**Summaries**

Catalysts (and specifically ligands)

* Catalysts can be small (one molecule/complex) or macro-molecular, like metalloenzymes or polymeric complexes.
* For a catalyst to reach its target inside the cell efficiently, and without being blocked by cellular barriers along the way, it often has a targeting functional group.
  + BODIPY (boron difluoride-dipyrromethene) motifs are lipophilic and fluorescent and allow internalization of the catalyst into microalgae lipid bodies1.
* The catalyst can be an artificial cofactor for an engineered holoenzyme.2
* 2 developed Streptavidin (SAV)-Ru-benzilydene:



* Modifying the enzyme's amino acids near the Ru allows optimization of the activity of the catalyst (how exactly does the amino acid change optimize the reaction?). For example, they can:2
  + Raise entropy
  + Reduce bulk and so minimize steric strain
  + Change hydrogen-bond interactions (and how does this help?)
* 3 developed HAS (human serum albumin)+Hoveyda-Grubbs metalloenzyme (Artificial Metalloenzyme – ArM), in which the negative charge of the protein prevents GSH (glutathione) interaction.
  + Next, they replaced the Cl on the Ru with I, and achieved better reactivity and great tolerance to blood components.
  + The better reactivity is achieved due to the steric hindrance of the bulky iodide makes the intermediates more stable in comparison to the reactant.
  + AquaMet is okay in water but sucks in blood
* Ruthenium is the most stable metal for OM in air and water (requires more sources).4
* Phosphine ligands can catalyze in water but they kind of suck.4

Synthesis of catalysts

* For a metalloenzyme – the biotin-streptavidin technology (what is it?)2
* Directed evolution through saturation mutagenesis on the amino acids close to the Ru can increase five-fold the cell-specific activity of the catalyst.2 This type of evolution can be perform substrate-specifically and thus we can develop the "ideal" enzyme for any type of reaction (personal observation, based on 2).

Reactions

* Artificial RCM reaction in 1 and 2; useful for assessing activity and efficiency (though it sucks in water2):



* "Natural" unsaturated fatty acids in microalgae are converted to olefin metathesis products with up to 79% conversion rate.
* The yield of most catalysts goes sharply down after incubation in blood because of deactivation (by GSH, for example); AlbRu-I achieves a good yield of 21% after incubation in blood for 24h.3
* AlbRu-I can be used to synthesize carboxylic acids.3
* One can split the available reactions into four groups:3
  + RCM
  + Sequential RCM/aromatization
  + Cross-metathesis
  + ROMP (ring-opening metathesis polymerization)
* Facilitate reaction:
  + Creation of 5/6 membered rings3
  + Protection of allylic hydroxyl group with pivalate group3
  + Sulfur-assisted metathesis mechanism ([??](https://pubmed.ncbi.nlm.nih.gov/21050005/)).
* Lower reaction yield:
  + Allylic hydroxyl group3

Why water sucks

Throughput, rate and byproducts (different types of selectivities)

* Both "free" and enzyme-bound Ru catalysts show Michaelis-Menten kinetics.2
* SAVmut outperforms both Hoveyda–Grubbs (HGII) and AquaMet (AQM) under some conditions.2
* AlbRu-I requires 1-5 mol% for efficient catalysis.3

Organisms and industry use-cases

* Microalgae offer high growth rate, minimal space and nutrient requirements and most importantly, are photoautotrophic and thus can produce by themselves reactants for olefin metathesis1.
* Unmodif1ied carbene ligands can lower cell viability in microalgae1.
* E. coli periplasm (the space between the two plasma membranes of the bacteria) is beneficial for olefin metathesis because it contains mostly glutathione disulfide and not glutathione, which is a metathesis inhibitor.2
* Non natural metabolic pathways1,2 (???).
* In-vivo drug synthesis in disease site enables avoiding side-effects due to harm to healthy tissues.3
* Design of transition metal catalysts to catalyze prodrug uncaging reactions in living humans is challenging because many components in the blood can deactivate them.3
* The antitumor-drug creating reaction in 3:



* Combining prodrug and the appropriate catalyst can achieve better activity (e.g. tumor growth suppression) than administering the drug itself, because of the site-specific activity.3
* The rarity of alkenyl groups in aqueous solutions in organisms allows OM to be very specific.4
* OM enables creating cross-linked peptide-mimics that are more stable than the "natural" ones.4

Papers and status

1. In Vivo Olefin Metathesis in Microalgae Upgrades Lipids to Building Blocks for Polymers and Chemicals – read and markered (not fully quoted)
2. Directed evolution of artificial metalloenzymes for in vivo metathesis – read, markered and quoted.
3. Catalytic olefin metathesis in blood – read, markered and quoted.
4. Olefin metathesis for chemical biology – read and markered (not fully quoted)
5. The Impact of Water on Ru-Catalyzed Olefin Metathesis: Potent Deactivating Effects Even at Low Water Concentrations
6. Grubbs-Hoveyda catalysts conjugated to a β-barrel protein: Effect of halide substitution on aqueous olefin metathesis activity

* Kinetic Protection of a Water‐Soluble Olefin Metathesis Catalyst for Potential Use under Biological Conditions.
* "Close-to-Release": Spontaneous Bioorthogonal Uncaging Resulting from Ring-Closing Metathesis
* Olefin metathesis catalysts embedded in β-barrel proteins: creating artificial metalloproteins for olefin metathesis
* Progress towards bioorthogonal catalysis with organometallic compounds
* Olefin cross-metathesis on proteins: investigation of allylic chalcogen effects and guiding principles in metathesis partner selection

**Schedule**

|  |  |  |
| --- | --- | --- |
| Task | Date | Status |
| 1. ~~In Vivo Olefin Metathesis…~~ 2. ~~Directed evolution of artificial metalloenzymes…~~ 3. ~~Catalytic olefin metathesis in blood~~ | 15.10.24 | Done |
| 1. Olefin metathesis for chemical biology 2. The Impact of Water on Ru-Catalyzed Olefin Metathesis: Potent Deactivating Effects Even at Low Water Concentrations 3. Grubbs-Hoveyda catalysts conjugated to a β-barrel protein: Effect of halide substitution on aqueous olefin metathesis activity   Answer Dr. Reem | 19.10.24 | Only if subject is accepted!  Was accepted :) |
|  | 23.10.24 |  |
|  | 31.10.24 |  |
|  | 2.11.24 |  |
| Start consolidating summaries and plan further | 9.11.24 |  |
|  | 16.11.24 |  |