DESIGN AND DISCOVERY OF NOVEL CATHEPSIN S LIGANDS AND THEIR EVALUATION AS POSITRON EMISSION TOMOGRAPHY AND FLUORESCENT PROBES FOR ONCOLOGY DIAGNOSIS

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Problem

?

- Human cathepsin S (**CatS**) is a cysteine protease
- Endopeptidase activity only
- Notably active at neutral pH
- Involved in physiological and pathophysiological processes
- Normally located in lysosomes and cell membrane
- Overexpressed in oncological and inflammatory diseases
- Found also in the extracellular matrix (**ECM**) in pathologies
- Remodeling into the tumor microenvironment (**TME**) [1]
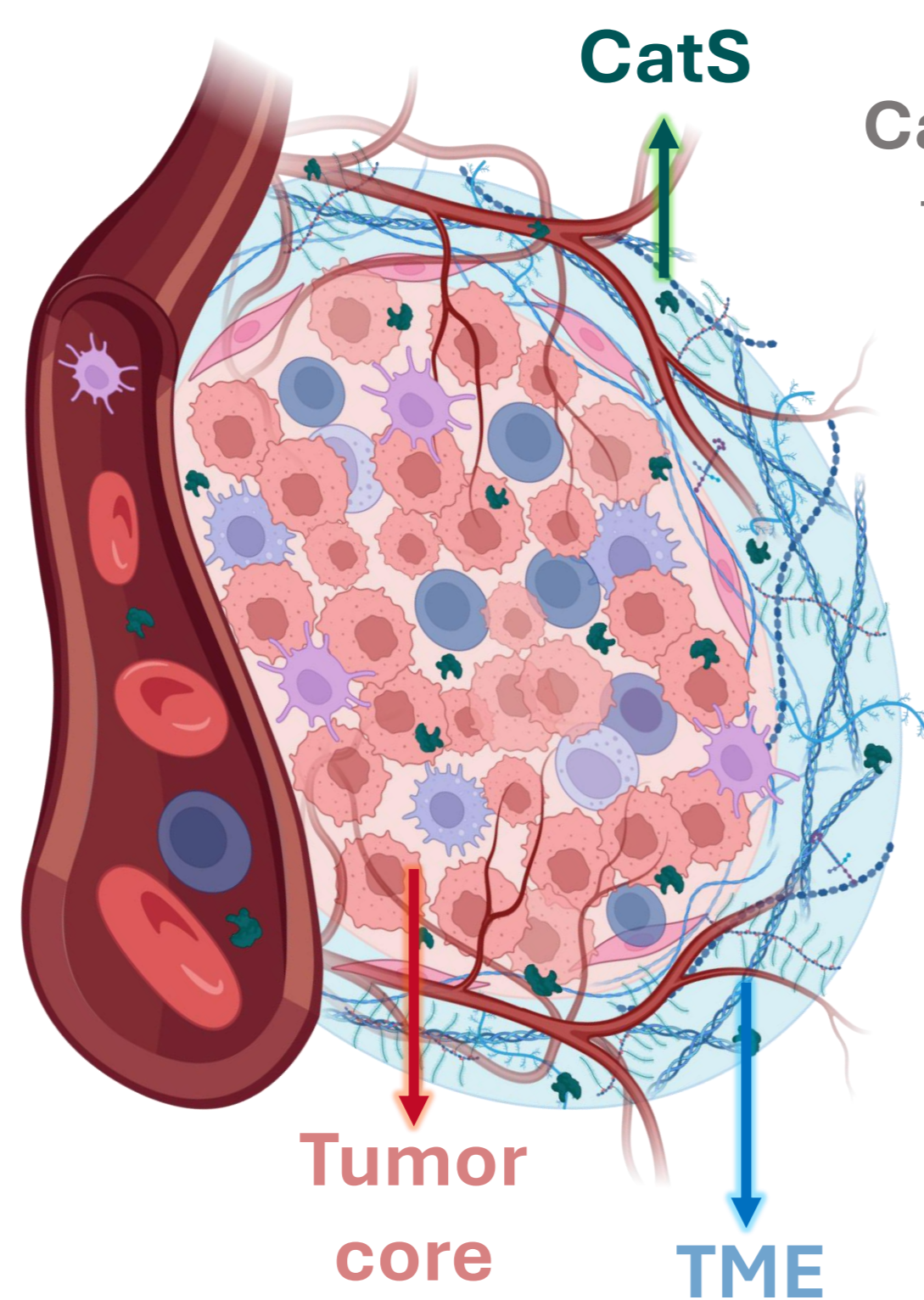
CatS
Tumor core
TME

CatS' role in (early) tumorigenesis?

Intra- and extracelullar localizations?

Relevance in prognosis and treatment?
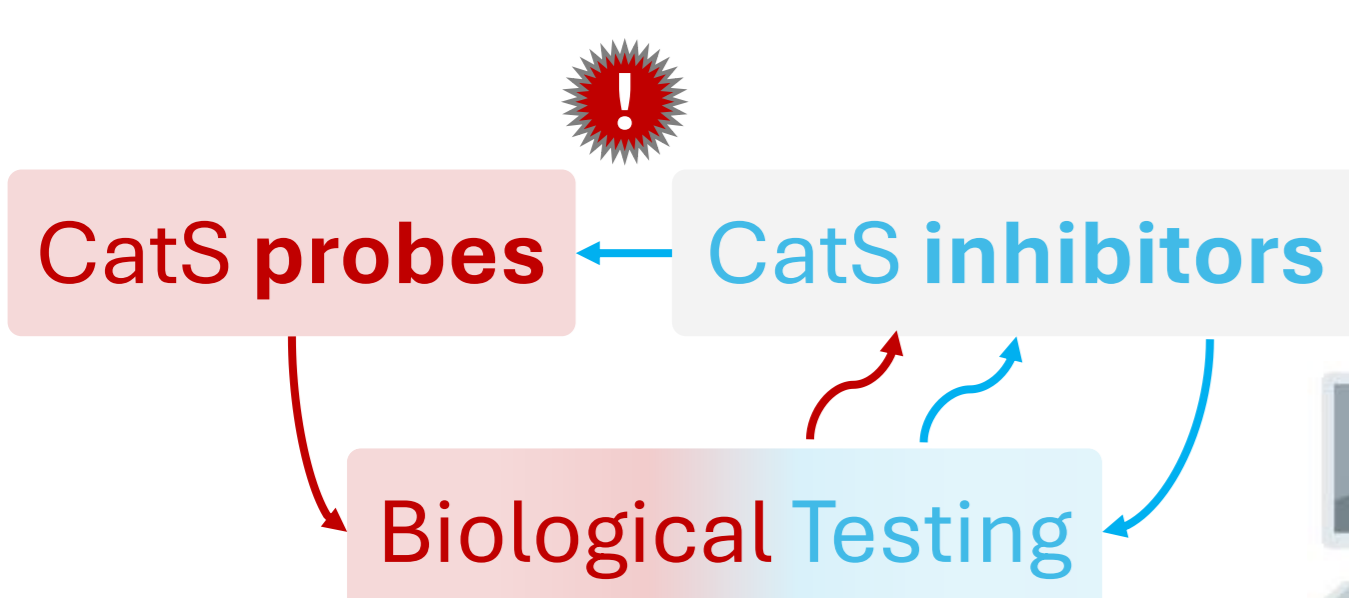
Diagnostic and therapeutic applications?

Approach

!

- **CatS** silencing or inhibition correlates with anti-tumor response [1]
- Diversity of in-house and published inhibitors [2,3]

H_2N— ... X = F (reversible, Ki 9.0 ± 2 nM, $SI_{CatB,L/S}$>1E3) or H (irreversible, to be determined)

- Flexible wide S2 (lock) [2]
- Solvent-exposed P1' and P3 [2]
- Benchmarks avaiable

P3 P1 P2 P1'
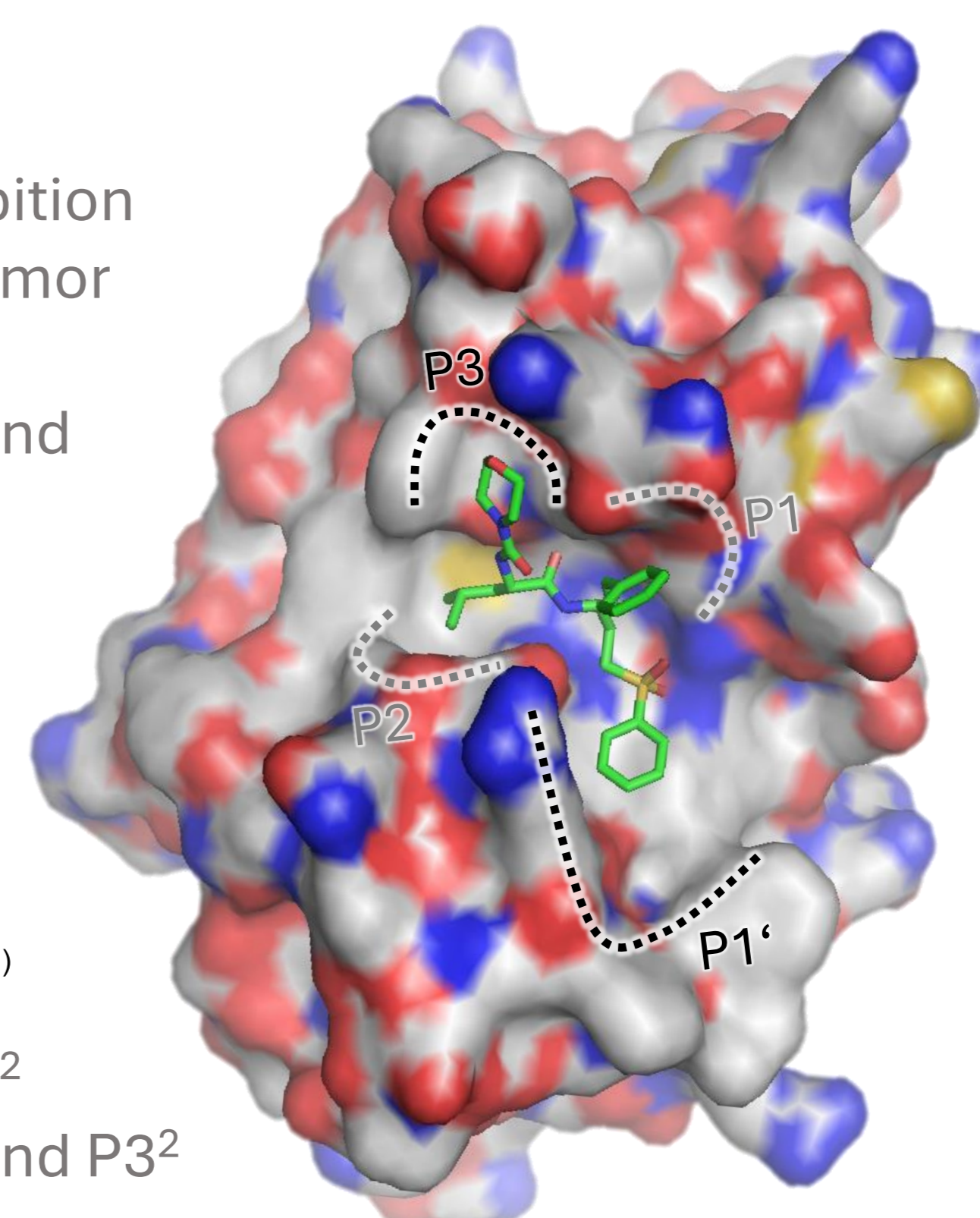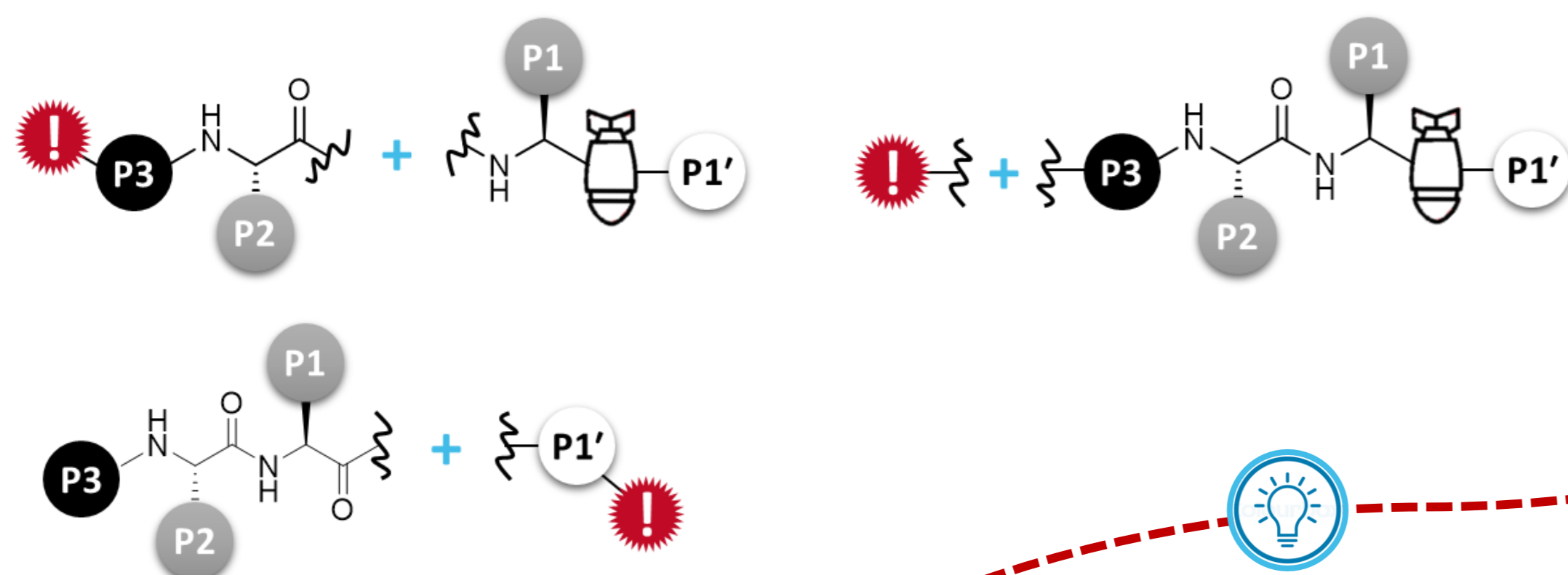
Selectivity over other cathepsins (B, L, K...)

Blood, lysosomal, ECM and TME stability

Cell permeability

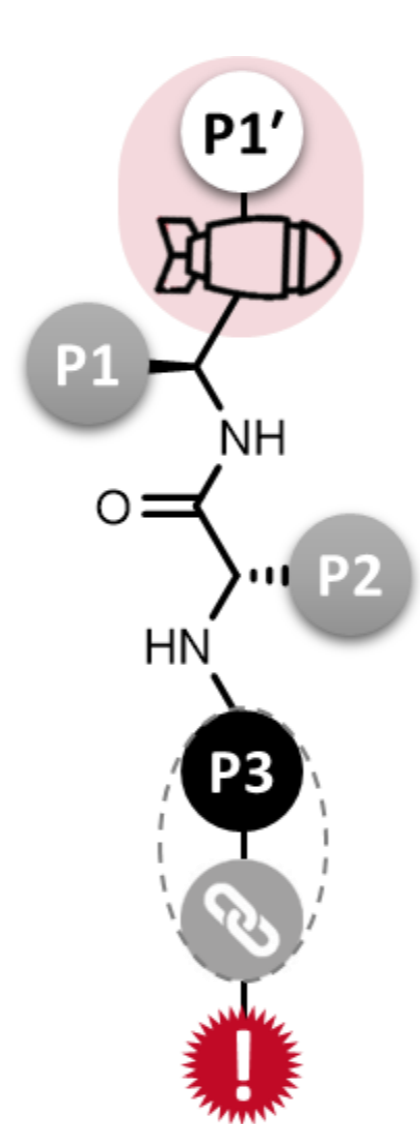Physicochemical, Pharmacokinetic and toxicological behavior
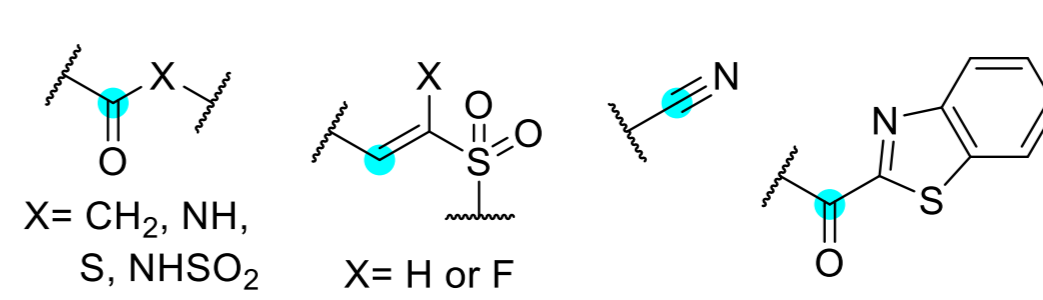
Deliver to disease-site

Design and Discovery

CatS probes ⇄ CatS inhibitors
Biological Testing

Synthesis in silico in vitro Optimize

CatS_I = Inhibitor

Optimization of the synthetic assembly possibilities

Probe

P1'
P1 NH
O
P2
NH
P3

Tuned reactive Warhead [3]
X= CH₂, NH, S, NHSO₂; X= H or F

Slow — Fast
Irreversible — Reversible
Non-covalent — Covalent
Inhibition mode

Peptidomimetic recognition unity [2]

Target specificity (complementarity) → **Selectivity**

Linker moiety [1,2]

Stability without compromising activity
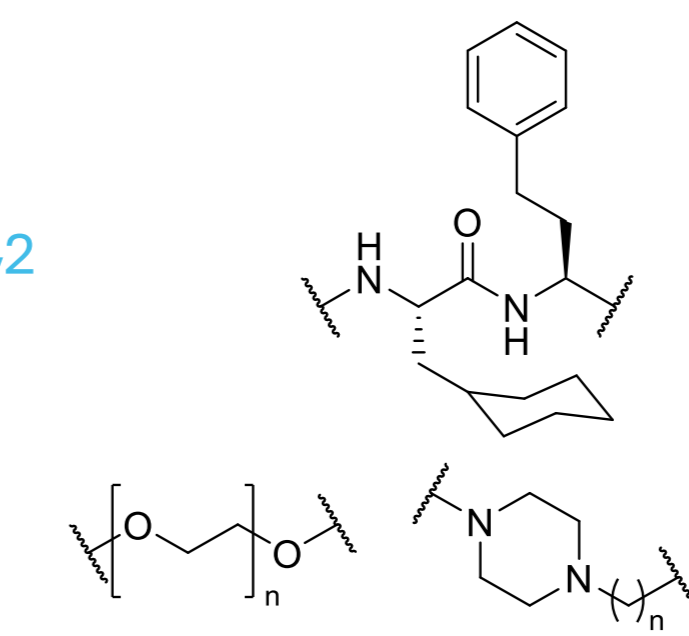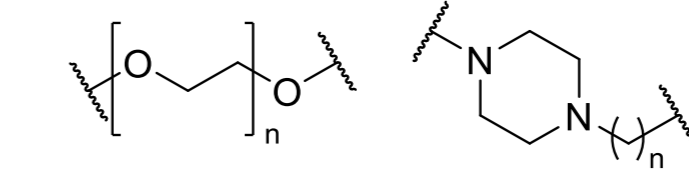
Tag [4]
Defines the kind of output or readout
X = CH₂, CO

Cy5

DOTA

Biotin

(Strept)avidin

99mTc SPECT | 68Ga PET | 177Lu Endoradiotherapy

Applications

Secondments

Collaborations

Flu

E.g., mouse with tumor

PET

Molecular tools

B7 (H)

Homing

Benchmark:
- Antibodies
- 2-step activity-based probes [4]

CellCarta

Testing

Immortalized cancer-associated fibroblasts

- FACS
- Blotting
- Cell and tissue staining

in vitro & ex vivo
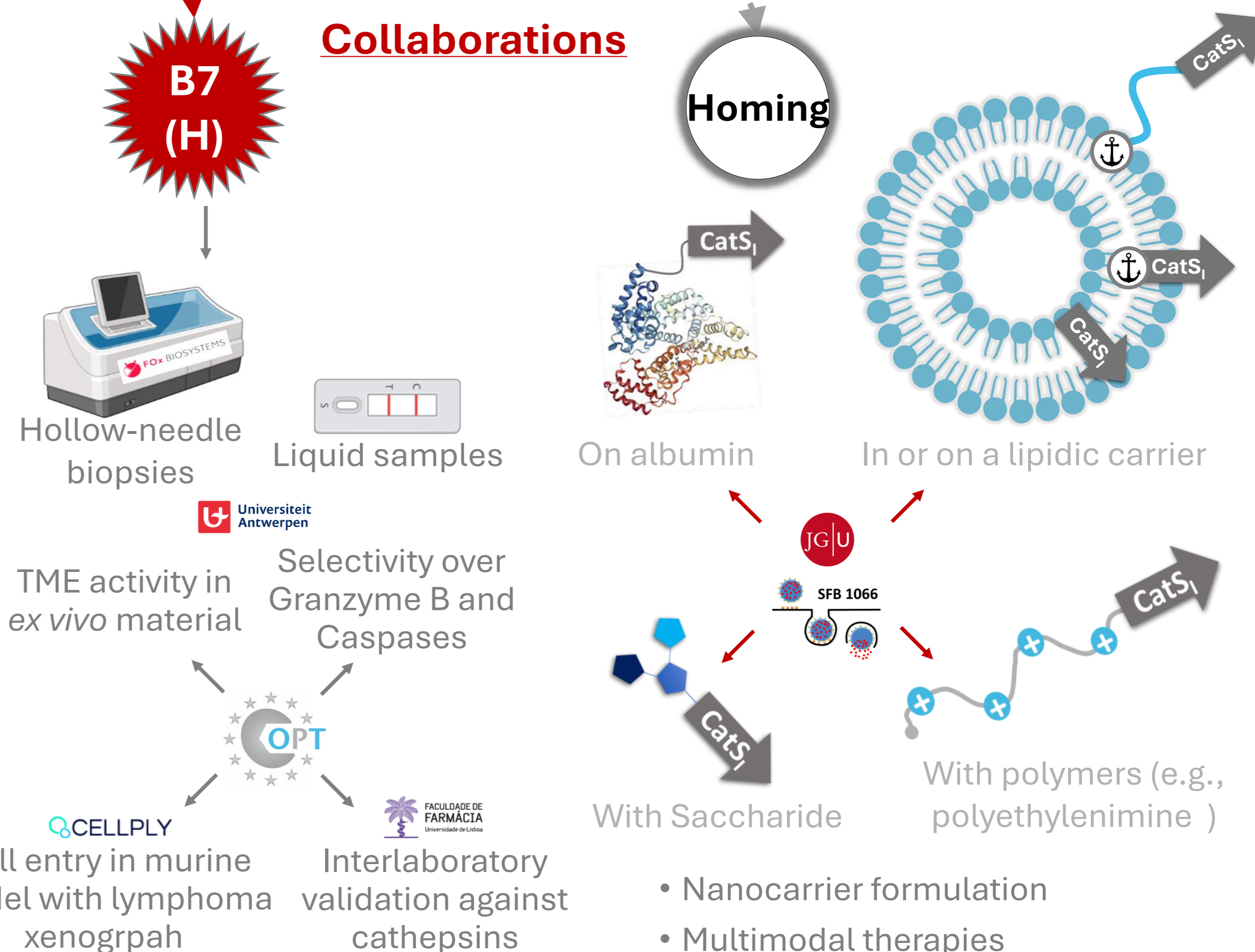p38 MAPK > TME proteases

Tumor sample

in vivo, GMP
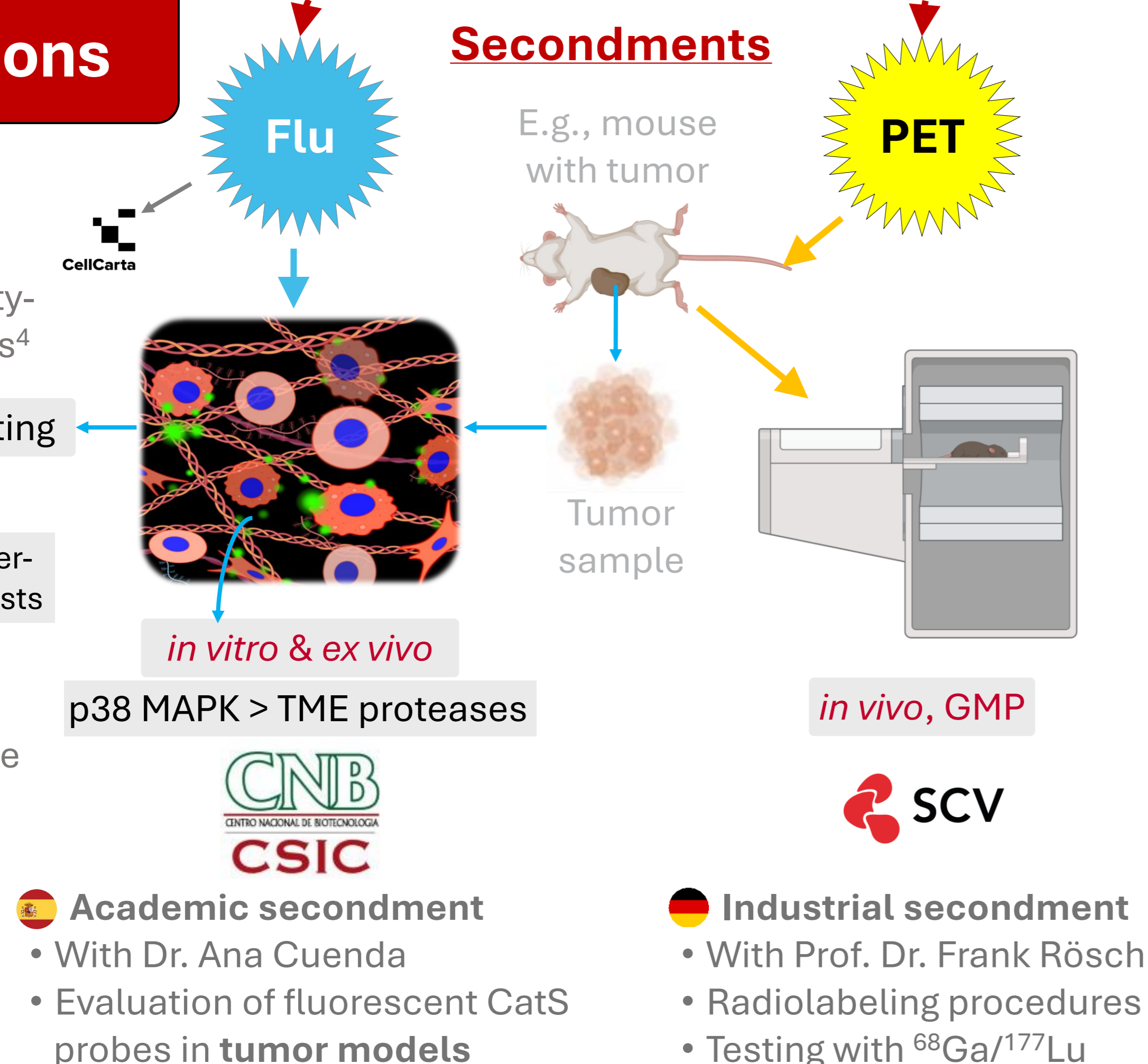
Diagnosis

Therapy

Theranostics

Hollow-needle biopsies

Liquid samples

TME activity in *ex vivo* material

Selectivity over Granzyme B and Caspases

On albumin

In or on a lipidic carrier

CatS_I

SFB 1066

With Saccharide

With polymers (e.g., polyethylenimine)

- Nanocarrier formulation
- Multimodal therapies

🇪🇸 **Academic secondment**
- With Dr. Ana Cuenda
- Evaluation of fluorescent CatS probes in **tumor models**

CNB CSIC
u UNIVERSITÄT BERN

🇩🇪 **Industrial secondment**
- With Prof. Dr. Frank Rösch
- Radiolabeling procedures
- Testing with 68Ga/177Lu

SCV

Universiteit Antwerpen

CELLPLY
Cell entry in murine model with lymphoma xenogrpah

OPT

FACULDADE DE FARMÁCIA
Interlaboratory validation against cathepsins

References

1. Fuchs & Meta *et al. Cells.* **2020** 9, 2021
2. Fuchs & Meta *et al. ChemMedChem.* **2023**, 15
3. Müller & Meta *et al. Int. J. Mol. Sci.* **2023** 24, 7276
4. van Dalen *et al, Front. Chem.*, **2021** 8

Figures created with MS Power Point, MS Paint 3D BioRender.com and ChemBioDraw Ultra.