THERACAT

Kickoff Meeting
Barcelona, May 31st 2018

The University of Basel

- The Basel Hub is (one of) the World's Headquarter of Life Sciences: Roche, Novartis, Actelion, Lonza, Syngenta etc

- University of Basel: oldest in Switzerland from Paracelsus to Wüthrich via Reichsten

- Dpt Chem. 15 Professors with a heavy focus on Life Sciences and Nanotechnology (15 mio CHF/year external funding); exceptional infrastructure, including newly renovated building

- Strong collaboration with the Bio-Systems Science Engineering Dpt of the ETHZ D-BSSE)

- Leading House of a National Centre of Competence in Research "Molecular Systems Engineering" (joint with the D-BSSE)

- Ward Group: 22 coworkers, (15 postdocs). Focus on *in vivo* catalysis and artificial metalloenzymes (Director of the NCCR Molecular Systems Engineering)

Research Center for
Functional Molecular Systems
Gravitation Program - The Netherlands

NCCR
Molecular Systems
Engineering

The THERACAT Concept

The main scientific objectives of THERACAT are:

S1 - Synthesis and characterization of bio-orthogonal (nano)catalysts (WP3)

S2 - Design of novel catalytically-activable prodrugs and prodyes (WP4)

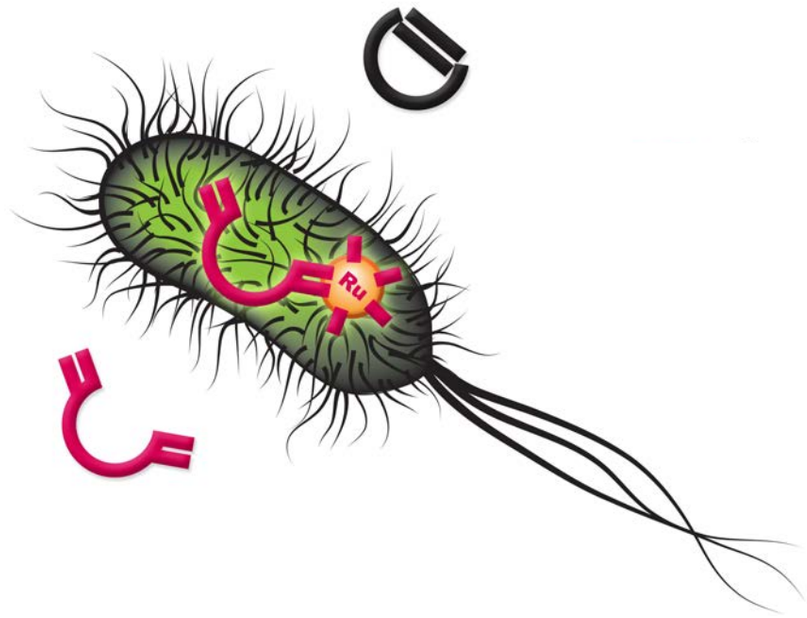
S3 - Effective strategies for the selective delivery of catalysts in the cancer site (WP5)

S4 - An in vivo validation of the antitumor strategy using imaging and animal models (WP6)

Our Approach

- Accumulate a metal catalyst on the surface of cancer cells
- A prodrug is uncaged thanks an *in vivo* compatible metal catalyst accumulated on the cancer cell
- human Carbonic Anhydrase IX (hCA IX) is over-expressed on the surface of various cancer cells
- Arylsulfonamides are high-affinity inhibitors for hCA IX
- Use hCA IX to localize and accumulate a metal catalyst on cancer cell to site-specifically uncage the drug

Prior Art



LETTER

doi:10.1038/nature19114

Directed evolution of artificial metalloenzymes for *in vivo* metathesis

Markus Jeschek[1], Raphael Reuter[2], Tillmann Heinisch[2], Christian Trindler[2], Juliane Klehr[2], Sven Panke[1]§ & Thomas R. Ward[2]§

Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal

Carbonic anhydrase II as host protein for the creation of a biocompatible artificial metathesase†

Cite this: DOI: 10.1039/c5ob00428d

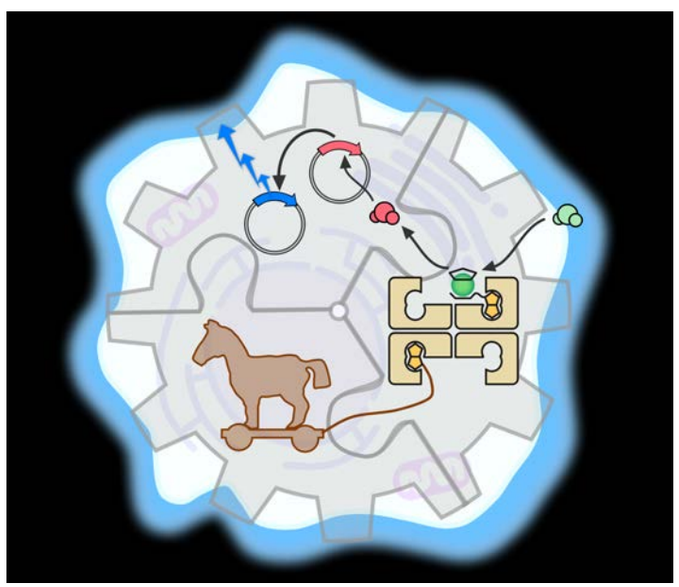
Chemical Science

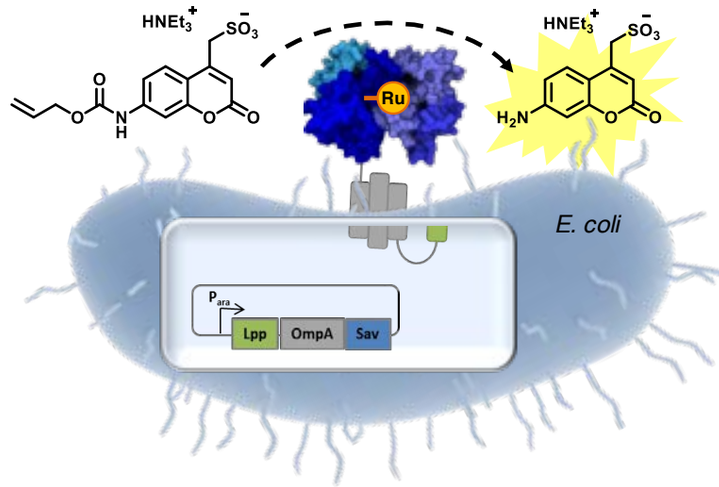
EDGE ARTICLE

Cite this: DOI: 10.1039/c8sc00484f

E. coli surface display of streptavidin for directed evolution of an allylic deallylase†

Tillmann Heinisch,‡[a] Fabian Schwizer,‡[a] Brett Garabedian,[a] Eszter Csibra,[b] Markus Jeschek,[c] Jaicy Vallapurackal,[a] Vitor B. Pinheiro,[b] Philippe Marlière,[d] Sven Panke[c] and Thomas R. Ward*[a]





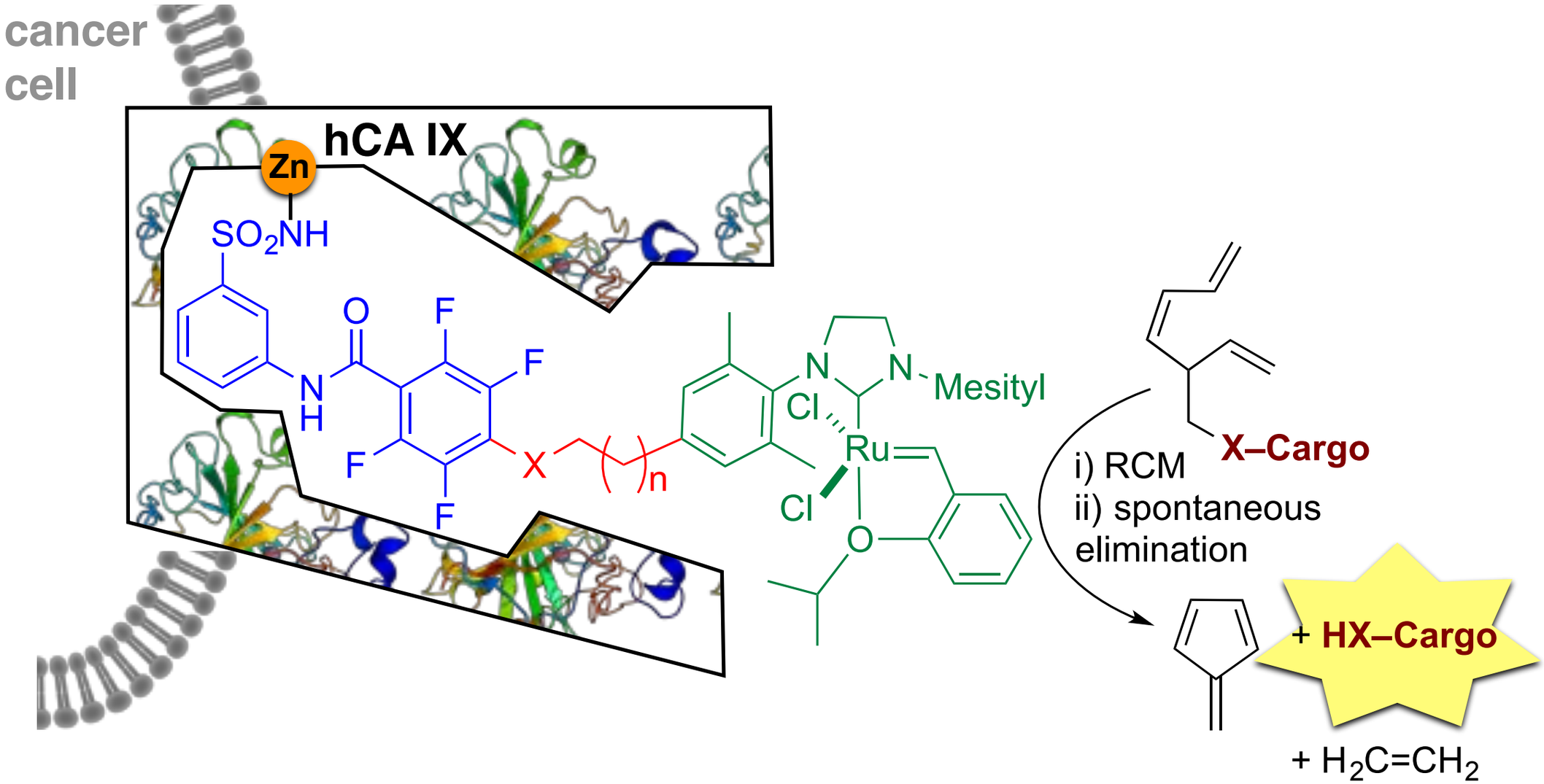
ARTICLE

DOI: 10.1038/s41467-018-04440-0 OPEN

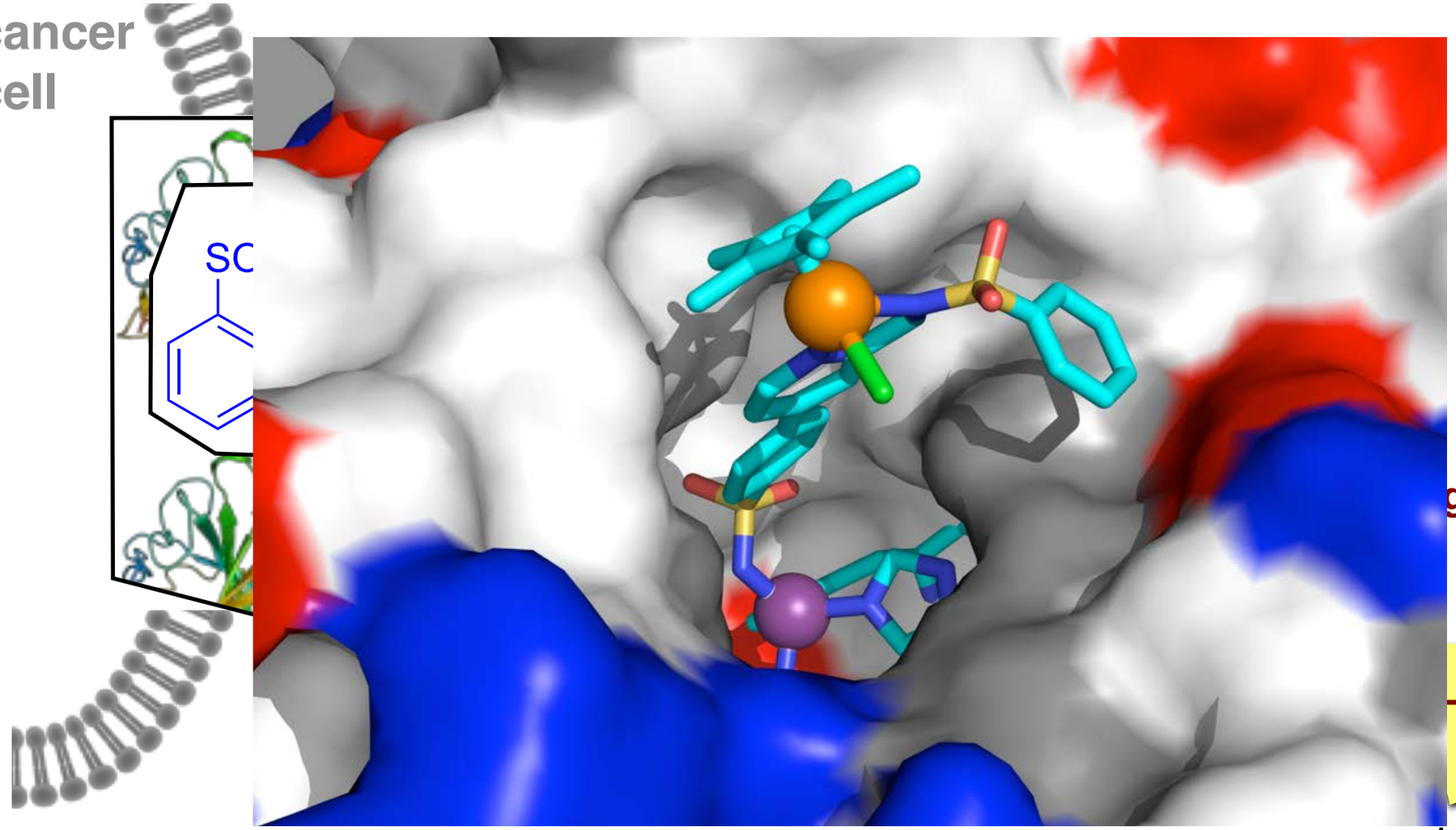
A cell-penetrating artificial metalloenzyme regulates a gene switch in a designer mammalian cell

Yasunori Okamoto[1], Ryosuke Kojima[2,3], Fabian Schwizer[1], Eline Bartolami[4], Tillmann Heinisch[1], Stefan Matile[4], Martin Fussenegger[2] & Thomas R. Ward[1]

hCA as Host Protein



hCA as Host Protein



ESR 10

- Synthesis of arylsulfonamide-bearing Hoveyda-Grubbs type metathesis catalysts: **$NH_2SO_2Ar(NHC)–Ru=CHR$** **Task 5.1**

- Synthesis of prodrugs which, upon RCM afford a drug or lead to the release of a drug

- Test the activity of **$NH_2SO_2Ar(NHC)–Ru=CHR$** towards the prodrugs in the presence of hCA IX using

  - surface displayed hCA IX on *E. coli*

  - cancer cells over expressing hCA IX on their outer surface **Task 5.3**

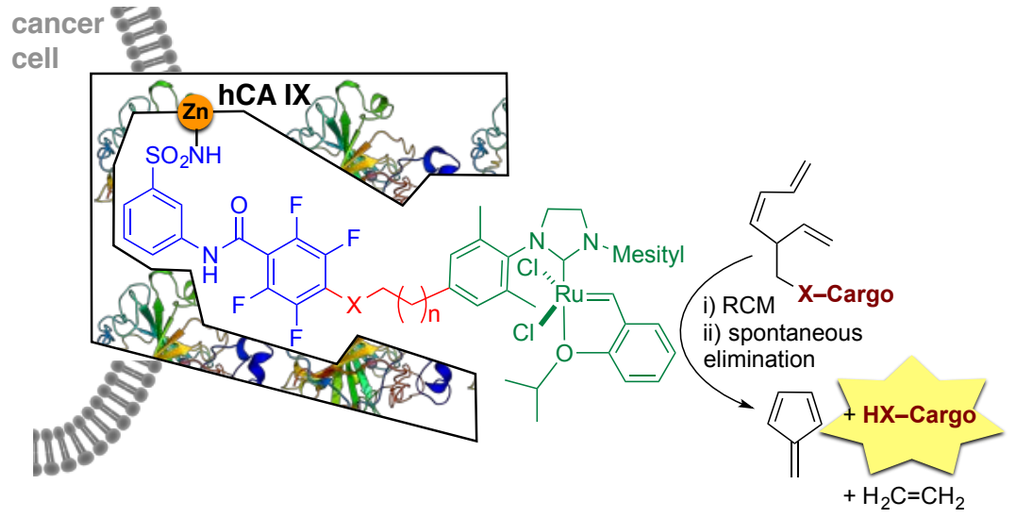
  - Localization efficacy **Task 6.3? (Secondment at Bas?)**

Secondments for ESR 10: **TEVA and EDI**

ESR 10

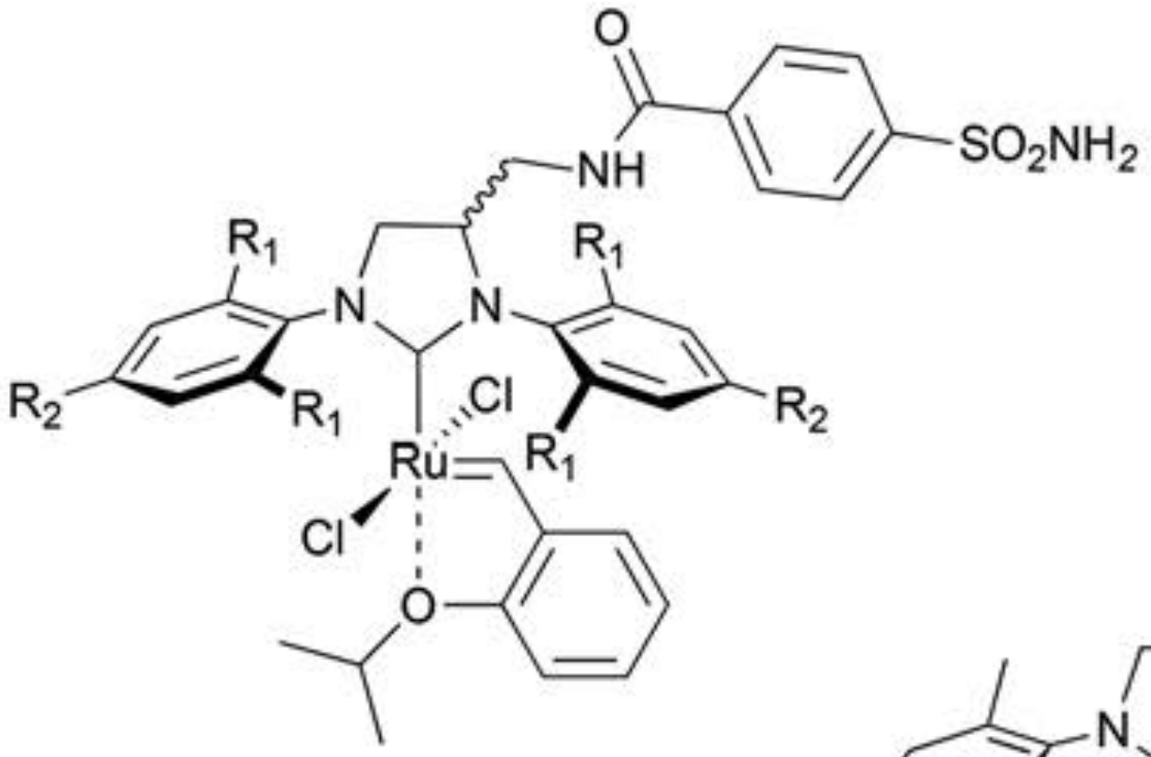
Objectives: **1.** Identify metathesis catalyst activated upon binding to hCA IX; **2.** Uncage cargo (fluorophore or drug) by ring-closing metathesis; **3.** Fluorophore- or Drug-release by ring-closing metathesis on the surface of cancer cell overexpressing hCA IX.

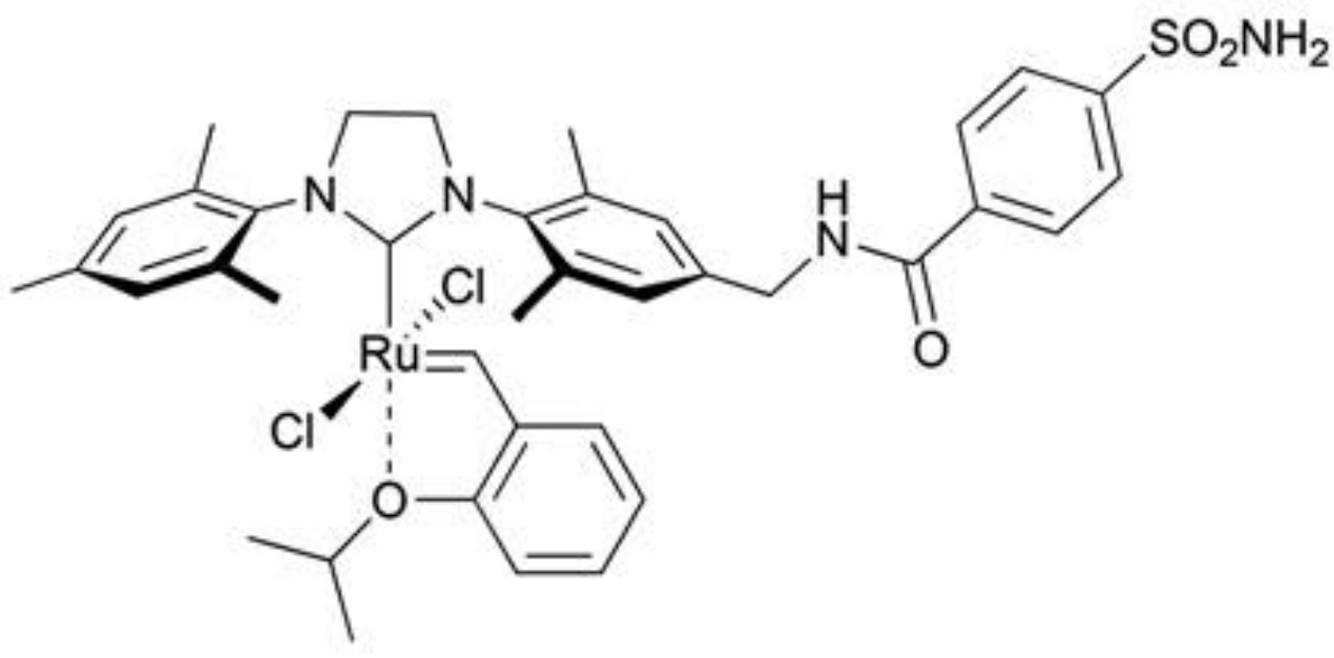
Description: In the past decade, the Ward group has developed a series of artificial metalloenzymes for a variety of bio-orthogonal reactions. For this purpose, a catalyst precursor (green) is activated upon incorporation within a host protein (ribbon display) via a high affinity anchor (blue). We have shown in the past that i) hCA II is an outstanding host for the creation of artificial metathases and ii) artificial metathases are fully biocompatible, air stable and can be performed *in vivo*.[4] To target the tumour site, it is proposed to exploit hCA IX to specifically accumulate and activate a metathesis catalyst on the surface of cancer cells. With this goal in mind, the fluorinated sulfonamide anchor will be linked via a spacer (red) to a metathesis catalyst. Initial experiments will be carried out with diallyl-*N*-tosylamide as model substrate. Having identified an active metathesis catalyst for incorporation within hCA IX, the artificial metathase will be screened for its RCM activity towards an heptatriene substrate bearing either a caged fluorophore or a caged drug. Upon RCM, a spontaneous elimination occurs via an aromatic transition state, thus uncaging the fluorophore or the drug. For the synthesis of the triene substrates, ESR10 will spend three months at EDI. Having identified a suitable precatalyst, activated upon incorporation in hCA IX, experiments will be performed in the presence of cells overexpressing hCA IX on their cell surface. To facilitate its delivery to cancer cells, the catalyst precursor will be non-covalently incorporated in a variety of delivery vectors including:, hydrogels, micelles, SCNPs, lipidic NPs. For this purpose, ESR10 will spend 3 months at TEVA to adapt their NPs to the delivery of the metathesis catalyst.



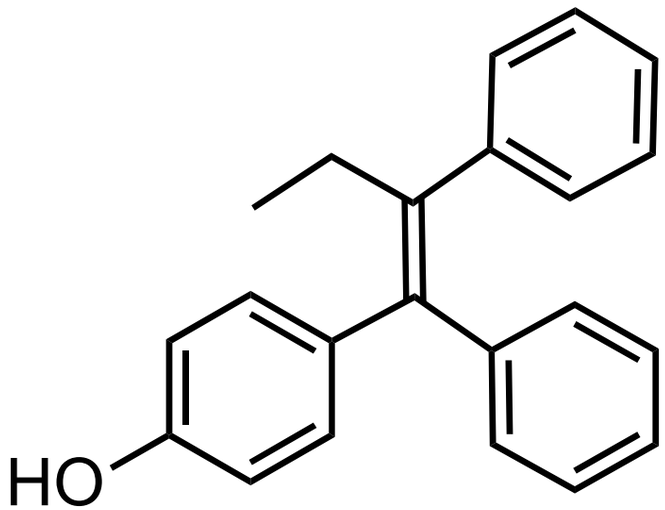
Planned secondments: EDI – synthesis of caged prodrugs (M27, 3 months); **TEVA** – encapsulate catalyst in lipid NP (M36, 3 months).	**Expected results (deliverables)**: synthesis of five metathesis catalysts (D5.1); reactivity profile of two caged fluorophores and one drug uncaged upon RCM (D5.3); reactivity profile upon incorporation within hCA IX (D5.3)

Sulfonamide-Bearing Metathesis Catalysts

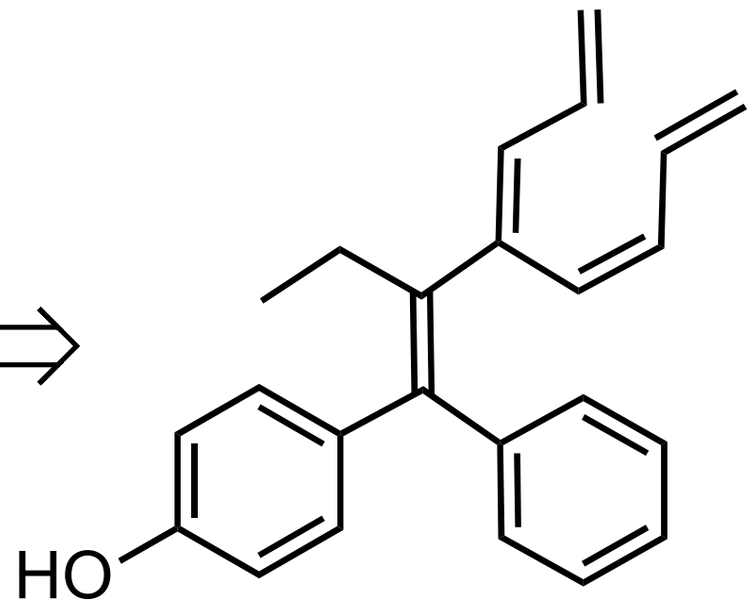




RCM to produce a drug



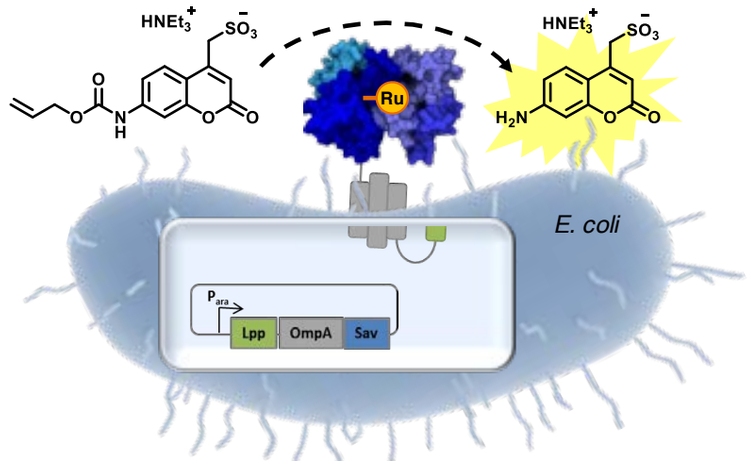
tamoxifen



tamoxifen-precursor
(non-cytotoxic?)

Contingency Plan

- Accumulate a metal catalyst on the surface of cancer cells
- human Carbonic Anhydrase IX (hCA IX) is over-expressed on surface of various cancer cells
- Arylsulfonamides are high affinity inhibitors for hCA IX
- Use hCA IX to localize and accumulate a metal catalyst on cancer cell
- A prodrug is uncaged thanks the metal catalyst accumulated on the cancer cell
- Rely on the CpRu-catalyzed de-allylation of doxorubicin



TRW's Involvement

- Recruiting committee (recruitement strategy, advertise positions, gender balance, conflict management)

- Coordination of Training event 1 (Month 12)

- Secondment: Activable PET Probe (M 28, 3 Months), Task 6.3?

Training Event at UniBas, Month 12

1 - Introducing the THERACAT Network & How to plan and start a PhD	M12, 4 days	2 ECTS	BAS
Content: The first training event will start with a general introduction of the network and its scientific and training goals. It will also include a comprehensive training of transferable skills aimed to accelerate the implementation of the ESRs into the training programme			
General introduction of the network and its scientific goals	**All PIs** (all nodes)	**SCI**	1 day
Introduction of the training programme	**All PIs** (all nodes)	**SCI**	½ day
Skills to start a successful PhD: Time management, team work, ethics, intercultural, gender and diversity awareness	**All PIs** (all nodes)	**COMP**	1 ½ day
Scientific communication: writing papers, the peer-review process, open science, oral and poster presentations	**IBEC** (Outreach office)	**COMP**	1 day
ESR Meeting 1	**ESR representatives**	**-**	½ day

Second event TRW presents: catalysis and ArM

Thank you!

See you in one year in Basel