SibFU-2
February 2012

# CHEMILUMINESCENCE

**BIOLUMINESCENCE** is an **ENZYME CATALYZED CHEMILUMINESCENCE**

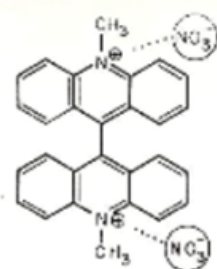# Chemical Light

- ## Only a 130-year history

- 1877 Radziszewski: Lophine/$O_2$
- 1928 Albrecht: Luminol/$O_2$
- 1963 Chandross: Oxalyl chloride/$H_2O_2$/fluorescent dye
- 1964 Weller: $e^-$ –transfer chemiluminescence
- 1982 Schaap: TMMPD/$OH^-$ (a dioxetanone)
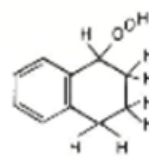
- These are the well-investigated cases
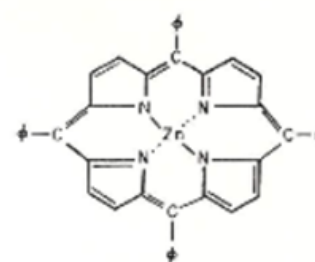
# Variety of CL reactions
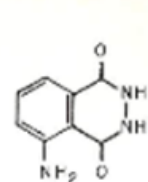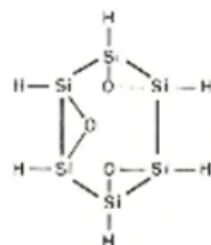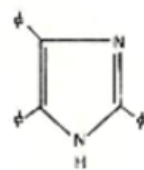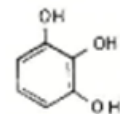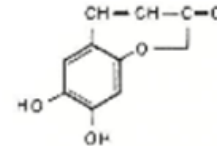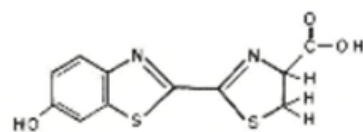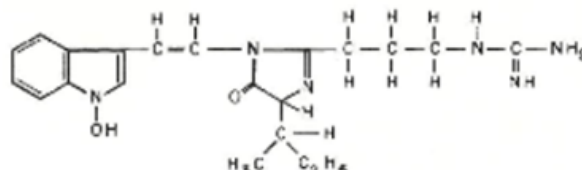
# AMPPD
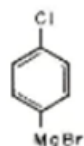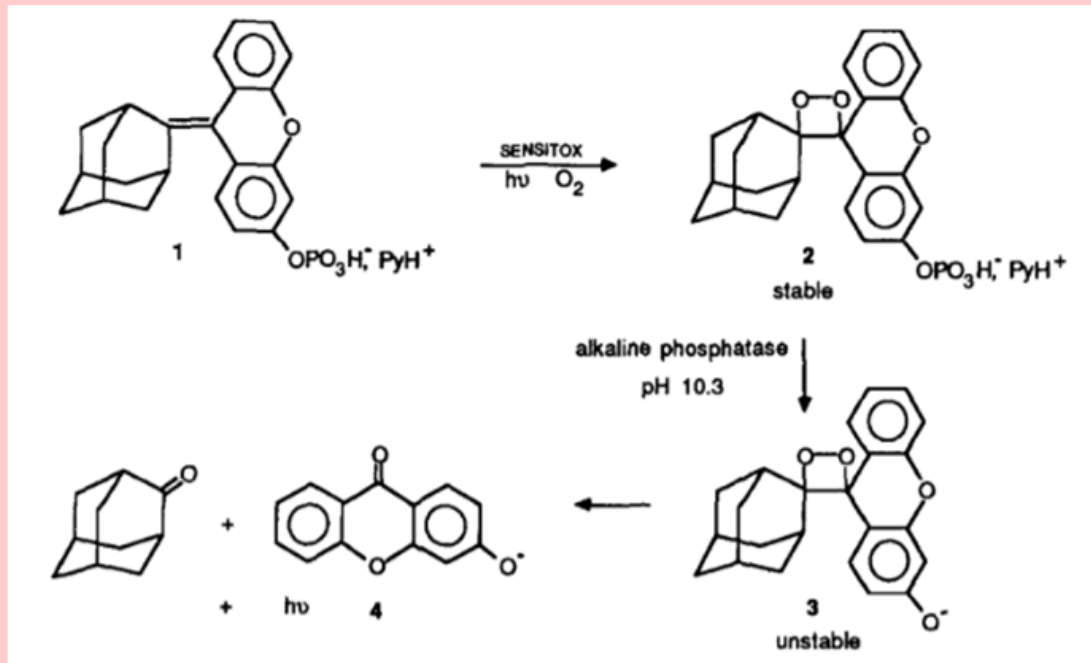


Application to any diagnostic protocol using alkaline phosphatase

# Proposed chemistry for Luminol chemiluminescence



luminol
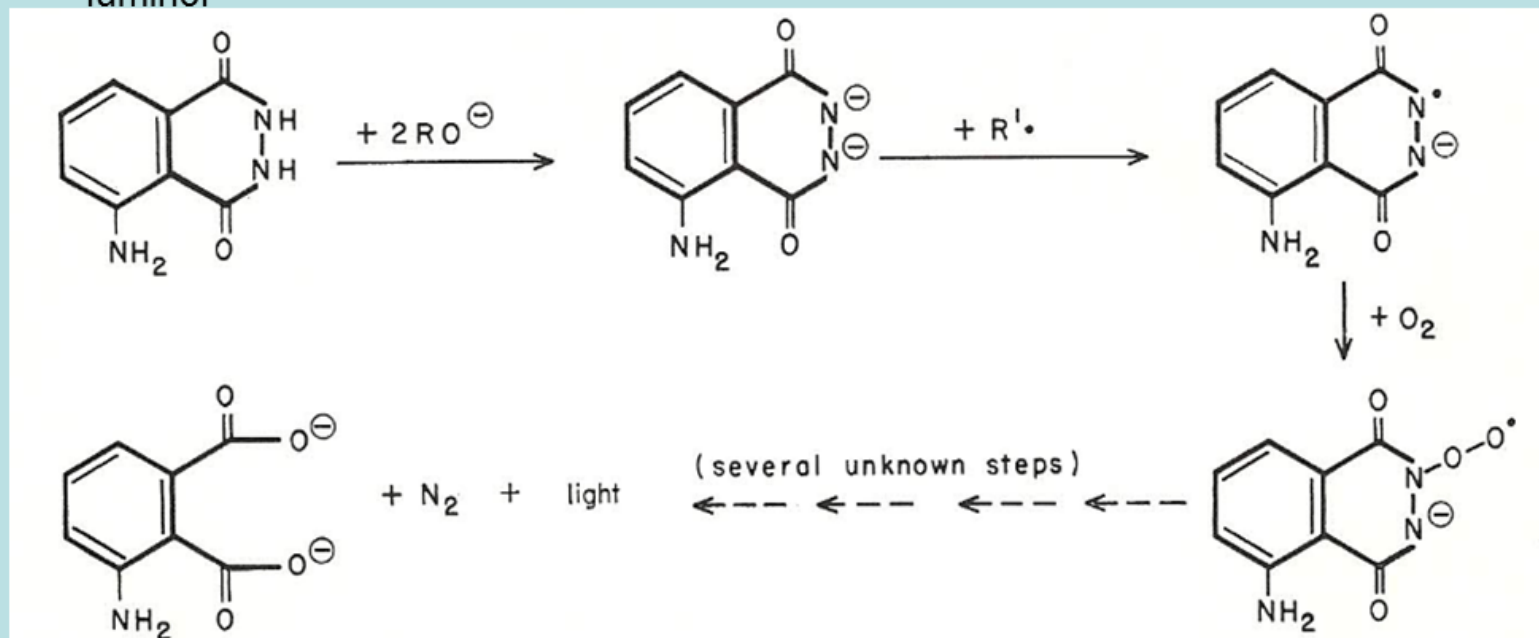
3-amino-phthalate

# Chemi-excitation

$$LH_2 + O_2 \longrightarrow L{=}O^* + H_2O$$
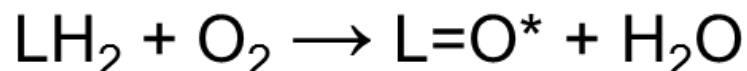
Chemical exothermicity must be sufficient to populate the singlet excited state, the same as photon excited L=O.

To identify L=O* as the CL emitter, its **fluorescence spectrum** should be close to a match to the CL spectrum.

# Luminol spectral match

The close match of the luminol chemiluminescence emission and the 3-aminophthalate fluorescence, indicates that the 3-aminophthlalate (AP) is the emitter.

# Abbreviations

$$LH_2 + O_2 \rightarrow H_2O + L{=}O^* \quad \text{(excited state)}$$
$$L{=}O^* \rightarrow L{=}O + h\nu \text{ (light)}$$
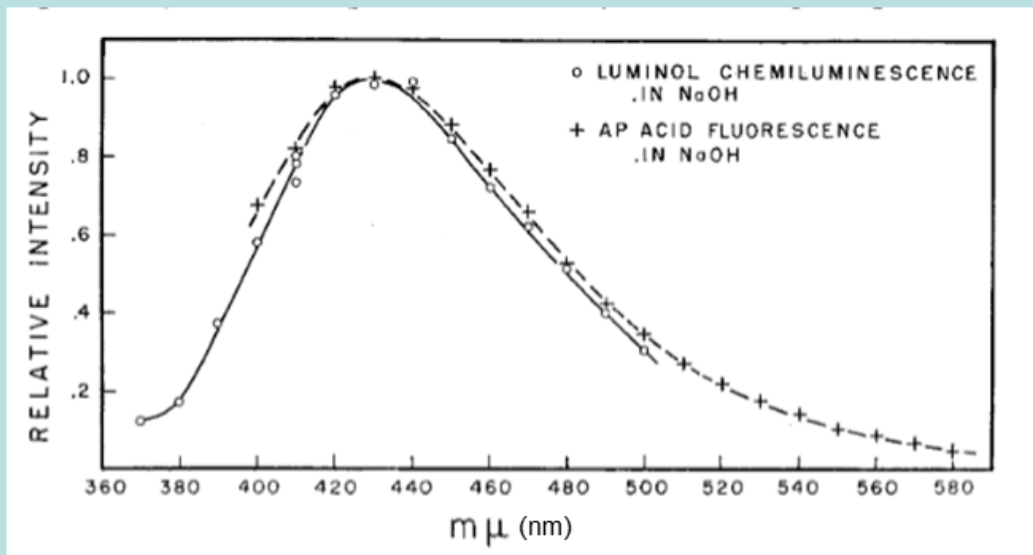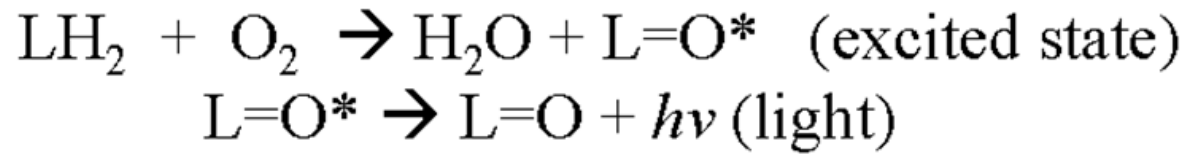
$Q_Y$ = chemical yield of L=O

$Q_F$ = fluorescence efficiency

$Q_E$ = excitation efficiency

$Q_C$ or $Q_B$ = quantum yield of CL or BL

$$\mathbf{Q_C = Q_Y \times Q_F \times Q_E}$$

# Absorption -> Fluorescence
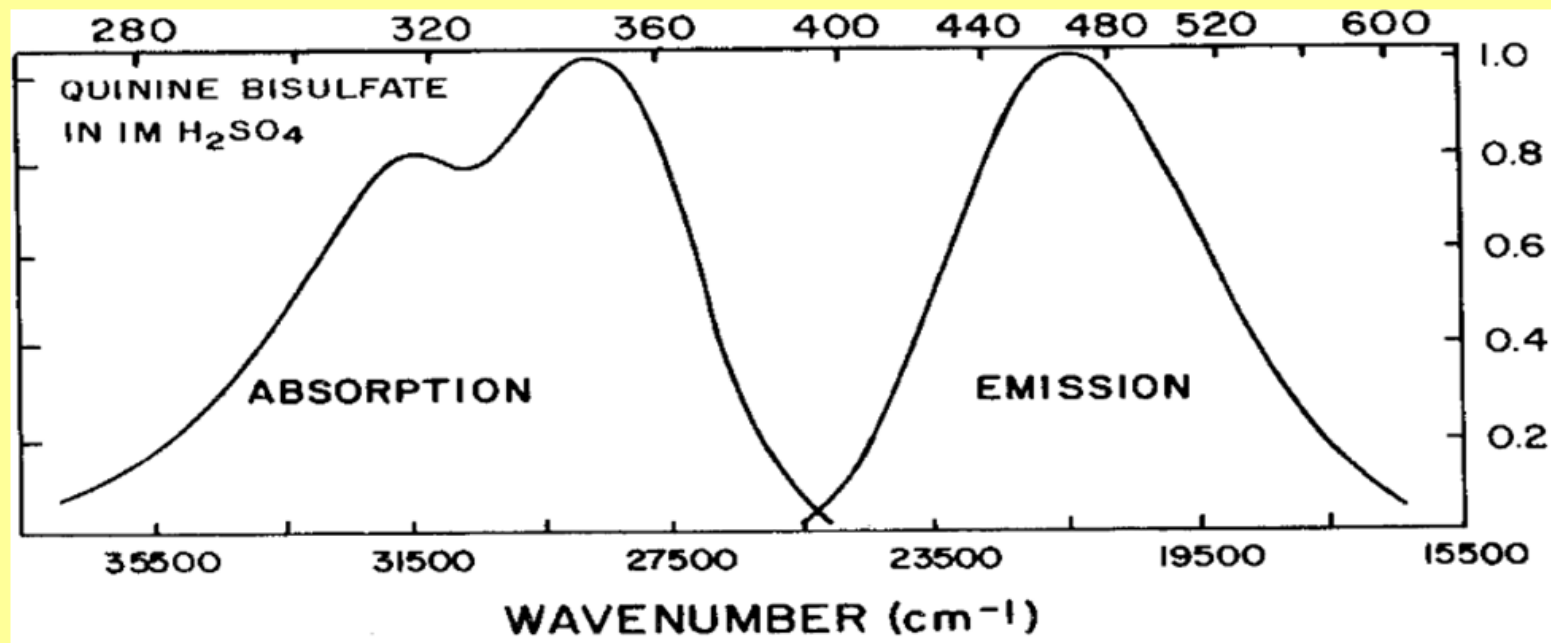


Light excitation in the absorption band produces fluorescence at longer wavelengths

# Franck-Condon Principle

1. Electronic transitions are essentially **instantaneous** ($10^{-16}$ s) on the time-scale of nuclear movement ($10^{-12}$ s).

2. These are called **vertical** transitions, from the ground to the Franck-Condon State.

3. Transition probability $\propto$ **overlap** of respective vibrational wave-functions.

# Vertical Transitions
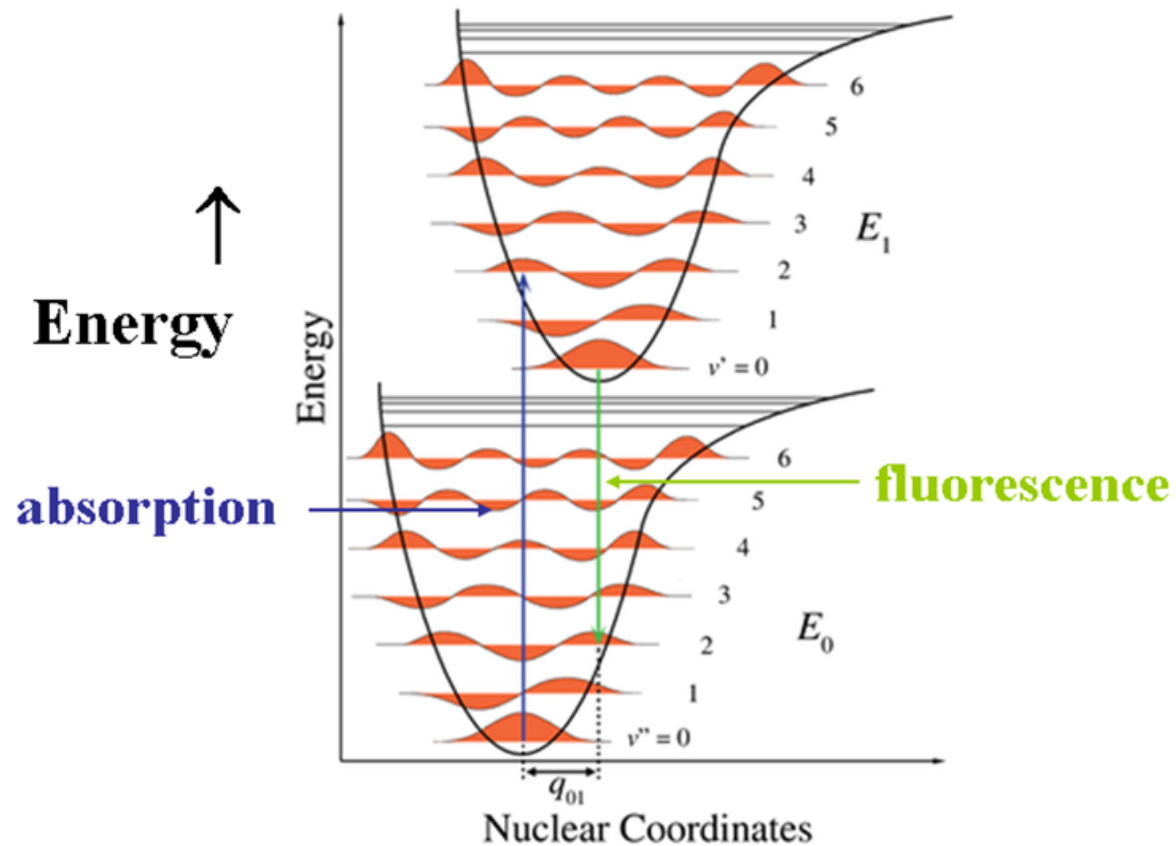


Molecular dimensions →

# Spectrum Analysis

1. The harmonic oscillator model for the potential well predicts the transition probabilities to be statistical, i.e., Normal or Gaussian distributions.

$$\frac{1}{\sigma\sqrt{2\pi}}\exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$$
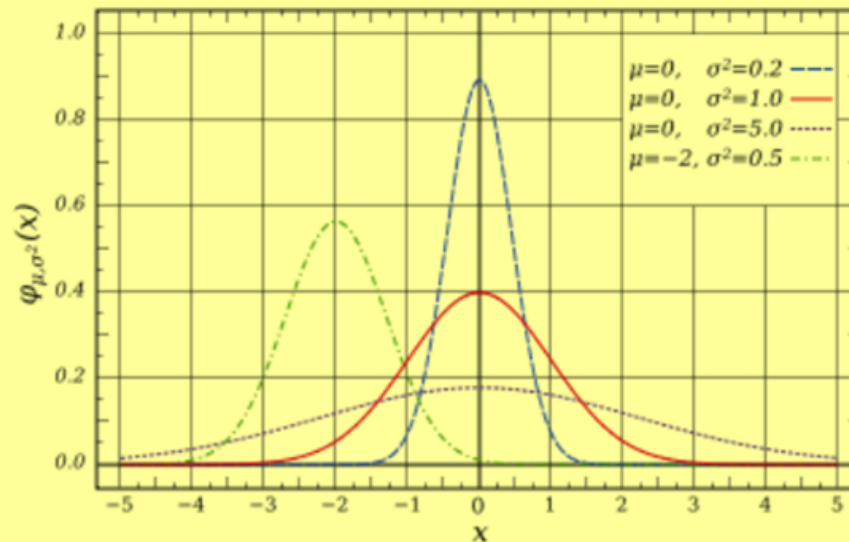
# Skewed Gaussians

In reality the well functions are anharmonic due to dissociation at the highest energies and are better approximated by the "Morse Function".
**Asymmetric** Gaussian fits are required.
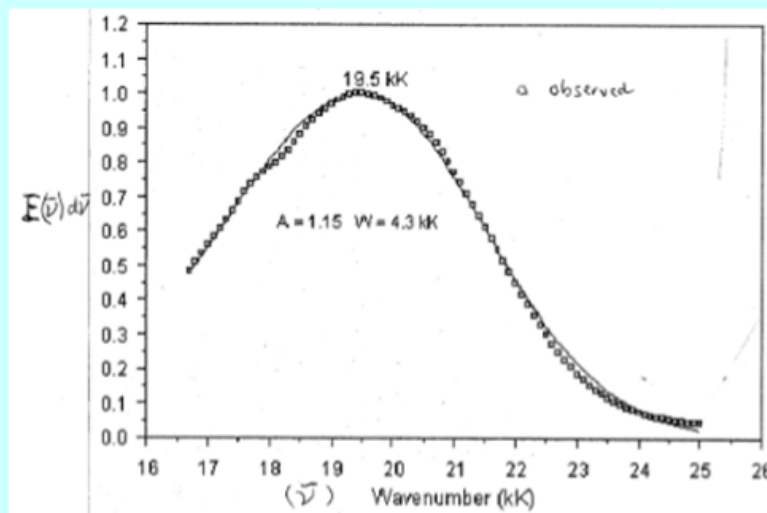
# Diffraction Grating Spectrometer

Spectra obtained experimentally need to be corrected for artifacts:

1. The dispersion is constant with $\lambda$ so the spectra are $F(\lambda)\Delta\lambda$ versus $\lambda$.

2. The grating is "blazed" to transmit maximally at say 500 nm and is also polarization dependent.

3. Chemiluminescence intensity decay $dF/dt$, must be corrected for, as the grating scans $d\lambda/dt$.

# Energy Spectra

Physically meaningful spectra are a plot of the function $\Delta E(\nu)/\Delta \nu = F(\nu)$. Experimental spectra however are collected as the function

$$\Delta F(\lambda)/\Delta \lambda = F'(\lambda)$$

frequency, $\nu = c/\lambda$

$$\Delta \nu = -c. \, \Delta \lambda / \lambda^2$$

$$\mathbf{F}(\nu) = \mathbf{-c.} \, \lambda^2 \, \mathbf{F'}(\lambda)$$

# Firefly analysis

Green-orange range of BL all have FWHM = 2600 cm$^{-1}$. The red BL with Zn$^{2+}$, is narrower, a different electronic form, FWHM = 1700 cm$^{-1}$.
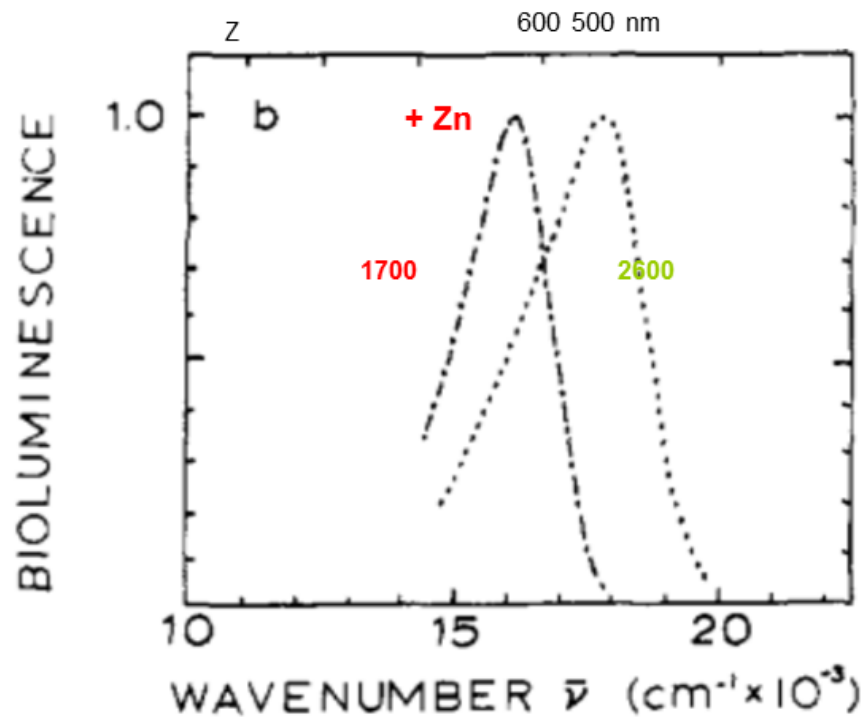
# Chemi-excitation

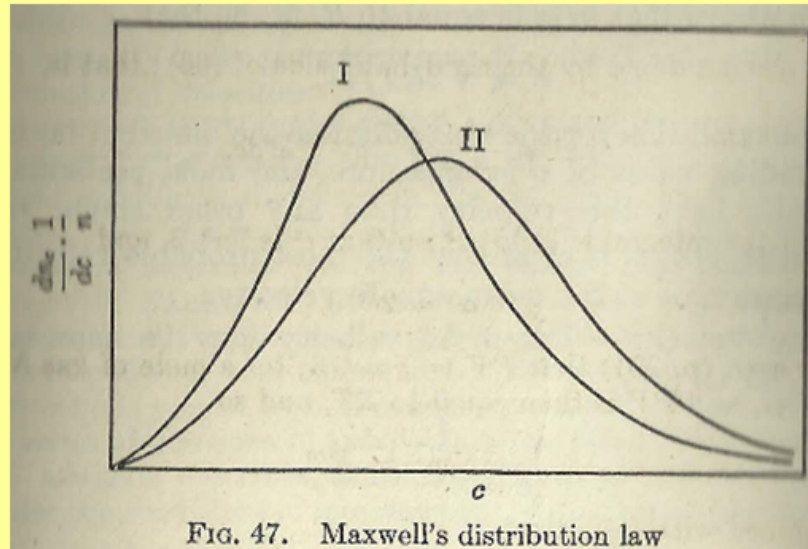- Chemi-excitation has a reverse Franck-Condon problem

$$A + B \longrightarrow C - D^* \longrightarrow CD + h\nu$$

- Chemical energy is released by bond breakage from slow movement of atoms.

- **Contradicts** the Franck-Condon principle.

# Energies of molecules
## Maxwell-Boltzman Distribution



FIG. 47.   Maxwell's distribution law

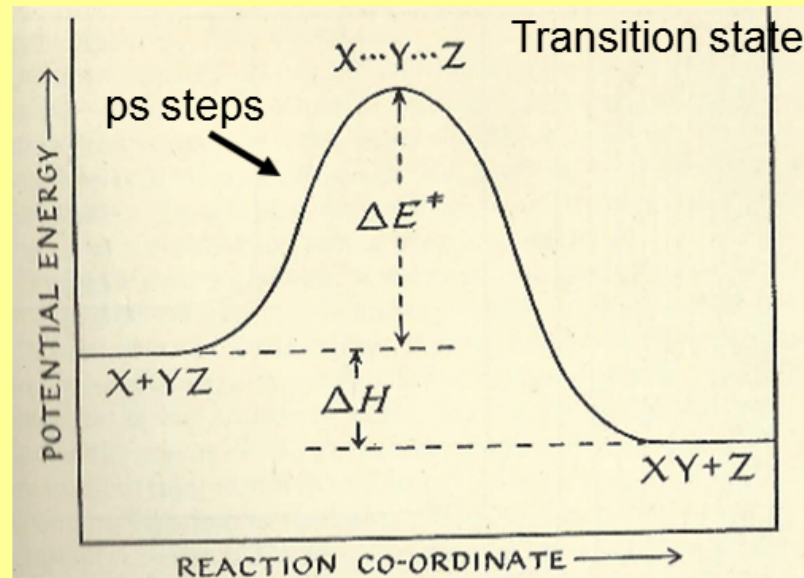In a system of N molecules, their velocities or energies ($\varepsilon$) are distributed according to probabilities:

$n = n_0 \exp(-\varepsilon/kT)$, where

$n$ = # with energy $\varepsilon$

$n_0$ = # in energy level $< \varepsilon$ .
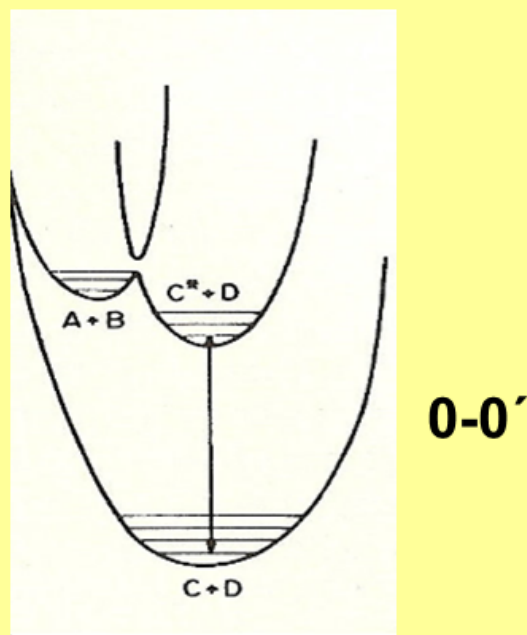
# Chemical Reaction Energy
## Transition State Theory



Molecular collisions occur in 1-10 ps steps and those that achieve an energy $> \Delta E^{\ddagger}$ (activation) jump over to products.
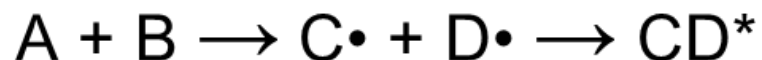$\Delta H$ = enthalpy

# 0-0´-Excitation Energy



0-0´

The reaction must release energy > **0-0´**

# Chemiexcitation Theories
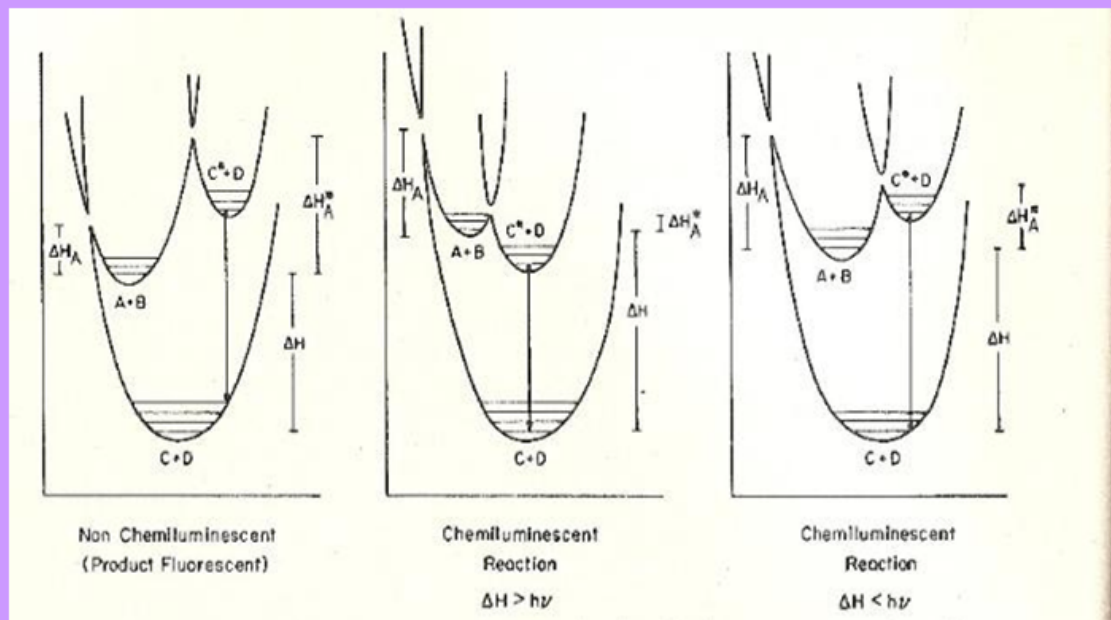
1. Radical recombination

A + B ⟶ C• + D• ⟶ CD*

This would be a "vertical" process

Electrochemiluminescence
Chemically induced electron exchange
luminescence (CIEEL)

2. Crossing of reaction potential energy surfaces
(R. Marcus, Nobel Chemistry, 1992)

# Marcus Theory



Marcus proposed reaction co-ordinate crossing near the transition state

# Oxalyl chloride CL

In 1963 Chandross discovered the bright chemiluminescence from the reaction of oxalyl chloride with $H_2O_2$ provided the mixture also contained a highly fluorescent dye. The emission was from the dye fluorescence.

$$Cl-O-\underset{\underset{O}{\|}}{C}-\underset{\underset{O}{\|}}{C}-O-Cl + H_2O_2 + dye \rightarrow dye^* + products$$
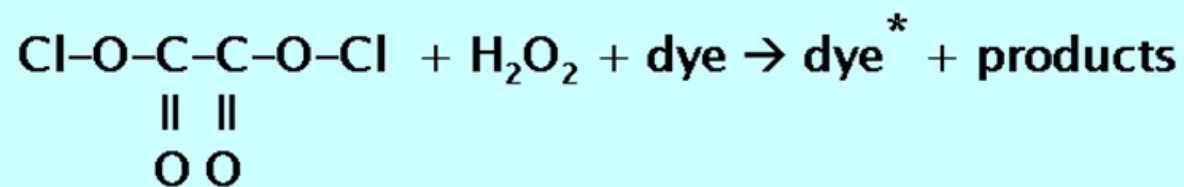
# Glow-stick Reaction
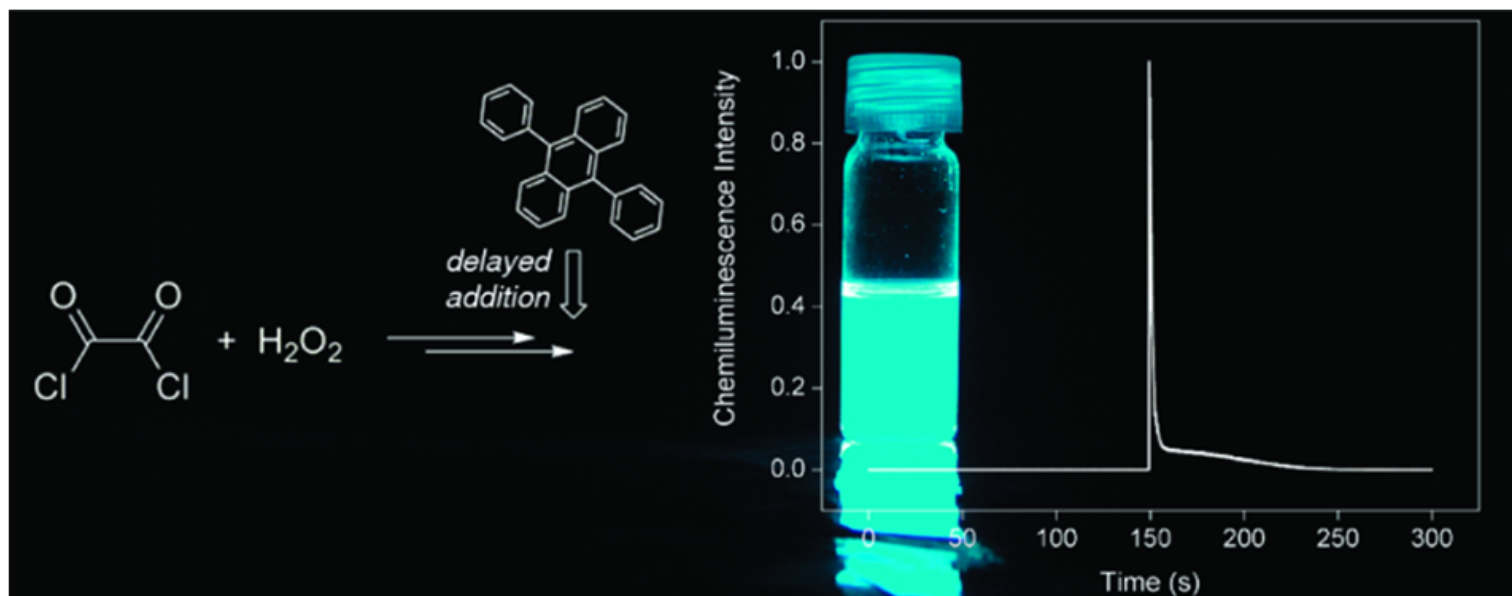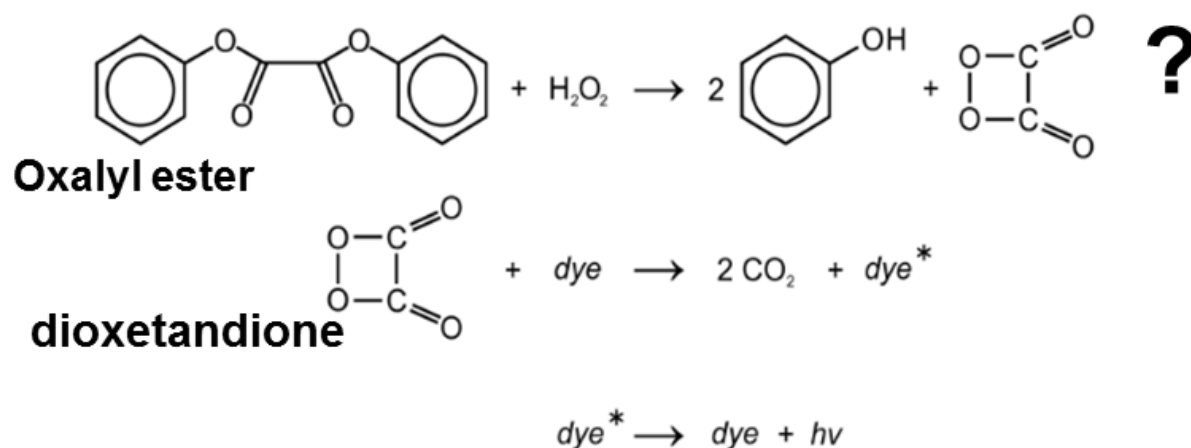


# A high-energy intermediate is stable for >150 s

# The Light Stick
## (Glowstick, Cyalum))

- Invented in 1965 at the American Cyanamide Co.
- High $Q_C = 0.34$
- Mechanism is intensely investigated 1965-2012
- Dioxetandione high energy intermediate proposed.



**Oxalyl ester**

**dioxetandione**

$$dioxetandione + dye \longrightarrow 2\ CO_2 + dye^*$$

$$dye^* \longrightarrow dye + h\nu$$

# Sensitized Chemiluminescence

• The light stick emitter is the excited state of the included dye.
• The mechanism of dye excitation is still a mystery
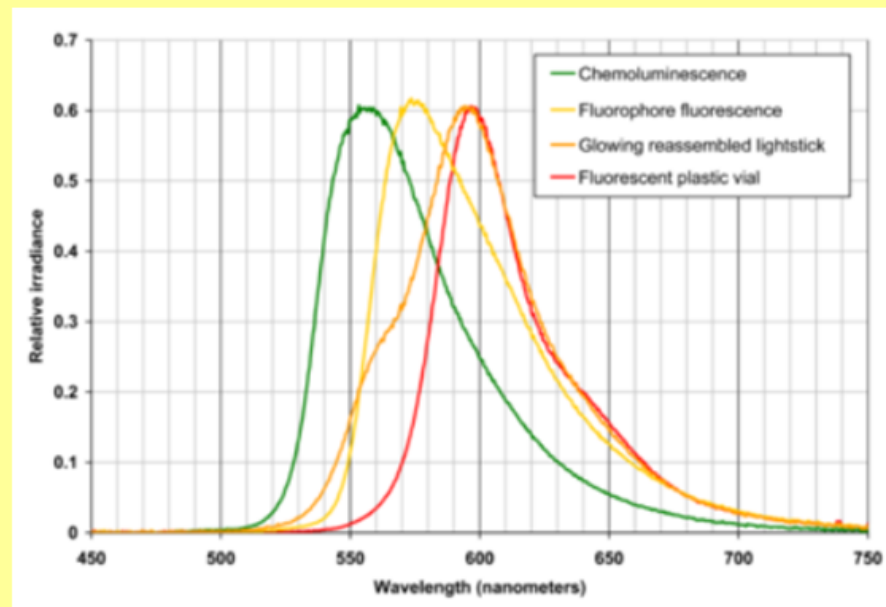
# Evidence for the dioxetandione

2008. The transient high energy intermediate was trapped and characterized by low temperature Carbon-13 Nuclear Magnetic Resonance.
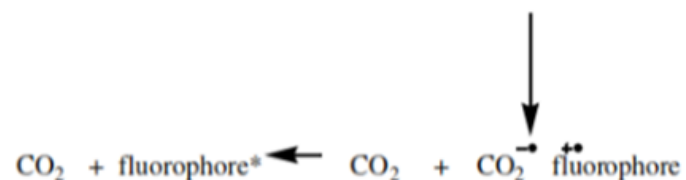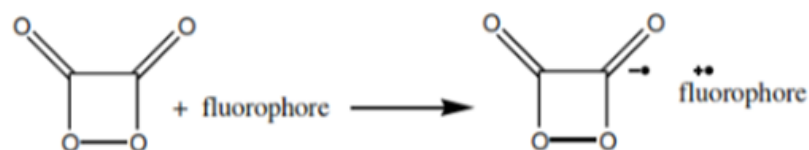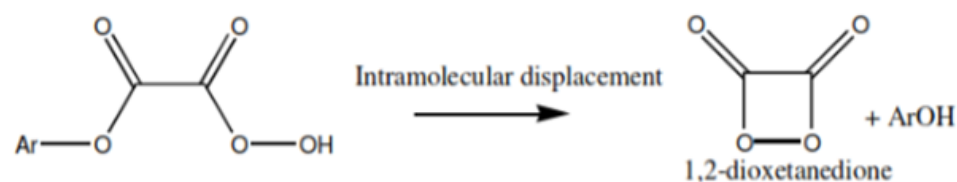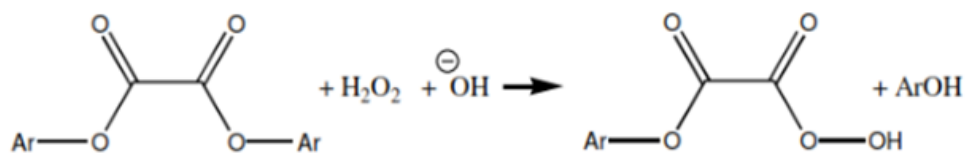


SCHEME 1. Elementary Peroxyoxalate Reaction Mechanism
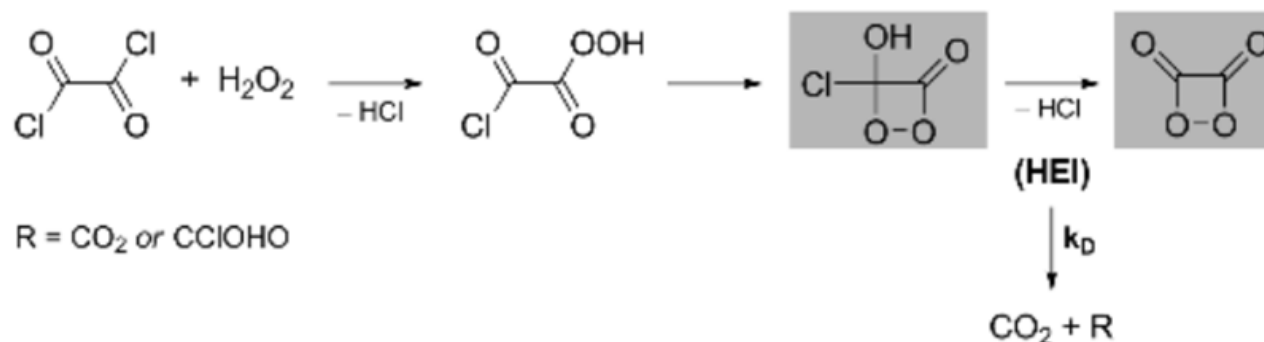
# $^{13}$C-NMR
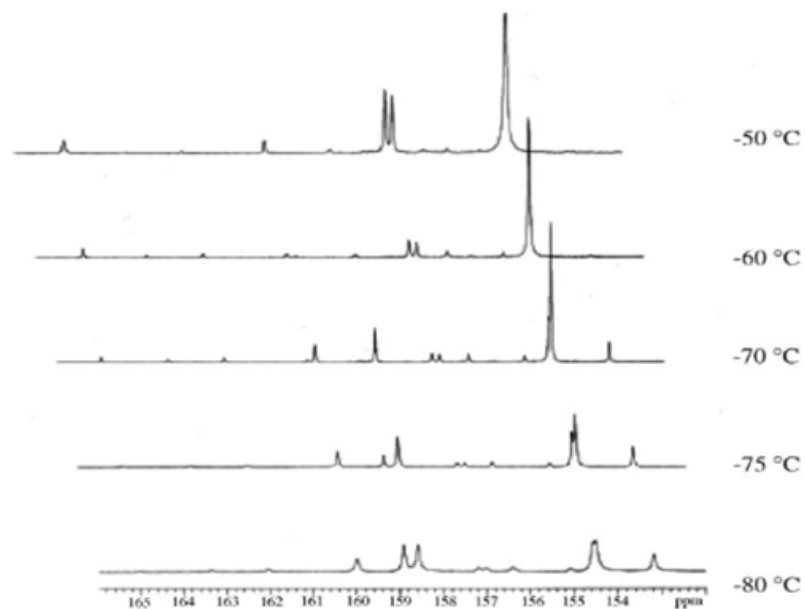
- **Bonding environment** of a C atom is reflected by the signal from the specifically enriched $^{13}$C.

- $^{12}$C (naturally abundant) gives no signal. $^{14}$C is radioactive.

- For $^{13}$C-NMR, the molecule is synthesized **enriched** with $^{13}$C.

# NMR of Carbon-13 enriched oxalyl ester

# ¹³C-NMR result

Reaction with $H_2O_2$ in tetrahydrofuran (THF) at -80 ºC in presence of dye, 9,10-diphenylanthracene.

# C-13 assignments

- Assignments: -80C oxalyl Cl 158.9
- -70C monoperoxyoxCl, doublets 153.9 and 159.3 is the unsymmetrical intermediate (2). These disappear by -60.
- -70C Dioxetanedione, singlet 154.5; disappears >-20C.
- -60 154.5 broadened indicating dynamic process and is reversible.
- >-60 160.4 unassigned
- <RT all show 125.6 $CO_2$ and 184.5 CO.

# Dioxetanes

First stable dioxetane synthesized in 1969 was found to be brightly chemiluminescent. Efficient preparation by reaction of **singlet oxygen** with an olefin, e.g.
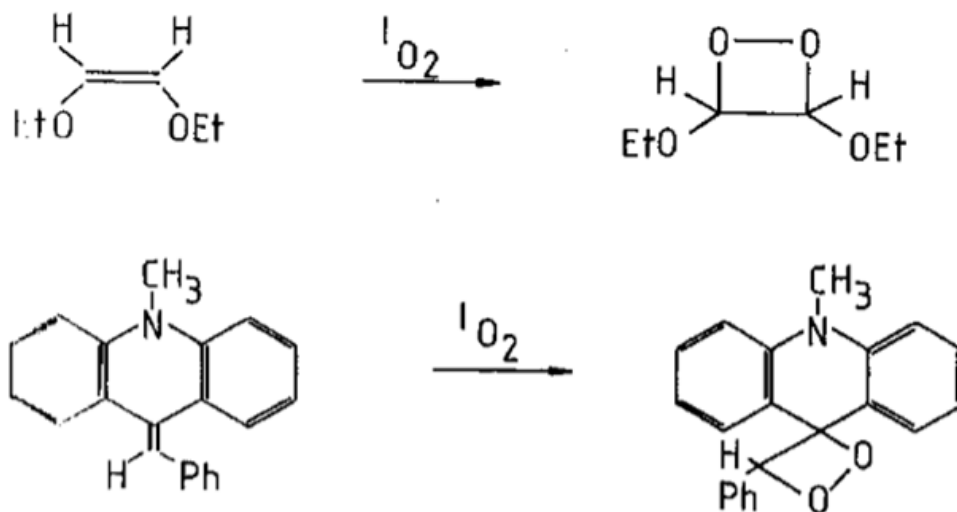
# Singlet Oxygen

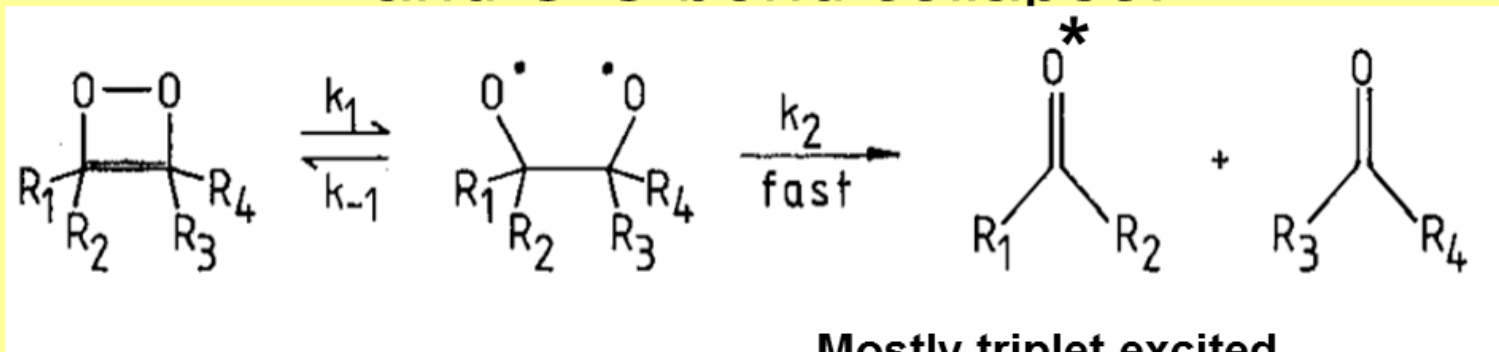Molecular oxygen by itself is not very chemically reactive due to spin conservation rules.

Singlet oxygen ( $O_2 {}^1\Delta_G$ ) is produced by photosensitization via a dye triplet state. **Methylene Blue** is popular.

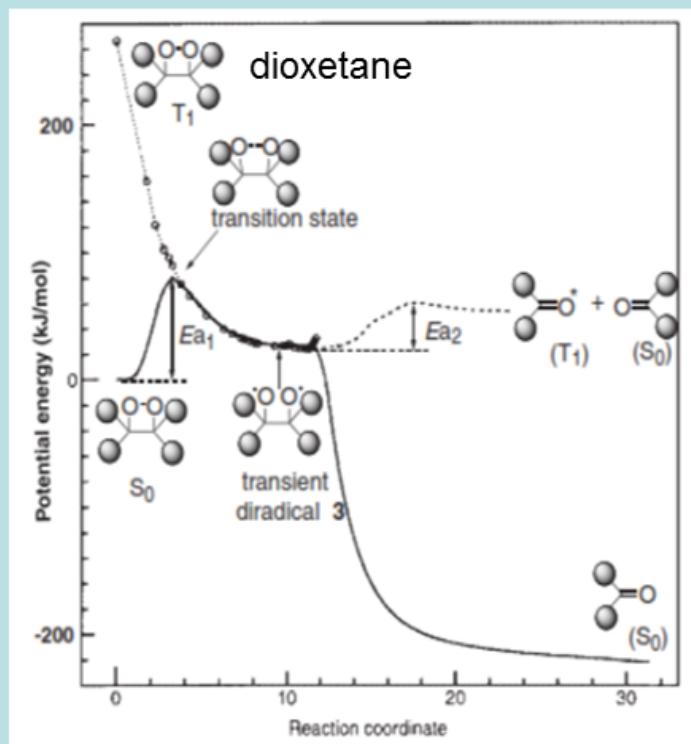$$^3MeB^* + {}^3O_2 \rightarrow MeB + O_2{}^1\Delta_G$$

# Diradical Excitation

Homolytic bond scission of the weaker O-O bond occurs, then simultaneous annihilation and C-C bond collapse.



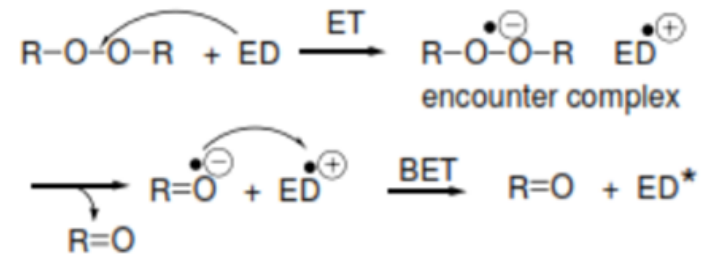**Mostly triplet excited states**

# Quantum Chemistry



After breakage of O-O, computation shows that the excited state ($T_1$) and transient are degenerate $\longrightarrow$ one $T_1$ ketone product and the other in the $S_0$ ground state.
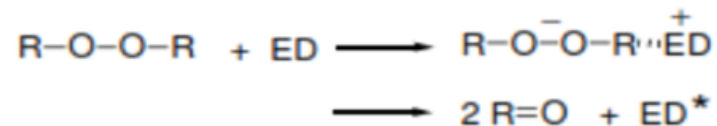
# Chemiexcitation
## CIEEL or CTIL?

**C**hemically **I**nitiated **E**lectron **E**xchange **L**uminescence
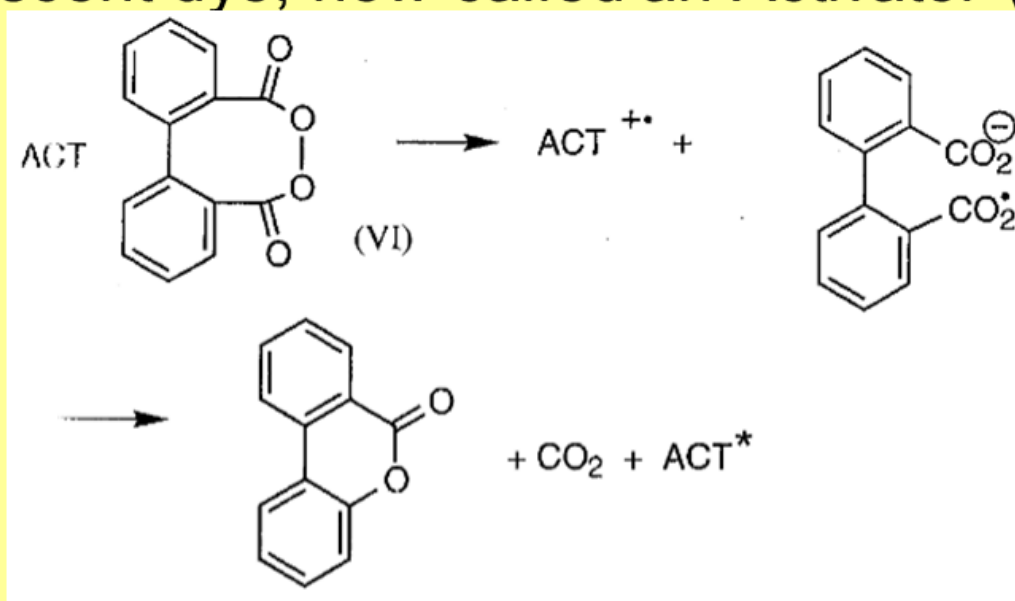1979



**C**harge **T**ransfer **I**nitiated **L**uminescence

# Diphenoyl Peroxide

The CIEEL hypothesis was based on the chemiluminescence intensity dependence on the oxidation potential and concentration of the fluorescent dye, now called an Activator **(ACT).**

# Imidazopyrazinone

The luciferins of many marine bioluminescent animals are derivatives of this central ring structure.
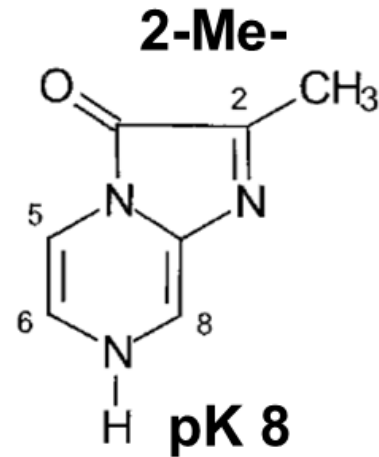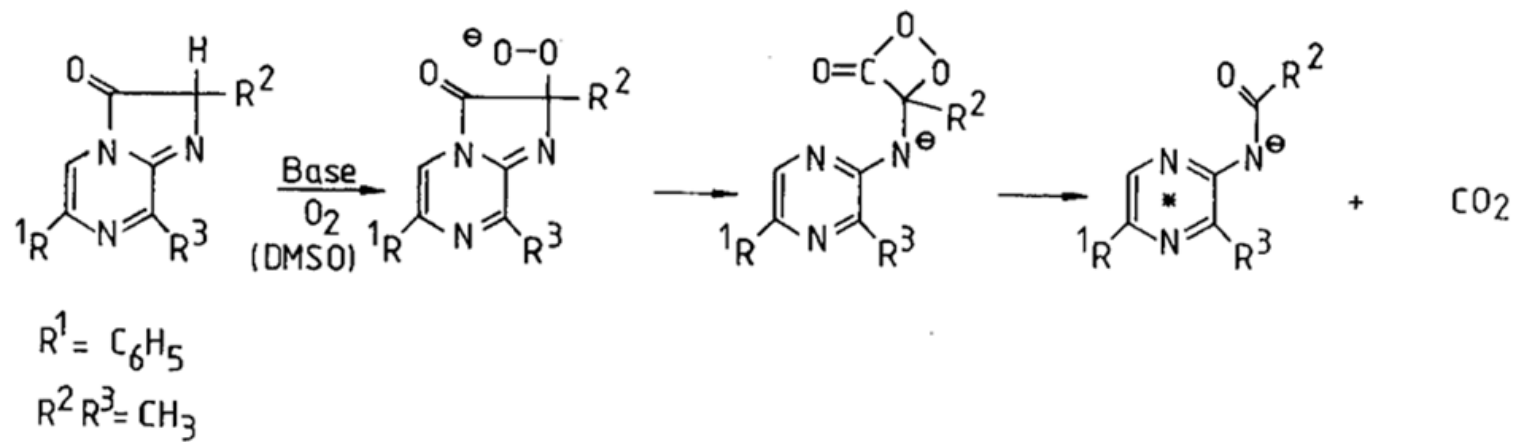


Chemiluminescence occurs spontaneously in DMSO with oxygen.

It is **base** catalyzed.

# Coelenterazine CL in basic DMSO

- Oxygen adds to the coelenterazine anion

- Product yield of coelenteramide = 85%

- Coelenteramide anion fluorescence in basic DMSO, $\lambda_F$ = 455 nm

- Chemiluminescence $\lambda_C$ = 455 nm
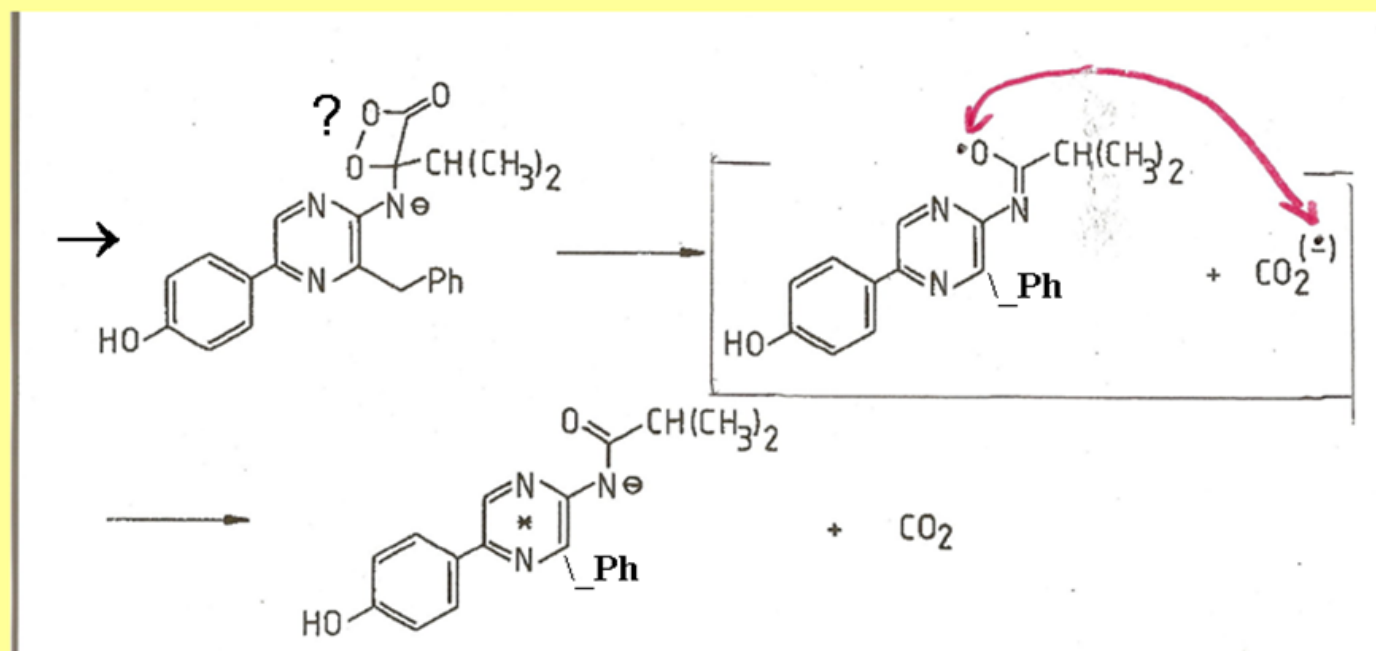
- Qc = 0.0003 < Qf ~ 0.1

# Chemiluminescence



$Q_Y = 0.89$ $Q_C = 0.0003$ $\lambda_C = 455$ nm

amide anion $\lambda_F = 455$ nm; neutral $\lambda_F = 398$ nm
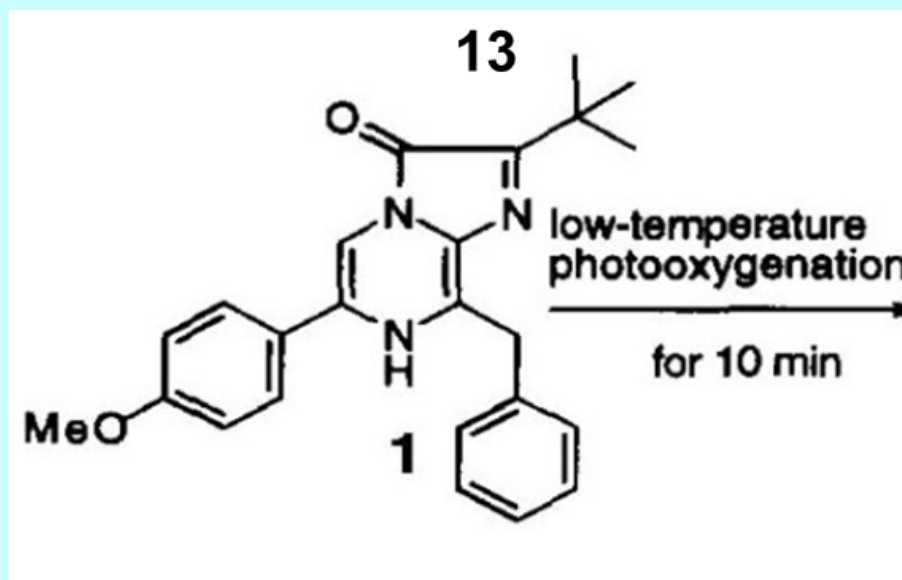$Q_F \sim 0.1$

# Coelenterazine Excitation



**1.** Possible CIEEL-type excitation

**2.** Product and emitter is the excited anion
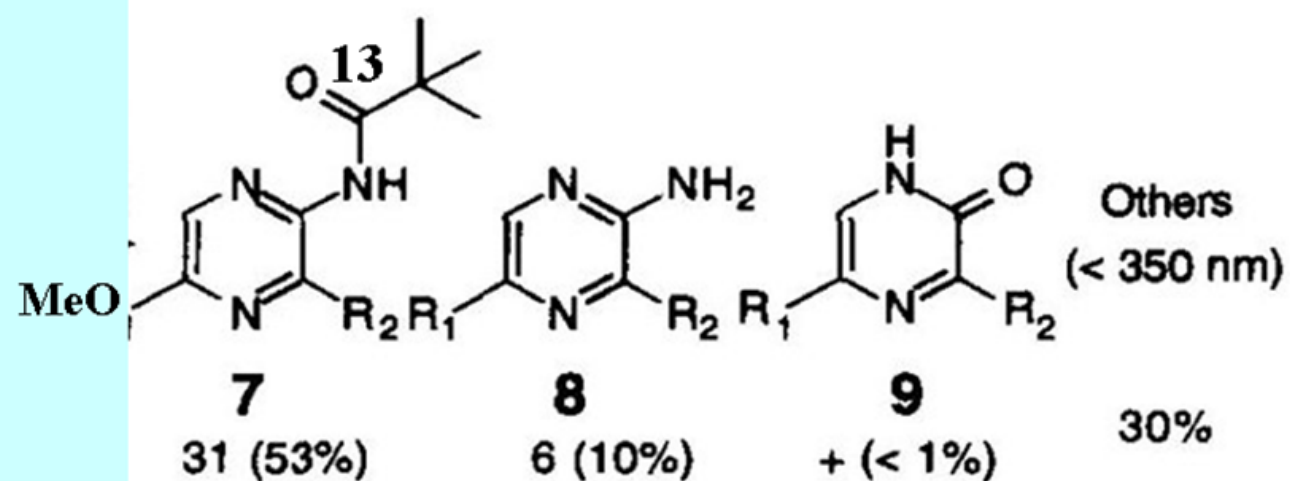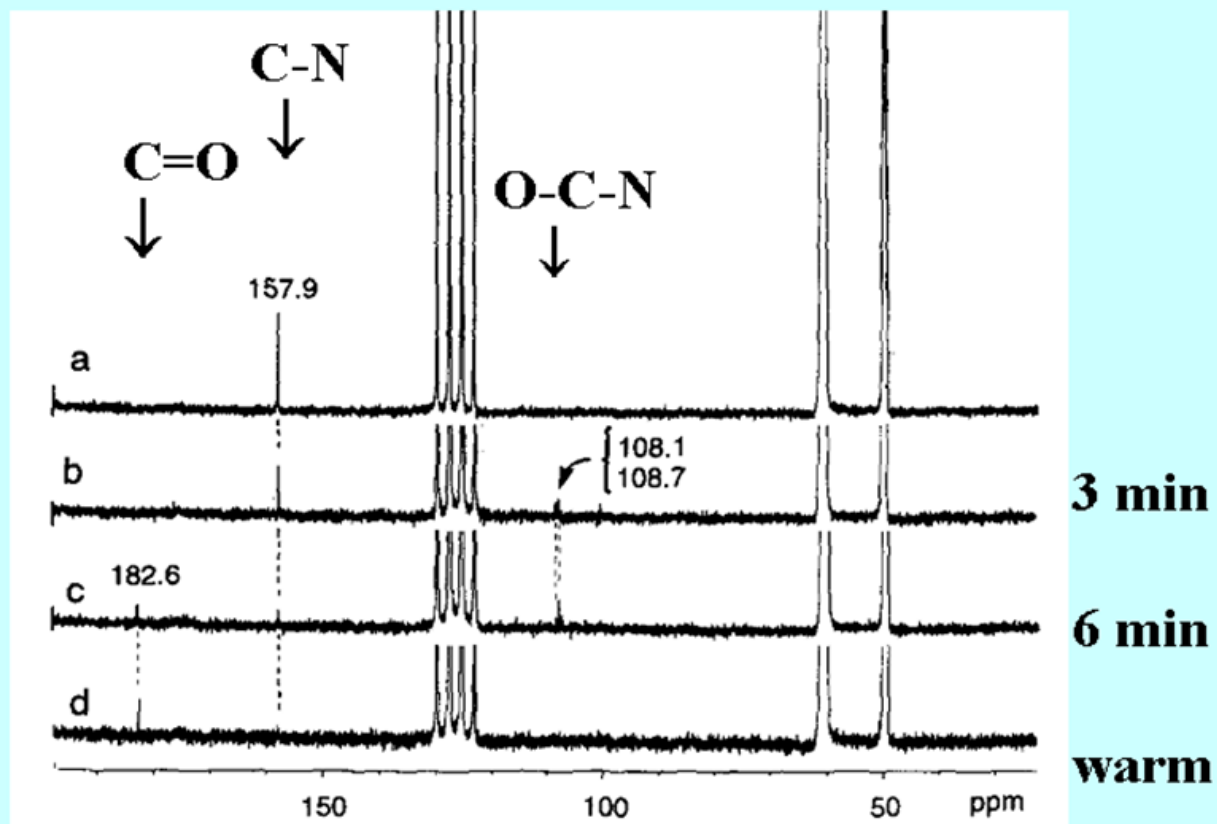
**3.** Amide or phenolate anion?

# Coelenterazine NMR



Stable (-78°C) peroxidic luminescent intermediate formed in $CF_3CH_2OHCH_3OH$

# Product Analysis

After warming



7
31 (53%)

8
6 (10%)

9
+ (< 1%)

Others
(< 350 nm)

30%
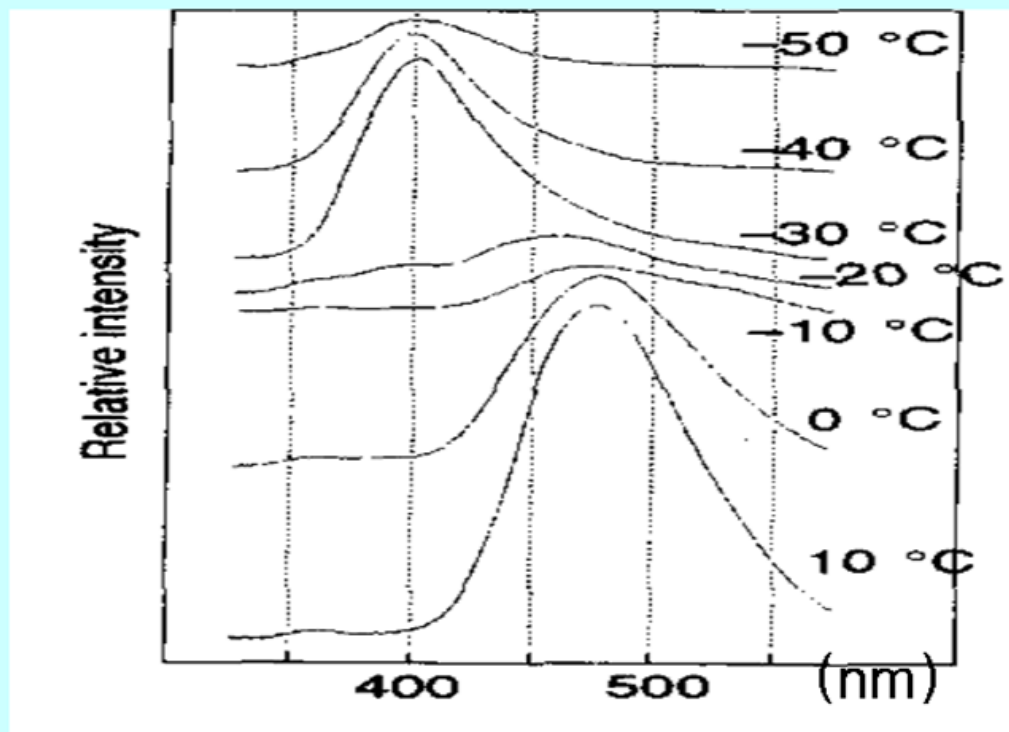
# Photooxidation $^{13}$C-NMR

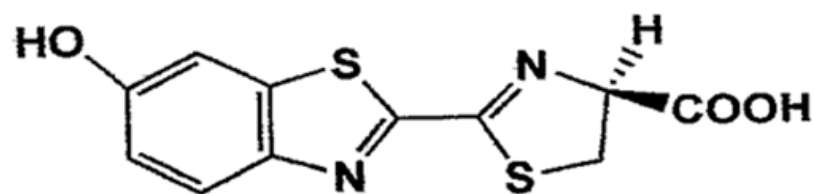# Chemiluminescence is emitted on warming

neutral product ↓      ↓anion

# Firefly Luciferin

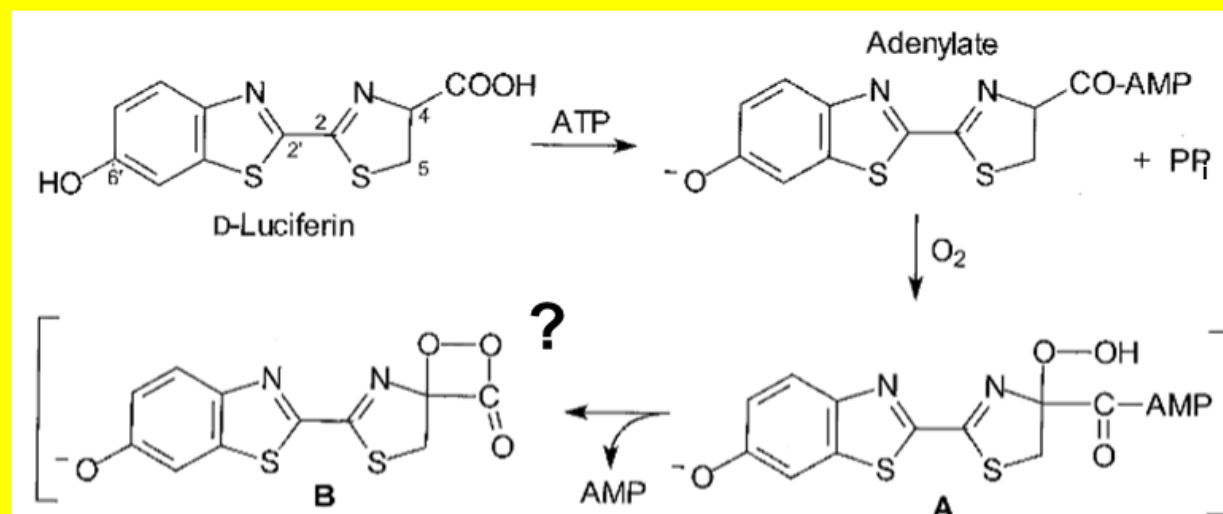Firefly luciferin was the first luciferin to have its structure fully determined (1961)



**D-Firefly Luciferin (LH$_2$)**

**Beetle Luciferin**

Notice the **asymmetric** position at C-4.
Only **D-isomer** produces bioluminescence.

# Firefly Chemistry

Chemiluminescence of model compounds in DMSO with tert-butoxide. Postulated dioxetanone intermediate (**B**)
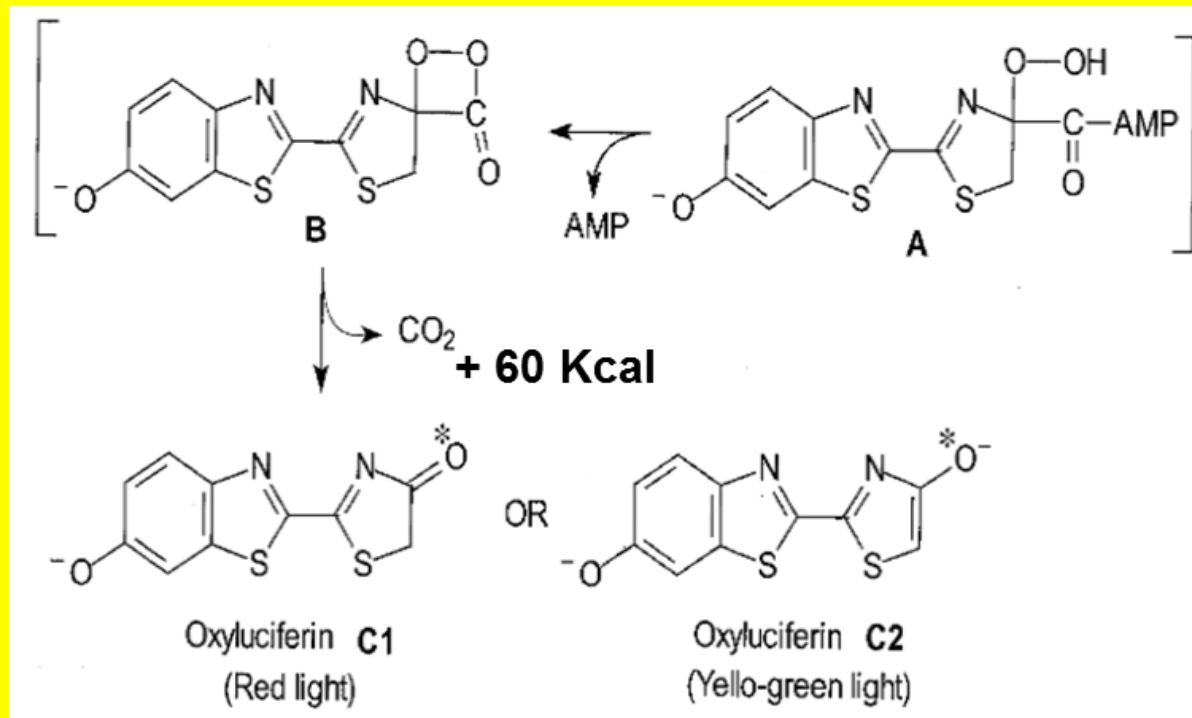
# Oxygen-18

Heavy atom labeling and detection by **mass spectra** supports the dioxetanone postulate. One oxygen-18 found in each product.
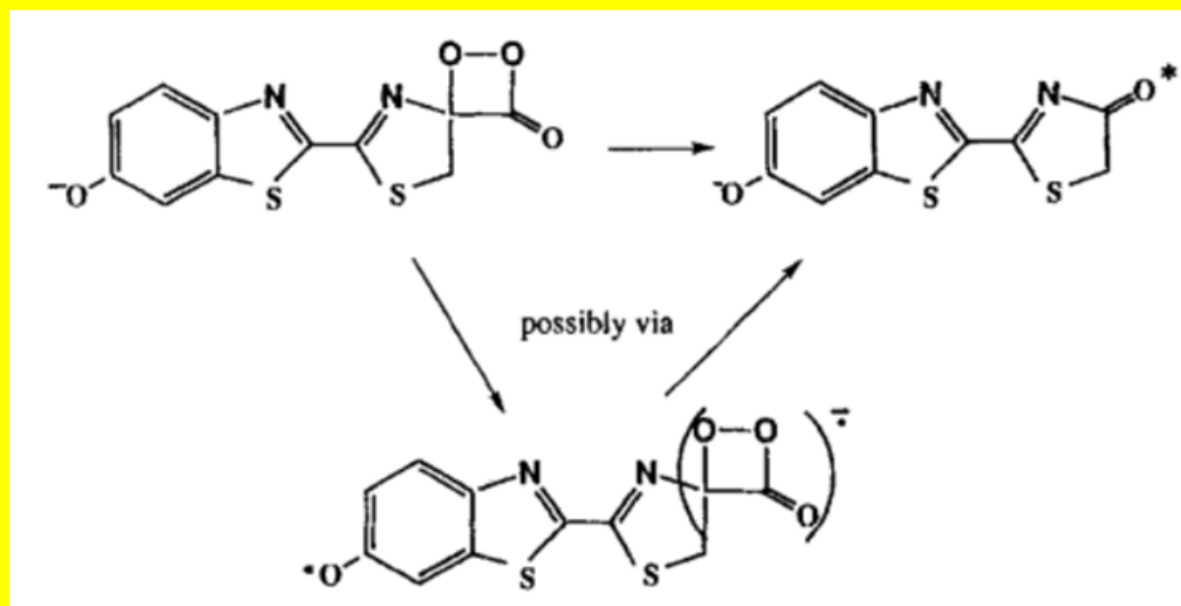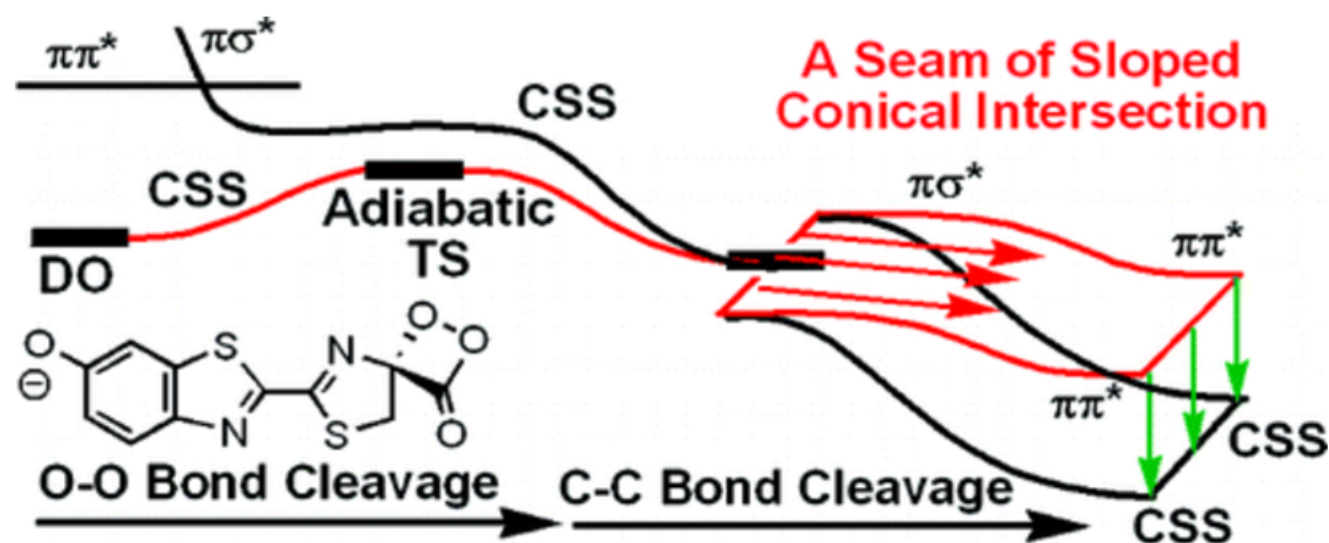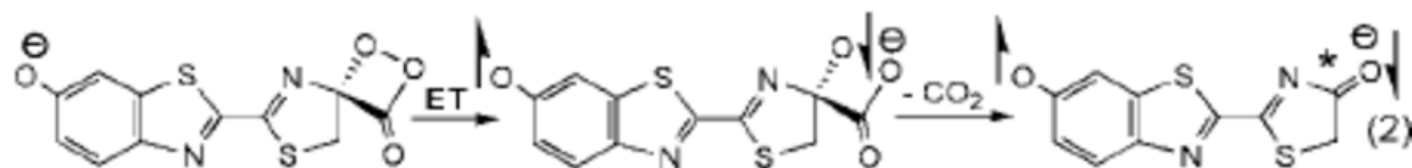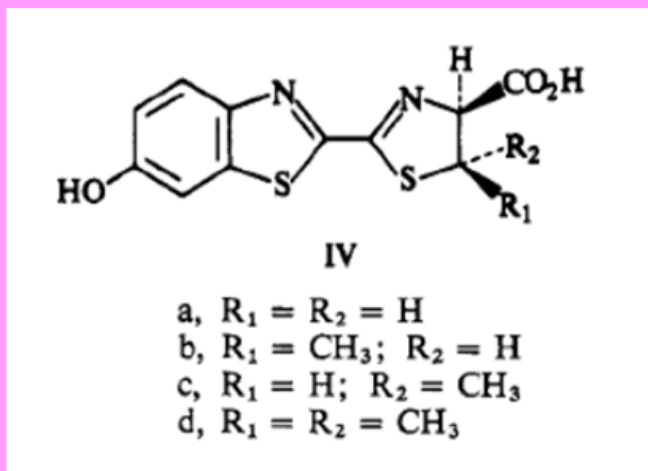
# Red or Yellow Emitters



Oxyluciferin **C1** (Red light)

Oxyluciferin **C2** (Yello-green light)
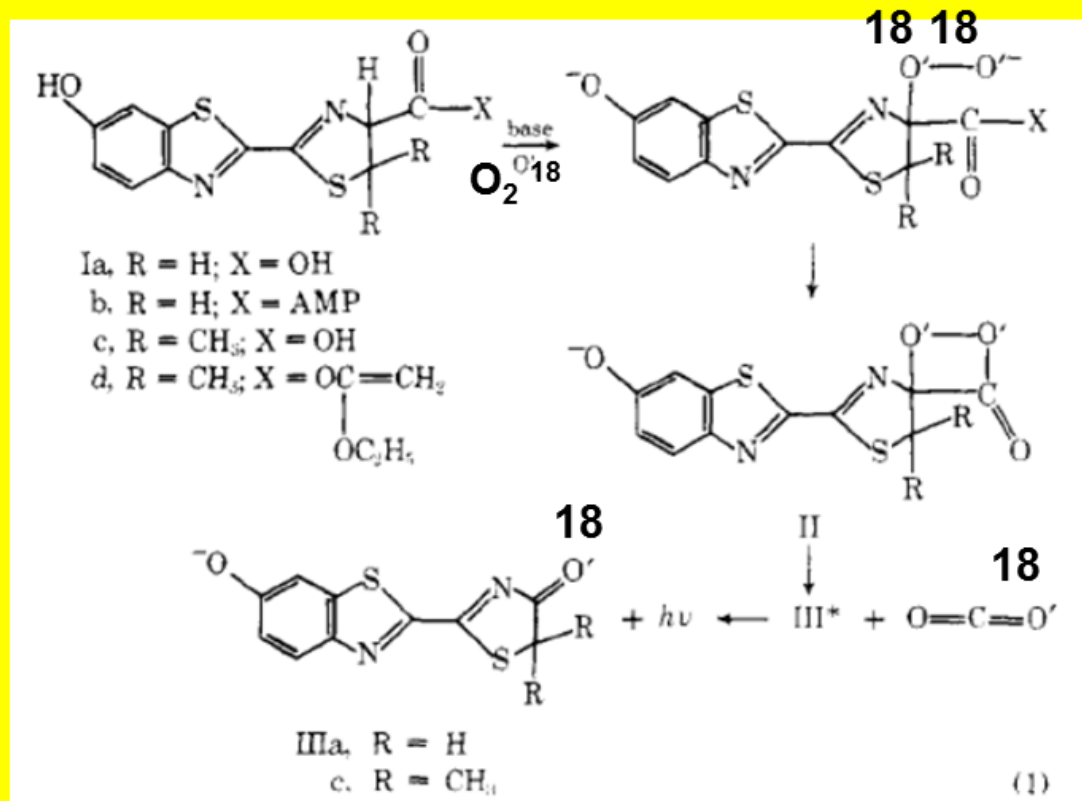
615 nm

560 nm

# Firefly CIEEL?



possibly via

# Firefly Quantum Mechanics!

# CL of Luciferin Analogs



IV

a, $R_1 = R_2 = H$
b, $R_1 = CH_3$; $R_2 = H$
c, $R_1 = H$; $R_2 = CH_3$
d, $R_1 = R_2 = CH_3$

Chemiluminescence: $a - c$ RED

stronger base $\longrightarrow$ 2 bands
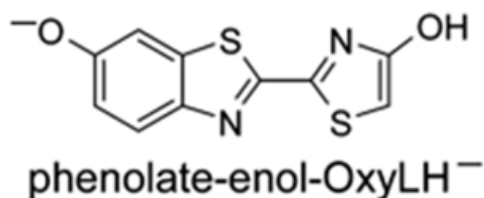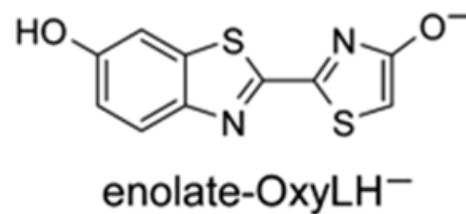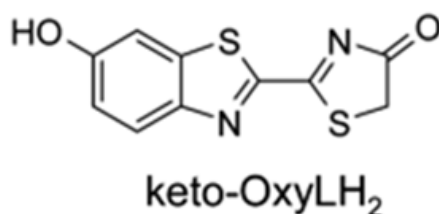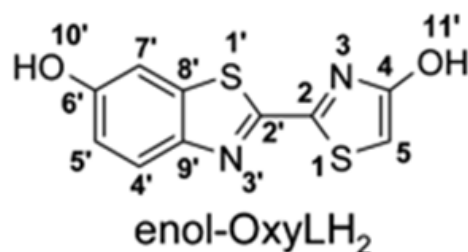$d$ RED only $Q_C = 0.1$


Product fluorescence: $a - c$ YELLOW
$d$ RED $Q_F = 0.6$

# Fluorescence of oxyluciferin ionic forms



**BLUE**       **YELLOW**

enol-OxyLH$_2$     keto-OxyLH$_2$     enolate-OxyLH$^-$

phenolate-enol-OxyLH$^-$     phenolate-keto-OxyLH$^-$     OxyL$^{2-}$
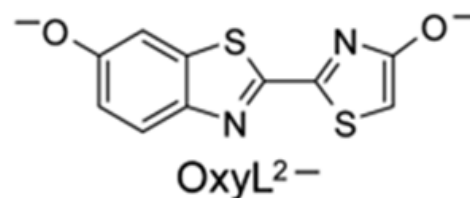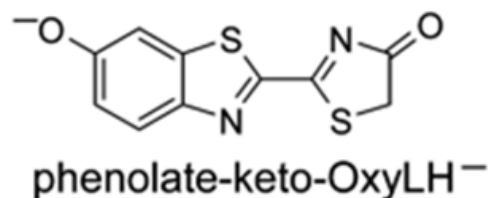
**YELLOW**       **RED**       **YELLOW**

# Literature

Seliger and Morton (1968) *Photophysiology* Vol.IV (Giese, A.C., Ed.). A physical approach to bioluminescence. (Academic Press, NY).

Gundermann and McCapra (1987) *Chemiluminescence in Organic Chemistry* (Springer- Verlag, Berlin).

Matsumoto (2004) Advanced chemistry of dioxetane-based chemiluminescent substrates originating from bioluminescence. *J. Photochem Photobiol C: Photochem. Reviews* 5: 27.