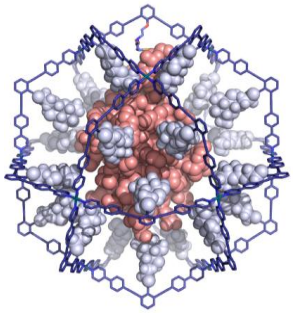
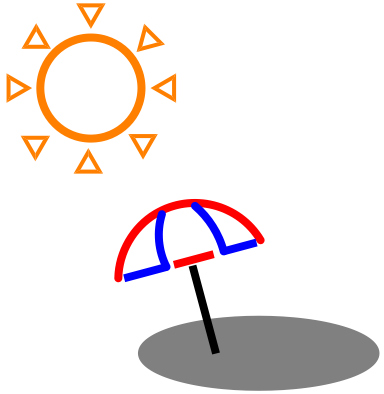
Molecular Capsule





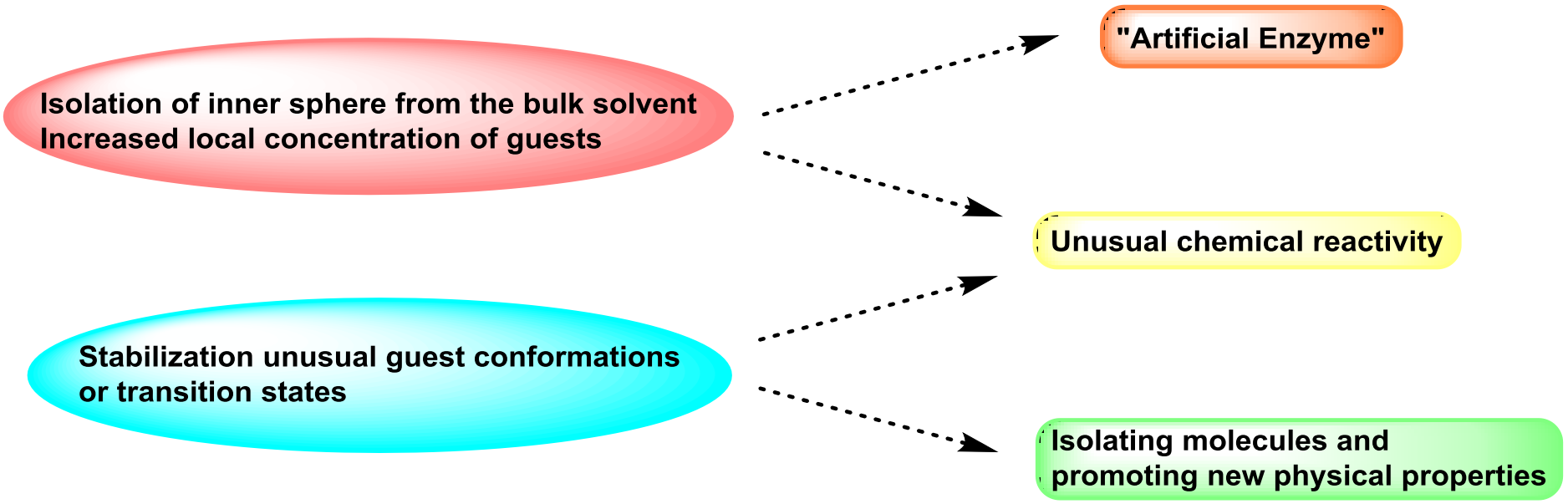
July 8, 2013
Haruka IDA

Contents

About Molecular Capsule

Molecular capsule

Artificial hosts controlling space to include guests.

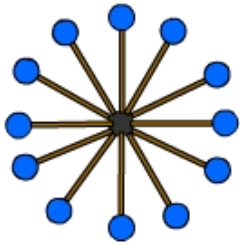


Categories of Molecular Capsule

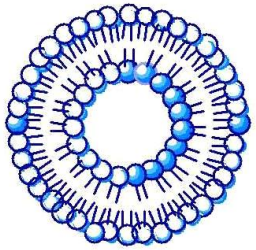
Self assembled system

(relatively simple building blocks)
<with Hydrophobic Interaction>

micelle

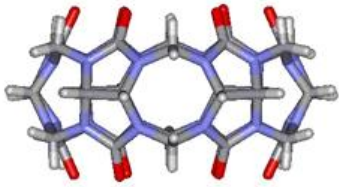


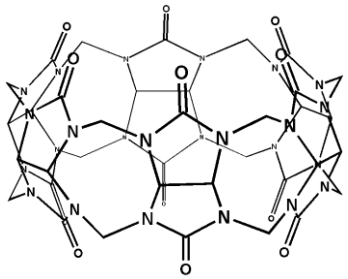
vesicle



Non-self assembled system

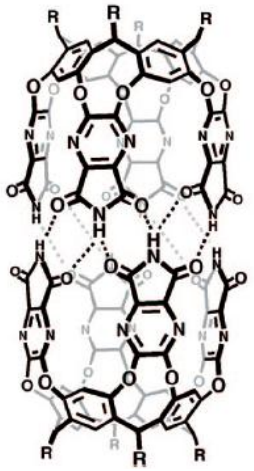
cucurbituril





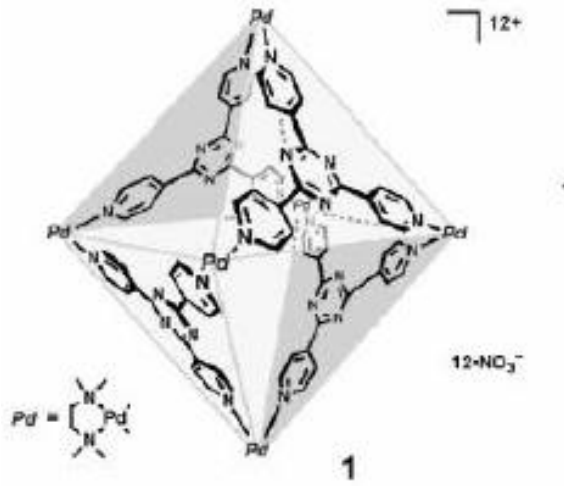
Self assembled system

<with Hydrogen Bond>

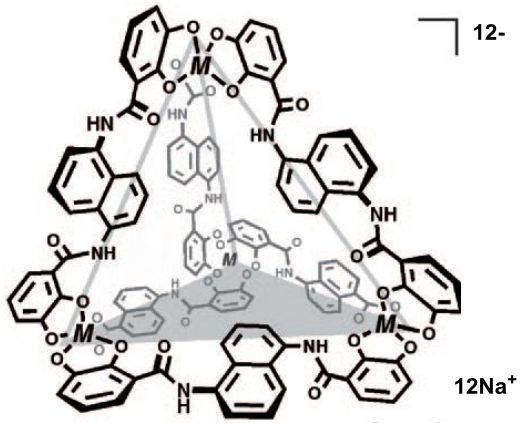


Cylindrical capsule

<with Cordination Bond>



Octahedral coodination cage

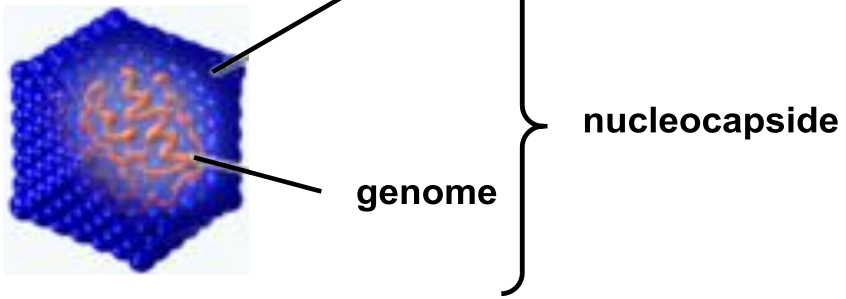


M= Ga^{3+}, Fe^{3+}, etc

Tetrahedral coodination capsule

Molecular Capsule in Biosystem

Inspired by virus...



Capside is the shell protecting and controling genome.

Consisting of capsomer that is small protein.

Functionalized interior.

Although seemingly complex,
the final capsid structures are limited by simple geometric constraints.

Researchers of Molecular Capsule

Prof. Kenneth N. Raymond

Department of Chemistry
University of California, Berkley

Supramolecular Coordination Chemistry
Luminescent Lanthanide Agents
MRI Contrast Agents

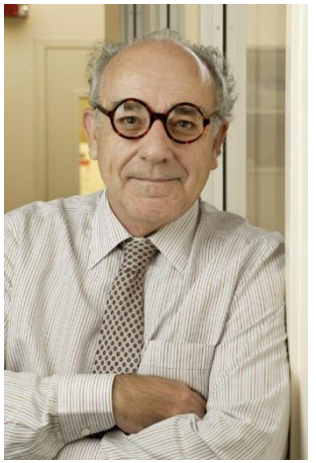
Prof. Makoto Fujita

School of Engineering
The University of Tokyo

Supramolecular
Coordination Chemistry

Prof. Julius Rebek , Jr.

Department of Chemistry
The Scripps Research Institute



Supramolecular
H-bonding Chemistry

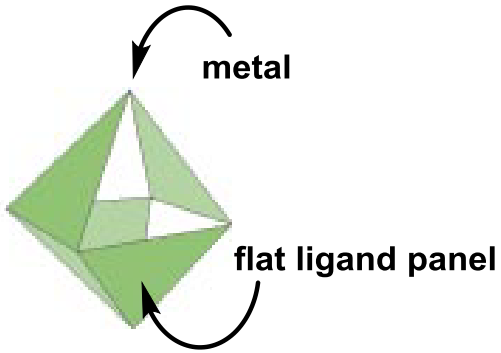
Capsule Using Coordination Bond

Coordination:

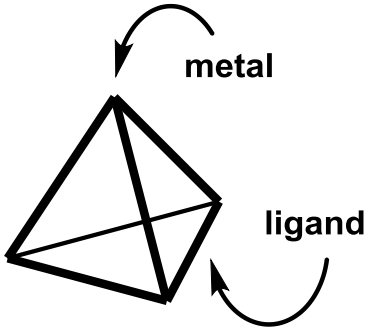
The formation of a covalent bond, the two shared electrons of which have come from only one of the two parts of the molecular entity linked by it.

Pure&Appl. Chem. **1994**, *66*, 1077.

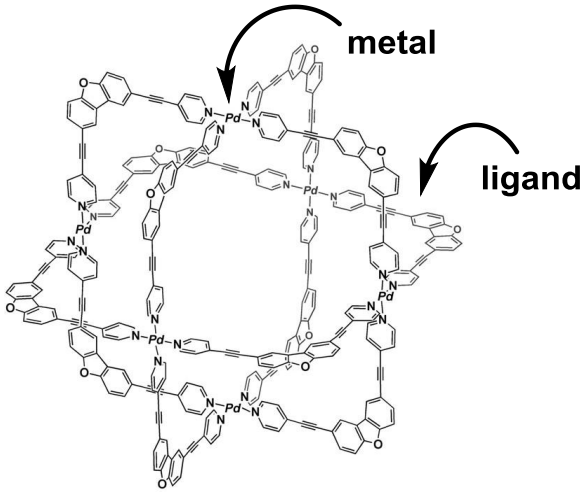
Molecular capsule using coordination bond



M_6L_4



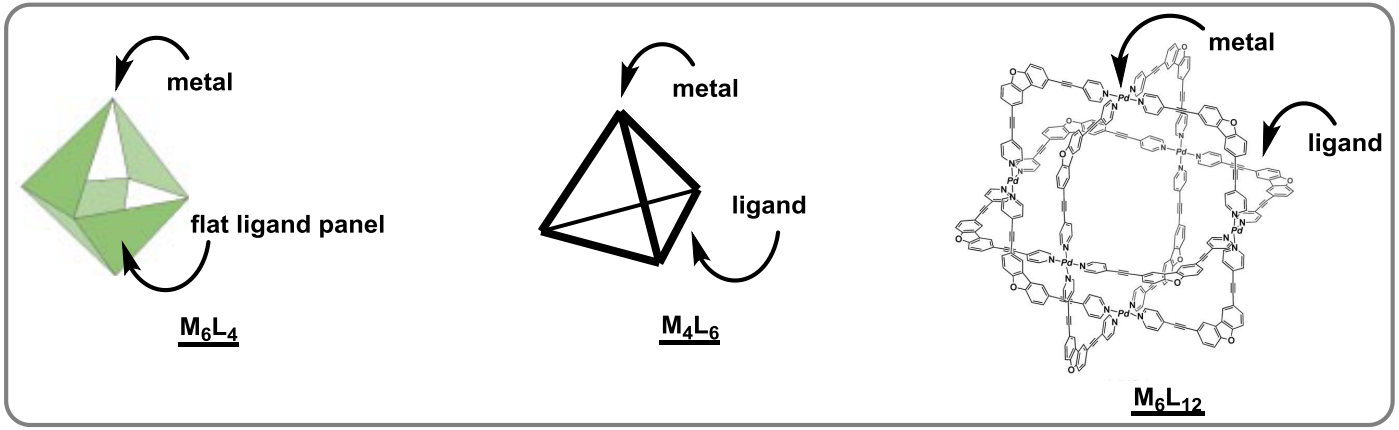
M_4L_6



M_6L_{12}

M: metal
L: ligand

Components of Molecular capsule

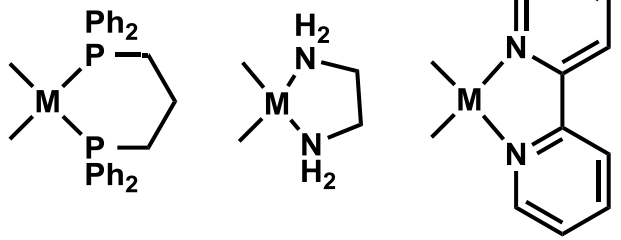


Metal

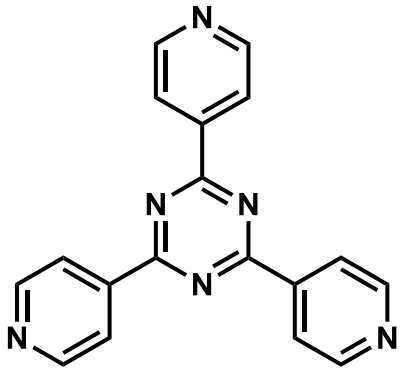
Pt(II), Pd(II): square planar metal ion

Pb(II), Ga(III), Al(III), Fe(III): octahedral metal ion

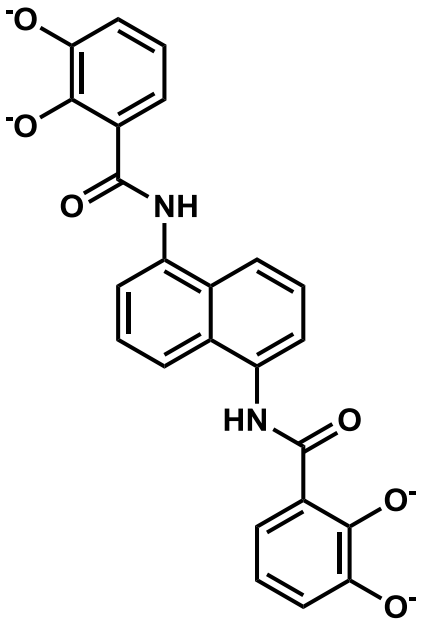
If needed...
***cis*-geometry two vacant orbitals on square planar metal ion is prepared by using bidentate ligand.**



Ligand



pyridine



phenoxide

Features of Molecular capsule using Coordination Bond

Water-soluble
Lipophilic inner sphere

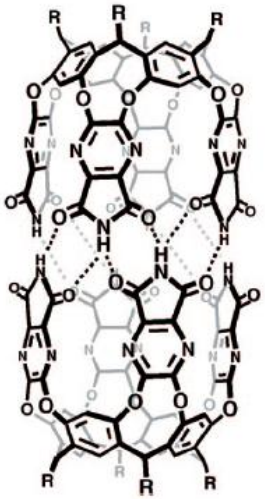
Selective self-assembly

Rubust structure compared with hydrogen-bond system
Stable to ligand-exchange

Modification of the structure is easier than micelle, vesicle and hydrogen-bond system.

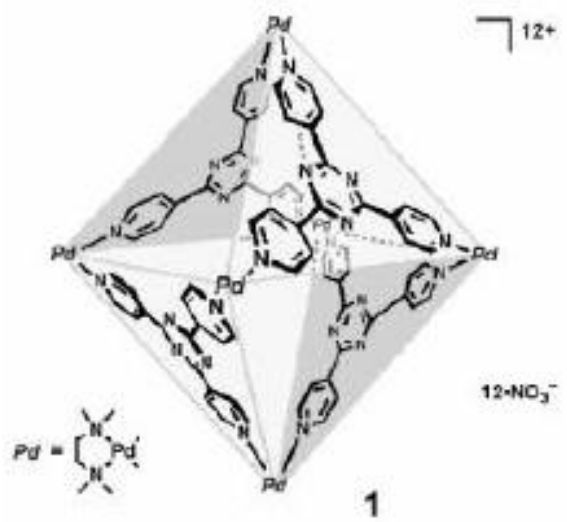
Self assembled system

<with Hydrogen Bonds>

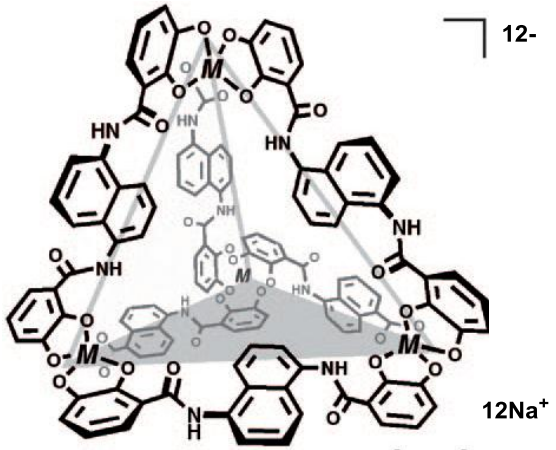


Cylindrical capsule

<with Cordination Bonds>



Octahedral coodination cage

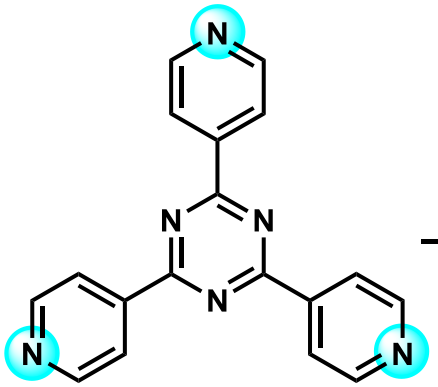


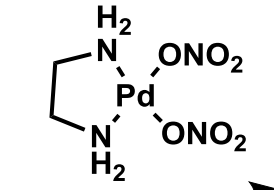
***M*= Ga^{3+}, Fe^{3+}, etc**

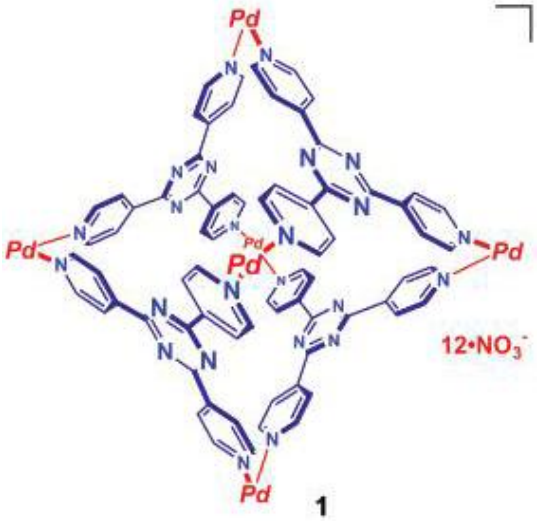
Tetrahedral coodination capsule

Ligand Structure - Form of Complex 1

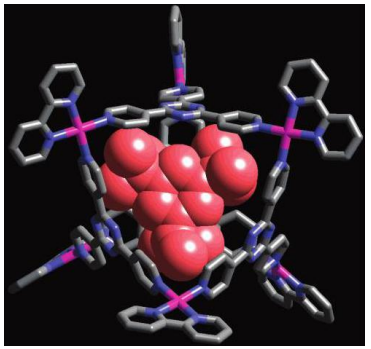
1-2. using Coordination Bond





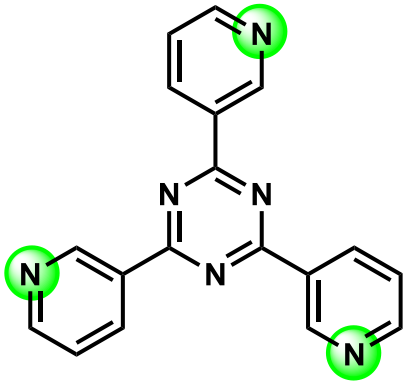


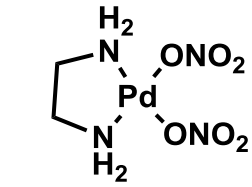
M_6L_4

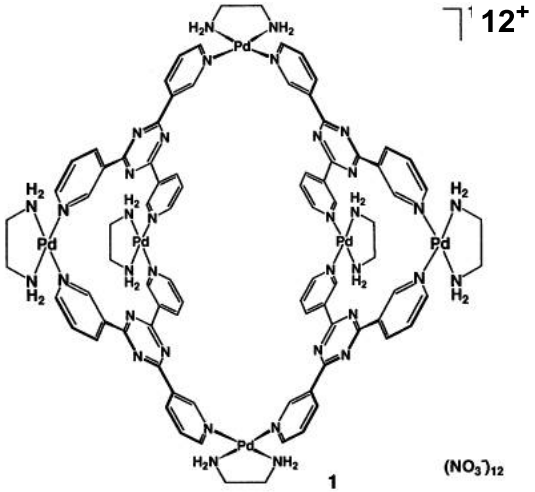


Octahedral cage

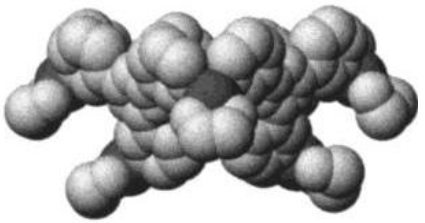
Regioisimer







M_6L_4

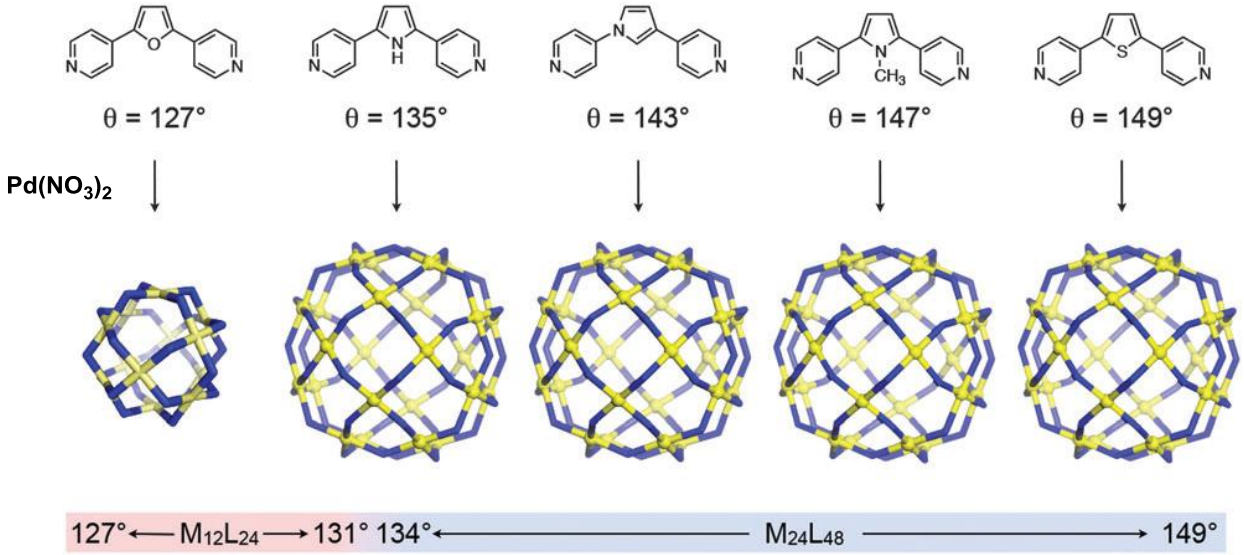


Bowl-shaped cage

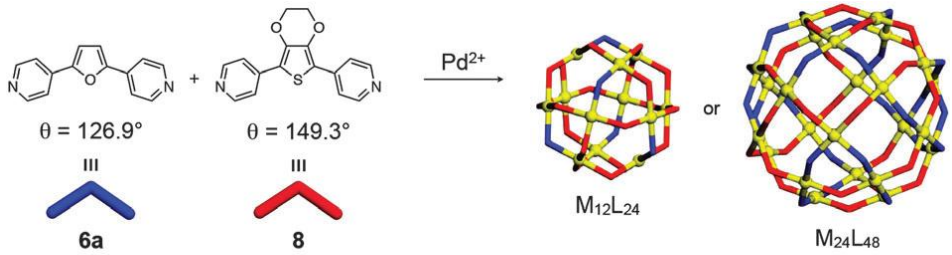
K. Ogura, *et al*, *Nature* **1995**, 378, 30.
T. Kusukawa and M. Fujita, *J. Am. Chem. Soc.* **2002**, *124*, 13576.
M. Fujita, *et al*, *J. Am. Chem. Soc.* **2000**, *122*, 2665.

Ligand Structure - Form of Complex 2

The ligand bent angle is an impotant factor to determine the form of complex.
Even a slight change in the angle critically switches the final structure.



Two kinds of ligand are mixed...



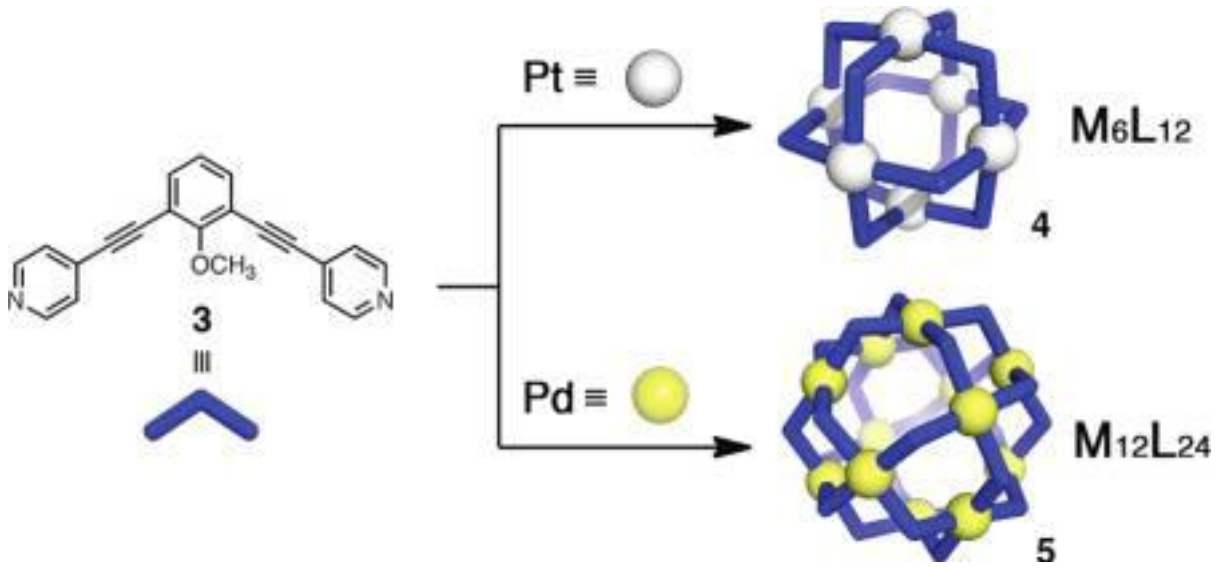
Mixing ratio (**6a**:**8**)	$\bar{\theta}$	Product
8 : 2	131.4	$M_{12}L_{24}$ only
7 : 3	133.6	$M_{24}L_{48}$ only

131° < threshold < 134°

Selective formation of the complex are observed.
The product is not the mixture of $M_{12}L_{24}$ and $M_{24}L_{48}$.

M. Fujita, *et al*, *Angew. Chem. Int. Ed.* **2012**, *51*, 3161.
M. Fujita, *et al*, *Science* **2010**, *328*, 1144.

Metal Effect to Form of Cage



The M_6L_{12} cube structure: a metastable local-minimum structure for the self-assembly process.

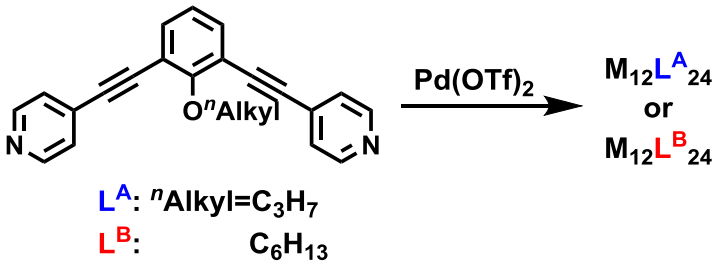
Pt(II)-pyridine interaction >> Pd(II)-pyridine interaction.

$Pt(II)_6L_{12}$ cube ⟶ ✕ $Pt(II)_{12}L_{24}$ structure

$Pd(II)_6L_{12}$ cube ⟶ $Pd(II)_{12}L_{24}$ structure

M. Fujita, *et al*, *J. Am. Chem. Soc.* **2011**, *133*, 13317.

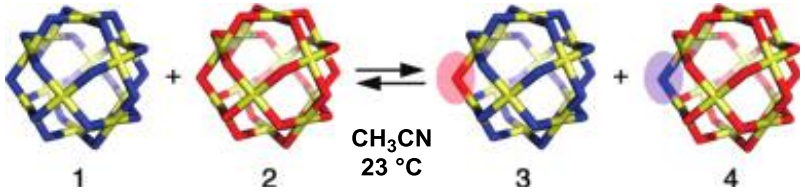
Stability of Cage



L^A: ^{n}Alkyl=C$_3$H$_7$

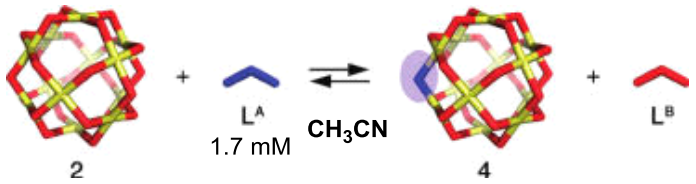
L^B: C$_6$H$_{13}$

1/2= 1/1 mixture



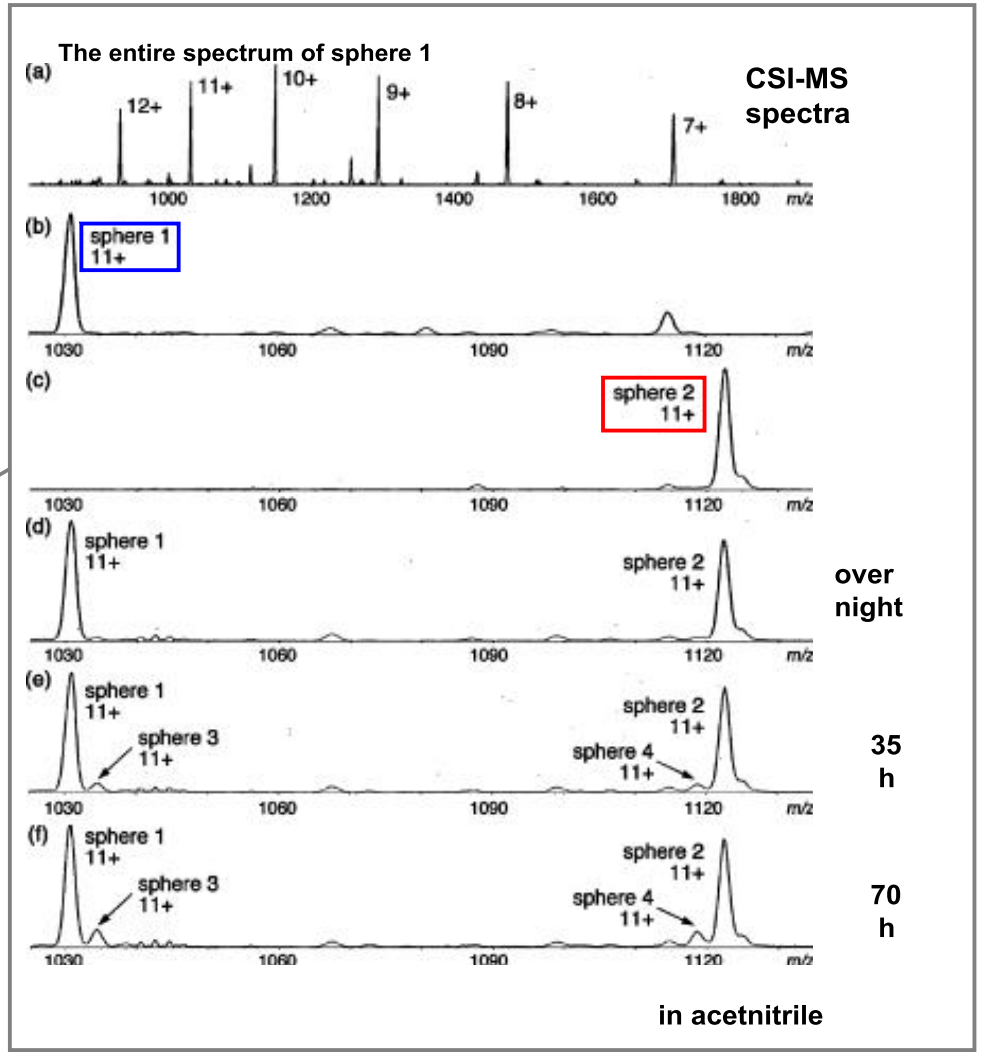
$t_{1/2}$= 20 days

36-component $M_{12}L_{24}$ self-assembly gains remarkable stability. >Supported by cooperation of 48 weak Pd(II)-pyridine interactions.



$t_{1/2}$= 23 min

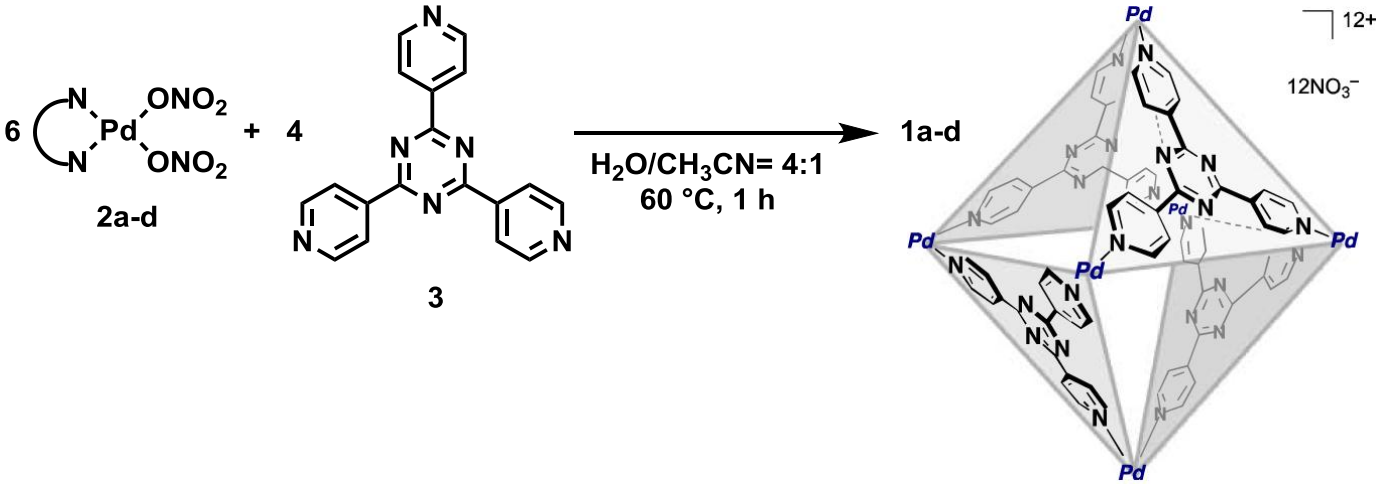
On the way to the completion of complexation (:there are many free ligands), the system equiblates quickly.

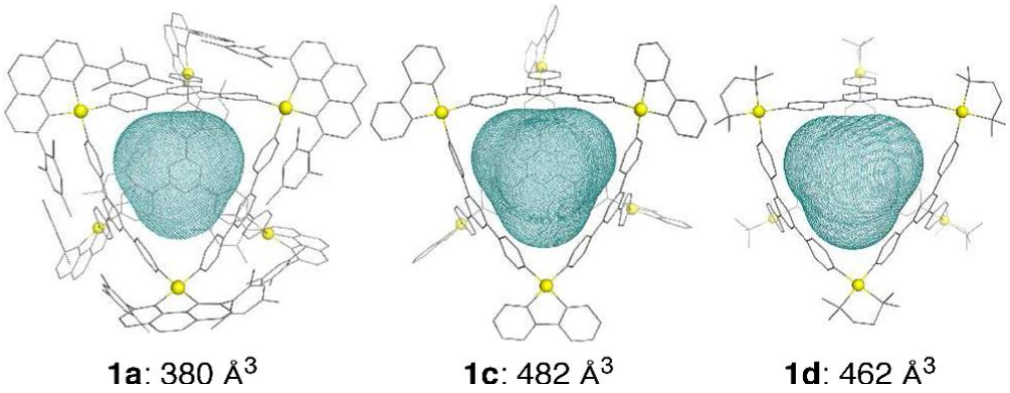


M. Fujita, *et al*, *J. Am. Chem. Soc.* **2009**, *131*, 6064.

Effect of the Remote Bulky Ancillary Groups

Formation of $M_{24}L_{48}$





The central void volumes (green mesh) in cages 1a, 1c, and 1d.
Calculated using *VOIDOO* program

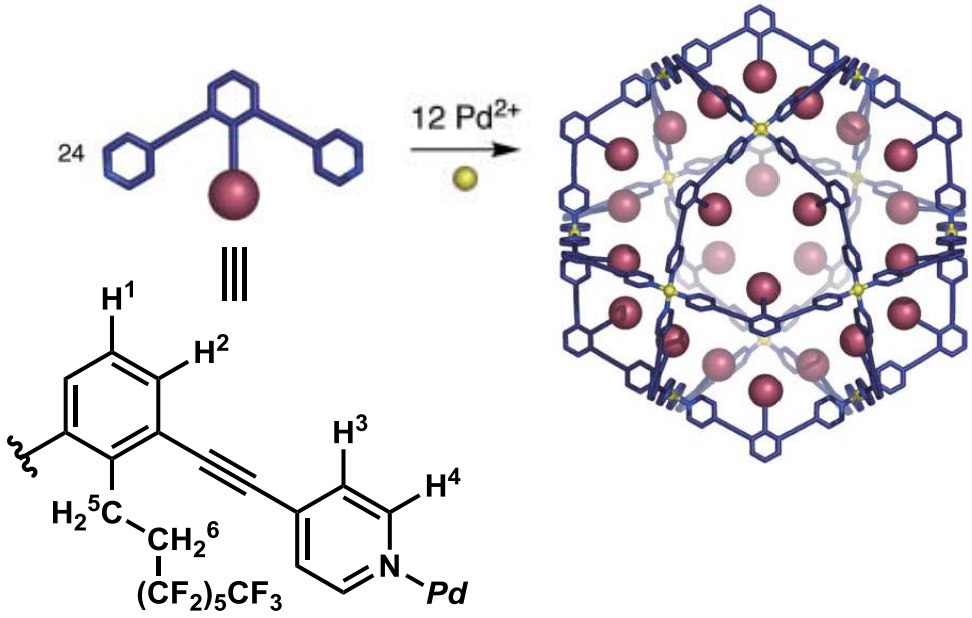
The bulky mesityl groups hang over the cavity, reducing the effective volume.
→ Controlling the guest binding and motion.

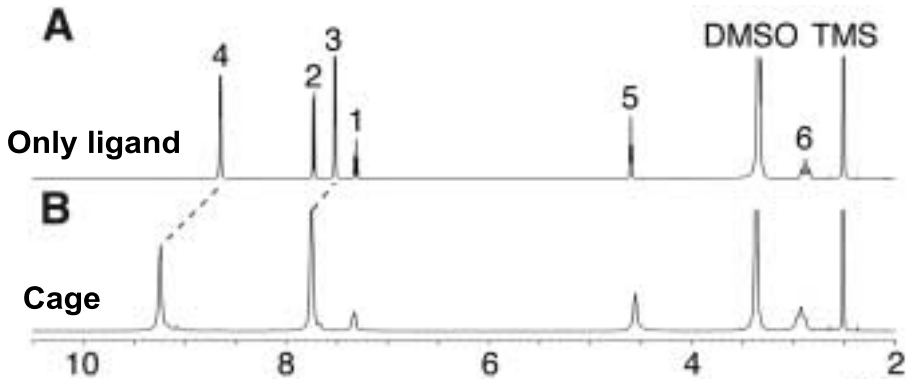
M.Fujita, *et al*, *J. Am. Chem. Soc.* **2013**, *135*, 613.

Methods to Check the Structure

^{1}H-NMR

Ligand composing the cage: downfield shift



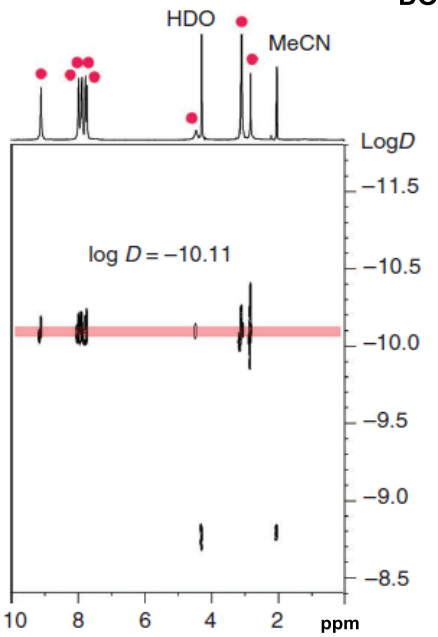


M. Fujita, *et al*, *Sciense* **2006**, *313*, 1273.
M. Fujita, *et al*,*Nature Commun.* **2012**, *3*, 1093.

DOSY: diffusion-ordered spectoscopy

Determine the value of self diffusion coefficient (*D* value)

Harada-san's lit.seminar 2006 (D2)
"DOSY-*NMR spectrometry-*"



*D value $D = \dfrac{k_B T}{6\pi\gamma\eta}$

(k_B: Boltzmann coefficient, T: absolute temp., γ: radius, η: medium viscosity)

X-ray crystalography

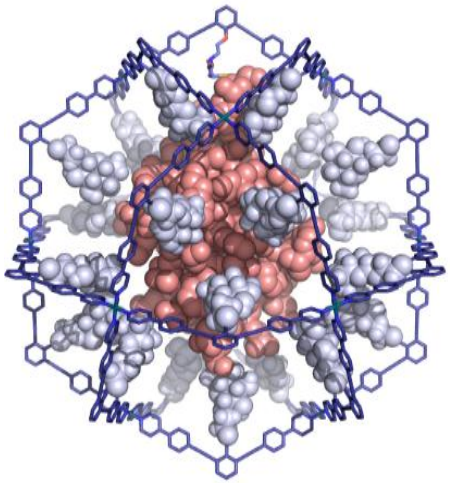
Mass spectrometry

NOESY:
Nuclear Overhauser enhancement and Exchange SpectroscopY

ROESY:
Rotating Overhauser enhancement and Exchange SpectroscopY

Encapsulation of Protein

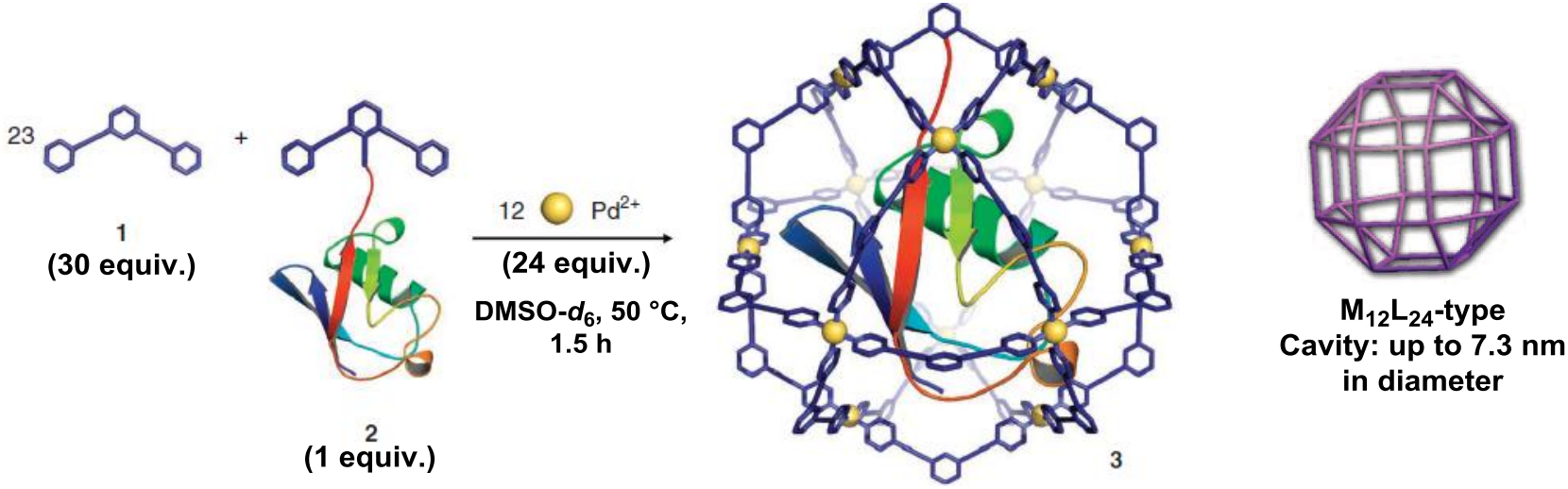
Protein encapsulation will enable...
- **Enclathration of biomolecules**
- **Simple cystalization of proteins**
- **Control of the structure and function of proteins**

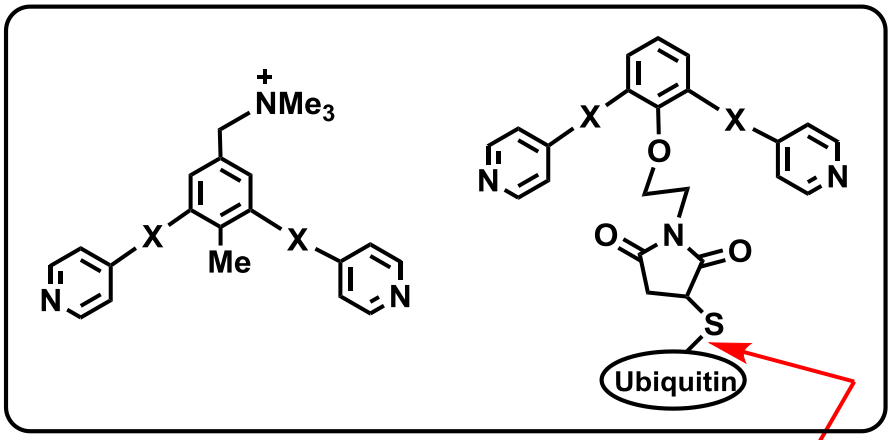


Protein encapsulation is difficult because...
- **Protein is large molecule**
- **Protein has sensitive nature**

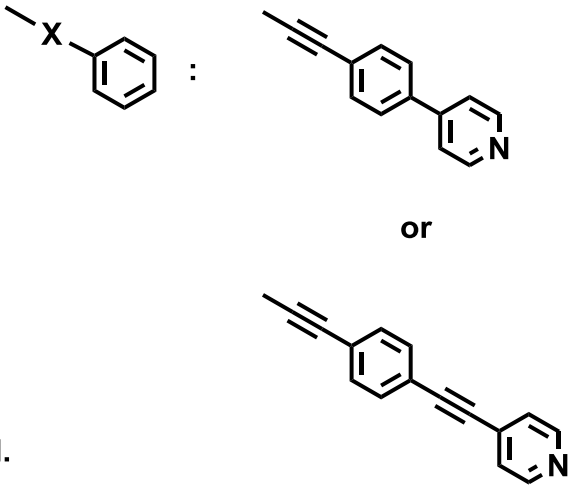
Method for protein encapsulation already reported (e.g. mosoporous silica, nanogel, reverse micelle...) includes problems about
- **Analysis**
- **Optimization of the structure of the capsule**

Encapsulation of Protein with Synthetic Host





Covalently attached.

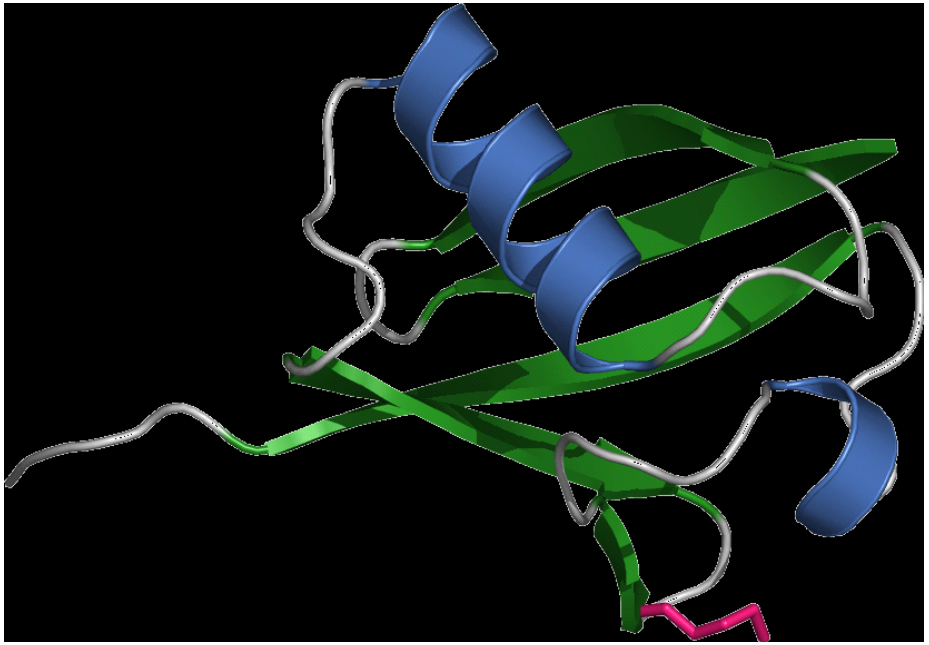


Pd^{2+} source: $[Pd(MeCN)_4](BF_4)_2$

M. Fujita, *et al*, Nature Communications **2012**, *3*, 1093.

Ubiquitin

Ubiquitin is present in all eukaryotic cells and investigated well.



Structure

- **76 amino acid residues, 8.6 kDa**
- **Small globular protein.**
- **Approximately 3.4 nm in diameter.**

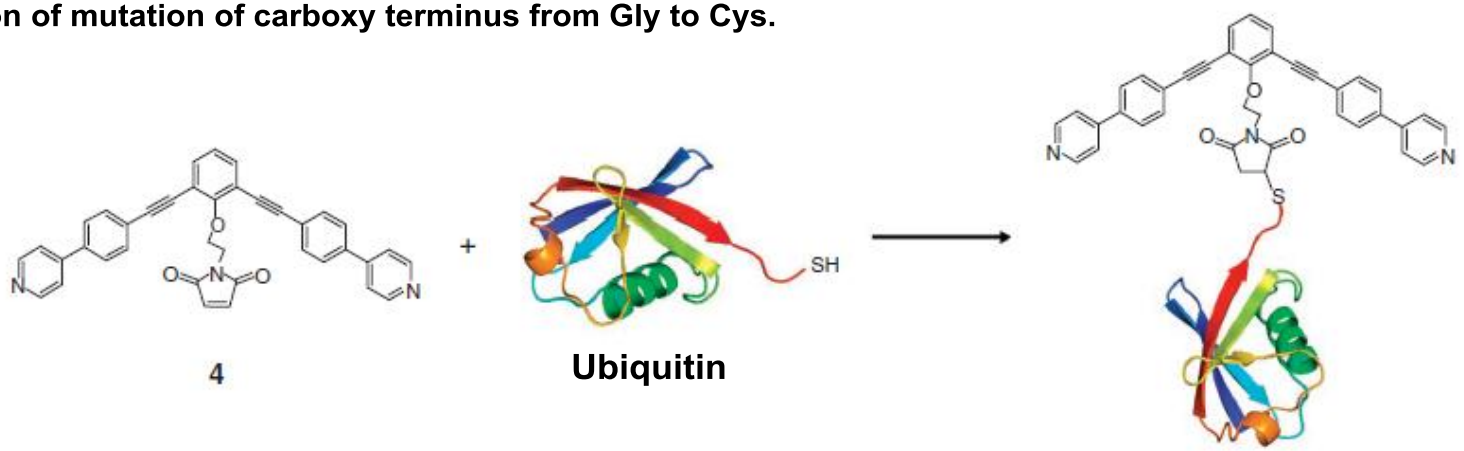
Functions

- **Degradation proteins**
- **DNA repair**
- **Control of translation**
- **Signal transduction**

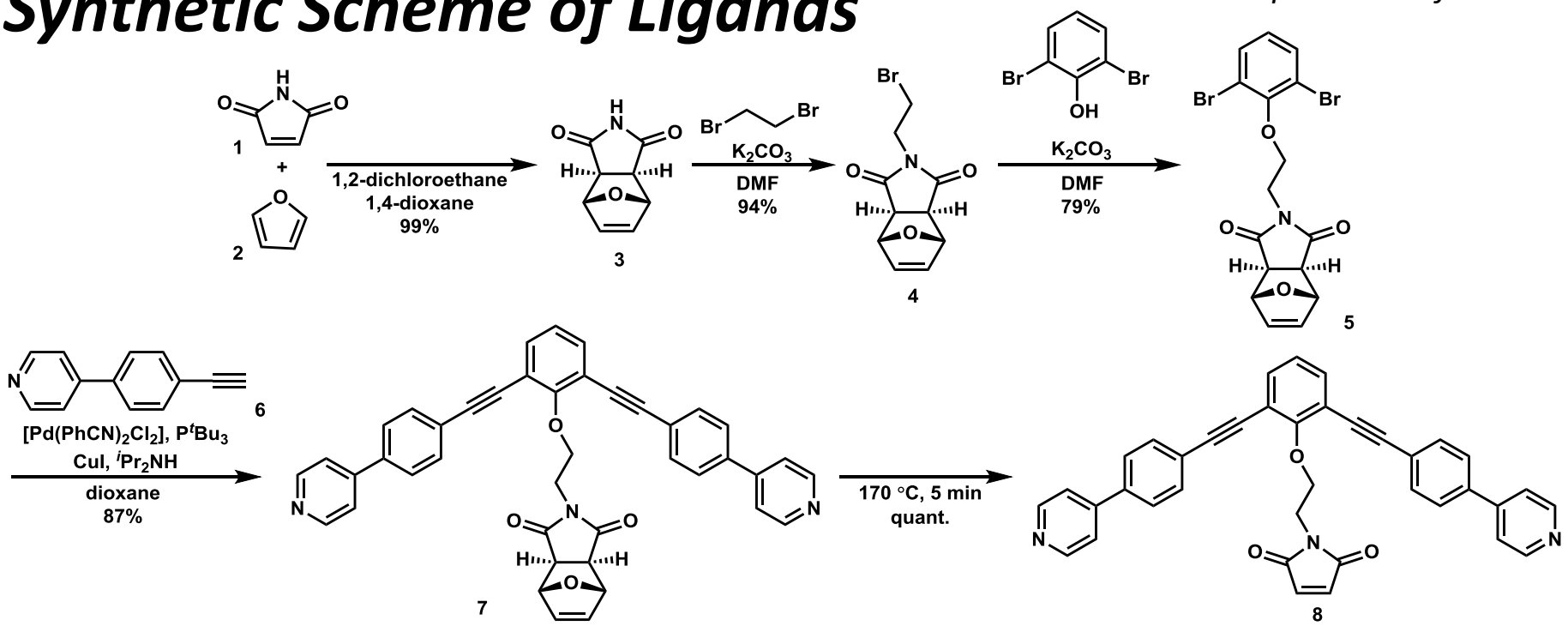
It is known that Gly76 residue at the carboxy terminus does not affect the protein structure.

Avram Hershko and Aaron Ciechanover, *Annu. Rev. Biochem.* **1998,** *67*, 425.
M. Fuiita. *et al*. *Nature Communications* **2012**. *3*. 1093.

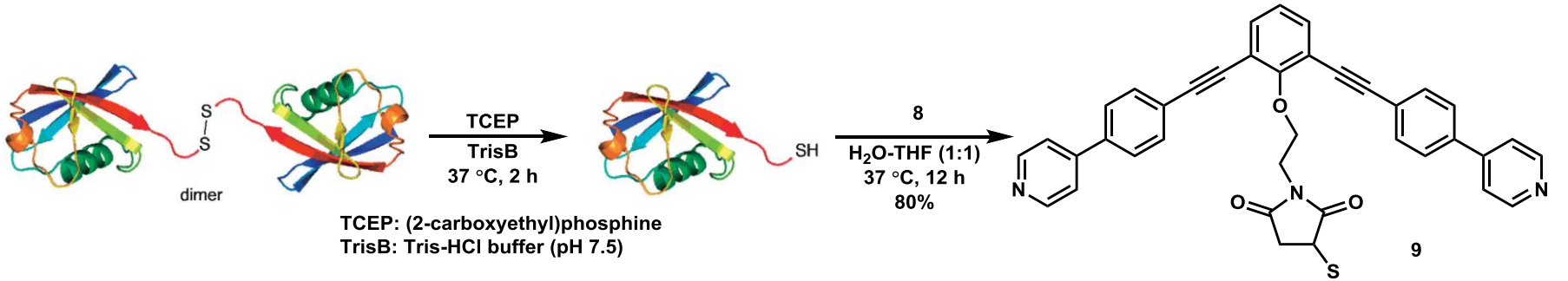
⇨ **Induction of mutation of carboxy terminus from Gly to Cys.**



Synthetic Scheme of Ligands



Scheme 1. Two synthetic routes to maleimide-coupled ligand 8.

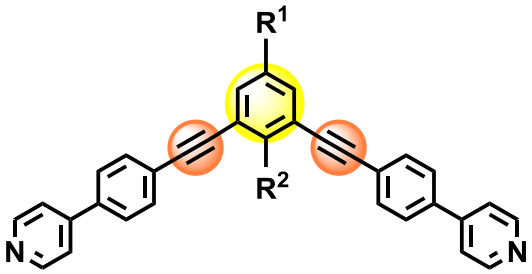


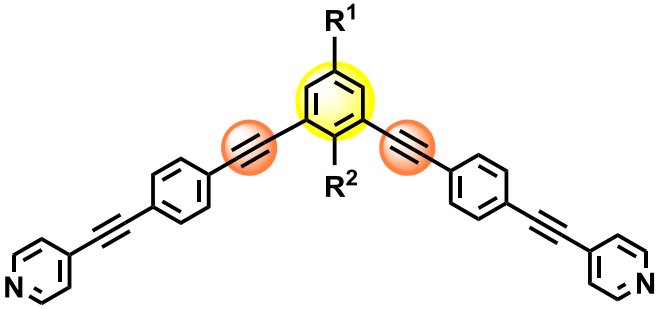
Scheme 2. Introduction of ubiquitin to the synthetic ligand through thiolmaleimide coupling.

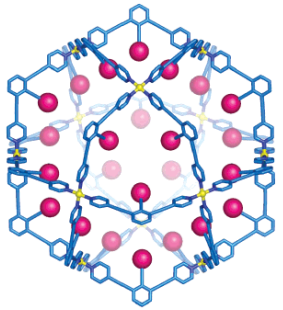
Characterization of 9 was carried out by ESI-MS, ^{1}H-NMR spectrometry and SDS-PAGE.

M. Fujita, *et al*, *Chem. Lett.* **2012**, *41*, 313.

Ligand Design



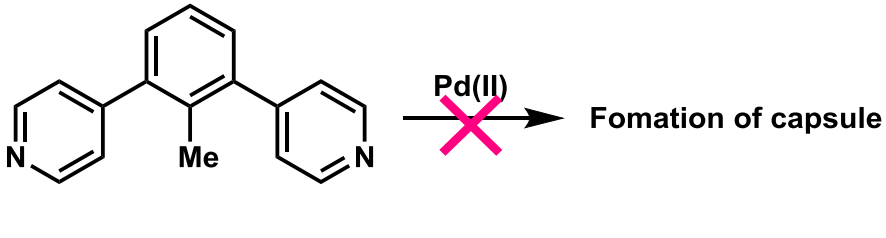




$M_{12}L_{24}$

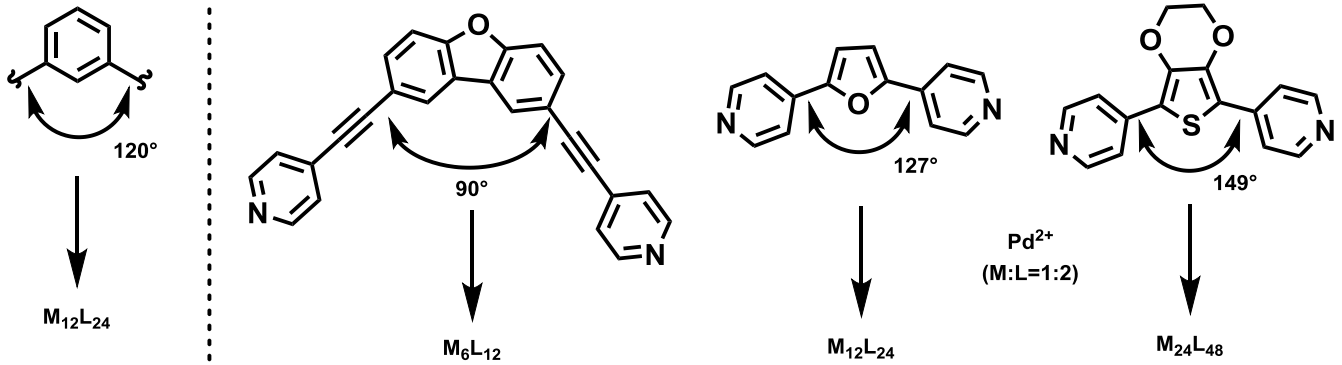
The important role of acetylene spacer

- **Prevention of the ligand from taking unfavorable nonplanar conformation.**



The meaning of selecting benzene ring at the ligand center.

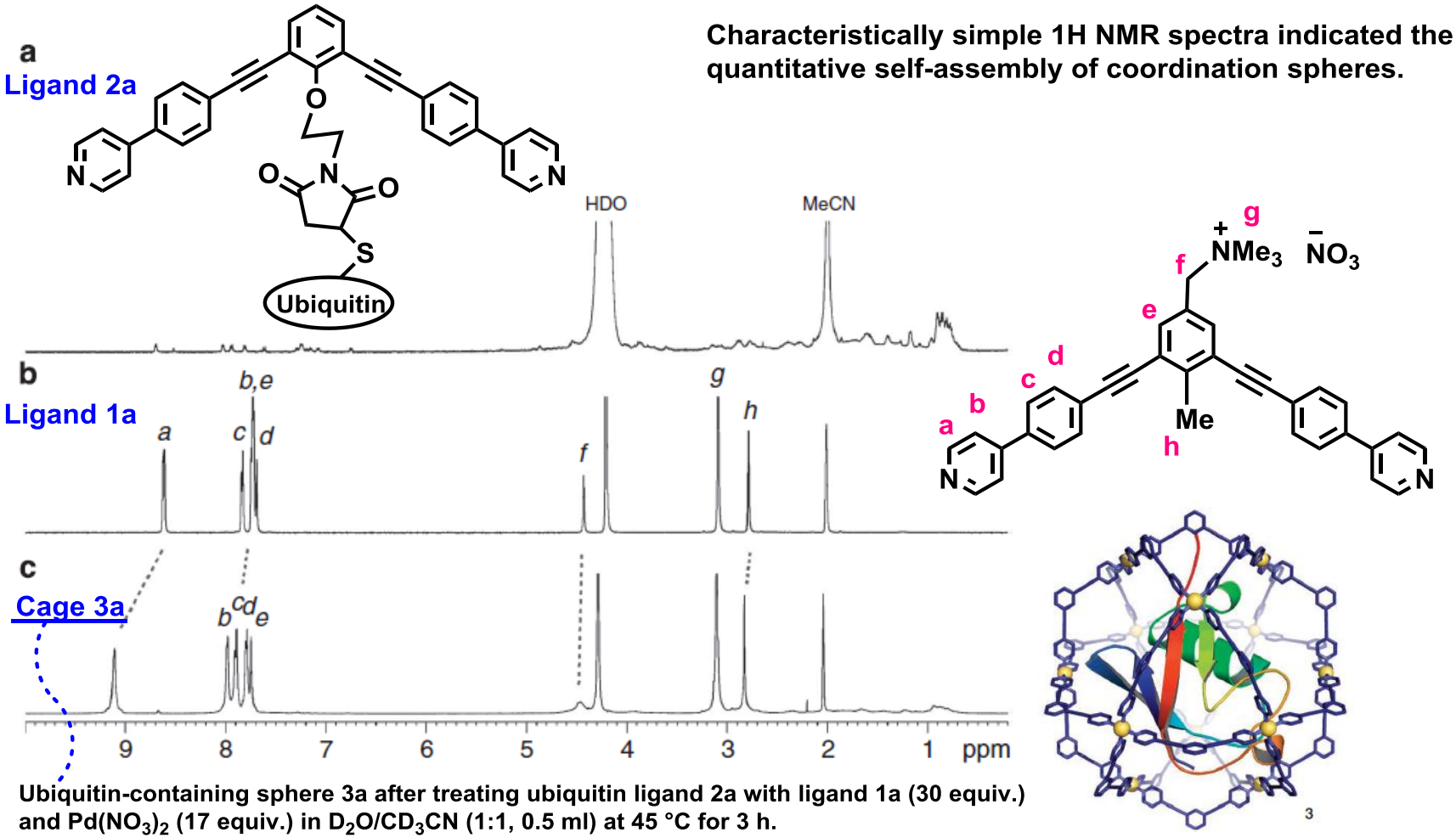
- **Fomation of the desired cage.**



M. Fujita, *et al*, *J. Am. Chem. Soc.* **2005**, *127*, 11950.
M. Fujita, *et al*, *Science* **2010**, *328*, 1144.

Checking Formation of Cage

♦ ^{1}H NMR spectra of the encapsulation of ubiquitin within coordination spheres.



Characteristically simple 1H NMR spectra indicated the quantitative self-assembly of coordination spheres.

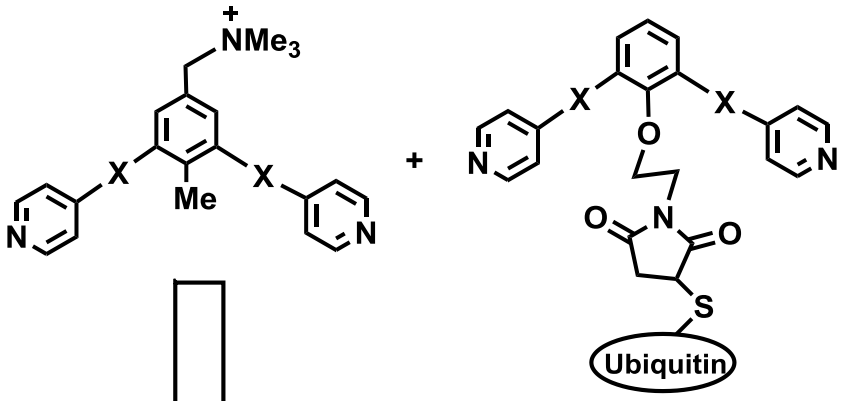
Ubiquitin-containing sphere 3a after treating ubiquitin ligand 2a with ligand 1a (30 equiv.) and $Pd(NO_3)_2$ (17 equiv.) in D_2O/CD_3CN (1:1, 0.5 ml) at 45 °C for 3 h.

♦ **diffusion-ordered NMR spectroscopy (DOSY)**

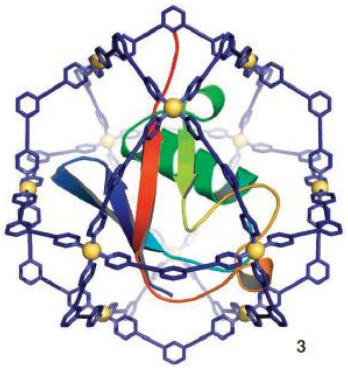
→Determination of the diffusion coefficient D→the presence of the giant coordination sphere

M. Fujita, *et al*, *Nature Communications* **2012**, *3*, 1093.

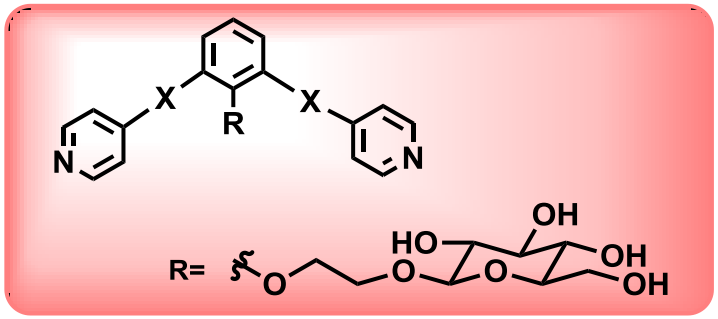
Introduction of Sugar-Functionalized Ligand



12 Pd^{2+}



Crystalyzation was unsuccessful
>Because of the high mobility of the dangling ubiquitin molecule in the cage.
>Fill the void in the cage.

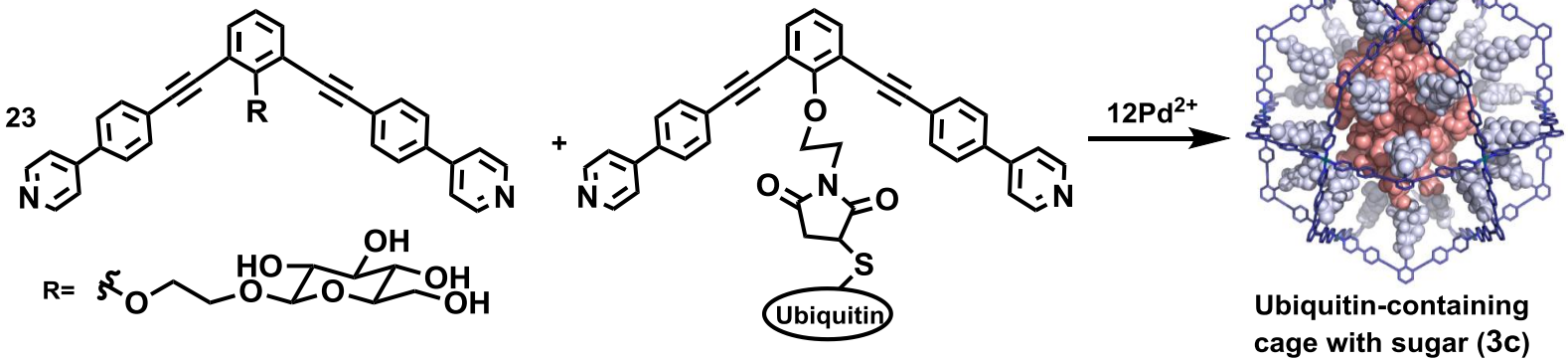


Encapsulation with ubiquitin-ligated Ligand

Ubiquitin-containing cage was formed.
(Confirmed by ^{1}H-NMR and DOSY)
X-ray quality single crystals were obtained.

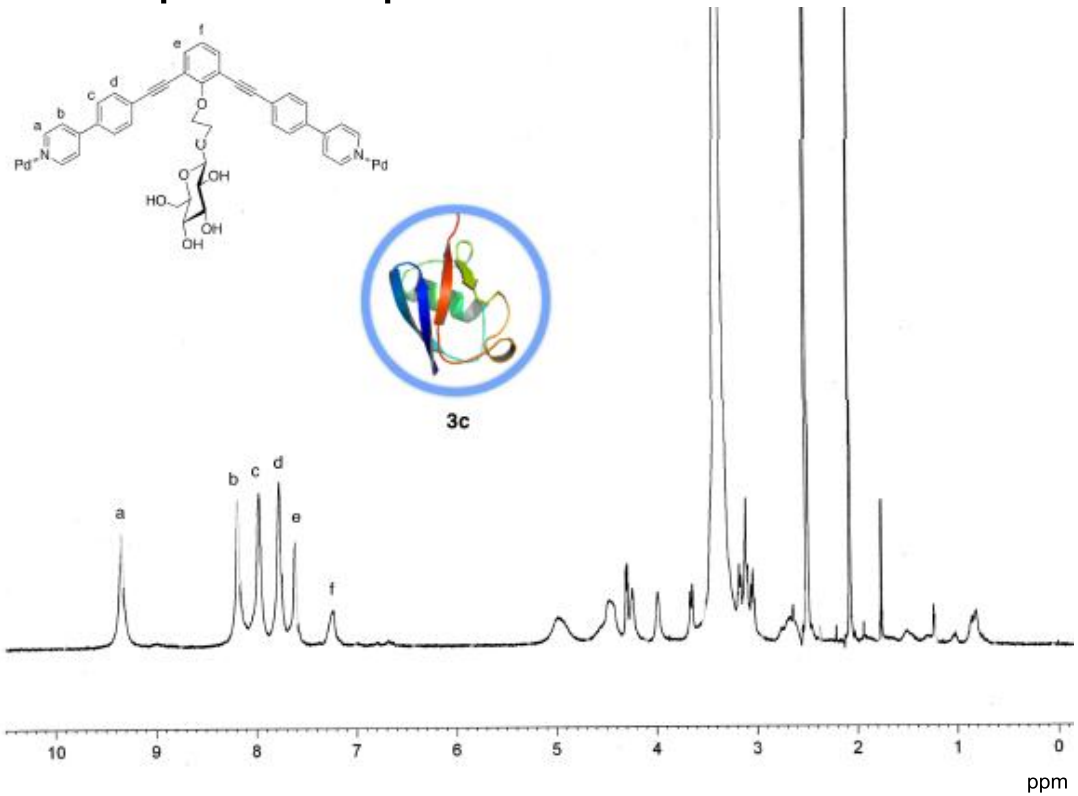
M. Fujita, *et al*, *Nature Communications* **2012**, *3*, 1093.

Checking Inclusion of Ubiquitin in Cage

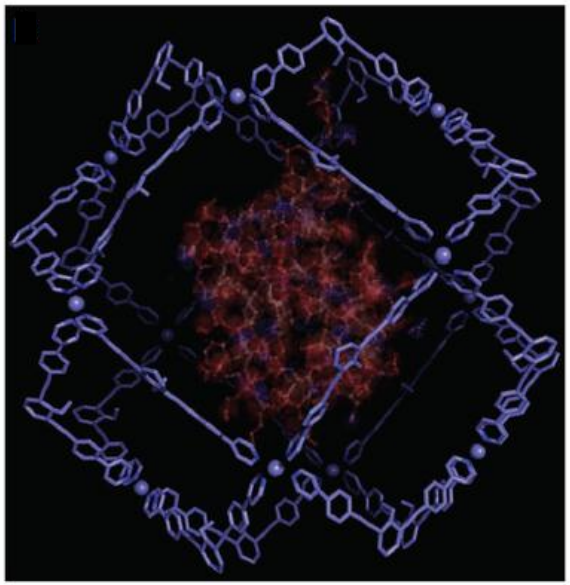


Ubiquitin-containing cage with sugar (3c)

^{1}H NMR spectrum of sphere 3c



MEM refinement of the structure of the ubiquitin-containing cage with sugar.



M. Fujita, *et al*, *Nature Communications* **2012**, *3*, 1093.

Encapsulation and Crystalization of Protein

Did molecular capsule make crystalization of protein easy??

There remain difficulties for this cage system to become useful method for crystalization of protein.

Screening of the condition for crystalization (e.g. method, pH of buffer, temperature, aditive)

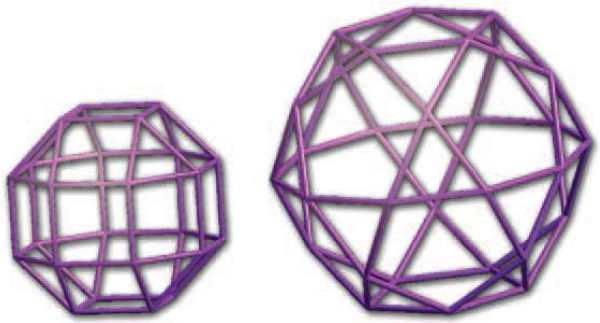
Preparation of the adequate amount of sample

The cage system is expected to organize the condition for crystalization of protein.

Necessities for General Method of Protein Encapsulation

Larger size of cupsule→Other structure of ligand

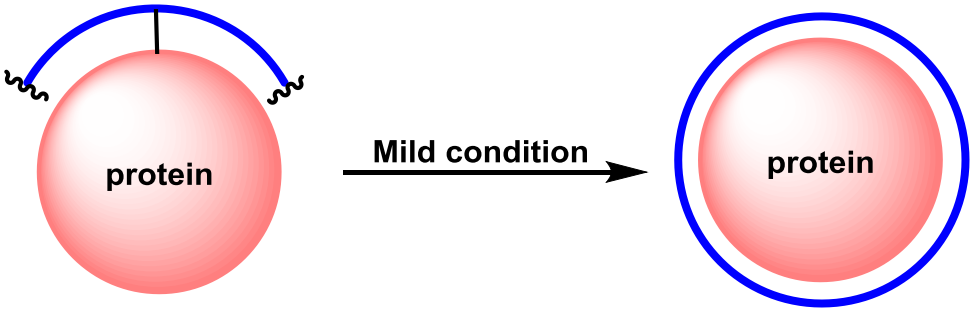
Most protein is larger than ubiquitin.



$M_{24}L_{48}$ $M_{30}L_{60}$

Bond cleavage between cage and protein

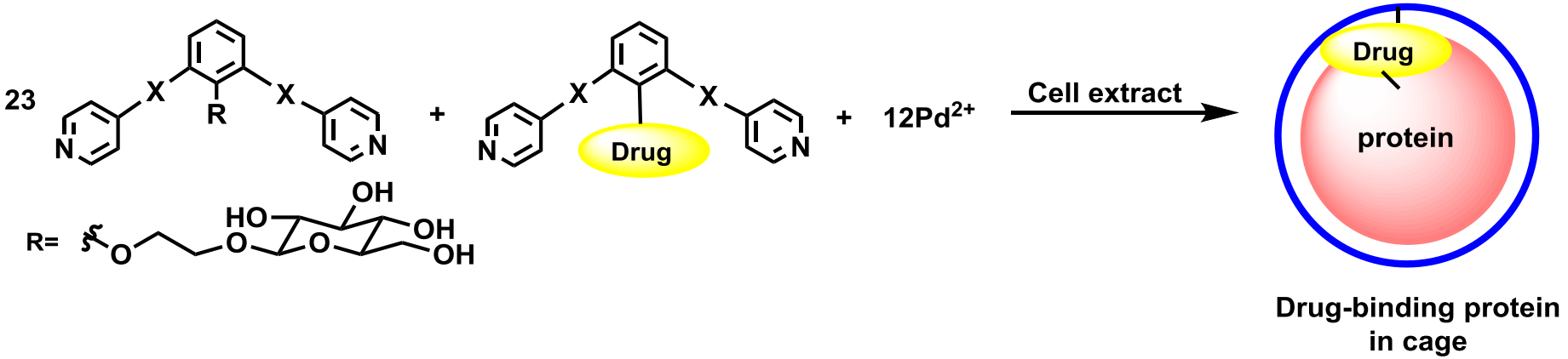
To guarantee the native structure.

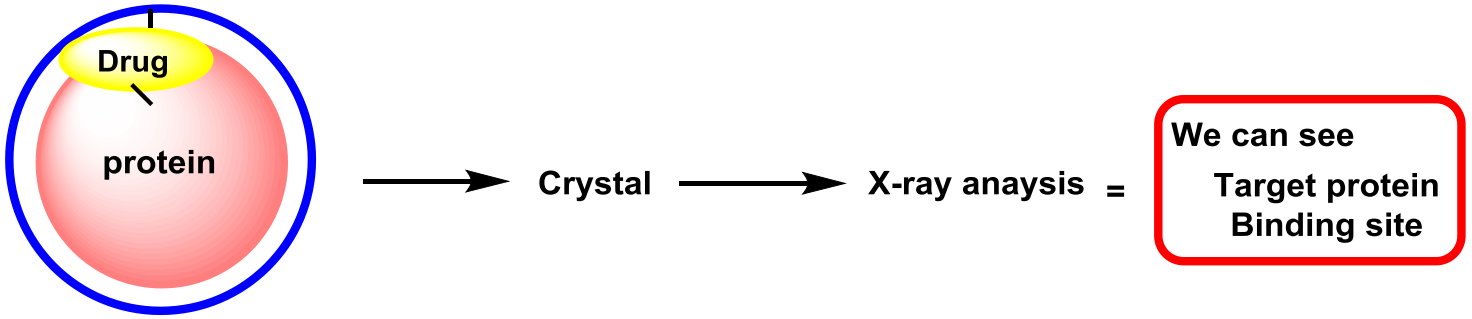


Application --Enclathration of Biomolecule

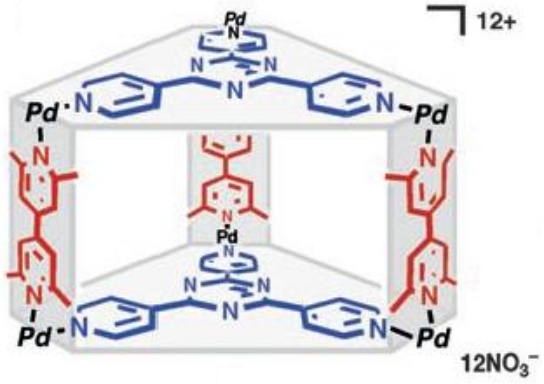
Drug → Modification of the structure

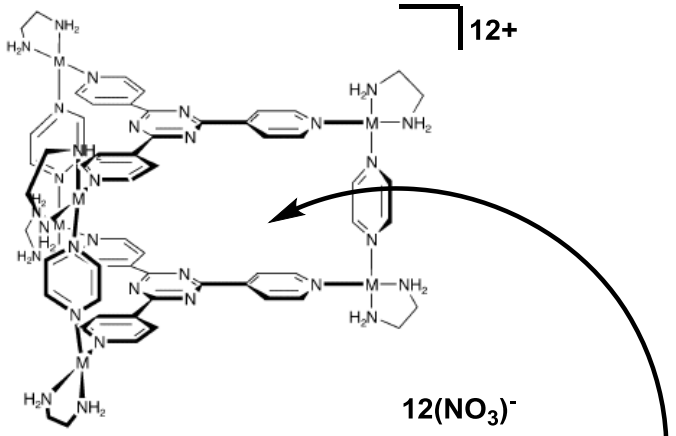
To identify the target protein of a drug, its isolation is important.





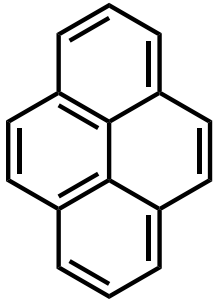
Prism-shaped Cage

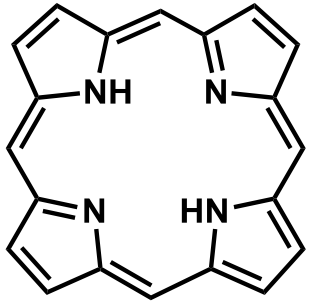


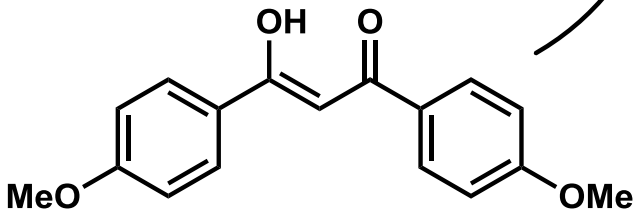


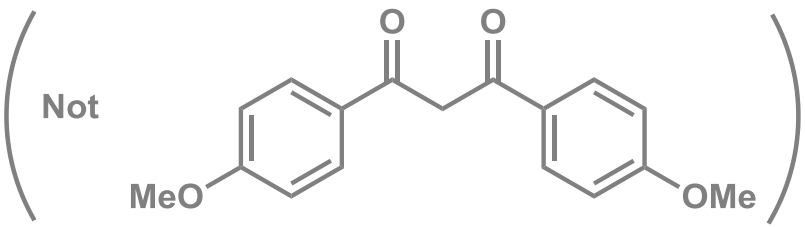
Using two types of ligands!!

Guest: polycyclic aromatic rings, planar structure



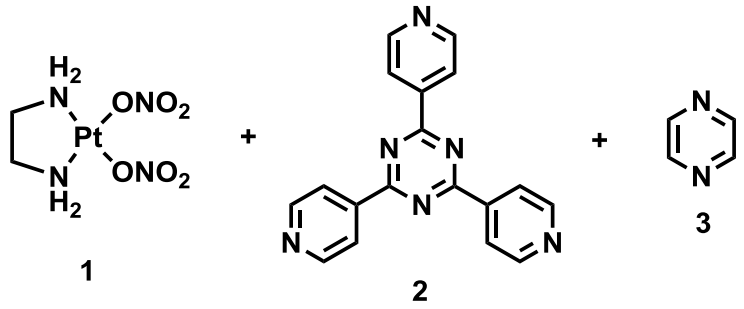


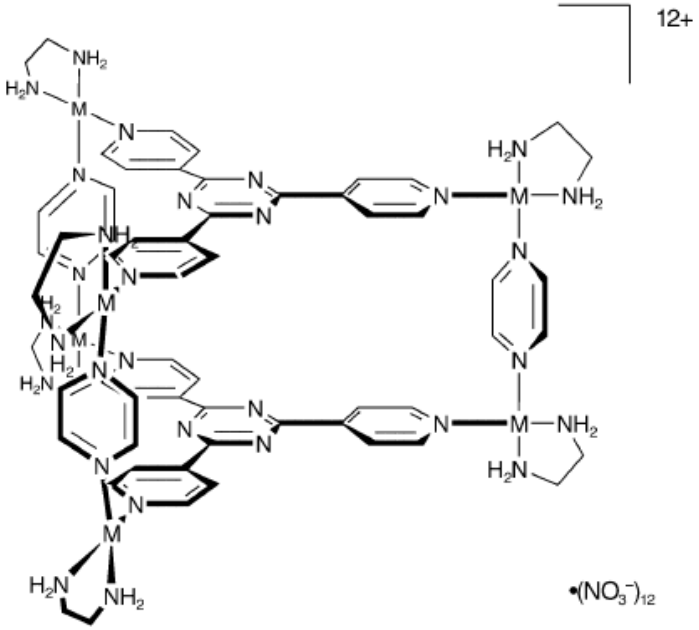




M. Fujita, *et al*, *Angew. Chem. Int. Ed.* **2003**, *42*, 3909.

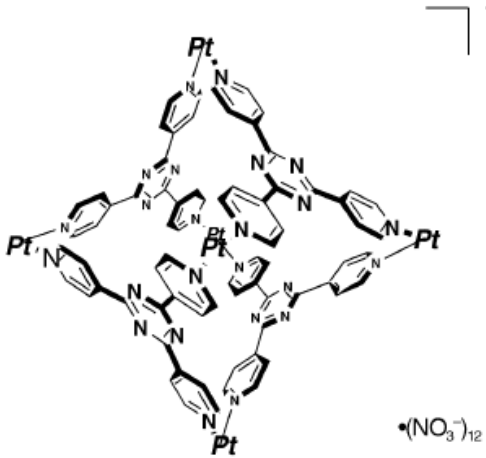
Synthesis of Prism-shaped Cage





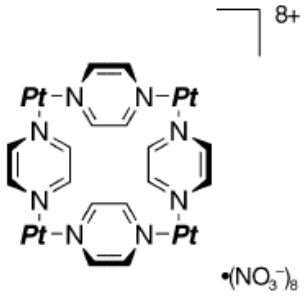
4 : M = Pt

$M_6L_2L'_3$



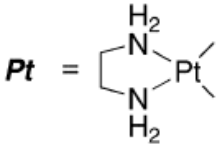
5

M_6L_4



6

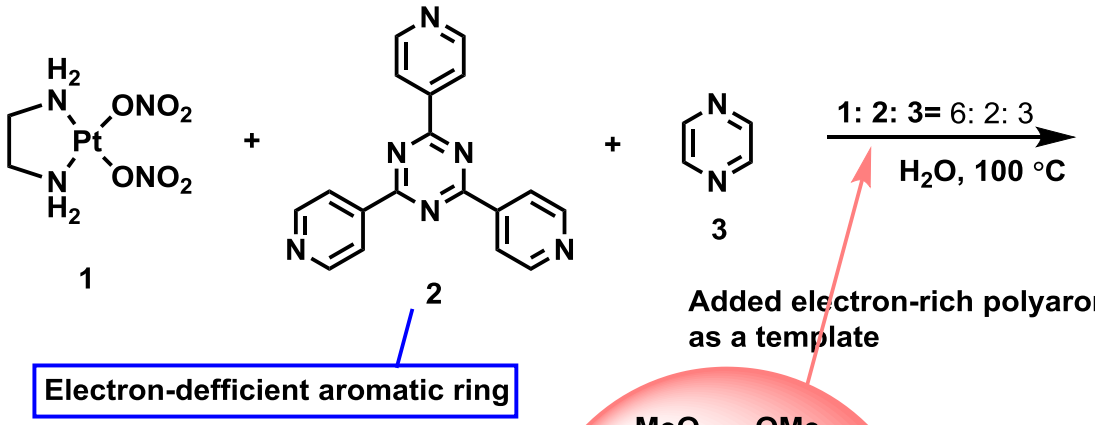
$M_4L'_4$

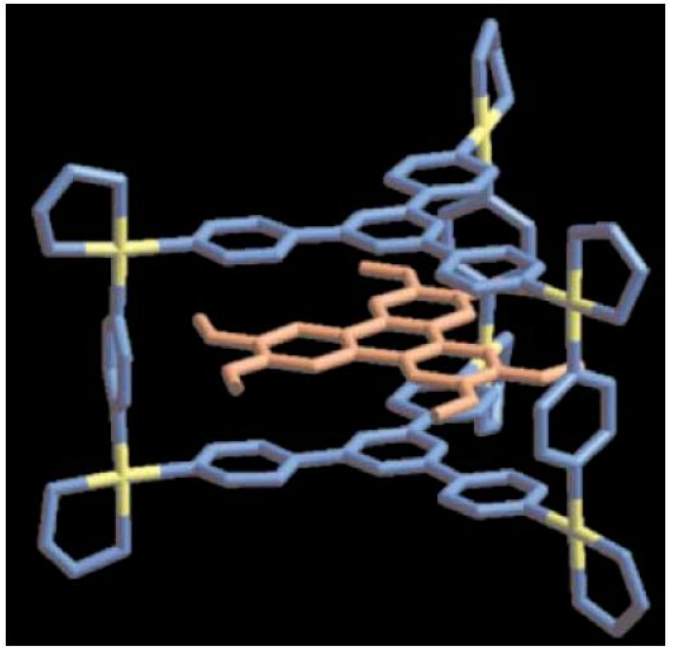


The products were the mixture of the three kinds of complexes.

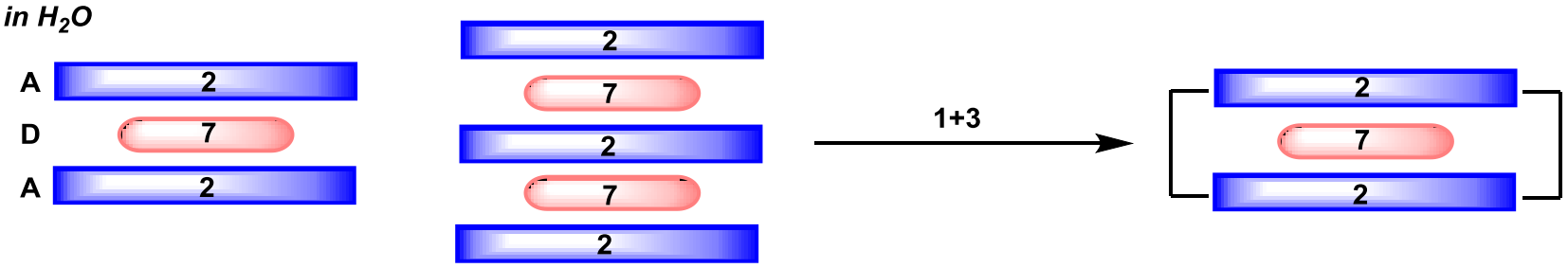
M. Fujita, *et al*, *Angew. Chem. Int. Ed.* **2003**, *42*, 3909.

Method for Selective Synthesis of Prism-shaped Cage



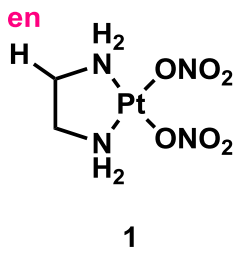


4⊂7

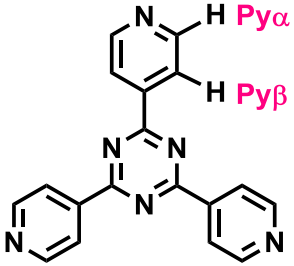


M. Fujita, *et al*, *Angew. Chem. Int. Ed.* **2003**, *42*, 3909.

Checking Formation of Cage



1



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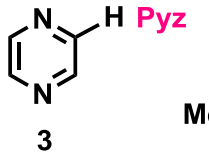

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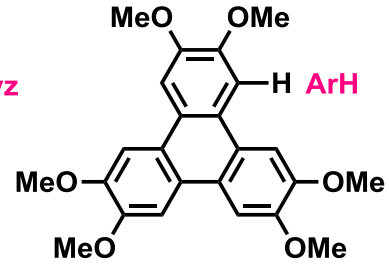
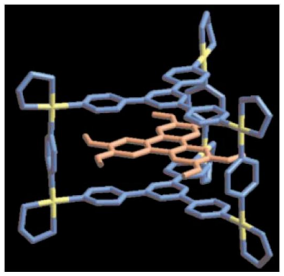
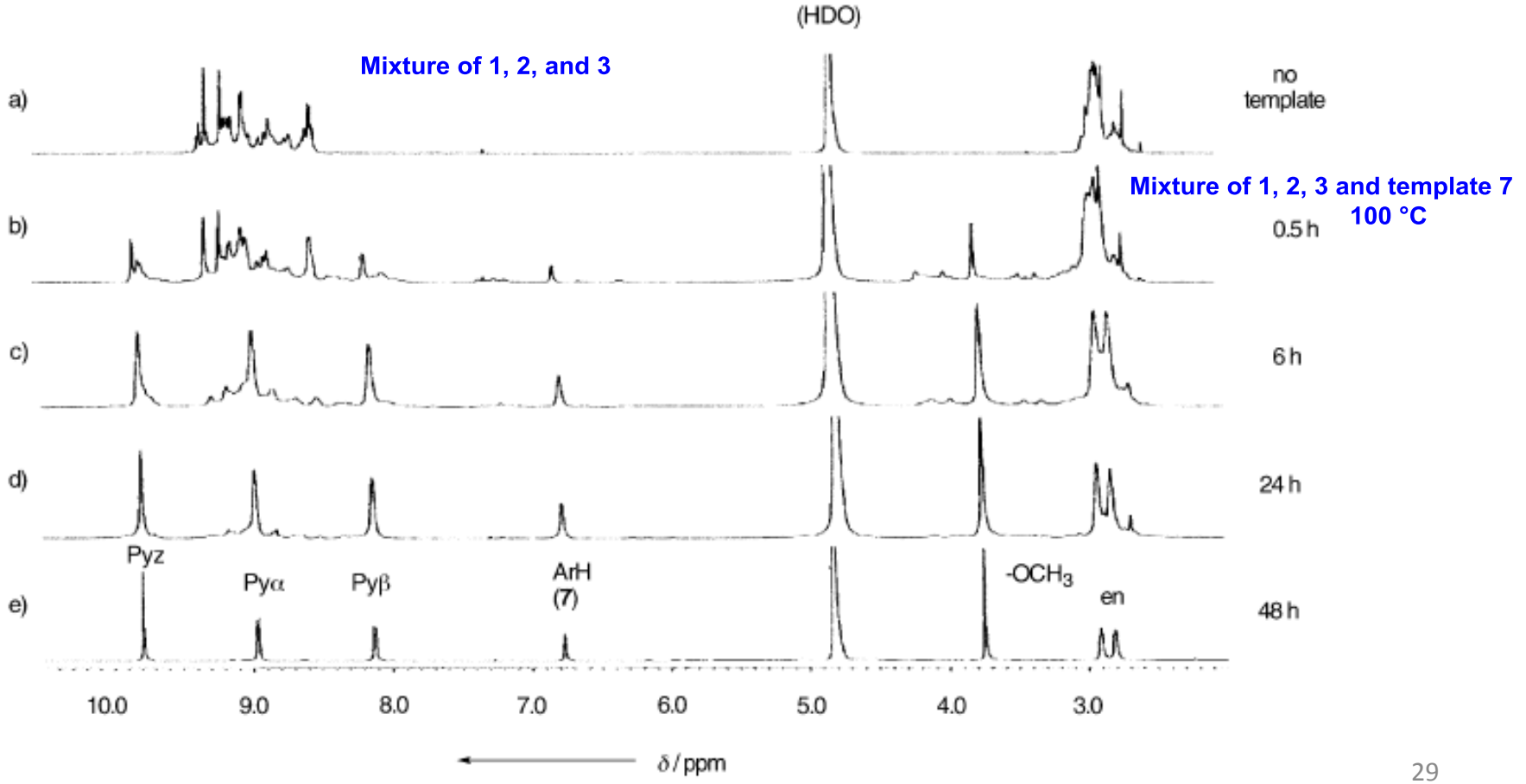


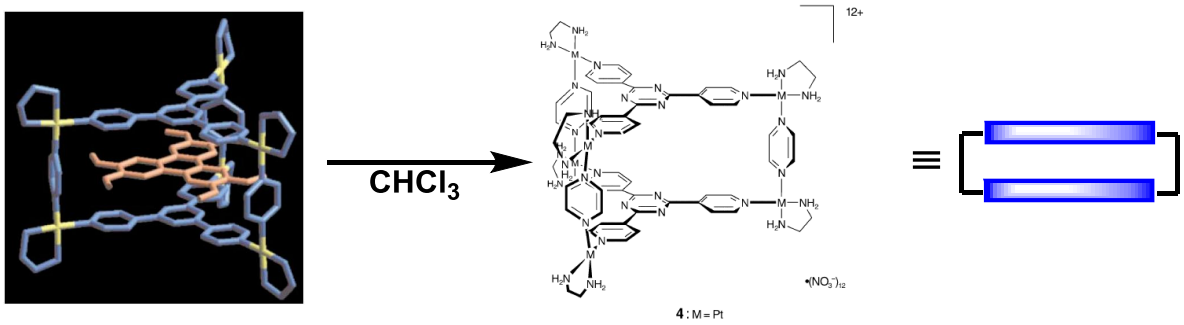
7

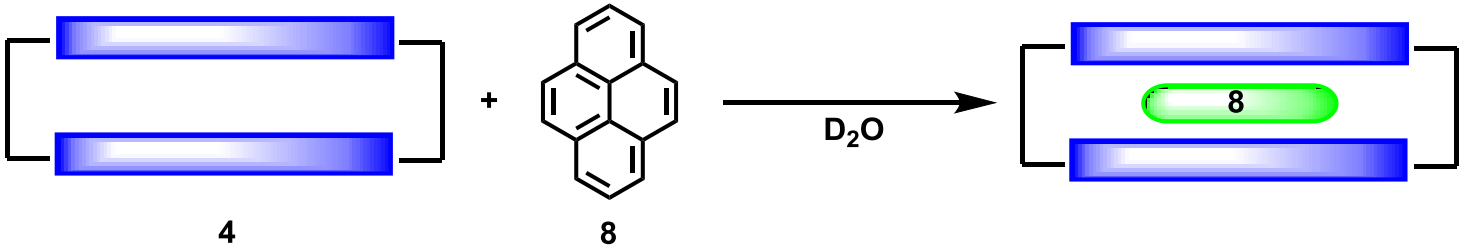


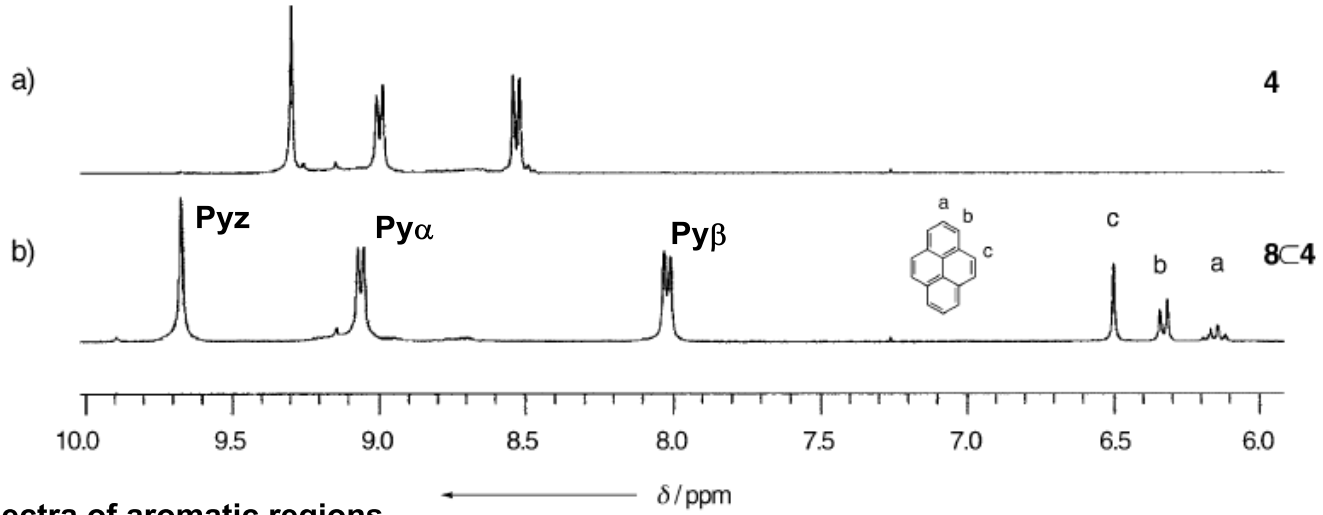


^{1}H-NMR spectra showing the guest-templated assembly of 7⊂4 complex (500 MHz, D_2O, 25 °C). Pyz=pyrazine.

Exchange of Guest Molecule



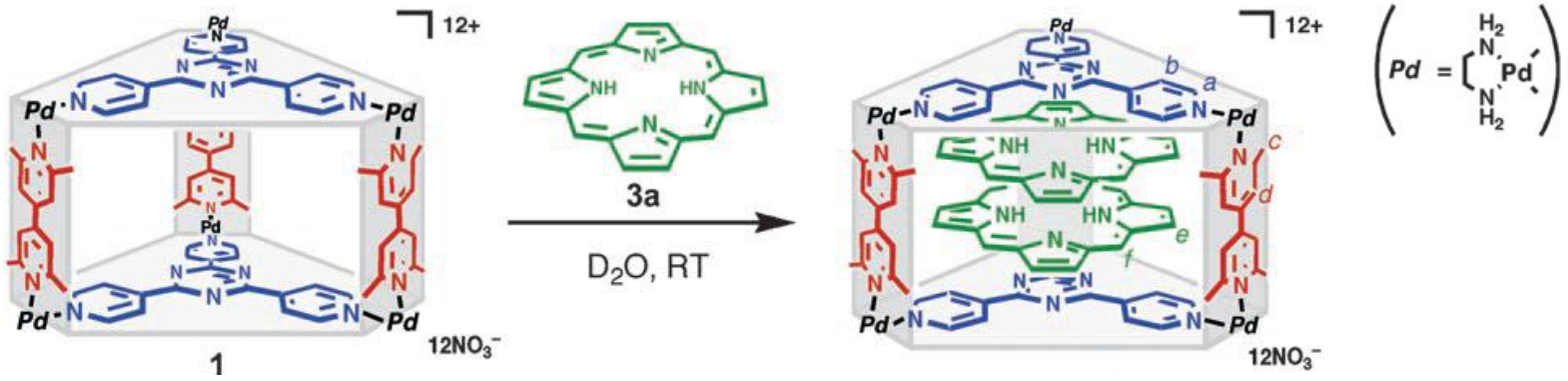




^{1}H-NMR spectra of aromatic regions

M. Fujita, *et al*, *Angew. Chem. Int. Ed.* **2003**, *42*, 3909.

Expansion of Void of Cage



Confirmed by ^{1}H-NMR, CSI-MS, X-ray crystalography

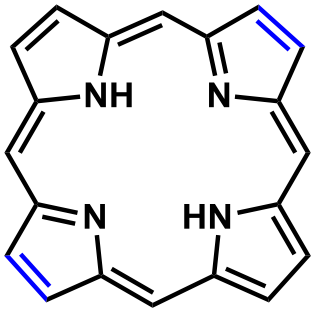
Extension of pillar ligands ⇨ Increase of the number of guests

Prism-shaped cage has potential to transfer porphyrine and analoges.

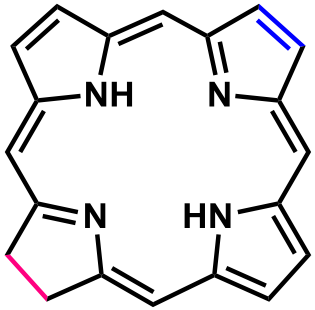
Used for therapy of cancer

M. Fujita, *et al*, *Angew. Chem. Int. Ed.* **2007**, *46*, 1803.

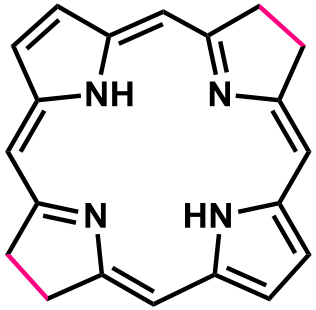
Photosensitizer for Treatment of Cancer



porphyrin
$22\pi e^-$

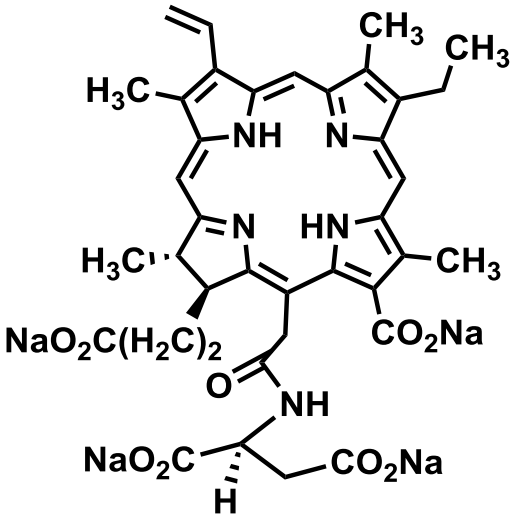


chlorin
$20\pi e^-$



bacteriochlorin
$18\pi e^-$

conjugate double bonds



Talaporfin sodium

Used for treatment of lung cancer

Side effect: skin photosensitivity

*One of the reason of skin photosensitivity: porphyria
= Too much photosensitizer occur some inflammation.

It is important to distribute chlorin core to target tissue selectively.

⇨ Drug delivery system (DDS)

Bruno Therrien, *Chem. Eur. J* **2013**, DOI: 10.1002/chem.201301348.

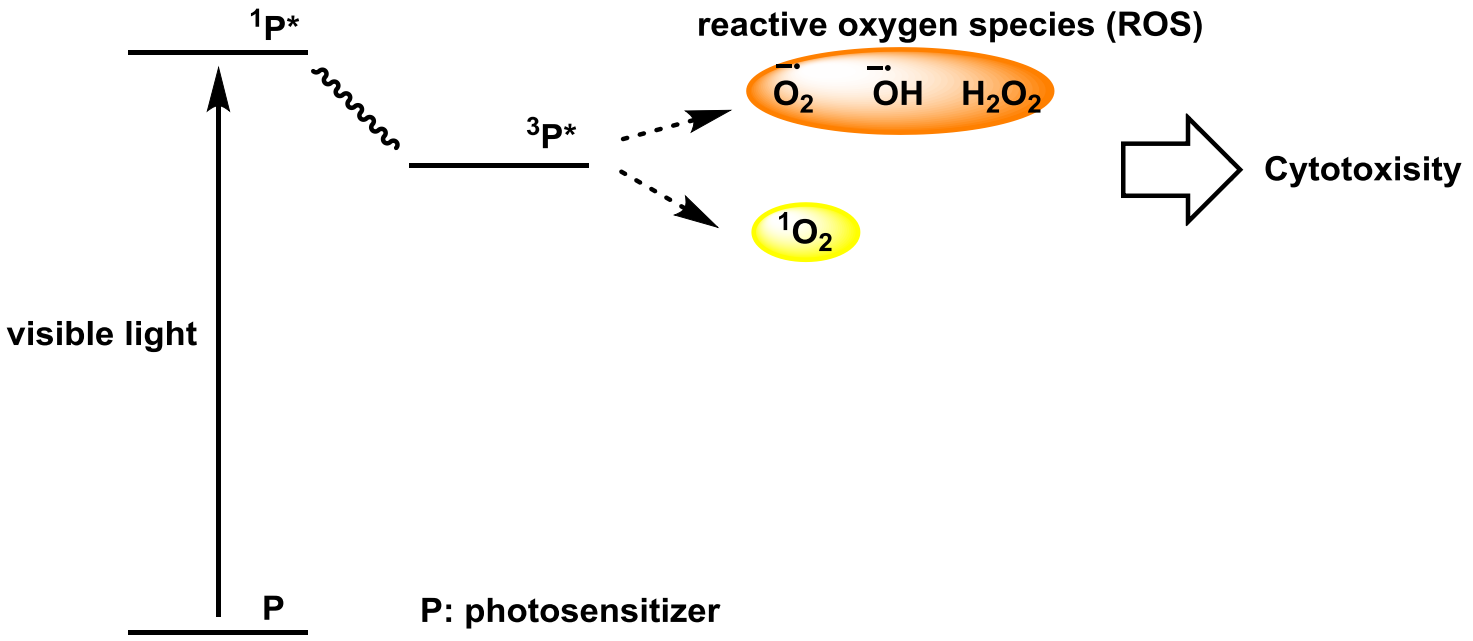
EPR Effect and PDT

Enhanced Permeability and Retention (EPR) effect

Enhanced permeability of blood vessels in tumor tissues.
Impaired clearance of molecules.

⇨ **Macromolecule are tend to accumulate in tumor tissue.**

Principle of Photodynamic Therapy (PDT)



H. Maeda, *Advan. Enzyme Regul.* **2001**, *41*, 189.
K. Takemura, *Hokkaido University Collection of Scholarly and Academic Papers*, http://hdl.handle.net/2115/24336.

Combination of PDT and DDS

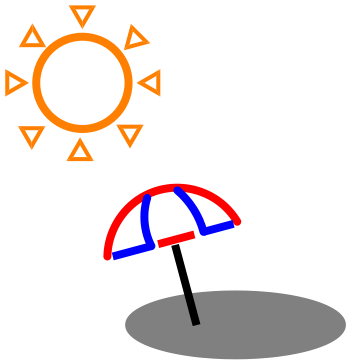
PDT

Pharmacological action of compunds can be controlled after medication.

× photosensitivity

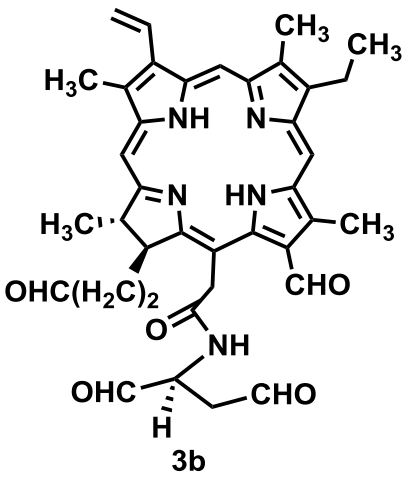
DDS

Selective transportation of drug to the target tissue can realize.



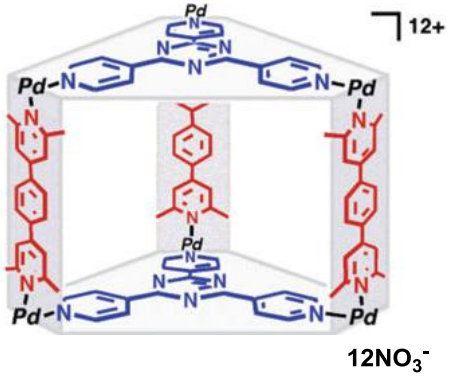
Inclusion of Photosensitizer

in vitro



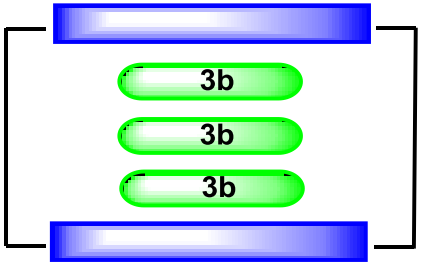
3b

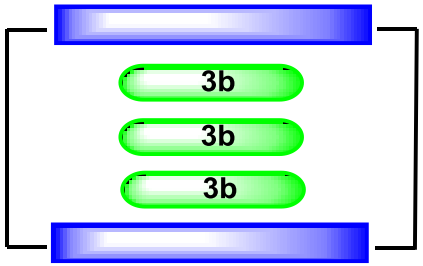
+



$12NO_3^-$

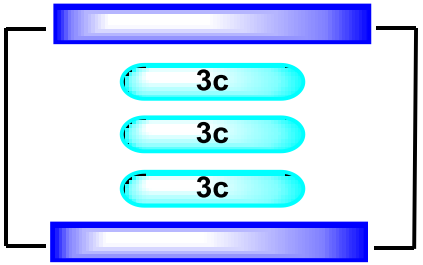
H_2O

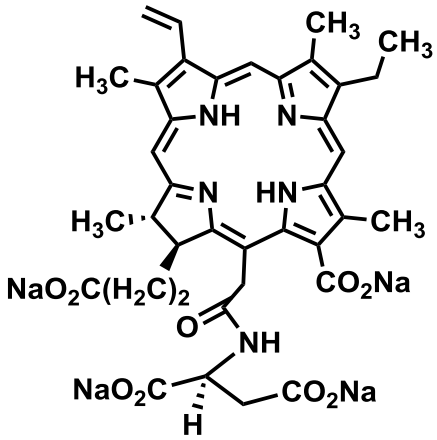




oxidation

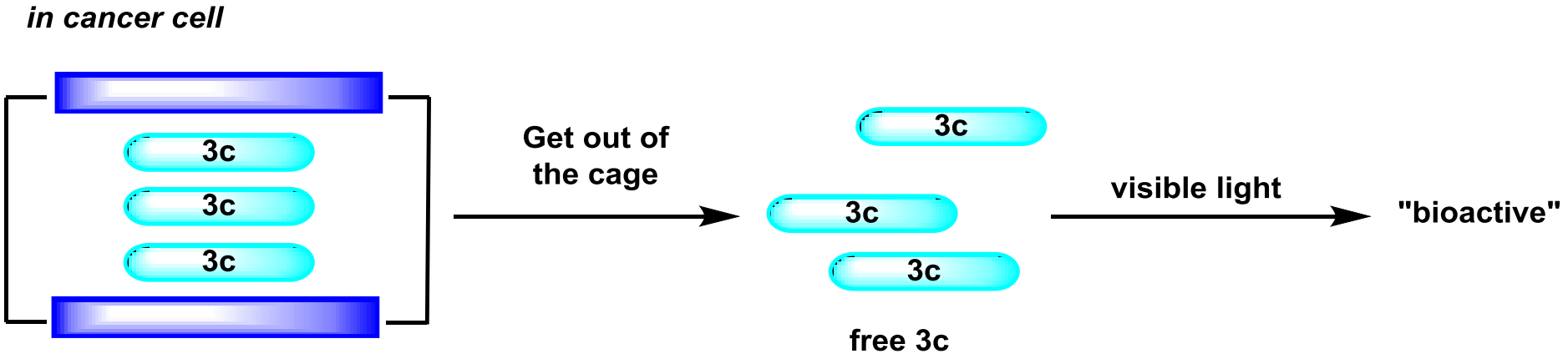
with excess of free 3b





3c

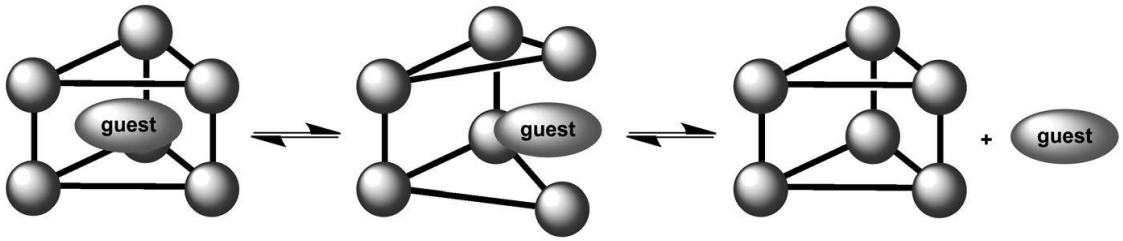
Exclusion of Photosensitizer



How to control the release of the guests in target cells??

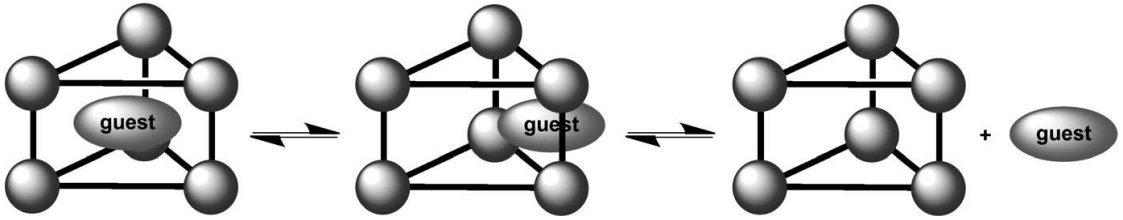
How to Release Guest?

<Via host rupture>



Further reagent will be needed to cleavage the coordintion bond.

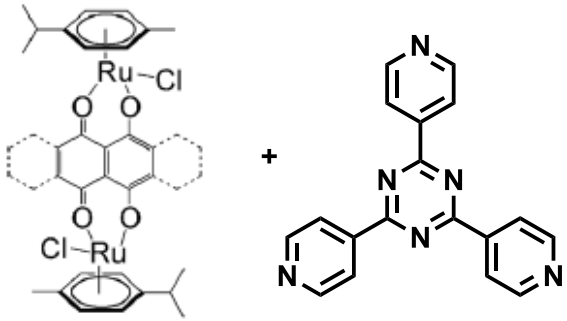
<Via host aperture>



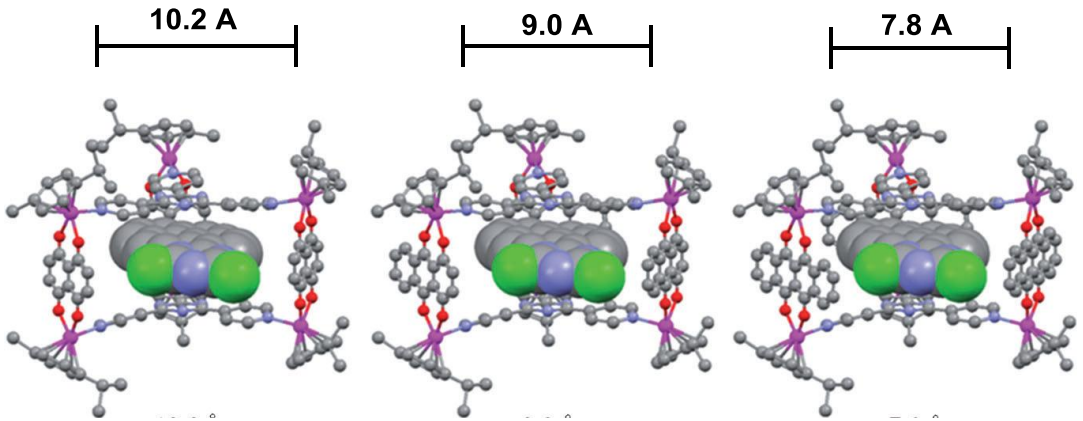
Stimuli change the portal size of cage and facilitate the guest release.

Bruno Therrien, *et al*, *Chem. Eur. J.* **2011**, *17*, 9669.

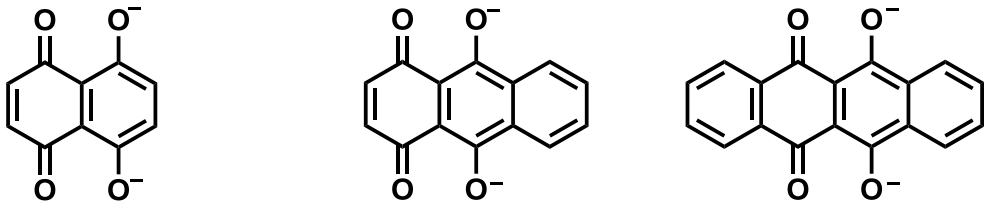
Control of Portal Size

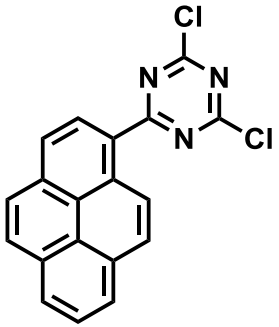


(HyperChem simulations of the metalla-cages)



$[pyrene\text{-}R\subset 1]^{6+}$ $[pyrene\text{-}R\subset 2]^{6+}$ $[pyrene\text{-}R\subset 3]^{6+}$



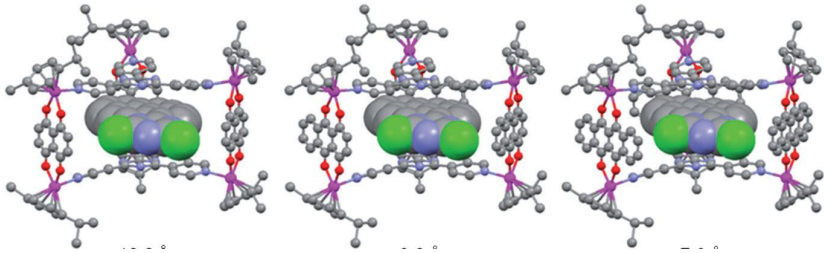
Guest = 

pyrene-R

These three cages have an equivalent size of cavity.
But the portal sizes are different.

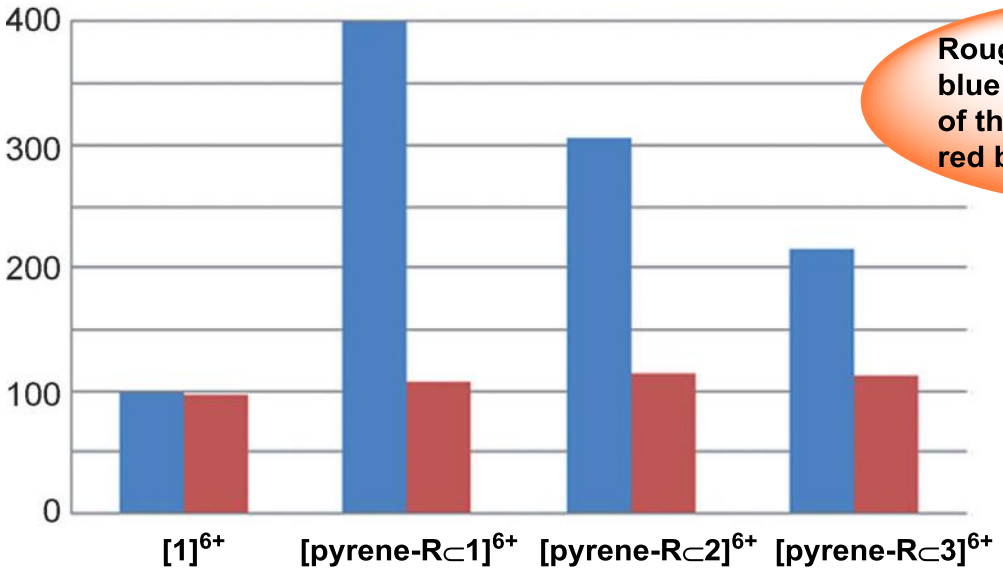
Bruno Therrien, *et al*, *Chem. Eur. J.* **2011**, *17*, 9669.

Portal Size and Release of Guest Molecule



[pyrene-R⊂1]$^{6+}$ [pyrene-R⊂2]$^{6+}$ [pyrene-R⊂3]$^{6+}$

*photochroism: 淡色効果
分子の規則的な配列によって
見かけ上の色がうすくなる効果



Roughly,
blue bar displays the amount
of the free pyrene-R and
red bar exhibits cage taking in.

blue: Fluorescence recorded by flow cytometry of [pyrene-R⊂1-3]$^{6+}$ indicating pyrene-R release from the host.
red: Ruthenium uptake determined by ICP-MS is also shown.
Cells were incubated with [1][CF_3SO_3]$_6$ and [pyrene-R⊂1-3][CF_3SO_3]$_6$ at 2 μM for 24 h.
Human A2780 ovarian carcinoma cells

This result shows things below.

Uptakes of cages in cell (red): There is almost no difference among 1, 2 and 3.

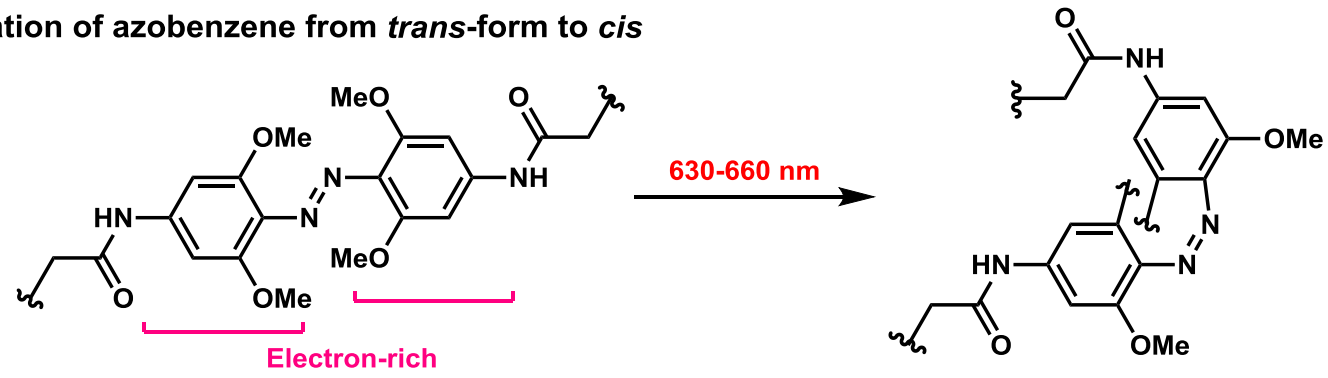
The amount of the released guest (blue): 1>2>3

→corelation between the portal size of the host and release of pyrane-R.

Bruno Therrien, *et al*, *Chem. Eur. J.* **2011**, *17*, 9669.

Expansion of Portal Size of Host

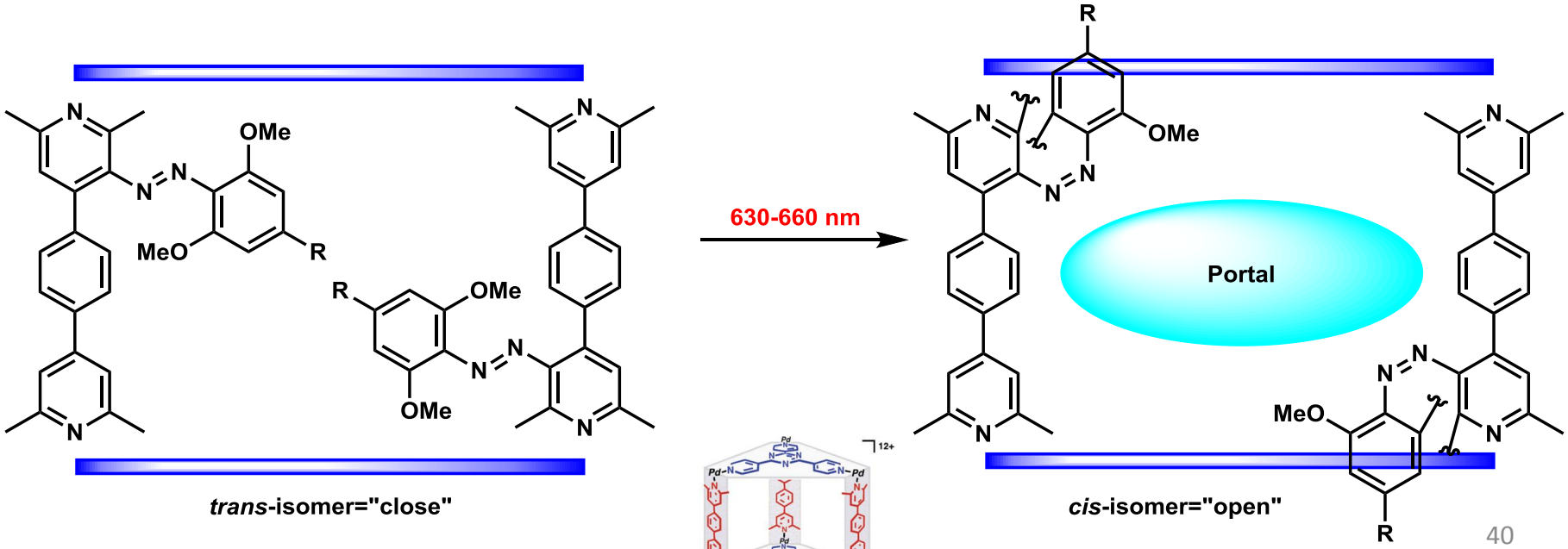
Isomerization of azobenzene from *trans*-form to *cis*



	UV light	Red light
Penetration of cells and tissues	×	○
Trigger of apoptotic events near to skin	○	×

Red light is better than UV light thinking about biological application.

G. Andrew Woolley, *et al*, *J. Am. Chem. Soc.* **2013**, doi.org/10.1021/ja402220t
G. Andrew Woolley, *et al*, *J. Am. Chem. Soc.* **2012**, *133*, 19648.



trans-isomer="close"

cis-isomer="open"

Side Effect Based on Cage

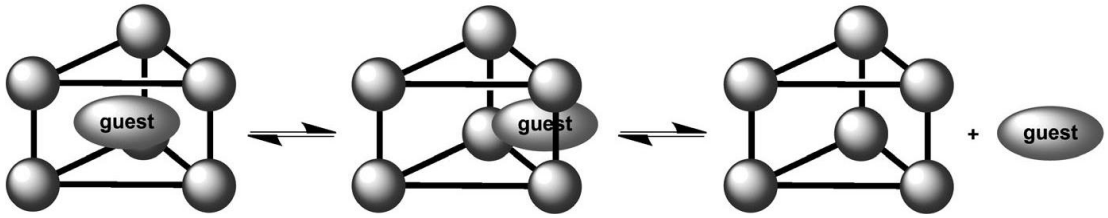
✖ **Drug drivery system potentially includes the compounds without the direct relation to drug efficacy.**

The compound is not only waste but also orijin of adverse event.

✔ **At least, this cage system is beneficial because**

the water-soluble cage is intact.= advantage in excretion

the ligands ocuupy the coodination sites of metal.= prevent the side effect owing to metal



Summary and Outlook

Coordination complexes (M_mL_n) can be easily synthesised.

Simple functionalization of ligand can change the property the inner sphere of the capsule and diverse molecules can be recognized selectively as guests.

Capsules having different size and shape were synthesized and we can select the good structure according to our sake.