Novel artificial metalloenzymes for olefin metathesis based on modified Grubbs-Hoveyda complexes

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Introduction: What are artificial metalloenzymes?

Artificial metalloenzymes represent an attractive approach for the design of biocatalysts by combining homogeneous catalysis with enzyme catalysis. A successful example are artificial metalloenzymes based on Grubbs-Hoveyda catalysts for olefin metathesis.[1,2] We designed artificial metalloenzymes with ***iodide substituted*** Grubbs-Hoveyda complexes and compared them to their chloride containing parents.

Reaction of choice: Ring-closing metathesis (RCM)

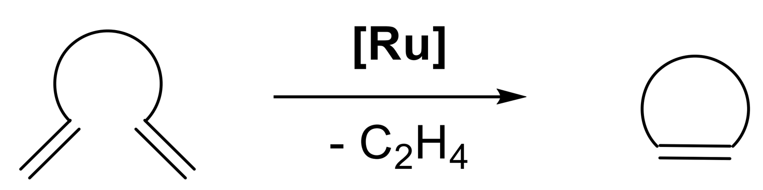


Figure 1. Ruthenium-catalyzed ring-closing metathesis

Artificial metalloenzymes

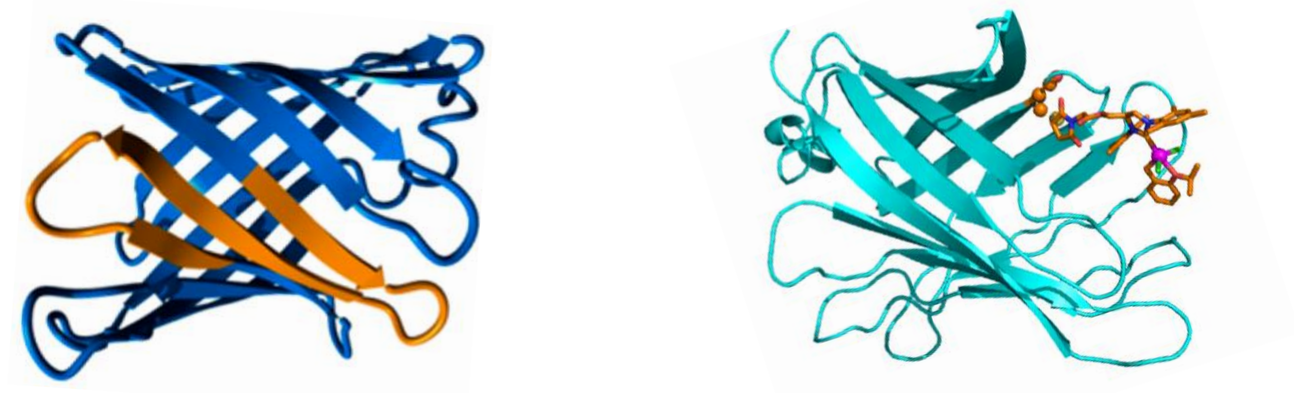
Biological perspective

- **Non-natural (biorthogonal) reactions in water**
- **Expanding reaction scope of enzymes / proteins**

Chemical perspective

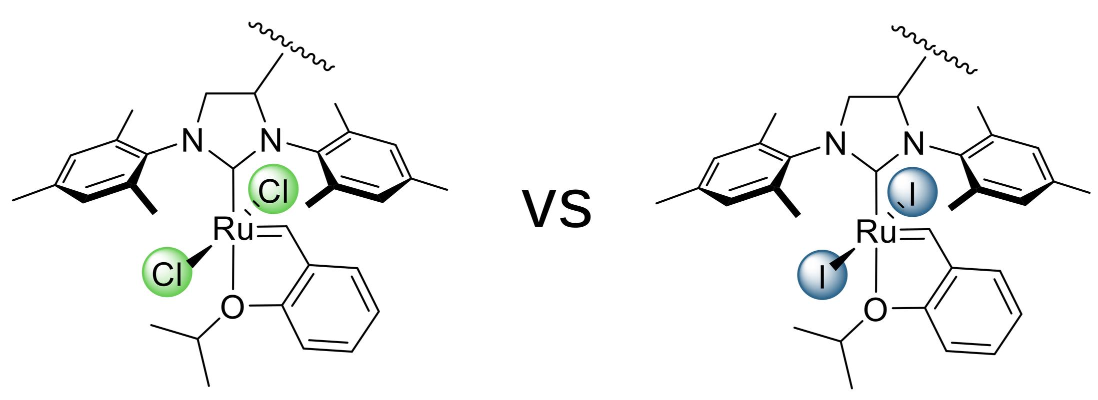
- **Solubilization of organometallic complexes**
- **Well-defined second coordination sphere around metal atom**
- **"Protection" of complexes from degradation**

Design of an artificial metalloenzyme for olefin metathesis



apo-NB4exp **Ru-X_2@NB4exp**

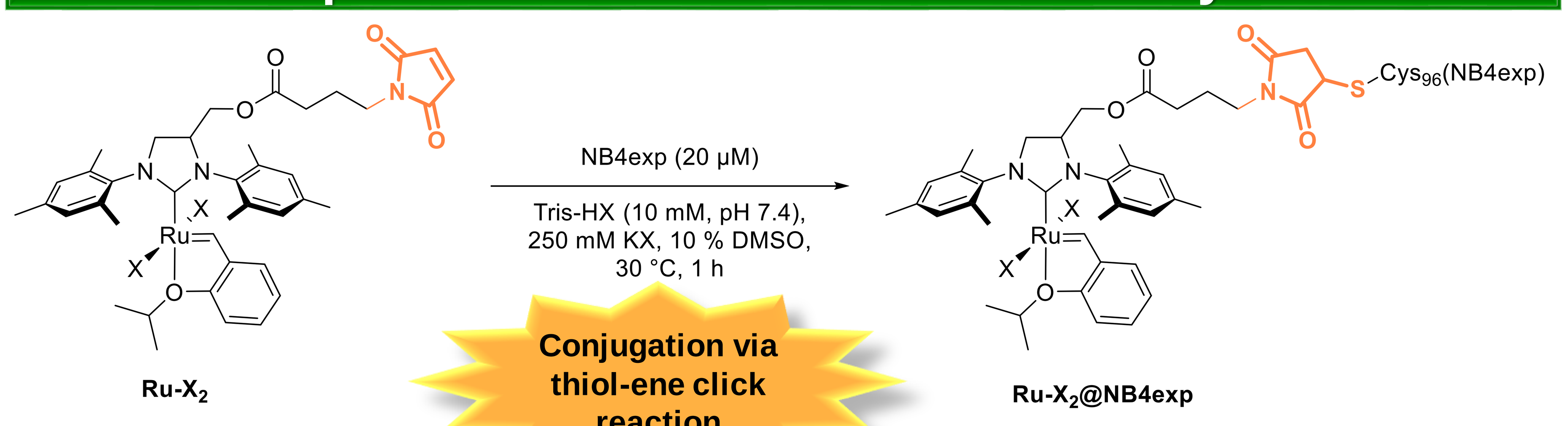
Figure 2. Engineered protein scaffold: Nitrobindin from *Arabidopsis thaliana* with two additional β-strands (NB4exp). Left: apo-NB4exp, right: NB4exp with conjugated Grubbs-catalyst (Ru-X_2@NB4exp).



Central questions:

- ***Impact of halide ligand?***
- ***Cross-interaction with protein?***

Preparation of the artificial metalloenzymes



Titration of free cysteine residues using fluorescence dye ThioGlo-1

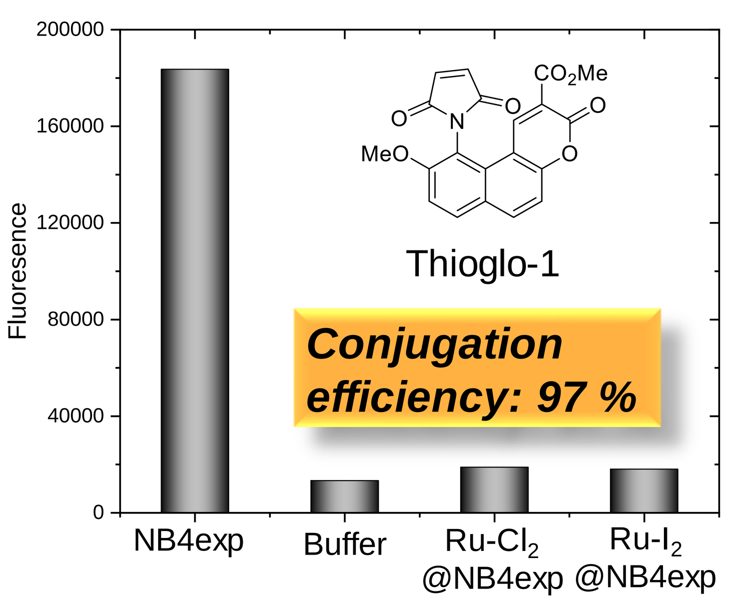


Figure 3. Fluorescence titration of apo-NB4 and conjugated variants using fluorescence dye ThioGlo-1.

CD-Spectroscopy

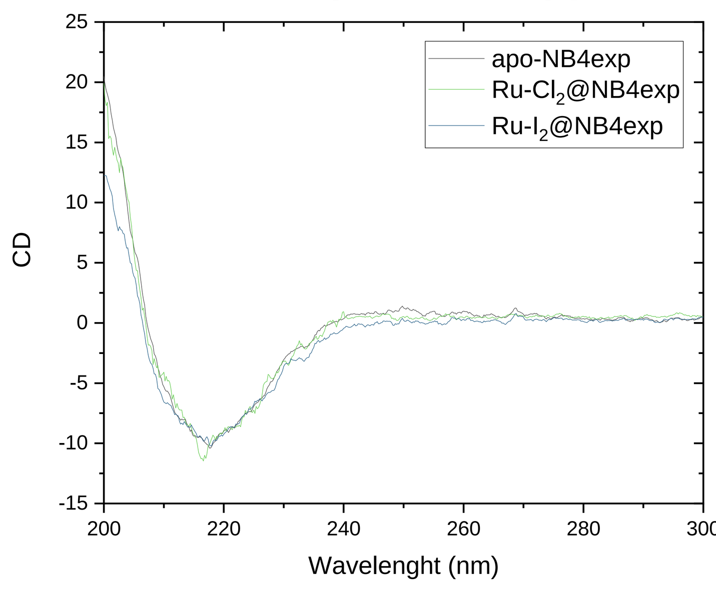
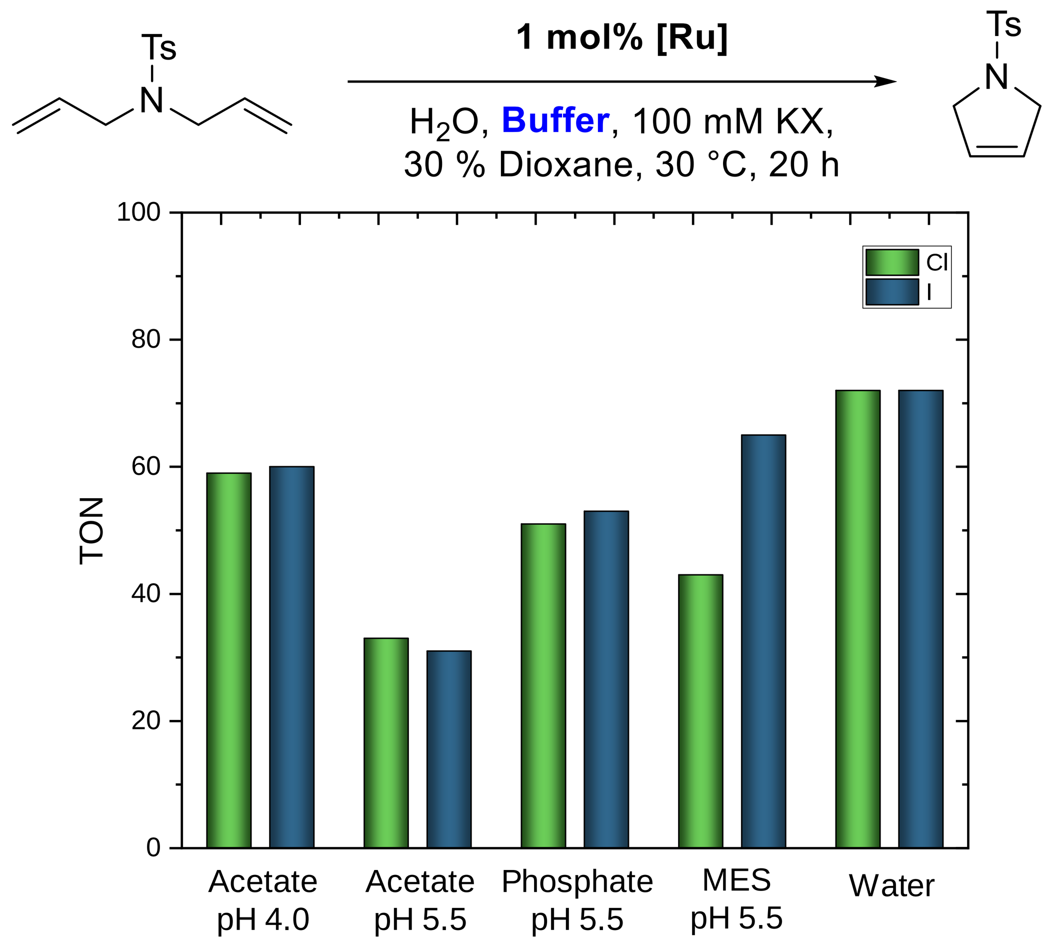
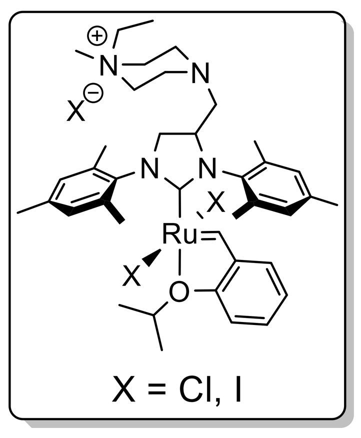


Figure 4. CD-spectrum of NB4exp and conjugated variants (5 µM). Black: apo-NB4exp, green: Ru-Cl_2@NB4exp, blue: Ru-I_2@NB4exp.

Conjugation of the Ru-complex and β-barrel fold confirmed.

RCM with "free" Ruthenium-complexes



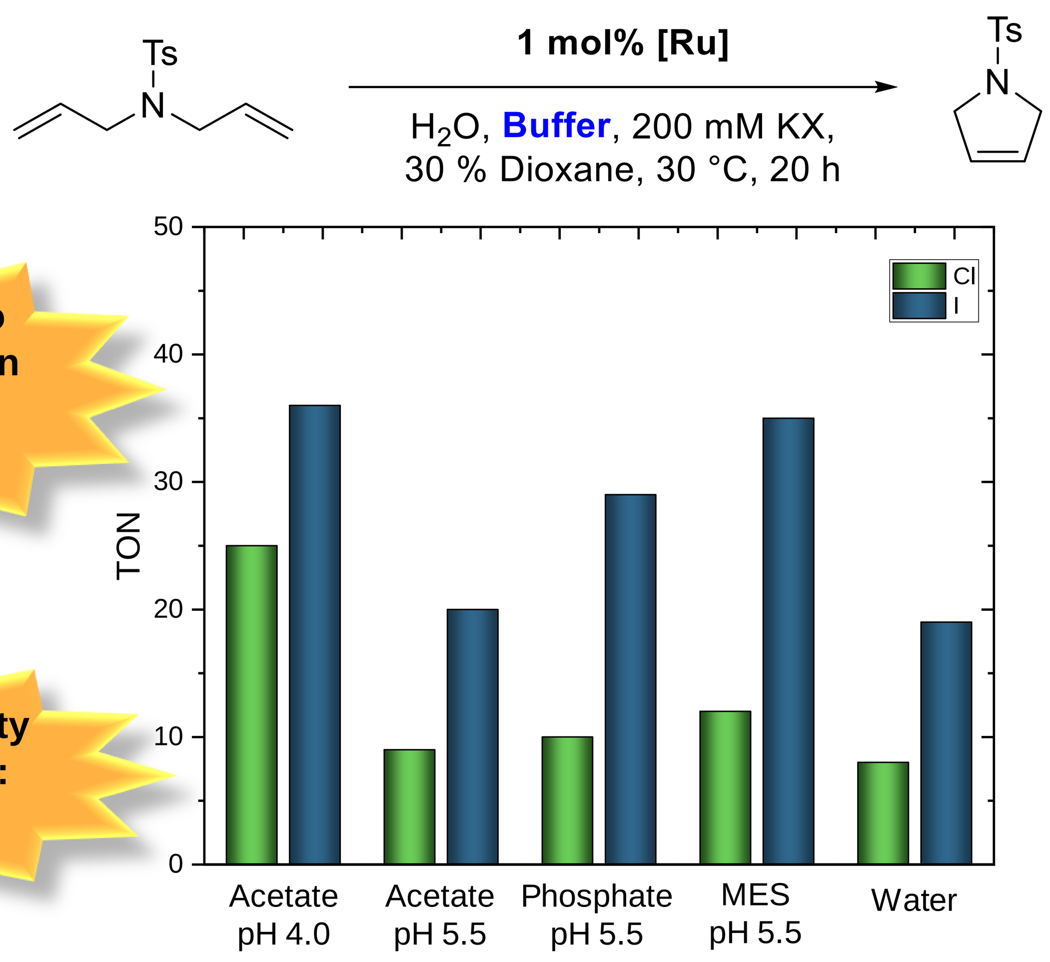


AquaMet®: X=Cl

Figure 5. TON for RCM of N,N-diallyltosylamide in different buffers using "free" Grubbs-Hoveyda complexes in solution.

Catalysis works better under acidic conditions.
Weaker coordinating buffers are better.
Only minor impact of halide ligand.

RCM with artificial metalloenzymes



Possibility to form hydrogen bonds: Cl >> I

Hydrophobicity of the cavity: I >> Cl

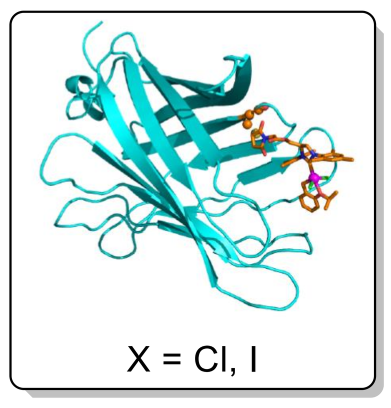
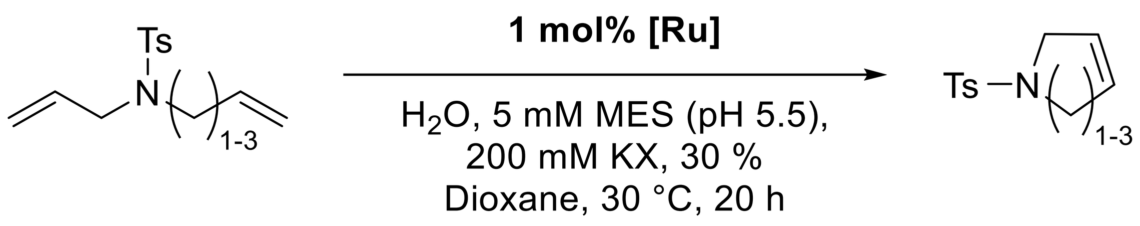
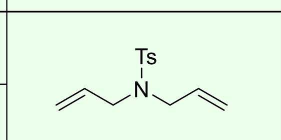
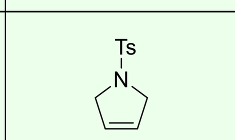
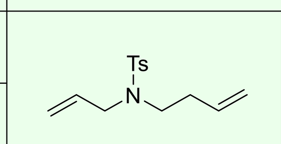
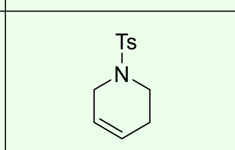
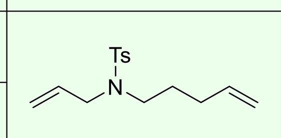
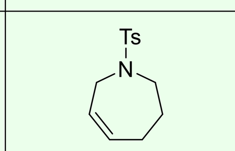


Figure 6. TON for RCM of N,N-diallyltosylamide in different buffers using Grubbs-Hoveyda complexes conjugated to NB4exp.

Different behavior compared to complexes in solution.
Conjugated iodide-complexes lead to higher TON.

RCM yielding larger ring sizes



Entry	Substrate	Product	Catalyst	TON
1			Cl	12
2			I	35
3			Cl	37
4			I	60
5			Cl	8
6			I	56

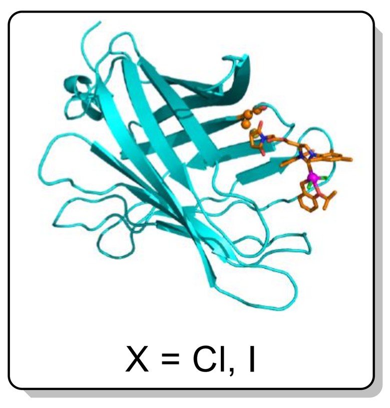


Figure 7. TON for RCM of different tosylamides using Grubbs-Hoveyda complexes conjugated to NB4exp.

Iodide complexes well suitable for synthesis of larger ring sizes.

Conclusion

Substituting chloride against iodide ligands in a Grubbs-Hoveyda catalyst embedded in nitrobindin improved the activity in ring closing metathesis in aqueous media.

Acknowledgement

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References:
1) D. F. Sauer et al., *ACS Catal.* **2015**, *5*, 7519-7522.
2) A. R. Grimm et al., *ACS Catal.* **2018**, *8*, 3358-3364.