

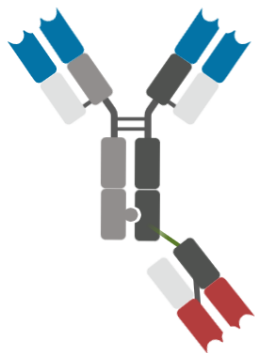
Design and CMC Considerations for Oligo-Functionalized Antibodies: The Case of Brainshuttle™-oligo Conjugates

CMC Strategy Forum Europe, Basel

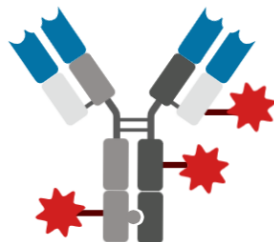
Felix Schumacher on behalf of the RNAHub teams

Multispecific antibodies - a growing family

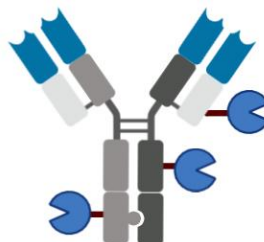
Multispecificity can be achieved through various engineering options



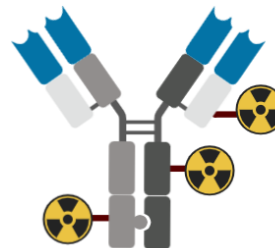
Bispecific



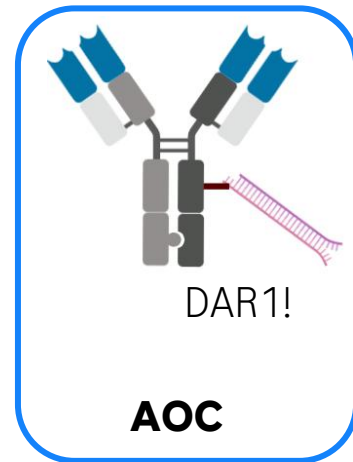
ADC



DAC



**Radioimmuno
conjugate**



AOC

DAR1!

On the
market*

19

18

0

2

0

In active
development*

1.667

1.086

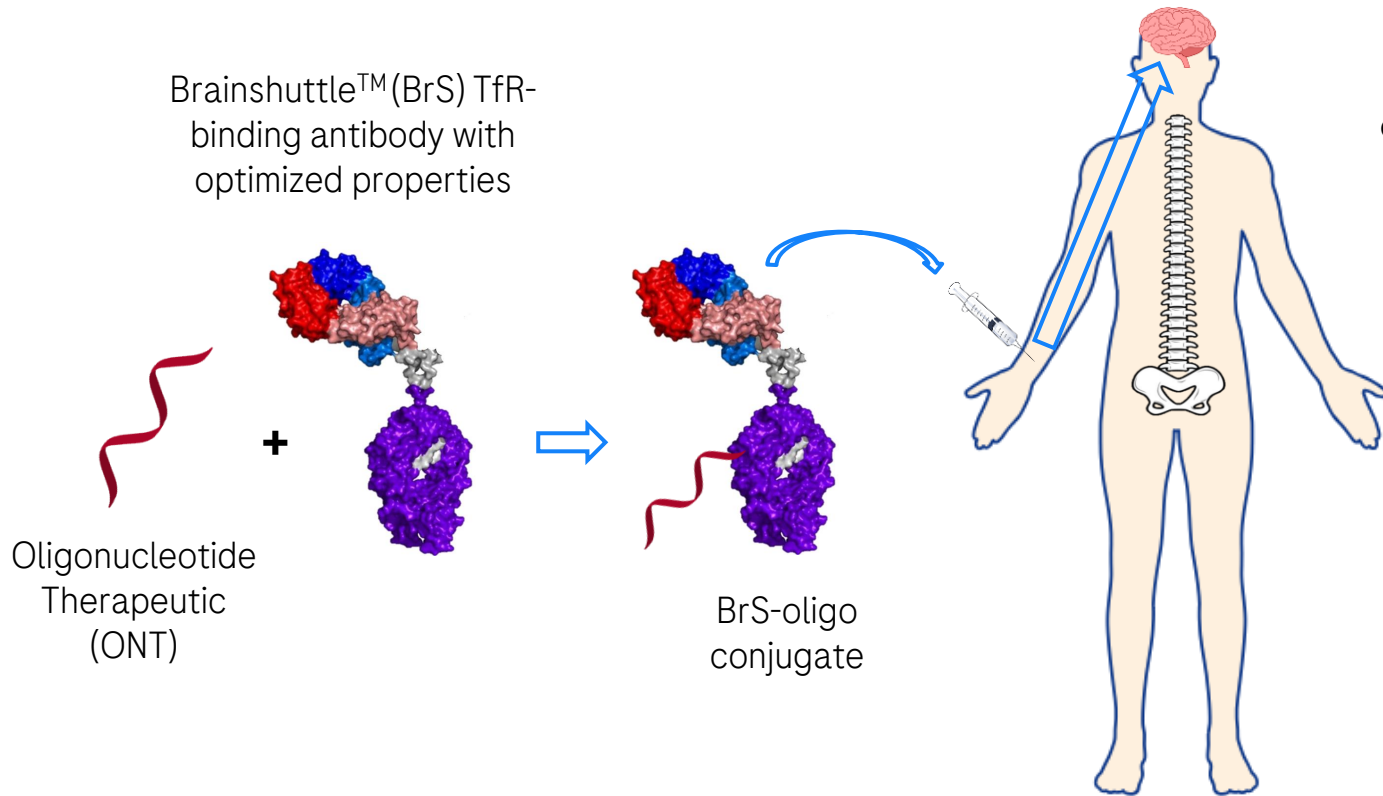
38

108

56

The concept: Brainshuttle™ based delivery of ONTs

Building on Roche's clinically validated brain delivery system



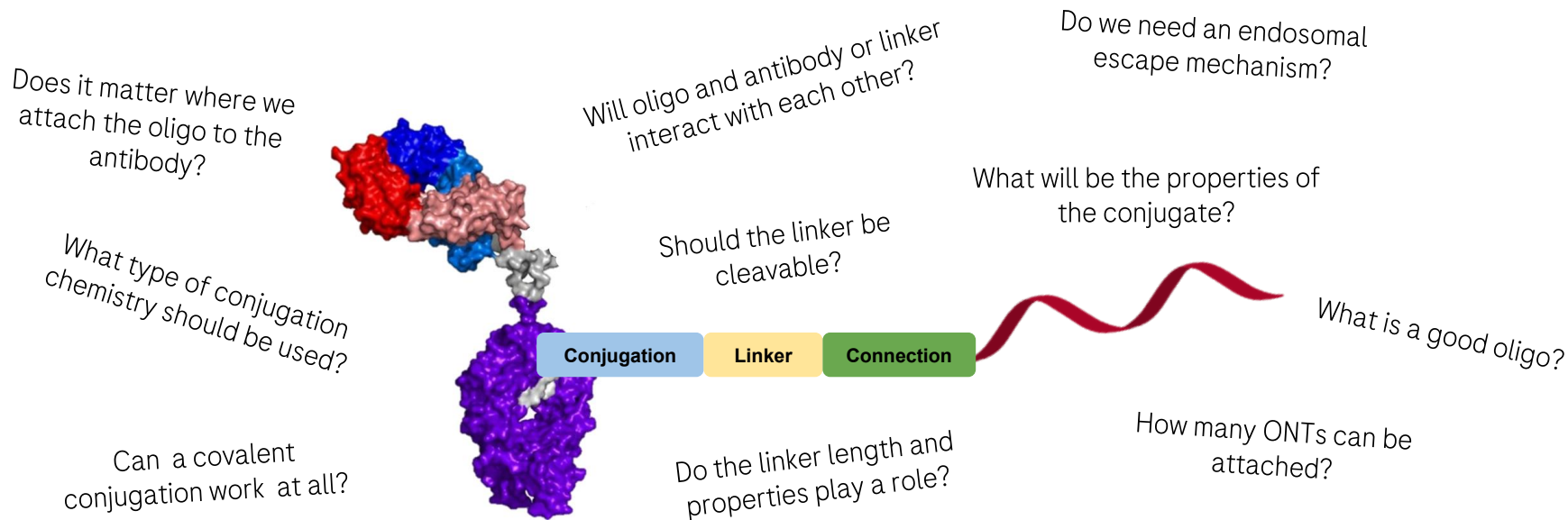
Promise 1: conjugates enable access to the CNS through **peripheral administration.**

Promise 2: the ability to “enter” the brain via all of the capillary system offers **excellent tissue biodistribution.**

Promise 3: oligos have a **long duration of action in the target tissue/ cells.**

Conjugate design: Mixing modalities offers plenty of choices

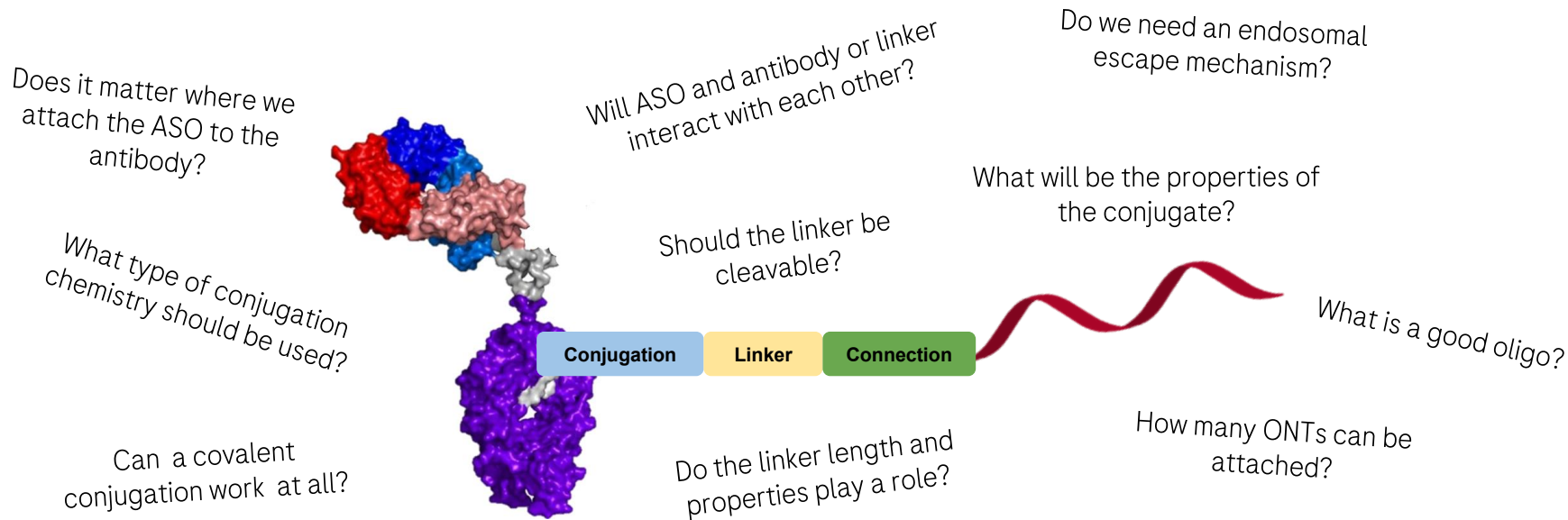
A multi-dimensional optimization challenge



Early steps - learning through failure

Conjugate design: Mixing modalities offers plenty of choices

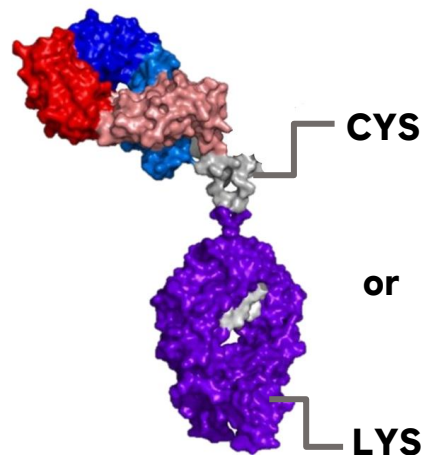
A multi-dimensional optimization challenge



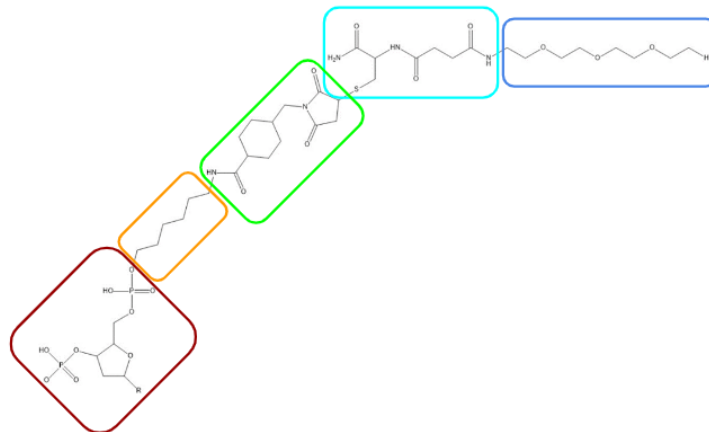
Initial decision: utilize readily available technologies and compounds

Early constructs

Random conjugation, “high” DAR and simplistic linkers



Random conjugation to lysines
or reduced disulfide bonds
(cysteine)



5-component linkers with a
cleavable component



ASO from ongoing in-house
program

Performance of early constructs

Poor PK profile and linker instability



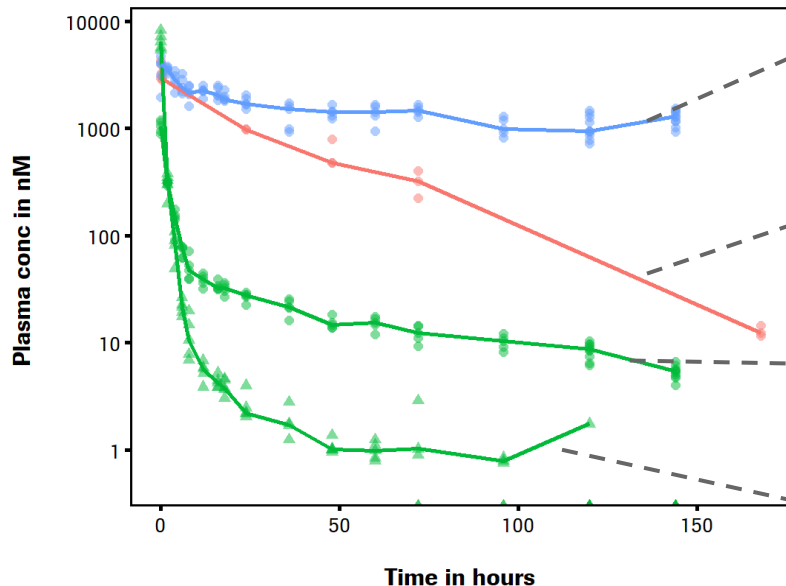
C57BL/6



30 mg/kg
SD IV



DAR ~2.5



Non-binding IgG = antibody PK without any target interaction

Target binding IgG = expected antibody PK, including target mediated drug disposition

Target binding conjugate
(analyte = mAb)

Target binding conjugate
(analyte = ASO)

➡ Issue: brain delivery is driven by exposure → poor PK profile prohibitive

CMC-inspired design considerations for AOCs

Back to the drawing board - AOC design

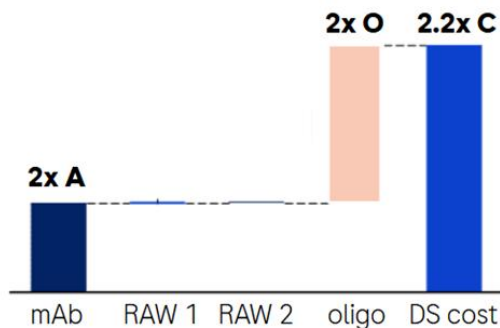
Major considerations

1. **Choice of conjugation technology** - control the DAR and multiple other design considerations
1. **Optimization of the conjugation process** - driver for the discovery process & future CMC “outlook”
1. **Selection of the conjugation site** - major determinant of conjugate performance
1. **Reality check and adoption to CMC** - fine tuning of the process

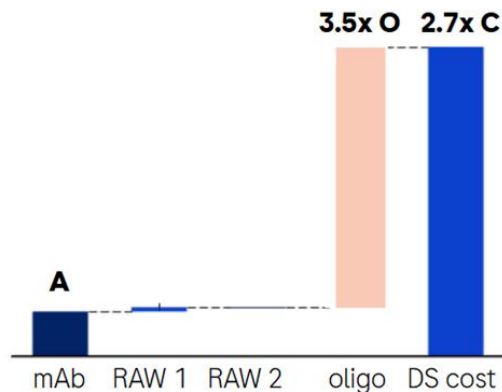
Choice of conjugation technology

Hypothetical production cost considerations as a major indicator of performance and CMC fitness

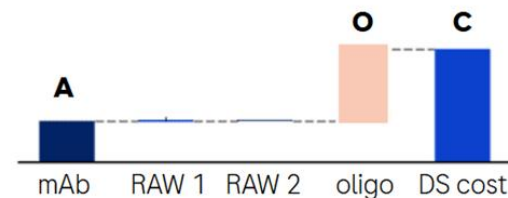
Conjugation approach 1



Conjugation approach 2



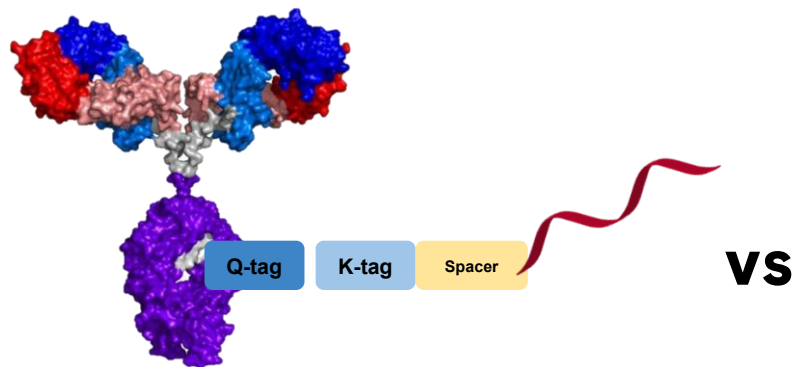
Conjugation approach 3



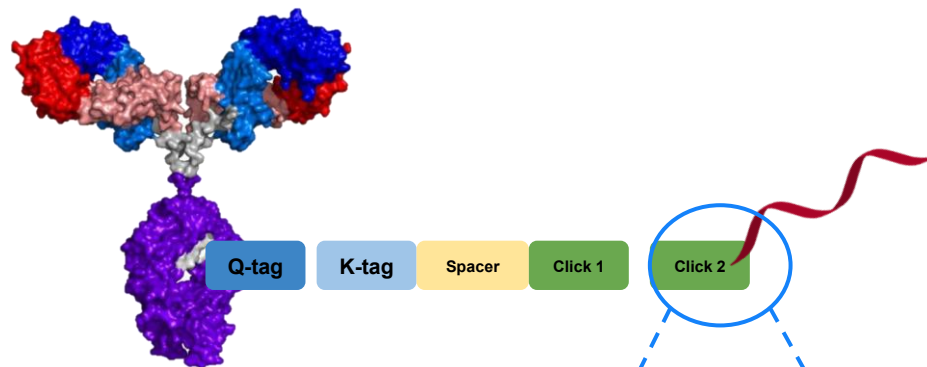
- Oligo and mAb (conjugation-ready!) as main cost drivers - other raw materials often negligible
- Efficient mAb production, excess of oligo required and reaction conversion rates are key factors
- Optimization of reaction conditions and (reduction of) purification steps essential
- Not shown here: facility fit considerations

Reality check and adoption to CMC - process design

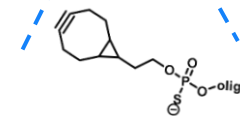
Research meets CMC



1-step conjugation process
(enzyme only)



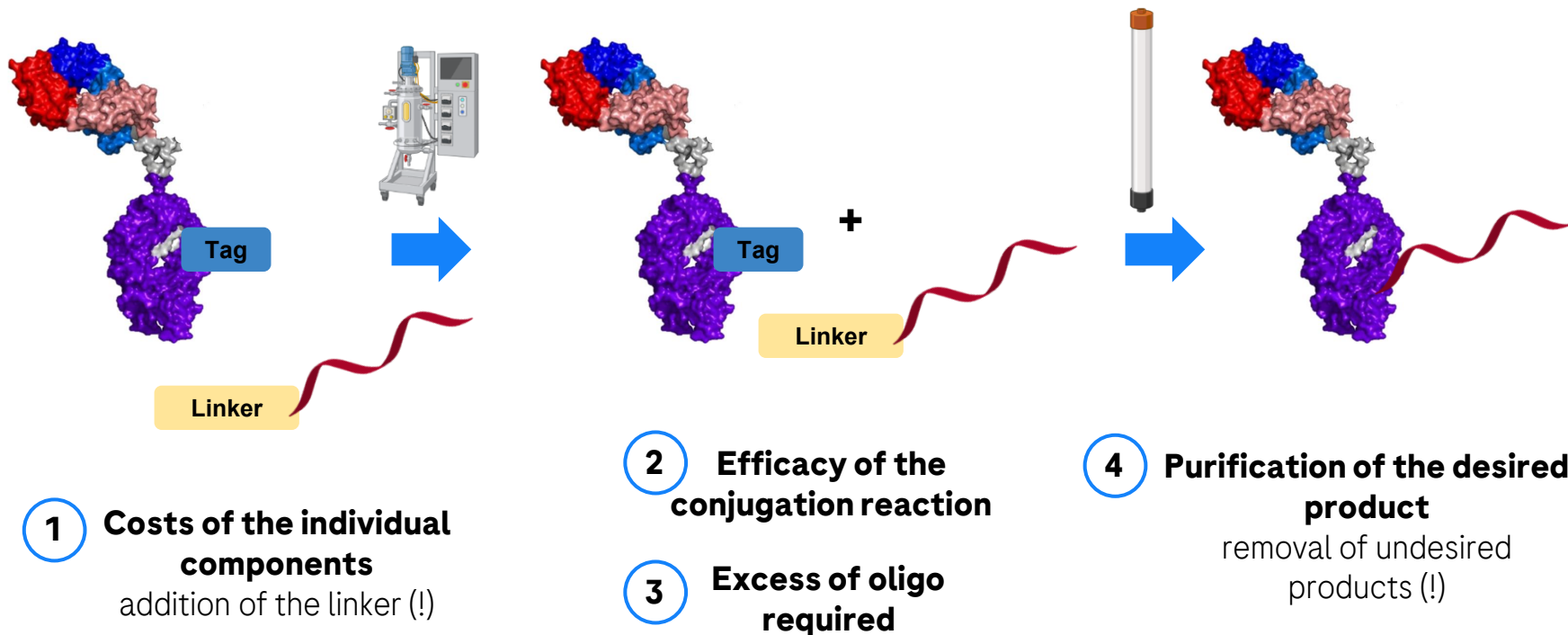
2-step conjugation process
(enzyme and click)



Kerstin Hofer, Kathryn Perez,
Jasmin Groeger, Michael
Tischler, Maximilian Hartl

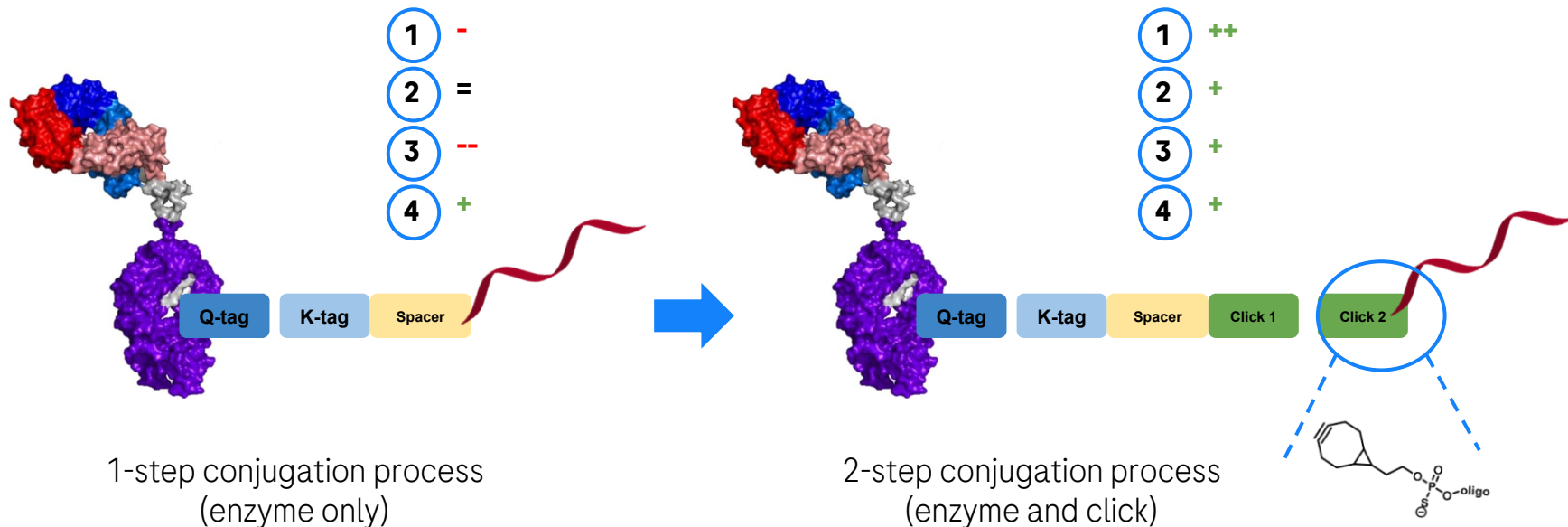
Reality check and adoption to CMC - key steps

Major aspects/ cost drivers



Reality check and adoption to CMC - outcome

Research meets CMC



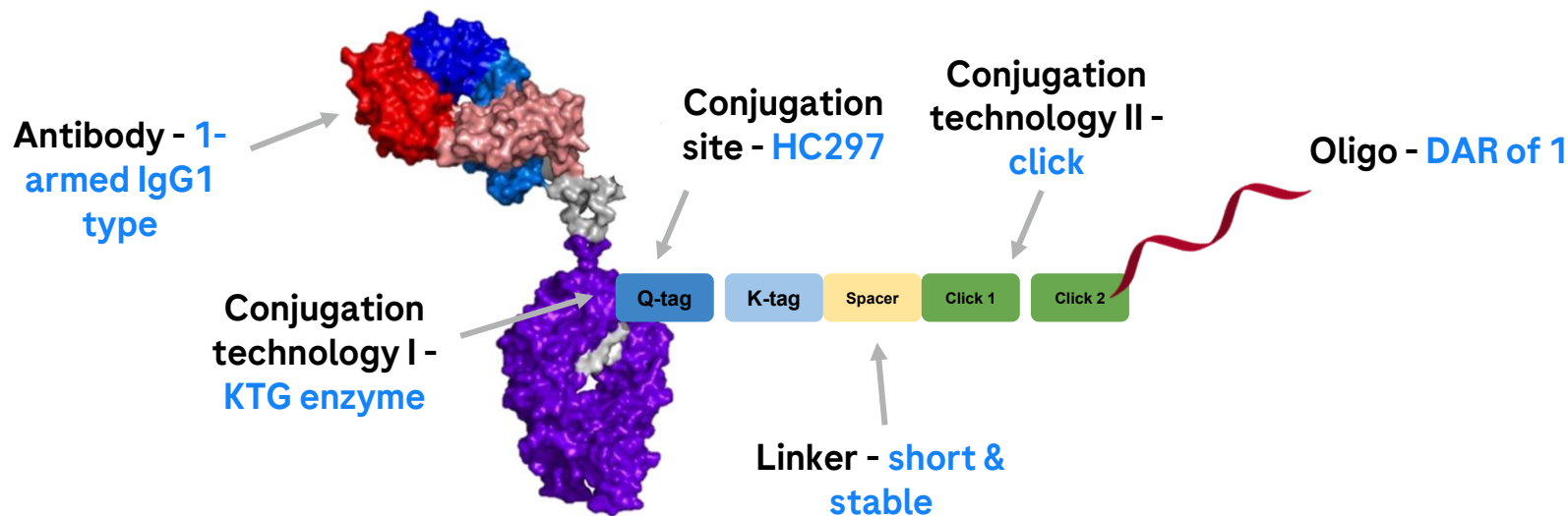
2-step approach enables the optimization of all important process steps

Kerstin Hofer, Kathryn Perez,
Jasmin Groeger, Michael
Tischler, Maximilian Hartl

Status

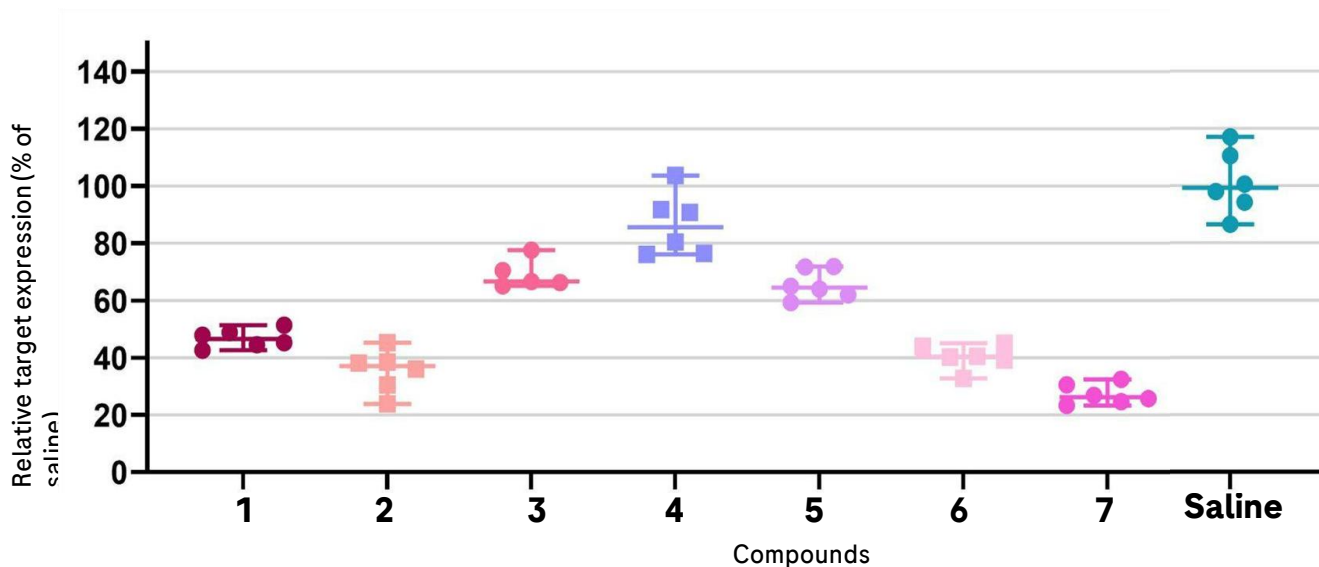
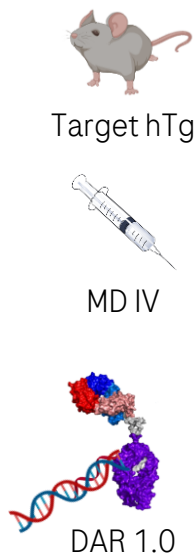
The current design of Roche's BrS-oligo conjugates

Tool “chassis” to explore important research questions



In vivo activity of Brainshuttle™-oligo conjugates

Continuously optimized designs show significant and sustained target reduction



Up to 70 % target reduction by selected oligo candidates

Acknowledgements



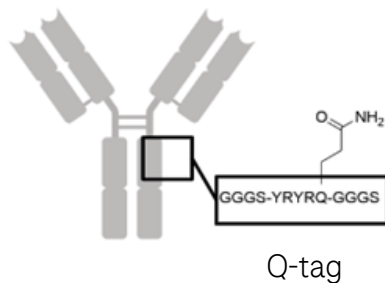
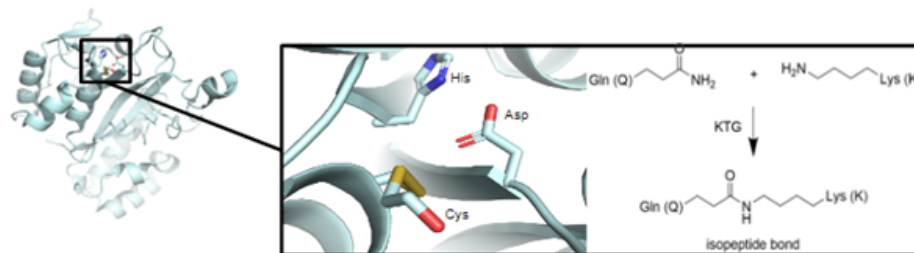
- + **Steffen Schulze**
- + **Maximilian Hartl**

Backup

KTG-based conjugation process

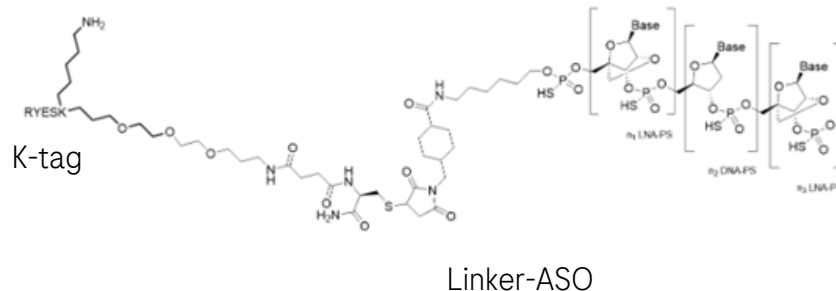
A unique member of the transglutaminase family

***Kutzneria albida*
transglutaminase**



KTG reaction

+



Doing now what patients need next