Exploiting HTRF for novel drug classes: Stabilizing 14-3-3 protein-protein interactions

The Lead Discovery Center (LDC)

- The Lead Discovery Center (LDC) was established in 2008 by the technology transfer organization of the Max-Planck Society, as a novel approach to capitalize on the potential of basic research for the discovery of new therapies for diseases with high medical need.

- As an independent company with an entrepreneurial outlook, the LDC closely collaborates with research institutions, universities and industry. Our aim is to transform promising early-stage projects into innovative pharmaceutical leads that reach initial proof-of-concept in animals.

LDC's drug discovery network

MAX-PLANCK-GESELLSCHAFT

- 80 institutes
  16.900 employees < 6.600 scientists
- >12.000 publications p.a.; 32 nobel laureates
- additional 7.700 young & guest scientists
- ~40 institutes with life science (biomedical) oriented research programs
- €1.73 Bio. annual research budget
- central tech transfer unit:

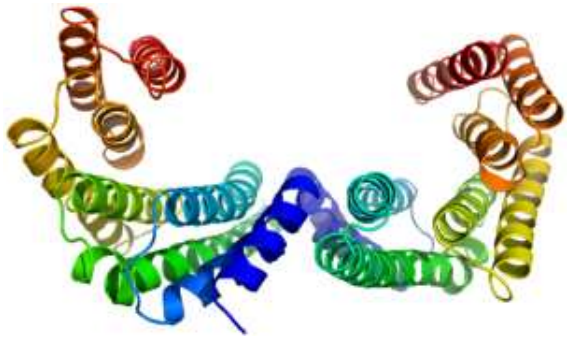
Max-Planck-**Innovation**

3.200 inventions
1.900 contracts
90 spin-offs

★ Max-Planck Institute
★ Academic Collaboration Partner
★ Pharma/Biotech Network

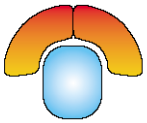
14-3-3 proteins

- ubiquitious in eucaryotes
- 7 isoforms in humans and higher plants
- highly conserved primary sequence
- physiological activity is mediated by direct protein-protein interactions
- key-regulators of signal transduction, cell cycle control, apoptosis, primary metabolism ….
- more than 500 interacting proteins described

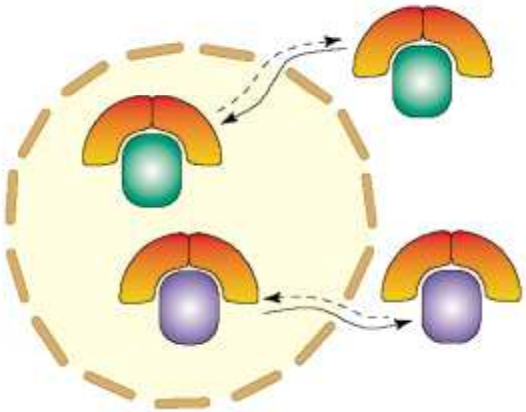


Modulation of the target activity by 14-3-3

1) enzymatic activity

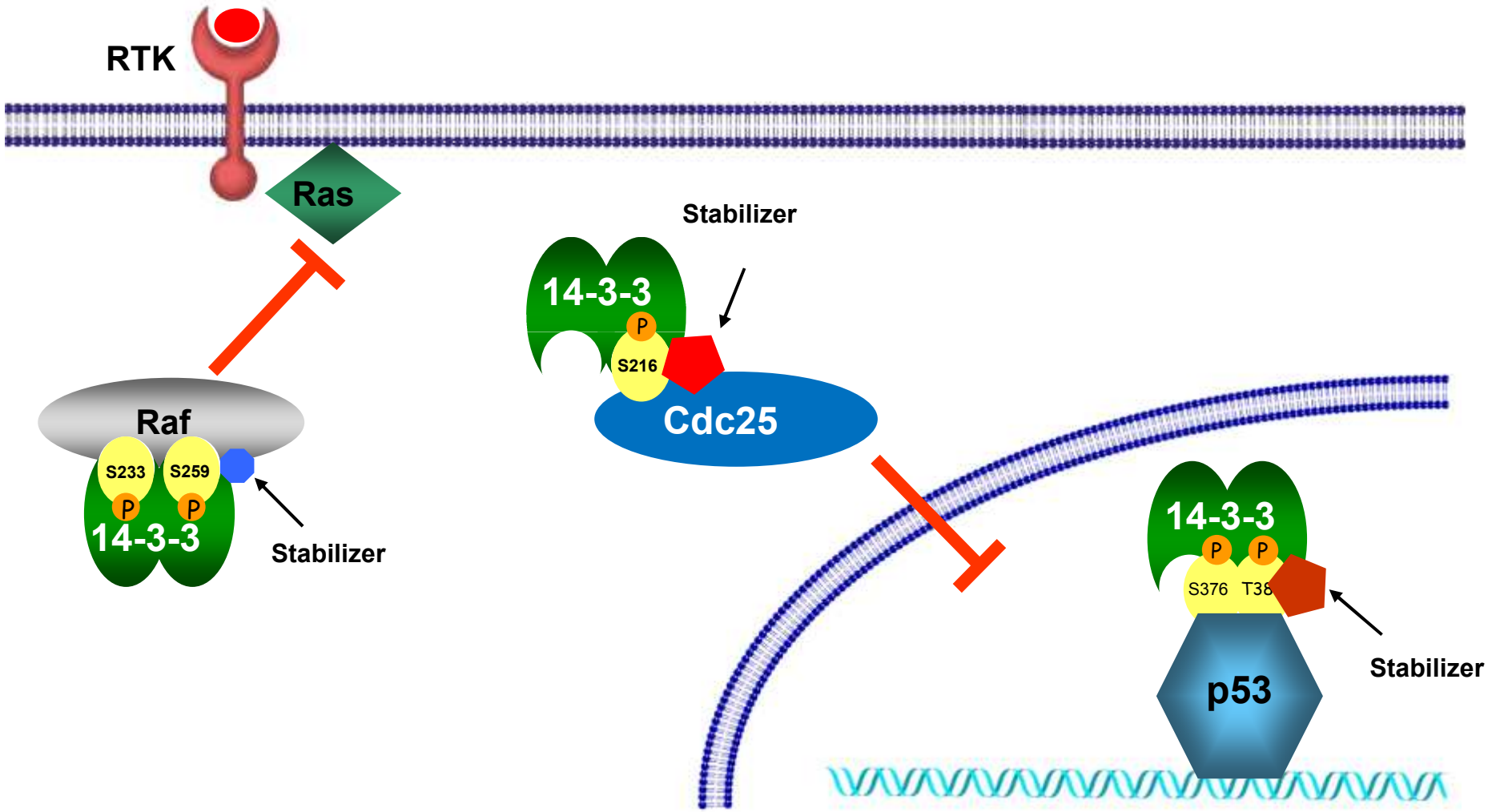


2) subcellular localization

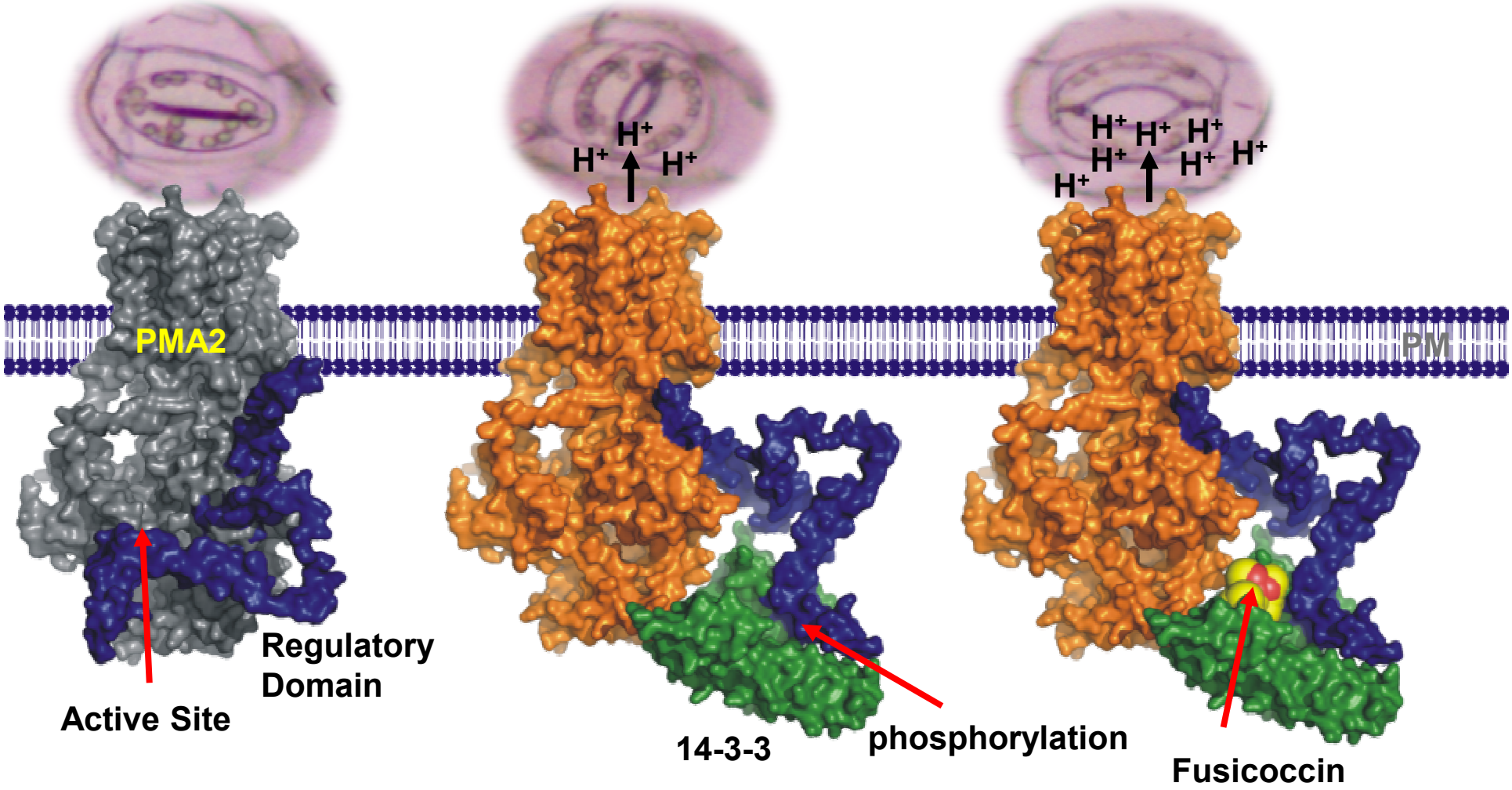


3) ability to interact with further protein partners

Why stabilize the 14-3-3 interaction



Nature‘s example:
Activation of the H^+-ATPase PMA2 by Fusicoccin

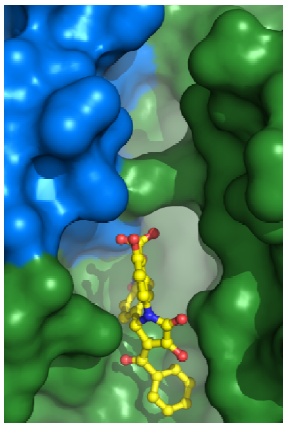


Structures courtesy by Christian Ottmann

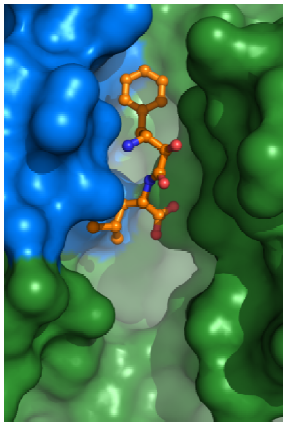
A feasibility study

Lead Discovery Center LDC

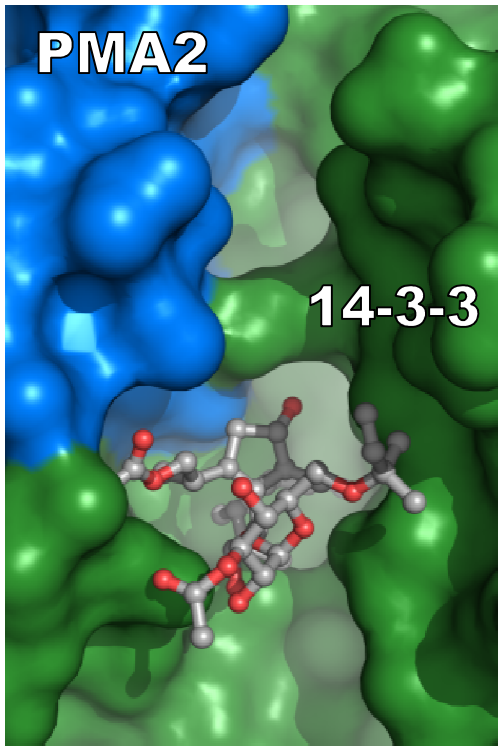
Pyrrolidone1

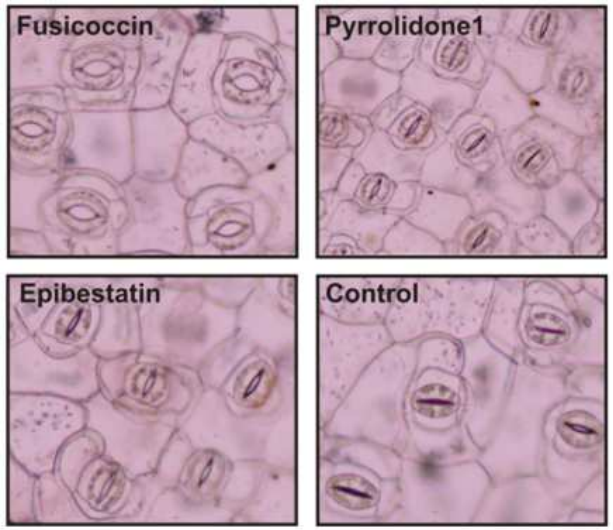


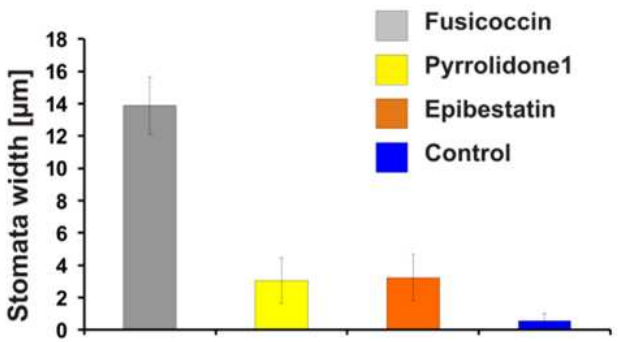
Epibestatin



Fusicoccin







Rose, R. et al: Angewandte 2010, *386*, 913-919.

HTS formats for PPI assays

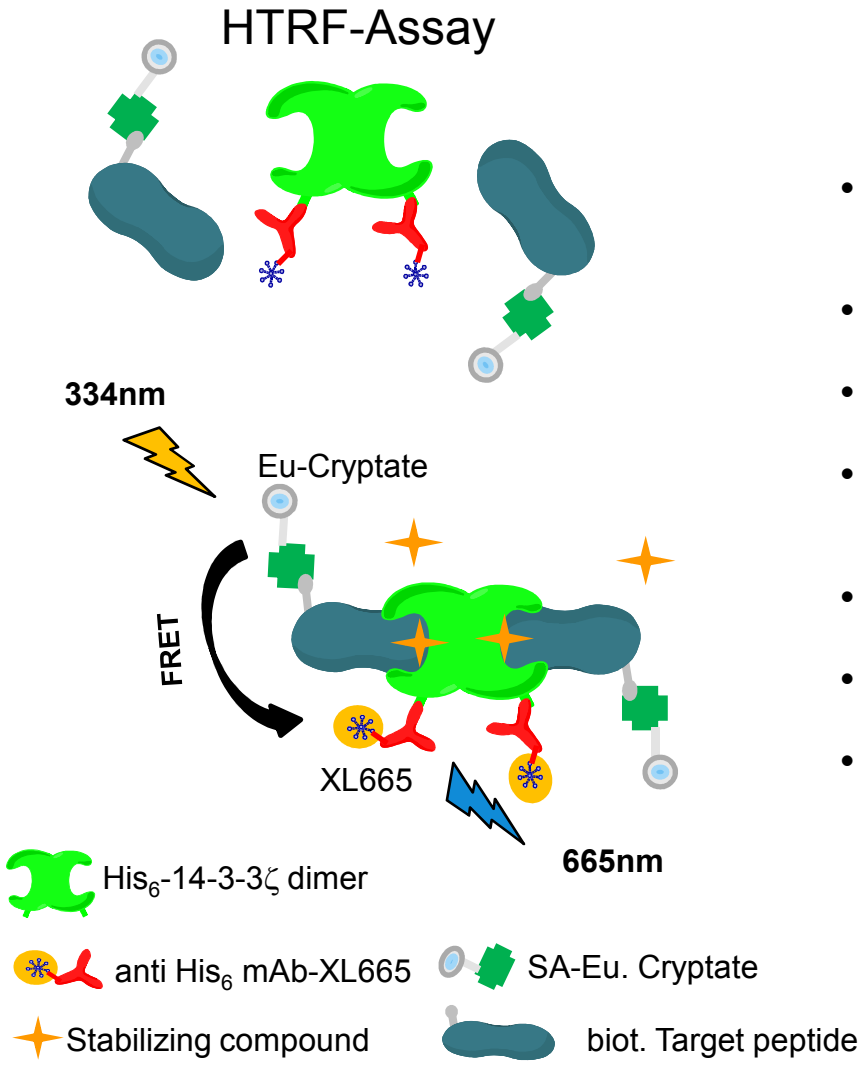
Prerequisites

- **Homogeneous („mix and read“)**
- **Sensitivity**
- **Miniaturizable to 384well and 1536well format**
- **Compatible to Screening-Hardware**

Common assay formats

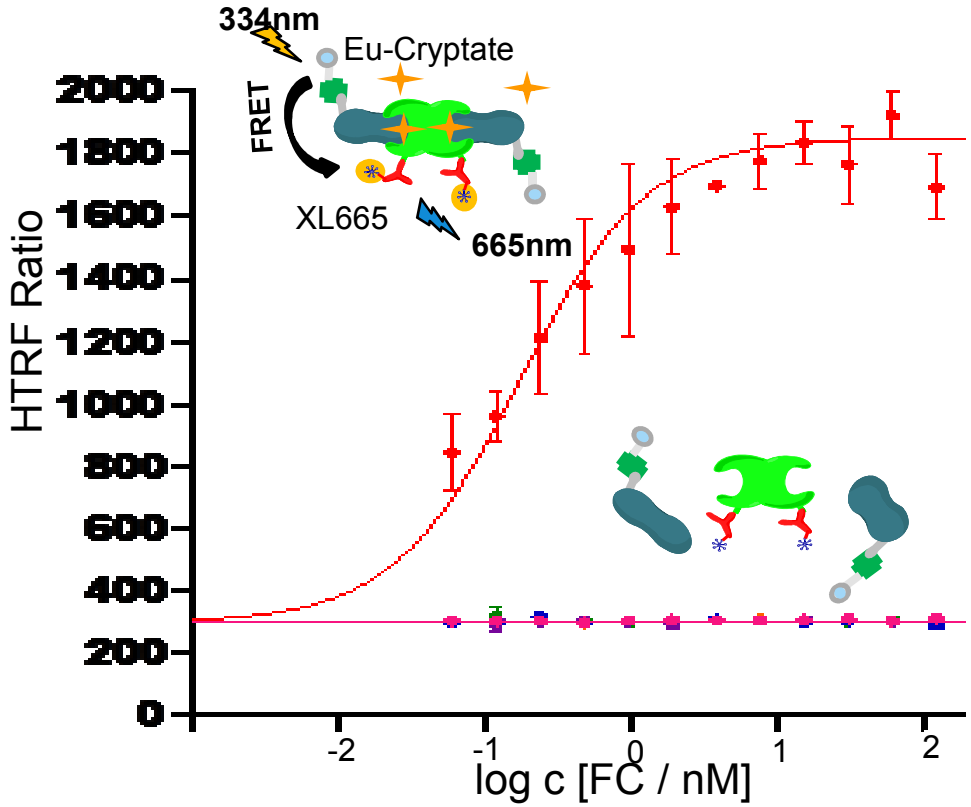
- **Fluorescence Polarization (FP)**
- **α-Screen**
- **Homogeneous Time Resolved Fluorescence (HTRF)**

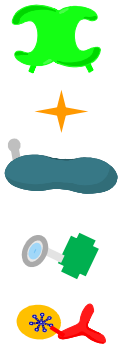
Assay development: Our choice HTRF



- **HTRF toolbox reagents give a good flexibility for assay development**
- **Signal stability**
- **Sensitive readout in the far red region (665nm)**
- **Donor Em. at 620nm can be used as an internal reference**
- **Ratiometric readout (665/620nm)**
- **More than one distributor**
- **Good support (trouble shooting)**

Assay development: HTRF assay for PMA2





His_6-14-3-3ζ dimer

Fusicoccin

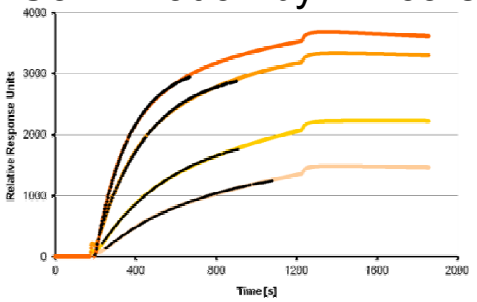
biot. PMA2 peptide

Strep.-Eu. Cryptate

anti His_6 mAb-XL665

- **HTRF assay performed for: 14-3-3 / PMA2 ± Fusicoccin**
- **K_d: < 3 nM**
- **Confirmation by BIAcore: K_d: 0.85 nM**

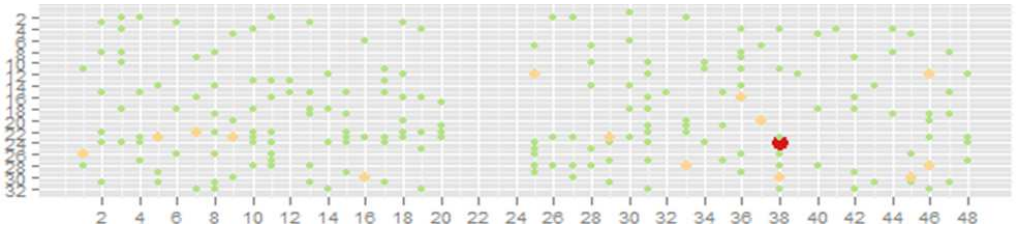
Confirmation by BIAcore

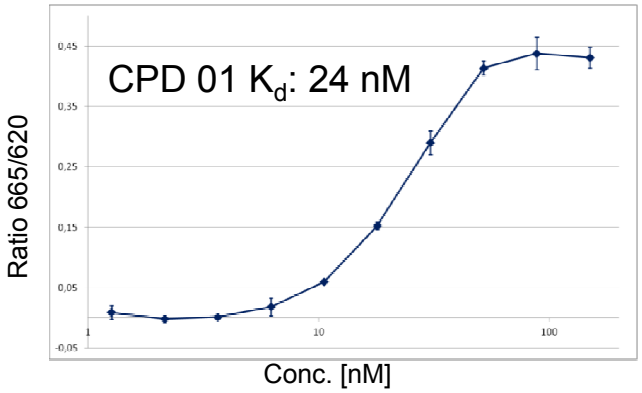


Assay development:
Adaptation to screening plattform

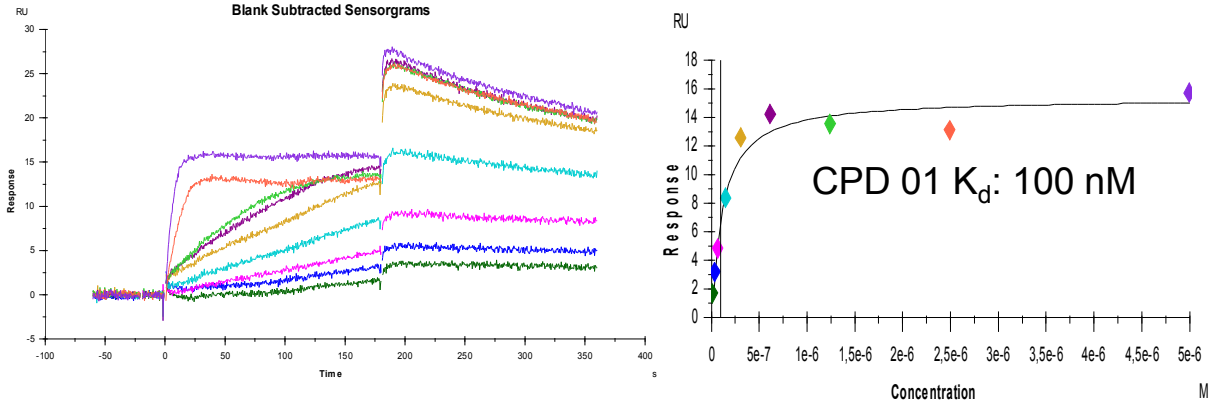
- **Scale down to 1536well format**
- **Total assay volume 8 µl**
- **Signal / Background ratio: 3**
- **Signal / Noise ratio: 25**
- **Z‘-factor: 0,76**

Identification of novel 14-3-3 PPI stabilizers





HTRF Assay

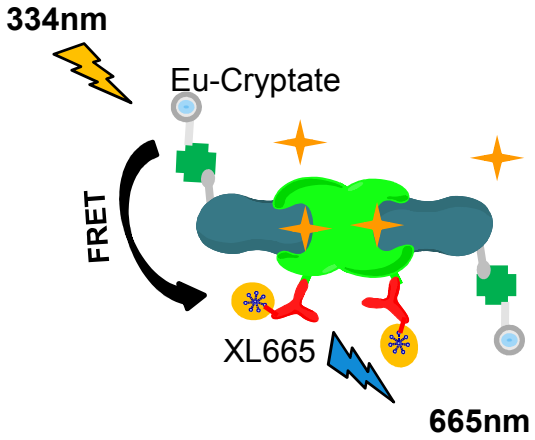


BIAcore Measurement

- **~150.000 compounds were screened**
- **320 compounds were picked as primary hits**
- **2 two scaffolds could be identified as new stabilizers for 14-3-3 PPI**
- **1 scaffold was verified by BIAcore with a nM K_d**

Summary

- HTRF is a robust generic assay technology used in HTS enviroments
- The HTRF toolbox reagents proved to be a versatile instrument to set up PPIs assays
- Modulation of 14-3-3 PPIs by small molecules is possible
- Could lead to novel ways of pharmacological intervention by targeting 14-3-3 PPIs
- Goal: Development of a screening-toolbox to probe the extensive 14-3-3 interactome for novel PPI stabilizers



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SEVENTH FRAMEWORK PROGRAMME

MAX-PLANCK-GESELLSCHAFT

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Thank you!

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