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研究課題名（和文）Application of gold-catalyzed 2-ethynylbenzamide cyclization for the tumor-localized in vivo release of anticancer drugs

研究課題名（英文）Application of gold-catalyzed 2-ethynylbenzamide cyclization for the tumor-localized in vivo release of anticancer drugs

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研究成果の概要（和文）：私達は、生体触媒反応を用いた２つのテーマに取り組んだ。１つ目のテーマでは、アルブミンの疎水性ポケットに金属を導入することで、グルタチオン存在下でも触媒反応が効率的に進むことを発見し、新しい人工金属酵素を開発した。ルテニウム触媒反応によるメタセシスをがん細胞で実施して、抗腫瘍活性を持つumbeliprenin天然物を合成してがんを選択的に殺傷した。さらに植物でもメタセシス反応を行って、エチレンホルモンの初めての生体イメージングに成功した。２つ目のテーマでは、金触媒によるエチニルベンズアミドの環化反応を開発し、抗腫瘍性のドキソルビシンやエンドキシフェンをがん細胞で活性化し、治療することに成功した。

研究成果の学術的意義や社会的意義
This is basic research done to develop new systems and reactions that can potentially be used to create new targeted drug therapies in the future. The research results were disseminated to the scientific community through refereed publications, as well as conferences.

研究成果の概要（英文）：In this project, we pursued two aspects related to biocatalysis. First, we focused on the development of albumin-based artificial metalloenzymes (ArM). We revealed that these systems were highly biocompatible due to their prevention of glutathione entry into the hydrophobic binding pocket of albumin. Using this technology, we developed a prodrug system that used ring-closing metathesis to synthesize umbelliprenin in cell cultures. In addition, we also adapted this system to design and develop an ethylene sensing probe. This probe was then used for the spatiotemporal detection of ethylene biosynthesis in fruits and leaves. Another aspect of biocatalysis that we explored was the development of an Au(I)-mediated ethynylbenzamide cyclization reaction. This reaction could be adapted to release amine containing drugs like doxorubicin and endoxifen. The activation of these prodrugs was shown to proceed effectively in various cell-based assays.

研究分野：Biocatalysis

キーワード：biocompatibility　artificial metalloenzyme　enzymes　gold catalysis　drug release

１．研究開始当初の背景

Interest in the synthetic scope of biocatalysts has expanded significantly over the past decade, largely due to the rapid advances made in molecular biology (i.e. directed evolution) and water-compatible transition metal catalysts. As such, future biocatalyst development may not only have substantial impact from an industrial standpoint, but could also find potential usage in creating new medical therapies via new-to-nature biocatalytic reactions.

２．研究の目的

There are two main objectives that arose from this research project:

The first objective was to develop a water-compatible, gold-catalyzed reaction that can lead to amine release. Thus far in literature, the bulk of examples have chiefly centered on transition metals like ruthenium and palladium. Likely, the reason for the lack of gold usage thus far is the dearth of water-compatible reactions that can lead to functional chemical moieties. As such, we aimed to fill this void via Au(I)-mediated alkynylbenzamide cyclization.

The second objective was to explore the use of the serum albumin protein scaffold for the development of artificial metalloenzymes (ArM). As a known drug transporter, albumin is known to possess many binding pockets; one being the Sudlow site I hydrophobic pocket. As such, we aimed to develop albumin-based ArMs with abiotic catalysts specifically anchored inside the hydrophobic binding pocket.

３．研究の方法

As this research project combines both aspects of chemistry and biology together, the methodology taken heavily reflects this. First, the focus was placed on the development of novel Au(I) catalyzed reactions. In parallel, the ligand-catalyst compounds needed for the development of ArMs were pursued. In the second part of this project, the focus instead shifted towards biological aspects. This would include assays related to enzyme activity and characterization, cell cytotoxicity, and fruit/plant imaging.

４．研究成果

The main results of our work are summarized in the following subsections, as extracted from the refereed papers related to this KAKENHI program:

1) Development of gold-catalyzed, drug release reactions: To explore novel metal-based uncaging reactions, this work introduces the 2-alkynylbenzamide (Ayba) moiety for the Au(I)-triggered release of secondary amines under mild and physiological conditions. As shown in Figure 1A, this reaction mechanistically proceeds via gold-activation of the alkynyl group, which then elicits nucleophilic attack from the proximal carbonyl oxygen. Endocyclization is then favored to generate the oxonium intermediate that is susceptible to base-dependent hydrolysis. As a result, a secondary amine-containing molecule can then be released. Studies were further performed to highlight some intrinsic benefits of the Ayba protecting group, which are 1) its amenable nature to derivatization for manipulating prodrug properties, and 2) its orthogonality with other commonly used transition metals like palladium and ruthenium. With a focus on highlighting its application for anticancer drug therapies, this study successfully showed that gold-triggered

conversion of Ayba-protected prodrugs into bioactive anticancer drugs (i.e. doxorubicin, endoxifen) can proceed effectively in cell-based assays (Figure 1B).

The paper presenting these findings has been finalized and is in the process of peer review/revision.

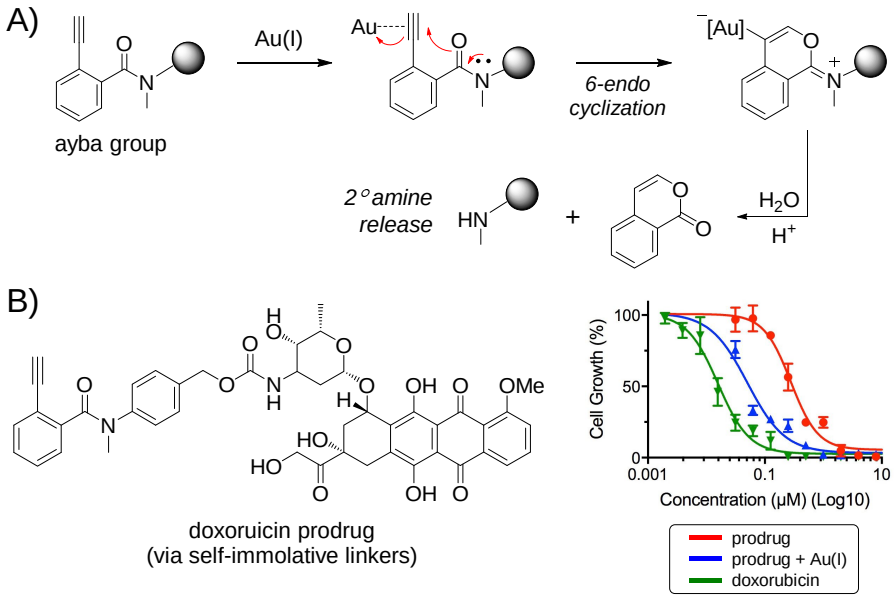

Figure 1. A) Proposed mechanism for Ayba deprotecton via Au(I)-catalysed cyclization. Subsequent hydrolysis then leads to the release of a secondary amine. B) Ayba-based doxorubicin prodrugs were then prepared, which were shown to be activated by Au(I) complexes in various cancer cell cultures (i.e. HeLa).

2) Development of artificial metalloenzymes: An albumin-based artificial metalloenzyme (ArM) was developed where coumarin-catalyst ligands were anchored into the hydrophobic binding pocket (Sudlow site I) of albumin. In terms of reactivity, we observed that the protein scaffold itself had a critical influence over substrate recognition. For example, the highest catalytic activities when performing ring-closing metastasis were observed only with hydrophobic substrates. In the case of charged or lipophilic substrates, these compounds generally displayed poor activity.

Another important observation from these studies was that the balance between the deep hydrophobic binding pockets of albumin and its negatively charged protein surface naturally repels entry to hydrophilic metabolites like glutathione (GSH). The significance of this effect speaks to one of the current challenges of in vivo metal usage, which is the general susceptibility of abiotic metals to be quenched by cellular thiols. As a result, studies showed that albumin-based ArMs could remain catalytically active even in the presence of GSH in solution. For example, using a 1,6-heptadiene-based substrate, metastasis activity was shown to proceed even in the presence of up to 1000× equivalents of GSH additive.

The next aim of this study shifted towards adaptation for cancer-selective prodrug activation. As depicted in Figure 2, glycosylated artificial metalloenzymes (GArMs) were developed in order to elicit localized transformation of the diallyl substrates **1a**/**2a** to coumarin derivatives **1b**/**2b** via ring closing metathesis. As discussed earlier, albumin ArMs generally display enhanced activities for hydrophobic substrates. As such, it came as no surprise that the farnesylated substrate **1a** showed a significantly higher

Kcat/KM than the simple methylated substrate **2a** (Figure 2A). Of particular note is that **1b** (umbelliprenin) is a known anticancer agent that functions primarily through inducing G1 cell cycle arrest. For cancer selective targeting, GArM-Ru complexes were decorated with an assembly of α(2,3)sialo-terminated glycans, which allowed them to gain strong affinity to SW620 cancer cells (Figure 2B). Subsequent assays were then done to show the accumulation of GArM-Ru for prodrug **1a** activated cytotoxicity in cultures of SW620 cancer cells (Figure 2C).

The paper presenting these findings has been finalized and published (*Nat. Catal.* **2019**, 2, 780-792).

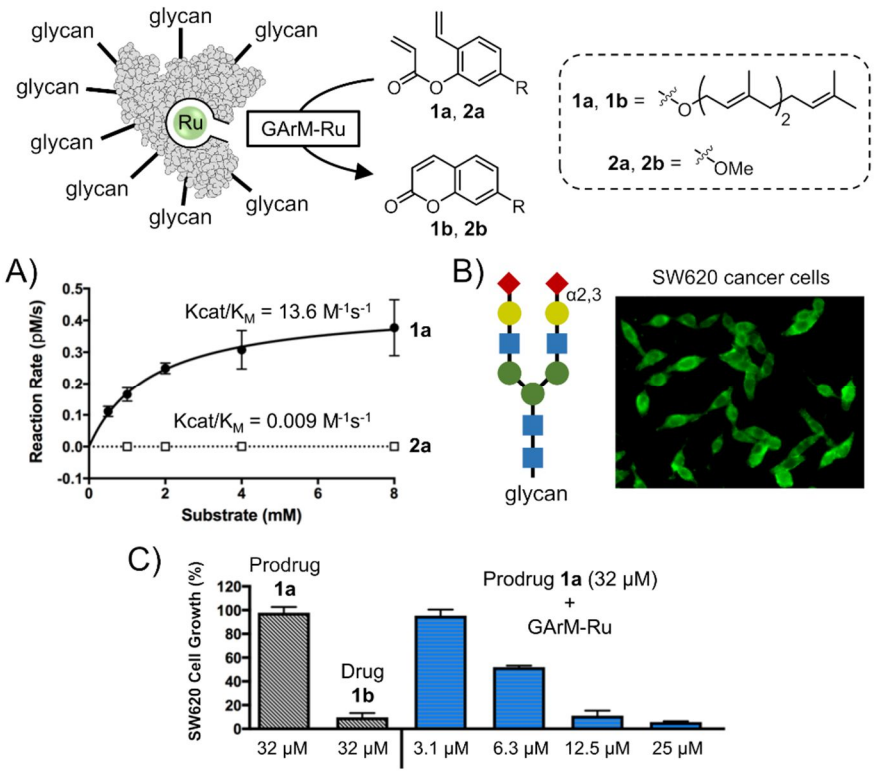

Figure 2. Cancer targeted prodrug activation via GArM-Ru. A) Enzyme kinetics for substrates **1a** and **2a**. B) Fluorescent imaging showing GArM-Ru affinity for SW620 cancer cells. C) Cytotoxicity of GArM-Ru/**1a** mixtures against SW620.

3) Development of ethylene biosensors: In this project, an ArM ethylene probe (**AEP**) was designed and developed. Ethylene gas is an essential plant hormone that plays a major role in regulating aspects of growth, immunity, and senescence. Since current ethylene detection methods have mainly employed analytical techniques like gas chromatography, electrochemical sensors, and laser-based techniques, there are no current means for spatiotemporal detection directly on samples. With this in mind, our group investigated the creation of an ethylene-sensing ArM biosensor (Figure 3A), which is based on using the albumin scaffold to solubilize and protect a quenched ruthenium catalyst. In the presence of ethylene, cross metathesis is then expected to occur, which leads to the removal of the quencher and the emission of a fluorescent signal.

Using the **AEP** probe, imaging studies were conducted on a variety of fruit and plant samples to validate detection produced by both exogenous and endogenous changes to ethylene biosynthesis. For example, the

spatial capabilities of **AEP** were shown in studies using slices of pears where specific regions were externally exposed to either an activator (ACC) or inhibitor (PZA) of ethylene biosynthesis. Shown in Figure 3B, these additives then led to either an increase or decrease in fluorescent intensity. In regards to endogenous ethylene changes, another part of this study focused on comparing images of unripe and ripe kiwifruit slices. Shown in Figure 3C, the **AEP** probe was used to detect changes in ethylene biosynthesis specifically in the outer pericarp of kiwifruit. Since this process is typically upregulated during the ripening process, comparative studies showed an increase in pericarp fluorescence for ripening kiwifruits as expected.

The paper presenting these findings has been finalized and published (*Nat. Commun.* **2019**, 10, 5746)

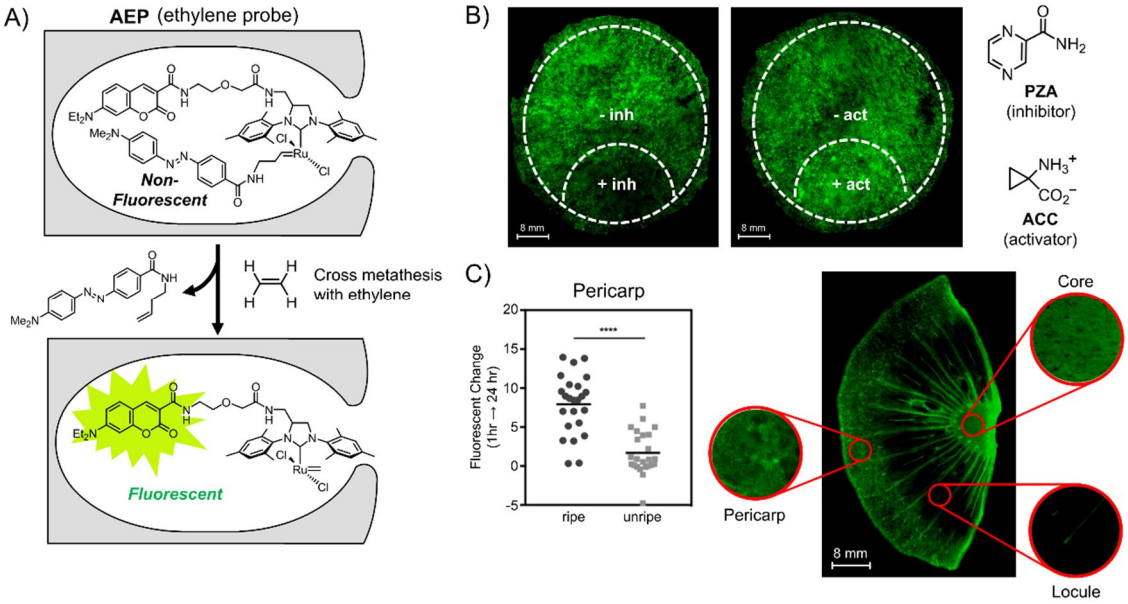

Figure 3. A) The mechanistic basis behind ArM ethylene probes (**AEP**) relies on the ethylene-triggered release of a quencher. B) Fluorescent images of pear slices to highlight the spatial imaging capabilities of **AEP** from exogenously induced ethylene. C) Fluorescent images of ripe kiwifruit slices to highlight the capabilities of **AEP** to detect endogenously induced ethylene.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　6件／うち国際共著　6件／うちオープンアクセス　1件）

１．著者名 A. Ogura, S. Urano, T. Tahara, S. Nozaki, R. Sibgatullina, K. Vong, T. Suzuki, N. Dohmae, A. Kurbangalieva, Y. Watanabe, K. Tanaka	４．巻 54
２．論文標題 Viable strategy for screening the effects of glycan heterogeneity on target organ adhesion and biodistribution in live mice	５．発行年 2018年
３．雑誌名 Chem. Commun.	６．最初と最後の頁 8693-8696
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/C8CC01544A	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Y. Lin, K. Vong, K. Matsuoka, K. Tanaka	４．巻 24
２．論文標題 2-Benzoylpyridine ligand complexation with gold critical for propargyl ester-based protein labeling	５．発行年 2018年
３．雑誌名 Chem. Eur. J.	６．最初と最後の頁 10595-10600
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/chem.201802058	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 S. Eda, I. Nasibullin, K. Vong, N. Kudo, M. Yoshida, A. Kurbangalieva, K. Tanaka	４．巻 2
２．論文標題 Biocompatibility and therapeutic potential of glycosylated albumin artificial metalloenzymes	５．発行年 2019年
３．雑誌名 Nat. Catal.	６．最初と最後の頁 780-792
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オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 K. Vong, S. Eda, Y. Kadota, I. Nasibullin, T. Wakatake, S. Yokoshima, K. Shirasu, K. Tanaka	４．巻 10
２．論文標題 An artificial metalloenzyme biosensor can detect ethylene gas in fruits and Arabidopsis leaves	５．発行年 2019年
３．雑誌名 Nat. Commun.	６．最初と最後の頁 5746
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-019-13758-2	査読の有無 有
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1．著者名 K. Tanaka, K. Vong	4．巻 96
2．論文標題 Unlocking the therapeutic potential of artificial metalloenzymes	5．発行年 2020年
3．雑誌名 Proc. Jpn. Acad. Ser. B.	6．最初と最後の頁 79-94
掲載論文のDOI（デジタルオブジェクト識別子） 10.2183/pjab.96.007	査読の有無 有
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/smll.201906890	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

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4．発表年 2018年

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４．発表年
2019年

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K. Vong, K. Tanaka	2019年
２．出版社	５．総ページ数
Elsevier	34
３．書名	
Kinetic Control in Synthesis and Self-Assembly	

１．著者名	４．発行年
K. Vong, K. Tanaka	2020年
２．出版社	５．総ページ数
Wiley	84
３．書名	
Handbook of In Vivo Chemistry in Mice: From Lab to Living System	

〔産業財産権〕

〔その他〕

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６．研究組織

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