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

Summarized enough

Summarized moderately

Lot of missing info

No info at all

Citation needed?

Citations are numbered by the Summaries order until I'll specify otherwise

# Introduction

* 1. Olefin metathesis

Formation of new carbon-carbon bonds is one of the major objectives in modern organic chemistry. Alongside mechanisms like the Wittig reaction and palladium-catalyzed coupling, olefin metathesis is an important tool for achieving this goal.

Olefin metathesis usually involves the exchange of partners between two double bonds, though the same concept has also been applied to reactions in enynes? (between the double and triple bond).

The mechanism of the catalytic cycle was proposed by Yves Chauvin in 1971. It involves initiation of the catalyst by a [2+2]-cycloaddition to create a metallacyclobutane intermediate that immediately undergoes cycloreversion to form a species with the metal atom of the catalyst bonded to the carbon atom of the first alkene. In the propagation step, another cycloaddition and cycloreversion cycle with the second alkene creates the metathesis product (figure 1). The release of small alkenes, like ethylene, can make the reaction entropically favorable.?

OM reactions are grouped by the type of the reactants and products (figure 2) – intermolecular cross-metathesis (2a) involves the exchange of double-bond partners between two separate molecules. Ring-closing metathesis (RCM; 2b) is an intramolecular reaction that can be driven by the relative stability of five- and six- membered rings?. Polymerization reactions include ring-opening metathesis polymerization (ROMP; 2c) and acyclic diene metathesis (ADMET; 2d), which competes with RCM in some cases.? A sentence about the regio- and stereo- selectivity of the reaction.

The evolution of metathesis catalysts includes two major progressions – choice of the central transition metal involved and its ligands. Early reactions included Ti?, Nb? and Ta? complexes as catalysts. Later, tungsten (W) and molybdenum complexes were introduced and enabled – especially in Mo complexes – superior catalytic activity.? However, their low functional group tolerance and sensitivity to moisture and oxygen were significant drawbacks for many reactions.? In 1992, Grubbs introduced the first ruthenium complex for olefin metathesis, exhibiting lower activity but a significant improvement in stability in oxygen- and water-containing environments and for various functional groups.?

The second factor determining the catalyst's characteristics – its ligands – has also progressed significantly. Most Ru catalysts contain the carbene ligand responsible for the initiation – usually benzylidene (?) – two anionic ligands like chloride and two neutral ligands. While in early catalysts these neutral ligands were phosphines – chosen for…

* 1. Principles of bioorthogonal chemistry

Bioorthogonal chemistry describes a set of reactions that can be used in biological contexts and include reactants or catalysts that are not commonly found in nature. Thus, their reactivity with endogenous biological molecules like proteins, sugars and nucleic acids can be limited. Other key characteristics of bioorthogonal reactions are high yields and reactions rate, water tolerance and generally small reaction partners, which minimize perturbance to the biological system.14 These requirements enable highly selective and efficient modification of molecules in biological environments.

Multiple bioorthogonal reactions have been reported, including native chemical ligation to create amide bonds in protein synthesis,? Copper-catalyzed azide-alkyne cycloaddition (CuAAC) that forms triazoles,? tetrazine ligation that forms bicyclic compounds? and photoinducible reactions, in which light activates relatively stable reactants.?

Bioorthogonal reactions are often modified forms of well-known reactions that were optimized for the strict requirements of biological systems. For example, the Staudinger reaction – between a phosphine and an azide – was described in 1919 but was not useful for the creation of an amide bond in an aqueous environment because of spontaneous hydrolysis. Changing the ligands of phosphine prevented this and uncovered a highly selective and biocompatible tool.20

Bioorthogonal techniques complement strategies like genetic engineering and protein tags for many applications – drug development? and delivery?, expansion of the genetic code by incorporation of unnatural amino acids?, targeted protein degradation? and cell imaging by tagging different kinds of target molecules. A striking example of the latter, for in-vivo imaging of glycans distribution, was achieved in 2008 – researchers labeled cell-surface glycans in a zebrafish cell line with an azide equivalent then reacted it with a fluorescent cyclooctyne variant (DIFO-488).15 The glycans were labeled distinctly through the zebrafish's development.

In this work, I shall summarize the recent advances in olefin metathesis in the context of bioorthogonal chemistry and focus on the techniques and catalysts used to progress from reactions in aqueous media to metathesis in living cells (in-vivo).

# Body

1. Reasons to attempt in-vivo metathesis and examples of specific reactions

Although bioorthogonal chemistry enabled significant progress in research and is even used in certain industry processes,? the reactions mentioned in the section above have a limited scope of reactants and products – most additions involve azides, nitrogen heterocycles or alkynes and the formation of amide or similar heteroatom-containing bonds.? Although these can be useful in biological contexts, there is still a need for reactions that create a new carbon backbone.

Olefin metathesis can be a valuable tool in this endeavor – its versatility enables synthesis of many biologic and biologically reactive molecules, such as amino acids, peptides, proteins, sugars, lipids nucleic acids and drugs. A couple of the researched and proposed usages are hereby presented.

Synthesis and modification of proteins is one of the best studied applications of bioorthogonal olefin metathesis. Not only can metathesis catalyze the formation of a protein similar to one found in nature, but through a careful choice of the reacting residues, the synthesized protein can be improved in terms of stability and ligand affinity in comparison to the "natural" form. For example

Sugars

Lipids (?)

DNA (and RNA?)

Drugs

* "Living factories" inside organisms
* Drug synthesis, transport and uncaging/deprotection
* Protein modification
* DNA modification
* Further examples
* Replacement of different bioorthogonal reactions (not OM)

1. Challenges and requirements

The things that currently prevent us from achieving in-vivo metathesis in industry scale.

* 1. General (limitations of every OM)

There must be alkenes…

Side reactions must be avoided…

Beta-hydrsomething and double bond migration…

Removing ruthenium from the final products…

* 1. Reaction-specific

Two ways I can explain this:

* + the common grouping of OM reactions – RCM, CM, ROMP and ADMET, which is better and which present challenges
  + effect of specific groups in biological reactants, such as OH in sugars, steric hindrance in proteins, side reactions and reactivity of products
  1. Water-related
  2. Biology-related

The reaction must be fast…

Low substrate concentration…

Specificity

That damned GSH

Poisoning the organism - Ru is usually considered toxic and carcinogenic :(

Catalyst poisoning, decomposition, chelation and aggregation

Probably more about it in my summaries

* 1. use-case-specific (e.g. blood/cancer environment)

componentization of the reaction to the correct organ/organelle inside the cell

1. Solutions (can include lessons from other biorthogonal reactions)
   1. Catalysts

Generally, why Ru is the best and the rest suck

* + 1. GHII (and III?) catalysts

Why carbenes are the best and phosphines suck

Short introduction to GHII, GHIII, AquaMet and Grela with comparative studies of their STABILITY, TON, TOF and selectivity in some reactions

* + 1. Charged catalysts

Cationic and anionic and what's good about them, should compare to previous point's catalysts in same/similar table

* + 1. Metalloproteins/metalloenzymes – design, synthesis and usage+examples
    2. Getting rid of the catalyst afterward
  1. Biologically relevant conditions and model reactions – choice of substrate and reaction partners

Pseudo-amino acids and how to make them

Metatheses in which one of the substrates is a peptide or a protein require the incorporation of alkene-containing amino acids, which are unnatural amino acids (UAAs). This can be achieved chemically – by modifying an existing residue – or genetically.

Common alkene containing amino acids are depicted in figure x.

Bla bla chemical modification

Genetic incorporation of UAAs exploits their similarity to natural amino acids which allows them to occupy the binding site in aminoacyl-tRNA synthetase. A study testing the incorporation of S and Se methionine analogues to E. Coli methionyl-tRNA synthetase revealed that the turnover was the highest for S-allylhomocysteine (Ahc). Ahc was also incorporated successfully to multiple proteins in-vivo while keeping their original secondary structure and function – look at notes. Cross-metathesis by GHII catalyst with allyl alcohol or the fluorescent tracer fluorescein was performed with moderate to high yields (55%-95%), depending on the protein and the reaction partner. The same UAA was also installed into the Fc region of immunoglobulin G in a human cell line and reacted with olefin-biotin, illustrating the potential use for biotin tagging (??).

The chalcogen effect

Steric optimizations

All the nice things that facilitate reactions

* 1. Modification of the environment/additional reagents

Should be careful that these reagents are chemically and biologically inert

* 1. Choice of the organism

In case we get to – a good place for lessons from other reactions

# Discussion

1. Recommended catalyst for each use-case
2. Most- and least-fitting OM reactions
3. Challenges that still aren't answered and if I have any possible solutions
4. More ideas for applications

# Conclusion

# References