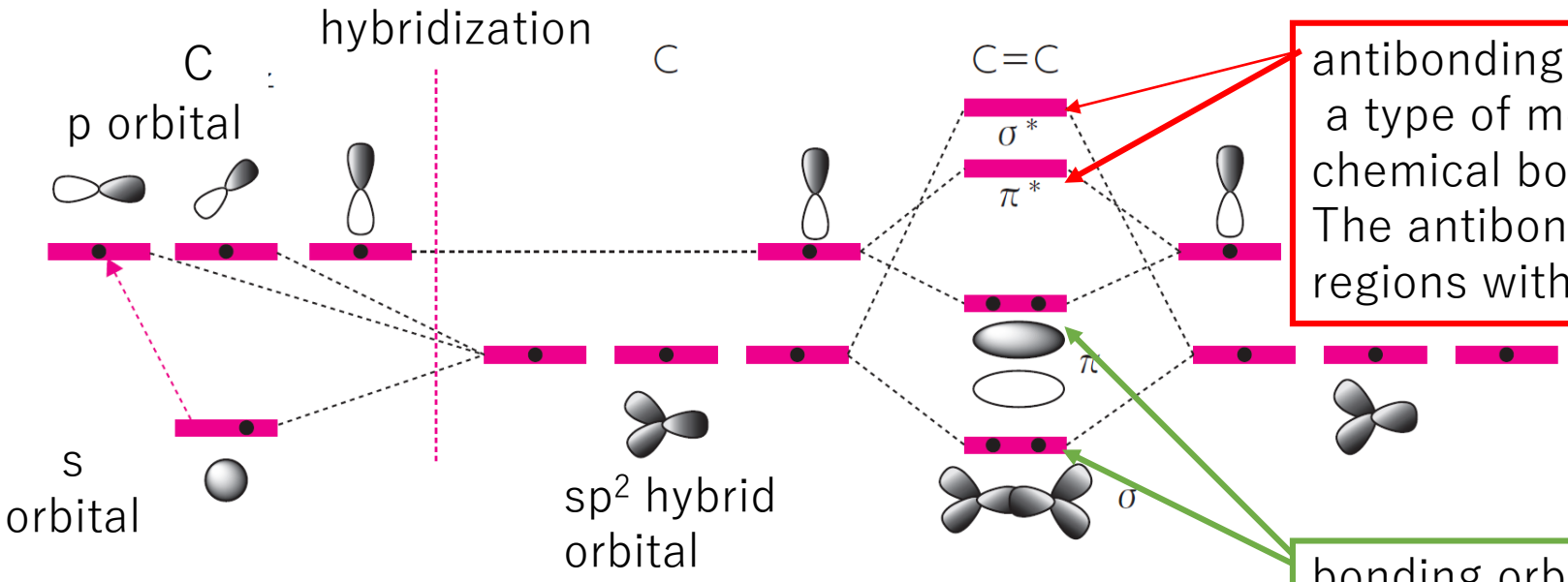
UV-VIS Spectroscopy 2

Ultraviolet and visible absorption of organic compounds



Molecular orbital resulting from sp^2 hy

antibonding orbital ; σ^*、 π^*
a type of molecular orbital that weakens the chemical bond between two.
The antibonding orbital has one or more "nodes", regions without electrons.

bonding orbital ; σ 、 π
bonding orbitals which have an energy lower than the energy of the atomic orbitals which formed them, and thus promote the chemical bonds which hold the molecule together

Molecules containing O oxygen, N nitrogen, S sulfur, etc.

non-bonding orbital; n-orbital, lone pair orbital
Since the electrons are not participating in bonds, they are neither stabilized nor destabilized.

→ Energy exists between bonding and antibonding orbitals

Transitions of Absorption

transition	wavelength (nm)	ε_{max} (mol^{-1} L cm^{-1})	kind of molecules	
σ-σ*				
σ-Rydberg	<200	10^3-10^5	all kind molecules	
n-Rydberg	(far-UV)			
n-σ*	180-200	10^3-	H_2O, NH_3	
	170-220	10^3-10^4	alkene, carbonyl, ketone	E_1-band
π-π*	170-800	10^4-10^5	aromatic, polyene	k- or E_2-band
	250-	10^2-10^3	aromatic	B- band
n-π*	250-	10-100	aromatics with non-bonding electron	

200 nm

Chromophore and auxochrome

$\pi \rightarrow \pi^*$　　$n \rightarrow \pi^*$

Electronic transitions observed at wavelengths longer than 200 nm

Absorption is observed in this region only for molecules with multiple bonds.

chromophore

C=C, C=N, C=O, C=S, N=N, N=O
(benzene ring、 porphyrin ring・・・)

When the functional group with lone pair of electrons binds to the chromophore, the absorption wavelength and intensity change.

auxochrome

chromophore

	compound	λ_{max} /nm	ε_{max} $mol^{-1}\,L\,cm^{-1}$	solvent condition	
R-CH=CH-R	ethylene	175	15000	gas	π-π*
R-C≡C-R	acetylene	185	21000	gas	π-π*
		196	7000	gas	n-3s Ryd
	acetone	279	15	hexane	n-π*
		180	10000	gas	n-3s Ryd
	acetaldehyde	290	20	hexane	n-π*
	acetic acid	208	32	ethanol	n-π*
	acetamide	220	63	water	n-π*
	nitromethane	278	20	ether	n-π*
	ethyl acetate	211	58	i-octane	n-π*
R-N=O	Methyl-2-nitrosopropane	300	100	ethanol	n-π*
	ethyl nitrate	270	12	1,4-dioxane	n-π*
R-O-N=O	amyl nitrite	219	1120	ether	n-π*

Shift by substituent

Benzene ring π - π * transitions have a longer wavelength shift and intensity increase due to auxochrome

	E_2-band		B-band		
substituent	λ_{max} /nm	ε_{max} /mol^{-1}Lcm^{-1}	λ_{max} /nm	ε_{max} /mol^{-1}Lcm^{-1}	solvent
H—	204	7,900	256	200	ethanol
CH_3—	207	7,000	261	200	ethanol
NH^{3+}—	203	7,500	254	200	acid
I—	207	7,000	257	700	ethanol
Br—	210	7,900	261	200	ethanol
Cl—	210	7,400	264	200	ethanol
OH—	211	6,200	270	1,500	water
CH_3—O—	217	6,400	269	1,500	water
COO^-—	224	8,700	268	560	water
CN—	224	13,000	271	1,000	water
COOH—	230	10,000	270	800	water
NH_2—	230	8,600	280	1,400	water
O^-—	235	9,400	287	2,600	base
SH—	236	10,000	269	700	hexane
CH≡C—	236	12,500	278	700	hexane
$CH_3C(O)$—	240	13,000	278	1,100	ethanol
CH_2=CH—	244	12,000	282	500	ethanol
C(O)H—	244	15,000	280	1,500	ethanol
C_6H_5—	246	20,000			
$(CH_3)_2N$—	251	14,000	280	2,100	hexane
NO_2—	269	7,800			

Molecules with larger conjugated bonds have absorption bands in longer wavelengths.

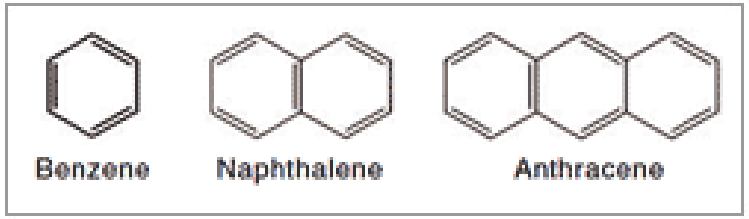


Fig.1 Structures of Benzene, Naphthalene, and Anthracene

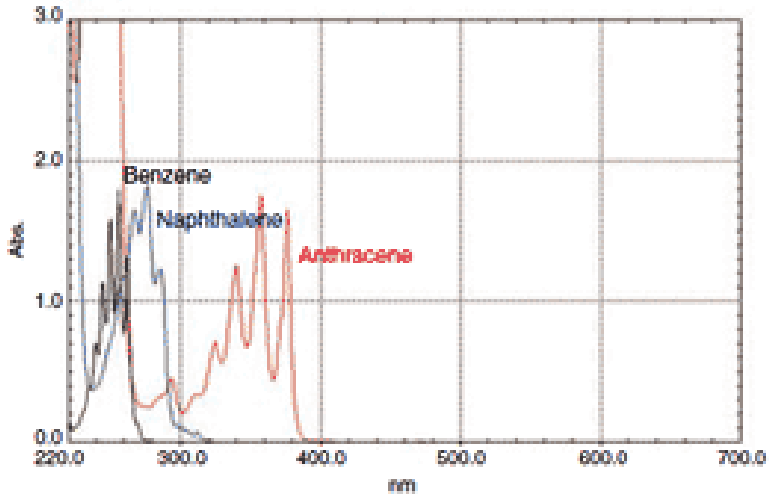
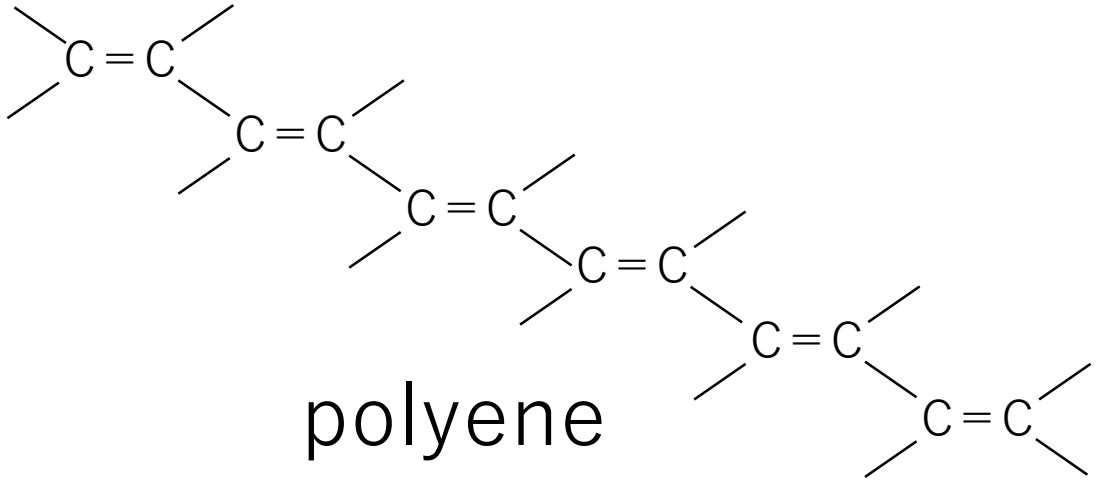


Fig.2 Absorption Spectra of Benzene, Naphthalene, and Anthracene

n	1	2	3	4	5	6
λ_{max}/nm	162	217	268	304	334	364
e /mol^{-1}dm^{3}cm^{-1}	10	21	34	64	121	138



polyene

$H(CH=CH)_nH$

https://www.shimadzu.co.kr/service-support/technical-support/technical-information/uv-ap/apl.html

Solvatochromism

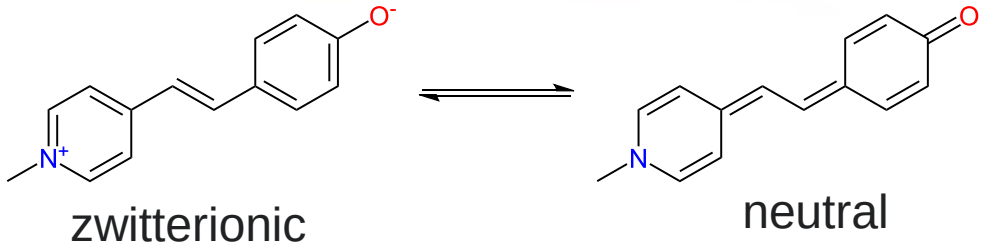
https://en.wikipedia.org/wiki/Brooker%27s_merocyanine

solvent	water	methanol	ethanol	2-propanol	DMSO	acetone	chloroform
color	yellow	red-orange	red	violet	blue-violet	blue-violet	blue
λmax/nm	442	509	510	545	572	577	618
Relative polarity	1	0.762	0.654	0.546	0.444	0.355	0.259

Brooker's merocyanine

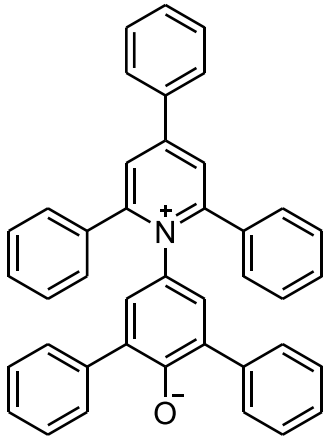
Negative solvatochromism

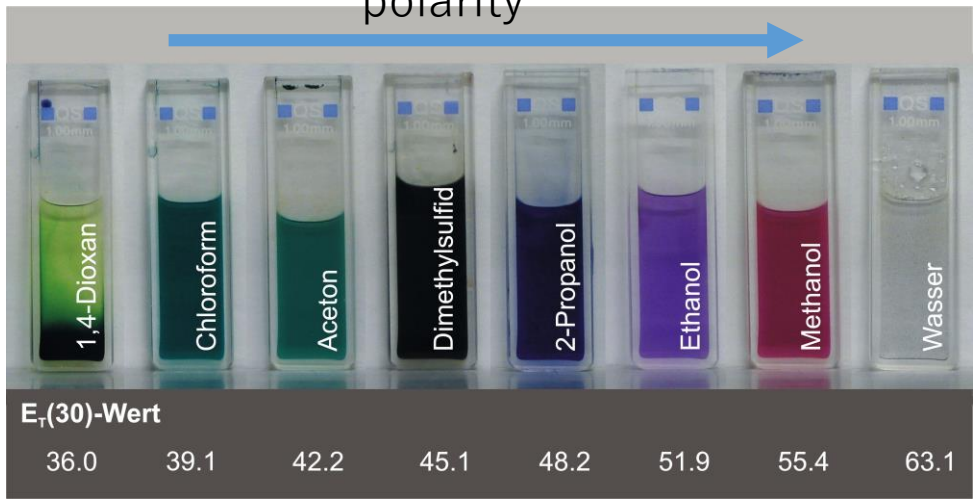
Shorter wavelength shift with increase polarity



Solvents give hydrogen bonding stabilize zwitterionic resonant form

Other example: Reichardt's dye

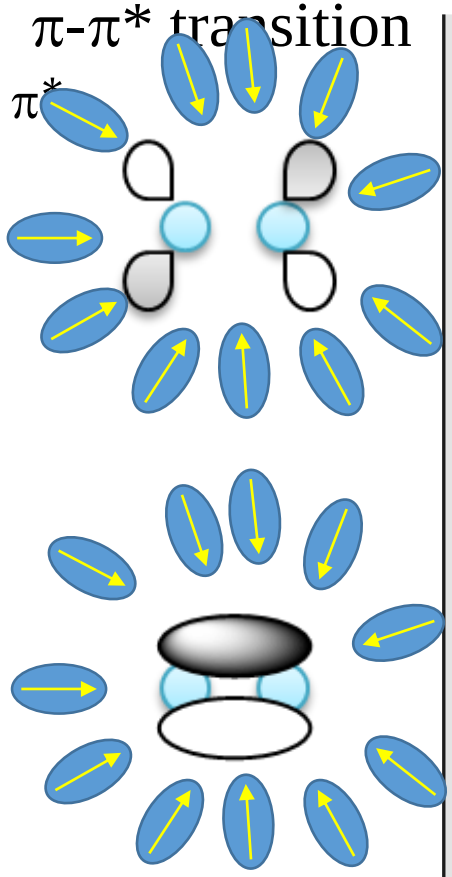




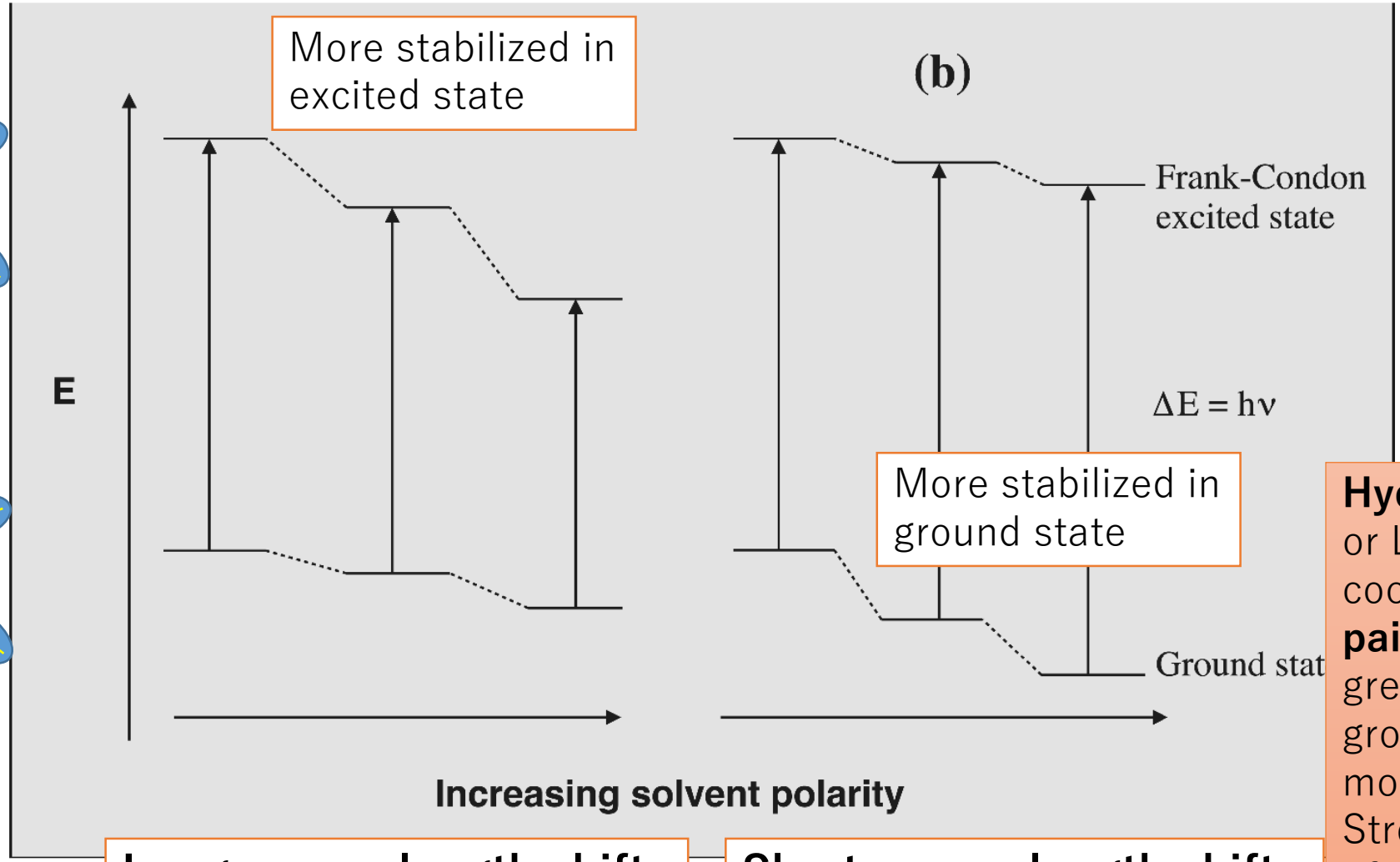
https://en.wikipedia.org/wiki/Reichardt%27s_dye

schematic qualitative representation of solvent effects on the electronic transition energy

π-π* transition

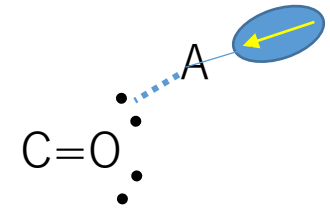


π* state that have larger dipole is more stabilized by polar solvent than π state



n-π* transition

Hydrogen bond (Lewise acid)



Hydrogen bonding or Lewis acid coordination **to lone pair electrons** greatly stabilizes the ground state of the molecule.
Strong acidity are often found in polarer solvents.

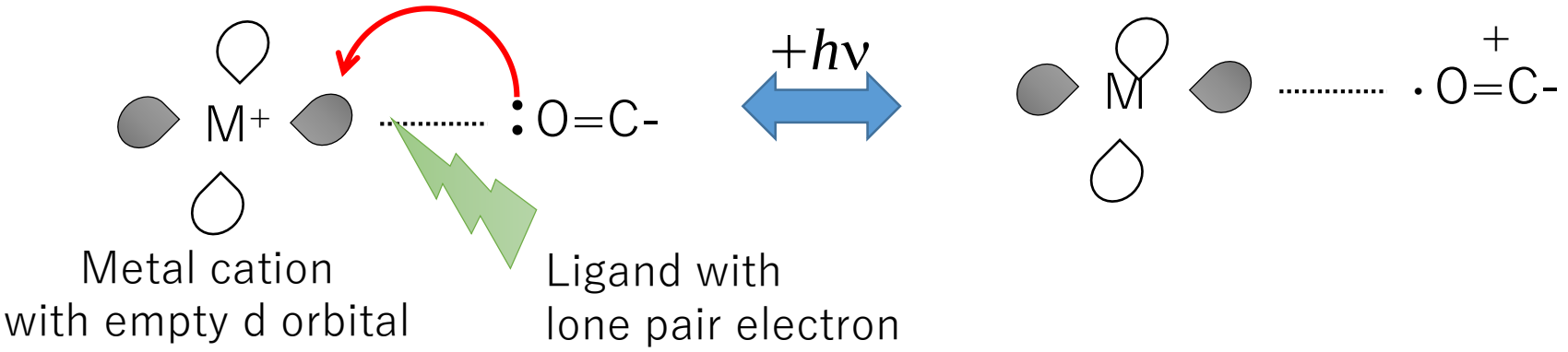
Longer wavelength shift =positive solvatochromic

Shorter wavelength shift =negative solvatochromic

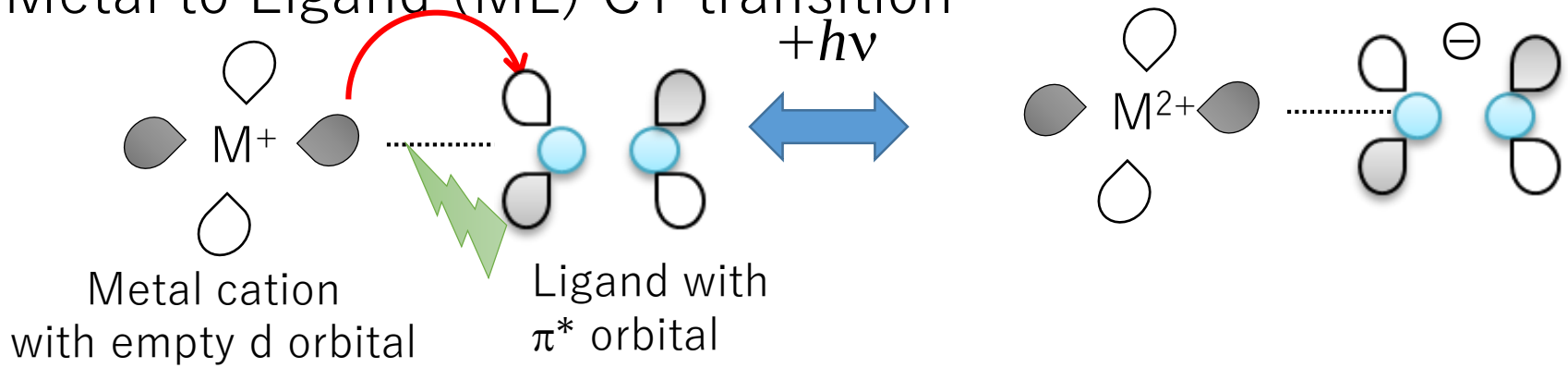
Ultraviolet and visible absorption of Metal complex compounds

(1) Charge transfer (CT) transition

Ligand toMetal (LM) CT transition



Metal to Ligand (ML) CT transition



Large molar extinction coefficient.
($\varepsilon = 10^4 \sim 10^5$ mol L^{-1} cm^{-1})

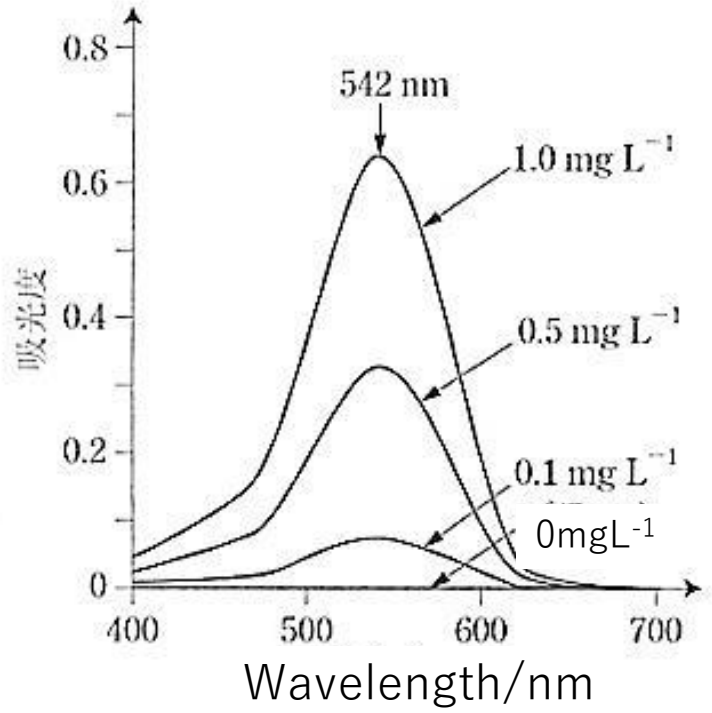
New absorption that appears due to complex formation. Since the absorption wavelength is different from that of the original solute/solvent, it is easy to accurately quantify the concentration of complexed molecules.

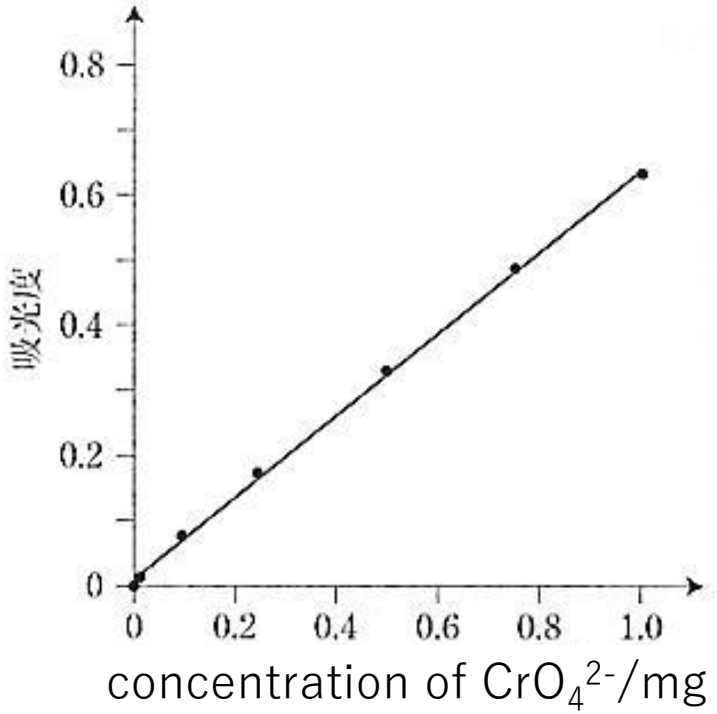
Quantitative analysis of heavy metal ions

Metal ions form complexes with coloring reagents (organic ligands).
Strong absorption due to CT transition appears

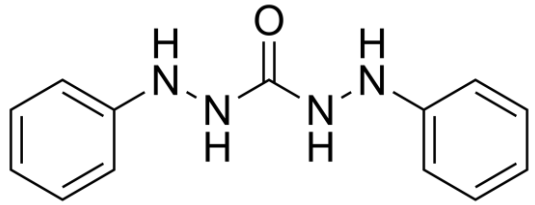
↳ New absorption that appears due to complex formation.

It is easy to accurately quantify the concentration of complexed molecules.





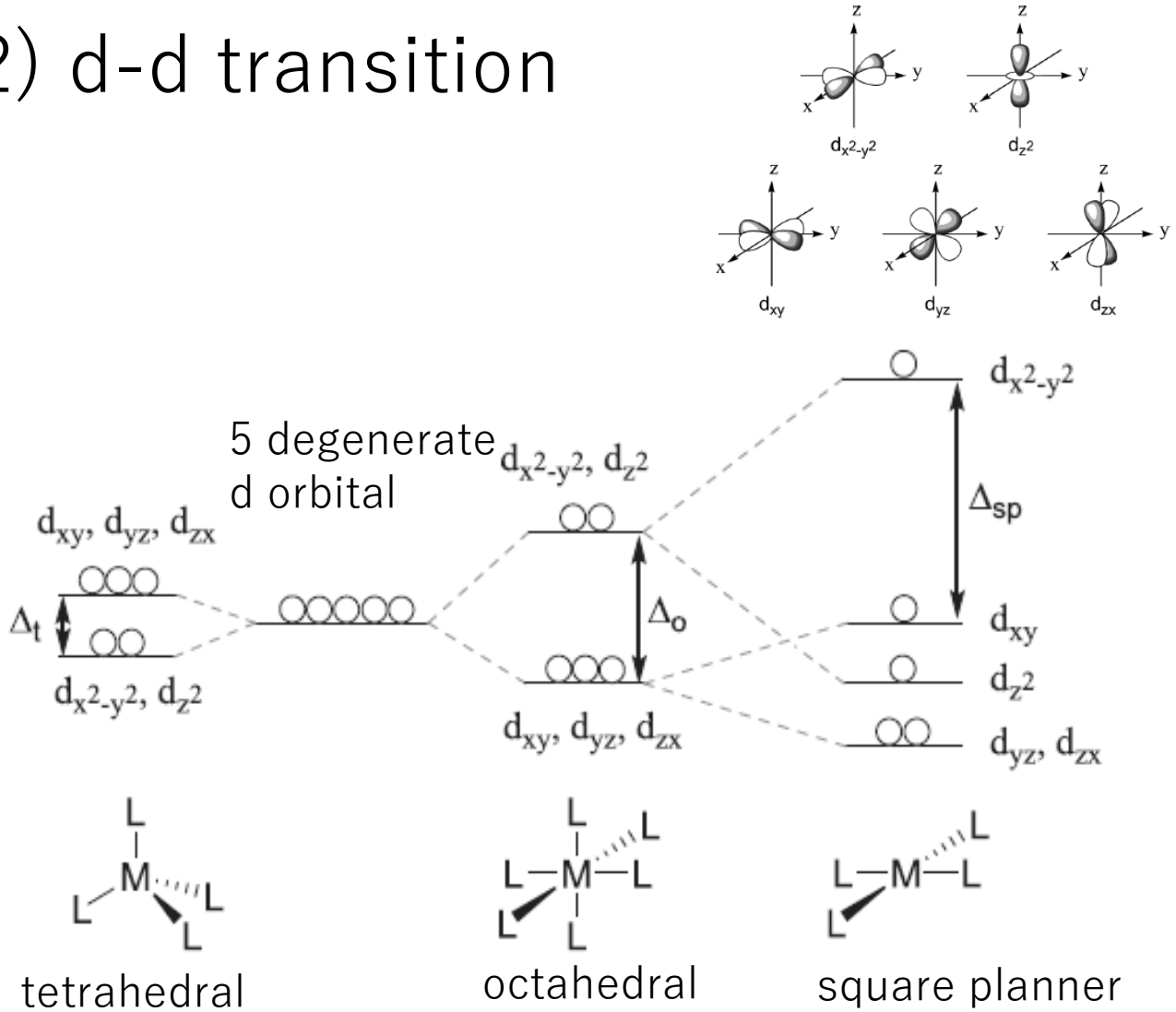
Quantitative analysis of CrO_4^{2-} using diphenylcarbazide (DPC),



This analysis has a calibration limit of 50 ppb

Ultraviolet and visible absorption of Metal complex compounds

(2) d-d transition



The ligand field splits the transition metal's d orbitals. The electronic transitions between the split orbitals are called the d-d transition

・Medium-small molar extinction coefficient.
($\varepsilon=10^2 \sim 10^3$ mol L^{-1} cm^{-1})

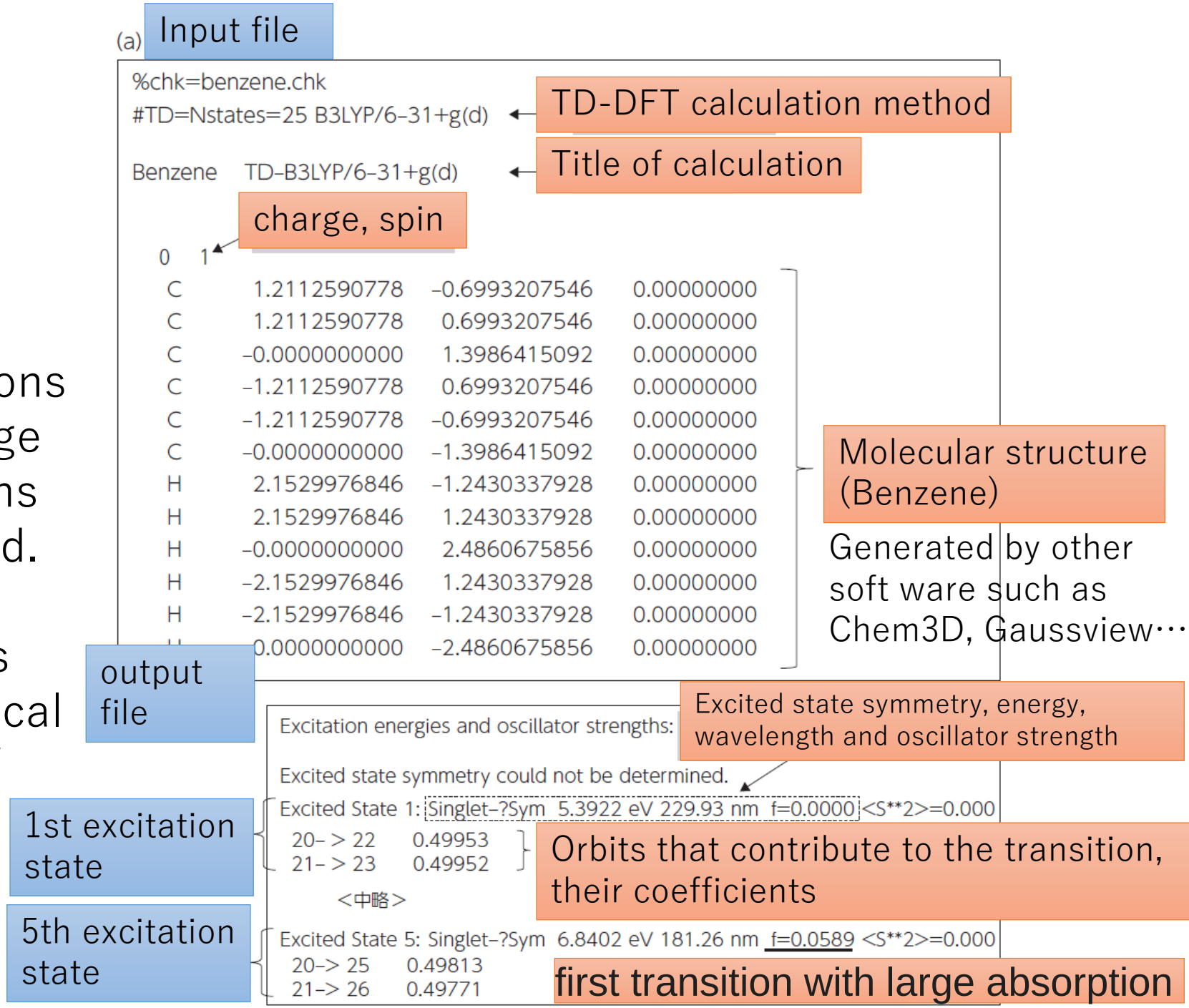
・Sometimes observed in the near-infrared region

・These transitions cannot occur in metal complexes where the d-orbital is completely empty (d^0) or completely full (d^{10}).

Quantum chemical calculation

Quantum chemical calculations predict the approximate range in which electronic transitions in molecules will be observed.

Even experimental scientists can perform quantum chemical calculations with the help of programs such as Gaussian.



UV-VIS absorption spectroscopy

- Qualitative analysis
  - Beer-Lambert law Sensitivity of about 10^{-6} mol L^{-1}
  - Simultaneous multi-component quantification

- Investigation of Electronic states
  - Observe the bonding and antibonding orbitals of molecules
    - HOMO-LUMO, Frontier Orbital
  - Molecular interaction
    - Effect of solvent on multiple bonds
    - Hydrogen bonding in a lone pair
    - Complex formation with metal ions

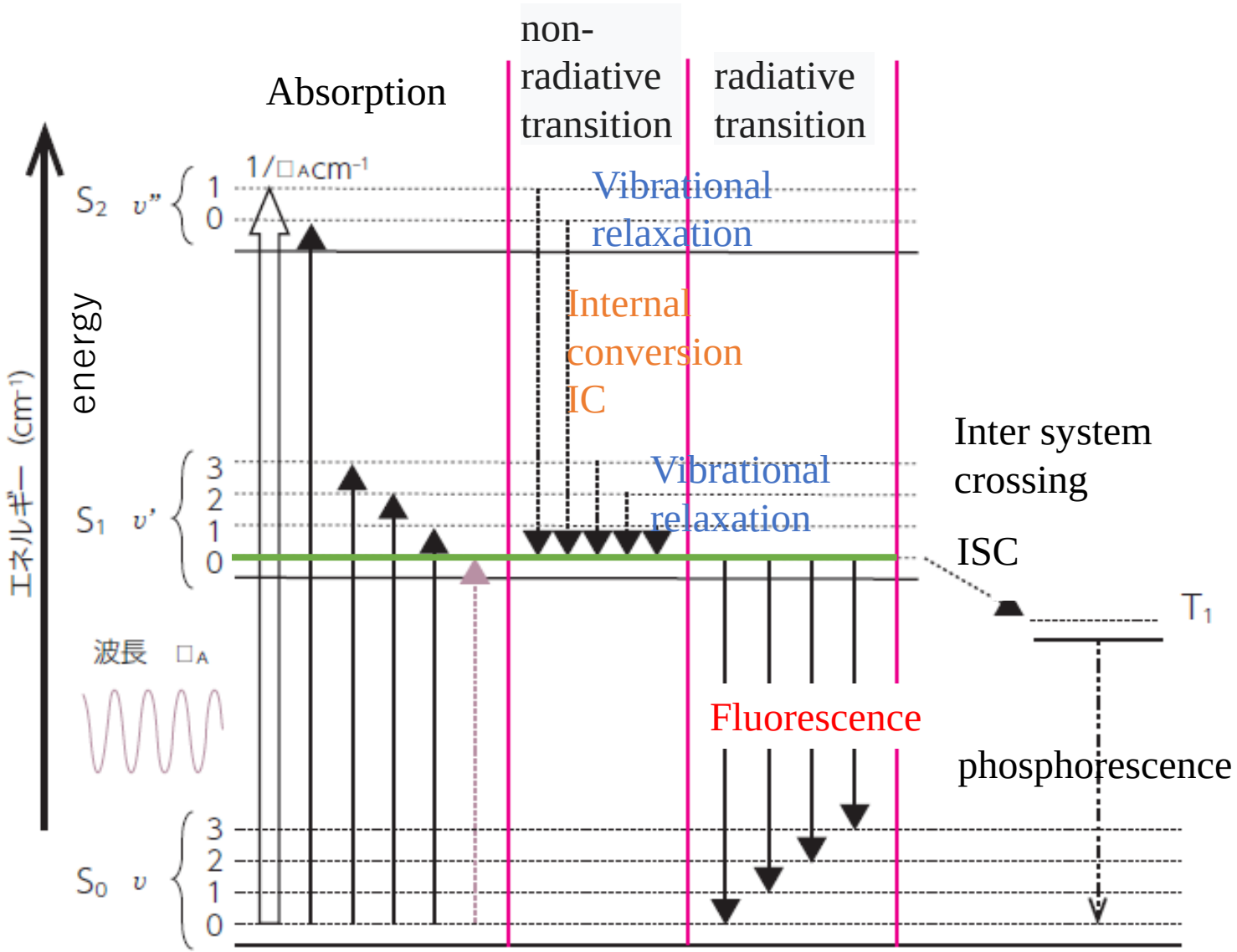
What can we learn from fluorescence spectroscopy?

- **Detection sensitivity and quantification**
- Detection sensitivity is 1 to 3 orders of magnitude higher because there is no background signal
- Quantitativeness is not good because it is influenced by various environmental factors.

- **Selective detection of analytes**
- Only molecules that absorb light at the incident wavelength emit fluorescence ⇒Excitation wavelength selectivity
- Limited analysis target ⇒Selectivity due to fluorescent labeling

- **Intermolecular interactions**
- Energy transfer from excited molecules to luminescent molecules may be visible
- Fluorescent label ⇒ Select by interaction

Fluorescence spectrum



Jablonski diagram

Molecules that absorb light
It loses energy through vibrational relaxation and internal transition (IC) and transitions to the singlet first excited vibrational ground state.

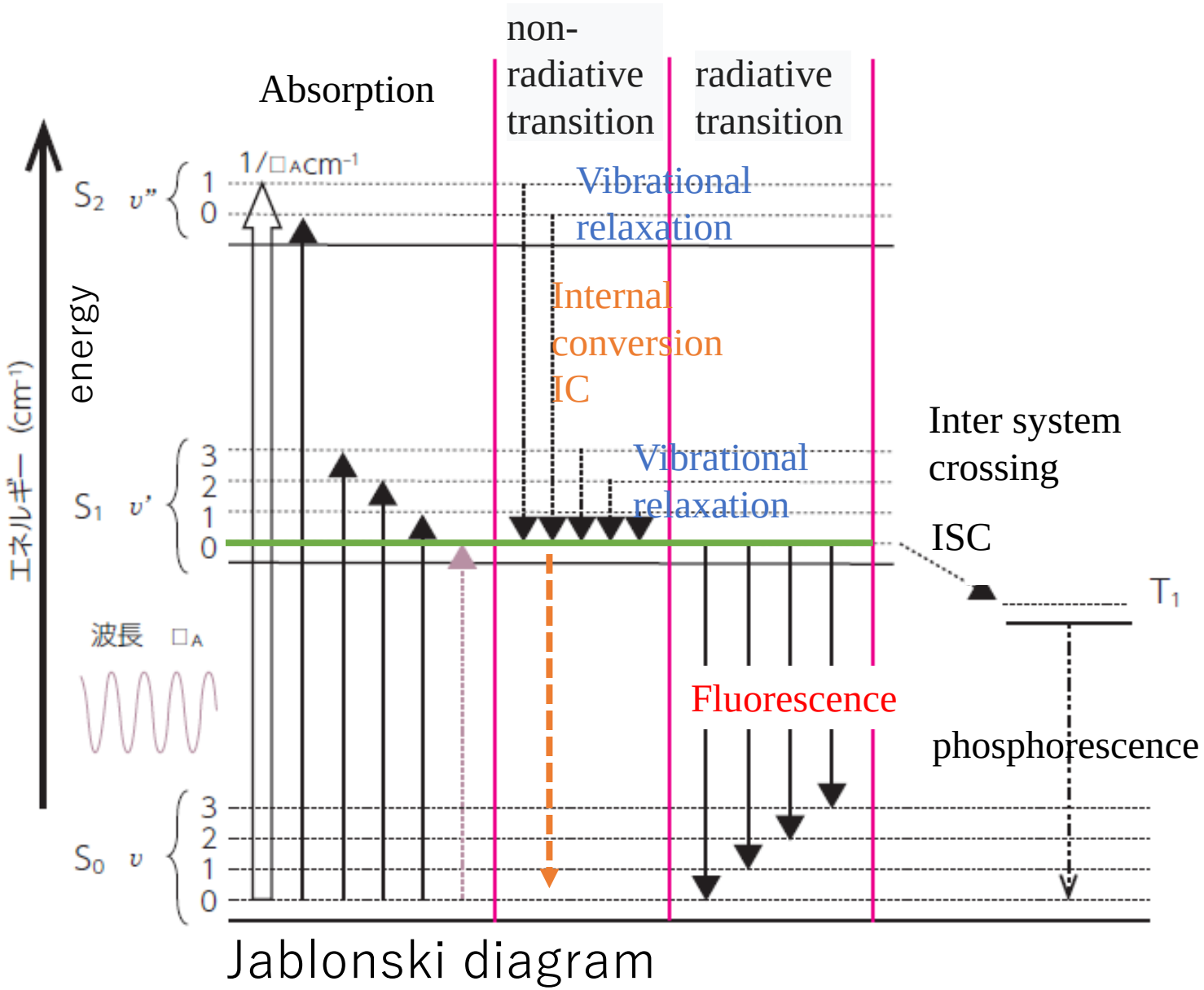
From there, energy is released as light → Fluorescence

蛍光の量子収率

$$\text{蛍光量子収率} = \frac{\text{(蛍光を発した分子数)}}{\text{(吸収された光子数)}}$$

量子収率

Fluorescence spectrum



Jablonski diagram

Molecules that absorb light
It loses energy through vibrational relaxation and internal transition (IC) and transitions to the singlet first excited vibrational ground state.

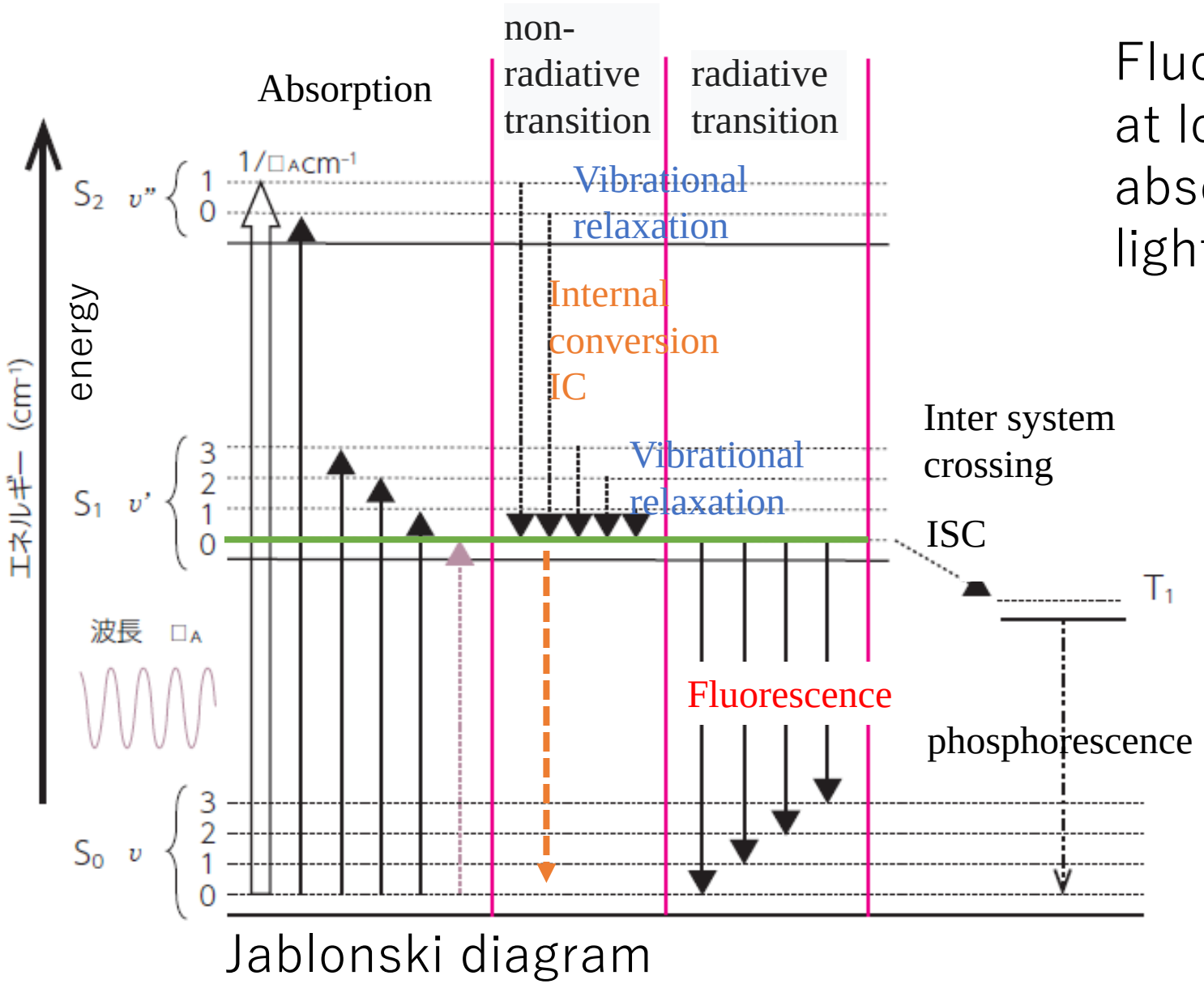
From there, energy is released as light → Fluorescence

Fluorescence quantum yield

Fluorescent molecules lost due to relaxation during non-radiative transition and ISC to triplet state.

$$\frac{(\#\text{ of molecules that emitted fluorescence})}{(\#\text{ of molecules that absorbed light})}$$

Stokes' law



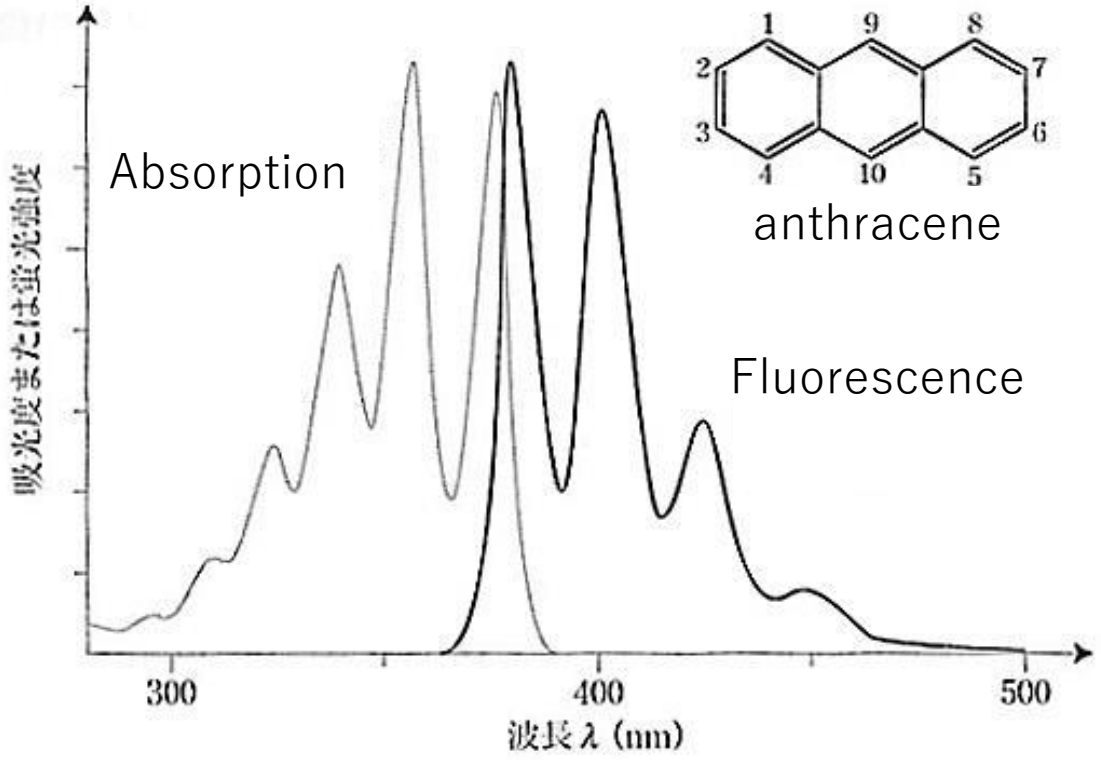
Jablonski diagram

Fluorescence spectrum is observed at longer wavelength than absorption spectrum (excitation light spectrum)

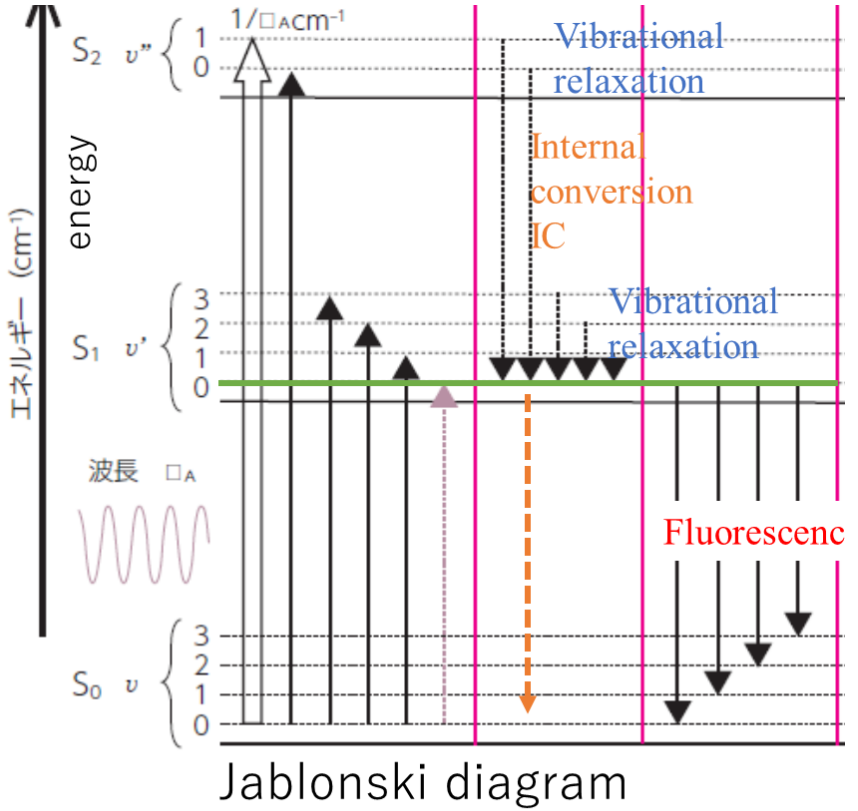
Fluorescence is emitted from the **singlet first excited vibrational ground state** after the energy absorbed from light is lost through non-radiative transition (vibrational relaxation).

Absorption spectrum and fluorescence spectrum

Fluorescence spectra are often observed as mirror images of absorption spectra.
But are they really the mirror image?



anthracene



Jablonski diagram

peak interval of absorption

peak interval of fluorescence

The spectral interval is not the same, but it is similar.
The structures of excited electronic state S1 and ground electronic state S0 are not the same, but they are similar.

Fluorescence intensity

Conditions: If it is a dilute solution

$F = k\varphi I_0 \varepsilon C x$

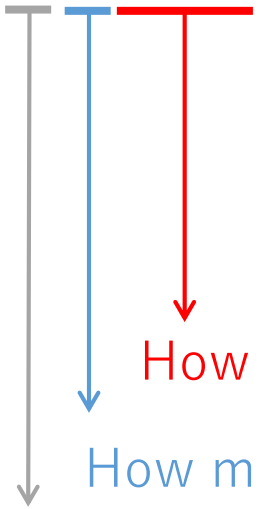
k ; Device constant (rate at which the device can collect trends)

φ ; Fluorescence quantum yield

Ratio of the number of photons emitted as fluorescence among the absorbed photons

I_0 ; light intensity of incident light

$\varepsilon C x$; absorption part



How many of the emitted photons were absorbed?

How many of the absorbed photons become fluorescent?

How many of the fluorescent photons were collected by the device?

Fluorescence intensity is proportional to concentration and quantitative analysis is possible only in dilute solutions.

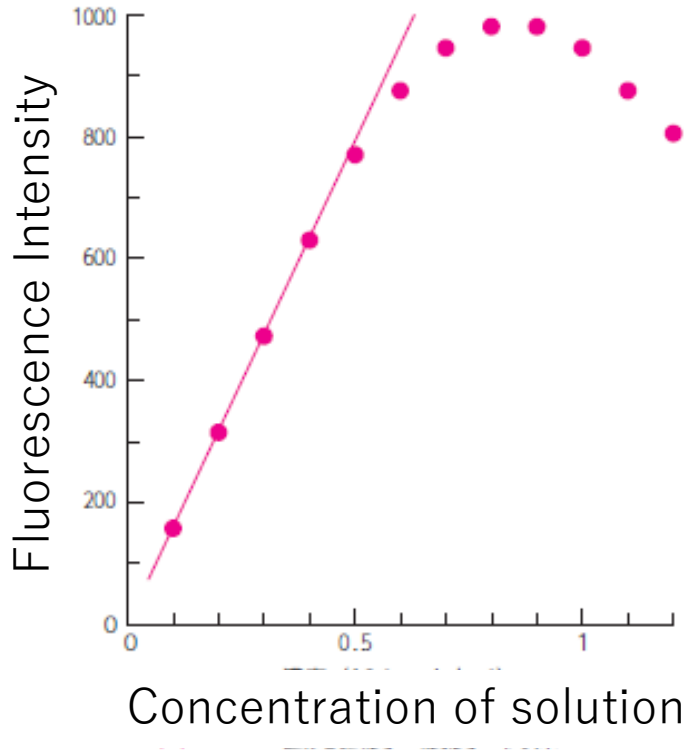
Quenching of fluorescence

Effect of temperature on quenching

Collisions with other molecules promote nonr
transitions. Thus, fluorescence intensity decr
temperature increase.

Concentration quenching

As the molecular concentration of the sample
the probability of (nonradiative) relaxation du
intermolecular interactions increases.



Self-absorption quenching

The emitted fluorescence is reabsorbed by the sample
and the fluorescence decreases.

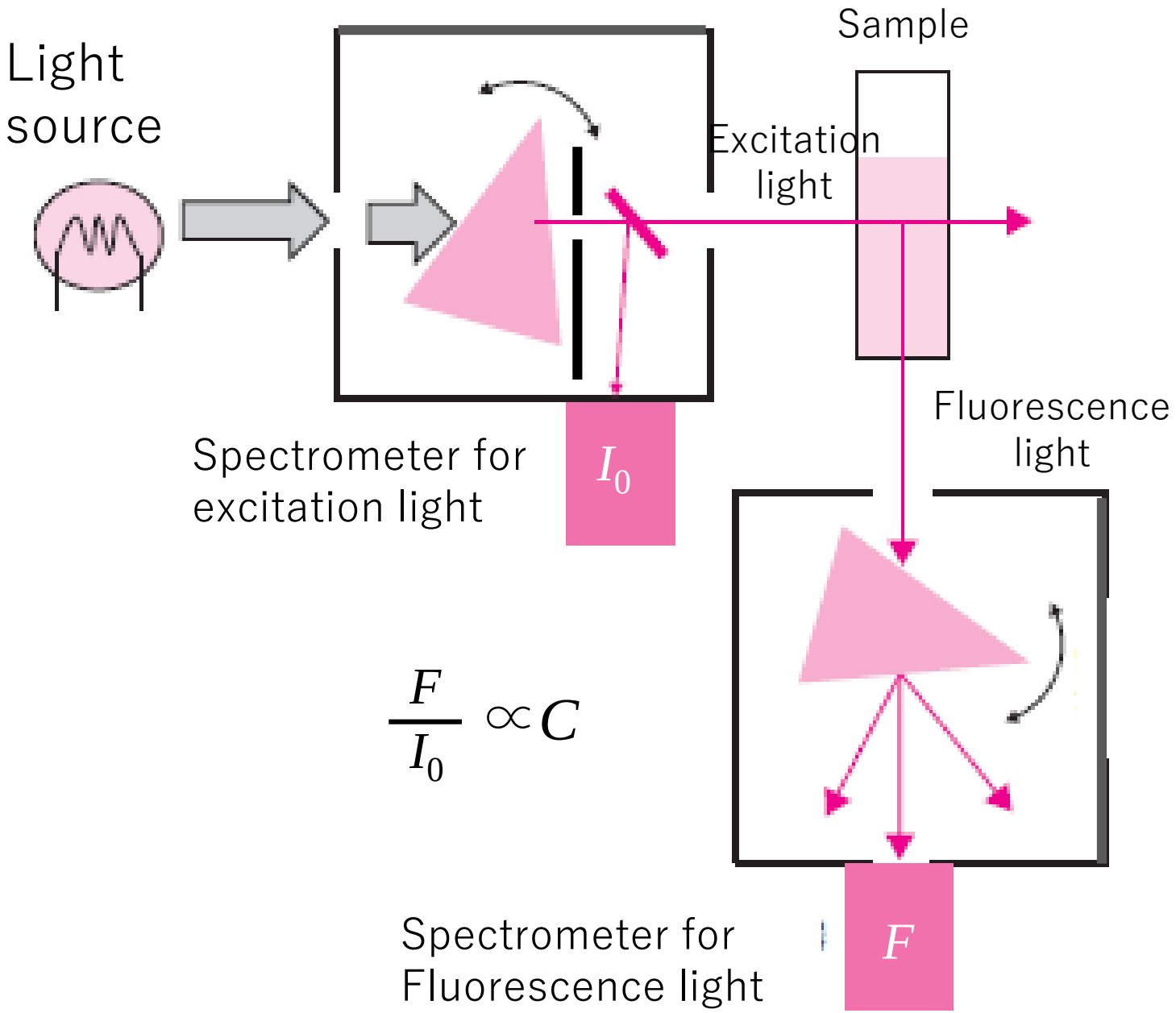
Quenching due to coexisting substances

The energy of the absorbed photon is transferred to the coexisting molecules.(ex. O_2, KI,)

Instruments of fluorescence spectroscopy

When using a lamp light source, a spectrometer for the excitation light source is required.
The light source can be monochromatic light such as a laser (rather preferable)

A sample cell with four transparent sides is used.
There are many instruments that collect fluorescence in the direction perpendicular to the excitation light.

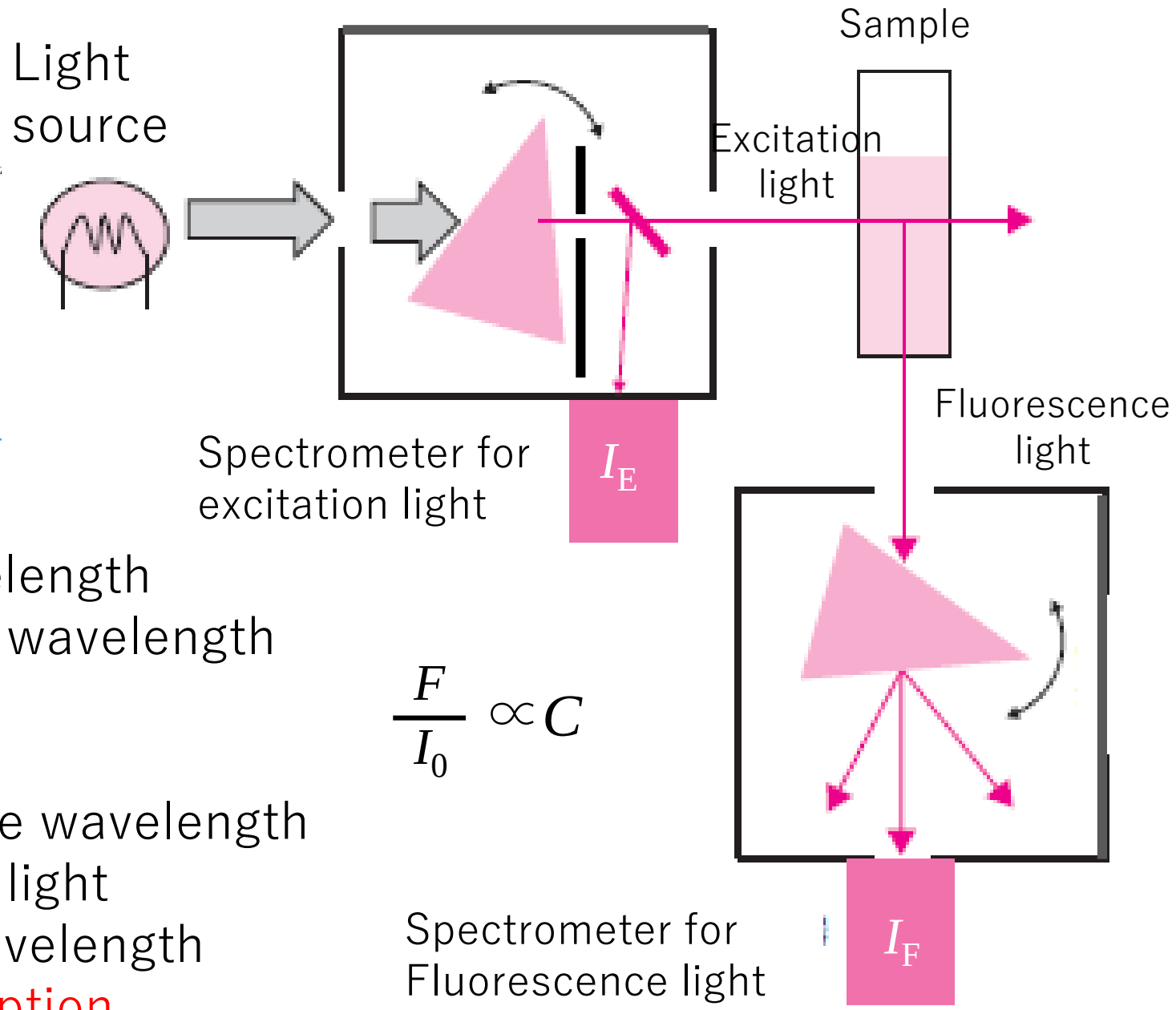


$$\frac{F}{I_0} \propto C$$

測り方で二種類の蛍光スペク
分光器部が二つある

Instruments of fluorescence spectroscopy

Since there are two spectrometers, there are two measurement methods in which only one spectrometer is scanned and fix the other wavelength.



Diffuse fluorescence spectrum

Scan the fluorescence wavelength by fixing the excitation light wavelength

$$\frac{F}{I_0} \propto C$$

Excitation fluorescence spectrum

While fixing the fluorescence wavelength and scanning the excitation light

→origin of fluorescence at that wavelength

Spectrum similar to absorption

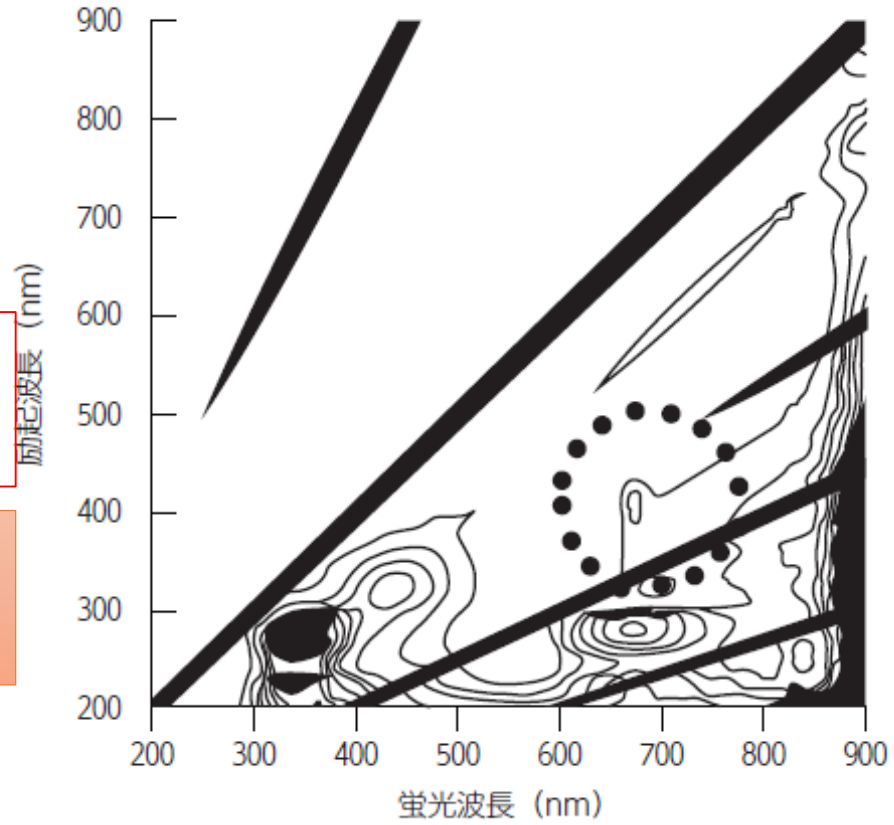
Excitation-emission matrix (EEM) (2D fluorescence,)

Measure diffuse fluorescence at various excitation wavelengths and stack them three-dimensionally (make two-dimensional contour lines)

Capable of spreading and analyzing spectra of complex mixtures in two dimensions

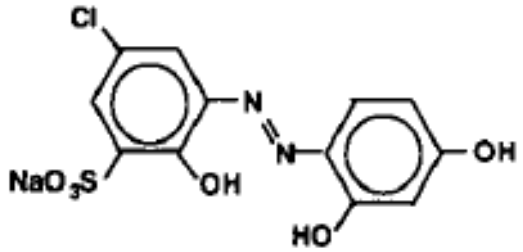
Diffuse fluorescence of different chemical species can be seen with each excitation light

EEM spectrum of
80% buckwheat flour and
20% wheat flour

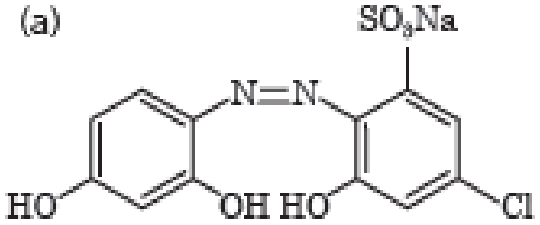


Introduction of polychromator shortens measurement time.
Applications such as machine learning are a research topic.

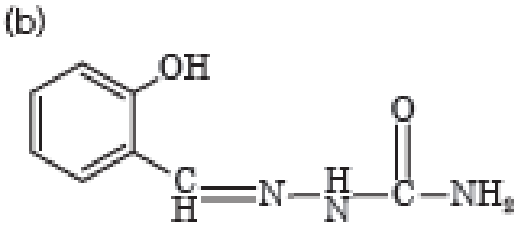
Fluorescent Labeling for metal ion



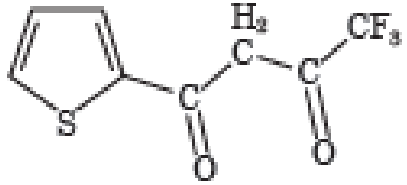
Lumogallion
(Al, Ga, and other)



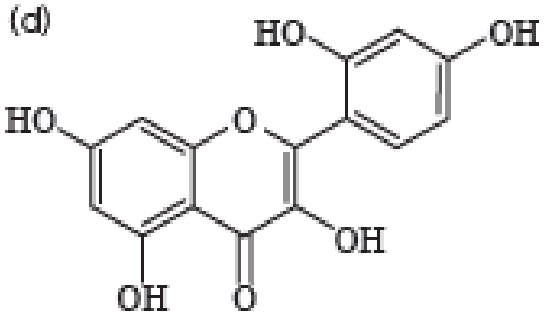
Alizarine Garnet
(Al,F-)



2-Hydroxybenzaldehyde semicarbazone
(Al, Ga, Sc, Y, Zn)



2-Thenoyltrifluoroacetone
(rear-earth ion)

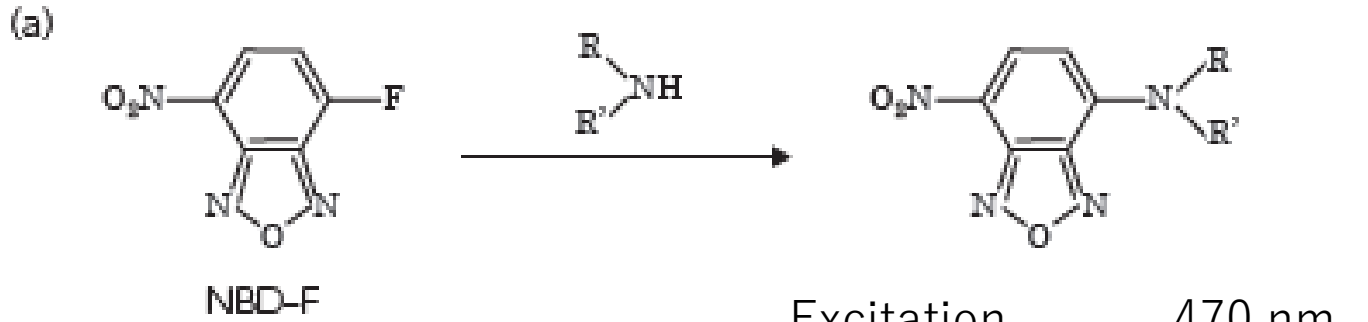


Morin
(Al, Be, Sc, Zr, Hf)

Emit unique fluorescence by forming a complex

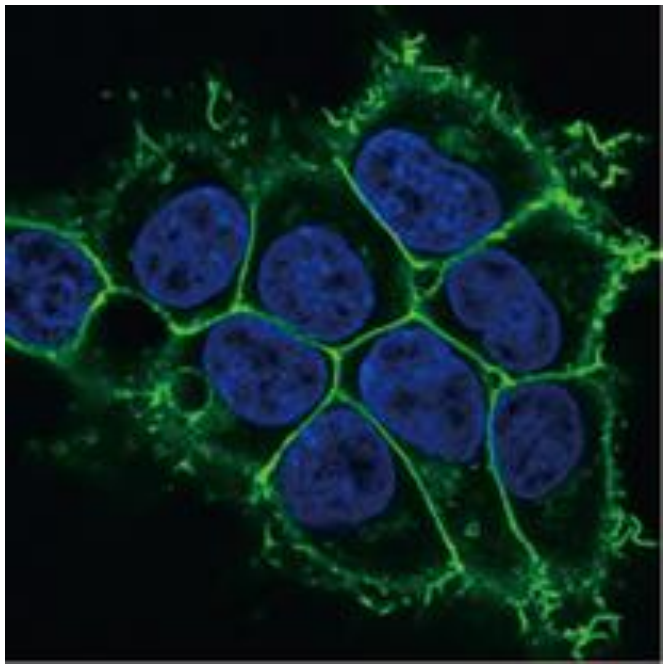
High sensitivity (nM, pM)

Fluorescent labeling for organic molecules (derivatization)

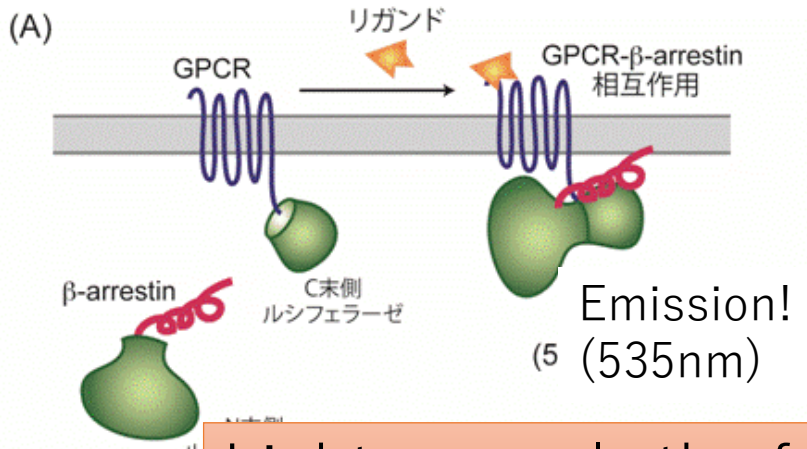


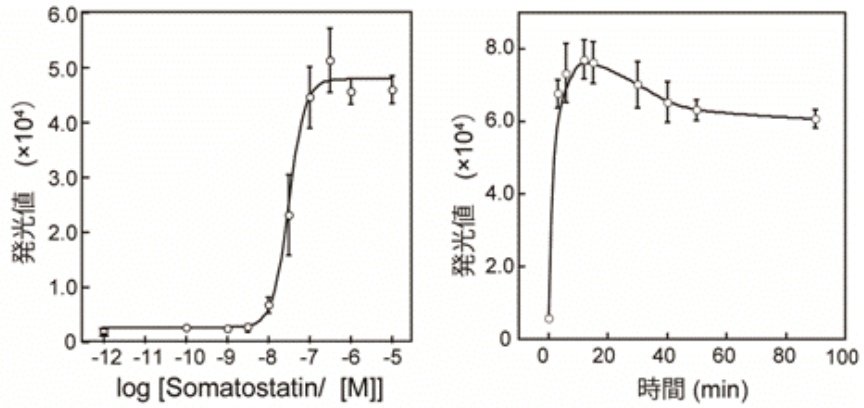
Analysis of biomolecules using fluorescence microscopy

Dividing the fluorophore luciferase into two
Luciferase reconstitutes only at the target site and emits light

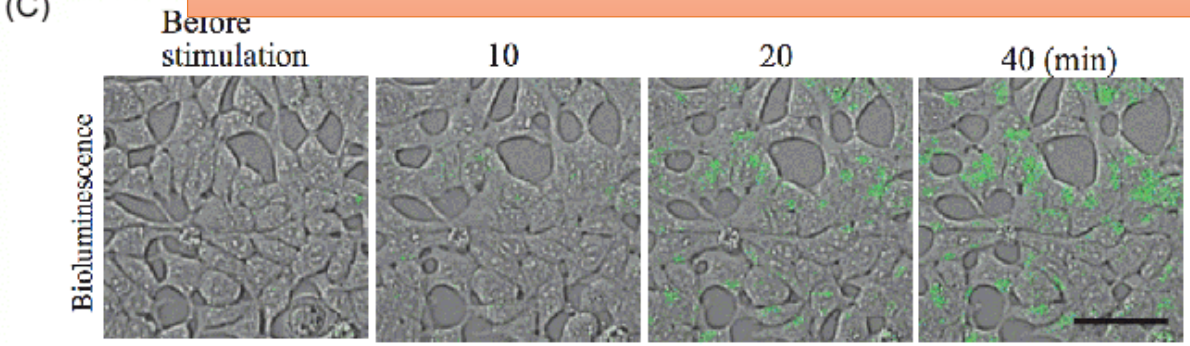


Staining different types of biomolecules with fluorescent labels of different colors





Lights up only the functional areas of biomolecules



Ozawa group in University of Tokyo http://www.chem.s.u-tokyo.ac.jp/users/analyt/research/

GPCR scriining method http://www.chem.s.u-tokyo.ac.jp/users/analyt/research/gpcr.html

Fluorescens Spectroscopy

Detection sensitivity and quantification

- Detection sensitivity is 1 to 3 orders of magnitude higher because there is no background signal
- Quantitativeness is not good because it is influenced by various environmental factors.--- quenching

Application

- EEM for complex mixture
- Selective detection --- Fluorescence Labeling
- Fluorescent microscope--- biomolecules