Kersten Lab

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Institute of Pharmaceutical and Biomedical Sciences

IQCB Institute for Quantitative & Computational Biosciences

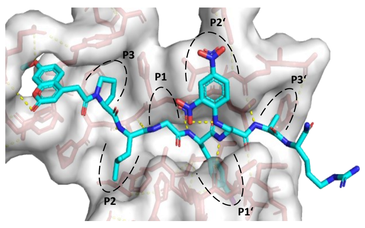
JG|U

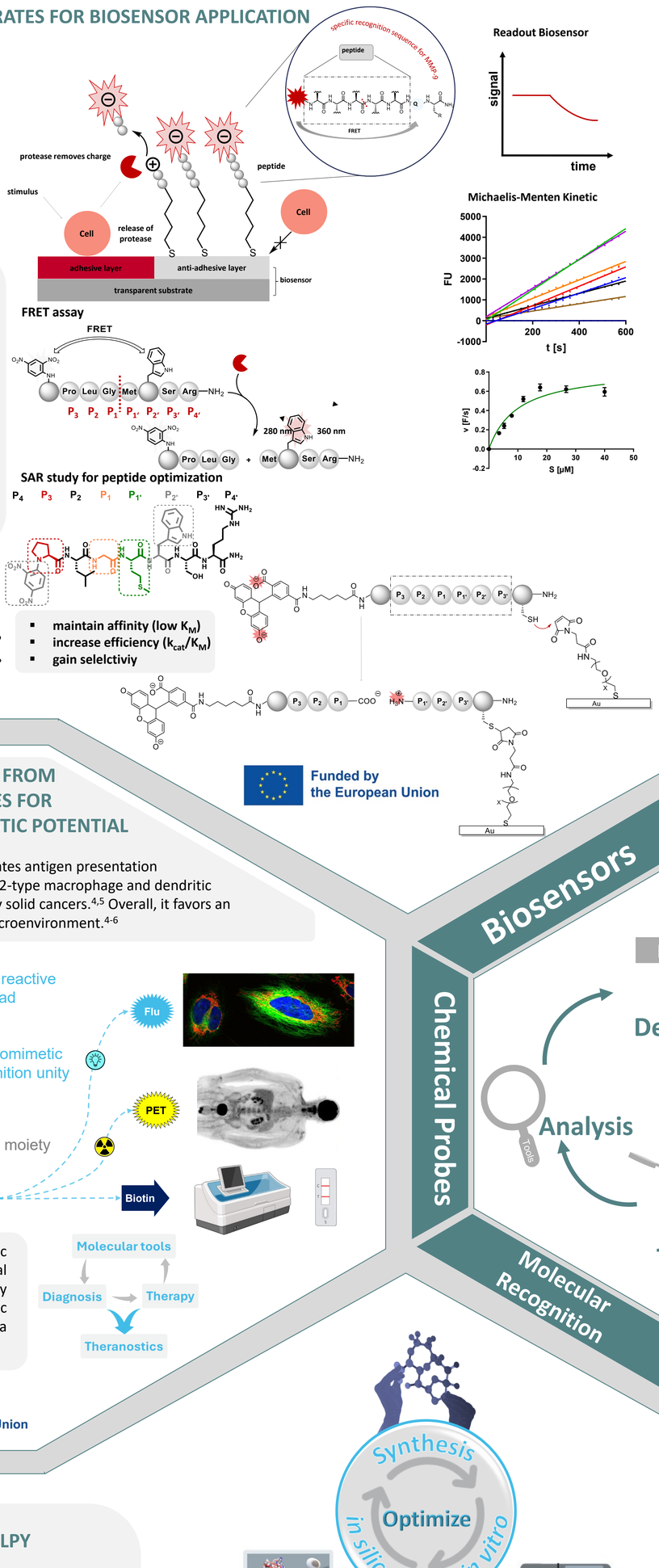
IPBW Institut für Pharmazeutische und Biomedizinische Wissenschaften

While rooted in the field of **Pharmaceutical/Medicinal Chemistry**, our work can be described best as **Molecular and Computational Biophysics**. We use, modify and combine computational tools with wet lab binding studies for medicinal chemistry applications. Here we identify and improve ligands and inhibitors of potential therapeutically relevant drug targets.[1-3] For selected model systems, we dissect binding events into details to not only understand binding **affinity**, but also binding **kinetics** and **thermodynamics** – always closely linked to **molecular interactions**.[4-7] This basic research helps to improve our understanding of molecular recognition and allows us to develop better computational models. While most current drugs target proteins, our current work focuses on the exploration of **RNA as a druggable target**.[8] This approach will open up novel treatment opportunities beyond the state of the art.

PEPTIDE-BASED PROTEASE SUBSTRATES FOR BIOSENSOR APPLICATION

Peptide Design





Protease detection can be achieved by peptides immobilized on a biosensor surface via C-terminal anchoring points, containing N-terminal fluorogenic charge carriers and specific protease recognition sequences. Computer-aided drug design and molecular docking optimize literature-known substrates to model affinity and characterize molecular interactions. FRET-based protease substrates for matrix metalloproteinase 9 (MMP-9) are then designed, synthesized, and analyzed for enzyme affinity and activity using a fluorometric assays.

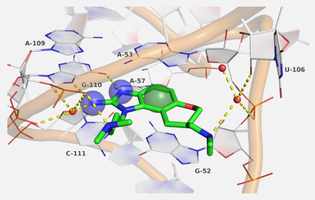
- maintain affinity (low K_M)
- increase efficiency (k_{cat}/K_M)
- gain selelctiviy

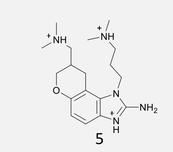
MultiSensE

MUNASET PART OF THE GRAPHENE FLAGSHIP

Funded by the European Union

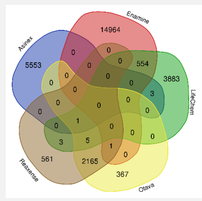
VIRTUAL SCREENING (VS)

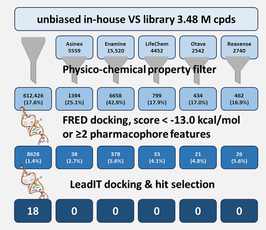




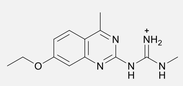
5

K_D=5.2±0.8 µM (MST)
EC_{50}=4.9±2.6 µM (FRET)

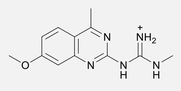




HCV IRES VS [9]
Hit compounds +results:



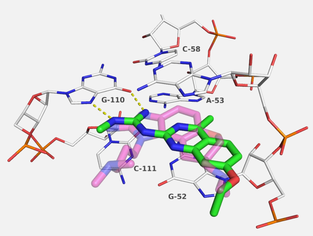
413 ± 97 µM EC_{50} (FRET)
≥440 µM K_D (MST)



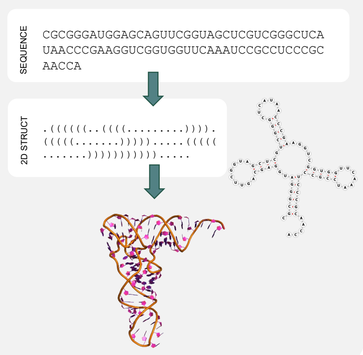
61 ± 19 µM EC_{50} (FRET)
88 ± 2 µM K_D (MST)

>10 fold weaker as **5, but:**
- Fragment-like size (HAC 18)
- LE 0.31 kcal mol-1
- Moderate lipophilicity
- Reduced basicity
- Selectivity

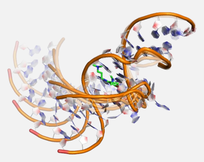
→ Good starting point for optimization
→ Proof of concept SBVS RNA targets

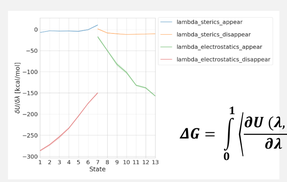


HOMOLOGY MODELING



MOLECULAR DYNAMICS (MD)





$$\Delta G = \int_0^1 \left\langle \frac{\partial U(\lambda, x)}{\partial \lambda} \right\rangle_\lambda d\lambda$$

Carl Zeiss Stiftung

PROTEASE COVALENT INHIBITION: FROM IMMUNOMODULATORS TO PROBES FOR CANCER BIOLOGY AND THERANOSTIC POTENTIAL

The cysteine protease cathepsin S (CatS) regulates antigen presentation in antigen presenting cells (APC), promoting M2-type macrophage and dendritic cell polarization. CatS is overexpressed in many solid cancers.[4,5] Overall, it favors an immuno-suppressive and tumor-promoting microenvironment.[4-6]

Research on protease inhibitors for therapeutic applications can be derived into chemical probes for cancer and inflammation biology studies. Later, these can foster novel diagnostic and therapeutic applications, ultimately, in a theranostic approach.

OncoProTools

Funded by the European Union

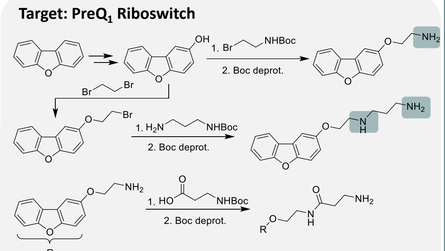
ISIDORe

eu:openscreen

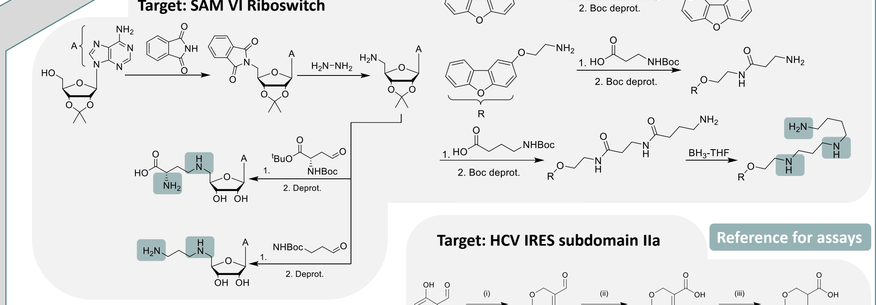
ORGANIC SYNTHESIS OF SMALL MOLECULES

Understanding impact of charge on affinity, selectivity and binding kinetics

Target: $PreQ_1$ Riboswitch



Target: SAM VI Riboswitch

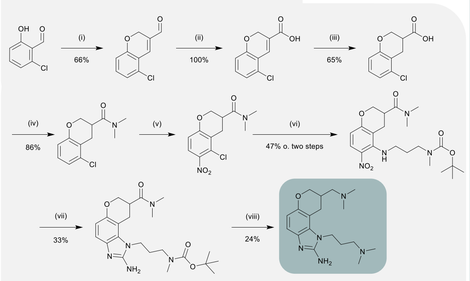


In Progress:

Target: SAM I Riboswitch
- SAR Analysis of Virtual Screening Hits
- Covalent ligands

Target: HCV IRES subdomain IIa

Reference for assays



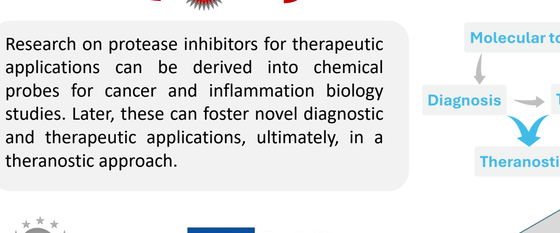
Reagents and conditons: (i) Acrolein, DABCO, dioxane, 95 °C; (ii) silver nitrate, NaOH, EtOH/H2O, 85° C; (iii) Na/Hg, NaOH/H2O, room temperature (rt); (iv) EDC HCl, HOBt, dimethylamine hydrochloride, N-Methyl morpholine, dichloromethane, rt; (v) sodium nitrate, trifluoro acetic acid, dichloroacetic acid, 0 °C-rt; (vi) N-(3-aminopropyl)-N-methylcarbamate, 1-methyl-2-pyrrolidinone, 75 °C; (vii) H2 Pd/C, EtOH, then cyanogen bromide/EtOH, rt; (viii) LAH, THF, 60 °C.

ENTROPY vs. ENTHALPY

Target-ligand interactions are complex. The free binding energy is the sum of entropic and enthalpic contributions:

$$\Delta G = \Delta H - T\Delta S$$

The entropic contributions to the binding energy can be optimized by macrocyclization of flexible ligands. By rigidizing the molecule, the degrees of freedom are reduced and a tighter bond to the target is achieved. If the binding pocket can now be better filled by the ligand and water is displaced, this can be entropically favorable.
Enthalpically favorable interactions can be lost in the process, so that it is necessary to consider which optimization leads to an actual improvement in the free bond energy.

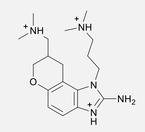


CONFORMATIONAL CHANGES

Both the target and the ligand are flexible and can adopt different conformations. These conformational changes can occur when the ligand binds due to interactions with it.

FÖRSTER RESONANCE ENERGY TRANSFER (FRET)

When a donor (Cy3) is excited and an acceptor (Cy5) is in close proximity, a non-radiative energy transfer occurs, leading to an increased emission of the acceptor. Conformational changes caused by a ligand can be detected.



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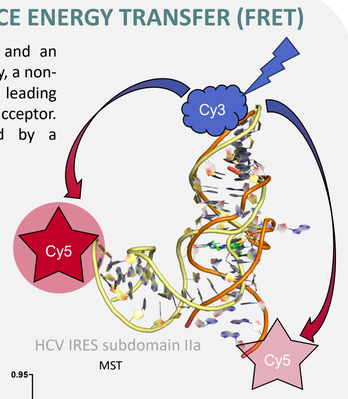
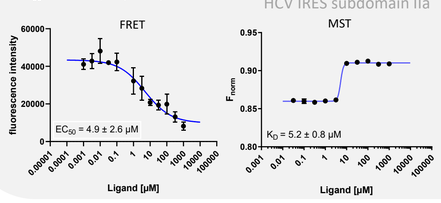
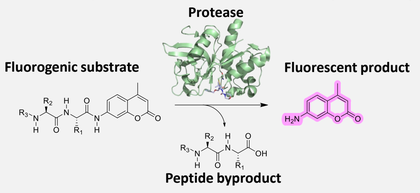




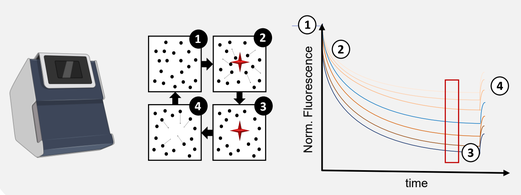
FLUOROMETRIC ACTIVITY ASSAY

Enzyme activity can be measured by the cleavage of a fluorogenic substrate. The same assay can be used to investigate the effect of other substances (like small molecules) over the enzyme activity.



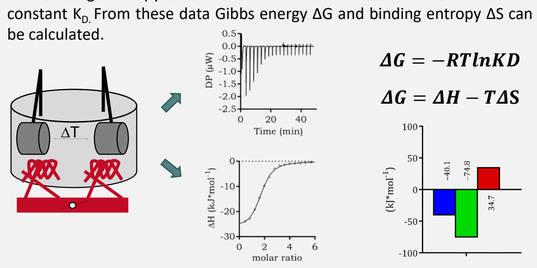
MICROSCALE THERMOPHORESIS (MST)

This method is used to determine binding affinity based on thermophoresis in a temperature gradient. Binding of a ligand, for example a small molecule to a labeled target molecule leads to a change in thermophoretic behavior which is dependent on size, charge, hydration shell and conformation.



ISOTHERMAL TITRATION CALORIMETRY (ITC)

During titration of a ligand to a target molecule the temperature change ΔT in the target cell compared to a reference cell is measured. It correlates with binding enthalpy ΔH and allows the determination of the dissociation constant K_D. From these data Gibbs energy ΔG and binding entropy ΔS can be calculated.



$$\Delta G = -RT\ln KD$$

$$\Delta G = \Delta H - T\Delta S$$

SELECTED PUBLICATIONS

[1] Zimmermann, R. A. et al. Int. J. Mol. Sci. 2023, 24 (7), 6109.
[2] Wettstein, L. et al. Commun. Biol. 2022, 5 (1), 681.
[3] Kersten, C. et al. J. Chem. Inf. Model. 2023, 63 (7), 2218–2225.
[4] Hammerschmidt, S. J. et al. Arch. Pharm. (Weinheim). 2023, 356 (4).
[5] Hammerschmidt, S. J. et al. RSC Med. Chem. 2023, 14 (5), 969–982.
[6] Johé, P. et al. J. Biol. Chem. 2021, 296, 100565.
[7] Kersten, C. et al. J. Med. Chem. 2020, 63 (5), 2095–2113.
[8] Kallert, E. et al. J. Chem. Inf. Model. 2022, 62 (17), 4134–4148.
[9] Kallert, E. et al RSC Med. Chem. 2024, 15, 1527-1538.

Rheinland-Pfalz Ministerium für Wissenschaft und Gesundheit

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