**In Vivo Olefin Metathesis – challenges, approaches and applications – summaries**

Work structure

* Introduction

1. Olefin metathesis overview
2. Principles of bioorthogonal chemistry

* Body:

1. Reasons to attempt in-vivo metathesis and examples of specific reactions
2. Challenges/requirements
   1. General (limitations of every OM)
   2. Reaction-specific
   3. Water-related
   4. biology-related
   5. use-case-specific (e.g. blood/cancer environment)
3. Solutions (can include lessons from other biorthogonal reactions)
   1. Biologically relevant conditions and model reactions
   2. Catalysts
      1. GHII (and III?) catalysts
      2. Metalloproteins – design, synthesis and usage
   3. Modification of the environment/additional reagents
   4. Choice of the organism

* Discussion

1. Recommended catalyst for each use-case
2. Most- and least-fitting OM reactions
3. More ideas for applications

* Conclusion

Basic knowledge

* Nitro-Grela catalyst: NHC-based catalyst, very efficient for many reaction types:

A chemical structure with letters and numbers

Description automatically generated

* AquaMet – another good GHII, can be used in metathesis of water-insoluble substrates in ′′classical′′ organic solvents and in reactions in ionic liquids (sigma-aldrich):



* Grubbs-Hoveyda second generation – an NHC catalyst with benzylidene ligands that have a chelating ortho-isopropoxy group attached to the benzene rings and without any phosphines. For example:



A deeper dive into GHII mechanism of reaction

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, probably because of the weaker hydrogen bonding (6). Similar results regarding iodide were reported in 6.
    - Bromide kind of sucks.6
  + 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 and also suppress CM.9
* In GH (Grubbs-Hoveyda) protein-conjugated catalysts, one of the halide ligands is directed toward the protein and the other toward the surface. That's why ligand (e.g. halide with hydroxide) exchange is possible and can lead to catalyst degradation.6
* Ways the protein protects the catalyst:
  + Preventing some of the ligand exchange.6
  + Making dimerization of the catalyst less favorable sterically.6
* NO3-AquaMet catalyst, in which NO3 replaces either one or both of the chloride ligands, can be generated from AquaMet and nitrate-containing species – it decomposes quickly alone but gives a greater yield than AquaMet for RCM of N,N-diallyltosyl amine specifically (but not to other metatheses tested).7
* GHII is stable in air and moisture.8 (look in quoted articles as well)
* Beta-barrel structure is a good anchoring for metalloproteins because the compact sheets provide robustness against degrading agents.8
* Membrane-spanning beta-barrel proteins are usually larger than their hydrophilic counterparts and can accommodate large GHII catalysts.8
* Schrock carbenes suck as ligands (for our use-cases) because they're not stable in oxygen and moisture.9 (look in quotes)
* NHC ligand donates elector and thus stabilizes the catalyst when catalyzing a reaction in proximity to S atoms, but phosphine ligands compete with the S atoms for coordination with the Ru.
* Schrock Mo catalysts can catalyze well CM of vinylglycine (press x for doubt), but its stability in air and moisture sucks.11
* For the catalyst to be soluble in water, it can contain a PEG or a charged group, like ammonium.11
* The ammonium also weakens the O–Ru coordination, resulting in fast initiation.11

Synthesis of catalysts

* For a metalloenzyme – the biotin-streptavidin technology2
  + The affinity between biotin and streptavidin is very high by nature, so when the metal catalyst is bonded to biotin it can be integrated to a streptavidin-derived protein.8 (look at quotes)
* 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).
* Metalloprotein with a dative bond – the protein donates both electors in the covalent bond – offer easy dissociation of catalyst from the protein but are hard to design.8
* Covalent anchoring offers precise positioning.8
* Anchoring can be based on creating on enlarging a cavity – an artificial active site – inside the protein (through mutations), to which the catalyst will bind.8
* A relatively long spacer between the protein and the catalyst may be needed to accommodate the bulky carbene groups.8
* Partial denaturation and then renaturation of the protein can be utilized to unfold it for the conjugation of the catalyst to inner amino acid residues.8

Reactions

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



* Another useful model RCM reaction:7



* "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 multiple groups:3
  + RCM
  + Sequential RCM/aromatization
  + Cross-metathesis:

Transferring an olefin to homoallylglycine didn't work but to Cys-like residues did work, probably because of the sulfur.9 (look in quoted article)

* + ROMP (ring-opening metathesis polymerization)
  + acyclic diene metathesis polymerization (ADMET)9
* 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/)) – the helpful chalcogen group can also be installed to on protein "artificially".9
  + Lower pH – increases TON.6
  + MgCl2 – the magnesium ions disrupt chelate formation.9
* Lower reaction yield:
  + Allylic hydroxyl group3
  + Water, of course (later on that)
  + GSH and histidine that coordinate to the metal center and can reduce the catalyst.7
  + Basic molecules – can deprotonate the metallocyclobutane and cause decomposition of the species – can be prevented by adding acids.7
  + Dimerization of the catalyst through the Ru.7
* The usage of pseudo-amino acids for metathesis allows the reaction to occur on the protein itself, but presents the additional challenge of conjugating the alkene to the protein
* Sac (s-allylcysteine) – a good residue for OM because the S coordinates with the Ru center and protects it from chelation while keeping it available for metallacyclobutane creation.10
  + The protein can be synthesized genetically or through direct allylation.10 Genetically, it's supplied externally and replaces a similar amino acid like methionine, or through amber stop codon reassignment.11
  + High yield substrates - allylic alcohols, ethers, and hexenyl glucoside, medium yield for allyl glycosides.10
  + Challenging substrates – bulky sugars with a short linking chain, alkenes with electron-withdrawing ammonium groups or acetamide.10
  + The metathesis partner needs to be slightly less reactive than the allyl sulfide.10
  + Linker-extended Sac is a bad bitch, both yield- and rate-wise,10 probably because the chain enables coordination with less steric strain than regular Sac.
  + Notably – this is not a metalloprotein case – the ligand is a "regular" GHII and the **substrate** is a protein.
* Se-allylselecocysteine (Seac) is even better than Sac because selenium is softer than sulfur and thus more fitting for the ruthenium, AND it's faster as well – also enhanced with a longer linker chain and succeeds with difficult substrats.10
* The reaction must tolerate chelating or reactive residues of the protein.11
* The protein must be accessible for the metathesis, sterically and conformationally.11
* Cross-metathesis can be templated by hydrogen bonding.11 (look in quoted article)
* Free OH group in a sugar in CM can damage yield.

Why water sucks

* Even in "organic solvent settings" in industry, water is a frequent, often unavoidable contaminant – even 100ppm water can cause 60% drop in yield.5
* Water form hydrogen bonds with NH2, Cl and I and the most sensitive catalyst will be the one with the weakest bonds.
* Water does not affect E/Z selectivity, but increases isomerization in the location of the double bond.5
* Hydrogen bonding destabilizes the reaction-ready conformer of some RCM reactants (increases its energy).5
* The effect is double – both on the catalyst and the reactant.
* Fast-initiating catalysts are more vulnerable to decomposition by water because the active state is the one attacked by water.5
* Catalyst decomposition in water =(mostly) halide exchange leading to dihydroxy complexes and binuclear species (6 quoting others).6

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
* The decomposed Ru catalyst can trigger double bond migration and DNA degradation (look for the quoted articles in 5).5
* High concentration of the catalyst can lead to biomolecular coupling and thus to a lower TON for some catalysts.5
* Annoying side reactions like olefin isomerization after beta-hydride elimination did not occur in 6.
* 6 achieved maximum TONs of 50-70 for some RCM reactions with the Ru-I2protein conjugated catalyst.
* An improvement in yield can be kinetic – for example, NO3-AM produces a greater yield than AM in some cases, though they decompose in the same rate because it initiates faster and improves the catalytic activity of the active species.7
* E/Z selectivity can be influenced by the anchoring protein:8

A diagram of a chemical structure

Description automatically generated

* Note to self – should find articles regarding metalloproteins with multiple catalysts per protein and whether they improve yield.
* Although a large spacer helps conjugation, it harms catalytic activity because the catalyst is free to move around (and it's bad) and coordinates to unwanted residues.8
* Large proteins -> large cavities -> more potential interactions -> less activity (personal observation based on the article).8
* Side reactivity is observed when installing a chalcogen group on a protein using reagents like MSH.9
* Self-metathesis of the product can ruin stuff, especially if it doesn't re-enter the catalytic cycle and the desired product does.10
* The products of catalyst decomposition in water – ruthenium hydrides – can promote unwanted side reactions like double bond isomerization and migration.11
* Coordination of carbonyl to ruthenium can promote OM instead of oligomerization.11

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.
* Unmodified 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
* Uncaging following RCM in a molecule connected to the olefin ([here](https://pmc.ncbi.nlm.nih.gov/articles/PMC6823642/)).
* Catalytically labeling or deactivating target molecules.
* The carbon-carbon double bond created can create analogues to proteins with disulfide bonds.9
* Requirements for protein-modifying reactions:9
  + Low-r.t.
  + Aqueous media
  + Chemoselectivity
  + The protein must contain an alkene group
* CM was used to mimic a certain post-translational modification of histones.9
* Genetic editing can be used to synthesize proteins that contain alkene groups.9
* Glycosylation and acetylation are biologically relevant modifications that can modify protein reactivity, affinity and cell communication.10
* Another reason for protein OM is creating a secondary structure with higher stability or affinity towards ligands (or other targets).11 Examples (all in 11 unless specified otherwise):
  + Beta-turn analogues.
  + Alpha-helices cross-linking.
  + "dicarba analogues" – mimicking disulfide bonds with the greater stability of the carbon-carbon bond – e.g. oxytocin.
  + Mimicking thioether bonds.
* PEGylation can increase the half-life of proteins.

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 – read, markered and quoted.
6. Grubbs-Hoveyda catalysts conjugated to a β-barrel protein: Effect of halide substitution on aqueous olefin metathesis activity – read, markered and quoted.
7. Kinetic Protection of a Water‐Soluble Olefin Metathesis Catalyst for Potential Use under Biological Conditions – read, quoted and markered.
8. Olefin metathesis catalysts embedded in β-barrel proteins: creating artificial metalloproteins for olefin metathesis – read, markered and quoted.

~~Progress towards bioorthogonal catalysis with organometallic compounds~~ – not OM :(

1. Modification of Proteins Using Olefin Metathesis - done, badly
2. Olefin cross-metathesis on proteins: investigation of allylic chalcogen effects and guiding principles in metathesis partner selection – read, markered and quoted.
3. Olefin Metathesis for Site-Selective Protein Modification

* Enabling olefin metathesis on proteins: chemical methods for installation of S-allyl cysteine (focus on the allyl-chalcogen effect) also:
  + Allyl sulphides in olefin metathesis: catalyst considerations and traceless promotion of ring-closing metathesis
* Metathesis in Peptides and Peptidomimetics
* Biocompatibility and therapeutic potential of glycosylated albumin artificial metalloenzymes
* In an Attempt to Provide a User's Guide to the Galaxy of Benzylidene, Alkoxybenzylidene, and Indenylidene Ruthenium Olefin Metathesis Catalysts
* On the Mechanism of the Initiation Reaction in Grubbs–Hoveyda Complexes
* Enabling olefin metathesis on proteins: chemical methods for installation of S-allyl cysteine
* Genetic Incorporation of Olefin Cross-Metathesis Reaction Tags for Protein Modification.
* Anionic Olefin Metathesis Catalysts Enable Modification of Unprotected Biomolecules in Water.

**General bioorthogonal stuff**

For reviews on metal-complex catalysis in biological systems, see a) L. Vigh, F. Jo\_ in Applied Homogeneous Catalysis with Organometallic Compounds, Vol. 3 (Eds.: B. Cornils, W. A. Herrmann),Wiley-VCH,Weinheim, 2002, pp. 1283 – 1289; b) \_.Csajb\_k, F. Jo\_ in Organometallic Chirality, Vol. 20 (Eds.: G. P\_lyi, C. Zucchi, L. Caglioti), Muchi Editore, Modena, 2008, pp. 69 – 86; c) P. Sasmal, C. Streu, E. Meggers, Chem. Commun. 2013, 49, 1581 – 1587. [3] For a review on palladium-mediated cross-coupling reactions in living systems, see J. Li, P. Chen, ChemBioChem 2012, 13, 1728 – 1731. [4] a) C. Streu, E. Meggers, Angew. Chem. 2006, 118, 5773 – 5776; Angew. Chem. Int. Ed. 2006, 45, 5645 – 5648; b) P. Sasmal, S. Carregal-Romero, A. Han, C. Streu, Z. Lin, K. Namikawa, S. Elliott, R. Klחster, W. Parak, E. Meggers, ChemBioChem 2012, 13, 1116 – 1120; c) P. Sasmal, S. Carregal-Romero, W. Parak, E. Meggers, Organometallics 2012, 31, 5968 – 5970. [5] a) R. Yusop, A. Unciti-Broceta, E. Johansson, R. S\_nchez-Mart\_n, M. Bradley, Nat. Chem. 2011, 3, 239 – 243; b) C. Spicer, T. Triemer, B. Davis, J. Am. Chem. Soc. 2012, 134, 800 – 803; c) N. Li, R. Lim, S. Edwardraja, Q. Lin, J. Am. Chem. Soc. 2011, 133, 15316 – 15319; d) J. Li, S. Lin, J.Wang, S. Jia, M. Yang, Z. Hoa, X. Zhang, P. Chen, J. Am. Chem. Soc. 2013, 135, 7330 – 7338; e) J. Weiss, J. Dawson, K. Macleod, W. Rybsko, C. Fraser, C. Torres- S\_nchez, E. Patton, M. Bradley, N. Carragher, A. Unciti- Broceta, Nat. Commun. 2014, 5, 3277; f) J. Li, J. Yu, J. Zhao, J. Wang, S. Zheng, S. Lin, L. Chen, M. Yang, S. Jia, X. Zhang, P. Chen, Nat. Chem. 2014, 6, 352 – 361; g) M. Yang, J. Li, P. Chen, Chem. Soc. Rev. 2014, DOI: 10.1039/c4cs00117f. [6] See also: a) K. Tishinov, K. Schmidt, D. H\_usinger, D. G. Gillingham, Angew. Chem. 2012, 124, 12166 – 12170; Angew. Chem. Int. Ed. 2012, 51, 12000 – 12004; b) Z. Chen, F. Vohidov, J. M. Coughlin, L. J. Stagg, S. T. Arold, J. E. Ladbury, Z. T. Ball, J. Am. Chem. Soc. 2012, 134, 10138 – 10145; c) K. K. Sadhu, T. Eierhoff,W. Rחmer, N.Winssinger, J. Am. Chem. Soc. 2012, 134, 20013 – 20016.

**Schedule**

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| --- | --- | --- |
| 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 :)  Done |
| 1. ~~Kinetic Protection of a Water‐Soluble Olefin Metathesis Catalyst for Potential Use under Biological Conditions.~~ 2. ~~Olefin metathesis catalysts embedded in β-barrel proteins: creating artificial metalloproteins for olefin metathesis~~ | 23.10.24 | Done |
| 1. ~~Modification of Proteins Using Olefin Metathesis~~ | 25.10.24 | Done |
| ~~Reread the relevant info in course textbook~~   1. ~~Olefin cross-metathesis on proteins: investigation of allylic chalcogen effects and guiding principles in metathesis partner selection~~ | 27,31.10.24 |  |
|  | 2.11.24 |  |
| Start consolidating summaries and plan further | 9.11.24 |  |
|  | 16.11.24 |  |