

# **Structure Determination**

**Literature Seminar**

**2024. 12. 27**

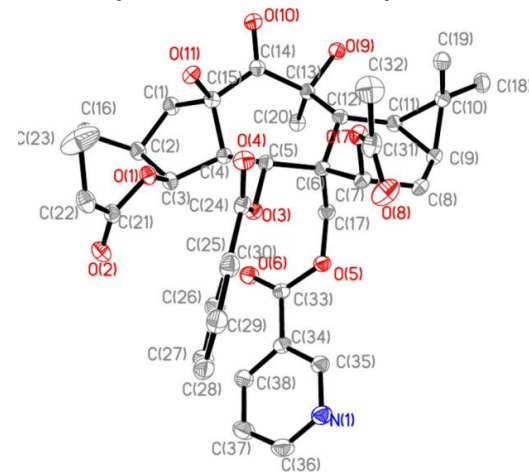
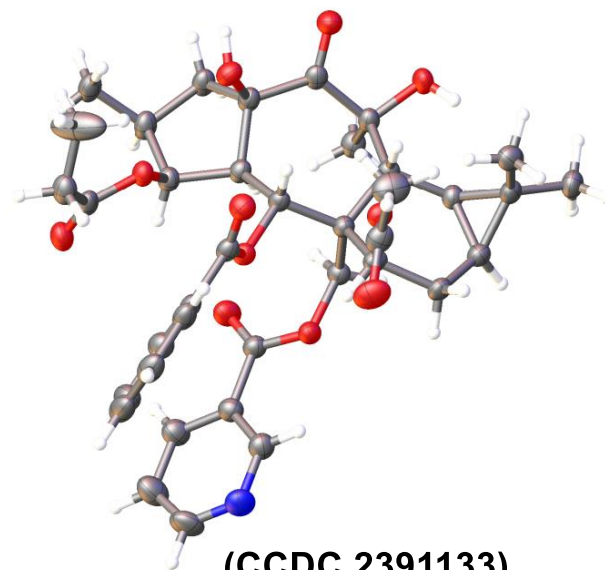
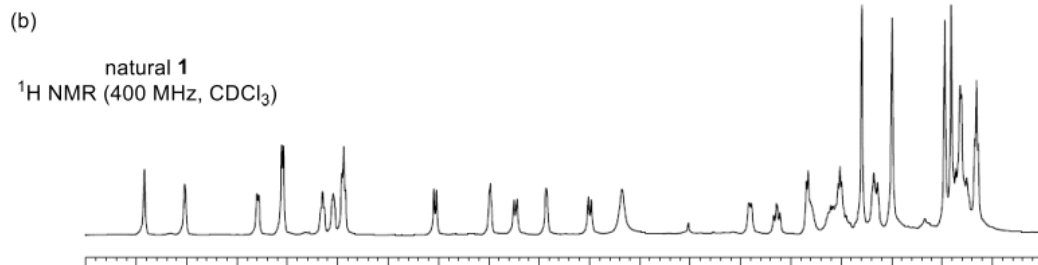
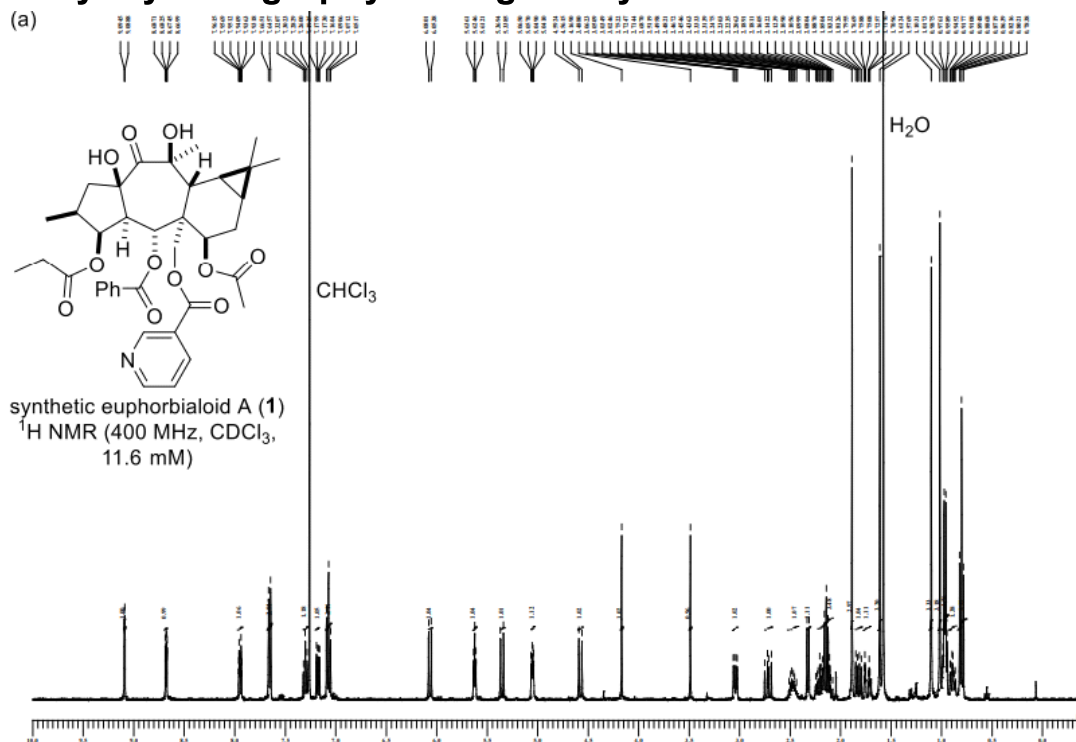
**Kyohei Takaoka**

**1. Introduction**

**2. Strategy for Crystal Analysis**

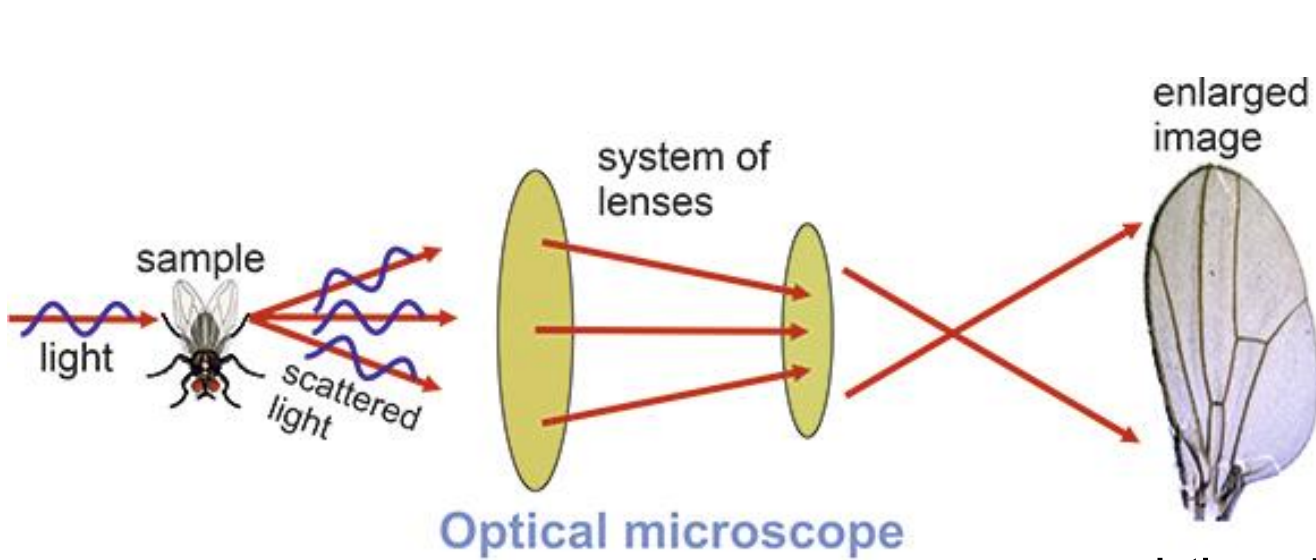
# X-Ray Crystallography as a Powerful Tool for Structure Determination

X-ray crystallography unambiguously determines the structure.



originally determined by  
X-ray crystallography

# Observing Substances in Atom Scale



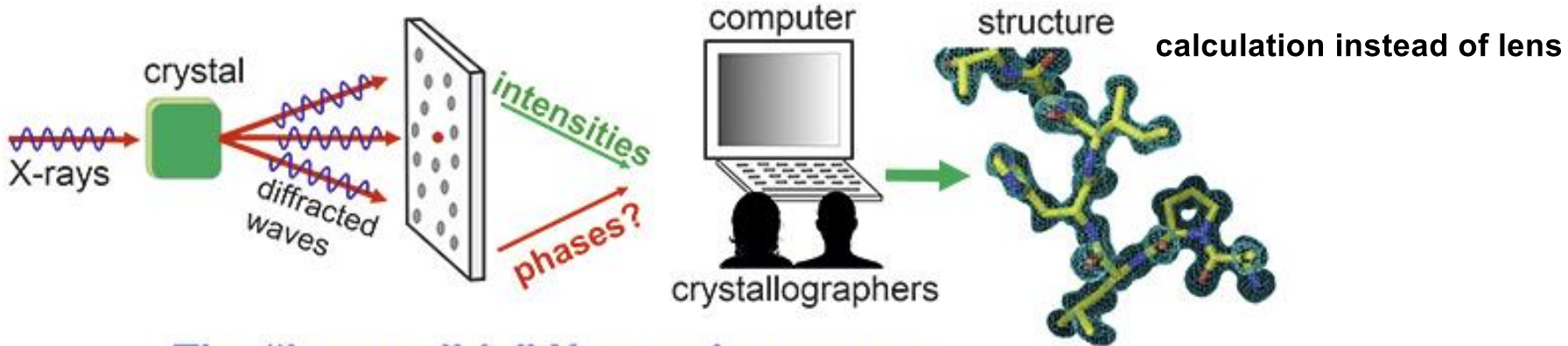
Optical microscope

resolution: ~ 700 nm

cf. electron microscope  
electron beam instead  
of light  
electric field instead of lens

resolution: < 0.1 nm

not suitable for small  
molecule observation



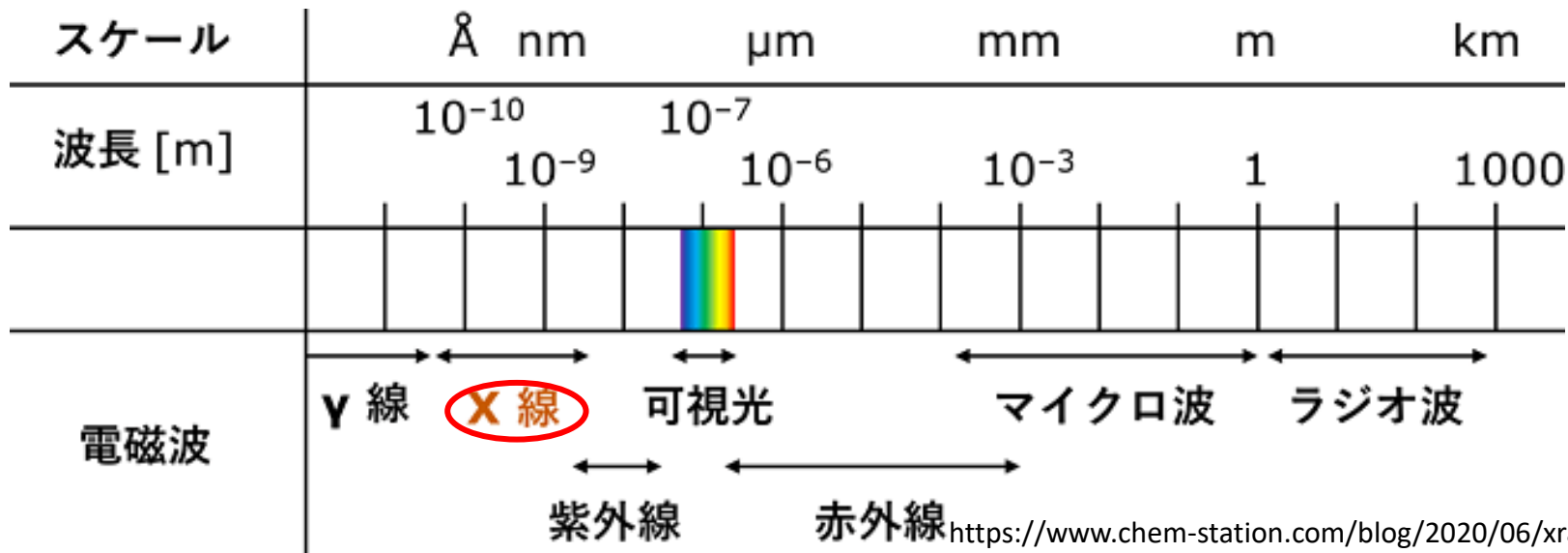
The "impossible" X-ray microscope

resolution: ~ 0.1 nm

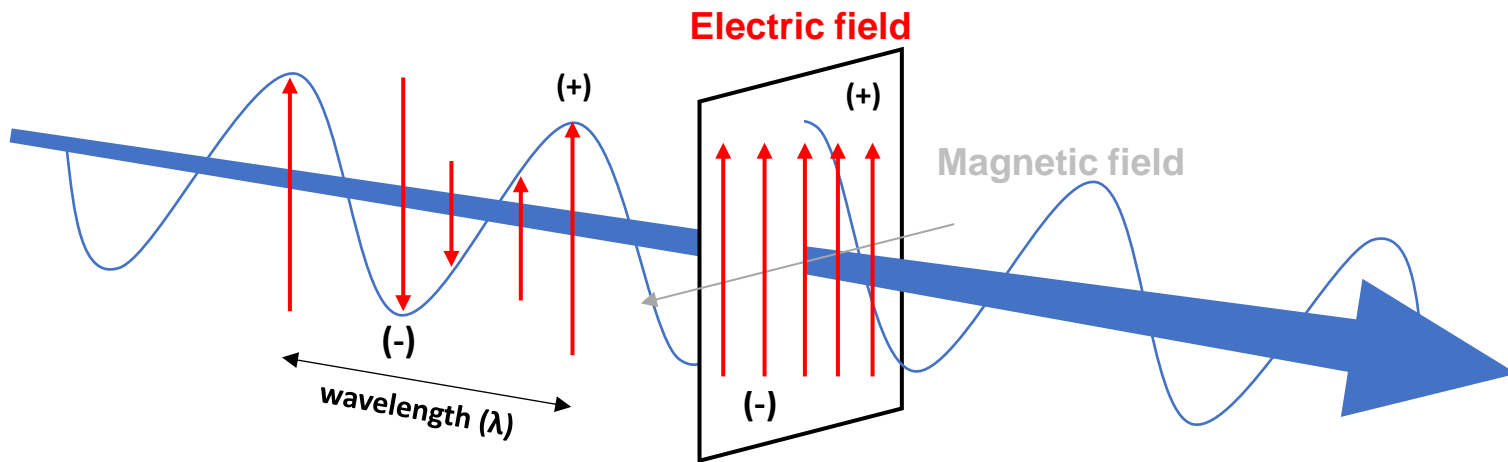
# What is X-Ray?

X-ray: A form of high-energy electromagnetic radiation

Wavelength ( $\lambda$ ) ~ 10 pm to 10 nm

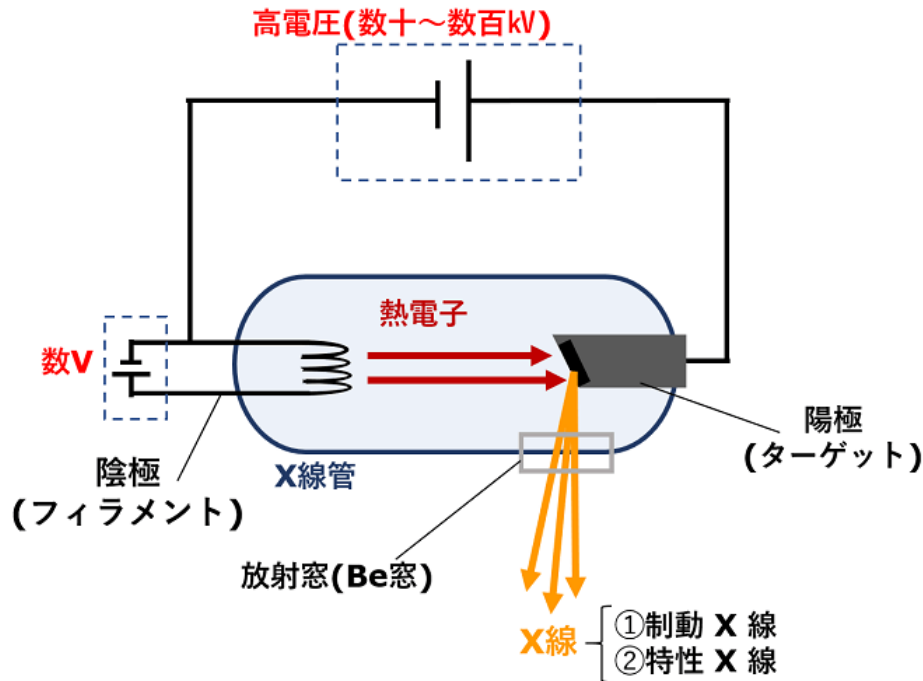


Electromagnetic waves can be regarded as the progression of the wavefront of an electricmagnetic field

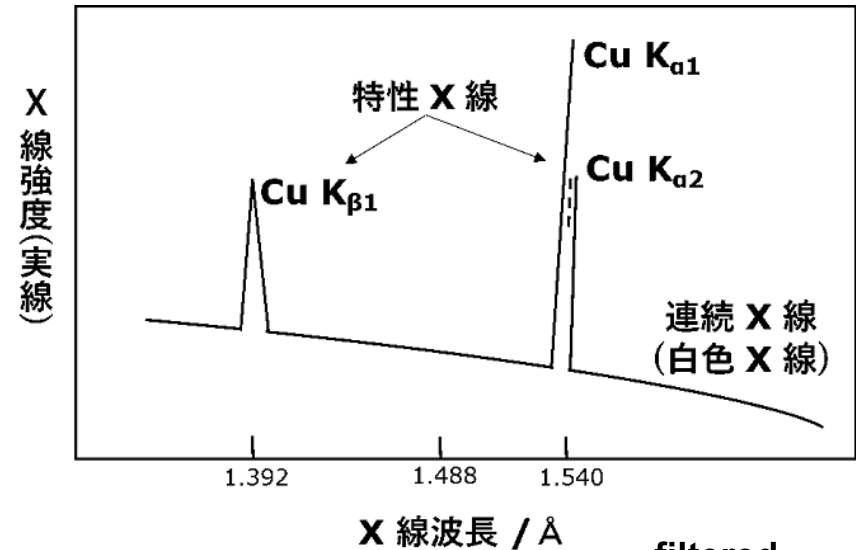


# X-Ray Generation

Example of X-ray tube:

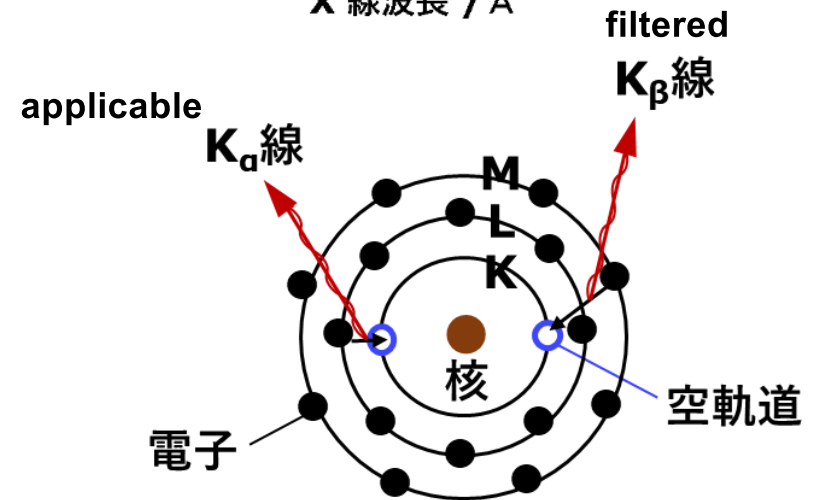


X-ray spectrum generated from Cu tube

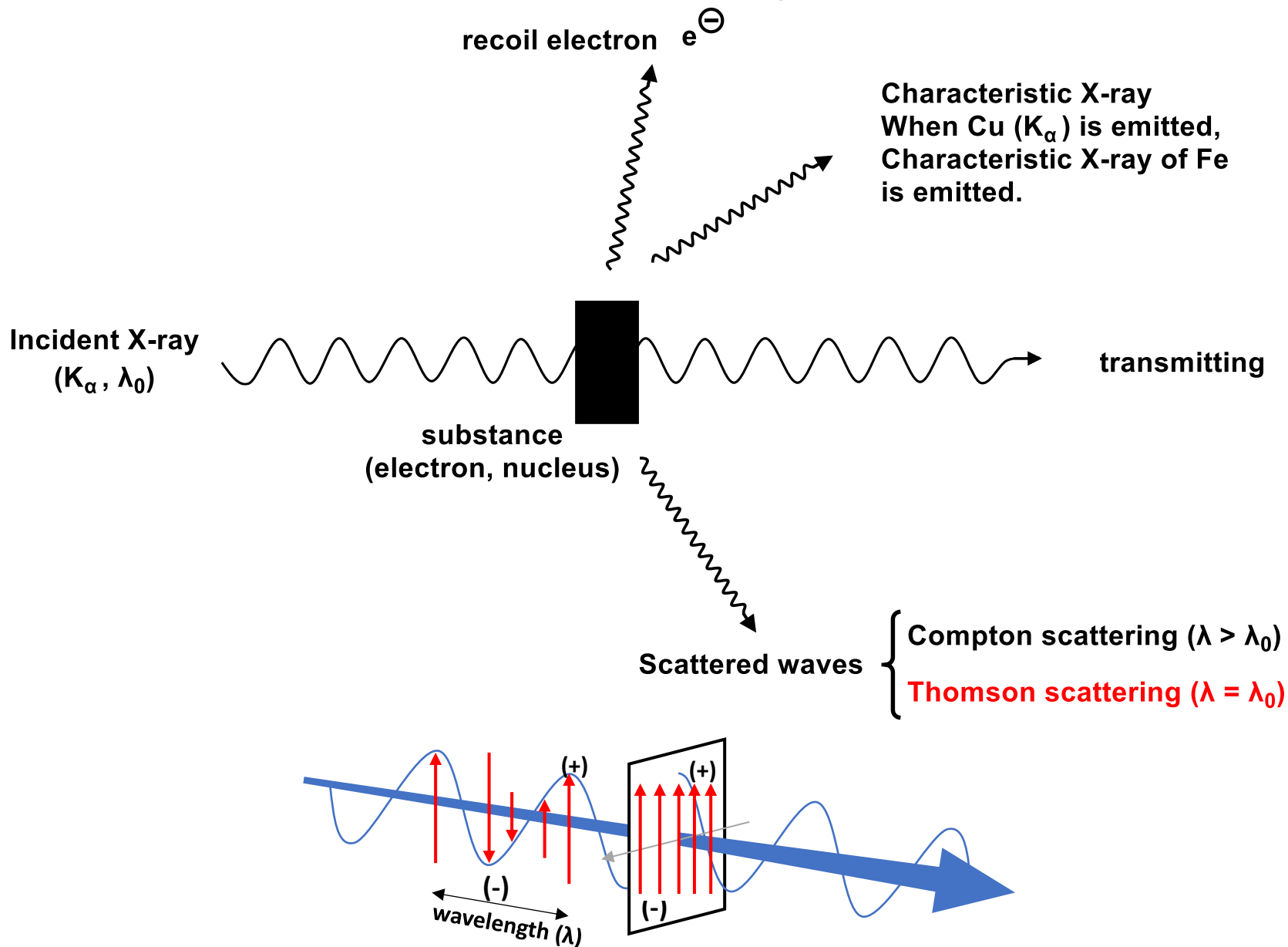


2 types of X-ray are emitted:

- ① Continuous X-ray (制動X線、連続X線)  
generated when electrons are decelerated as they pass near the nuclei of target atoms  
not used in X-ray crystallography = filtered
- ② Characteristic X-ray (used in X-ray crystallography, 特性X線)  
generated when high-energy electrons collide with inner-shell electrons of target atoms, causing them to be ejected.  
**used in X-ray crystallography ( $K_{\alpha}$ )** e.g. Mo: 0.711 Å, Cu: 1.542 Å

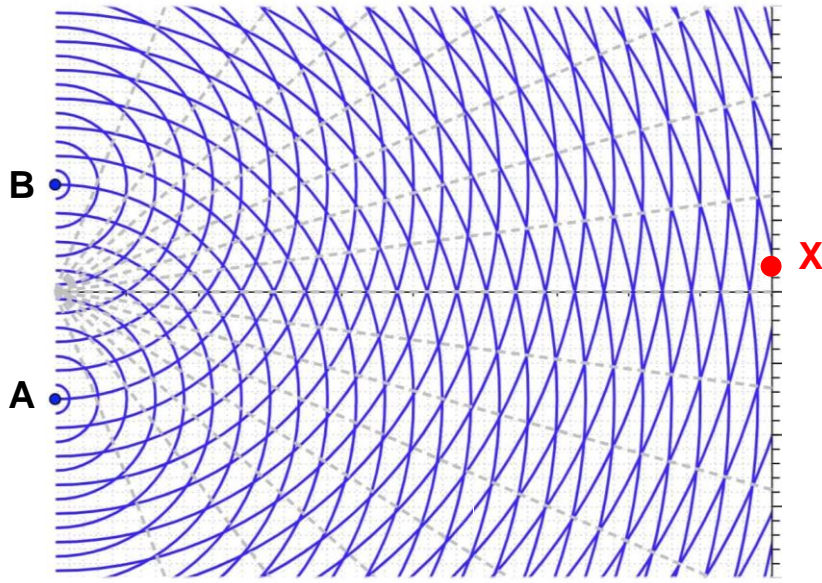


# Interaction between X-ray and Substance



# Wave Interaction

Wave interaction will be observed when waves have same frequency



Supposing waves from A and B are same and phase difference between A and B at point X is  $2\pi\delta$ , Wave  $E_A$  and  $E_B$  can be described as

$$E_A = A_0 e^{2\pi i \left( vt - \frac{x}{\lambda} \right)}$$

$$E_B = A_0 e^{2\pi i \left( vt - \frac{x}{\lambda} + \delta \right)}$$

$A_0$ : amplitude of wave A and B  
 $x$ : distance between point X and A  
 $v$ : wave frequency  
 $\lambda$ : wavelength  
 $t$ : time

Composite waves E can be described as

$$E = E_A + E_B = A_0 e^{2\pi i \left( vt - \frac{x}{\lambda} \right)} \underline{(1 + e^{2\pi \delta i})}$$

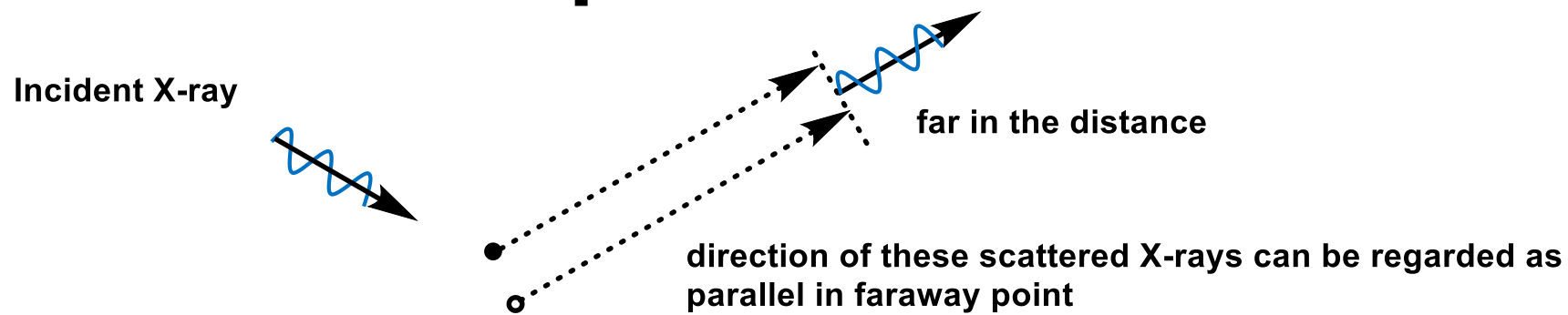
determined by underlined formula

Wavelength of Thomson scattered X-ray from each electron is the same. Therefore, these X-ray must be interacted.

When considering composite wave, we only need to consider phase difference of each wave.

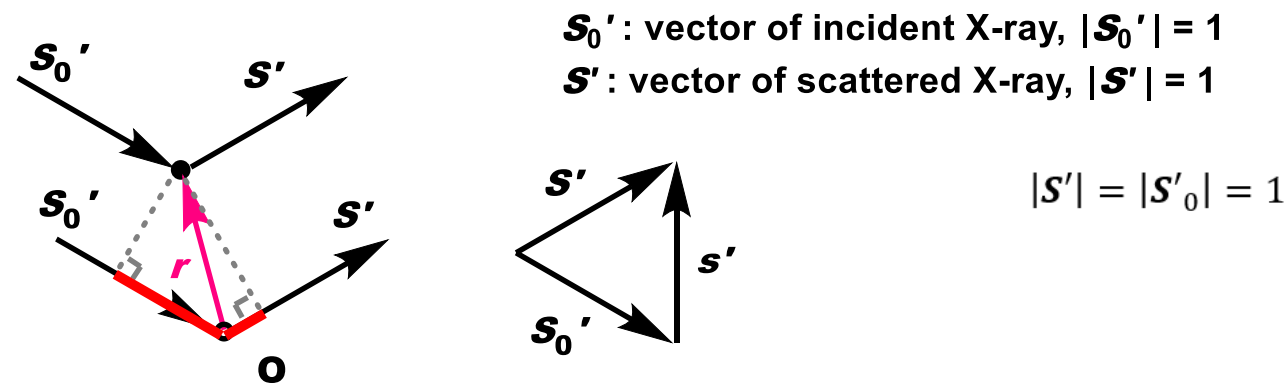


# Phase Difference can be Expressed as Simple Formula



How can we express path difference and phase difference?

- Path difference can be described as **below red lines**.



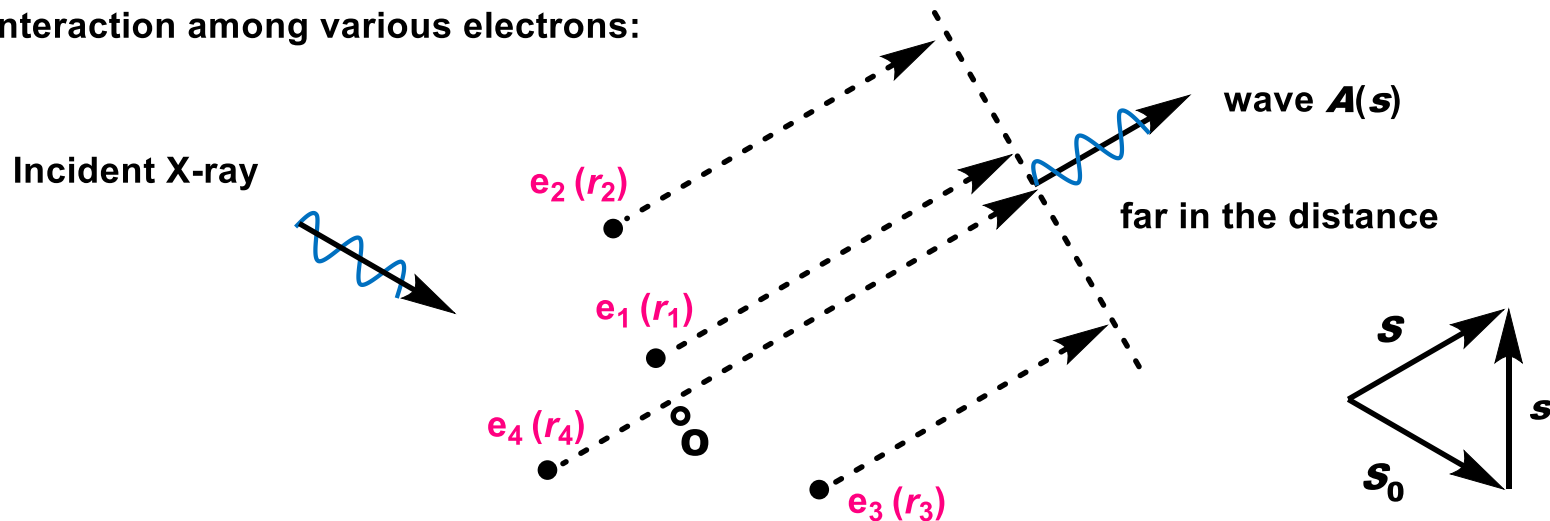
path difference =  $|r| \times |\mathbf{S}'| \times \cos(\text{r と } \mathbf{S}' \text{ の角度}) - |r| \times |\mathbf{S}'_0| \times \cos(\text{r と } \mathbf{S}'_0 \text{ の角度}) = \mathbf{r} \cdot \mathbf{S}' - \mathbf{r} \cdot \mathbf{S}'_0 = \mathbf{r} \cdot (\mathbf{S}' - \mathbf{S}'_0)$

When  $\mathbf{S}_0$  and  $\mathbf{S}$  are defined as  $\mathbf{S}_0 = \mathbf{S}_0'/\lambda$ ,  $\mathbf{S} = \mathbf{S}'/\lambda$ , phase difference parameter  $\delta(\mathbf{r})$  is described as:

$$\delta(\mathbf{r}) = \frac{\text{pathdifference}}{\lambda} = \frac{\mathbf{r} \cdot (\mathbf{S}' - \mathbf{S}'_0)}{\lambda} = \mathbf{r} \cdot \frac{(\mathbf{S}' - \mathbf{S}'_0)}{\lambda} = \mathbf{r} \cdot (\mathbf{S} - \mathbf{S}_0) = \mathbf{r} \cdot \mathbf{s} \quad (\mathbf{s} = \mathbf{S} - \mathbf{S}_0)$$

# Relationship between Wave and Electron Density

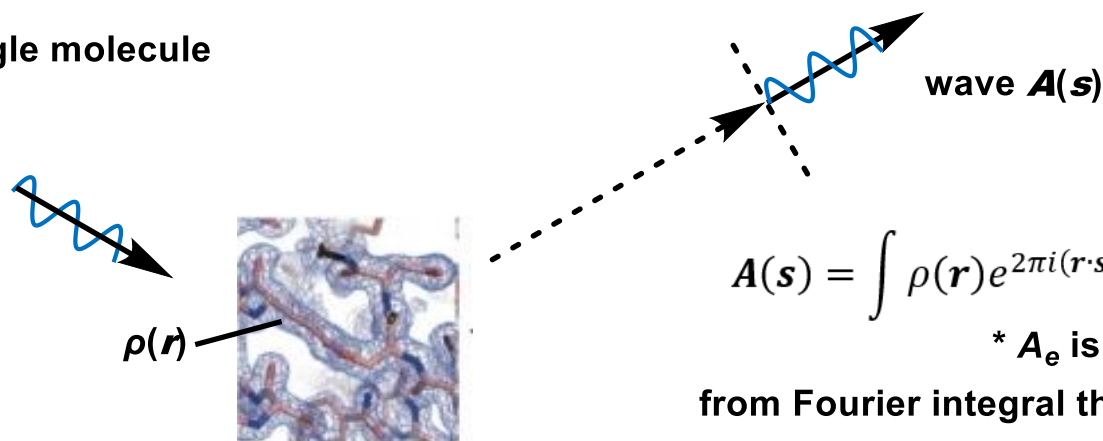
Wave interaction among various electrons:



$$A(\mathbf{s}) = A_e e^{2\pi i(\mathbf{r}_1 \cdot \mathbf{s})} + A_e e^{2\pi i(\mathbf{r}_2 \cdot \mathbf{s})} + \dots = \sum_{n=1,2,\dots} A_e e^{2\pi i(\mathbf{r}_n \cdot \mathbf{s})}$$

Wave interaction in single molecule

Incident X-ray

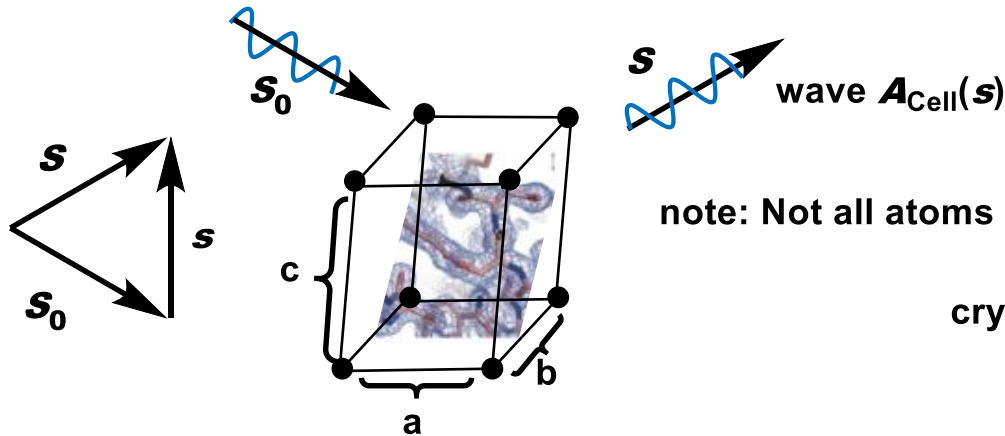


$$A(\mathbf{s}) = \int \rho(\mathbf{r}) e^{2\pi i(\mathbf{r} \cdot \mathbf{s})} dV_r$$

\*  $A_e$  is omitted from Fourier integral theorem...

$$\rho(\mathbf{r}) = \int A(\mathbf{s}) e^{-2\pi i(\mathbf{r} \cdot \mathbf{s})} dV_s$$

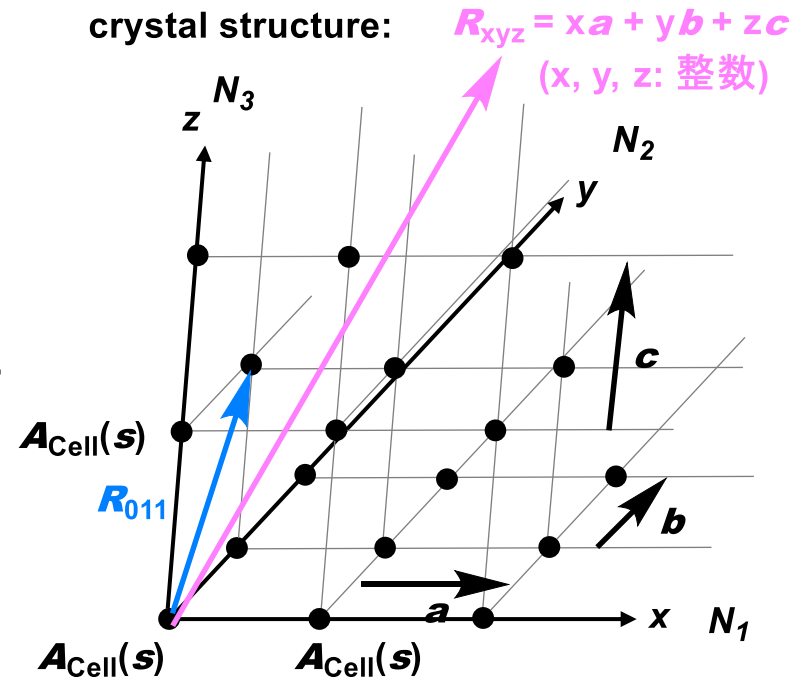
# Mechanism of X-Ray Diffraction (1)



note: Not all atoms in a molecule are packed in the unit cell.

Composite waves of crystal  $\mathbf{A}_{\text{crystal}}(\mathbf{s})$  is regarded as the sum of each unit cell's composite waves  $\mathbf{A}_{\text{Cell}}(\mathbf{s})$ .

$$\begin{aligned}
 \mathbf{A}_{\text{crystal}}(\mathbf{s}) &= \mathbf{A}_{\text{Cell}}(\mathbf{s})e^{2\pi i\{(a+b+c)\cdot\mathbf{s}\}} + \mathbf{A}_{\text{Cell}}(\mathbf{s})e^{2\pi i\{(2a+b+c)\cdot\mathbf{s}\}} + \dots \\
 &= \sum_{x=-\frac{N_1-1}{2}}^{\frac{N_1-1}{2}} \sum_{y=-\frac{N_2-1}{2}}^{\frac{N_2-1}{2}} \sum_{z=-\frac{N_3-1}{2}}^{\frac{N_3-1}{2}} \mathbf{A}_{\text{Cell}}(\mathbf{s})e^{2\pi i\{(xa+yb+zc)\cdot\mathbf{s}\}} \\
 &= \mathbf{A}_{\text{Cell}}(\mathbf{s}) \left\{ \frac{\sin N_1\pi(\mathbf{a}\cdot\mathbf{s})}{\sin\pi(\mathbf{a}\cdot\mathbf{s})} \cdot \frac{\sin N_2\pi(\mathbf{b}\cdot\mathbf{s})}{\sin\pi(\mathbf{b}\cdot\mathbf{s})} \cdot \frac{\sin N_3\pi(\mathbf{c}\cdot\mathbf{s})}{\sin\pi(\mathbf{c}\cdot\mathbf{s})} \right\}
 \end{aligned}$$



# Mechanism of X-Ray Diffraction (2)

X-ray intensity is proportional to the square of the amplitude.

Composite wave  $A(\mathbf{s})$  is complex number, so X-ray intensity  $I_{\text{crystal}}(\mathbf{s})$  is expressed as

$$I_{\text{crystal}}(\mathbf{s}) \propto A_{\text{crystal}}(\mathbf{s}) \cdot A_{\text{crystal}}^*(\mathbf{s}) = |A_{\text{Cell}}(\mathbf{s})|^2 \left\{ \frac{\sin^2 N_1 \pi (\mathbf{a} \cdot \mathbf{s})}{\sin^2 \pi (\mathbf{a} \cdot \mathbf{s})} \cdot \frac{\sin^2 N_2 \pi (\mathbf{b} \cdot \mathbf{s})}{\sin^2 \pi (\mathbf{b} \cdot \mathbf{s})} \cdot \frac{\sin^2 N_3 \pi (\mathbf{c} \cdot \mathbf{s})}{\sin^2 \pi (\mathbf{c} \cdot \mathbf{s})} \right\}$$

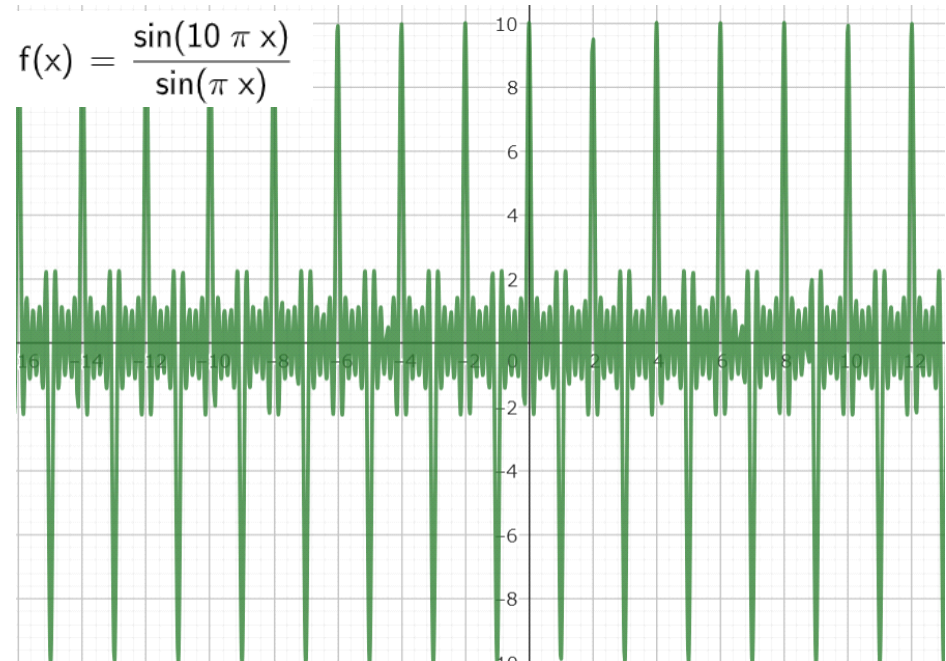
According to the graph of right,  $I_{\text{crystal}}(\mathbf{s})$  becomes strong when the following rules are satisfied.

$$(\mathbf{a} \cdot \mathbf{s}) = h, \quad (\mathbf{b} \cdot \mathbf{s}) = k, \quad (\mathbf{c} \cdot \mathbf{s}) = l$$

$h, k, l$ : integer (整数) \*Laue equation

Otherwise,  $I_{\text{crystal}}(\mathbf{s})$  would be neglected

→ Scattered X-rays are observed only at certain points.  
X-ray diffraction!



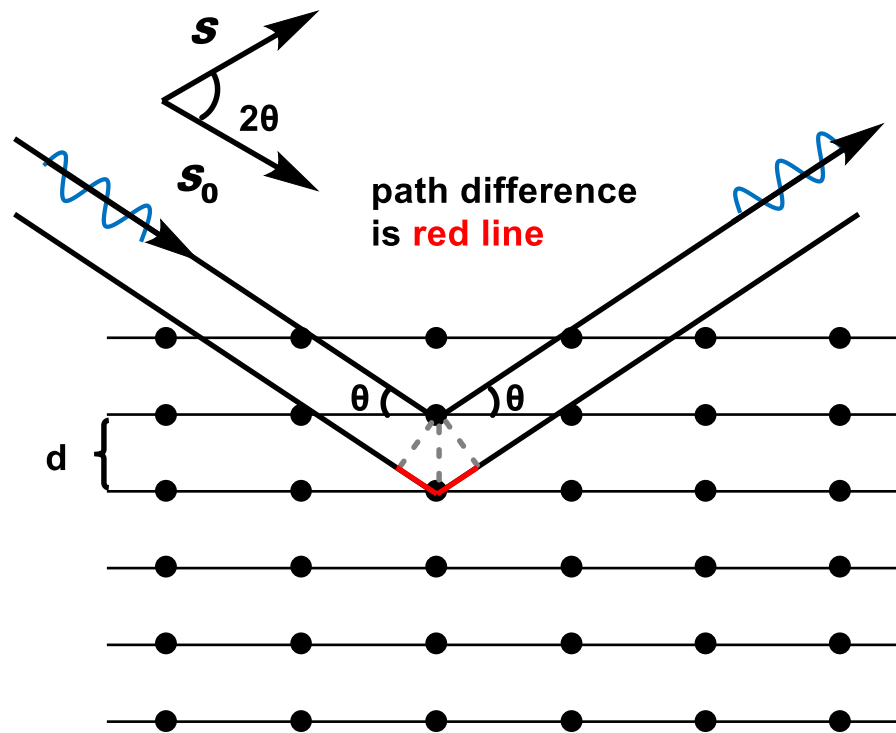
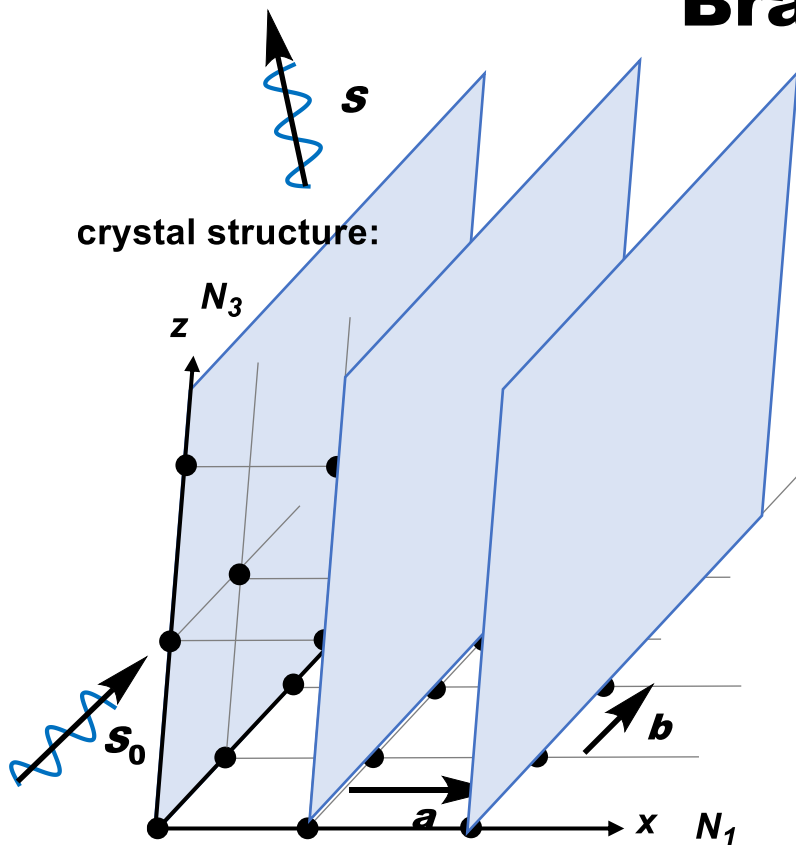
$$\mathbf{r} = x\mathbf{a} + y\mathbf{b} + z\mathbf{c}$$

$$\mathbf{r} \cdot \mathbf{s} = (x\mathbf{a} + y\mathbf{b} + z\mathbf{c}) \cdot \mathbf{s} = x(\mathbf{a} \cdot \mathbf{s}) + y(\mathbf{b} \cdot \mathbf{s}) + z(\mathbf{c} \cdot \mathbf{s}) = hx + ky + lz$$

$$A_{\text{Cell}}(\mathbf{s}) = \int \rho(\mathbf{r}) e^{2\pi i (\mathbf{r} \cdot \mathbf{s})} dV_{\mathbf{r}} = \iiint \rho(x, y, z) e^{2\pi i (hx + ky + lz)} V dx dy dz = \underline{F(h, k, l)}$$

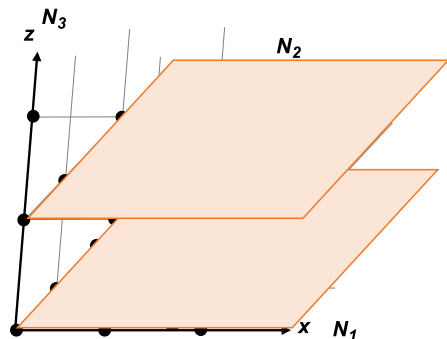
structure factor

# Bragg's law

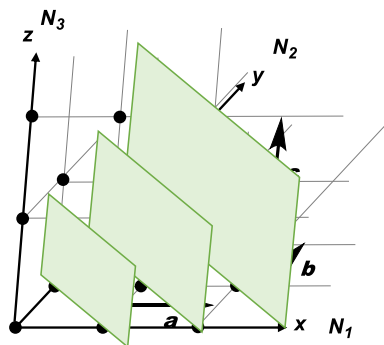


$$2d\sin\theta = n\lambda \quad (n = 1, 2, 3, \dots) \quad \text{Bragg's law}$$

crystal structure:



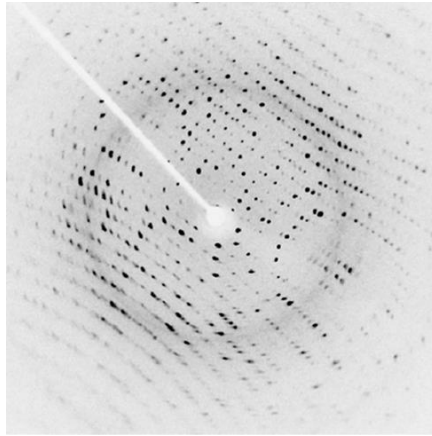
crystal structure:



Such face can be drawn in various way.  
 → Many spots are observed in X-ray diffraction

# Introduction of Reciprocal Lattice

diffracted images:



definition of reciprocal lattice vector ( $\mathbf{a}^*$ ,  $\mathbf{b}^*$ ,  $\mathbf{c}^*$ ):

$$\mathbf{a}^* \cdot \mathbf{a} = \mathbf{b}^* \cdot \mathbf{b} = \mathbf{c}^* \cdot \mathbf{c} = 1$$

$$\mathbf{a}^* \cdot \mathbf{b} = \mathbf{a}^* \cdot \mathbf{c} = \mathbf{b}^* \cdot \mathbf{a} = \mathbf{b}^* \cdot \mathbf{c} = \mathbf{c}^* \cdot \mathbf{a} = \mathbf{c}^* \cdot \mathbf{b} = 0$$

note: The reciprocal of the reciprocal lattice is the original lattice.

Defining  $\mathbf{H}$  as the vector toward the reciprocal grid point, the following relationships hold

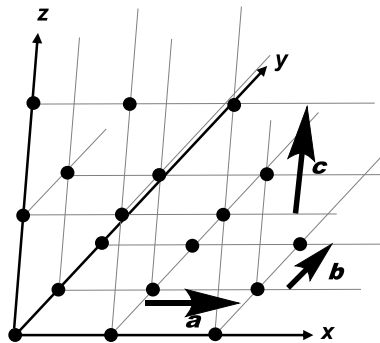
$$\mathbf{H} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^* \quad (h, k, l: \text{整数})$$

$$\mathbf{a} \cdot \mathbf{H} = \mathbf{a} \cdot (h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*) = h\mathbf{a} \cdot \mathbf{a}^* + k\mathbf{a} \cdot \mathbf{b}^* + l\mathbf{a} \cdot \mathbf{c}^* = h$$

$$\mathbf{b} \cdot \mathbf{H} = k, \mathbf{c} \cdot \mathbf{H} = l$$

→  $\mathbf{H}$  satisfies Laue equation:  $(\mathbf{a} \cdot \mathbf{s}) = h, (\mathbf{b} \cdot \mathbf{s}) = k, (\mathbf{c} \cdot \mathbf{s}) = l$

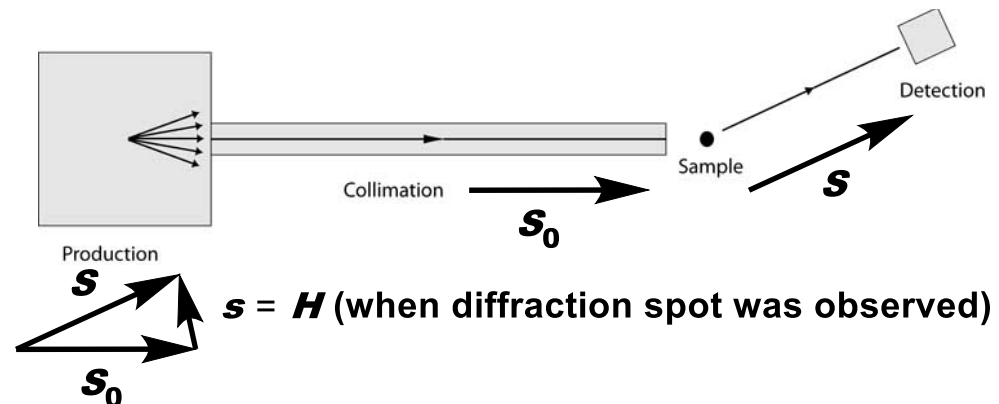
crystal lattice:



We know the direction of incident and scattered X-rays, therefore we can determine vector  $\mathbf{H}$  from each diffraction spot.

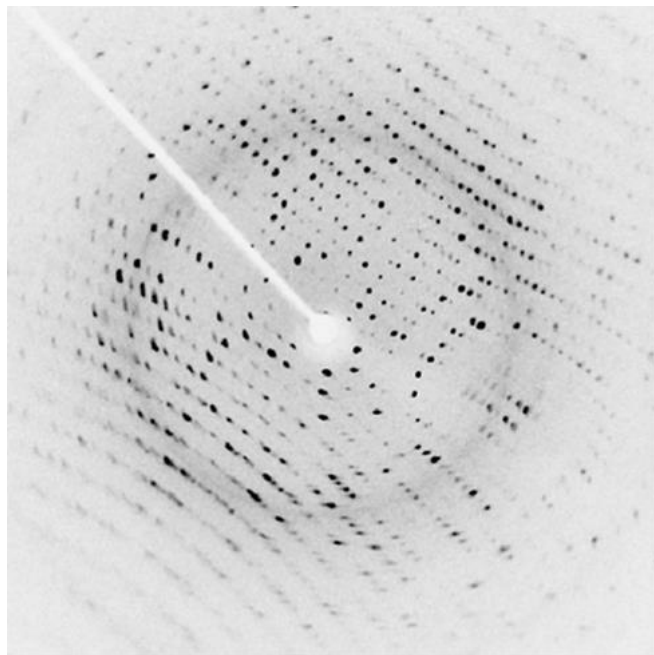
→ Reciprocal lattice can be decided.

= original (crystal lattice) can be decided.



1. X線解析入門 第3版 角戸正夫・笹田義夫著 東京化学同人
2. <https://www.physics.upenn.edu/~heiney/datasqueeze/basics.html>

# Phase Problem in X-ray Analysis



X-ray diffraction images

$$F(h, k, l) = \iiint \rho(x, y, z) e^{2\pi i(hx + ky + lz)} V dx dy dz$$



Fourier integral theorem...

$$\begin{aligned} \rho(x, y, z) &= \frac{1}{V} \sum \sum_{-\infty}^{\infty} \sum F(h, k, l) e^{-2\pi i(hx + ky + lz)} \\ &= \frac{1}{V} \sum \sum_{-\infty}^{\infty} \sum \frac{|F(h, k, l)|}{\underbrace{\phantom{|F(h, k, l)|}}_{\text{available from}}} e^{-i\{2\pi(hx + ky + lz) - \underbrace{\phi(h, k, l)}_{\text{unavailable from}}\}} \end{aligned}$$

available from  
observable  
intensity

unavailable  
from diffraction  
images!

**"Phase problem"**

- Direct (*ab initio*) method: Developed by Hauptman and Karle
- Solve phase problem by using mathematical processing
- Commonly used in recent X-ray analysis

In determination of absolute configuration of the structure, the effect of anomalous scattering (異常散乱) is utilized. Anomalous scattering was mainly observed on heavy atoms, so introduction of heavy atom is recommended.



Hauptman



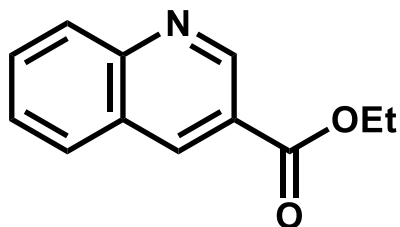
Karle

The Nobel Prize in  
Chemistry 1985

# Limitation of X-ray Crystallography

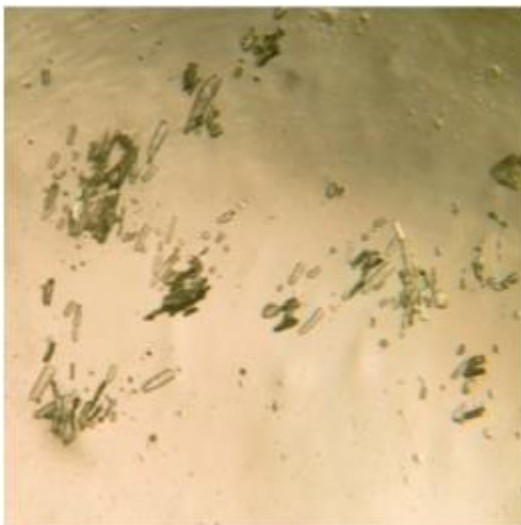
Limitation:

Crystal with proper size and good quality is required for X-ray analysis



3-carboethoxyquinoline

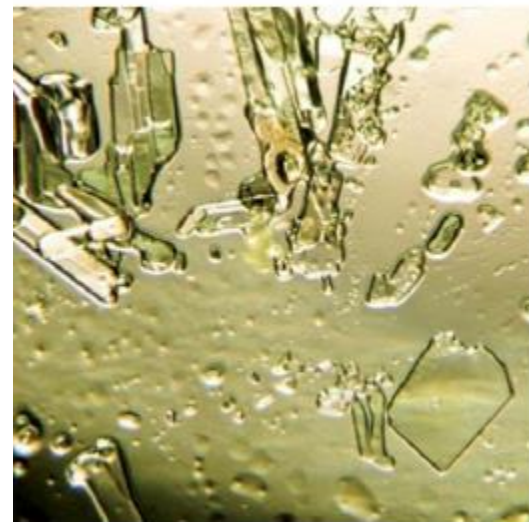
1 mm



Too small for X-ray analysis



Too thin for X-ray analysis



Proper crystal

3 approaches will be explained:

- Micro ED
- Crystalline Sponge
- Structure modification for better crystallinity



1. Introduction

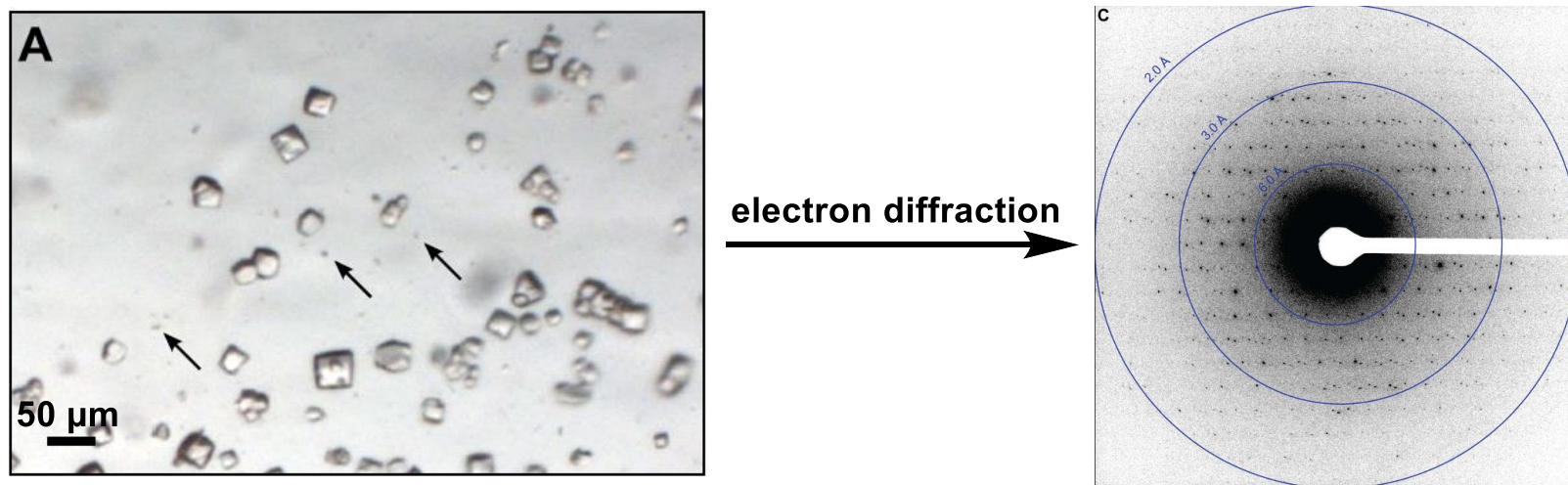
**2. Strategy for Crystal Analysis**

# Micro ED

micro ED: Use electron beam (interacts with nucleus and electrons)

Interactions are stronger than X-ray (interacts with only valence electron),  
so stronger diffraction patterns can be obtained.

→ Small amount of molecule is needed for sufficient analysis



Usually, electron beam is too strong for small molecules, so Gonen's group reduced the electron dose to 1/200 compared with usual method.

After obtaining the diffraction pattern, the operation is the same as for X-ray crystallography.

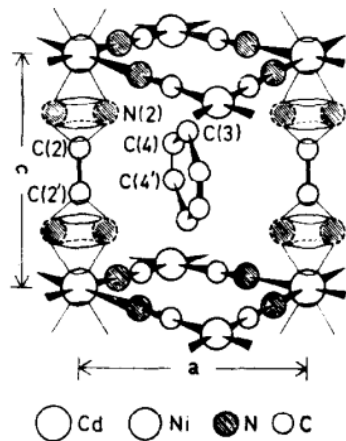
See also 240420\_LS\_Manaka\_Matsumoto

1. Jones, C. G.; Martynowycz, M. W.; Hattne, J.; Fulton, T. J.; Stoltz, B. M.; Rodriguez, J. A.; Nelson, H. M.; Gonen, T. *ACS Cent. Sci.* **2018**, *4*, 1587.
2. Shi, D.; Nannenga, B. L.; Iadanza, M. G.; Gonen, T. *eLife*, **2013**, *2*, e01345.

# Crystalline Sponge: Background

crystalline sponge method: Absorb the compounds to crystalline sponge

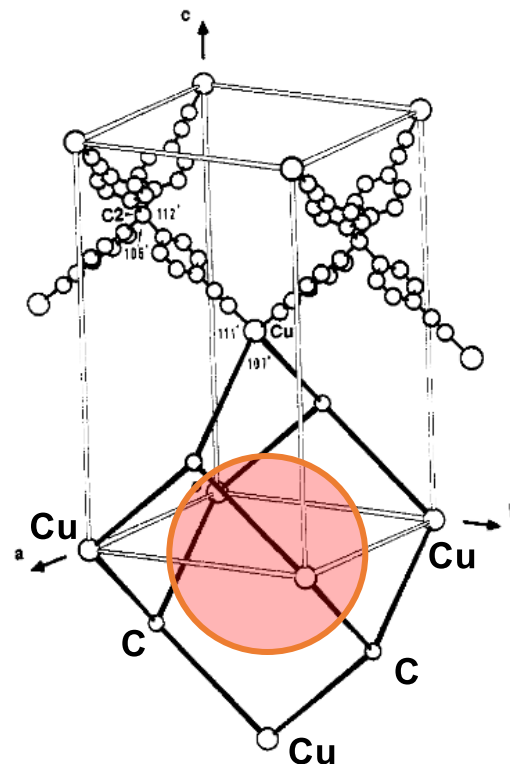
→ Crystallization is not necessary. Oil compounds can be applied to X-ray analysis in this method.



Some crystal structure has cavities (pores) in the network.

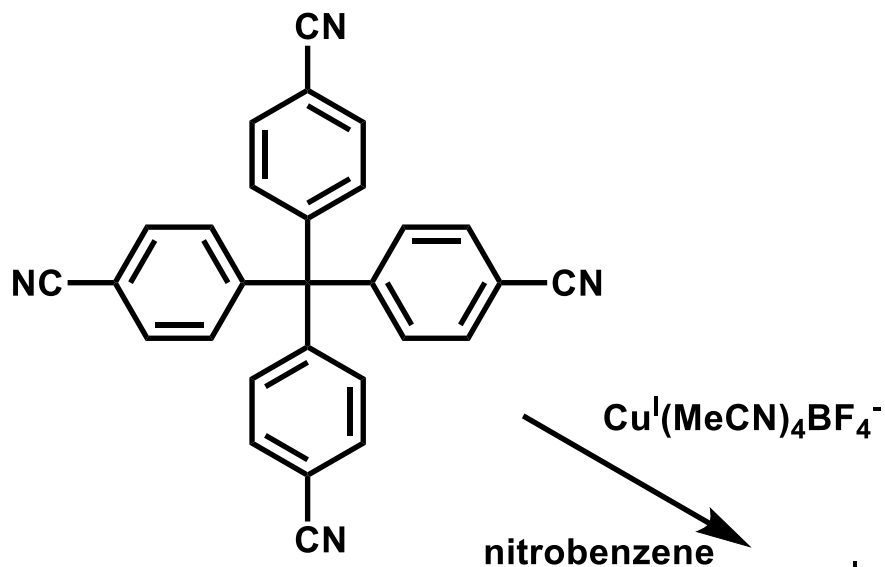
← Structure of  $\text{Cd}(\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2)\text{Ni}(\text{CN})_4$ <sup>1)</sup>

benzene is in the pore (guest molecule)



The pores were filled with nitrobenzene and  $\text{BF}_4^-$

When adding  $\text{NBu}_4^+\text{PF}_6^-$ , anion change in the pores were observed.<sup>2)</sup> (Guest exchange)

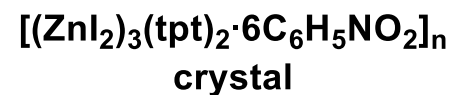
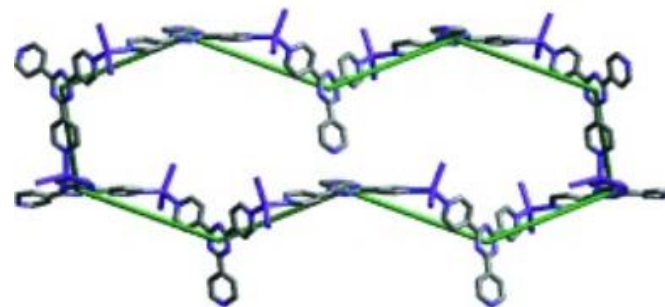
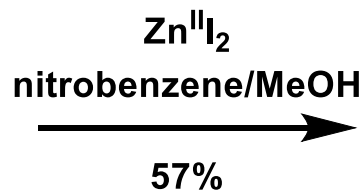
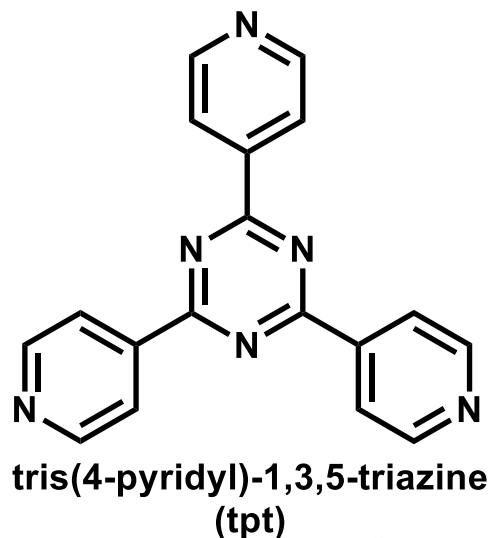


1. Miyoshi, T.; Iwamoto, T.; Sasaki, Y. *Inorg. Chim. Acta.* **1972**, *6*, 59.

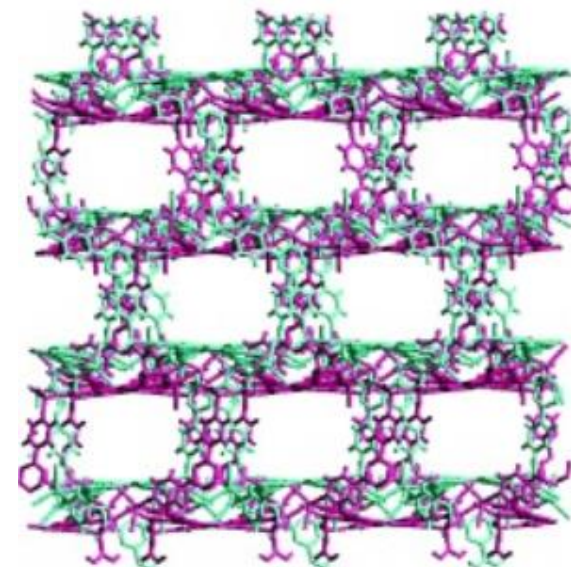
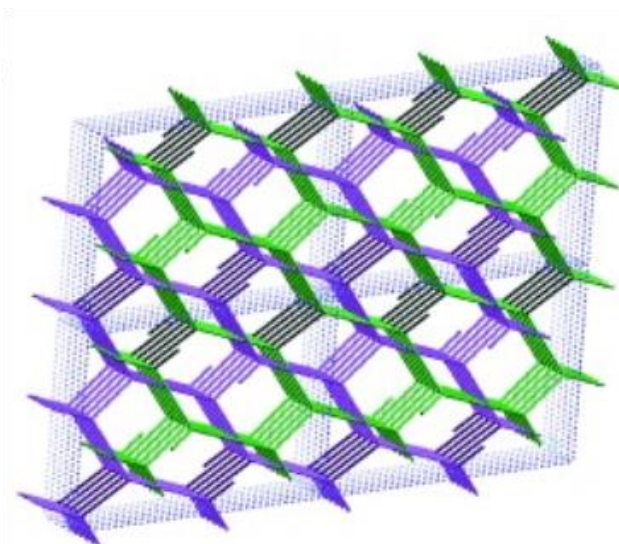
2. Hoskins, B. F.; Robson, R. *J. Am. Chem. Soc.* **1990**, *112*, 1546.

# MOF with Flexibility and Robstness (1)

Interpenetrated network MOF developed by Fujita's group:

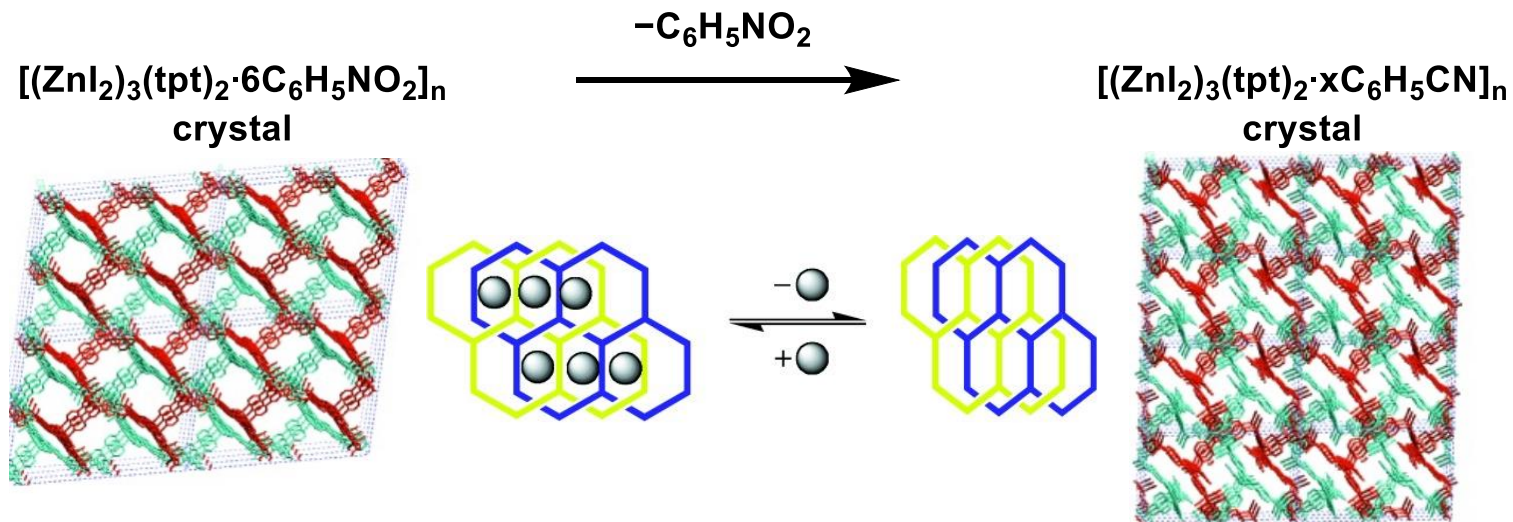


They found that two types of networks (green and purple) are interpenetrated in crystal structure



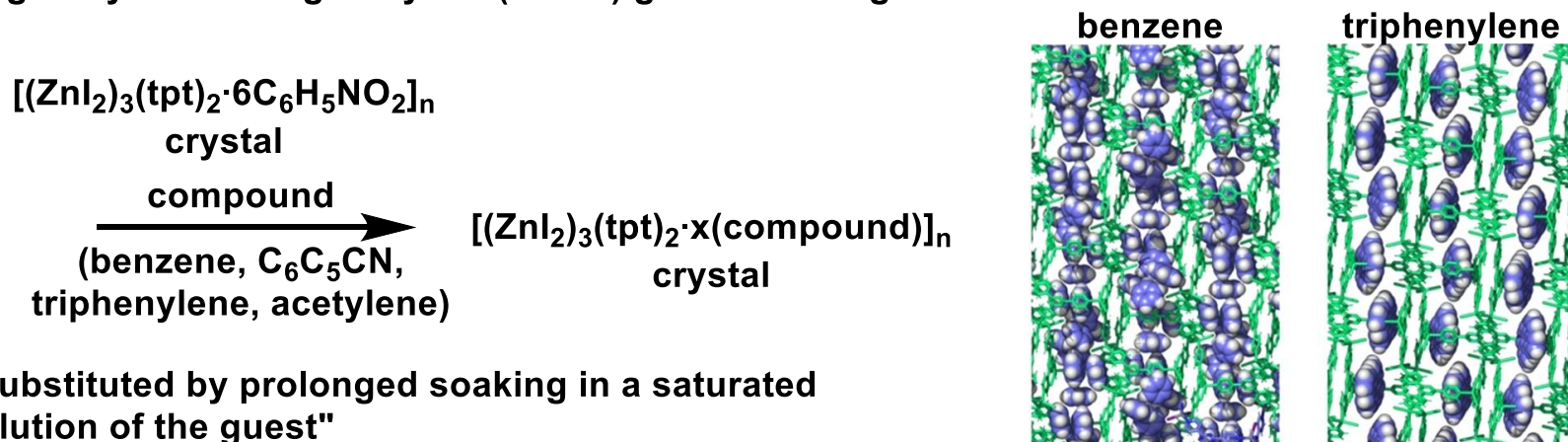
# MOF with Flexibility and Robustness (2)

- Compression change of the networks "without destroying the crystalline nature "



The guest molecule is released from the crystal and unit-cell parameters changed.

- Single-crystal-to-single-crystal (SCSC) guest exchange

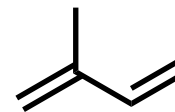
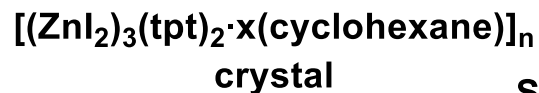
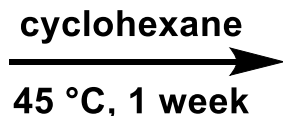
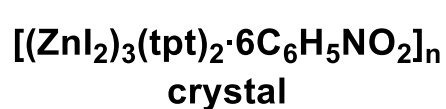


1. Zigon, N.; Duplan, V.; Wada, N.; Fujita, M. *Angew. Chem. Int. Ed.* **2021**, *60*, 25204.
2. Biradha, K.; Fujita, M. *Angew. Chem. Int. Ed.* **2002**, *41*, 3392.
3. Ohmori, O.; Kawano, M.; Fujita, M. *J. Am. Chem. Soc.* **2004**, *126*, 16292.

# Crystalline Sponge

Crystalline sponge: Developed by Fujita's group

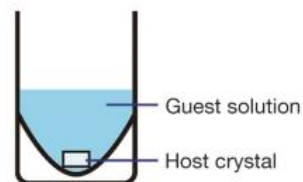
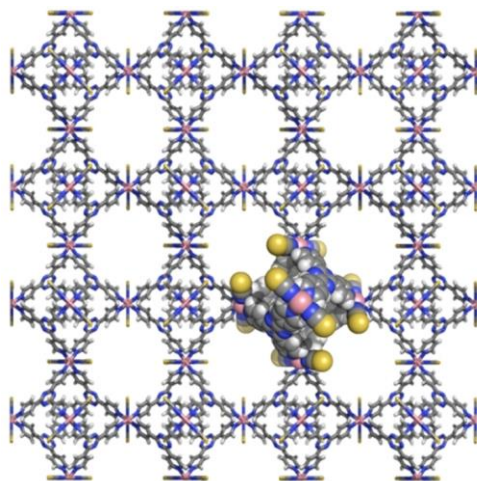
Enabled X-ray analysis of non-crystal compound at nano gram scale



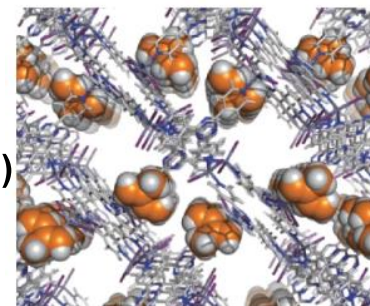
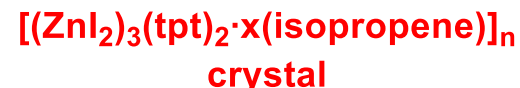
Simple operation

"Any guest that shows stronger host-guest interactions than hydrocarbons can, in principle, be absorbed."

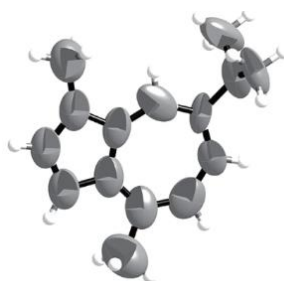
Other crystalline sponge in the paper:



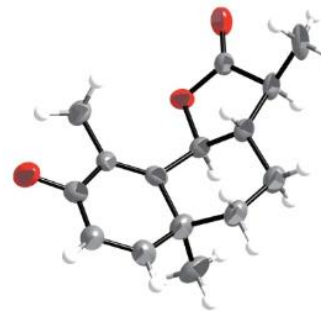
(cyclohexane/CH<sub>2</sub>Cl<sub>2</sub> solution)



Other examples:



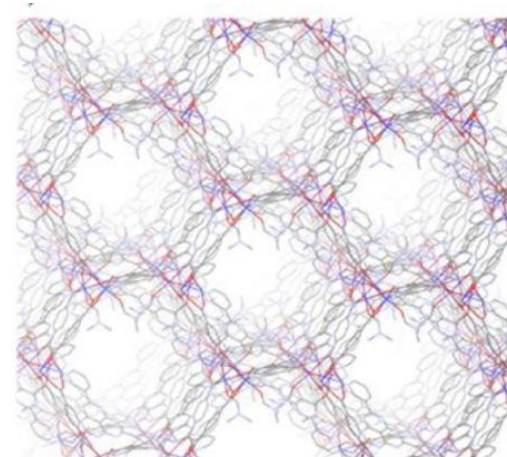
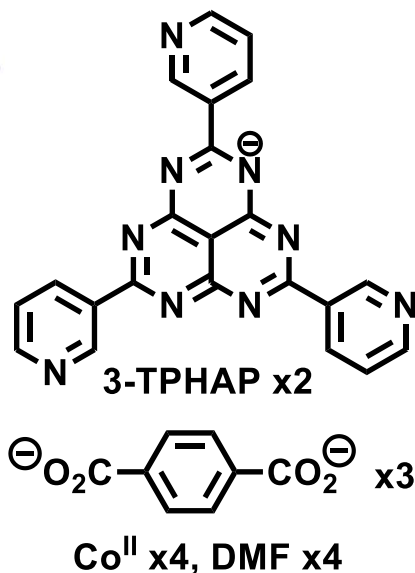
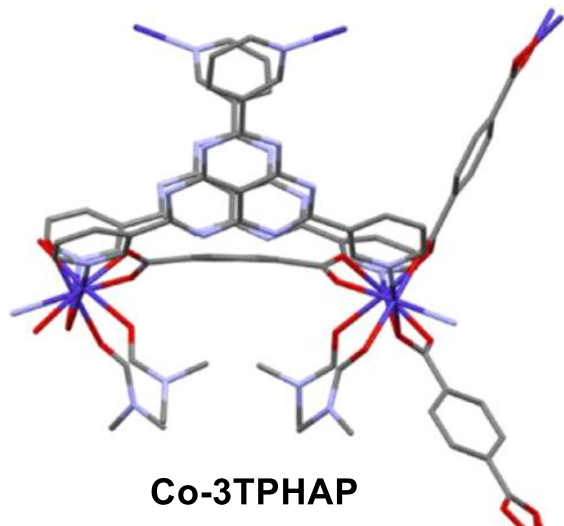
guaiazulene  
(from 80 ng)



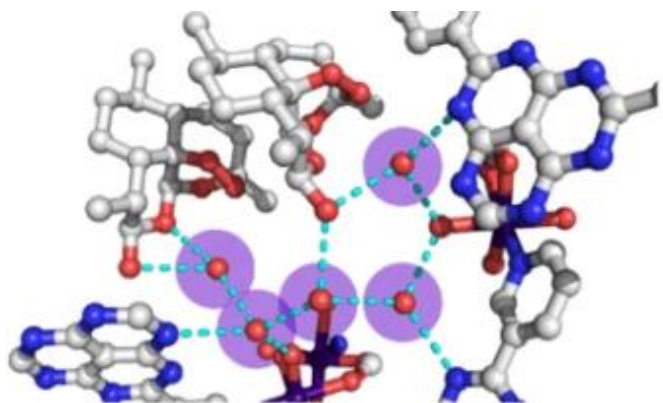
Santonin (from 15 µg)  
absolute configuration  
unambiguously determined

1. Inokuma, Y.; Yoshida, S.; Ariyoshi, J.; Arai, T.; Hitora, Y.; Takada, K.; Matsunaga, S.; Rissanen, K.; Fujita, M. *Nature*, **2013**, 495, 461.
2. Zigon, N.; Duplan, V.; Wada, N.; Fujita, M. *Angew. Chem. Int. Ed.* **2021**, 60, 25204.

# Crystalline Sponge: Recent Progress

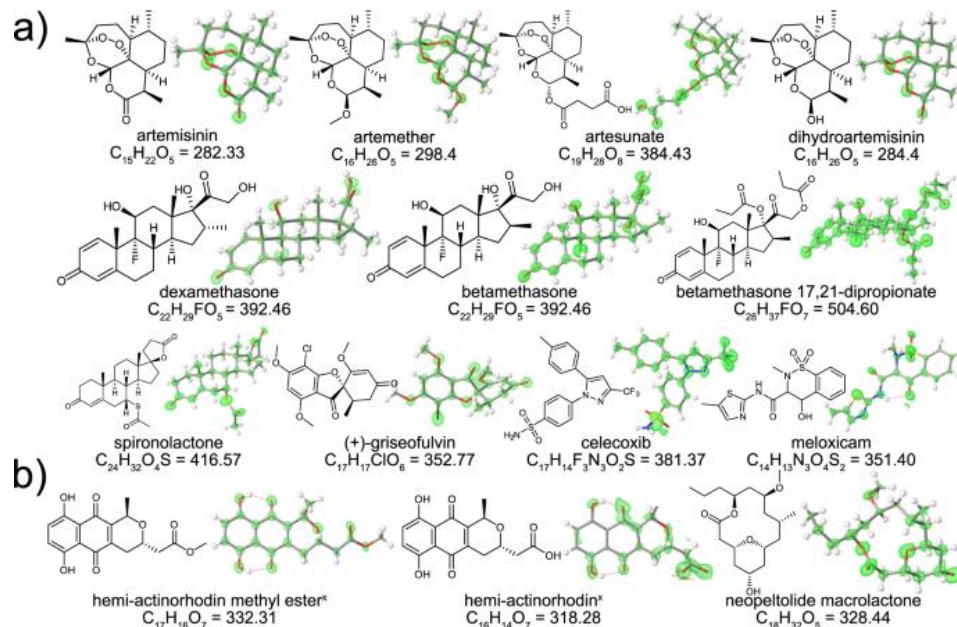


pore size: 12.5 x 8.3 Å



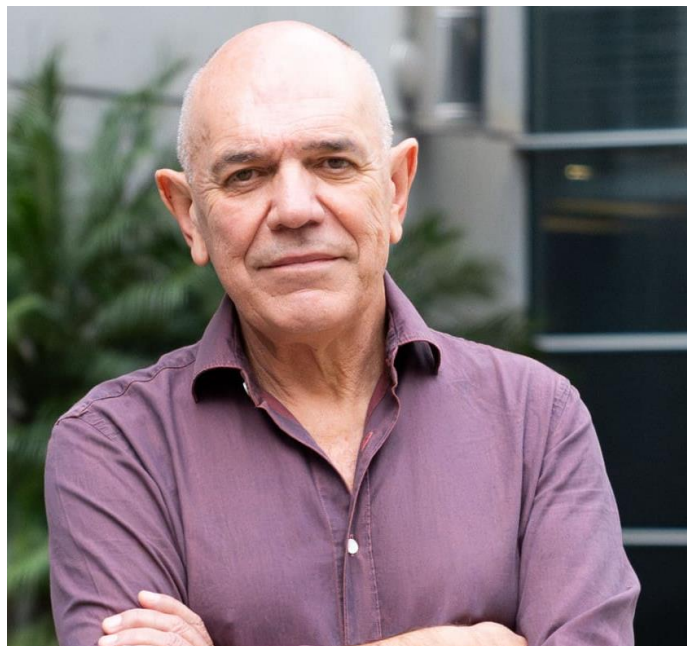
hydrogen bond interaction  
stabilize the guest

→ High resolution data, wide range



# Prof. Antonio M. Echarvarren

Prof. Antonio M. Echarvarren



## Career:

- 1982: Ph. D. @The Universidad Autónoma de Madrid (Prof. F. Fariña)
- 1982-1984: Postdoctoral Fellow @Boston College (Prof. T. R. Kelly)
- 1984-1986: Assistant professor @The Universidad Autónoma de Madrid
- 1986-1988: NATO-fellow @The Colorado State University (Prof. J. K. Stille)
- 1988-1992: Institute of Organic Chemistry of Spanish National Council (CSIC) researcher
- 1992-2009: Professor @The Universidad Autónoma de Madrid
- 2004-: Research Professor @CSIC
- 2004-: Group Leader @Institute of Chemical Research of Catalonia (ICIQ)

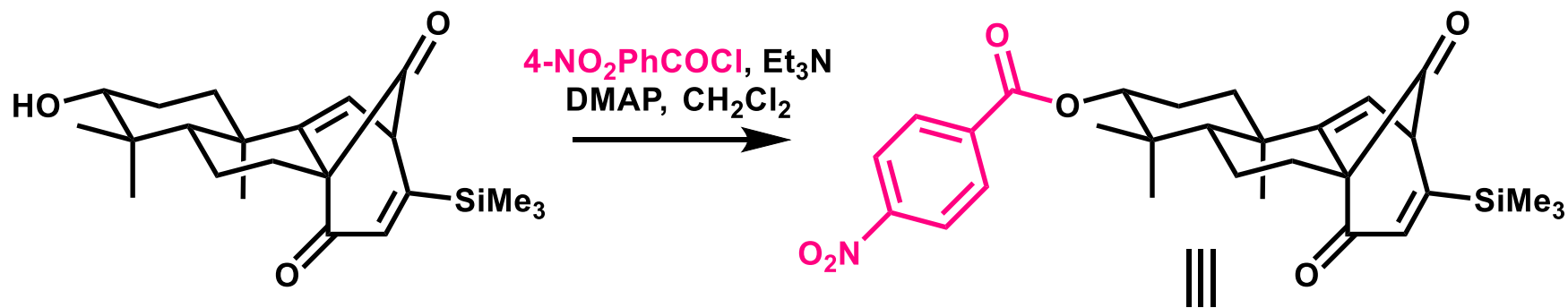
## Research Interest:

C-H activation, Total synthesis (Au catalyzed)

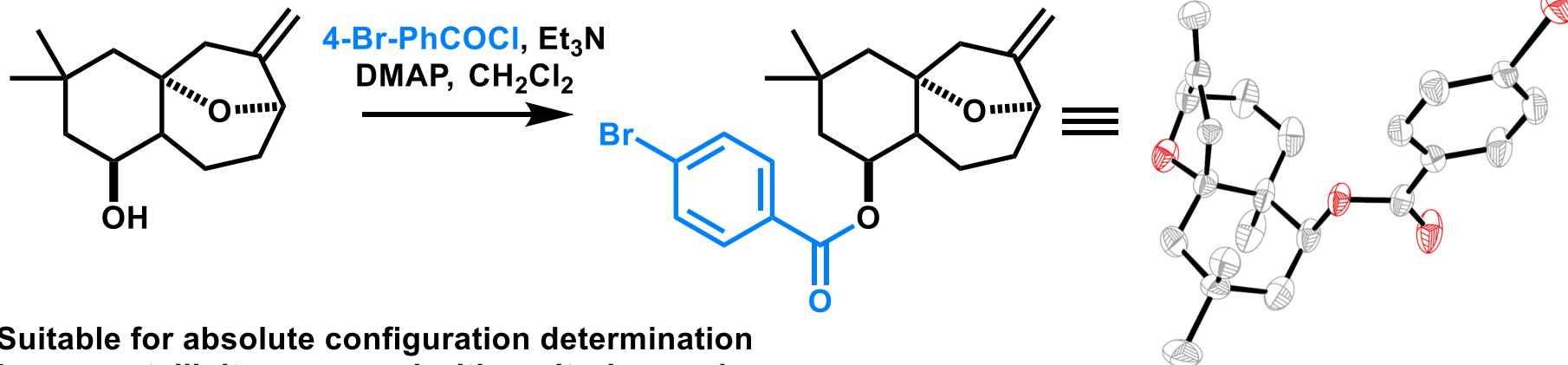
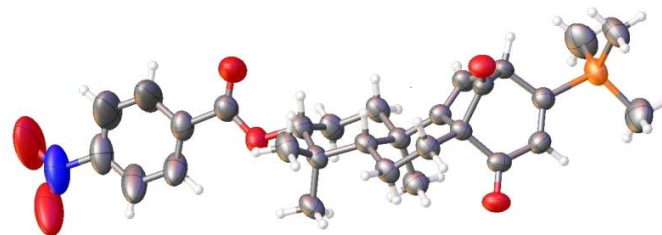


# Structure Modification for Better Crystallinity

Acylation of alcohol was sometimes conducted for better crystallinity of substrates. Intermolecular interactions (hydrogen bond, electrostatic interactions) increase the crystallinity



Not suitable for absolute configuration determination.

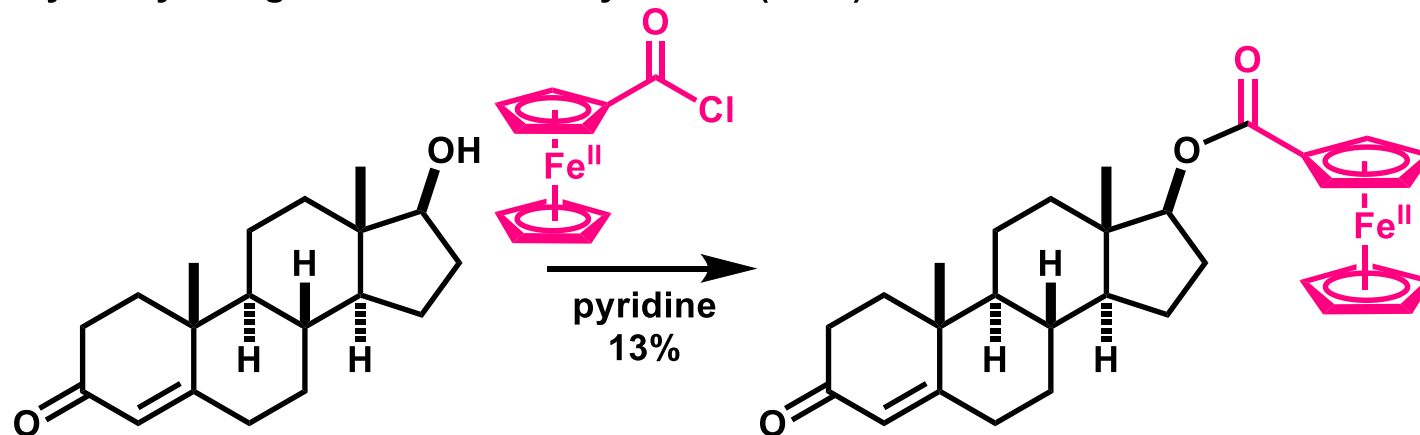


Suitable for absolute configuration determination  
Less crystallinity compared with *p*-nitrobenzoyl

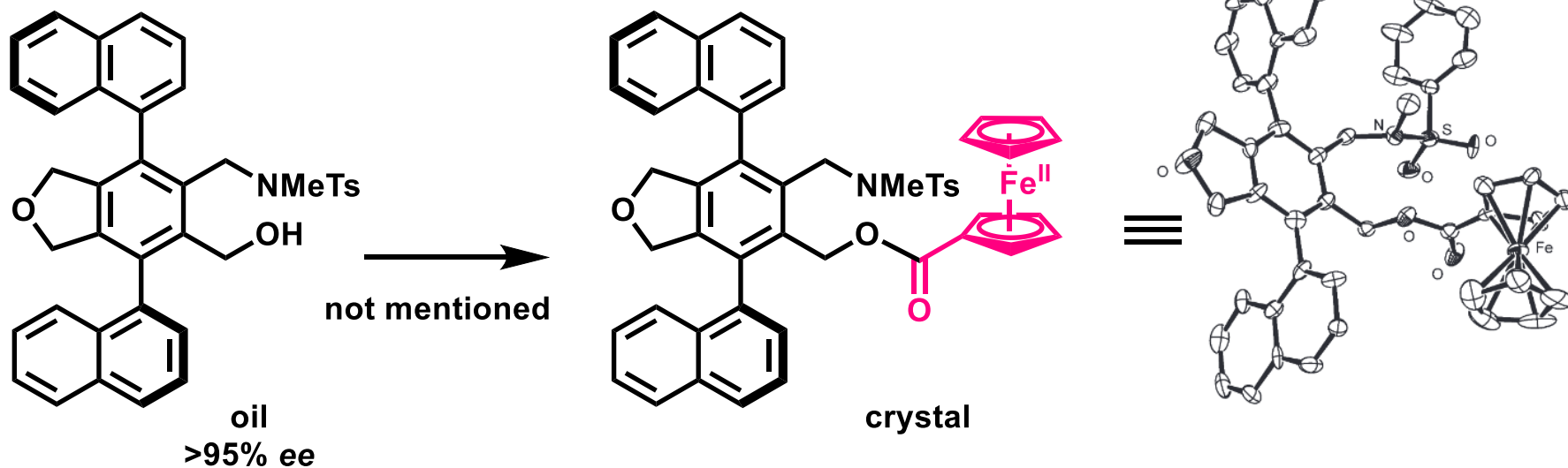
1. Song, L.; Zhu, G.; Liu, Y.; Liu, B.; Qin, S. *J. Am. Chem. Soc.* **2015**, *137*, 13706.
2. Qin, X.-Q.; Wu, Q.-L.; Han, F.-S. *Org. Lett.* **2021**, *21*, 8570.

# Prior Work Using Ferrocene Carboxylic Acid

Early study using ferrocene carboxylic acid (1980)

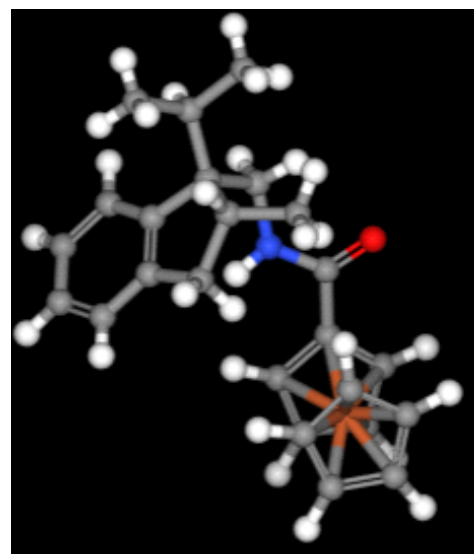
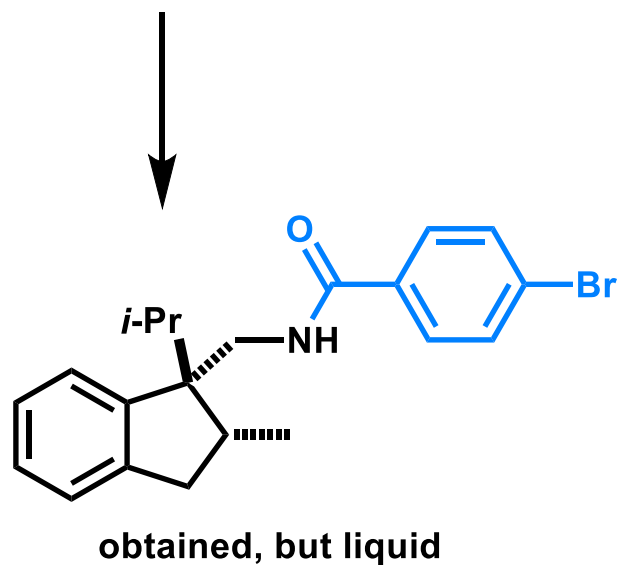
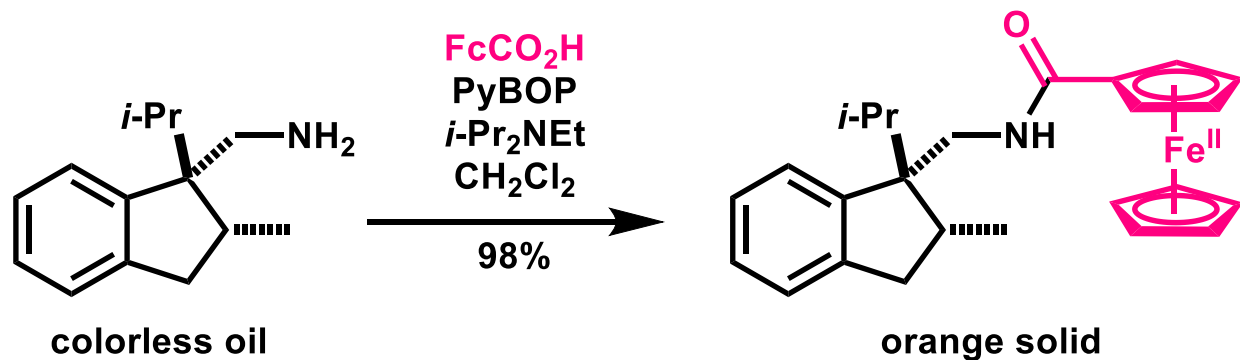


Previous studies of ferrocenyl esters as crystallizing reagents

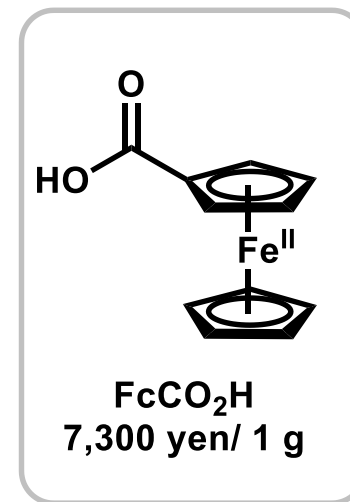


1. Hoffman, K.; Riebelmann, B.; Wenzel, M. *Liebigs Ann. Chem.* **1980**, 1980, 1181.
2. Shibata, T.; Arai, Y.; Takami, K.; Tsuchikama, K.; Fujimoto, T.; Takebayashi, S.; Takagi, K.; *Adv. Synth. Catal.* **2006**, 348, 2475.

# Proof of Concept



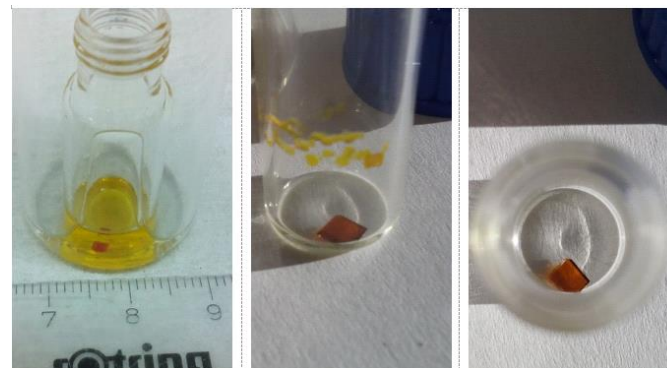
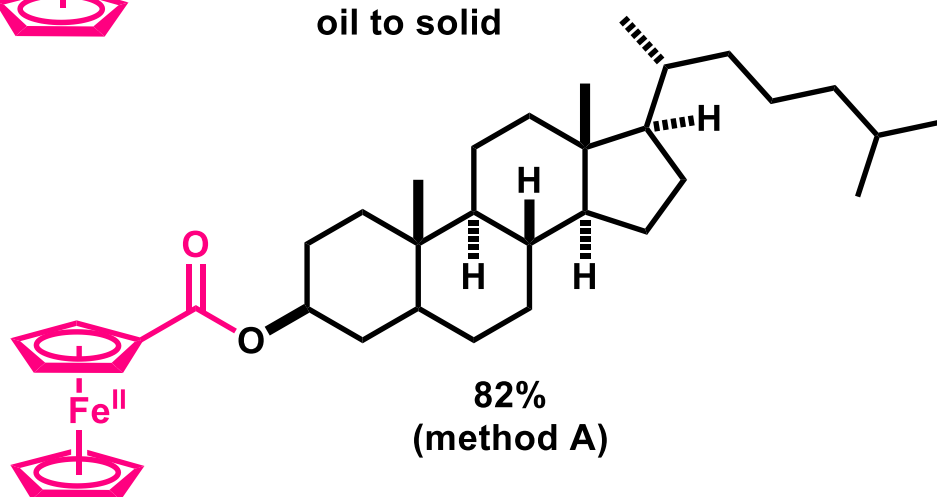
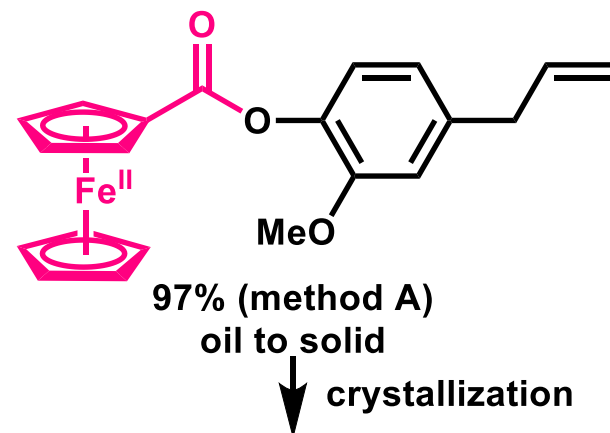
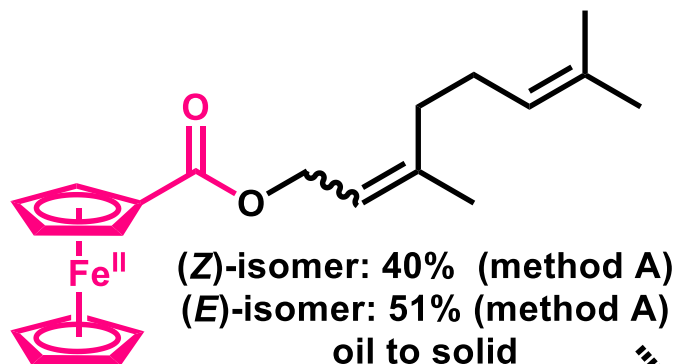
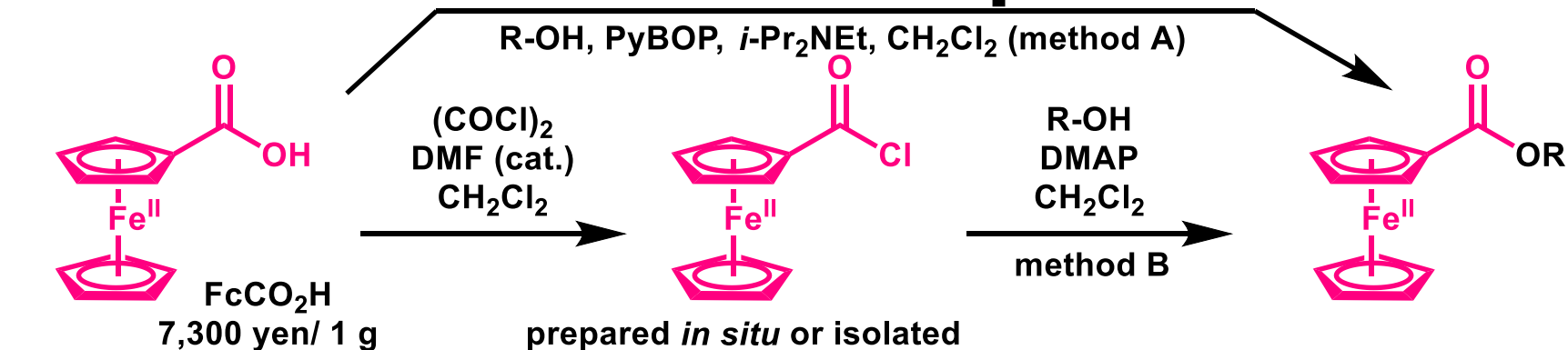
absolute configuration  
unambiguously determined



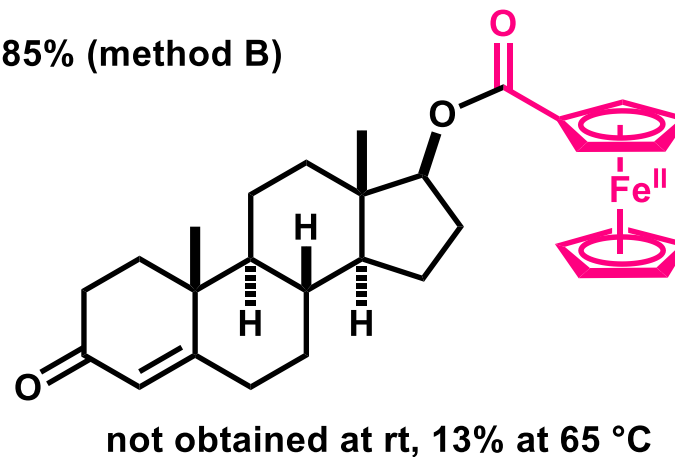
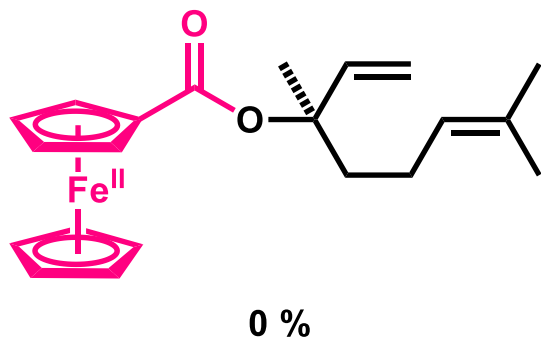
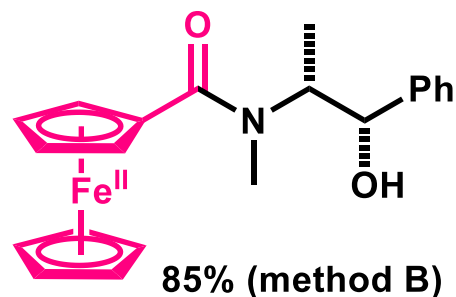
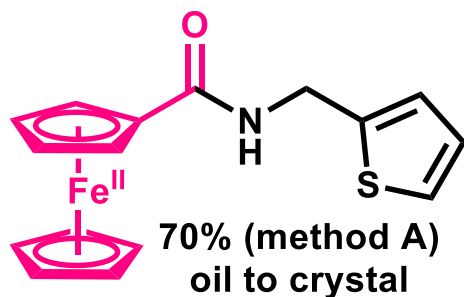
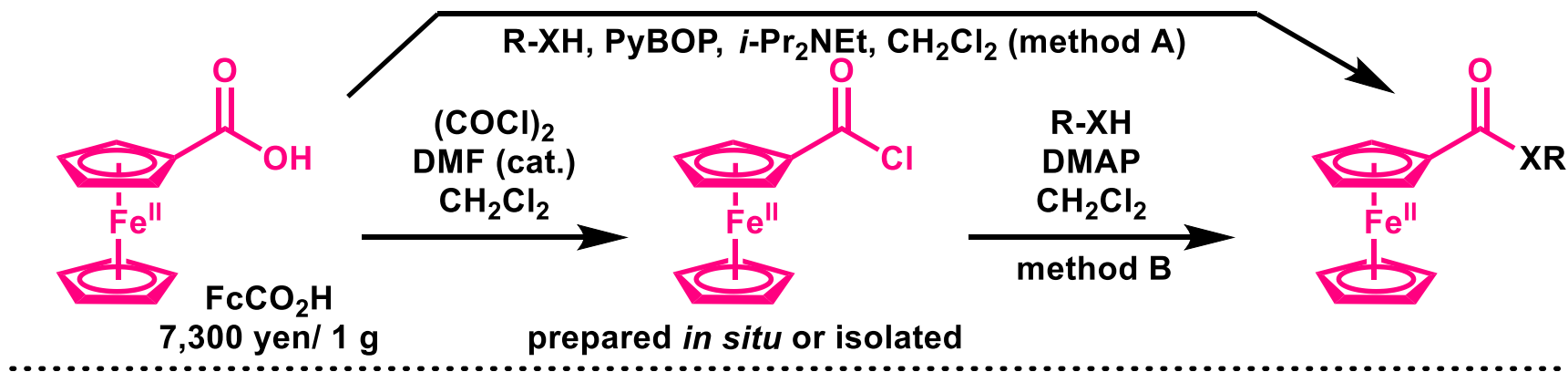
Characteristic of FcCO<sub>2</sub>H:

- Highly crystalline
- Contains heavy atom
- Stable
- Orange color
- Relatively inexpensive

# Substrate Scope



# Substrate Scope and Limitation

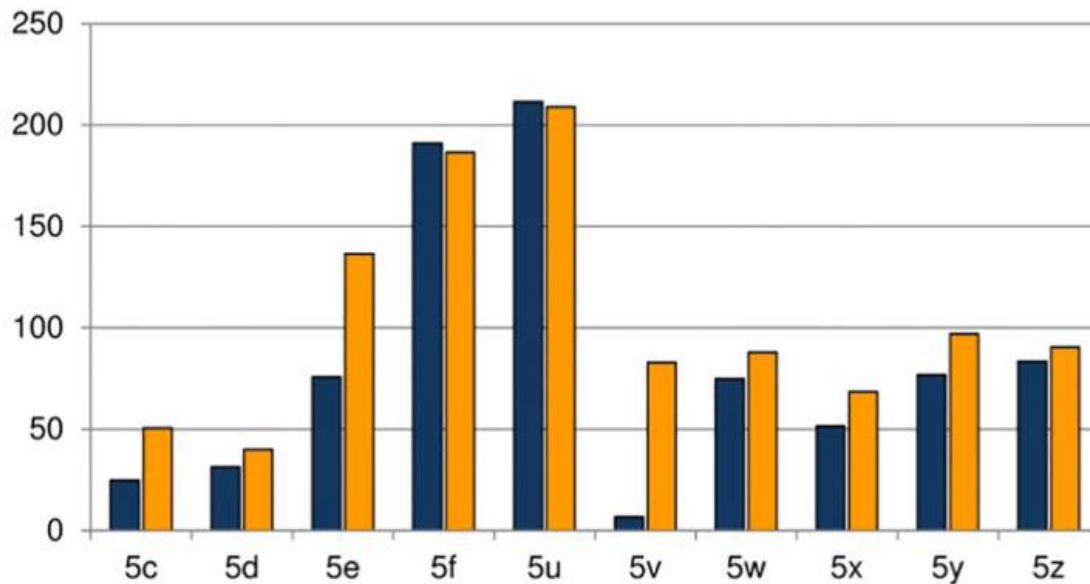


"it should be mentioned that a small number of ferrocene derivatives remained liquid"

1. Hoffman, K.; Riebelmann, B.; Wenzel, M. *Liebigs Ann. Chem.* **1980**, 1980, 1181.
2. Holstein, P. M.; Holstein, J. J.; Escudero-Adan, E. C.; Baudoin, O.; Echavarren, A. M. *Tetrahedron Asymmetry*, **2017**, 28, 1321.

# Comparison of the Melting Point

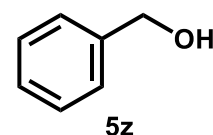
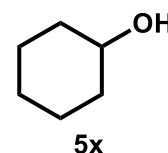
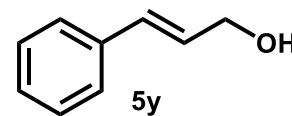
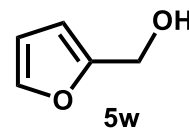
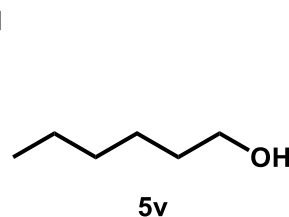
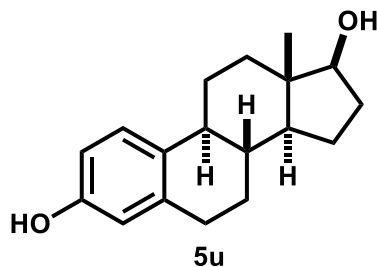
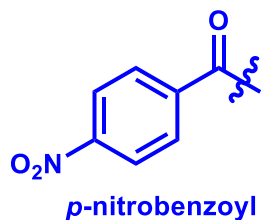
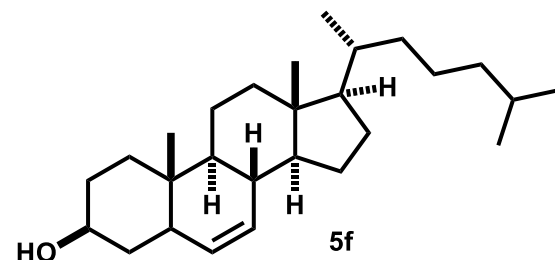
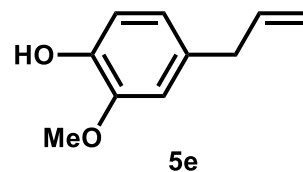
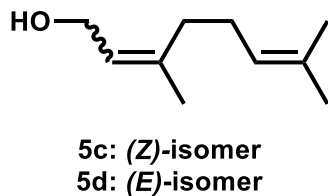
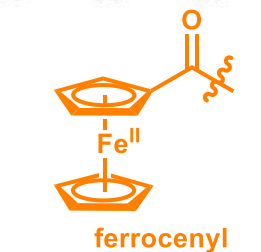
m.p. in °C



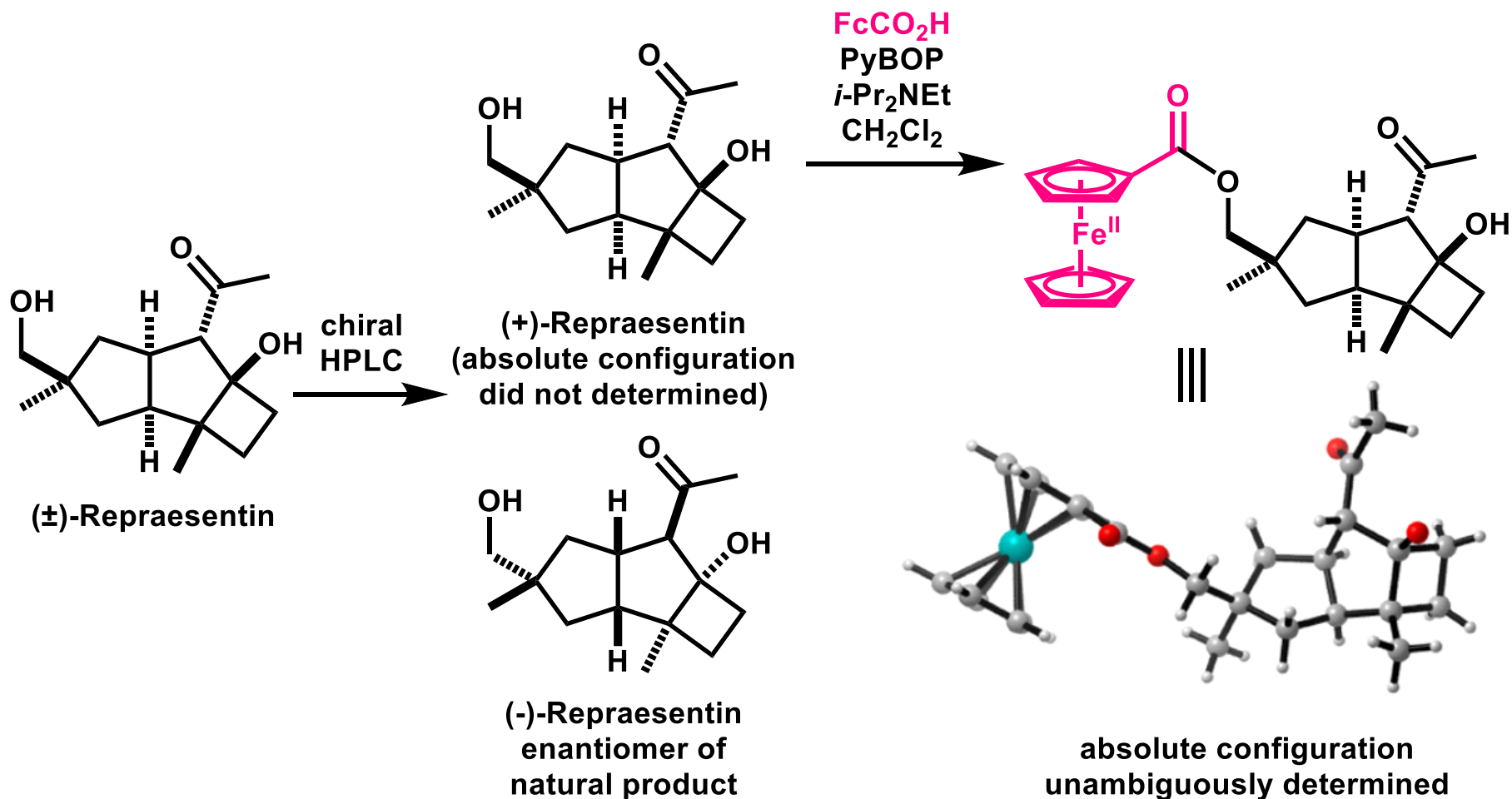
blue: *p*-nitrobenzoyl derivatives  
orange: ferrocenyl derivatives

similar melting point was observed.

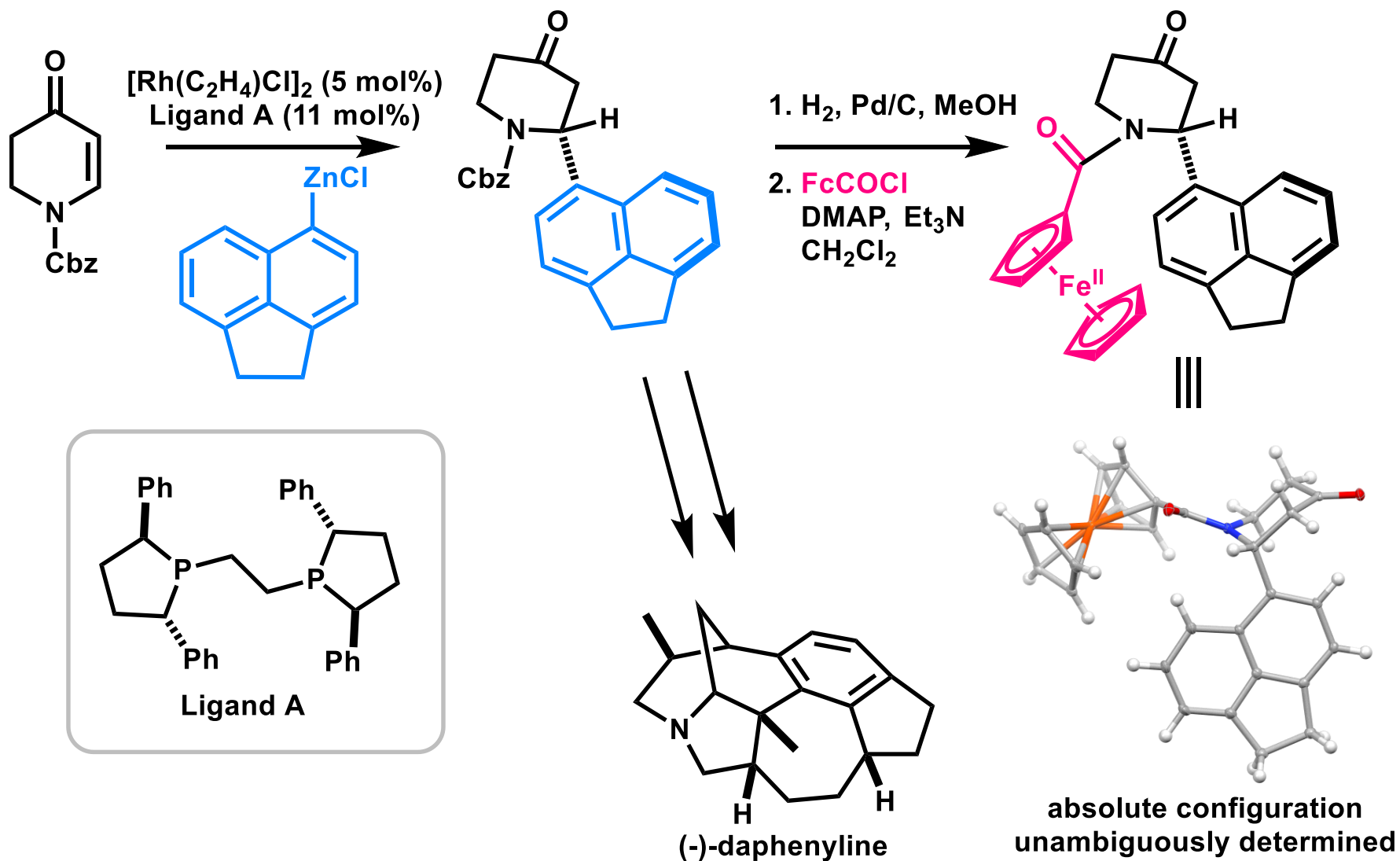
Indicating that ferrocenyl derivative is as good crystallinity as *p*-nitrobenzoyl derivative.



# Application in Total synthesis (1)



# Application in Total Synthesis (2)





# Summary

Method	NMR	X-ray crystallography	Micro ED	Crystalline sponge
Range	◎ (almost all)	× (crystal with good size, quality)	△ (crystal with good quality)	potentially ○ (size of molecule)
Certainty of the structure	○	◎	◎	Potentially ◎
Comment	~ mg order is required for data collection	Structure modification is sometimes effective	~μg scale would be possible	Further development will be expected