**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, 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.

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.

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

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

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 – in progress

* Grubbs-Hoveyda catalysts conjugated to a β-barrel protein: Effect of halide substitution on aqueous olefin metathesis activity
* The Impact of Water on Ru-Catalyzed Olefin Metathesis: Potent Deactivating Effects Even at Low Water Concentrations
* 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

**Schedule**

|  |  |  |
| --- | --- | --- |
| Task | Date | Status |
| 1. ~~In Vivo Olefin Metathesis…~~ 2. ~~Directed evolution of artificial metalloenzymes…~~ 3. Catalytic olefin metathesis in blood | 15.10.24 |  |
|  | 19.10.24 | Only if subject is accepted! |
|  | 23.10.24 |  |
|  | 31.10.24 |  |
|  | 2.11.24 |  |
| Start consolidating summaries and plan further | 9.11.24 |  |
|  | 16.11.24 |  |