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

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 of 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), in which … is formed.

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 several types of photoinducible reactions, in which light activates 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, nowadays called the Staudinger ligation.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

## Use cases for in-vivo metathesis

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.? These can be useful in biological contexts, but 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, when an ethylene bridge replaces the disulfide bond in protein this can result in greater conformational rigidity and stability. A study comparing the activity and half-lives of oxytocin agonists and antagonists with these "dicarba analogues", that were synthesized through RCM, found that the substitution increases the half-life while retaining the biological activity of this peptide hormone.19

Post-translational modifications can alter proteins' stability, lifecycle and characteristics, and are vital to their function. Therefore, changing them through metathesis is another effective way to modulate biological activity. Histone acetylation is a well-known modification that regulates gene expression.? This modification was successfully mimicked by cross-metathesis on an alkene-bearing histone and even recognized by an antibody that naturally binds to this epigenetic marker. Next, the acetyl group was removed by elimination to restore the alkene. This unique "write-read-erase" cycle, though harder to perform in a living cell, is a prime example of the flexibility and switch mechanism metathesis reactions enable.

Protein degradation?? no examples yet

Sugars

Lipids (?)

DNA (and RNA?)

Metathesis in-vivo can be a valuable tool in drug delivery, as the bond forming or breaking process can liberate bioactive molecules from a cage in the required tissue or microenvironment. A study of virus-like particles as carrier molecules for peptide drugs developed the use of ROMP as the uncaging process. The particles were functionalized with NHS-activated norborene, and so a ROMP reaction that created a bond between two norborene molecules drove the disassembly of the vesicle and release of the cargo peptide.23 This generic mechanism may be used for other types of drugs and for other vesicles, as long as they can bind metathesis substrates.

Instead of protecting the bioactive compound of a drug in a cage, drugs can be administered in their inactive form – prodrug – and then converted to the bioactive form inside the body, in the target tissue. This approach can both minimize side effects, because the active drug will not reach unnecessary tissues, and increase the activity, because the drug will only act in the target tissue or cells. Olefin metathesis is especially suitable for such syntheses because it changes the carbon backbone of a molecule, and this can lead to different affinity and binding.

Prodrug activation was investigated with the antitumor drug SW620. An artificial metalloenzyme (ArM) was synthesized, containing a Grubbs-Hoveyda second-generation (GHII) catalyst anchored to human serum albumin protein. Localization to tumors was achieved by incorporation of the pentapeptide cGRD, which interacts with integrin, a protein that's overexpressed in cancer cells. cGRD was bound to the surface of the ArM so metathesis only proceeded near tumors. The metalloenzyme managed to catalyze the formation of the drug from its precursor through RCM in a solution containing blood, with moderate yield (39%). This technique was next tested in-vivo, in mice with implanted tumors. The prodrug administered with the metalloenzyme proved effective in halting tumor growth and achieved better results than direct administration of the drug itself, suggesting that increased specificity does enhance the effectiveness, at least for this drug.

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

Bioorthogonal olefin metathases can also be used in basic research, since it's ideal for creating artificial metabolic or signaling pathways. A study investigating abiogenesis and the development of modern cells employed this strategy in protocells consisting of only DNA. These cells were loaded with ArMs – a Grubbs-Hoveyda catalyst conjugated to streptavidin through biotin. The ArM catalyzed an RCM reaction that resulted in the release of fluorescent – and thus self-reporting – molecules, and molecules that intercalate into double-stranded DNA and lead to cell swelling by dynamization of the DNA membrane. More cellular effects – cell growth, fusion and functional adaptation – were mimicked without any "natively biological" reactions, illustrating the way abiotic reactions can be adopted by pre-cellular systems that could have evolved into modern cells.

In all the examples above, olefin metathesis was utilized in cells for the products to affect the organisms. But even when synthesizing non-biologically active molecules, cells may provide a good environment for the reaction to take place. Photoautotrophic organisms, like unicellular microalgae, are attractive candidates because they naturally produce unsaturated fatty acids, which can react in olefin metathesis. A recent study utilized CAAC and NHC ruthenium catalysts to catalyze cross-metathesis of these unsaturated acids to alkenes or diesters. Since these substrates are stored in lipid bodies inside the cells the catalysts were conjugated to a lipophilic fluorescent BODIPY moiety that localized them to these organelles. The conversion rate inside the cells was high – 72-29% (should check) – suggesting that sustainable and efficient polymer synthesis may be achieved using this type of cellular factories.1

## Challenges and requirements

A major challenge in utilizing olefin metathesis reactions in biological systems stems from nature of their biorthogonality – unconjugated alkenes, and especially terminal ones, are the common reactant in metathesis and may not be found in the cells where the desired metathesis should occur.4 Thus, in most cases the reactants should be administered with the catalyst or synthesized in-situ from a naturally occurring compound. Those olefins should be stable in the cellular environment and not cause harm to the cell.

Two common ways to increase the yield and rate of olefin metathesis reactions are lowering the pH and increasing the temperature.6,16,21 These settings, although easily controlled in a lab solution, usually cannot be modified in cell culture or inside an organism – biologically-regulated homeostasis will attempt to return the acidity and temperature to their original state and more importantly, such a change is likely to harm the cell, by causing protein denaturation or dynamizing lipid membranes, for example.

Another important factor that influences reactivity is the solvent. For most metatheses with ruthenium complexes, organic solvents like toluene were found to provide the best results.4,16 Cell cytoplasm, however, is an aqueous solution and cannot be modified.

Optimizing catalyst reactivity in aqueous conditions often involves addition of a cosolvent – DMSO, tBuOH or PEG compounds. 2,17,22 The cosolvent increases the solubility of the catalyst and prevents aggregation.21 However, just like for the solvent, in living systems a cosolvent cannot be added without serious damage to the cell.

Even without challenging substrates and biological condition, olefin metathesis can lead to undesired side reactions, the most common of which is isomerization – migration of the product's double bond as a result of beta-hydride elimination. Isomerization occurs with most common catalysts – though with varying degrees – and not only lowers the yield of the desired product but can also lead to the formation of harmful compounds.

### Reaction-specific challenges

Two ways I can address 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

### Water-related challenges

As mentioned before, the reaction solvent highly affects the rate, selectivity and yield of the reaction. In-vivo reactions must proceed in aqueous environments, which are far less-than-ideal for olefin metathesis.

The main effect of water is catalyst decomposition – the anionic ligands of the metathesis catalyst interact with the polar groups of hydrogen-bonding water molecules to form a hydrous species that's inactive in metathesis, or binuclear ruthenium complexes.6 quoting others,12 (?) Ruthenium hydroxide and hydride species – the result of exchange of the halide for hydroxide – do not catalyze metathesis but cause double bond migration and isomerization, leading to unexpected side products.5,11 (look at quote)

This pathway can lower yield significantly even when water is only a contaminant or cosolvent, but is most prominent when it's the main solvent. Fast-initiating catalysts are more susceptible to decomposition because the active form is more likely to interact with water. Therefore, the design of catalysts that can achieve efficient TONs while being soluble and water tolerant is a major challenge of aqueous olefin metathesis.

Water can also lower metathesis yield by changing the reactant's conformation – hydrogen bonding to water molecules changes the free energy of the alkene. For some RCMs tested, interaction with water destabilized the precyclic conformation by disrupting π-orbital stacking arrangement so that the free energy of the state increased by several kcal/mol. This makes the reaction less thermodynamically favorable and lowers the yield.5

### Biology-related

Lessons learned from other bioorthogonal reactions provide further constraints for olefin metathesis – since the reaction must proceed in both low substrate and low catalyst concentration (to minimize harm to the cell) and interact with biological molecules, which often undergo very rapid enzymatic changes, the reaction rate must be maximized as well. The decomposition of metathesis catalysts in water exacerbates this demand, because the limited lifetime of every catalyst molecule requires that it catalyzes as many cycles as possible before being inactivated.

It's nearly impossible to set a guideline for the desired reaction rate in TON, TOF or catalytic units like M/s, since every use case and reaction has different constraints, and most metathesis catalysts don't follow Michelis Menten kinetics used for comparison with enzymes.? We do know that most metathesis reactions suffer a significant rate decrease when organic solvents are exchanged for water,? and should therefore be optimized to achieve effective yields in-vivo.

Low substrate and catalyst loading in the cellular environment mean that the catalyst must be very specific and not interact with different molecules in the cell, which may be in a higher concentration. Since olefin metathesis is a bioorthogonal reaction not many cell-native terminal alkenes are expected to compete with the substrates, but ruthenium complexes demonstrate an ability to bind to DNA, which can inactivate them.

Specificity of the metathesis reaction does not mean only that the metathesis should proceed for desired reactants, but also in a specific cell or tissue, and in some cases, a specific cellular compartment. Localization, or selective activation of the catalyst, may be crucial for a successful synthesis that maximizes the effect of the synthesized product, provides a good metathesis micro-environment (e.g. an organelle with a lower pH) or prevents metathesis in sensitive tissues. To achieve this, the catalyst must contain a localizing moiety that binds preferentially near the reaction environment.

A unique biological challenge is the peptide glutathione (GSH). GSH is present in high concentrations (1-2 mM) in most cells and servers as an important antioxidant that aids in the removal of peroxides.yay  This tendency (?) for reduction makes it a potent inhibitor for metathesis complexes – it coordinates to the metal center and deactivates it. The amino acid histidine has similar properties and damages yield in a comparable manner.7 Since removal of these two vital molecules will damage the cell, overcoming the deactivation should be achieved by preventing interaction between the catalyst and GSH.

Removal of the catalyst from the reaction site is a challenge for all reaction, but in this case the set of available tools is much smaller – most techniques employ additives like phosphines, DMSO, supercritical fluid etc. or tagging the catalyst with a group that binds it to another material (e.g. solid support) followed by washing.last paper in textbook Obviously, none of these can be utilized if the cellular environment should remain living and functioning. An alternative should be found to scavenge the catalyst while keeping the cell intact, or to use such small loads that the cumulative effect of the transition metal complexes on the cell would be minimal. Since ruthenium complexes are known to have toxic and mutagenic effects, special care should be taken to avoid its aggregation (?).

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

Catalyst poisoning, decomposition, chelation and aggregation

Probably more about it in my summaries

Removing ruthenium from the final products (look at textbook)…

## Solutions (can include lessons from other biorthogonal reactions)

Most of the challenges and limitations presented above can be resolved, or at least minimized, by a careful design of reaction components (?) and settings. Since limited data on in-vivo metathesis was reported to date, I will also include results regarding aqueous media that may apply to living systems.

### Catalysts

* + 1. GHII 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

Choosing the catalyst is one of the most important decisions when planning a synthesis, and in the case of in-vivo olefin metathesis it must fit the strict requirements of the reaction – rapid and efficient catalysis in low concentration, specificity for reactants and selectivity for products, being biologically inert and non-toxic and localization to the relevant tissues and cellular components.

Ru complexes are established as the most stable and functional group- and water- tolerant olefin metathesis catalysts,4?? and most recent research in the field of aqueous olefin metathesis involved different ruthenium compounds.? Their early versions, with phosphine ligands, were very air sensitive and could catalyze a limited range of metathesis reactions,9? even when equipped with cationic groups to increase solubility.4 quoted? Replacing one phosphine ligand with NHC and the other with an isopropoxy group bound to the benzylidene creates the Grubbs-Hoveyda second generation catalyst (GHII), blab la bla (figure Xa)

Despite these advantages, GHII catalysts have glaring drawbacks – the cellular toxicity of ruthenium, poor solubility in aqueous solutions and rapid decomposition of the catalyst. The basic structure of the GHII should be optimized to fit the conditions inside cells.

* + 1. Charged catalysts

Article 21

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

Some downsides of neutral, hydrophobic catalysts can be resolved by attaching ionic moieties. AquaMet is a GHII catalyst in which a quaternary ammonium group is attached to the N-heterocyclic carbine, improves the solubility in water and weakens the coordination of the isopropoxy group to the ruthenium, resulting in faster initiation11 quotes. It was successfully employed in physiological conditionsand is moderately cytotoxic, significantly impacting HeLa cells viability in concentrations above 0.2 mg/mL and not harming 1MEA cells even in greater concentrations.23

However, cationic catalysts like AquaMet have drawbacks as well – they may acidify the water around the ruthenium complex, accelerating their decomposition, and fail to catalyze substrates on nucleic acids because of the attraction between the cationic group and the negatively-charged phosphate backbone (12 quotes).

AqueMet reactivity for specific RCM reactions can be improved by replacing one of the chloride ligands with nitrate. This new catalyst – AM-NO3 – decomposes in a similar rate but offers "kinetic protection" – because its initiation is faster more product is formed before the catalyst is inactivated and the total yield is greater.7

Anionic tags are an attractive alternative to cationic ligands – cyclic alkylaminocarbene (CAAC) ligands functionalized with a sulfonate tag showed greatly improved stability in water – the AquaMet aqua species, which is formed immediately in water, decayed over 2 hours whereas the anionic catalyst remained over 24 hours.11 They also managed to achieve ?? TONs (70-1520, depending on the reaction) for RCM and CM of various challenging substrates like the sugar galactopyranoside and uridine (a sentence about potential applications). Another significant improvement in comparison to AquaMet is the suppression of isomerization, enabling selective metathesis with little side-products. The reactions for these reported TONs proceeded in 70˚C and in the presence of NaCl and should therefore be tested in more realistic biological conditions.

* + 1. Metalloproteins/metalloenzymes – design, synthesis and usage+examples

Article 21

Preserving the structure and reactivity of the catalyst can be achieved by anchoring it to a protein. Metalloproteins or artificials metalloenzymes (ArMs) offer several advantages – minimizing the cytotoxic effect of free ruthenium species while protecting the catalyst from decomposition and side reactions and directing it to the relevant cell and site.

An appropriate anchoring protein and conjugation strategy of the catalyst to the amino residues are vital to the success of the metalloprotein. β-barrel proteins are good candidates because of the rigidity of the β-sheet motif and compact barrel structure that can protect the catalyst from degradation. More specifically, membrane-spanning β-barrel proteins are relatively large and can fit bulky catalysts in their pores or binding sites.8 and quotes?

A good example for this type of protein is ferric hydroxamate uptake protein component A (FhuA) - a transmembrane β-barrel protein isolated from E. coli. After attaching a maleimide linking unit and a cysteine residue to the NHC ligand of a GHII catalyst, the catalyst was successfully anchored to the protein and catalyzed a ROMP reaction in aqueous conditions of oxanorborene with high TONs (300-500) but without cis/trans selectivity. It's worth noting that for such a large protein the conjugation required genetically engineering a protease cleavage site, denaturing and digesting the protein and renaturing it afterwards to achieve refolding to the original secondary structure.28

Streptavidin is a small bacterial β-barrel protein with very high affinity to biotin.? This enables non-covalent conjugation of a biotinylated GHII catalyst to the protein. A metalloenzyme created this way outperformed both classic GHII catalyst, AquaMet and the free biotin-Ru species for RCM reactions inside E. coli cells.2

Exploitation of existing binding-site affinity was also used for synthesis of human carbonic anhydrase II (hCAII) ArM. Since this enzyme natively contains a Zn ion, installing an arylsulfonamide anchor on a GHII catalyst promoted dative binding of the catalyst to the protein and catalyzed RCM in aqueous conditions with TON of 28 in physiological conditions.25

Regardless of the type of protein or reaction, the length of the spacer between the catalyst and the protein's peptide is significant to the reactivity and selectivity of the reaction as it affects the second ligand sphere – the amino acids that the catalyst contacts and the substrate's steric constraints for contact with the catalyst. The study on FhuA protein compared multiple lengths of the chain linking the catalyst to the FuhA protein. It was found that the shortest spacer led to the highest TONs and a slightly higher cis/trans ratio of the product – it may be concluded that in this case a longer spacer enables coordination with hydrophilic amino acid residues on the anchoring protein that compete with the metathesis reaction.28 However, when the same catalyst was incorporated to the smaller β-barrel protein nitrobindin, a longer spacer was required both for successful synthesis of the metalloenzyme – to accommodate the bulky NHC ligand – and achieved the highest TONs in ROMP reaction.8+its quotes Therefore the design of a metalloenzyme must take into account the balance between minimizing contact with nearby amino acids residues and enabling interaction with the substrate, which depends on the protein, the catalyst and the catalyzed reaction.

The stability and reactivity of the metalloenzyme may be affected by the anionic ligand bound to the ruthenium. A study comparing three different halides – chloride, iodide and bromide – in a GHII catalyst conjugated to nitrobindin discovered that iodide complexes had the highest TONs for several RCM reactions in aqueous environment. Chloride complexes were the second best, with bromide metalloenzymes having the lowest TONs and failing to catalyze in some conditions. The improvement in reactivity for iodide-substituted metalloenzymes is attributed to the hydrophobicity of this ligand in comparison to chloride. Since it doesn't form hydrogen bonds in the aqueous environment, it's less susceptible to halide-hydroxide ligand exchange the leads to the decomposition of the catalyst. The higher stability over a long reaction time enables higher TONs.6 The same effect – both for catalyst stability and RCM yield – was observed for a different metalloenzyme containing albumin, suggesting that iodide substitution will be helpful for any ArM in which the catalyst is exposed to the aqueous environment.3

The synthesis of the FhuA ArM required mutagenesis to insert a cleavage site for the incorporation of the GHII catalyst;28 experiments employing the smaller protein nitrobindin introduced mutations to construct a hydrophobic cavity for catalyst anchoring.8+quotes The most striking usage of mutagenesis for ArM optimization was exhibited in the directed evolution of a streptavidin (SAV)-based metathase. In contrast to the previous examples, the SAV research employed multiple consecutive rounds of mutagenesis that improved the cell-specific activity of the ArM for a specific reaction. The mutations included substitutions of amino acids close to the catalysis site to increase the flexibility of the tertiary structure, reduce steric hindrance or increase the entropy of reaction (??? Check that I'm not lying). Different mutants of the protein performed best for different metathesis reactions, suggesting that a global "ideal metathase" for all reactions may not be developed, but each use-case can benefit from engineering of a customized mutant through directed evolution.2

Another promising technology for catalyst uptake is through a polymersome – an artificial organelle composed of a polymer and the catalyst. In a recent study, a polyethylene glycol (PEG) derivative was conjugated – either covalently or through hydrophobic interactions – to HGII catalyst to form polymersomes. These were successfully internalized by HeLa cells, localized in the lysosome and catalyzed RCM formation of umbelliferone inside the cell. The combined effect of encapsulation and localization inside an intracellular organelle both protects the cell from the cytotoxicity of free GHII catalysts and prevents decomposition of the catalyst by cellular compounds like GSH. It should be noted that the conversion rate of this reaction was quite low, perhaps due to said encapsulation isolating the catalyst from the substrate.22 Further optimization is necessary to adapt this interesting technique to other metathesis reactions and on a wider scale.

* 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.

Chemical modification can be achieved by direct allylation with allyl halides. This method was used to convert cysteine to S-allylcysteine (Sac), and showed(?) a high conversion rate with mild conditions and without denaturing the protein. Since allyl halides may also react with other amino acids – such as lysine and histidine – a more specific allylation protocol was developed for cysteine in which the amino acid was first converted to Se-allyl selenenylsulfide using allyl selenocyanate and then underwent rearrangement to form Sac. Both methods provided an active protein that also reacted in olefin metathesis with allyl alcohol.

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 + selenium?

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

* 1. Purification and removal of the catalyst

# Discussion

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

RCM is king

1. Challenges that still aren't answered and if I have any possible solutions
2. More ideas for applications

Ubiquitylation -> protein degradation

# Conclusion

# References