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Cover Letter

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Dear Editor Dr. Phillip Steindel in JoVE,

The manuscript entitled "Considerations for the development of heterogeneous enantioselective catalysts using chiral Metal-Organic Frameworks (MOFs)" authored by J. Han, S. Kim, M. S. Lee, N. Jeong, and myself has been revised and resubmitted to *Journal of Visualized Experiments* via the web for your final decision.

All reviewer's comments were very constructive. We have attached our responses to all queries and questions raised by reviewers and editorial office. We have highlighted our changes with green color during the revision process, and highlighted with yellow color for movie clip. The whole manuscript was proofread and checked by professional editorial service.

We are, confident that our findings, discussion, and visualized results with animations would draw great interests from scientists whose research relates to organic chemistry, catalysis and organometallic chemistry along with inorganic chemistry.

Thanks for your kind handling this manuscript.

Sincerely yours,

Min Kim

TITLE:

Development of heterogeneous enantioselective catalysts using chiral Metal-Organic Frameworks (MOFs)

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KEYWORDS:

heterogeneous catalysts, metal-organic frameworks, carbonyl-ene reaction, particle size effect, enantiomeric excess, chiral environment

SUMMARY:

Here, we present a protocol for active site validation of metal-organic framework catalysts by comparing stoichiometric and catalytic carbonyl-ene reactions to find out whether a reaction takes place on the inner or outer surface of metal-organic frameworks.

ABSTRACT:

Substrate size discrimination by the pore size and homogeneity of the chiral environment at the reaction sites are important issues in the validation of the reaction site in metal-organic framework (MOF)–based catalysts in an enantioselective catalytic reaction system. Therefore, a method of validating the reaction site of MOF-based catalysts is necessary to investigate this issue. Substrate size discrimination by pore size was accomplished by comparing the substrate size versus the reaction rate in two different types of carbonyl-ene reactions with two kinds of MOFs. The MOF catalysts were used to compare the performance of the two reaction types (Zn-mediated stoichiometric and Ti-catalyzed carbonyl-ene reactions) in two different media. Using the proposed method, it was observed that the entire MOF crystal participated in the reaction, and the interior of the crystal pore played an important role in exerting chiral control when the reaction was stoichiometric. Homogeneity of the chiral environment of MOF catalysts was established by the size control method for a particle used in the Zn-mediated stoichiometric reaction system. The protocol proposed for the catalytic reaction revealed that the reaction

mainly occurred on the catalyst surface regardless of the substrate size, which reveals the actual reaction sites in MOF-based heterogeneous catalysts. This method for reaction site validation of MOF catalysts suggests various considerations for developing heterogeneous enantioselective MOF catalysts.

INTRODUCTION:

MOFs are considered a useful heterogeneous catalyst for chemical reactions. There are many different reported uses of MOFs for enantioselective catalysis[1-19]. Still, it has yet to be determined whether the reactions take place on the inner or outer surface of the MOFs. Recent studies have raised questions concerning the utilization of the available surface and reduced diffusion[20-23]. A more striking issue is that the chiral environment varies with the location of each cavity in the MOF crystal. This heterogeneity of the chiral environment implies that the stereoselectivity of the reaction product depends on the reaction site[24]. Thus, designing an efficient enantioselective catalyst requires identification of the location where the reaction would take place. To do so, it is necessary to ensure that the reaction occurs either only on the inner surface or only on the outer surface of the MOF while leaving the interior intact. The porous structure of MOFs and their large surface area containing chiral environment active sites can be exploited for enantioselective catalysis. For this reason, MOFs are excellent replacements of solid-supported heterogeneous catalysts[25]. The use of MOFs as heterogeneous catalysts needs to be reconsidered if the reaction does not occur inside them. The location of the reaction site is important, as well as the size of the cavity. In porous materials, the size of the cavity determines the substrate based on its size. There are some reports of MOF-based catalysts that overlook the cavity size issue[25]. Many MOF-based catalysts introduce bulky catalytic species (e.g., $Ti(O\text{-}i\text{Pr})_4$) to the original framework structure[3,8,13]. There is a change in the cavity size when bulky catalytic species are adopted in the original framework structure. The reduced cavity size caused by the bulky catalytic species makes it impossible for the substrate to fully diffuse into the MOFs. Thus, discrimination of substrate size by the cavity size of the MOFs needs to be considered for these cases. The catalytic reactions by MOFs often make it difficult to support evidence of reactions taking place inside the MOF cavity. Some studies have shown that substrates larger than the MOF cavities are converted to the expected products with ease, which seems contradictory[8,13]. These results can be interpreted as a contact between the functional group of the substrate and catalytic site initiating the catalytic reaction. In this case, there is no need for the substrate to diffuse into the MOFs; the reaction occurs on the surface of the MOF crystals[26] and the cavity size is not directly involved in the discrimination of the substrate based on its size.

To identify the reaction sites of MOFs, a known Lewis-acid promoted carbonyl-ene reaction was selected[2]. Using 3-methylgeranial and its congeners as substrates, four types of enantioselective carbonyl-ene reactions (**Figure 1**) were studied[27]. The reactions, which have been previously reported, were classified into two classes: a stoichiometric reaction using a Zn reagent and catalytic reactions using a Ti reagent[27]. The reaction of the smallest substrate requires a stoichiometric amount of Zn/**KUMOF-1** (KUMOF = Korea University Metal-Organic Framework); it has been reported that this reaction takes place inside of the crystal[27]. Two kinds of MOFs were used in this method, Zn/**KUMOF-1** for the stoichiometric reaction and Ti/**KUMOF-1** for the catalytic reaction. Owing to the distinct reaction mechanisms of these two kinds of MOFs, a comparison between the reaction rate versus substrate size is possible[2,28,29]. The effect of particle size on the carbonyl-ene reaction with Zn/**KUMOF-1**[27] demonstrated that, as seen in the previous report, the chiral environment of the outer surface was different from the inner side of the MOF crystal[24]. This article demonstrates a method that determines the reaction sites by comparing the reactions of three kinds of substrates with two classes of catalysts and the effect of particle size as reported in the previous paper[27].

PROTOCOL:

1. Preparation of (*S*)-KUMOF-1 crystals in three sizes

NOTE: Each step follows the experimental section and supplementary information of previous reports[2,24,27]. Three different sizes of (*S*)-**KUMOF-1** were prepared: large (*S*)-**KUMOF-1**-(L), medium (*S*)-**KUMOF-1**-(M), and small (*S*)-**KUMOF-1**-(S) with particle sizes >100 µm, >20 µm, and <1 µm, respectively. When out of the solvent, (*S*)-**KUMOF-1** dismantles. Therefore, the crystals should always be kept wet while in use.

1.1. Synthesis of small size (*S*)-**KUMOF-1**-(S)

1.1.1. In a 10 mL cell, dissolve $Cu(NO_3)_2 \cdot 3H_2O$ (0.2 mg, 0.0008 mmol) and (*S*)-2,2'-dihydroxy-6,6'-dimethyl-[1,1'-biphenyl]-4,4'-dicarboxylic acid[2] (0.24 mg, 0.0008 mmol) in 4 mL of DEF/MeOH (DEF = *N,N*-diethylformamide, 1/1, v/v).

NOTE: It is best to use newly prepared DEF and MeOH (methanol). (*S*) in (*S*)-**KUMOF-1** means that the stereochemical configuration of the ligand used in KUMOF synthesis is *S*.

1.1.2. Cap the reaction cell with a PTFE (polytetrafluoroethylene) cap and place it into a microwave reactor (65 °C, 100 psi, 50 W, 20 min).

NOTE: To obtain the required number of crystals, repeat the above steps (1.1.1. and 1.1.2.) several times.

1.1.3. Whisk gently with a small spatula to float the obtained blue cubic crystals (45% yield).

1.1.4. Pour the floating crystals on filter paper, and wash 3x with 3 mL of hot DEF.

1.1.5. Exchange the solvent 3x with 3 mL of anhydrous dichloromethane (DCM) for storage.

NOTE: Every step requiring DCM in the protocol is DCM distilled over CaH_2.

1.2. Synthesis of medium size (*S*)-**KUMOF-1**-(M)

1.2.1. Dissolve $Cu(NO_3)_2 \cdot 3H_2O$ (7.2 mg, 0.030 mmol) in 1.5 mL of MeOH and (*S*)-2,2'-dihydroxy-6,6'-dimethyl-[1,1'-biphenyl]-4,4'-dicarboxylic acid (9 mg, 0.030 mmol) in 1.5 mL of DEF.

NOTE: The compounds and solvents mentioned are for one vial set. Scaling up is needed to obtain the required number of MOFs for catalytic use. Multiply the scales in this step and make stock solutions for each compound. Then subdivide the stock solutions into each vial.

1.2.2. Combine the two solutions in a 4 mL vial.

1.2.3. Cover the 4 mL vial with PTFE tape and punch the cover with a needle to make a hole.

1.2.4. Put this small vial into a 20 mL vial and add 1.0 mL of *N*,*N*-dimethylaniline into the space between the small and large vials.

1.2.5. Cap the large vial tightly and place in an oven at 65 °C for 1 day.

1.2.6. Whisk gently with a small spatula to float the obtained blue cubic crystals.

1.2.7. Pour the floating crystals on a filter paper and wash 3x with DEF/MeOH (3 mL/3 mL).

NOTE: After pouring the floating crystals, tilt the vial above the filter paper. Then eject the solvent with a syringe to wash down every crystal remaining in the vial.

1.2.8. Exchange the solvent 3x with 3 mL of anhydrous DCM for storage.

1.3. Synthesis of large size (*S*)-**KUMOF-1**-(L)

1.3.1. Use the same procedure as in section 1.2, except at step 1.2.3, leave the 4 mL vial open.

NOTE: The yield of the obtained crystal is based on the ligand used. The yield for the medium and large size (*S*)-**KUMOF-1** were almost the same (35% yield) after final washing.

2. Preparation of Zn/(*S*)-KUMOF-1 in three sizes

NOTE: Each step follows the experimental section and supplementary information of previous reports[2,24,27].

2.1. Add dimethylzinc (0.68 mL, 1.2 M in toluene, 0.81 mmol) to a suspension of (*S*)-**KUMOF-1** (102 mg, 0.27 mmol) in 2 mL of DCM at -78 °C and shake 3 h at this temperature.

CAUTION: All steps at -78 °C are done with a cryogenic cooling bath (dry ice with acetone). Always be careful when handling this equipment.

NOTE: All shaking procedures are done using a plate shaker (180 rpm).

2.2. Decant the supernatant and wash with 3 mL cold DCM several times for complete removal of unreacted dimethylzinc.

NOTE: Three sizes of Zn/**KUMOF-1** are required for the carbonyl-ene reaction. Follow the same steps as described for the three sizes of **KUMOF-1**. The number of catalytic sites is calculated assuming that one catalytic site is present in a Cu and a ligand pair. For this reason, the Zn/Cu and Ti/Cu ratios of the prepared crystals were determined as in the previous report using inductive coupled plasma atomic emission spectroscopy (ICP-AES)[27]. The amounts of Zn and Ti reagents used in this protocol were the same as those used in our previous study[27].

3. Preparation of Ti/(*S*)-KUMOF-1 in three sizes

NOTE: Each step follows the experimental section and supplementary information of previous reports[2,24,27].

3.1. Add Ti(O-*i*Pr)$_4$ (59 µL, 0.20 mmol) to a suspension of (*S*)-**KUMOF-1** (24 mg, 0.063 mmol) in 2 mL of DCM and shake for 5 h at room temperature.

3.2. Decant the supernatant and wash with 3 mL of cold DCM several times for the complete removal of residual Ti(O-*i*Pr)$_4$.

4. **Carbonyl-ene reaction using the prepared MOFs**

NOTE: Prepare a series of substrates according to the method described in our previous report[27]. All three substrates are used individually in each carbonyl-ene reaction except for the particle size effect determination, in which only the smallest substrate (**1a**) is used[27]. Each step follows the experimental section and supplementary information of previous reports[2,24,27].

4.1. Heterogeneous stoichiometric carbonyl-ene reaction by Zn/(*S*)-**KUMOF-1**.

4.1.1. Add the substrate solution (0.089 mmol) in 0.1 mL of DCM to a suspension of Zn/(*S*)-**KUMOF-1** (102 mg, 0.27 mmol) in 2 mL of DCM at -78 °C.

4.1.2. Warm the reaction mixture slowly to 0 °C and shake for 3.5 h at this temperature.

4.1.3. Quench the reaction mixture with 3 mL of an aqueous solution of 6 N HCl.

4.1.4. Filter the resultant mixture through a diatomaceous silica pad.

4.1.5. Concentrate the filtrate in vacuo and purify the residue by flash chromatography (*n*-hexane/ethyl acetate 10:1).

NOTE: Silica gel 60 (230–400 mesh) and an appropriate *n*-hexane/ethyl acetate mixture as the eluent are used for flash chromatography. The product is a pale yellow oil. Optical purity of all products in this protocol were determined as described previously[27]. The same procedure should be followed for the three sizes of Zn/(*S*)-**KUMOF-1**.

4.2. Heterogeneous catalytic carbonyl-ene reaction by Ti/(*S*)-**KUMOF-1**.

4.2.1. Add the substrate solution (0.29 mmol) in 0.1 mL of DCM to a suspension of Ti/(*S*)-**KUMOF-1** (12 mg, 0.029 mmol) in DCM (2 mL) at 0 °C, and shake for 36 h at this temperature.

4.2.2. Collect the supernatant and wash the resultant crystals 3x with 3 mL of DCM.

4.2.3. Concentrate the collected supernatant in vacuo and purify the residue by flash chromatography (*n*-hexane/ethyl acetate 10:1).

REPRESENTATIVE RESULTS:

The enantioselective carbonyl-ene reaction using the Zn reagent is stoichiometric because of the difference in the binding affinities of the alkoxy and carbonyl groups to the metal (**Figure 2**). For this reason, the substrates were converted into the products at the reaction site and remained there. The desired products were obtained by dismantling the crystals, as detailed in section 4 of the protocol. The results of the heterogeneous enantioselective carbonyl-ene reaction of substrates by Zn/(*S*)-**KUMOF-1** (**Table 1**) showed that the smallest substrate (**1a**) could diffuse inside the crystal and convert to the product in a high yield (92%), proving that all reaction sites of the MOF were available. The yield and enantiomeric excess (ee) decreased as the substrate size increased, which suggests that the larger substrates could not access the reaction sites inside the MOF crystal. The largest substrate (**1c**) did not undergo the reaction in this system. It is plausible that the reaction channel was blocked by the corresponding reaction products in this case (**Figure 3**). When the size of the substrate is sufficiently small in comparison to the size of the void, additional substrates can penetrate the crystal. If the size of substrate is too large, the surface reaction site makes the first contact and directly blocks the entrance of the channel, which makes it impossible for other substrates to penetrate (**Animation 1**). As the reaction takes place near the surface, the ee is lower[24] and the blockage of the reaction site decreases the reaction yield.

Particle size effect results (**Table 2**) showed that the larger crystals were better than the small crystals in utilizing the reaction sites inside the crystal, clearly demonstrating the identification of the reaction site in this system. The yields in the carbonyl-ene reaction of **1a** using the three sizes of Zn/(*S*)-**KUMOF-1** were similar, which indicates that the efficacies of the three MOFs are identical. The optical purity dramatically decreased with the decreasing size of the crystals because their surface area increased. In contrast, a larger sized crystal had much lower surface area, which allowed **1a** to penetrate deeply and have better access to the inner reaction sites.

Unlike the Zn-mediated system, the Ti-catalyzed system provided more information about the events occurring at the catalytic reaction sites. The results of the heterogeneous catalytic carbonyl-ene reaction by Ti/(*S*)-**KUMOF-1** (**Table 3**) revealed no discrimination by the substrate size; indeed, the effect of the substrate size on the yield was marginal. The optical purity of **2a** was much lower compared to the product obtained via the Zn-mediated reaction. Most of the product was found in the reaction solution, and the amount inside the crystal was negligible. These results indicate that most reactions occurred on or beneath the surface and the products were immediately removed to the solution (**Figure 4**) (**Animation 2**). The substrate that is larger than the cavity size undergoes the reaction upon contact with the reaction site on the surface. The product dissociates quickly from the catalytic site without penetrating the crystal.

Based on these results, the reaction sites of MOFs can either be on the outer surface or the inner side of MOFs. However, as previously reported, the chiral environment of the reaction site varies by its location. A reaction that is catalytic with MOFs should follow the method proposed in this article to determine the location of the reaction site. Therefore, if the reaction is catalytic, claims of the reaction occurring inside the channel should be reconsidered.

FIGURE AND TABLE LEGENDS:

Figure 1: Two classes of enantioselective carbonyl-ene reactions. Lewis acid Cat I and II were used for a homogeneous model reaction in a previous report[27]. This figure has been reprinted with permission from Han et al.[27]

Figure 2: Possible mechanism of the homogeneous stoichiometric carbonyl-ene reaction. Difference of binding affinity between the alkoxy and carbonyl group to metal makes the Zn-mediated carbonyl-ene reaction stoichiometric. This figure has been reprinted with permission from Han et al.[27]

Figure 3: Schematic presentation of the heterogeneous stoichiometric carbonyl-ene reaction. Pink colored molecules represent the substrates while greens indicate the products attached to the reaction sites. **(A) 1a** is small enough to penetrate the crystal regardless of blockage by **2a**. **(B) 1b** suffers from blockage of **2b** but still diffuses into the channel. **(C) 1c** makes first contact with the reaction site at the surface and directly blocks the entrance of the channel by **2c**, which makes it impossible for another substrate to penetrate. This figure has been reprinted with permission from Han et al.[27]

Figure 4: Schematic presentation of the heterogeneous catalytic carbonyl-ene reaction. Pink colored molecules represent the substrates while greens indicate the products. **(A)** to **(D)** illustrate the steps of the reaction. Dissociation of the products from the reaction site is very fast and penetrating the crystal is not necessary. This figure has been reprinted with permission from Han et al.[27]

Table 1: Heterogeneous stoichiometric carbonyl-ene reaction of 1. NR = no reaction, under detection limit; NA =not applicable. This table has been reprinted with permission from Han et al.[27]

Table 2: Result of particle size effect determination. Only **1a** was used for this determination.

Entries 1–3 correspond to large, medium, and small sized particles, respectively. This table has been reprinted with permission from Han et al.[27]

Table 3: Heterogeneous catalytic carbonyl-ene reaction of 1. NA = not applicable. This table has been reprinted with permission from Han et al.[27]

Animation 1: Animated illustration of the heterogeneous stoichiometric carbonyl-ene reaction.

Animation 2: Animated illustration of the heterogeneous catalytic carbonyl-ene reaction.

DISCUSSION:

After the synthesis of (*S*)-**KUMOF-1**, crystals in some vials seem to be powdery and are not appropriate for use in catalysis. Therefore, proper crystals of (*S*)-**KUMOF-1** need to be selected. The yield of (*S*)-**KUMOF-1** is calculated using only those vials in which it was successfully synthesized. When withdrawn from the solvent, (*S*)-**KUMOF-1** dismantles. Therefore, the crystals should always be kept wet. For this reason, weighing of intact (*S*)-**KUMOF-1** crystals dipped into the solvent is difficult. The amount of (*S*)-**KUMOF-1** needs to be determined before its use in catalysis. By synthesizing (*S*)-**KUMOF-1** on a massive scale and abandoning some crystal samples in the vial, a statistical calculation of the yield was possible. The yield was calculated by weighing perfectly dried samples per vial. Samples were selected randomly from the population of synthesized (*S*)-**KUMOF-1** averaged by the number of vials. This method gave a statistically defined amount of (*S*)-**KUMOF-1** in one vial. The amount of (*S*)-**KUMOF-1** required for catalysis was prepared by collecting the crystals in vials (e.g., the required amount of (*S*)-**KUMOF-1** = amount of (*S*)-**KUMOF-1** per vial x number of vials). Subdivision of (*S*)-**KUMOF-1** floating in the solvent is incorrect for matching the calculated equivalents of substrates; the amount of substrate used is calculated from the calculated amount of (*S*)-**KUMOF-1**. The crystal structure and characteristics have been reported previously[2,27].

Homogeneous carbonyl-ene reactions with Zn and Ti catalysts have been performed previously to prove that there is no discrimination by substrate size in the homogeneous reaction. At this point, the influence of the substrate size on the reaction efficiency can be neglected as the same as in the heterogenous reaction. The temperature required for the carbonyl-ene reaction using Ti/(*S*)-**KUMOF-1** is 0 °C. Owing to the smashing problems of crystals, all reactions should be performed by shaking and not stirring. However, a low temperature shaking incubator chamber was not available. Instead, a polystyrene foam icebox was used. A stainless steel wire test tube rack was installed in the icebox and tightly sealed reaction vials were fixed into the rack. Water was poured to ~1 cm height into the icebox, and ice cubes were added. The lid-covered icebox was put on the shaker and fixed with adhesive tape. New ice cubes were added to replace the melting ice. For the carbonyl-ene reaction using Zn/(*S*)-**KUMOF-1**, the reaction vial was kept in a cryogenic cooling bath (dry ice with acetone) before adding the substrate to the solution. After adding the substrate, the reaction vial was moved to the icebox described above.

Additional well-marked data for the reaction site validation of Zn/(*S*)-**KUMOF-1** and Ti/(*S*)-**KUMOF-1** used in the carbonyl-ene reaction can be visualized by Two-Photon Microscopy (TPM) measurements[27]. Characterization of (*S*)-**KUMOF-1** crystals by TPM has been previously reported. To gauge the cavity size of newly synthesized MOFs, TPM measurements with various size of dyes are available[30].

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DISCLOSURES:

The authors have nothing to disclose.

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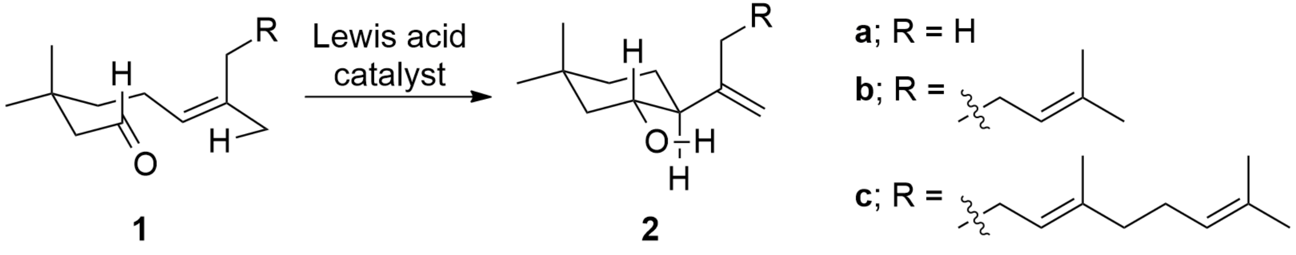
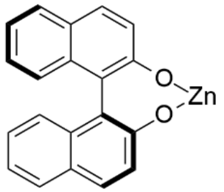
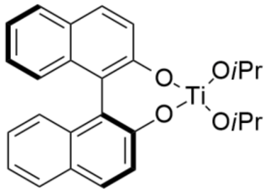
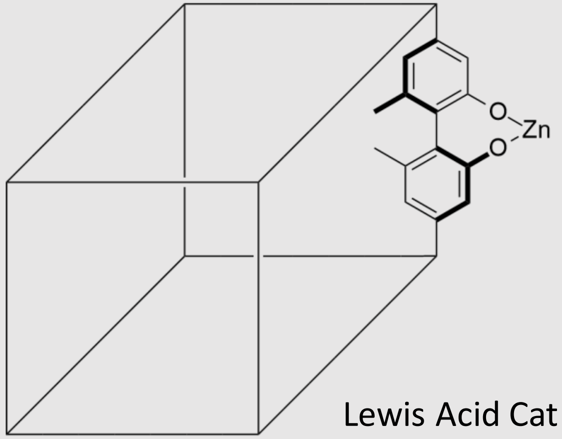
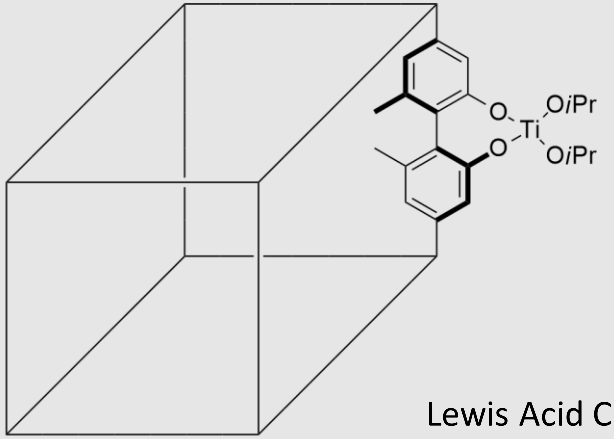
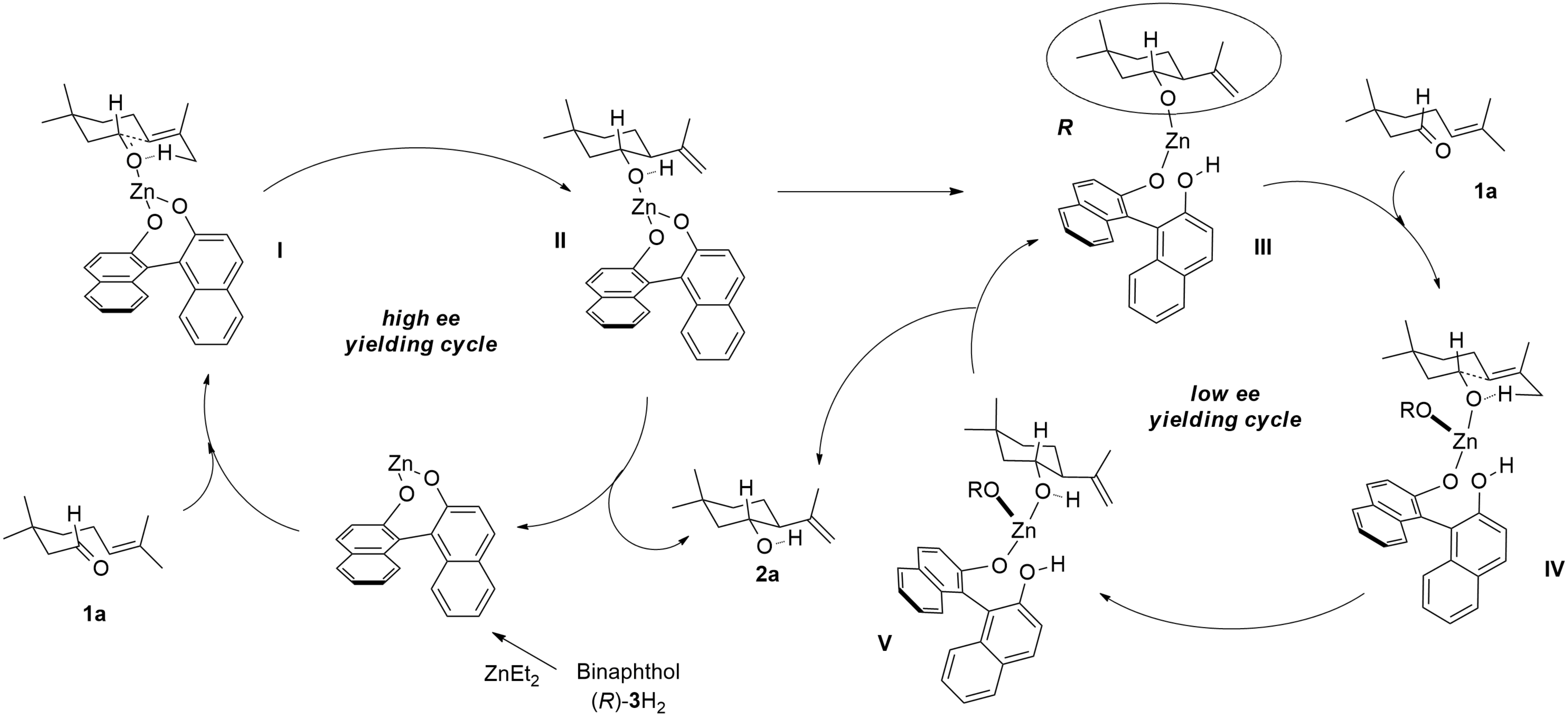
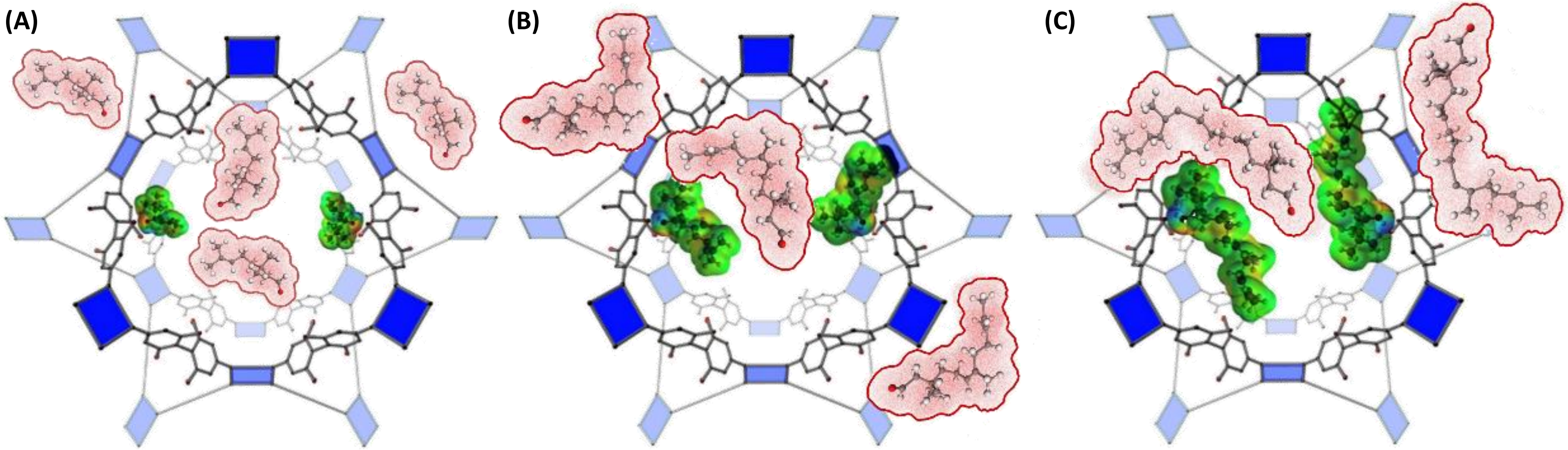
30. Shin, S. M., Lee, M. S., Han, J. H., Jeong, N. Assessing the Guest-Accessible Volume in MOFs Using Two-Photon Fluorescence Microscopy. *Chemical Communications.* **50**, 289–291 (2014).



	Zn catalyst [**stoichiometric** reaction]	**Ti** catalyst [**catalytic** reaction]
Homogeneous reaction [model reaction]	 Lewis Acid Cat **I**	Lewis Acid Cat **II** 
Heterogeneous reaction [model reaction]	 Lewis Acid Cat **III**	 Lewis Acid Cat **IV**


I
II
III
IV
V
1a
2a
R
RO
Zn
O
H
high ee
yielding cycle
low ee
yielding cycle
ZnEt$_2$
Binaphthol
(R)-**3**H$_2$


(A)
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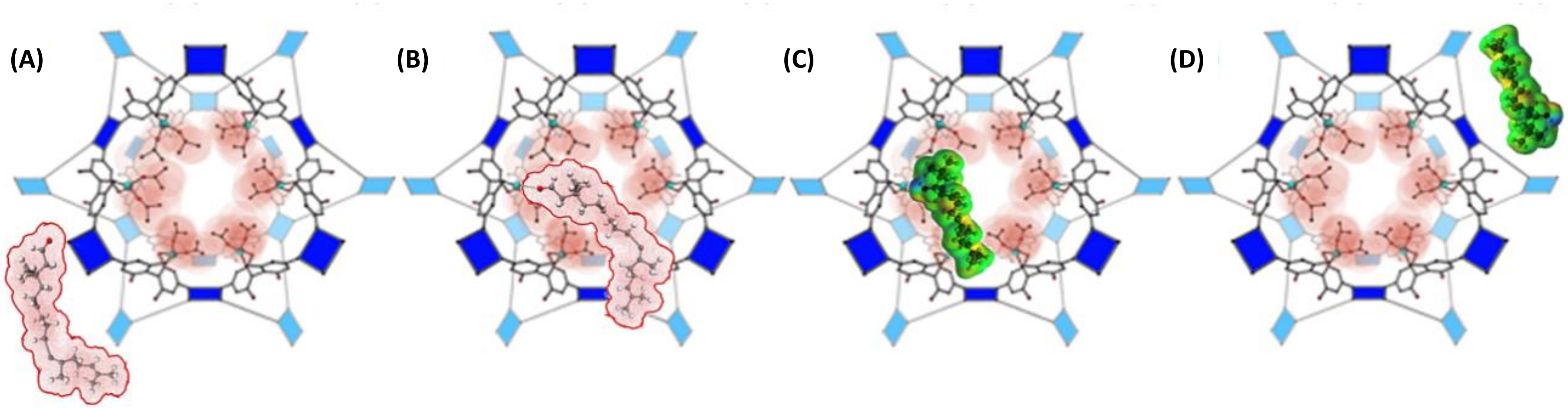
(C)


(A)
(B)
(C)
(D)

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Animation 2.mp4

entry	substrate	t (h)	yield (%)	ee (%)
1	1a	3.5	92	50
2	1b	3.5	52	5
3	1c	20	NR	NA

entry	catalyst	yield (%)	ee (%)
1	Zn/(*S*)-**KUMOF-1**-(L)	92	70
2	Zn/(*S*)-**KUMOF-1**-(M)	89	50
3	Zn/(*S*)-**KUMOF-1**-(S)	91	0

		product obtained from solution		product obtained from MOF	
entry	substrate	yield (%)	ee (%)	yield (%)	ee (%)
1	1a	85	24	2.8	NA
2	1b	89	7	0.7	NA
3	1c	83	0	0.2	NA

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Acetone	Daejung	1009-4110	
Analytical Balance	Sartorius	CP224S	
Copper(II) nitrate trihydrate	Sigma Aldrich	61194	
Dichloromethane	Daejung	3030-4465	
Dimethyl zinc	Acros	377241000	
Ethyl acetate	Daejung	4016-4410	
Filter paper	Whatman	WF1-0900	
Methanol	Daejung	5558-4410	
Microwave synthesizer	CEM		Discover SP
Microwave synthesizer 10 mL Vessel Accessory Kit	CEM	909050	
N,N -Diethylformamide	TCI	D0506	
N ,*N* -Dimethylaniline	TCI	D0665	
n-Hexane	Daejung	4081-4410	
Normject All plastic syringe 5 mL luer tip 100/pk	Normject	A5	
Pasteur Pipette 150 mm	Hilgenberg	HG.3150101	
PTFE tape	KDY	TP-75	
Rotary Evaporator	Eyela	243239	
Shaker	DAIHAN Scientific	DH.WSO04010	
Silica gel 60 (230-400 mesh)	Merck	109385	
Synthetic Oven	Eyela	NDO-600ND	
Titanium isopropoxide	Sigma Aldrich	87560	
Vial (20 mL)	SamooKurex	SCV2660	
Vial (5 mL)	SamooKurex	SCV1545	

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Author(s): Jeehwan Han, Seongwoo Kim, Mi Sun Lee, Min Kim, Nakcheol Jeong

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CORRESPONDING AUTHOR

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Institution:	Chungbuk National University
Title:	Associate Professor

Signature:

Date: 2019-07-31

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Editorial comments:

Q1. (General) Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

A1. Thank you for suggestion. We have carefully proofread the whole manuscript with all authors and professional editing service. We do not highlight general changes including grammar issues and rephrasing for better understandings.

Q2. Please define all abbreviations before use, e.g., KUMOF, PTFE.

A2. We have inserted full define for all abbreviations in the revised manuscript such as KUMOF = Korea University Metal-Organic Framework, PTFE (polytetrafluoroethylene), ICP-AES (Inductive Coupled Plasma Atomic Emission Spectroscopy), hexane/ethyl acetate, and Two-Photon Microscopy (TPM).

Q3. (Summary) Please rephrase the Summary to clearly describe the protocol and its applications in complete sentences between 10 and 50 words, e.g., “Here, we present a protocol to …”

A3. We have modified Summary section according to Editorial comment.

(Original) Prejudice with enantioselective catalytic reaction using MOF catalysts; size discrimination of substrate and homogeneity of the chiral environment of reaction sites was able to be broken by using two different chiral MOF catalysts in two different reaction system (stoichiometric and catalytic carbonyl-ene reaction).

(Revised version) Comparison for substrate size *vs* reaction rate and particle size effect in the carbonyl-ene reaction of MOF-based catalyst was studied. Here, we present a protocol for active site validation of MOF catalyst by comparing stoichiometric and catalytic carbonyl-ene reaction whether reaction takes place inner or outer surface of MOF.

Q4. (Protocol) For each protocol step/substep, please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

A4. We have revised “Protocol” section to answer “how” question, and divided to single sentence for each step/substep.

Q5. (Specific Protocol steps) 1.1.2 Note: Do you mean steps 1.1.1 and 1.1.2? What is the usual yield for each prep? 2.1, 3.1, 4.1.2, 4.2.1: How fast do you shake (e.g., in rpm) during these steps?

A5. We have modified 1.1.2 Note, and mentioned for detail information such as yields and rpm.

Q6. (Figures) Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account.

A6. We have obtained copyright permission to reuse all figures from our previous publications.

Q7. (Reference) Please do not abbreviate journal titles.

A7. We have modified all journal names in the reference section without abbreviation.

Q8. (Table of Materials) Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

A8. We have modified 'Table of Materials' to include all materaials and equipment used. For example, information of copper nitrate is changed; Copper(II) nitrate trihydrate, catalog # : 61194, Hexane → n-Hexane, *N,N*-Diethylforamide → *N,N*-Diethylformamide

Reviewer #1:

The proposed work deals with the determination of the location of active sites in MOF catalysts. The topic is interesting and the methodology deserves visualization. However, I have important remarks as well as some minor comments concerning the paper. They are listed according to the appearance in the text. Therefore, I recommend a major revision before publication.

We thank the referee for his/her kind comments and endorsement for publication in JoVE.

Q9. Lines 8, 9: The affiliations provided are not full.

A9. Thank you for suggestion. We have inserted city, country and ZIP code for the affiliations.

Q10. Line 34. The abstract should be modified in order to focus on the methodology. Now it is rather oriented to the scientific part of the paper.

A10. Thank you very much for constructive comments. As Referee's comment, our original abstract was focused "why" this research is important, now we have modified abstract section to "why this work is important" and "how this question is handled".

(Original) Substrate size discrimination by the pore size and homogeneity of the chiral environment at the reaction sites are important issues in validation of the reaction site in MOF-based catalysts in an enantioselective catalytic reaction system. Ideas to confirm the upright perspective for these issues are the goal of this study. Breaking stereotype in substrate size discrimination by pore size was accomplished by comparing substrate size versus reaction rate in two different types of carbonyl-ene reaction with two kinds of MOFs. MOF catalysts were used to compare in two different media the performance of two reaction types (Zn-mediated stoichiometric carbonyl-ene reaction and Ti-catalyzed carbonyl-ene reaction). It clearly demonstrates that the whole MOF crystal is working, and the inside of crystal pore is important part for chirality controls when the reaction is stoichiometric. By controlling the size of particle used in a desired reaction system (Zn-mediated stoichiometric reaction), novel concept for homogeneity of chiral environment of MOF catalysts is suggested. In case of catalytic reaction, reaction mainly occurs on the surface regardless of the substrate size, and this gives information on actual reaction site of MOF-based heterogeneous catalysts.

(Revised version) Substrate size discrimination by the pore size and homogeneity of the chiral environment at the reaction sites are important issues in the validation of the reaction site in MOF-based catalysts in an enantioselective catalytic reaction system. Therefore, a method of validating the reaction site of MOF-based catalysts is necessary to gain perspective on this issue. Substrate size discrimination by pore size was accomplished by comparing the substrate size versus reaction rate in two different types of carbonyl-ene reactions with two kinds of MOFs. The MOF catalysts were used to compare the performance of the two reaction types (Zn-mediated stoichiometric and Ti-catalyzed carbonyl-ene reactions) in two different media. By following the proposed method, it was observed that the entire

MOF crystal participated in the reaction, and the interior of the crystal pore played an important role in exerting chiral control when the reaction was stoichiometric. Homogeneity of the chiral environment of MOF catalysts was established by the size control method for a particle used in the Zn-mediated stoichiometric reaction system. The protocol proposed for the catalytic reaction revealed that that reaction mainly occurred on the catalyst surface regardless of the substrate size, which gives evidence of the actual reaction sites in MOF-based heterogeneous catalysts. This method for reaction site validation of MOF catalysts suggests various considerations for developing heterogeneous enantioselective MOF catalysts.

Q11. Line 52: Several different cases reported cases -> Several different reported cases / Line 57: product depend -> product dependent / Line 59: to make the reaction occur -> to make the reaction to occur

A11. Thank you for kind comments. Theses contents have been corrected.

Q12. Line 59: General comment. The authors write: "reaction occur only in the inside of MOFs or only at the surface leaving the inside intact." This is not clear. I propose to use the widely accepted for porous materials terms inner and outer surface. In fact, the reaction could occur only at the outer surface or at both, the inner and outer surface. This is not clear from the presented material. The remark is valid also for the following text.

A12. Thank you very much for suggestion. We have modified the above sentence to “To resolve the issue, it is necessary to make the reaction to occur only on the inner side of MOFs or only on the outer surface leaving the inner intact which can specify the reaction site inner or outer surface.”. In addition, we have revised the whole manuscript with inner and outer concept.

Q13. Line 66: Many MOF-based catalysts introduce bulky catalytic species to original framework structure - the sentence is not clear / Line 81. The term KUMOF should be defined (most of the readers do not know that it refers to Korea University MOF). The same is valid for (s)-KUMOF-1 / Line 101. Diethylforamide -> Diethylformamide / Line 160. Three sizes of Zn/KUMOF-1 are needed for carbonyl-ene reaction. The sentence is not correct. / Line 185. Warm the reaction mixture slowly to 0 °C slowly -> Warm the reaction mixture slowly to 0 °C

A13. Thank you for kind comments. We have corrected these contents in the revised manuscript.

Q14. Line 290. I am not satisfied by the Discussion section and suggest rewriting with a focus on the protocol. In particular, the information that " When placed out of the solvent, (S)-KUMOF-1 dismantles" should be moved before the Discussion section.

A14. Thank you for suggestion. We prepared the Discussion section based on the JoVE’s guide line and instruction to include troubleshooting of the methods. Dismantling problem of KUMOF-1 makes hard for its storage and weighing for catalytic applications. Therefore, we mentioned and suggested the solutions for this problem in Discussion section. We have also mentioned this dismantle issues before the Discussion section.

Reviewer #2:

The Authors have studied the enantioselectivity of chiral MOF catalysts. In order to demonstrate the same, they have considered 4 types of Lewis-acid promoted model carbonyl-ene reactions considering Zn and Ti reagents. Two kinds of reactions were considered a) stoichiometric and b) catalytic. The Zn-based MOF was used for the stoichiometric reactions and the Ti-based MOF was used for the catalytic reactions. They have considered 3 different sizes of (S)-KUMOF-1 crystal: small, medium and large. The stoichiometric reaction based on Zn-reagent suggests that the larger crystal is better than the small one, but the optical purity decreases with the decreasing crystal size. However, the ee remains almost the same. On the other hand, the catalytic reaction seems to remain marginally affected by the altering substrate sizes. The optical purity is lesser than the Zn-mediated reaction. Most of the product was found in the solution as compared to the products inside the crystals. These results indicate that the Ti-mediated reactions happen on or beneath the surface and the product goes to the solution as soon as they are formed. Their main motive is to show that the chiral MOF can takes place on either location (surface or inside the MOF). The preparation method and demonstration looks clear to me and the results were very clearly presented. the animation was also understandable.

Therefore, I recommend its publication.

We thank the referee for his/her kind comments and endorsement for publication in JoVE.

Reviewer #3:

Despite the growing interest in MOF catalysis in the community, it remains unclear whether reactions occur inside MOFs or on the surface. In this work, the authors addressed this important question of MOF enantioselective catalysis, by studying the carbonyl-ene reaction in Zn/KUMOF-1 and Ti/KUMOF-1, which exhibited different selectivity patterns and thus provided insights into the location of the catalytic site. The reader of this journal will learn much from this work.

We thank the referee for his/her kind comments and endorsement for publication in JoVE.

Reviewer #4:

It took me a while to realize that the manuscript submitted to JoVE is mainly the visualized demonstration of key experiments that have already been previously published in ACS Catalysis by the same group of authors. It's eye-opening for me to read about the difference between the carbonyl-ene reactions happening on MOF surface versus inside cavities of chiral MOFs. The results with the catalytic Ti/KUMOF-1 are indeed surprising. The work challenges assumptions that many researchers take for granted without in-depth analysis. It provides a valid protocol for thorough evaluation of new MOF-based catalytic systems and makes practitioners in the field want to review and reexamine results that have already been published. I consider the work to be significant and suitable for publication in JoVE. With my overall positive evaluation, I think there are deficiencies with the manuscript that should be properly addressed before publication.

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Q15. On line 87 the word "introduce" should be changed to "demonstrate" or another word of similar strength. The word "introduce" gives a sense of bringing out something new, but the results have already been "introduced" in the previous publication (ref. 27).

A15. Thank you for suggestion. We have corrected this issue.

Q16. The previous publication tells a complete story with comprehensive data, which is absent in the current paper. I would like the authors to provide more data and comparison on the three kinds of KUMOF-1, namely, large, medium, and small. Other than TPM imaging, what are the surface area and pore size distribution of the three MOFs before and after catalyst installation? These data can be easily obtained from standard measurements using liquid nitrogen or other gases. Is surface area the crucial factor in making the difference? I don't see such discussion in the ACS Catalysis paper, and it would be the perfect place for the current paper to address the issue.

A16. Thank you for comments. As Referee's suggestion, we have added more, detail information for KUMOF-1 such as yields and size. However, based on the aim and theme of JoVE journal, the present manuscript is more focused on the protocol of KUMOF-1 catalysis along with preparation. The detail characterizations were performed and focused in the previous publications. In the present manuscript and previous publications, we didn't focus on the surface area analysis (such as BET and/or Langmuir) rather than TPM experiment since we performed the catalysis in the solution level and KUMOF-1 is not highly stable in the desolvated condition under vacuum.

Q17. line 51 the word "several" should be changed to many or a significant number of. Nineteen references are not just several.

A17. Thank you for suggestion. We have corrected this word.

Q18. On lines 70 to 76, the sentences create an impression that all catalytic reactions by MOFs happen at the surface, which is certainly not true. Revision is needed to clarify the scope to the current work and acknowledge reports in which catalytic reactions did happen inside MOF cavities with excellent enantioselectivity.

A18. Thank you for suggestion. We have revised related part.

(Original) Thus, discrimination of substrate size by MOFs cavity needs to be considered. The catalytic reaction by MOFs have limitations in providing evidence of reactions taking place inside of MOFs cavity. Some studies also illustrate that substrates larger than the cavities in size were converted to expected products easily, which seems to be contradicted.[8,13] *Contact between functional group of the substrate and catalytic site initiates catalytic reaction. Thus, there is no need for the substrate to diffuse into the MOFs. Reaction occurs on the surface of the MOFs crystals*[26] *and the cavity size is not directly involved in the discrimination of substrate size.*

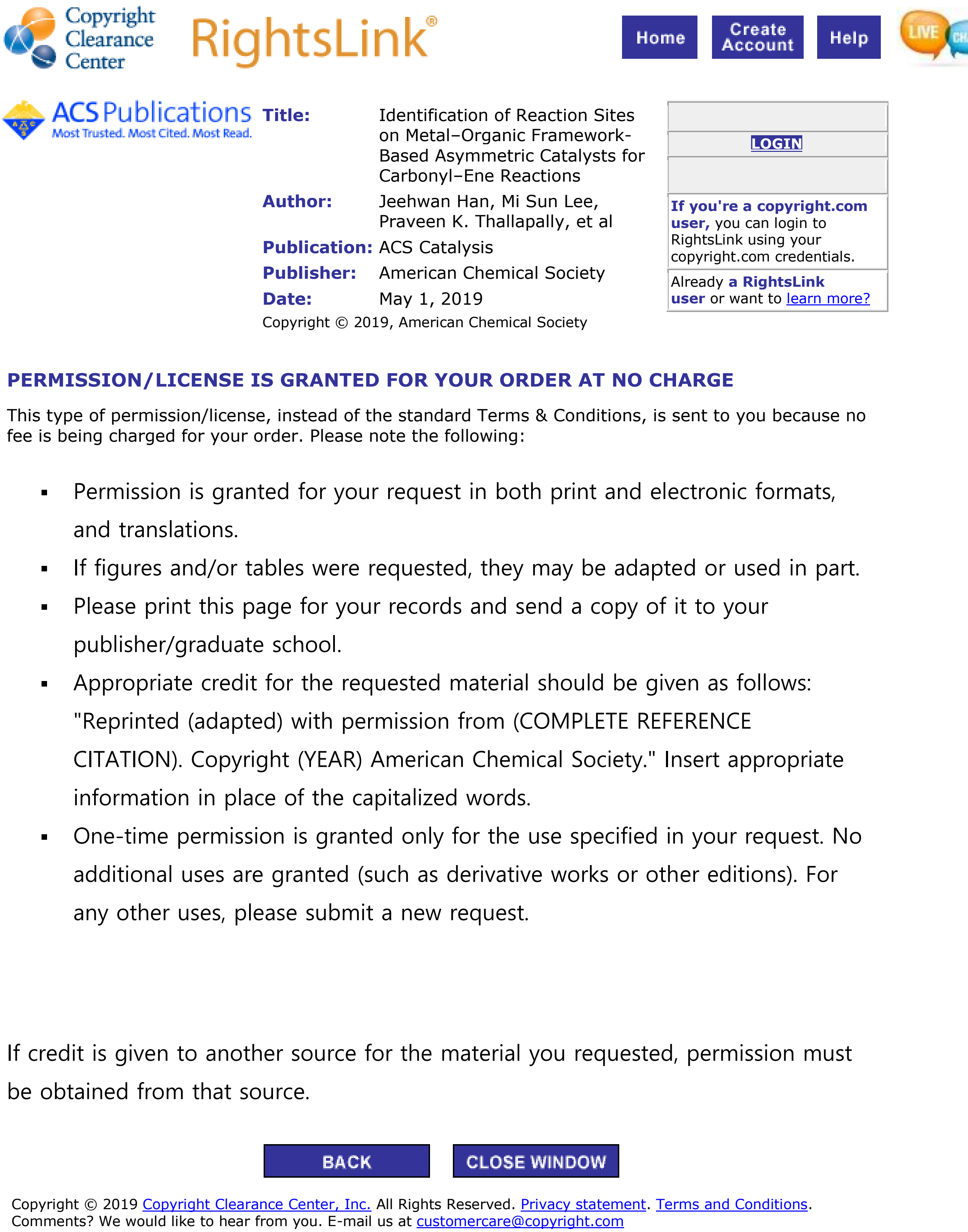
(Revised version) Thus, discrimination of substrate size by the cavity size of the MOFs needs to be considered for these cases. The catalytic reactions by MOFs often make it difficult to support evidence of reactions taking place inside the MOF cavity. Some studies have shown that the substrates that are larger in size than the MOF cavities are converted to the expected products with ease, which seems contradictory.[8,13] These results can be interpreted as a contact between the functional group of the substrate and catalytic site initiating the catalytic reaction. In this case, there is no need for the substrate to diffuse into the MOFs; the reaction occurs on the surface of the MOF crystals[26] and the cavity size is not directly involved in the discrimination of the substrate based on its size.

Q19. On line 144 it's not clear what "step 3" is referring to.

A19. We have modified to step 1.2.3.

Q26. The paper mentions three sizes (line 196) and "different sizes" at different places. What are the exact physical measurement numbers? It's nonprofessional to simply say small, medium, and large.

A26. Thank you for comments. We have added particle size information in protocol section with related reference: Three different sizes of (*S*)-**KUMOF-1** were prepared: (*S*)-**KUMOF-1**-(L) with a particle size of >100 μm, (*S*)-**KUMOF-1**-(M) with a particle size of >20 μm, and (*S*)-**KUMOF-1**-(S) with a particle size of <1 μm.[27]



Title: Identification of Reaction Sites on Metal–Organic Framework-Based Asymmetric Catalysts for Carbonyl–Ene Reactions

Author: Jeehwan Han, Mi Sun Lee, Praveen K. Thallapally, et al

Publication: ACS Catalysis

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