博士論文

“Bio-Adhesive” Covalent Organic Framework for Bioapplications

(生医学応用を志向した生体接着性共有結合性有機構造体)

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【1】 Introduction

Porous polymeric materials with high surface area and porosity are an attractive materials for diverse applications. Since these properties facilitate the storage of small molecules or the transfer of ions, the porous material has a strong potential for bio-applications that can act as a drug carrier. A wide range of porous materials for bio-applications have been developed including zeolite, mesoporous silica, and metal-organic frameworks (MOFs). However, it has long been a challenge to develop designable porous materials where precise and simultaneous control over pore geometries and chemical functionalities is possible, which can be leveraged to tailor the functions for designed bio-applications. Thus, if we can fabricate the interior/surface of the pore precisely for specific purposes such as target-selective drug release, the scope of bio-applications of the porous materials will be further broadened. In this regard, covalent organic framework (COF; Figure 1), which is crystalline porous organic polymers having tunable pores, permanent porosity, high surface area, and metal-free back bone, provide an emerging platform for researchers to design favorable materials.(1) Since COF has the advantage of having a wide range of ligand selection, allowing for the rational design and facile post-synthetic treatment,(2) researchers are able to fabricate them at a molecular level toward bio-applications. Previously, our group developed "molecular glue" having multiple guanidinium ions (Gu^+) that strongly adheres to oxyanionic moiety of biomolecules by salt-bridge interactions (Figure 2).(3) Molecular glue shows great potential for diverse bio-applications such as selective drug release and enzymatic activity control.

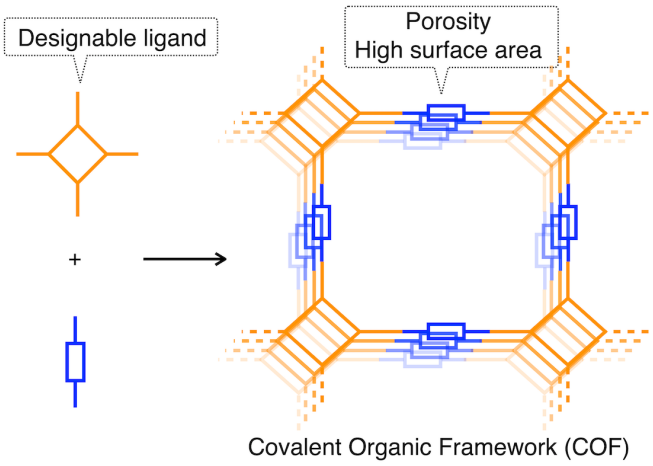

Figure 1. A schematic image of assembly of covalent organic framework with designable ligands.

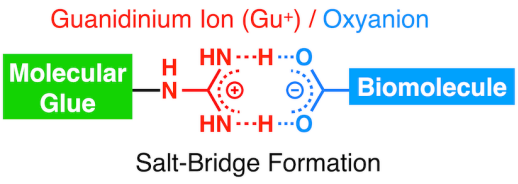

Figure 2. A salt-bridge between guanidinium ion (Gu^+) of molecular glue and an oxyanion on biomolecular surfaces.

Inspired by previously developed molecular glue in our group, I mainly focused on development new class of COFs by functionalizing guanidinium ion (Gu^+) and its bio-applications.

1) We newly developed a "bio-adhesive" covalent organic framework ^{Glue}COF, which allows noncovalent incorporation of a proteinous capping module (Cap) onto the guest-loaded 1D nanopores that can spatiotemporally release drugs in response to endogenous disease signals.

2) We developed a photosensitizer loaded ^{Glue}COF, which enables substance transfer by light irradiation.

【2】Spatiotemporal Guest Release

Spatiotemporal drug release selectively at diseased sites is one of the most awaited functions for next-generation drug carriers. An ideal carrier design would feature a particular capping module that can strongly adhere and block the guest-loaded nanopores for stable guest entrapping but change its conformation, upon selective binding with a signaling species, to allow the guest release.

We newly developed "bio-adhesive" covalent organic framework ^{Glue}COF (Figure 3), which allows noncovalent incorporation of a capping module (Cap) onto its guest-loaded 1D nanopores, affording ^{Glue}COF⊃^{Cap}Guest (Figure 3b). As shown in Figure 3a, ^{Glue}COF is densely functionalized with a large number of guanidinium ion (Gu^+) pendants that can be salt-bridged with oxyanionic species. Therefore, ^{Glue}COF possibly adheres to biomacromolecules such as proteins via a multivalent salt-bridging interaction. As a proof-of-concept study, we chose calmodulin (CaM), which is known to bind to Ca^{2+} (K_d = 0.1–1.0 μM) and change its conformation. Intrinsically, CaM has a high binding affinity

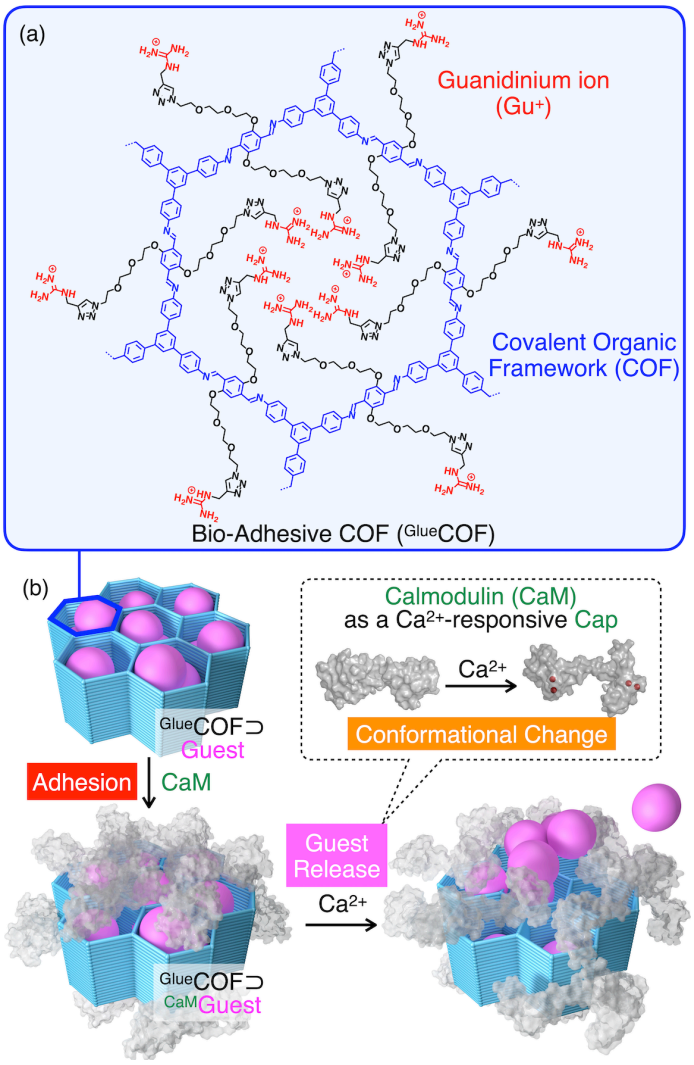

Figure 3. (a) Schematic illustration of Ca^{2+}-responsive guest release from ^{Glue}COF⊃^{CaM}Guest, whose guest-loaded nanopores are blocked by calmodulin (CaM) as a "capping module". (b) Molecular structure of bio-adhesive ^{Glue}COF bearing multiple guanidinium ion (Gu^+) pendants as an adhesive unit for proteins.

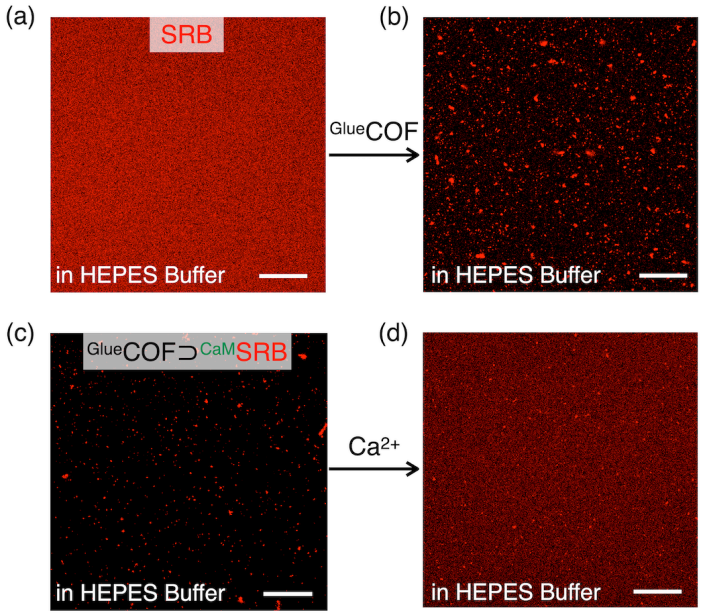

Figure 4. (a, b) Confocal laser scanning microscopy (CLSM; λ_{ex} = 550 nm) images of a HEPES (20 mM, pH 7.2) buffer solution of SRB before (a) and (b) after 3 h incubation with ^{Glue}COF. Scale bars = 20 μm. (c, d) CLSM images of a HEPES buffer suspension of ^{Glue}COF⊃^{CaM}SRB before (c) and (d) after 48 h incubation with Ca^{2+} ([$CaCl_2$ = 8 mM]).

toward Ca^{2+}, an ionic biosignal originating from bone diseases such as multiple myeloma, where the local concentrations of Ca^{2+} at diseased sites are usually higher than 4 mM. Upon treatment with Ca^{2+}, ^{Glue}COF⊃^{CaM}Guest actually released its guest as a consequence of the Ca^{2+}-induced conformational change of the CaM cap.

^{Glue}COF was characterized by several analytical methods such as XPS, FT-IR, PXRD, solid state NMR and TEM. These analyses indicated that ^{Glue}COF have 2D hexagonal geometry with 1D pores and the conversion of precursor into ^{Glue}COF as 82%. Guest loading and release property of ^{Glue}COF was examined using a fluorescent dye such as negatively charged sulforhodamine sodium salt (SRB, 1.6 × 1.2 nm) as a model drug. ^{Glue}COF showed very high loading capacity (0.18 g/g), which is comparable to those reported for other COF-based carriers (9.7–32.5 wt%).

As shown in Figure 4, confocal laser scanning microscopy (CLSM; λ_{ex} = 552 nm) successfully visualized the trapping and release of SRB: The initial bright fluorescence due to SRB in the solution phase (Figure 4a) became dark upon addition of ^{Glue}COF (Figure 4b), while the ^{Glue}COF particles became fluorescent. CLSM also showed that the solution phase of the suspension of ^{Glue}COF⊃^{CaM}SRB in HEPES buffer (Figure 4c), after the addition of Ca^{2+}, again turned entirely fluorescent (Figure 4d) as a possible consequence of the release of SRB from ^{Glue}COF⊃^{CaM}SRB.

After mixing with CaM (1 mM) to form ^{Glue}COF⊃^{CaM}SRB ([^{Glue}COF] = 5 μg/mL, [SRB] = 1.6 μM), we detected the leakage of only a negligible amount of SRB (Figure 5a, red), compared to that of ^{Glue}COF⊃SRB. Of particular interest, when Ca^{2+} ([$CaCl_2$] = 8 mM) was added to the suspension of ^{Glue}COF⊃^{CaM}SRB, SRB was readily released (Figures 5a, green). Figure 5b shows that the addition of Ca^{2+} to a HEPES buffer suspension of guest-free ^{Glue}COF⊃^{CaM}None ([^{Glue}COF] = 0.5 μg/mL, [CaM] = 50 μM) resulted in changing its circular dichroism (CD) spectral profile in a manner analogous to that observed for free CaM, suggesting that Ca^{2+} was bound to the CaM capping module in ^{Glue}COF⊃^{CaM}None to change its conformation. When Mg^{2+}

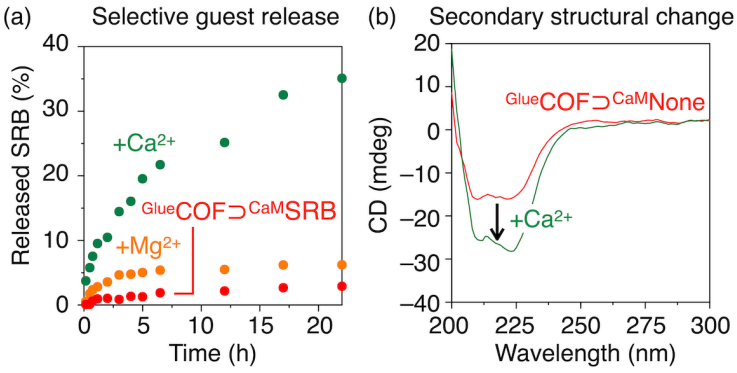

Figure 5. (a) SRB release profiles of ^{Glue}COF⊃^{CaM}SRB in the absence (red) and presence of Ca^{2+} (green) and Mg^{2+} (orange). (b) Circular dichroism (CD) spectra of a HEPES (20 mM, pH 7.2) buffer suspension of ^{Glue}COF⊃^{CaM}None before and after the addition of Ca^{2+}.

($[MgCl_2]$ = 8 mM) that has been reported to interact only weakly with CaM, instead of Ca^{2+}, was added to a HEPES buffer suspension of ^{Glue}COF⊃^{CaM}SRB, the release of SRB from ^{Glue}COF⊃^{CaM}SRB barely resulted (Figure 5a, orange). We also found that ^{Glue}COF⊃^{CaM}SRB can enter living cells by means of confocal laser scanning microscopy, and it was non-cytotoxic and is therefore potentially usable as a carrier for drug delivery.

In conclusion, we have shown that our newly developed bio-adhesive ^{Glue}COF with regularly arranged Gu^+-appended nanopores spatiotemporally released drugs in response to endogenous disease signals. As demonstrated in this study, a Ca^{2+}-responsive protein CaM adheres to and blocks the ^{Glue}COF nanopores for stable guest entrapping. However, a conformational change of CaM, upon selective binding with Ca^{2+}, causes the guest release.

【3】Substance Transport

Biological ion channels are molecular gatekeepers that facilitate transport across cell membranes. Development of artificial channel is scientifically exciting relevant to substance transport. However, fabricating synthetic channels in a spatiotemporal manner remains a significant challenge.(5)

In a previous study in 【2】, we found that proteins strongly adhered to ^{Glue}COF⊃Guest through a salt bridge interaction. Based on it, here, we developed the substance transfer between liposomes adhered by rose bengal (RB) loaded ^{Glue}COF (^{Glue}COF⊃RB) upon irradiation (Figure 6a). RB is known to generate singlet oxygen ($^1O_2$) species and peroxidize the lipid of the membrane.(6) Therefore, the generation of $^1O_2$ from ^{Glue}COF⊃RB, which connects the liposomes, induce the substance transfer upon light irradiation.

The adhesion of liposomes takes place upon addition of ^{Glue}COF. As shown in Figure 6b, confocal laser scanning microscopy (CLSM; λ_{ex} = 405 nm) shows the fluorescence due to ^{Glue}COF in the boundary part of adhered liposomes, suggesting that ^{Glue}COF can be a mediator to connect the liposomes due to a strong salt-bridge interaction between phosphate (PO_4^-) groups of lipid and the Gu^+ pendants.

In order to confirm whether RB loaded ^{Glue}COF plays a channel for substance transfer, we mixed guest-encapsulated liposomes and non-capsulated liposomes. After addition of ^{Glue}COF, two different kinds of liposomes adhered to each other (Figure 6c). When irradiated with light for 15 minutes, fluorescence due to the guest in liposomes became weak, while the other liposome turned fluorescent, indicating the guest was transferred from one liposome to another. In sharp contrast, when the light was not applied, the substance transfer did not occur. This result indicated that the generation of $^1O_2$ upon light irradiation induces a substance transfer while ^{Glue}COF without rose bengal, which generates $^1O_2$, did not show any substance transfer.

In summary, we have shown that RB loaded ^{Glue}COF (^{Glue}COF⊃RB) has the potential to be an artificial channel that can spatiotemporally transfer guest upon irradiation. As demonstrated in this study, ^{Glue}COF adheres to liposomes and is located in the boundary. A generation of singlet oxygen from RB, upon light irradiation, causes the substance transfer.

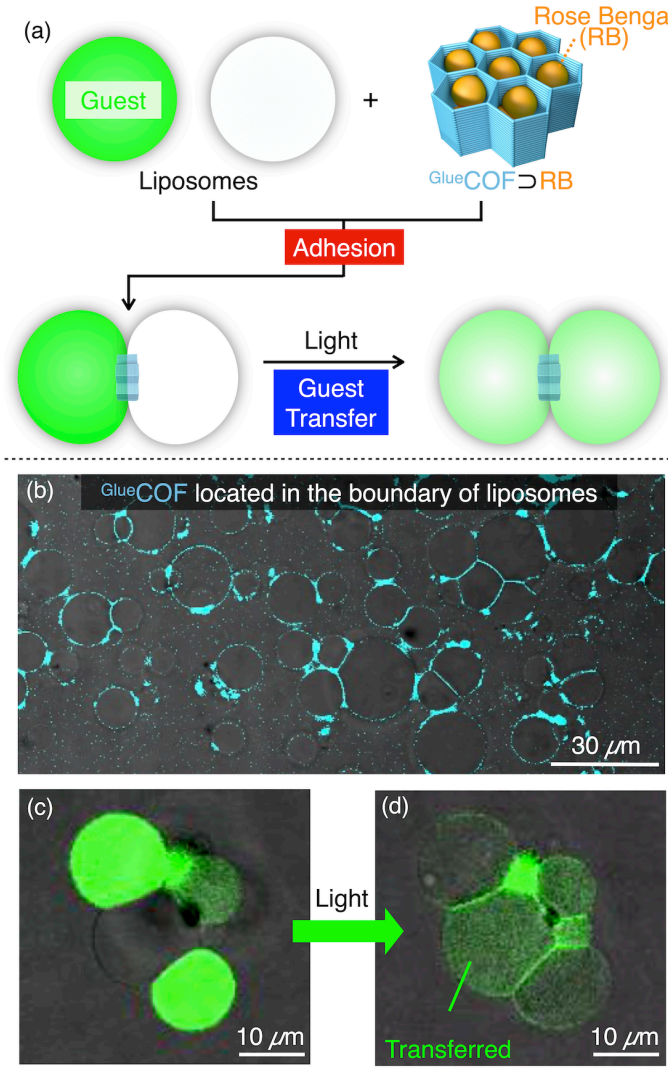

Figure 6. (a) Schematic illustration of guest transfer mediated by ^{Glue}COF⊃RB upon light irradiation. (b, c, d) Merged images of confocal laser scanning microscopy (CLSM; λ_{ex} = 405 nm) and transmission images of adhered liposomes (b) after addition of ^{Glue}COF. Merged images of CLSM (λ_{ex} = 488 nm) of mixture of guest-encapsulated liposomes and non-encapsulated liposomes with ^{Glue}COF (c) before and (d) after light

【4】Conclusion

In this study, we developed a bio-adhesive COF by functionalizing a guanidinium (Gu^+) moiety that shows strong adhesion to the surface of biomolecules such as proteins and lipid membranes. Especially, we succeeded in releasing guests or transferring substances in a spatiotemporal manner. The technology we pioneered in this study was realized through the combination of the advantages of COF and the universality of adhesiveness, thus it can be applied to a wide range of bio-applications. Considering the

significance toward its applicability, it is expected that the understanding of biological phenomena at the molecular level and the development of disease treatments will be achieved.

【5】References

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【6】Publications

[1] H, Jo, T. Aida, K. Okuro*. to be submitted.*

[2] H. Jo, T. Kitao, A. Kimura, Y. Itoh, T. Aida, K. Okuro, *submitted.*

[3] H. G. Lee,† H. Jo,† S. Eom, D. W. Kang, M. Kang, J. Hilgar, J. D. Rinehart, D. Moon, and C. S. Hong, *Cryst. Growth Des.* 2018, 18, 3360–3365. († Equally contributed)

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