

**M5052**

**CHARACTERIZATION OF MATERIALS AND NANOMATERIALS**

*Graduate Program in Nanotechnology*

# **FLUORESCENCE**

## **(LUMINESCENCE SPECTROMETRY)**

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**A**



**Photon  
Absorption**

### **LUMINESCENCE METHODS**

#### **Photoluminescence**

Chemical species reaches excited state by photon absorption, and relaxes emitting photons with a characteristic spectrum

**A\***



$h\nu \rightsquigarrow \rightsquigarrow \rightsquigarrow$

**A**

**Characteristic Emission Spectrum**

**Chemical  
Reaction**

**A**

**Qualitative  
Analysis**

(from trace amounts)

**Quantitative  
Analysis**

(not as commonly used, other techniques may be more convenient)

#### **Chemiluminescence**

Reaction product forms in an excited state and emits photons with a characteristic spectrum



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# LUMINESCENCE METHODS

- Based on measurement of photons emitted by analytes, either as a characteristic spectrum or at a specific wavelength

- **Photoluminescence (Fotoluminiscencia)**:

- Photons are absorbed, then other photons are emitted in two ways:

1. **Fluorescence (Fluorescencia)**: excited states are short lived ( $10^{-10}$ – $10^{-5}$  s)
  - Fluorometric measurements are more common than phosphorescence or chemiluminescence methods
2. **Phosphorescence (Fosforescencia)**: long lived excited states ( $10^{-4}$ – $10$  s, even minutes)

- Disadvantages of luminescence methods:
  - High probability for non-radiative deactivation makes the method more susceptible to interference

- **Chemiluminescence (Quimioluminescencia)**

- Based on emission of radiation, by excited species formed in a chemical reaction, with two options:

1. Analyte may be a reaction product that emits photons, or a reaction (e.g. oxidation) with the analyte produces a chemiluminescent species
2. Analyte catalyzes or inhibits a chemiluminescence reaction

- Advantages of luminescence methods:

- High sensitivity (1-3 orders of magnitude greater than absorption methods)
  - Qualitative analysis from trace amounts, even down to individual molecule detection
- Large linearity range for quantitative analysis

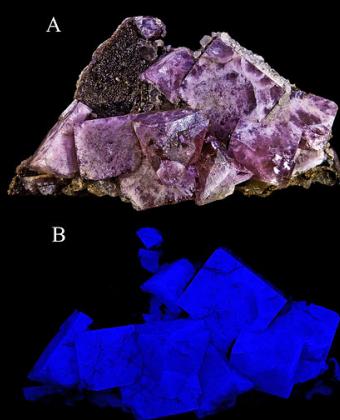


## FLUORESCENCE

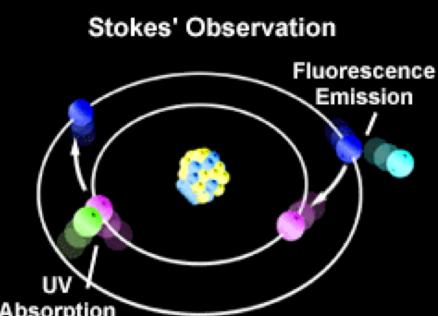
- Phenomenon first quantified in the middle of the 19<sup>th</sup> century by George G. Stokes

- Stokes observed that the mineral fluorite emitted light with a longer wavelength than that of the excitation source (UV light)
- Stokes coined the term fluorescence
  - *NOTE: fluorescence of fluorite is due to impurities, is not observed for pure CaF<sub>2</sub>*

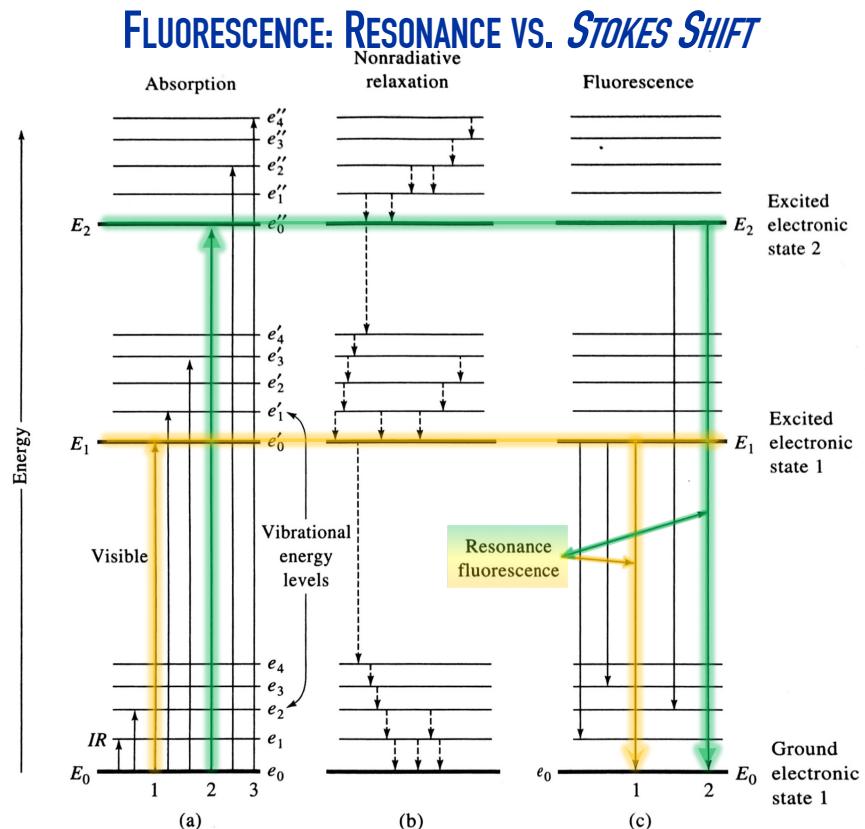
- Shift to longer wavelength (lower energy) is known as “Stokes shift”
  - Re-emission of photons with no change in wavelength is called *resonance radiation* or resonance fluorescence



Fluorite under visible light and under UV light



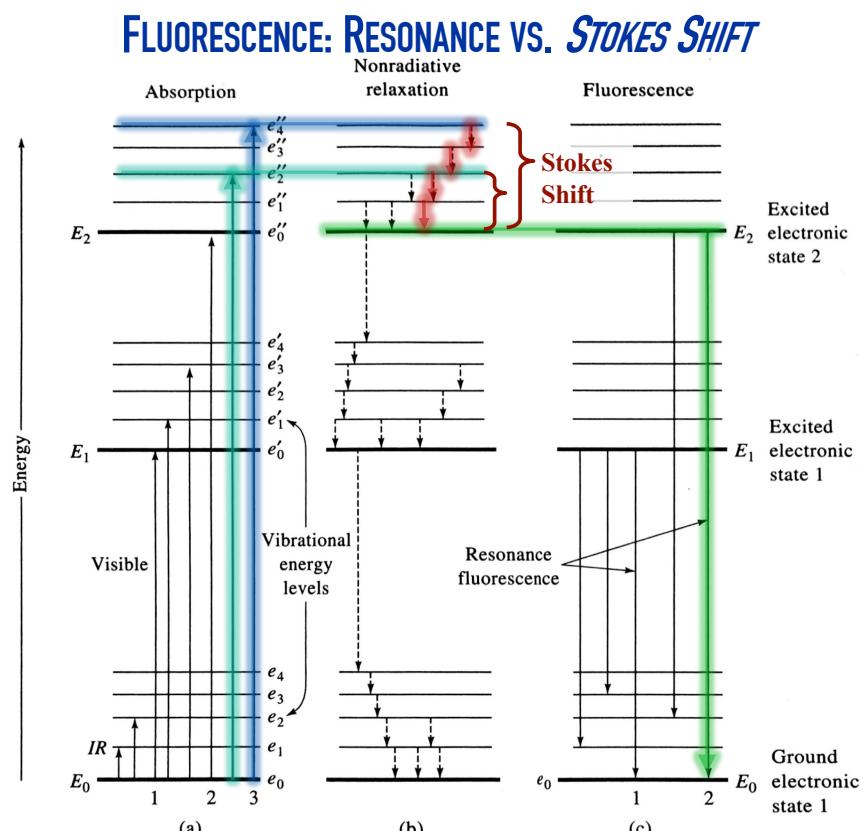
- Resonance Fluorescence : fast re-emission of absorbed light, no change in wavelength
  - Electrons jump back to ground state without losing energy
- Stokes Shift: electrons loose energy before returning to ground state
  - Vibrational levels are short-lived, fast losses of energy
  - Lifetimes of electronic states: on the order of  $10^{-8}$  s
  - Lifetimes of vibrational states on the order  $10^{-15}$  s
  - Excess vibrational energy is transferred to solvent or other molecules in very short times
  - That energy loss originates the Stokes Shift



**Figure 6-20** Partial energy-level diagrams for a fluorescent organic molecule.

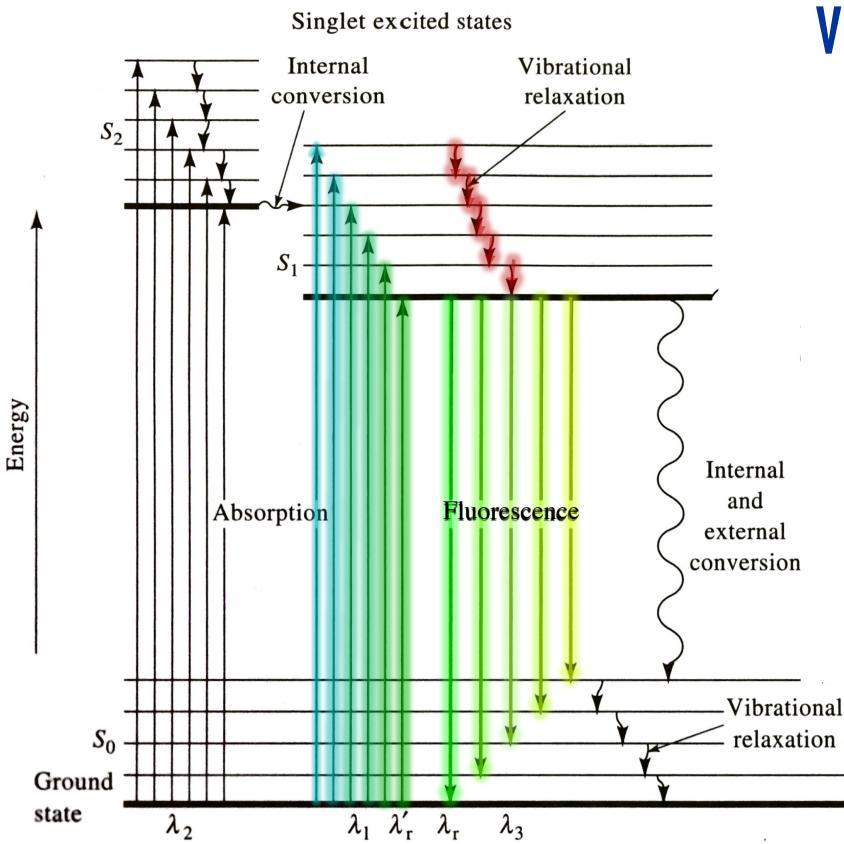


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**Figure 6-20** Partial energy-level diagrams for a fluorescent organic molecule.





## VIBRATIONAL RELAXATION AND FLUORESCENCE (STOKES SHIFT)

- Due to existence of vibrational sub-levels absorption peak of molecules is broadened
- Emission peak of molecules is broadened due to vibrational levels of the ground state

*Modified from:* D.A. Skoog, F.J. Holler, T.A. Nieman "Principles of Instrumental Analysis" 5th. Edition, Orlando, Florida : Harcourt Brace College Publishers,1998.



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## ELECTRONIC TRANSITIONS AND PHOTOLUMINESCENCE



Fluorescent Minerals, images taken from:  
<http://news.minerals.net/2015/04/default>



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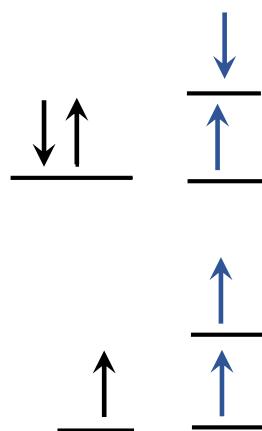
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# FLUORESCENCE, PHOSPHORESCENCE, AND SPIN

Transition between electronic states involves quantum mechanical phenomena related to spin

**Electronic Spin:** Two electrons in the same orbital must have opposite spins

Pauli exclusion principle: electrons can not have the same four quantum numbers ( $n, l, m, s$ ).  
There can only be 2 electrons in one orbital



**Diamagnetism:** When all spins are paired the molecule has no net magnetic moment and does not interact with a magnetic field

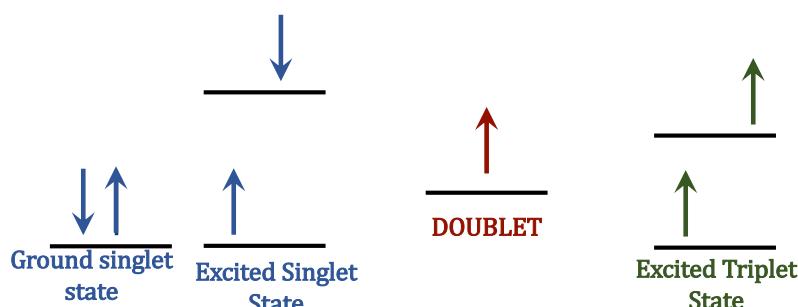
**Paramagnetism:** Unpaired electrons in a molecule result in a magnetic moment

Molecule or ion can interact with magnetic fields

Examples: free radicals, some metals in the  $f$ -block



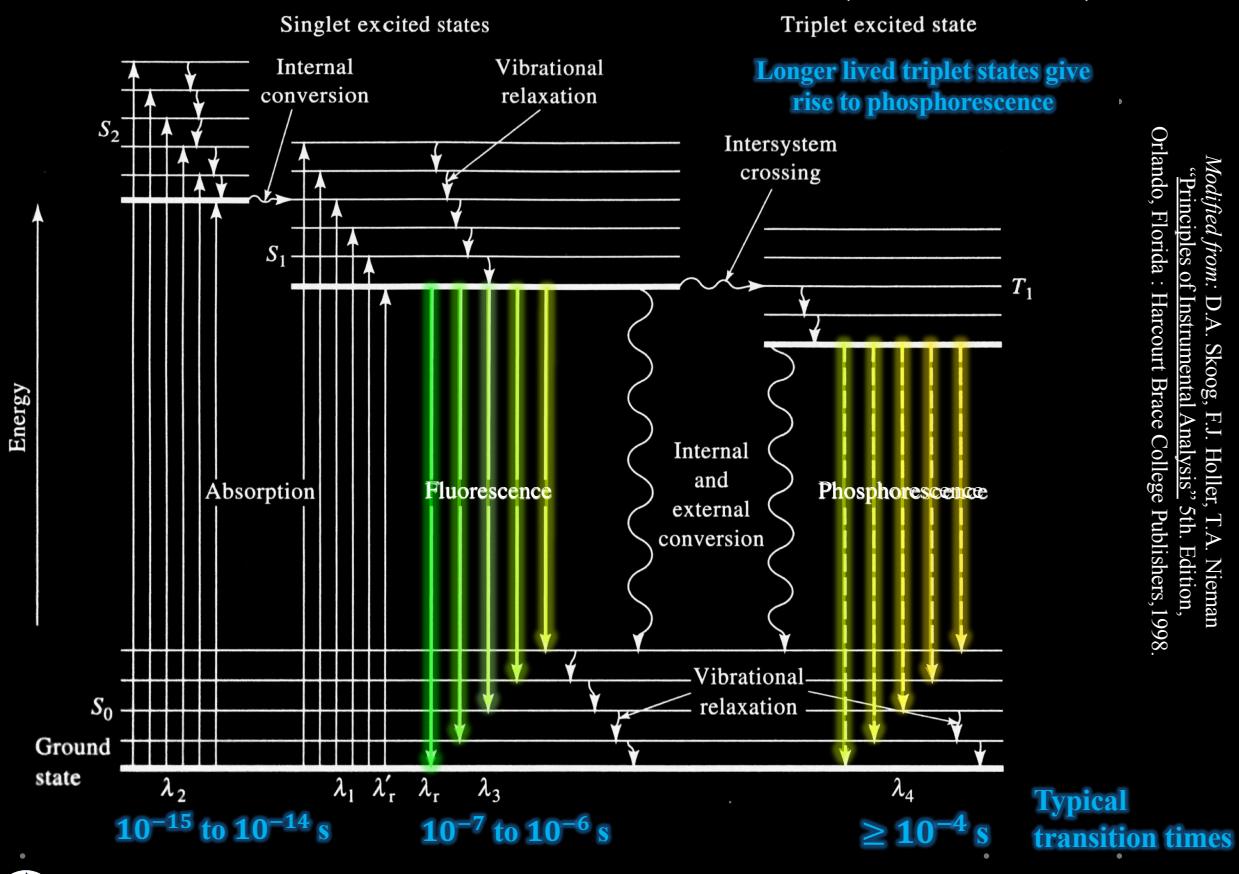
## SINGLET, DOUBLET AND TRIPLET STATES



- **SINGLET.** All electron spins are paired, total spin is zero, only one excited state
  - Molecule energy levels do not split when exposed to a magnetic field
  - Electron in excited state remains antiparallel to the electron in the ground state
- **DOUBLET.** Unpaired electron can have one of two ( $\downarrow\uparrow$ ) orientations in a magnetic field
  - The two spin states ( $-1/2$  or  $+1/2$ ) have slightly different energy levels
  - Ground state of a free radical
- **TRIPLET.** Parallel electron spins (unpaired spins)
  - In a magnetic field signal splits in three energy levels (total spin with three possible values:  $-1, 0, 1$ )
  - Slightly lower energy than doublet, but lower probability since it implies flipping a spin
- *These three states are different in their multiplicity*



## PARTIAL ENERGY DIAGRAM FOR A PHOTOLUMINESCENT SYSTEM (JABLONSKI DIAGRAM)



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## EXCITED STATE DE-ACTIVATION

- Photoluminescence is not the only way to release the excess energy
- Fastest relaxation processes are more likely
  - Rates of relaxation process vs. excited state lifetime
  - Velocity of non-radiative relaxation processes should be slow enough to allow kinetics of photoemission to compete
- The combination of photoluminescent-molecule + solutes + solvent determines the rates of de-activation processes

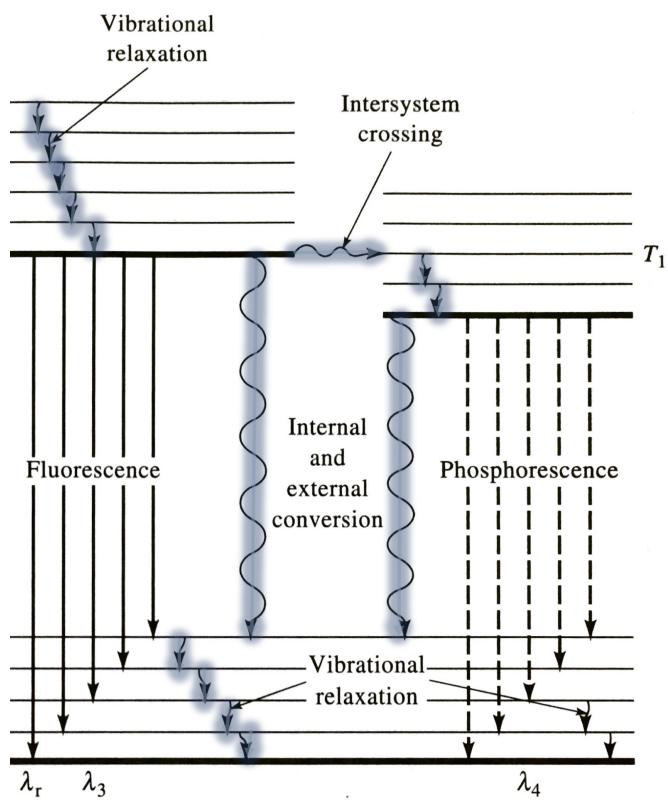


Figure Modified from: D.A. Skoog, et al.  
"Principles of Instrumental Analysis"



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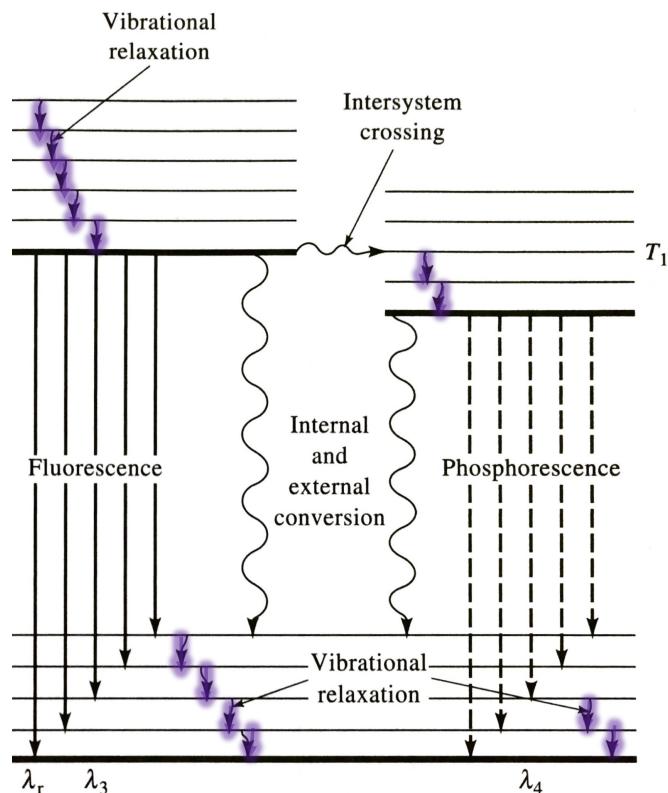
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# EXCITED STATE DE-ACTIVATION (2)

## Vibrational Relaxation

- Energy lost in collisions (momentum transfer) of excited species with solvent molecules
  - Slight increase in solvent temperature
  - Typical lifetime of vibrational excited states  $10^{-15} - 10^{-12}$  s
  - Due to this, fluorescent emission (lifetime:  $10^{-10} - 10^{-5}$  s) occurs from the lowest level of the excited electronic state
- This displaces fluorescent emission band to longer wavelengths (Stokes shift)

Figure Modified from: D.A. Skoog, et al.  
“Principles of Instrumental Analysis”



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# EXCITED STATE DE-ACTIVATION (3)

## Internal Conversion

- Non-radiative, intramolecular energy transfer processes
- Crossing between states of the same multiplicity (singlet–singlet, triplet–triplet)
- Can happen if the electronic excited state level overlaps with vibrational levels of a lower energy electronic state
- In some molecules (e.g. aliphatic molecules) vibrational levels overlap with excited electronic state and they do not fluoresce

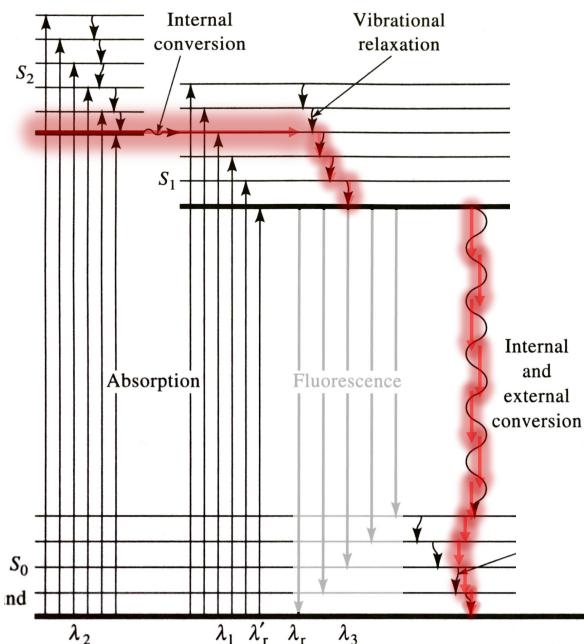


Figure Modified from: D.A. Skoog, et al.  
“Principles of Instrumental Analysis”



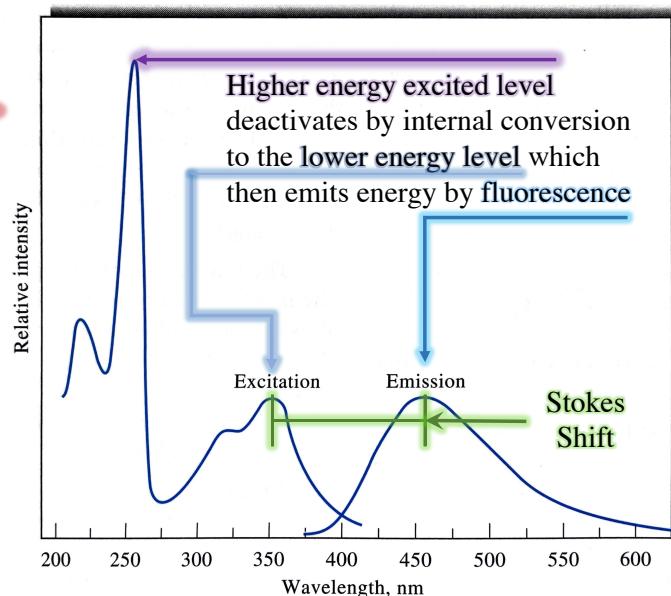
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# EXCITED STATE DE-ACTIVATION (3B)

- Internal Conversion
  - Non-radiative, intramolecular energy transfer processes
  - Crossing between states of the same multiplicity (singlet–singlet, triplet–triplet)
  - Can happen if the electronic excited state level overlaps with vibrational levels of a lower energy electronic state
  - In some molecules (e.g. aliphatic molecules) vibrational levels overlap with excited electronic state and they do not fluoresce
  - If internal conversion from one level (e.g. S<sub>2</sub>) to a lower one (e.g. S<sub>1</sub>) is very efficient there may be two absorption bands but only one fluorescence band

- Example: Quinine



**FIGURE 15-3** Fluorescence excitation and emission spectra for a solution of quinine.

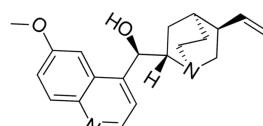


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“Principles of Instrumental Analysis”

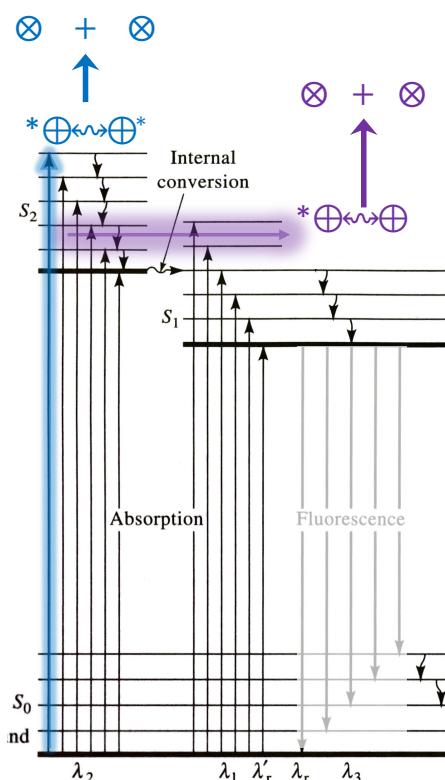
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# EXCITED STATE DE-ACTIVATION (4)

- Dissociation
  - The absorbed radiation excites the bonding electrons of the chromophore to a vibrational level high enough for the bond to break
  - Dissociation happens independently of internal conversion, but also competes with fluorescence processes



- Pre-Dissociation by Internal Conversion

- When energy is transferred to an excited state that leads to rupture of bonds
- If the vibrational energy of such level is large enough bonds can break
- Chromophore group may absorb energy and transfer it to a weaker bond



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# EXCITED STATE DE-ACTIVATION (5)

- External Conversion

- Energy transferred from excited state of molecule to solvent or other solutes without emission of radiation
- Solvent effect on fluorescence intensity: some solvents interact more strongly than others with the fluorescent molecules

- External conversion effects are reduced under conditions that reduce intermolecular collisions (Lower Temperature or High Viscosity)

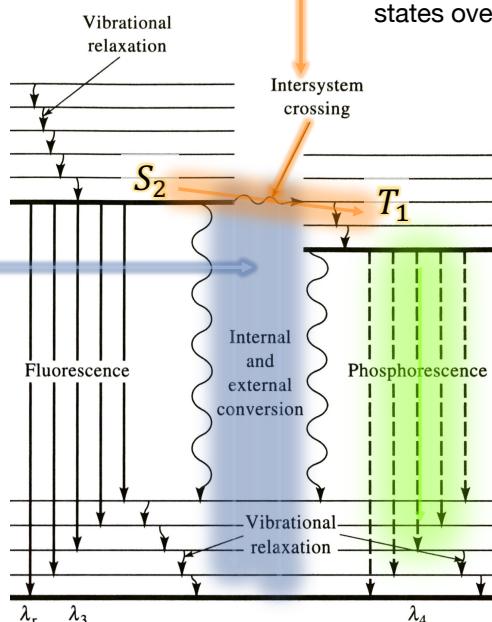
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- Intersystem Crossing

- Energy transferred to another excited level together with spin inversion
- Crossover to state with different multiplicity can occur if vibrational levels of the two states overlap

- Intersystem crossing can lead to **Phosphorescence**

- Heavy atom effect: Intersystem crossing is more common in molecules with heavy atoms (e.g., Br, I)
  - These increase spin and orbital interaction
- Paramagnetic species (e.g. molecular oxygen) enhance intersystem crossing and decrease fluorescence



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## VARIABLES AFFECTING FLUORESCENCE AND PHOSPHORESCENCE

- Quantum Yield

$$\phi = \frac{\text{No. Photons Emitted}}{\text{No. Photons Absorbed}}$$

$$\phi = \frac{k_f}{k_f + k_i + k_{in} + k_{ex} + k_{pr} + k_d}$$

**Chemical Structure Effects:**

$$k_f, k_{pr}, k_d$$

Fluorescence, Predissociation, Dissociation

**Chemical Environment Effects:**

$$k_i, k_{in}, k_{ex}$$

Intersystem crossing, Internal and External Conversion

- Type of transition

- $n-\pi^*$  or  $\pi-\pi^*$ , depending on which of the levels has lower energy
- $\sigma-\sigma^*$  and  $n-\sigma^*$  transitions are usually very energetic and lead to dissociation or pre-dissociation instead of fluorescence

- Larger quantum yield for  $\pi-\pi^*$  transitions



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# SOME OTHER FACTS ABOUT FLUORESCENCE

- Fluorescence from molecules usually will only happen for absorption with  $\lambda > 250$  nm
  - Higher energy radiation can break bonds
    - 200 nm radiation energy is equivalent to 140 kcal/mol, higher than some bond energies
- For organic molecules presence of aromatic rings increases likelihood of fluorescence
- Addition of some functional groups to a molecule may decrease fluorescence
- Heavy atoms (e.g. halogens) as substituents can decrease fluorescence
  - These atoms promote intersystem crossing (they may enhance phosphorescence from triplet states)
  - Heavy atoms in solution can also promote intersystem crossing
- Rigid molecular structures may fluoresce more effectively
  - Similar molecules that are more flexible have more opportunities for vibrational relaxation de-excitation



## FLUORESCENCE AND STRUCTURE: SUBSTITUTION EFFECTS

TABLE 15-1 Effect of Substitution on the Fluorescence of Benzene

Compound	Formula	Wavelength of Fluorescence, nm	Relative Intensity of Fluorescence
Benzene	C <sub>6</sub> H <sub>6</sub>	270–310	10
Toluene	C <sub>6</sub> H <sub>5</sub> CH <sub>3</sub>	270–320	17
Propylbenzene	C <sub>6</sub> H <sub>5</sub> C <sub>3</sub> H <sub>7</sub>	270–320	17
Fluorobenzene	C <sub>6</sub> H <sub>5</sub> F	270–320	10
Chlorobenzene	C <sub>6</sub> H <sub>5</sub> Cl	275–345	7
Bromobenzene	C <sub>6</sub> H <sub>5</sub> Br	290–380	5
Iodobenzene	C <sub>6</sub> H <sub>5</sub> I	—	0
Phenol	C <sub>6</sub> H <sub>5</sub> OH	285–365	18
Phenolate ion	C <sub>6</sub> H <sub>5</sub> O <sup>-</sup>	310–400	10
Anisole	C <sub>6</sub> H <sub>5</sub> OCH <sub>3</sub>	285–345	20
Aniline	C <sub>6</sub> H <sub>5</sub> NH <sub>2</sub>	310–405	20
Anilinium ion	C <sub>6</sub> H <sub>5</sub> NH <sub>3</sub> <sup>+</sup>	—	0
Benzoic acid	C <sub>6</sub> H <sub>5</sub> COOH	310–390	3
Benzonitrile	C <sub>6</sub> H <sub>5</sub> CN	280–360	20
Nitrobenzene	C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>	—	0

- Benzene is a simple example of how relatively small changes in chemical structure and composition can change fluorescence intensity and wavelength



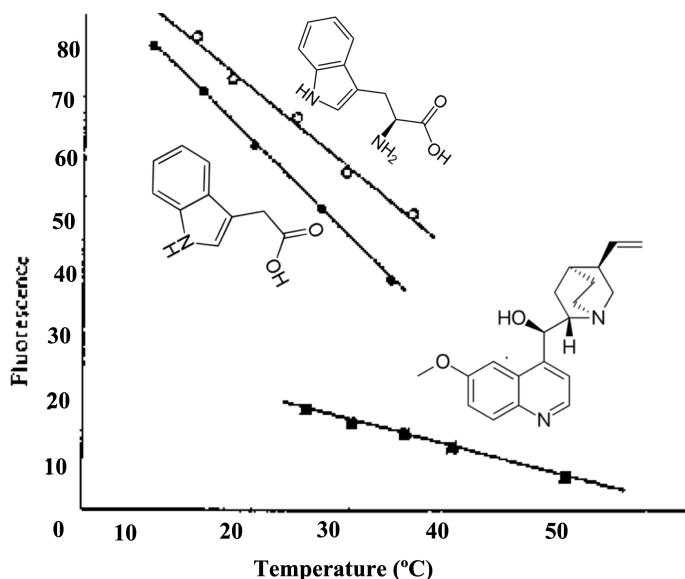
# FLUORESCENCE AND CHEMICAL ENVIRONMENT

- Temperature

- Increased temperature reduces fluorescence intensity
- Increased collision frequency increases deactivation by external conversion

- Solvent effects

- Solvents or solutes with heavy atoms reduce fluorescence
  - Examples:  $\text{CCl}_4$ ,  $\text{C}_2\text{H}_5\text{I}$
  - Orbital spin interactions increase conversion to triplet states
  - Effect can be used to increase phosphorescence



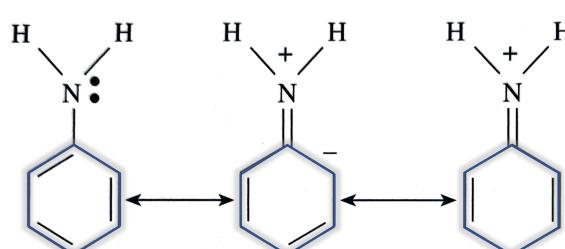
Variations in fluorescence intensity for some substances as function of temperature  
○ Tryptophan, ● Indole acetic Acid, ■ Quinine



## CHEMICAL ENVIRONMENT: EFFECT OF pH

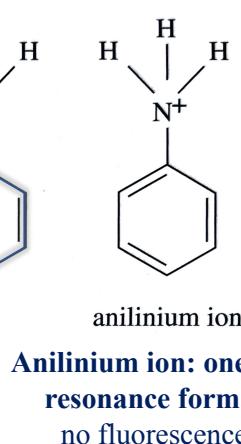
- Conjugated or aromatic molecules with acid or basic groups will have different resonance structures depending on the state of those group
- Protonation or deprotonation change with pH

- Some forms of molecules may not fluoresce with pH change
  - Example: Aniline-Aniliinium
- Wavelength and emission intensity of fluorescence may change with protonation
  - Example: Phenol-Phenolate



resonance forms of aniline

**Aniline fluoresces in the UV:**  
first excited state is more stable due to resonance structures



aniliinium ion

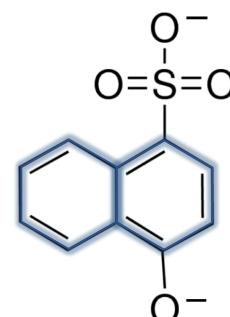
**Aniliinium ion: one resonance form, no fluorescence**

	$\lambda > 310-400 \text{ nm}$ Rel. Intensity: 1
	$\lambda > 285-365 \text{ nm}$ Rel. Intensity: 1.8



## CHEMICAL ENVIRONMENT: PH EFFECT

- pH may shift the position of fluorescent peaks from the UV to the visible due to protonation/deprotonation
  - This can be used to detect the endpoint of acid-base titrations of such compounds
  - Example: 1-naphthol-4-sulfonic acid, fluoresces in the UV.
    - The phenolate form in basic pH fluoresces in the visible
- Change may occur at a different pH than expected from the dissociation constant of the compound
  - The excited molecule has a different dissociation constant than the ground state molecule
  - Such differences can be as large as 4–5 orders of magnitude



## CHEMICAL ENVIRONMENT: QUENCHING AND OXYGEN EFFECT

- Quenching: suppression of fluorescence intensity
  - Desactivación Fluorescente, también “atenuación” o “supresión”
  - Note: in materials science the word “quenching” has a different meaning
- Dissolved Oxygen reduces fluorescence intensity
- In some cases this is due to photochemical oxidation of the fluorescent species
  - Molecule in excited state may be more reactive
- Quenching may be due to the paramagnetism of molecular oxygen
  - Unpaired spin of molecular oxygen may promote intersystem crossing
  - Excited molecules get converted into the triplet state instead of fluorescing
  - Other paramagnetic species also tend to quench fluorescence

The blue fluorescence of quinine (right side) is quenched by dissolved chloride ions (left side)



Image modified from

[https://commons.wikimedia.org/wiki/File:Quenching\\_of\\_Quinine\\_fluorescence\\_by\\_chloride\\_ions.JPG](https://commons.wikimedia.org/wiki/File:Quenching_of_Quinine_fluorescence_by_chloride_ions.JPG)

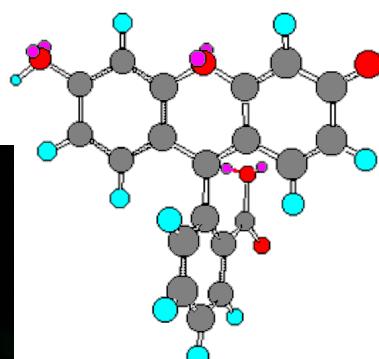


# CONCENTRATION EFFECT ON FLUORESCENCE INTENSITY

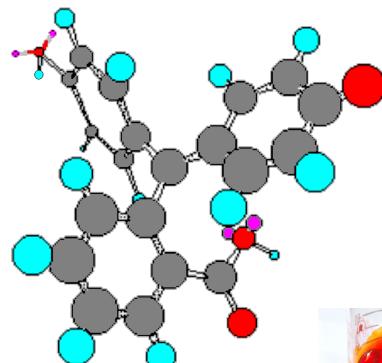
- Primary Absorption: Linear relation of Fluorescence to concentration is lost with high concentration
  - Fluorescence as function of absorbance is calculated by an exponential series
  - In this series, for low absorbance values ( $A<0.05$ ) quadratic and cubic terms are negligible
- Self-Absorption effect (secondary absorption)
  - Light emitted by fluorescence is re-absorbed by other analyte molecules and by other species in solution (emitted wavelength overlaps an absorption band)
- Dynamic (Collisional) Quenching
  - Increasing concentration increases the probability of collisions that dissipate energy as heat
  - Concentration of quenching agent large enough for collisions to occur in the lifetime of the excited state
- Other types of Quenching
  - Static quenching: formation of a non-fluorescing complex between analyte and a quencher
  - Long-Range Quenching: Energy transfer by dipole interactions, without collisions
    - Called *Förster Quenching*



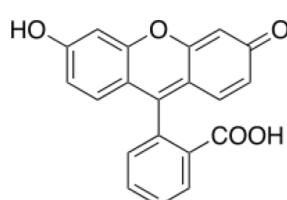
## STRUCTURAL RIGIDITY AND FLUORESCENCE



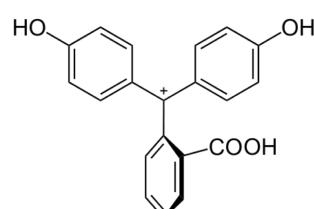
Fluoresceína



Fenolftaleína



- Fluorescein is similar in structure to protonated phenolphthalein, but the latter does not fluoresce



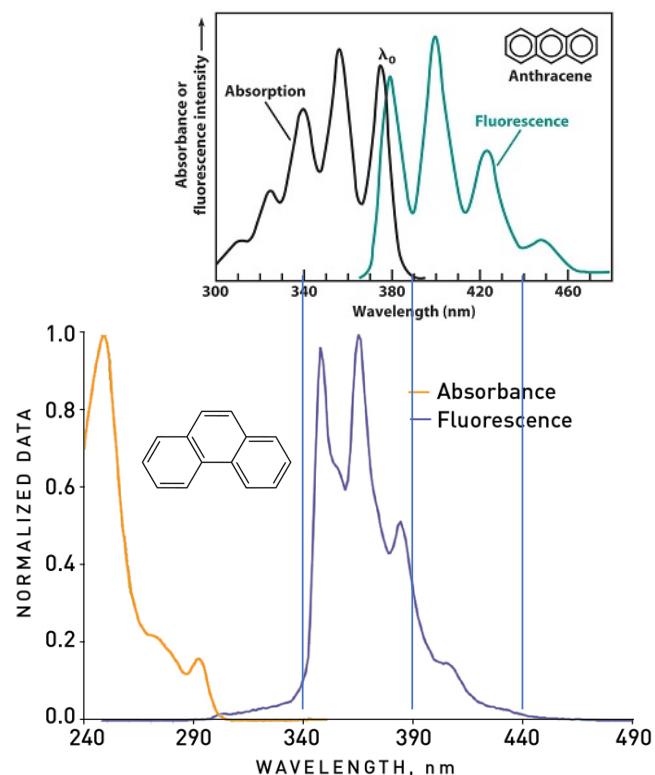
# MOLECULAR STRUCTURE AND FLUORESCENCE

- Relative arrangement of aromatic rings can change intensity and wavelength of absorption and fluorescence
  - Example: Absorption and Fluorescence spectrum of anthracene compared to phenanthrene

Spectra taken from: Anthracene: <  
<http://archive.cnx.org/contents/81bb0311-98ee-4fcf-b3c8-0eab6aeace37@2/photoluminescence-spectroscopy-and-its-applications>>  
Phenanthrene: <  
<https://www.watersonline.com/doc/addressing-environmental-regulations-with-uvc-leds-0001>>



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## EXAMPLE:

# CARBON DOTS

## Quantum Dots made of Carbon



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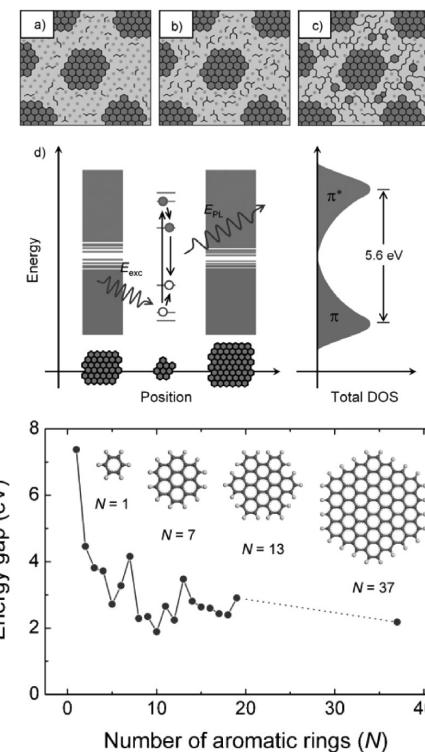
# CARBON DOTS

- Fluorescence of carbon dots is a very active area of research
- Computational chemistry studies show that the separation between the  $\pi$  and  $\pi^*$  levels changes with the number of fused aromatic rings
- In practice mixtures of C-dots of different structures and with structural defects are produced from reduction of graphene oxide or from carbonization of organic precursors
- Fluorescence peak color can be controlled even without full structural control at the molecular level

Figure from: L. Cao et al. "Photoluminescence Properties of Graphene versus Other Carbon Nanomaterials" Acc. Chem. Res., 46 (2013) 171, DOI: 10.1021/ar300128j

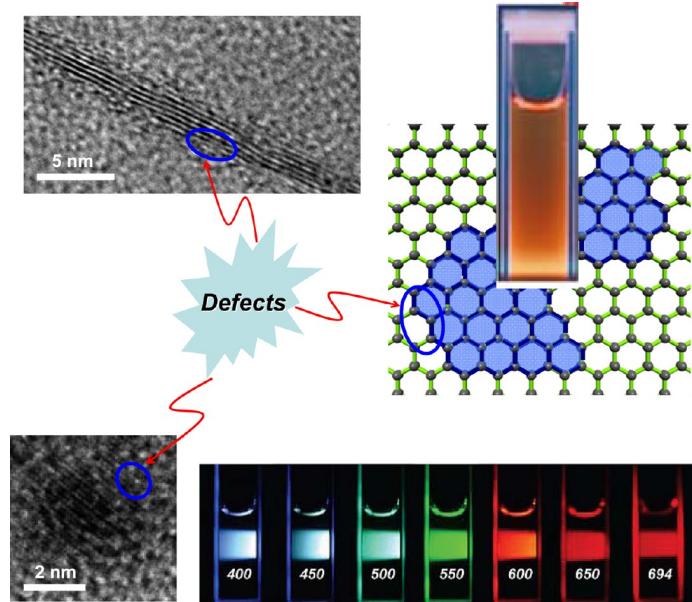


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**FIGURE 2.** Structural models of GO at different stages of reduction (upper), and the energy gap of  $\pi-\pi^*$  transitions calculated based on DFT as a function of the number of fused aromatic rings (lower). Adapted from ref 2 with permission. Copyright 2010 Wiley-VCH.

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**FIGURE 1.** Upper: Isolated sp<sup>2</sup> islands in a graphene sheet and a photo showing bandgap fluorescence in solution (right, ref 7), and a multiple-layer graphene piece (left). Lower: a carbon nanoparticle with surface defects (left), and emission color variations in carbon dots (right, ref 9). Adapted from refs 7 and 9 with permission. Copyright 2011 American Chemical Society and Copyright 2006 American Chemical Society.

- Fluorescent emission color of carbon dots depends on size and on the presence of structural defects

Figure from: Acc. Chem. Res., 46 (2013) 171, DOI: 10.1021/ar300128j



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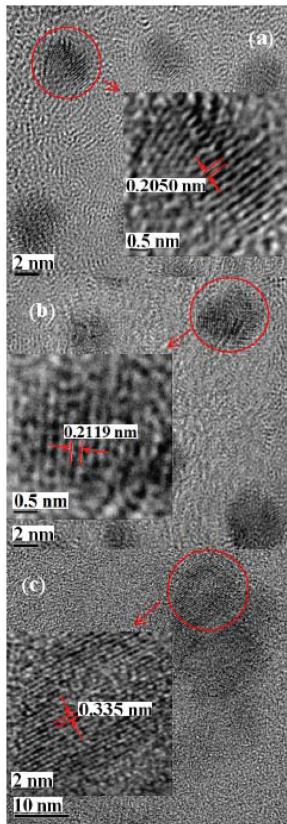


Fig. 3 High resolution TEMs of C-dots. The inter-planar spacing is indicated on the figures, the scale bars are in black.



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## SPECTROSCOPIC CHARACTERIZATIONS OF C-DOTS FROM FRUCTOSE

- Carbon dots made from reduction of fructose in a basic solution
- Raman shows that a carbonaceous material was formed
- FTIR shows the functional groups in the C-dots and how they differ from those in the precursor
- UV-vis shows the absorption bands of the C-dots
- Fluorescence spectroscopy shows how the fluorescence peak shifts with the excitation wavelength

Green Chem. **16** (2014) 2566

DOI: 10.1039/c3gc42562b

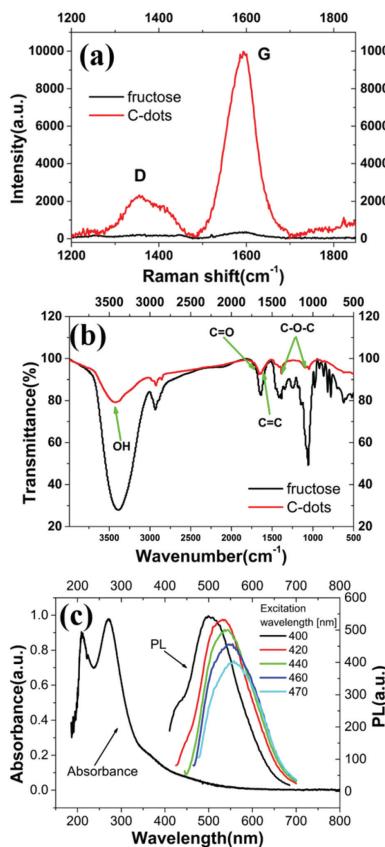


Fig. 4 (a) Raman and (b) FTIR spectra of fructose and C-dots. (c) UV-Vis absorption and photoluminescence spectra for C-dots.

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# EQUIPMENT FOR MEASURING FLUORESCENCE (AND PHOSPHORESCENCE)

Fluorometers, Spectrofluorometers,  
Phosphorimeters

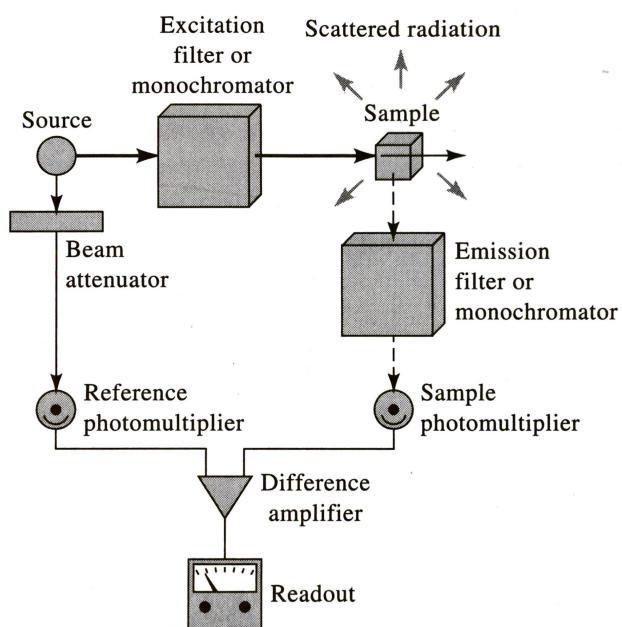


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# MEASURING FLUORESCENCE

- **Fluorometers:** use filters to select  $\lambda$ 
  - Measure only certain bands, not the whole spectrum
  - Less expensive, used for concentration measurements
  - Also spelled *fluorimeter*
- **Spectrofluorometers:** uses two monochromators to isolate  $\lambda$ 
  - One monochromator used to choose excitation wavelength
  - Other used to separate the emission wavelengths
- **Hybrid Spectrofluorometers:** use filters to select some excitation wavelengths, but with a monochromator to separate emission
- True Spectrofluorometers can produce a *fluorescence excitation* spectrum and a *fluorescence emission* spectrum

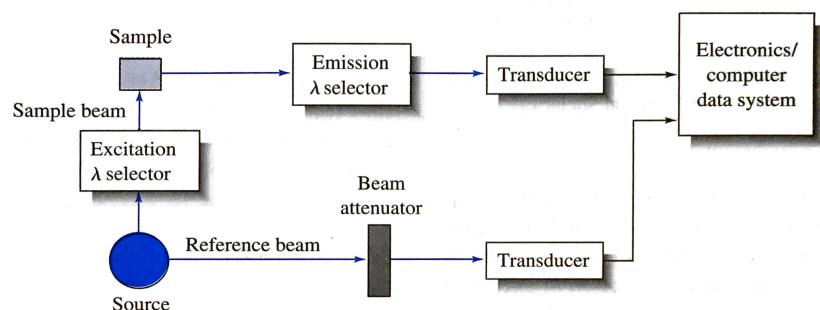


**Figure 15-4** Components of a fluorometer or a spectrofluorometer.



## FLUORESCENCE AND PHOSPHORESCENCE INSTRUMENTATION

- Double beam configurations used to compensate for variations in radiant power of the light source
  - Attenuator required so that relative intensities are not orders of magnitude apart (typical attenuation factor  $\geq 100$ )
- Detector in a right angle to the excitation beam
  - Configuration minimizes contributions from scattering and interference from incident beam
- Components of fluorometers and spectrofluorometers similar to those of UV-vis

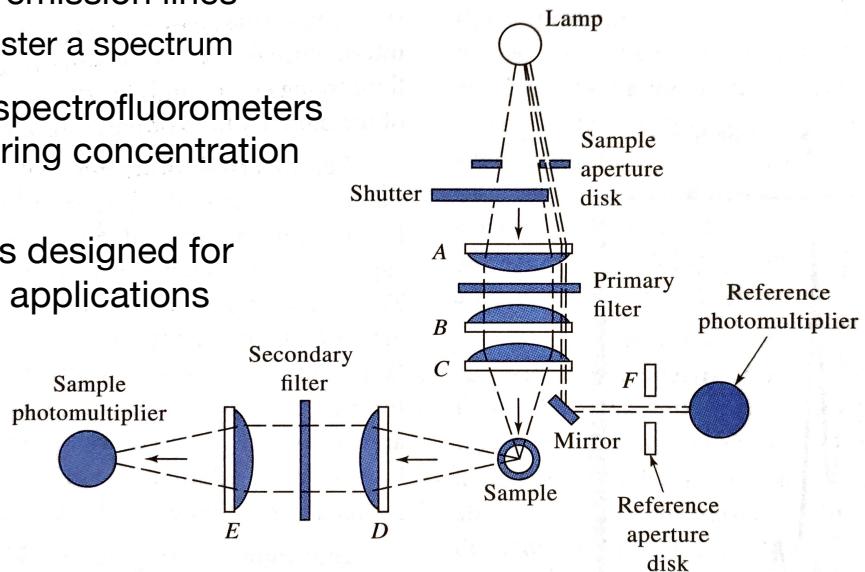


**FIGURE 15-8** Components of a fluorometer or spectrofluorometer. Source radiation is split into two beams. The sample beam passes through the excitation wavelength selector to the sample. The emitted fluorescence is isolated by the emission wavelength selector before striking the transducer. The reference beam is attenuated before striking the transducer. The electronics and computer system compute the ratio of the fluorescence intensity to the reference beam intensity, which cancels the effect of source intensity fluctuations.



# SCHEMATIC DIAGRAM OF A FLUOROMETER

- Uses specific filters to select some excitation lines and observe some specific fluorescence emission lines
  - Does not allow to register a spectrum
- Less expensive than spectrofluorometers and suffice for measuring concentration by fluorometry
- There are fluorometers designed for specific and selective applications
- NOTE: there are “hybrid spectrofluorometers” which can be used for emission spectra but only use excitation with specific lines using filters



**FIGURE 15-10** A typical fluorometer. (Courtesy of Farrand Optical Components and Instruments Division of Ruhle Companies, Inc.)



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## SOME FLUORIMETERS



Fluorometer for nucleic acid and protein quantification (Thermo Fisher Scientific)



QuantaMaster 400 Fluorimeter  
(PTI / Horiba)



QFX Fluorometer ,  
DeNovix Inc.



Fluorometer for chlorophyll measurement to monitor algae growth in water pipes (Turner Designs)



Fluorometer for absorbance and turbidity measurements (Turner Designs)



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# DIAGRAM OF A SPECTROFLUOROMETER

- Used to obtain both the excitation spectrum and the fluorescent emission spectrum

- To measure the excitation spectrum fluorescence intensity (at a fixed wavelength) is measured as the excitation wavelength is changed with the monochromator

- Absolute Excitation spectrum is usually similar in appearance to the absorption spectrum
- Corrections made for detector response at different wavelengths and for variations in lamp intensity

- To measure the fluorescence spectrum a fixed excitation wavelength is used (e.g. a UV line) and the monochromator scans the emission wavelengths

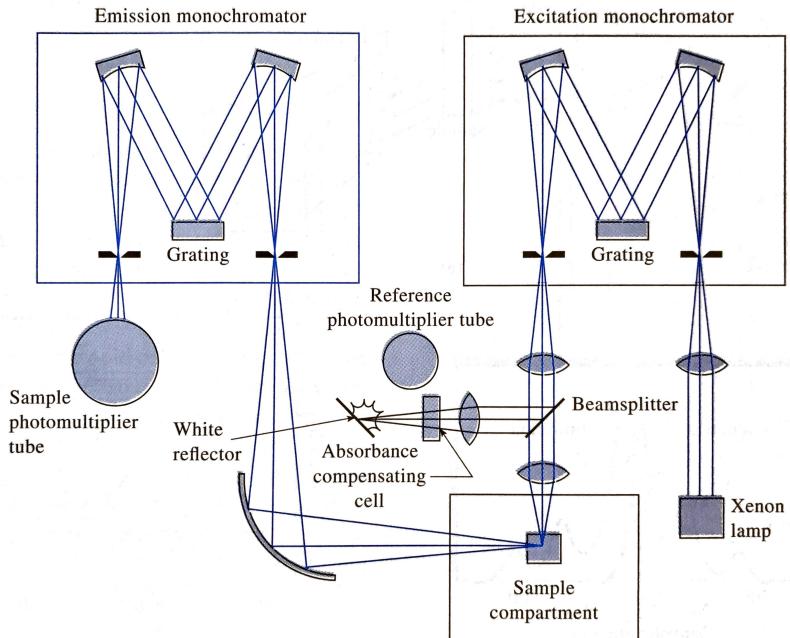


FIGURE 15-11 A spectrofluorometer. (Courtesy of Horiba Jobin Yvon, Edison, NJ.)



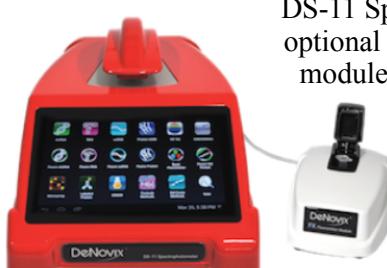
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## SOME SPECTROFLUOROMETERS



Fluorolog Spectrofluorometers with modular design, configurable for several applications (Horiba)



DS-11 Spectrometer with optional FX Fluorometer module, DeNovix Inc.

Hitachi Fluorescence spectrometer F-2700



PerkinElmer LS 45 Fluorescence spectrometer



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# COMPONENTS OF FLUOROMETERS AND SPECTROFLUOROMETERS: LIGHT SOURCES

- **Lamps**
  - Intensity usually higher than in UV-vis measurements, fluorescence intensity is proportional to incident power
- **Lamps for Fluorometers**
  - Mercury vapor lamp with fused silica window
  - Useful lines emitted at 254, 302, 313, 546, 578, 691 and 773 nm
    - Filters used to select individual lines
- **Lamps for Spectrofluorometers**
  - Source of continuum radiation required for excitation spectra
  - High pressure Xenon arc lamp, continuum spectrum 300–1300 nm
  - Pulsed lamps can be used to provide flashes for excitation
- **Blue Light Emitting Diodes**
  - LEDs emitting at 450–475 nm can be excitation source for some fluorophores
- **Lasers**
  - Tunable dye lasers allow selecting excitation wavelength
    - “Pumped” with pulsed nitrogen laser or Nd:YAG laser to excite the dyes responsible for lasing
  - More expensive, not so common
- **Fixed Wavelength Laser**
  - Used in fluorescence detectors for chromatography and electrophoresis
    - Laser can be focused in small spaces for small sample volumes
  - Used in remote sensing
    - Collimated nature of laser allows exciting a remote spot for detecting hydroxyl radicals in the atmosphere or chlorophyll in bodies of water
  - Used as highly monochromatic excitation source (minimize interference)



# COMPONENTS OF FLUOROMETERS AND SPECTROFLUOROMETERS

- **Filters and/or Monochromators**
  - Filters for specific lines in fluorometers
  - Grating Monochromator(s) for spectrofluorometers
- **Transducers**
  - Photoluminescence intensity is low, highly sensitive detectors needed
  - Photomultiplier Tubes
  - CCD (*charge coupled devices*) for Spectrofluorometers
- **Sample compartment (cuvette holder)**
  - Should be designed to minimize stray and scattered photons from reaching the detector
  - Sometimes deflector is added to block scattered light
- **Data Analysis**
  - Specialized computer programs can be used for specific applications (kinetics, mixture analysis, detection in chromatography)



# SAMPLE HOLDERS FOR FLUOROMETRY AND SPECTROFLUOROMETRY

- Cuvettes (Sample Cells)

- Cylindrical or rectangular cuvettes, made of glass or silica (fused quartz)
- Important: grease from fingerprints may fluoresce and interfere with measurements (use gloves!)
- Low-volume micro-cells and flow cells are available
- Detection at 90 ° is the most common configuration, but other angles can be used
- All walls should be transparent

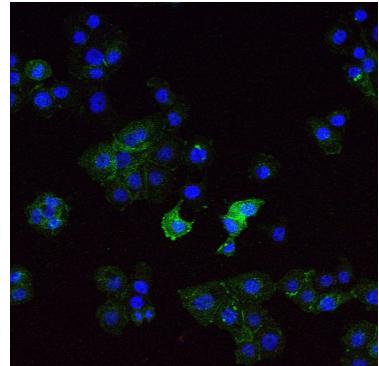


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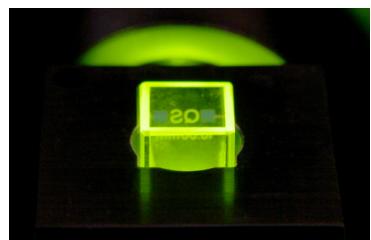
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## SPECTROFLUOROMETRY APPLICATIONS

- High selectivity of spectrofluorometry is important in characterizing electronic and structural properties of fluorescent molecules and quantum dots
  - Maximum absorption peak in UV-vis can be used to select a suitable excitation wavelength
- High selectivity is also useful for qualitative and quantitative analyses
- Low limits of detection: Fluorescence intensity can be increased by increasing the radiant power of the excitation source
  - Linear relation of source power to luminescence
    - This is not helpful for absorbance as it depends on the ratio of incident power to that of transmitted light



Quantum dots (fluorescent nanoparticles) used as labels for cell components



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## SPECTROFLUOROMETRY EXAMPLES

- Anthracene:** Emission Spectrum is almost a mirror image of the excitation spectrum, since separation between vibrational states of the excited state is very similar to the ground state

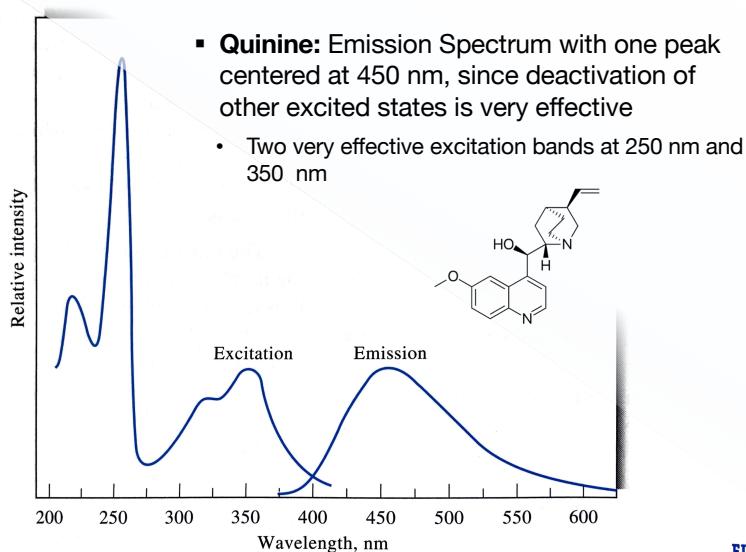


FIGURE 15-3 Fluorescence excitation and emission spectra for a solution of quinine.

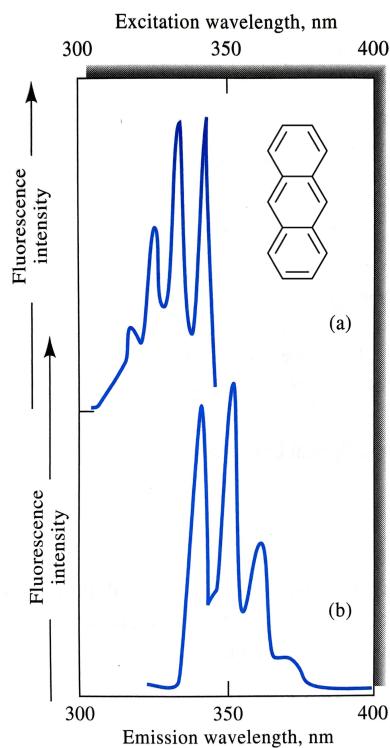


FIGURE 15-9 Fluorescence spectra for 1 ppm anthracene in alcohol: (a) excitation spectrum and (b) emission spectrum.



## APPLICATIONS: DETERMINATION OF INORGANIC SPECIES

- Measurement of fluorescing chelates of ions
- Measurements via *quenching* of fluorescence: typical for anions

TABLE 15-2 Selected Fluorometric Methods for Inorganic Species

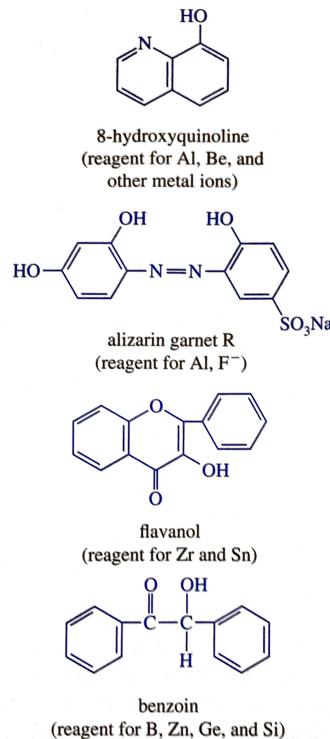
Ion	Reagent	Wavelength, nm		LOD, $\mu\text{g/mL}$	Interferences
		Absorption	Fluorescence		
$\text{Al}^{3+}$	Alizarin garnet R	470	500	0.007	Be, Co, Cr, Cu, $\text{F}^-$ , $\text{NO}_3^-$ , Ni, $\text{PO}_4^{3-}$ , Th, Zr
$\text{F}^-$	Quenching of $\text{Al}^{3+}$ complex of alizarin garnet R	470	500	0.001	Be, Co, Cr, Cu, Fe, Ni, $\text{PO}_4^{3-}$ , Th, Zr
$\text{B}_4\text{O}_7^{2-}$	Benzoin	370	450	0.04	Be, Sb
$\text{Cd}^{2+}$	2-( <i>o</i> -Hydroxyphenyl) benzoxazole	365	Blue	2	$\text{NH}_3$
$\text{Li}^+$	8-Hydroxyquinoline	370	580	0.2	Mg
$\text{Sn}^{4+}$	Flavanol	400	470	0.1	$\text{F}^-$ , $\text{PO}_4^{3-}$ , Zr
$\text{Zn}^{2+}$	Benzoin	—	Green	10	B, Be, Sb, colored ions

From J. A. Dean, *Analytical Chemistry Handbook*, New York: McGraw-Hill, 1995, pp. 5.60–5.62.



# FLUOROMETRIC CHELATING AGENTS

- Fluorescent chelates are more useful for non-transition metals
  - Many transition metal ions are paramagnetic
  - Increased rate of intersystem crossing to triplet state
- Transition metals have many closely spaced energy levels
- Internal conversion deactivation is more likely



**FIGURE 15-15** Some fluorometric chelating agents for metal cations. Alizarin garnet R can detect Al<sup>3+</sup> at levels as low as 0.007 µg/mL. Detection of F<sup>-</sup> with alizarin garnet R is based on fluorescence quenching of the Al<sup>3+</sup> complex. Flavanol can detect Sn<sup>4+</sup> at the 0.1-µg/mL level.



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## ORGANIC SPECIES DETERMINATION

- Fluorescence can be used for direct determination of organic and biochemical substances
- Over 200 substances can be routinely analyzed by fluorescence
  - Nucleic acids, proteins, aminoacids
  - Aromatic compounds, including aspirin, antibiotics and others
  - Many medicinal agents
  - Numerous physiologically important compounds fluoresce
- Fluorometry is used in the analysis of food products, pharmaceuticals, clinical samples, natural products
  - High sensitivity and selectivity make it a valuable technique for these fields

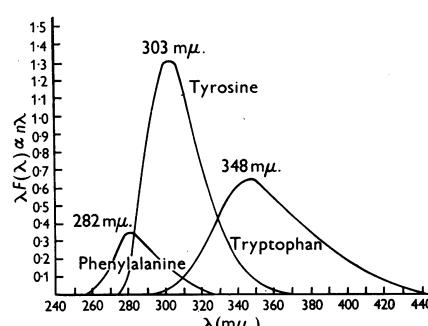


Fig. 7. Fluorescence spectra of the aromatic amino acids in water. Abscissa: wavelength (m $\mu$ ). Ordinate: relative number of quanta.

Figure from F. W. J. Teale, G. Weber “Ultraviolet fluorescence of the aromatic amino acids” *Biochem J.* 65 (1957) 476–482.

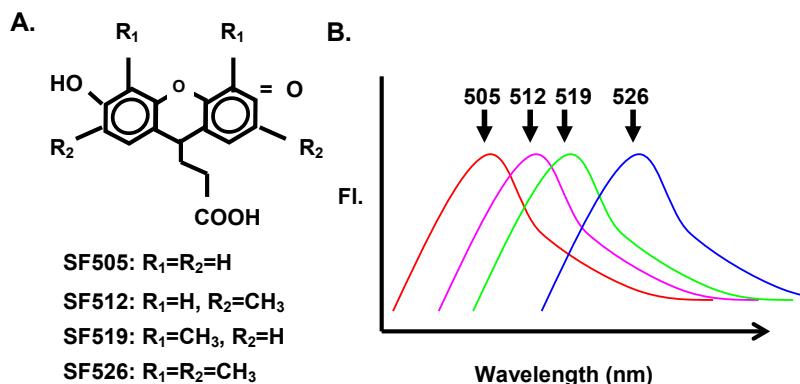


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# FLUORESCENCE IN DNA SEQUENCING

- Automated sequencing of DNA is based on attaching fluorophores to DNA fragments, a different one for each of the four bases (A, T, C, G)
  - In the example below, emission wavelength is tuned by changing the substituent groups
- A detector reads emission wavelengths as DNA pieces pass in a capillary tube
- Fragments are previously separated by size through electrophoresis

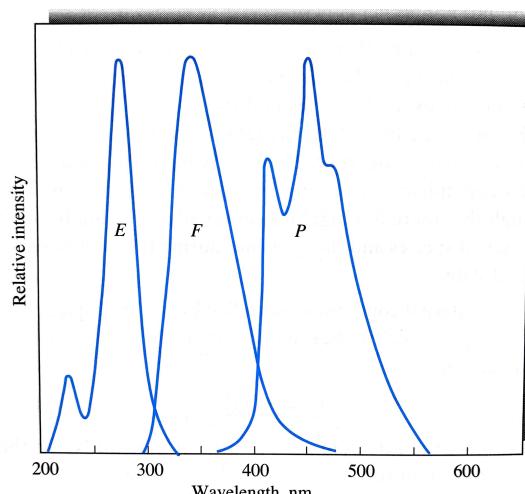


**Figure 5.** A. Chemical structure of the four succinylfluorescein dyes developed at DuPont. B. Normalized fluorescence emission spectra for each of the four dyes following excitation at 488nm. Shifts in the spectra were achieved by changing the side groups R<sub>1</sub> and R<sub>2</sub>.



# EMISSION AND EXCITATION SPECTRA

- Excitation spectrum: measure luminescence at fixed wavelength, scan excitation wavelengths
- Fluorescence and Phosphorescence spectra: fixed wavelength for excitation, record emission intensity as function of wavelength



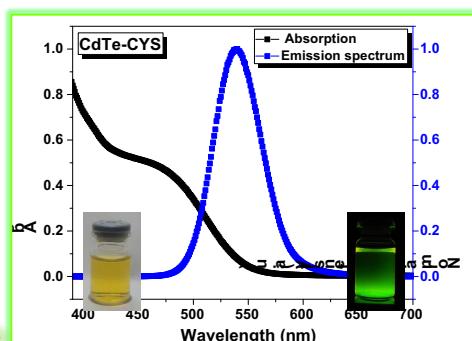
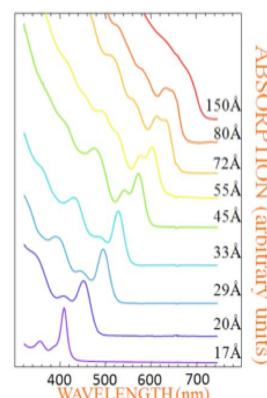
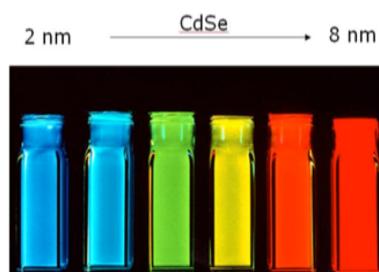
**FIGURE 15-5** Spectra for tryptophan: E, excitation; F, fluorescence; P, phosphorescence. (Adapted from G. G. Guilbault, *Practical Fluorescence*, New York: Marcel Dekker, 1973, p. 164. Courtesy of Marcel Dekker, Inc.)



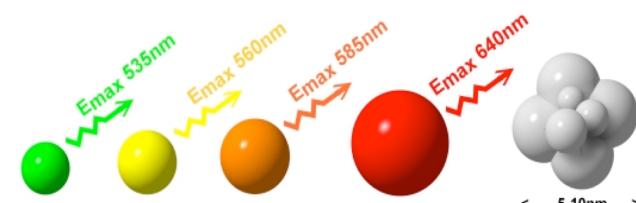
# QUANTUM DOTS

Quantum Confinement

- Electron confinement effects make the bandgap of semiconductor quantum dots change with size
- Their fluorescence maximum (and maximum UV-vis absorption peak) changes with size

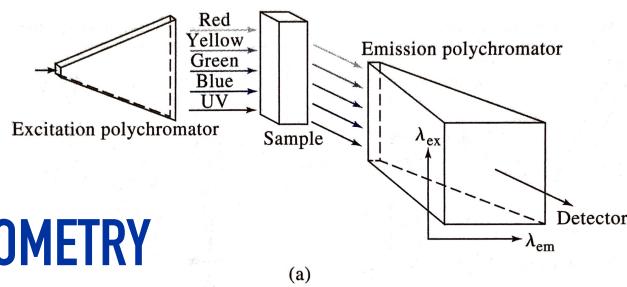


Absorption (UV-vis) and emission (fluorescence) spectra of CdTe quantum dots (stabilized by cysteamine)  
Vanessa F.A. Lima, D.V. Freitas, M. Navarro, F.J. Rodríguez-Macías



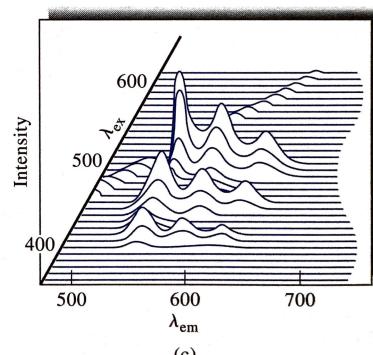
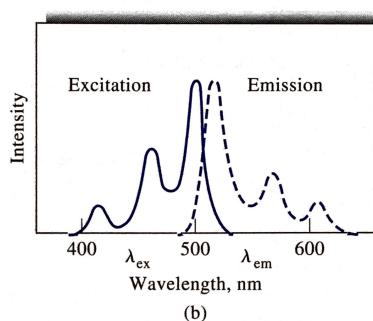
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## 3D SPECTROFLUOROMETRY

- Measurement of intensity of emission in different wavelengths by excitation with different wavelengths



**FIGURE 15-12** Three-dimensional spectrofluorometer. (a) Schematic of an optical system for obtaining total luminescence spectra with a CCD detector. (With permission from G. W. Suter, A. J. Kallir, and U. P. Wild, *Chimia*, **1983**, *37*, 413.) (b) Excitation and emission spectra of a hypothetical compound. (c) Total luminescence spectrum of compound in (b). (With permission of D. W. Johnson, J. P. Callis, and G. C. Christian, *Anal. Chem.*, **1977**, *49*, 747A, DOI: 10.1021/ac50016a769. Figure 3, p. 749A. Copyright 1977 American Chemical Society.)

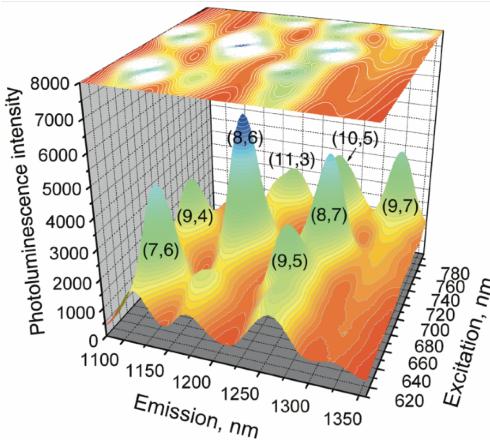


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## 3D SPECTROFLUOROMETRY EXAMPLE: PHOTOLUMINESCENCE MAPS OF SINGLE WALLED CARBON NANOTUBES (SWCNT)

- Fluorescent emission wavelength of single-walled carbon nanotubes depends on their molecular structure
  - SWCNT can be metallic or semiconducting depending on chirality (how carbon hexagon rings are rolled around the tube axis)
- Only semiconducting nanotubes show photoluminescence due to separation of electron-hole pairs, followed by emission on recombination
  - In metallic nanotubes any holes of excited states get filled by other electrons
- An emission-excitation matrix scan allows identifying nanotubes by chirality depending on wavelength of photoluminescence



**Fig. 3.** Emission-excitation matrix scan of a mixture of SWNTs recorded with the NanoLog®. Chirality of each species is presented as (n,m). The white lines on the upper surface of the 3-D plot are from a simulation of the same matrix performed by the Nanosizer™ software.

Figure from Horiba Scientific “Fluorescence Spectra from Carbon Nanotubes”, Application Note brochure (PDF format) downloaded from <http://www.horiba.com/scientific/products/fluorescence-spectroscopy/application-notes/> [accessed September 2017]

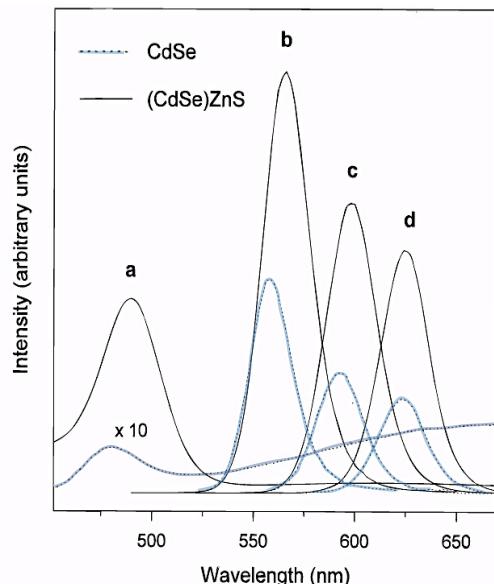
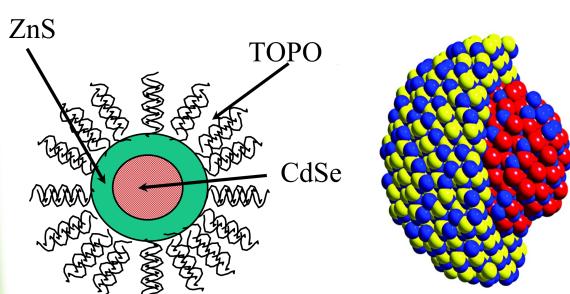


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## EXAMPLE: CORE-SHELL (CdSe)ZnS QUANTUM DOTS

- Photoluminescence spectra show that coating CdSe with ZnS improves quantum yield
  - Coating passivates surface states that lead to non-radiative de-excitation
  - Larger band-gap of shell increases confinement of electrons in core



**Figure 2.** Photoluminescence (PL) spectra for bare (dashed lines) and ZnS overcoated (solid lines) dots with the following core sizes: (a) 23, (b) 42, (c) 48, and (d) 55 Å in diameter. The PL spectra for the overcoated dots are much more intense owing to their higher quantum yields: (a) 40, (b) 50, (c) 35, and (d) 30.

B.O. Dabbousi, J. Rodriguez-Viejo, F.V. Mikulec, J.R. Heine, H. Mattoussi, R. Ober, K.F. Jensen, M.G. Bawendi “(CdSe)ZnS Core-Shell Quantum Dots: Synthesis and Characterization of a Size Series of Highly Luminescent Nanocrystallites” J. Phys. Chem. B 101 (1997) 9463



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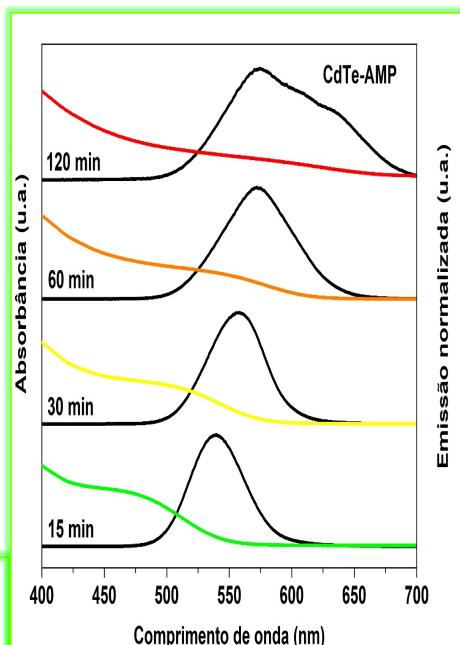
# EXAMPLE: QUANTUM DOTS

- Fluorescence maximum (and maximum UV-vis absorption peak) change with size due to electron confinement effects

CdTe quantum dots (stabilized by mercaptopropionic acid) under visible light (A) and under UV light (B), samples taken after synthesis times of 0, 15, 30, 60 and 120 minutes



Shifts in the absorption band and the fluorescence peak due to increase in QD size with synthesis time



Images taken from: Vanessa Ferreira de Araujo Lima, "Síntese Eletroquímica de Nanocompósito de Pontos Quanticos de CdTe Ancorados em Nanotubos de Carbono" (*Electrochemical Synthesis of Nanocomposites of CdTe Quantum Dots Anchored on Carbon Nanotubes*), Masters Thesis, Graduate Program in Materials Science, Universidade Federal de Pernambuco, Recife, Brasil, 2017 Thesis Advisors: Prof. F.J. Rodríguez-Macías, and Prof. M. Navarro .

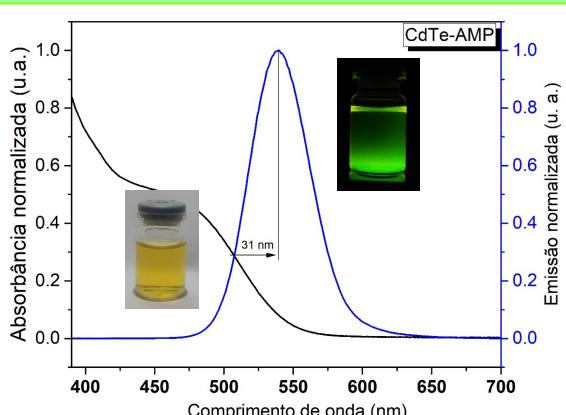


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## CNT+QUANTUM DOTS

Absorption (UV-vis) and emission (fluorescence) spectra of CdTe quantum dots (stabilized by cysteamine)  
Vanessa F.A. Lima, D.V. Freitas, M. Navarro, F.J. Rodríguez-Macías



CdTe quantum dots (stabilized by mercaptopropionic acid) synthesized *in situ* with Multi-Walled Carbon Nanotubes, under visible light (left) and under UV light (right), thermal treatment times of 0, 15, 30, and 45 minutes were used to change the QD diameter



Images taken from: Vanessa Ferreira de Araujo Lima, "Síntese Eletroquímica de Nanocompósito de Pontos Quanticos de CdTe Ancorados em Nanotubos de Carbono" (*Electrochemical Synthesis of Nanocomposites of CdTe Quantum Dots Anchored on Carbon Nanotubes*), Masters Thesis, Graduate Program in Materials Science, Universidade Federal de Pernambuco, Recife, Brasil, 2017. Thesis Advisors: Prof. F.J. Rodríguez-Macías, and Prof. M. Navarro

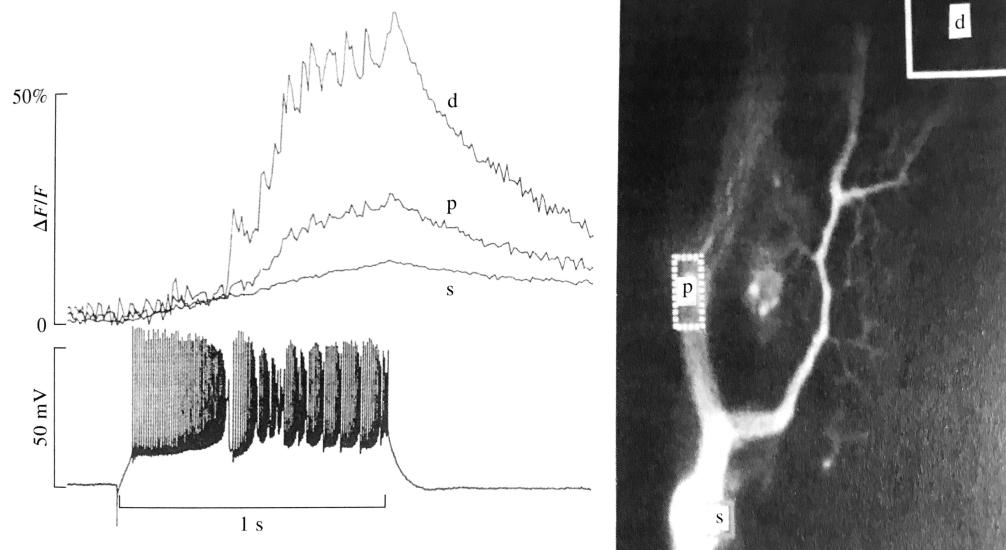


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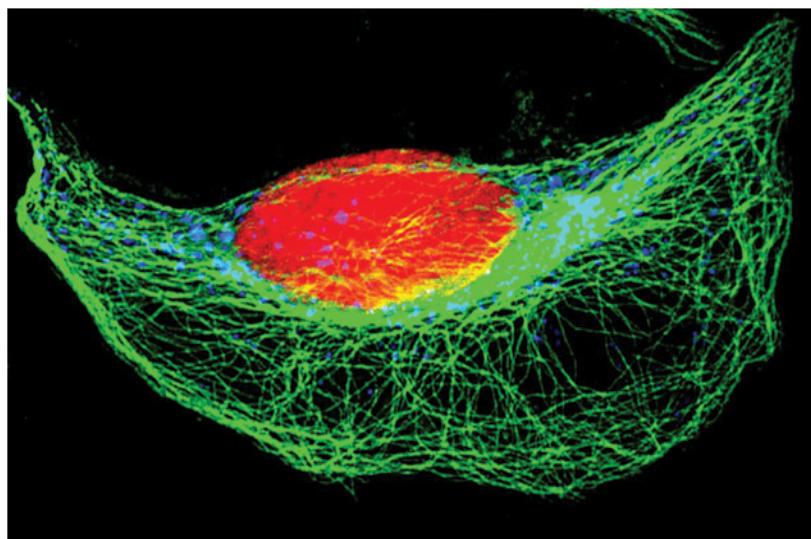
# FLUORESCENCE IMAGING

- Microscopy image of fluorophores inside biological matrices (tissues or cells)
- Fluorescent indicators used when analyte is not fluorescent



**FIGURE 15-16** Calcium transients in a cerebellar Purkinje cell. The image on the right is of the cell filled with a fluorescent dye that responds to the calcium concentration. Fluorescent transients are shown on the top left recorded at areas d, p, and s in the cell. The transients in region d correspond to the dendrite region of the cell. Specific calcium signals can be correlated to the action potentials shown on the bottom left. (From V. Lev-Ram, H. Mikayawa, N. Lasser-Ross, W. N. Ross. Calcium Transients in Cerebellar Purkinje Neurons Evoked by Intracellular Stimulation, *J. Neurophysiol.*, **1992**, *68*, 1170-1177, figure 2A.)

## APPLICATION OF QUANTUM DOT FLUORESCENT MARKERS IN MICROSCOPY



Three-color staining of HeLa cells using fluorescent Qdot® nanocrystal conjugates. The intracellular structures in fixed HeLa cells were visualized using a red-fluorescent Qdot® 655 F(ab')2 goat anti-mouse IgG (Q11021MP, Q11022MP) (nuclei), a yellow-fluorescent Qdot® 585 F(ab')2 goat anti-rabbit IgG (Q11411MP) (Golgi complex), and a green-fluorescent Qdot® 525 streptavidin conjugate (Q10141MP) (microtubules).

# SOME EXAMPLES OF FLUORESCENT NANOSTRUCTURES AND THEIR APPLICATIONS

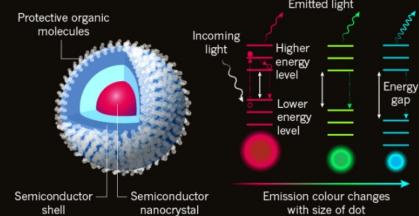
- X. Lim, “*The nanolight revolution is coming*”, Nature 531 (2016) 26–28 DOI:10.1038/531026a
  - <http://www.nature.com/news/the-nanolight-revolution-is-coming-1.19482>
- K. Bourzac, “*Quantum dots go on display*”, Nature 493 (2013) 283; DOI:10.1038/493283a
  - <http://www.nature.com/news/quantum-dots-go-on-display-1.12216>
- K. Sanderson “*Quantum dots go large*” Nature 459 (2009) 760–761 DOI:10.1038/459760a
  - <http://www.nature.com/news/2009/090610/full/459760a.html>
- G. Morrison “*Quantum dots: How nanocrystals can make LCD TVs better*” CNET, 26-enero-2015
  - <<http://www.cnet.com/uk/news/quantum-dots-how-nanocrystals-can-make-lcd-tvs-better/>> (accesado Marzo, 2016)

## BRIDGE THE GAP

Some virus-sized particles can be tailored to absorb light and fluoresce at specific frequencies. Light kicks electrons up to higher energy levels, and the glow is emitted when the electrons fall back past a wide energy gap.

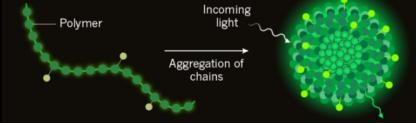
### QUANTUM DOTS

These particles contain layers of crystalline semiconductor material. The colour they emit depends on the particle's size.



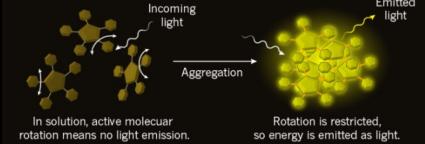
### P-DOTS

These particles are made of semiconducting polymers that emit light when they aggregate. They can be brighter than a quantum dot of the same size, but the glow fades if the strands bunch too tightly.



### AIE-DOTS

These molecules will not fluoresce unless they are tightly packed, which restricts the energy they lose through internal motion.



### UPCONVERSION PARTICLES

Particles made from layers of heavy metal lanthanide elements can accumulate energy from infrared light and re-emit it as visible or ultraviolet light.

©nature bit.ly/Nanolights

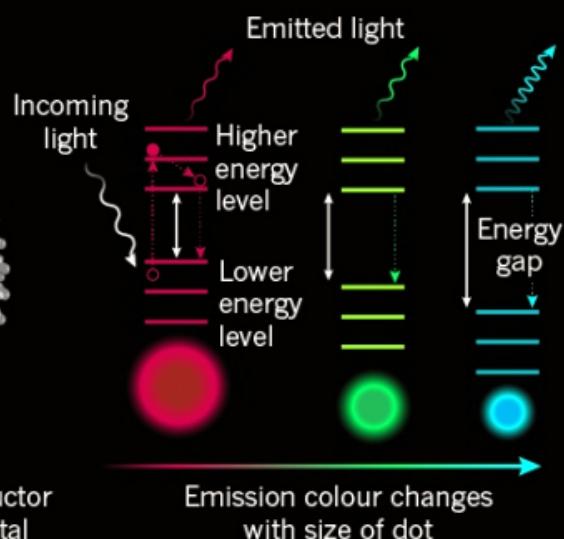
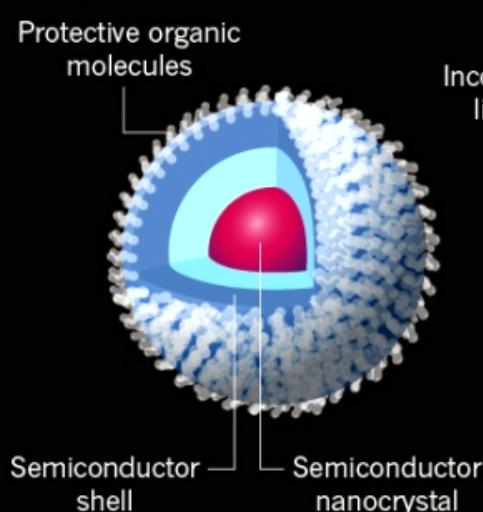


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# PHOTOLUMINESCENT NANOSTRUCTURES

## QUANTUM DOTS

These particles contain layers of crystalline semiconductor material. The colour they emit depends on the particle's size.



Modified from image taken from <http://www.nature.com/news/the-nanolight-revolution-is-coming-1.19482>



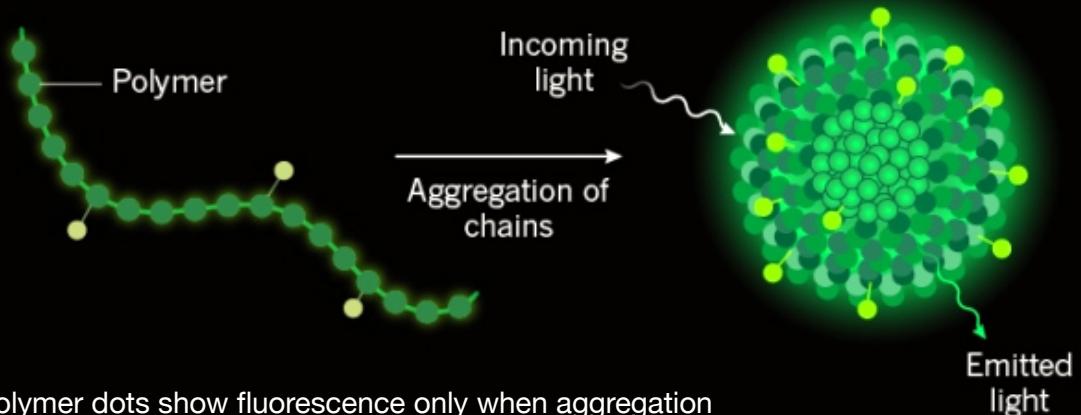
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# PHOTOLUMINESCENT NANOSTRUCTURES

## P-DOTS

These particles are made of semiconducting polymers that emit light when they aggregate. They can be brighter than a quantum dot of the same size, but the glow fades if the strands bunch too tightly.



- Polymer dots show fluorescence only when aggregation reduces quenching from interaction with the environment
- But if polymer chains in aggregate are too close there is self-quenching

Modified from image taken from <http://www.nature.com/news/the-nanolight-revolution-is-coming-1.19482>



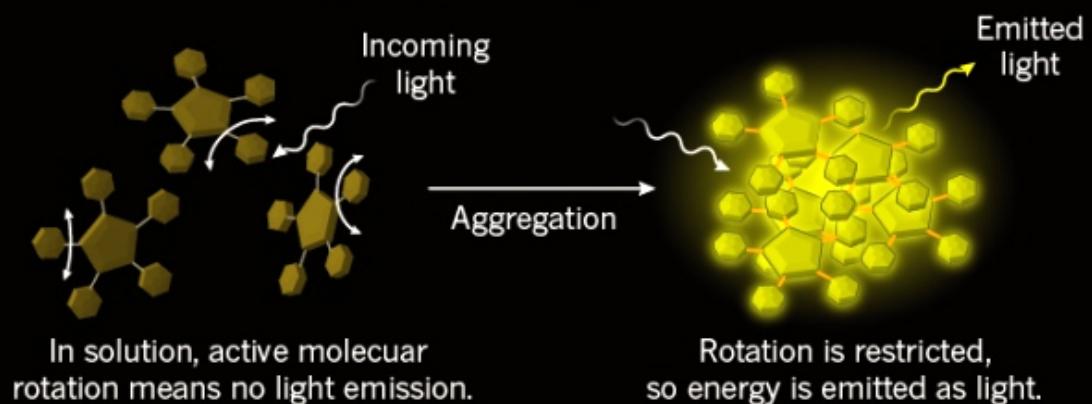
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# PHOTOLUMINESCENT NANOSTRUCTURES

## AIE-DOTS

These molecules will not fluoresce unless they are tightly packed, which restricts the energy they lose through internal motion.



- When these molecules aggregate internal conversion is not possible and the agglomerates show fluorescence

Modified from image taken from <http://www.nature.com/news/the-nanolight-revolution-is-coming-1.19482>



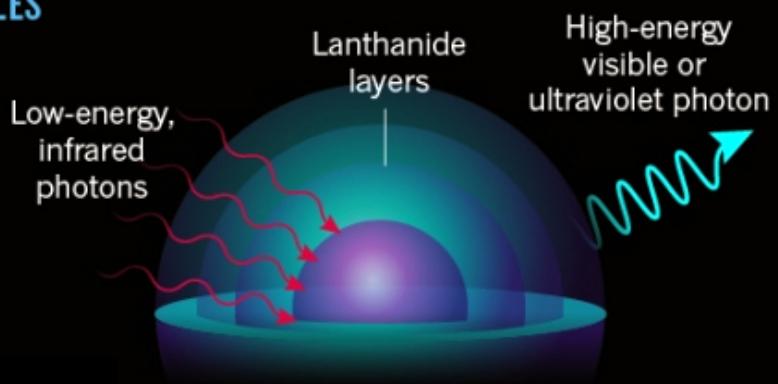
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# PHOTOLUMINESCENT NANOSTRUCTURES

## UPCONVERSION PARTICLES

Particles made from layers of heavy-metal lanthanide elements can accumulate energy from infrared light and re-emit it as visible or ultraviolet light.



- In upconversion, energy of two absorbed photons puts molecules or nanostructures in a state emitting higher energy photons
- Upconversion can be important in photoemitting structures, and also in photovoltaic devices

Modified from image taken from <http://www.nature.com/news/the-nanolight-revolution-is-coming-1.19482>

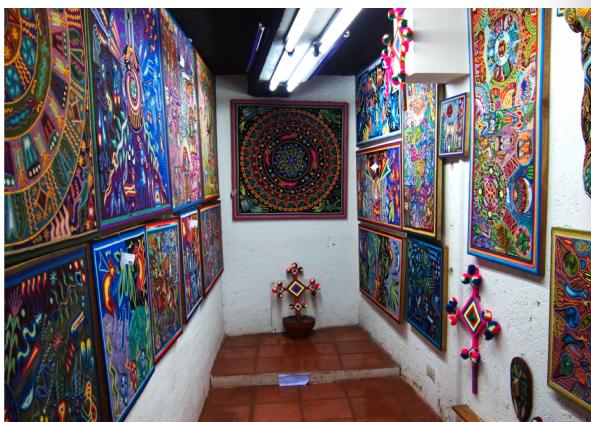


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## AN EXAMPLE OF *CREATIVE* USE OF FLUORESCENCE

- People from the Huichol tribe create elaborate designs using yarn of different colors
- They now incorporate yarns dyed with fluorescent molecules



- Under UV light the yarns fluoresce with a variety of colors



Photos © Fernando JRM, under a CC-BY-NC-SA license

Photo above taken under "black" light lamps



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# BONUS CONTENT:

# PHOSPHORESCENCE &

# CHEMILUMINESCENCE

Note 1: There are phosphorescent nanostructures, but fluorescent nanostructures are much more common

Note 2: Chemiluminescence can be important for chemical analyses.

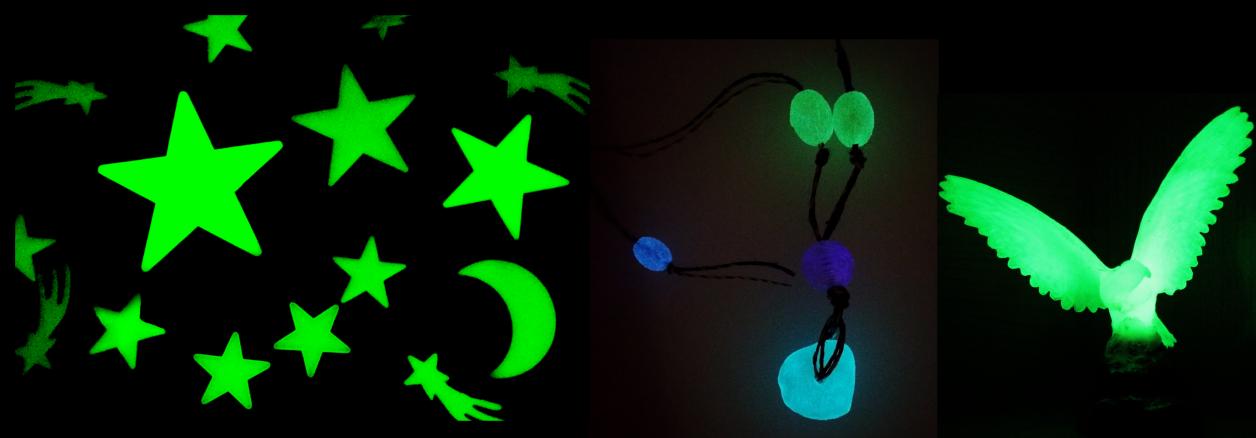
In nanotechnology its main applications are in Bionanotechnology



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## PHOSPHORIMETRY: PHOSPHORESCENCE MEASUREMENT



Stars and collar photos from: <https://sciencenotes.org/what-are-glow-in-the-dark-stars-made-of/>  
Phosphorescent eagle from: <https://www.boundless.com/physics/textbooks/boundless-physics-textbook/introduction-to-quantum-physics-28/applications-of-quantum-mechanics-183/fluorescence-and-phosphorescence-676-4913/>



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# PHOSPHORIMETRY

- Phosphorescence is measured in instruments similar to fluorometers and spectrofluorometers plus two additional components
  1. A device to alternate sample irradiation and measure emission after a delay
  2. A sample cooling cell (Dewar flask) to measure at low temperature

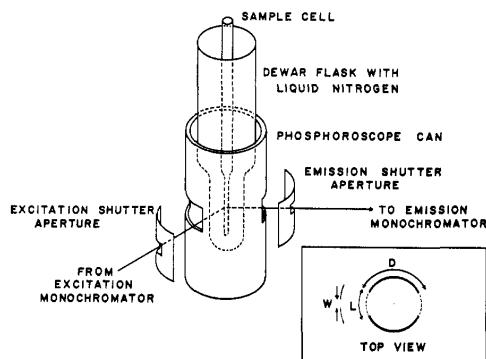


Figure 1. Schematic diagram of rotating-can phosphoroscope

- Delay between excitation and measurement is needed to differentiate between phosphorescence (long-lived states) and fluorescence (short-lived states) originating from the same sample
- Mechanical designs are an option
- Electronic delay: excitation by pulse from Xenon lamp. Data acquisition system only activated after delay
  - User can specify delay for a time when it is known that fluorescence has decayed to a very small value
  - Luminescence signal is integrated after lamp is off

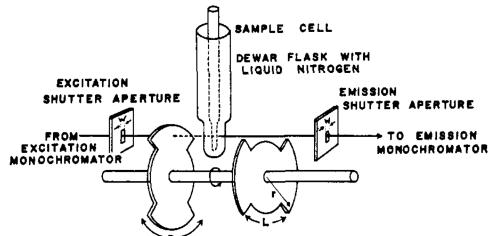


Figure 2. Schematic diagram of rotating disk phosphoroscope

Mechanical shutter designs for phosphoroscopes

From: *Anal. Chem.*, 1966, 38 (4), pp 602–607 DOI: 10.1021/ac60236a019

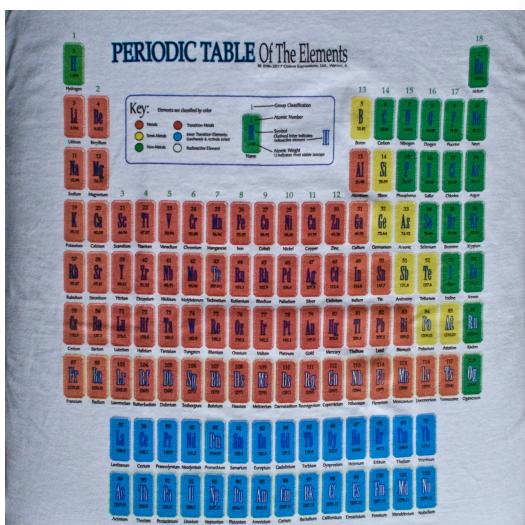


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## AN EXAMPLE OF *CREATIVE* USE OF PHOSPHORESCENCE

- Periodic Table T-shirt with radioactive element squares marked with a phosphorescent material



Photos © Fernando JRM under CC-BY-NC-SA license



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- Phosphorescent material glows in the dark for several minutes



Photo above taken in the dark, with a long(ish) exposure a few minutes after exposing the t-shirt to sunlight for about 40 minutes

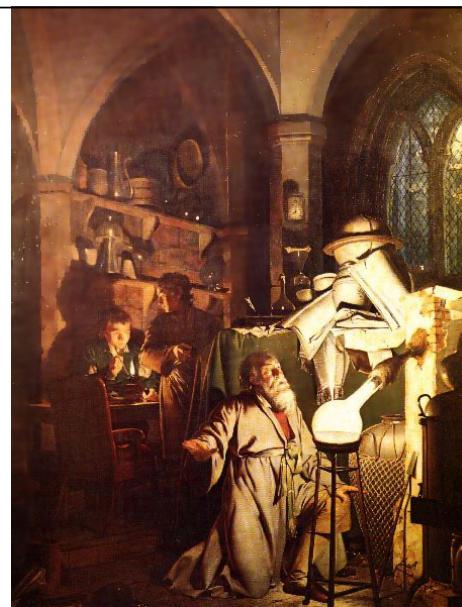
# CHEMILUMINESCENCE

- Some chemical reactions produce chemiluminescence
  - Chemiluminescence from biological systems (e.g. fireflies) is called *bioluminescence*
- General reaction can be represented as:



A reaction product is formed in an excited state  $C^*$  which emits a photon

- Highly selective method and with high sensitivity for any molecule that chemiluminesces
  - Sensitivity in the ppb or ppm range
- Chemiluminescence Intensity (photons/ second) depends on reaction rate ( $d[C]/dt$ ), and quantum efficiency of reaction ( $\phi_{CL}$ , photons emitted divided by number of reacted molecules)



Painting representing the Discovery of Phosphorus

by German alchemist Hennig Brand

Elemental phosphorus undergoes an oxidation reaction forming chemiluminescent transient species  $HPO$  and  $P_2O_5$

Photo of painting by Joseph Wright: "The Alchymist, In Search of the Philosopher's Stone, Discovers Phosphorus, and prays for the successful Conclusion of his operation, as was the custom of the Ancient Chymical Astrologers" taken from: <https://en.wikipedia.org/wiki/File:JosephWright-Alchemist.jpg>

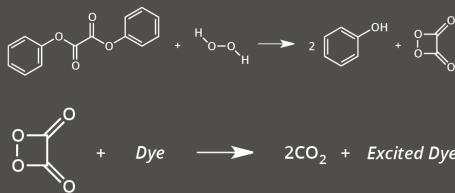
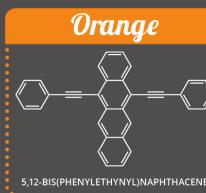
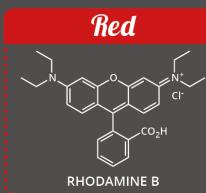


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## CHEMILUMINESCENCE: EMERGENCY (OR RECREATIONAL) LIGHT SOURCE

### THE CHEMISTRY OF GLOW STICK COLOURS



#### How do glow sticks produce light?

When glow sticks are bent, the inner glass tube is broken, releasing hydrogen peroxide solution. This then reacts with a diphenyl oxalate, producing 1,2-dioxetanedione; this product is unstable, & decomposes to carbon dioxide, releasing energy. The energy is absorbed by electrons in dye molecules, which subsequently fall back to their ground state, losing excess energy in the form of light.



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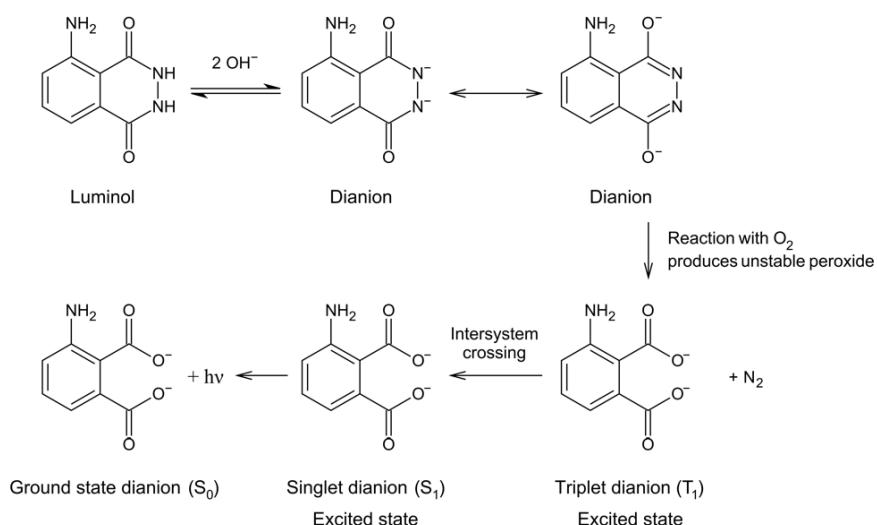


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# APPLICATIONS OF CHEMILUMINESCENCE: LUMINOL

- Luminol oxidizes emitting blue light with a peak centered at 425 nm
  - Reaction in basic solution with oxygen, hydrogen peroxide, permanganate, or hypochlorite
- Intensity proportional to concentration of oxidant, catalyst or luminol itself
  - Catalyst may be required for a useful reaction rate
    - Determination of Co(II), Cr(III), Cu(II) ions by catalyst effect on hydrogen peroxide reaction
  - Detection of cations that inhibit oxidation also possible
- Iron in blood acts as catalyst, allowing detection of blood in forensic science



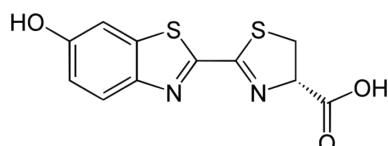
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# BIOLUMINESCENCE

## EXAMPLE: FIREFLIES IN ILLINOIS, USA

- Chemiluminescence from biological systems is called bioluminescence



- Structure of Firefly Luciferin
- Enzymatic Oxidation leaves luciferins in an excited state which emits light as it decays to the ground state

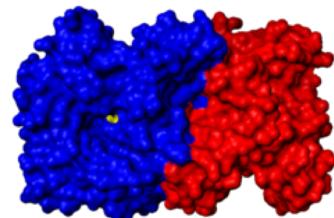
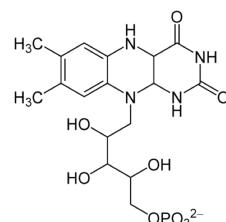
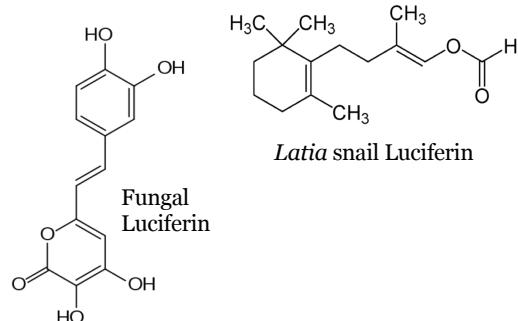


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# LUCIFERIN + LUCIFERASE

- The luciferin+luciferase (substrate/enzyme) system has been used to obtain chemiluminescence images in biological systems
- Luciferase gene inserted by genetic engineering linked in a way that it acts as “reporter” for the activity of other genes
- Reporter gene gets expressed at the same rate as the gene of interest
- Luciferase reaction has a high quantum efficiency (88%)
- For every 100 oxidized luciferin molecules, 88 photons are emitted
- R. Créton, L.F. Jaffe “Chemiluminescence Microscopy as a Tool in Biomedical Research” BioTechniques 31:1098-1105 (November 2001)



Bacterial Luciferin structure and 3D model of luciferase enzyme (from some genus in the Gammaproteobacteria class)

Images from Wikimedia Commons:< https://commons.wikimedia.org/wiki/File:Firefly\_luciferin.svg > < https://commons.wikimedia.org/wiki/File:Fungal\_luciferin.svg > < https://commons.wikimedia.org/wiki/File:Luciferin\_Latia.png > < https://commons.wikimedia.org/wiki/File:Luciferin\_bacterial.png > < https://commons.wikimedia.org/wiki/File:Bacterial\_luciferase.png >



# LUCIFERIN + LUCIFERASE ASSAYS

- Commercial reporter assays are available to insert luciferase genes with other genes in bacteria
- Applications in biotechnology and bionanotechnology

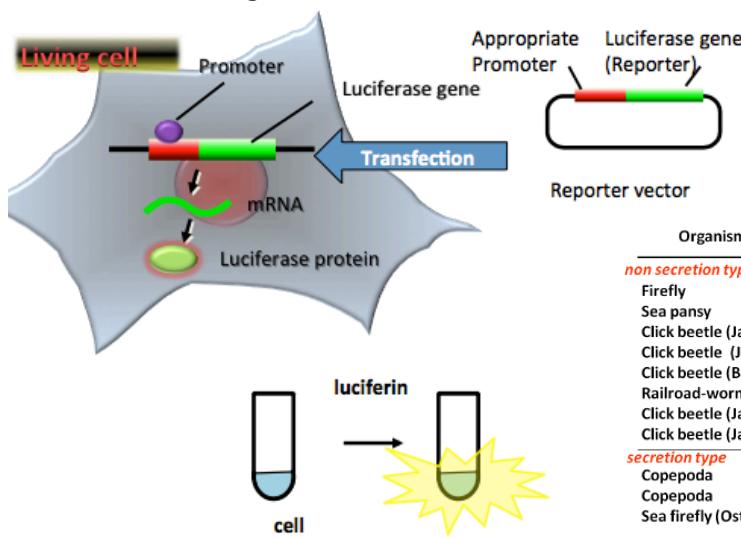


Image and table taken from:  
<http://photobiology.info/Ohmiya.html>

Organism	Gene symbol	Luciferin	Mass (kDa)	I <sub>max</sub> (nm)	Company
<i>non secretion type</i>					
Firefly	luc(+), luc2	firefly luciferin	61	562	Promega
Sea pansy	Rluc	coelenterazine	36	480	Promega
Click beetle (Jamaica)	CBGluc	firefly luciferin	60	537	Promega
Click beetle (Jamaica)	CBRluc	firefly luciferin	60	613	Promega
Click beetle (Brazil)	ELuc	firefly luciferin	61	538	TOYOBO
Railroad-worm	SLR	firefly luciferin	61	630	TOYOBO
Click beetle (Japan)	SLG	firefly luciferin	60	550	TOYOBO
Click beetle (Japan)	SLO	firefly luciferin	60	580	TOYOBO
<i>secretion type</i>					
Copepoda	GLuc	coelenterazine	20	480	NEB
Copepoda	MetLuc	coelenterazine	24	480	Clontech
Sea firefly (Ostracod)	CLuc	cypridinid luciferin	61	465	ATTO
					NEB

Promoter activity = Luciferase activity / Cell number or cellular enzyme activity

