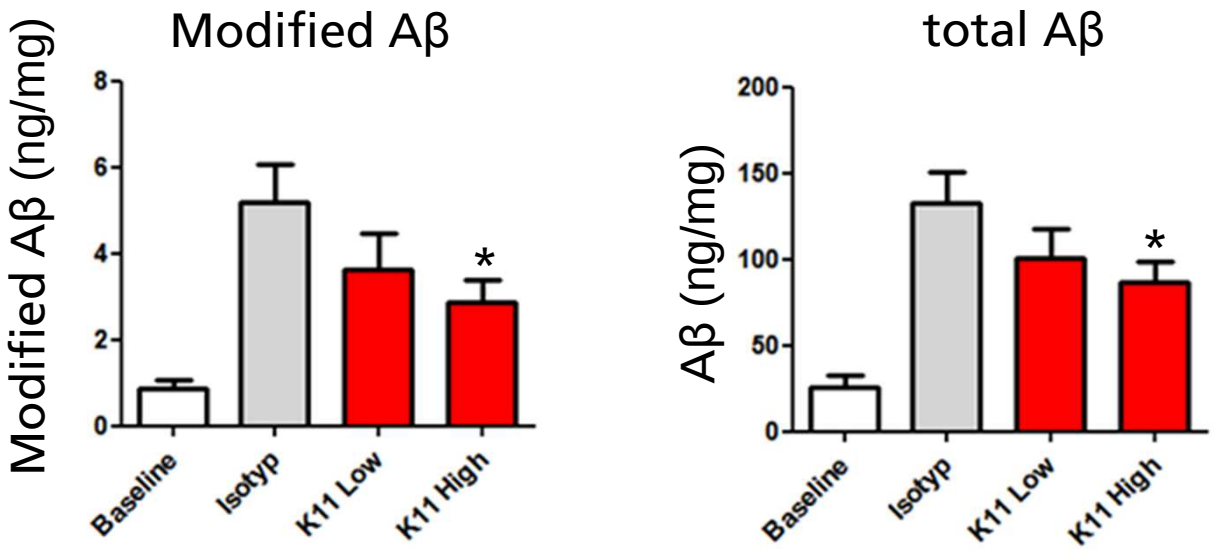
Treatment of 5xFAD tg Mice

Antibody: Antibody A, IgG2a

Mice: Female, 26 mice in treatment, 6 mice for baseline control

Duration: 12 weeks, start at 3 mo of age

Route: I.p. injection weekly (~100 µl; 150 and 500µg; 500µg isotype control)



Treatment of transgenic mice - reduction of modified and total Aβ → Targeting a minor Species (4%) mediates significant therapeutic effects

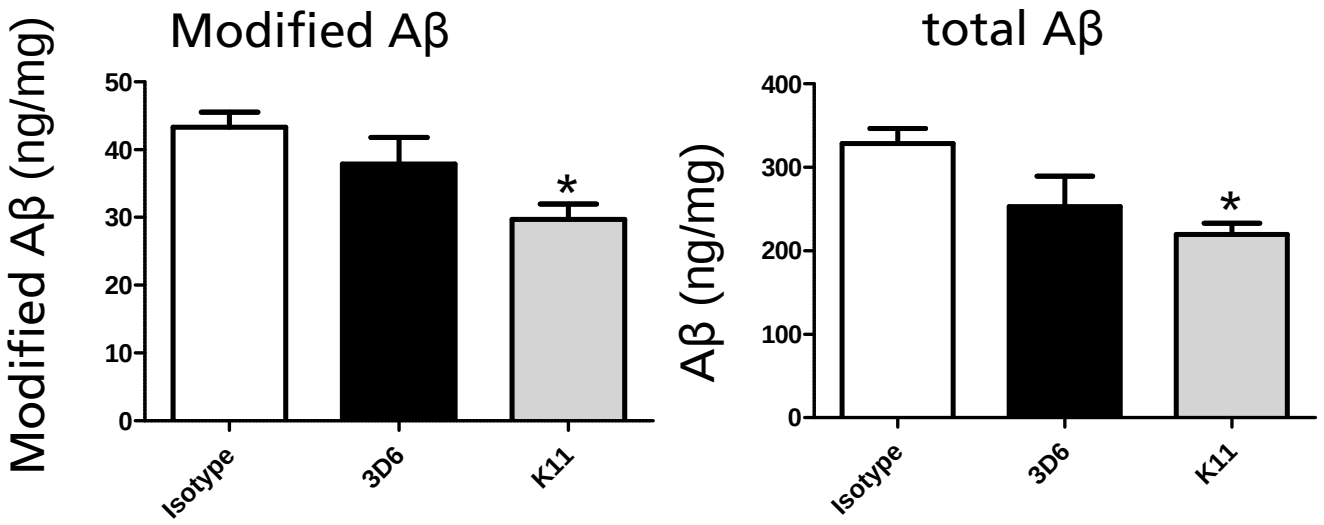
2nd POC Study in Transgenic Mice

Antibody: isoAsp-Antibody (K11), IgG2a; 3D6, IgG2a; Isotype control

Mice: Female, 45 animals in 3 treatment groups

Duration: 3 -11 months of age

Route: Injection (*i.p.*) weekly (~100 µl; 300µg; 300µg isotype control)



IsoAsp-antibody K11- more pronounced reduction of Aβ compared to 3D6 (murine version of bapineuzumab)

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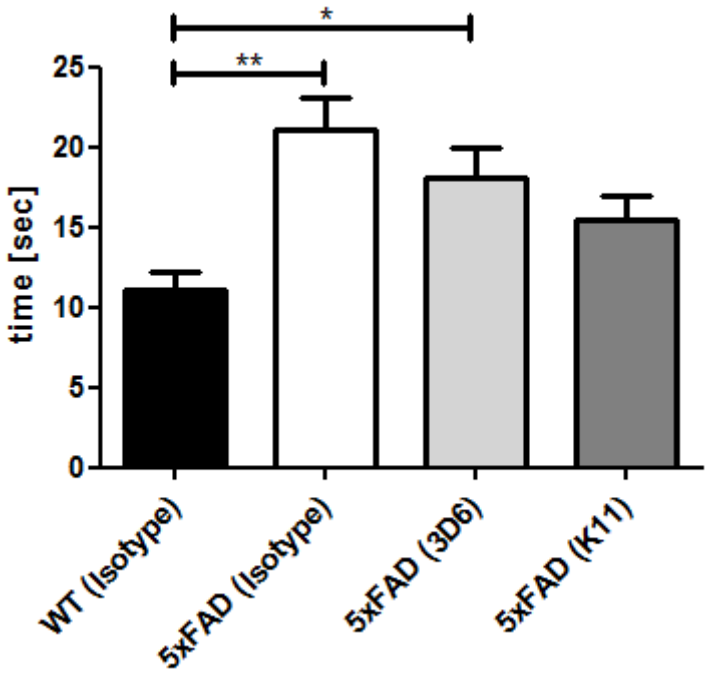
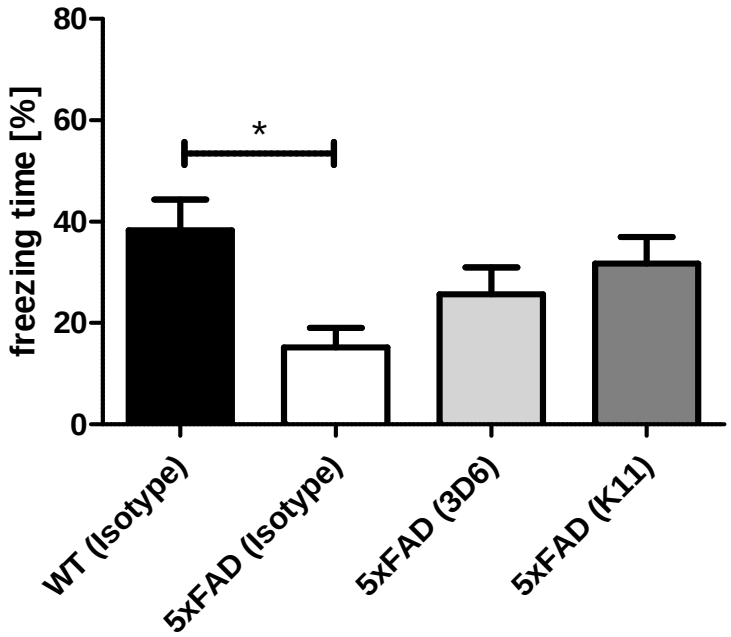
Behavioral assessment

Morris Water Maze



Fear Conditioning



Mean ±SEM, ANOVA followed by Bonferroni post-hoc analysis

IsoAsp-antibody K11- treatment results in a significant improvement compared to isotype control in several cognitive tests

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Project Status and Future Directions

Current topics

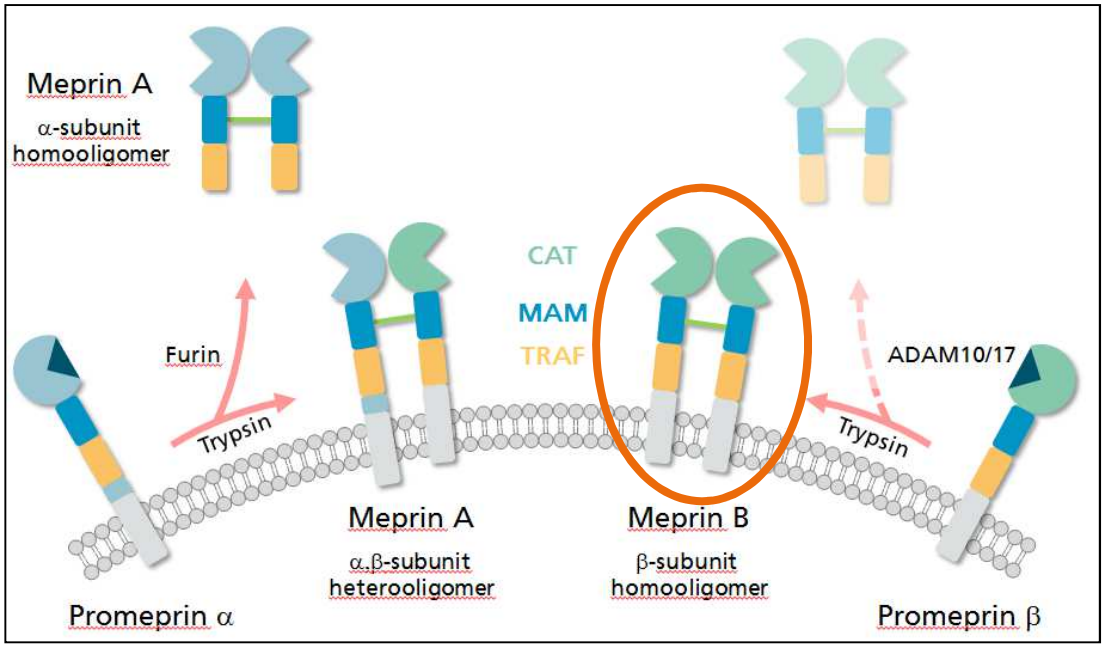
- IP protection (composition of matter, use for therapeutic and diagnostic approaches)
- Ongoing treatment in rodent models – introduction of CDC mutation (no complement activation)

USPs over other monoclonal antibodies (e.g. aducanumab, bapineuzumab)

- characterized profile in animal model(s)
- much higher specificity for „disease-specific“ amyloid
- no mobilization of amyloid peptides to plasma
- Lower epitope density – lower incidence of ARIA expected

Selective Meprin-inhibitors for the treatment of fibrosis

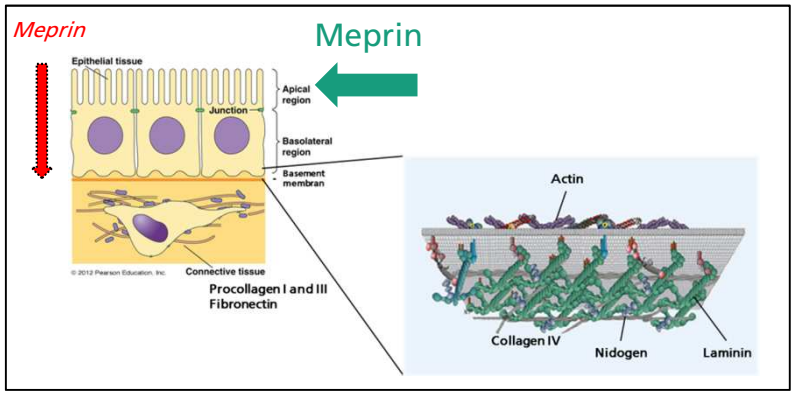
Meprin β program
Target enzyme



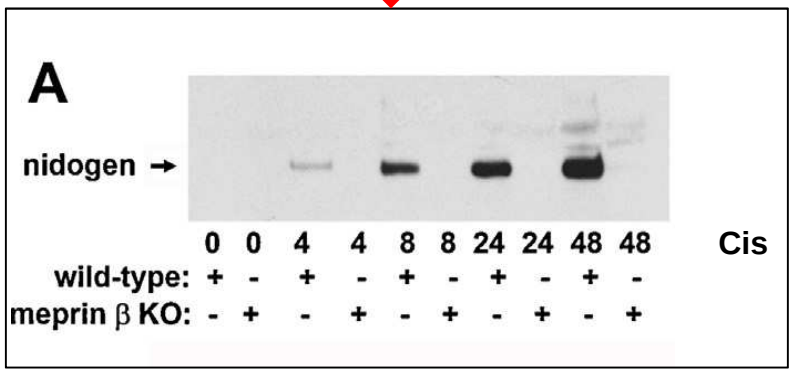
- Extracellular zinc-dependent metalloendoproteinase, member of the astacin family
- Highly expressed in kidney*/bowel ephithelial cells as well as in skin, lung, intestinal leukocytes and some cancer cells
- Two isoforms (type 1 membrane protein meprin β, secretory meprin α)
- Substrates include bioactive peptides and proteins of ECM

* Brush-border membrane of the kidney: 5% of total protein content

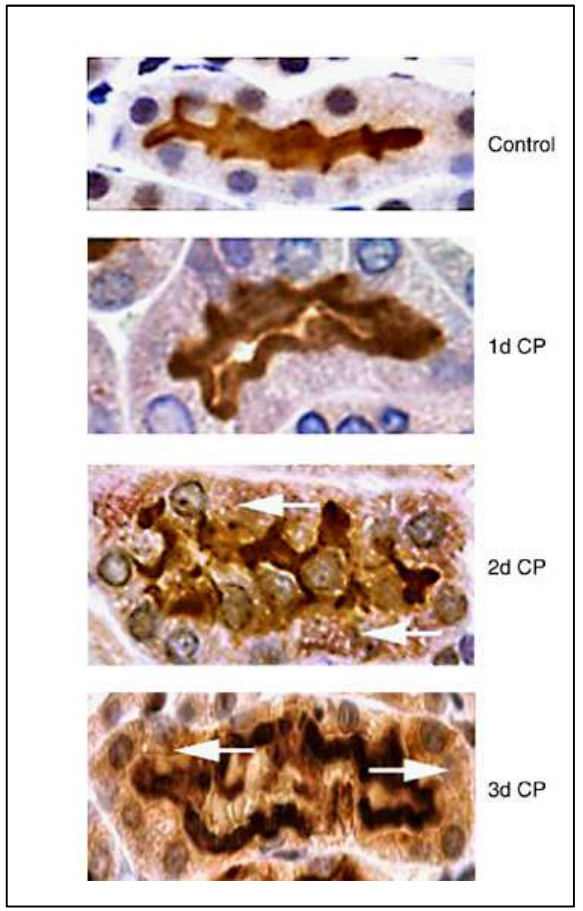
Meprin β program
Rationale for meprin inhibition in kidney diseases/AKI



Relocalization of meprin-β after toxic trigger brings the enzyme in contact with a number of (new) substrates



Cisplatin-induced nidogen release in wt but not in mep-β KO mice#

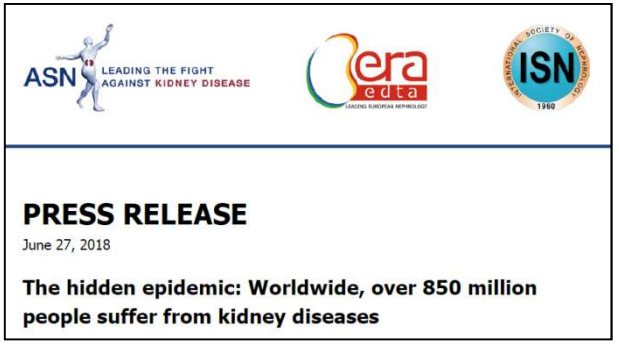


Cisplatin (CP) -induced relocalization of meprin β from epithelial tissue apical side to basolateral membrane*

* C. Herzog et al, Kidney International (2007); # C. Herzog et al., Toxicology Letters (2015)

Fraunhofer IZI

Meprin β program
Target indication acute kidney injury (AKI)



- Fast kidney function decline caused by **ischemic, toxic** or **septic** triggers (doubles sepsis mortality)
  - → Up to 30% of patients[1] require chronic dialysis after initial AKI
- **Only supporting therapies available** (volume substitution, kidney support by dialysis, cardiovascular stabilization, diuretics etc.)
  - → Urgent medical need for new therapy approaches addressing the tissue-degrading AKI effects
- **Meprin KO mice are less susceptable to AKI[2] and meprin inhibitors were able to reduce kidney injury in vivo to some extent[3]**
- **Therapeutic kidney protection to block development of chronic nephropathies and fibrotic kidney disease** (long term consequence of chronic kidney diseases)
- Prophylactic treatment to enlarge the therapeutic window for nephrotoxic drugs (chemotherapy, antibiotics etc.)

1: http://nephrologie.uniklinikum-leipzig.de/nephrologie.site,postext,das-nierenversagen,a_id,522.html
2: Tan et al., 2018; Takayama et al., 2008; Ongeri et al., 2011; Walker et al., 1998
3: Herzog et al., 2015; Martin et al., 2016

Fraunhofer IZI

Meprin β program
AKI market opportunity

- Annual incidence of AKI in the UK is 577/100.000[1]
- Prevalence of chronic kidney diseases worldwide is around 11%[2]
- Prevention or early treatment at the onset of AKI **may substantially reduce dialysis and/or kidney transplantation needs**
  - Dialysis
    - Most important therapeutic option: Hemodialysis (costs p.a. 2.1 billion EUR Germany; 42 billion USD USA[3])
    - 66% of dialysis indications are interstitial (8%), vascular (23%) and diabetic (35%) nephritis = susceptible for nephro-protective meprin beta inhibitors[4])
  - Kidney transplantation
    - Germany: Ca. 2.100 transplantations p.a., up to 65.000 EUR per transplantation plus long term follow-up costs up to 20.000 EUR p.a.

1: Holmes et al., 2016; 2: ASN press release 27.06.2018
3: GKV_Kennzahlen_Booklet_Q4-2016_300dpi_2017-03-13; 56; USRDS Chapter 5: Acute Kidney Injury; 4: Bündnis Niere (2008) Mehr als nur Überleben, Dialysetherapie in Deutschland. Broschüre (http://www.diamed.de/files/1513/6923/1719/broschuere_niere_2008.pdf

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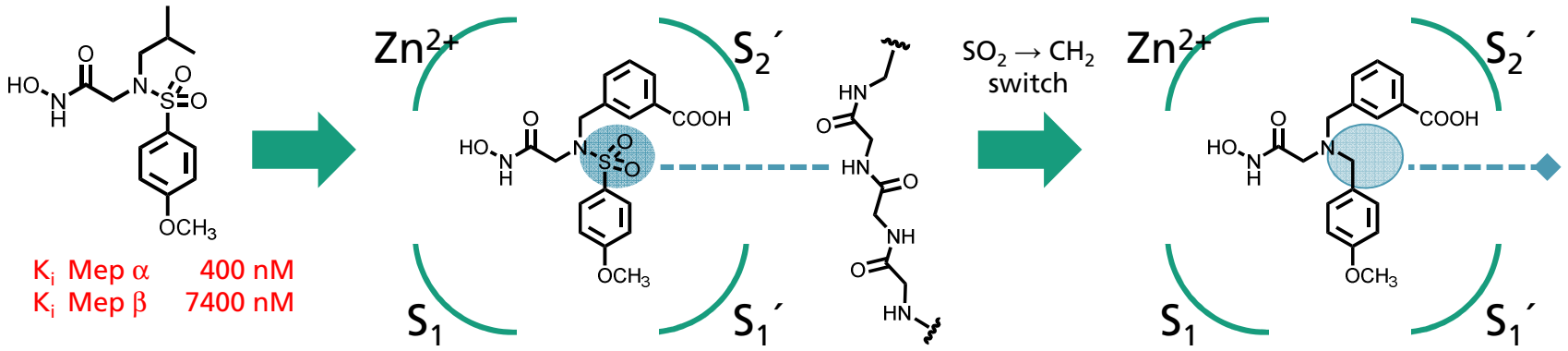
Meprin β program
Development of selective meprin β inhibitors

Starting point NNGH → **1st generation** → **2nd generation**



K_i Mep α 400 nM
K_i Mep β 7400 nM

MWT-S-00…	163	277
K_i Mep α [nM]	1340 ± 226	*IC_{50} 3.420 ± 325 nM*
K_i Mep β [nM]	22 ± 1	39 ± 0.2 nM
MMP2 RA @ 200µM [%]	<1 (IC_{50}=2 nM)	68
MMP9 RA @ 200µM [%]	2 (IC_{50}=49 nM)	85
MMP13 RA @ 200µM [%]	<1 (IC_{50}=0.3 nM)	94
ADAM10 RA @ 200µM [%]	2 (IC_{50}=11700 nM)	87
ADAM17 RA @ 200µM [%]	<1 (IC_{50}=1240 nM)	60

Indispensable hydroxamic acid Zinc-binding group → but sulfonamide group can get modified by replacing the sulfonyl group with a methylene group → substantial increase of selectivity

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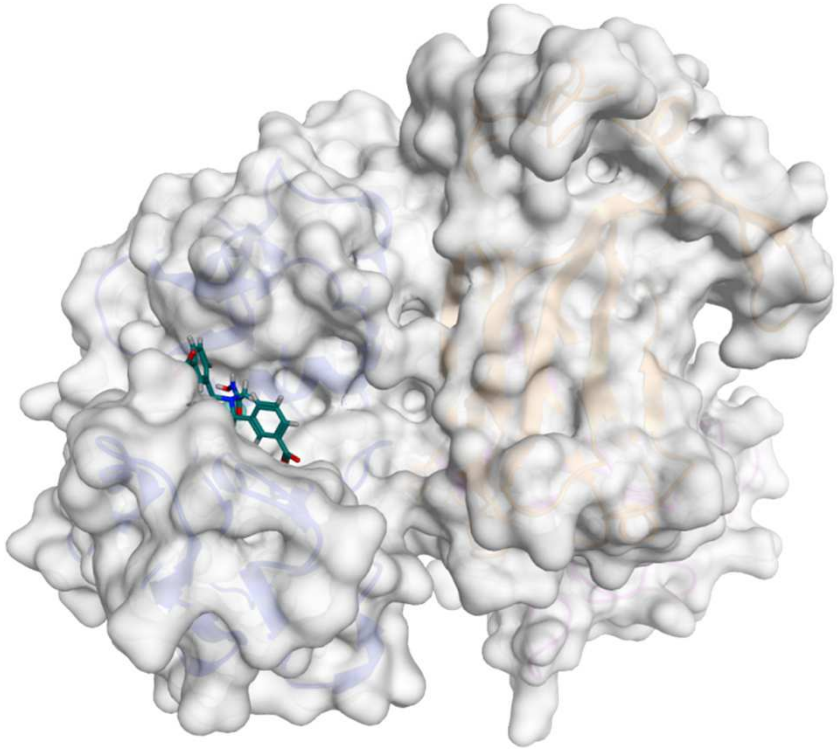
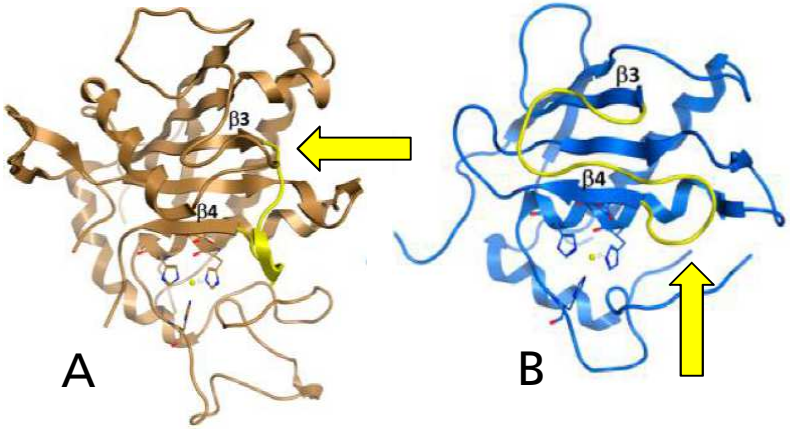
Meprin β program
Crystal structure

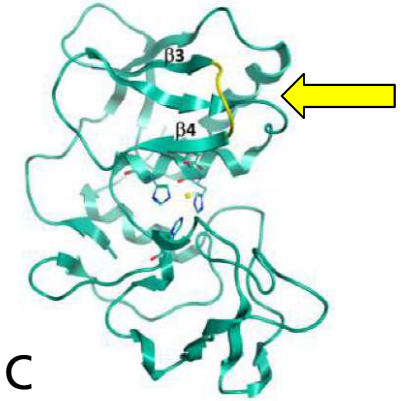


1st crystal structure of meprin β in complex with inhibitor solved → Critical step for substantial increase of meprin selectivity

S-shaped loop adjacent to the active site, bound inhibitors interact with this loop via hydrogen bonds



The loop in Meprin β was shown to be much smaller and cannot form such interactions



Structural comparison of ADAM17 (pdb code: 2DDF, A), MMP13 (pdb code: 3ZXH, B), and meprin β (pdb code: 4GWN, C), Rambsbeck et al., 2018

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Meprin β program
Lead MWT-S00270 – Profiling

Parameter	Value	Parameter	Value
K_i Mep α [nM]	**10410 ± 999**	F_{abs} p.o. PK, rat, 5 mg/kg	13%
K_i Mep β [nM]	**18 ± 3**	C_{max} p.o. PK, rat, 5 mg/kg	0,335 mg/l
Sol [µM]	**> 200**	T_{max} p.o. PK, rat, 5 mg/kg	0,17 h
Viability SY-5Y [%]	**97 (@ 100µM)**	$T_{½}$ p.o. PK, rat, 5 mg/kg	5h
Viability Hep-G2 [%]	**97 (@ 100µM)**	V_D p.o. PK, rat, 5 mg/kg	49,5 ml
MMP2RA @ 200 µM [%]	**64**	F_{abs} i.p. PK, rat, 10 mg/kg	**83,9%**
MMP9RA @ 200 µM [%]	**74**	C_{max} i.p. PK, rat, 10 mg/kg	**6,46 mg/ml**
MMP13 RA @ 200 µM [%]	**78**	T_{max} i.p. PK, rat, 10 mg/kg	**0,12 h**
ADAM10 RA @ 200 µM [%]	**93**	$T_{½}$ i.p. PK, rat, 10 mg/kg	**1,98 h**
ADAM17 RA @ 200 µM [%]	**76**	V_D i.v. PK; rat, 3 mg/kg	**0,073 L**
PPB (mice plasma, 5µM cpd)	**82,2%**	$T_{½}$ i.v. PK; rat, 3 mg/kg	**2,5 h**
EC50 (cellular assay, APP, EC50	**2 µM**	F_{abs} i.p. PK, mouse, 10 mg/kg	**94%**
CYP2C9 Inhibition (% RA@25µM)	**94%**	C_{max} i.p. PK, mouse, 10 mg/kg	**29,81 mg/ml**
CYP3A4 Inhibition (% RA@25µM)	**93%**	T_{max} i.p. PK, mouse, 10 mg/kg	**0,13 h**

No red flags detected yet

Fraunhofer IZI

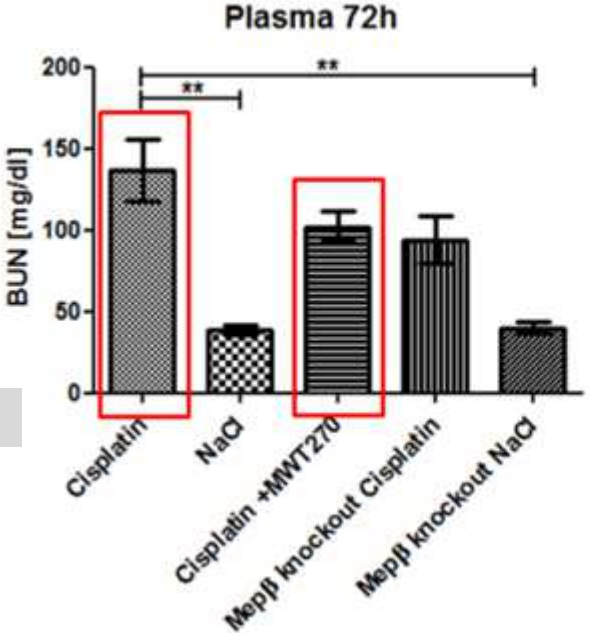
Meprin β program
PoP study cisplatin-induced kidney failure

Dosage MWT-270: 50 mg/kg i.p. every 12h, or 10/25/100 mg/kg; first dose 11h before cisplatin treatment

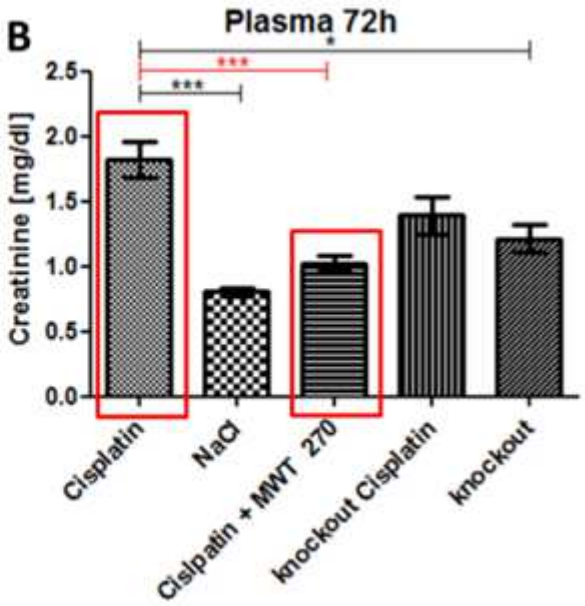
Dosage cisplatin: One dose i.p. 20mg/kg

NaCl: Control group

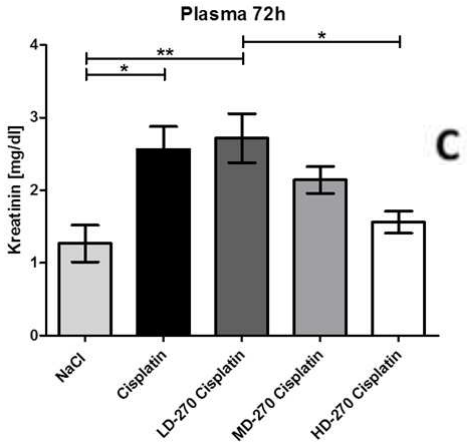
Blood Urea Nitrogen (BUN)



Creatinine



Dose-response-study, plasma creatinine



MWT-270 reduces BUN/creatinine levels in CP-mice, dose-dependency for serum creatinine levels demonstrated

Fraunhofer IZI

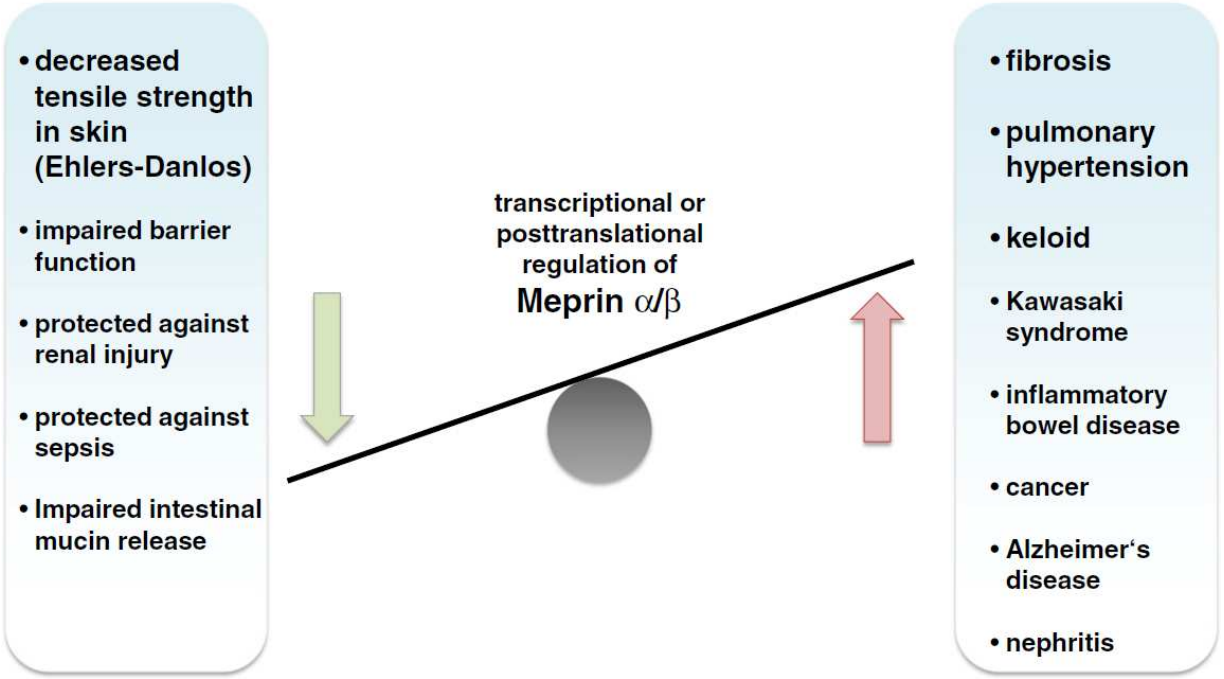
Meprin β program
Summary and Outlook

- There is a good rationale for meprin β playing a significant role in the pathogenesis of kidney diseases, fibrotic disorders, Alzheimer disease, cancer and IBD
- We successfully developed selective meprin β inhibitors and demonstrated PoP in an animal model of acute kidney injury*
- Based on promising in vivo data are we going to continue with the preclinical development of MWT-270 for treatment of AKI and other diseases including **fibrosis** (i.v. route intended)
- A recently discovered alternative scaffold class with extraordinary potency and enhanced physico-chemical properties is going to be developed for oral application and further indications
- We seek industry partners for further joint development

* Schlenzig et al., 2015; Ramsbeck et al., 2017; Ramsbeck et al., 2018; Tan et al., 2018; Schulze et al., 2018; Schlenzig et al., 2018

Fraunhofer IZI

Meprins and other Astacin Proteases: Attractive Targets for Drug Discovery



Prox et al., Matrix Biology 2015; 44-46, 7-13

Meprin: Endoproteases involved in cytokine activation and procollagen/collagen cleavage
Fraunhofer IZI – development of first selective inhibitors

Fraunhofer IZI