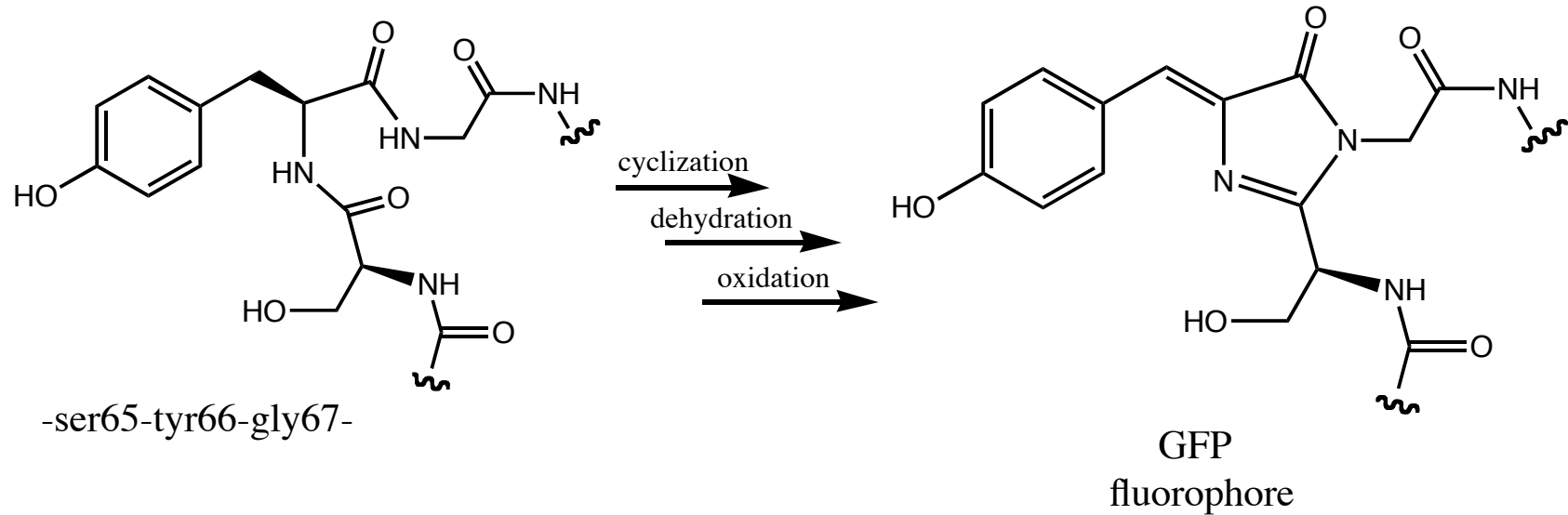


Chem184

Chemistry of ZS Yellow fluorophore

Green Fluorescent Protein (GFP)

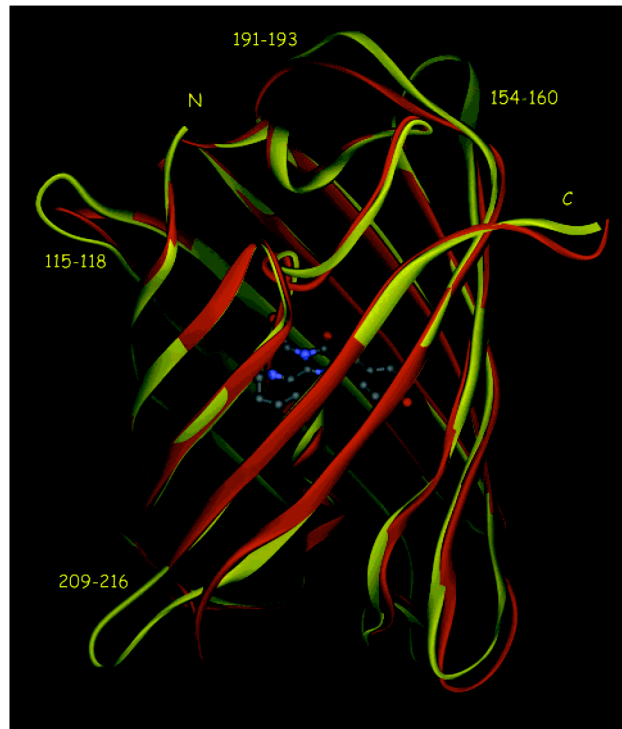


- protein makes its own fluorophore out of amino acids

Green Fluorescent Mouse

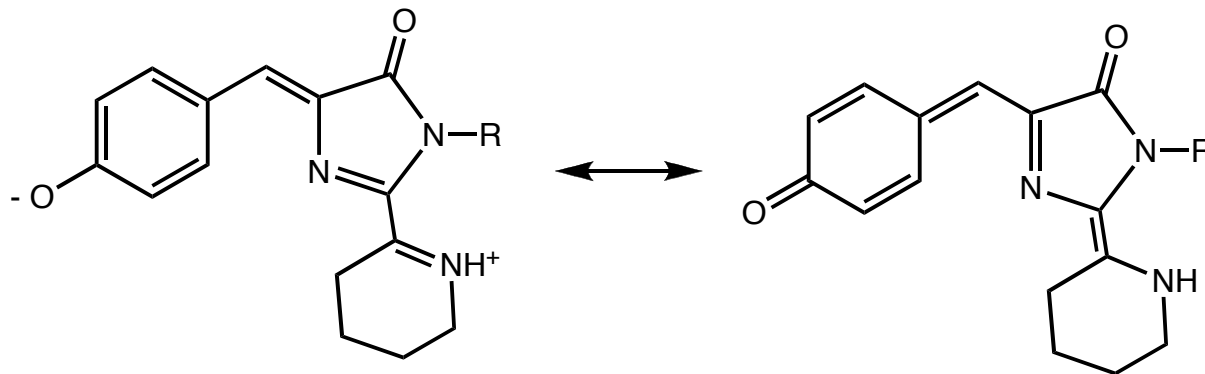
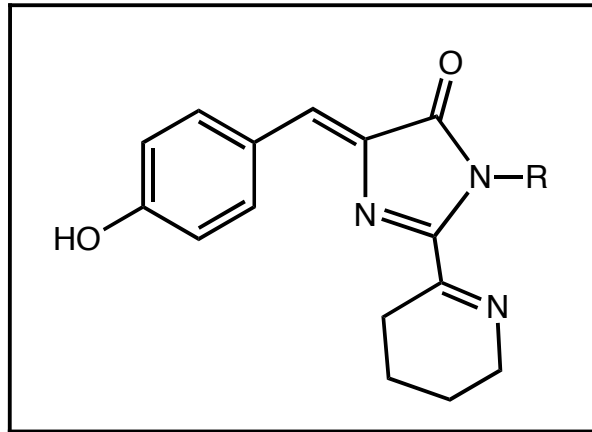


ZS Yellow Protein



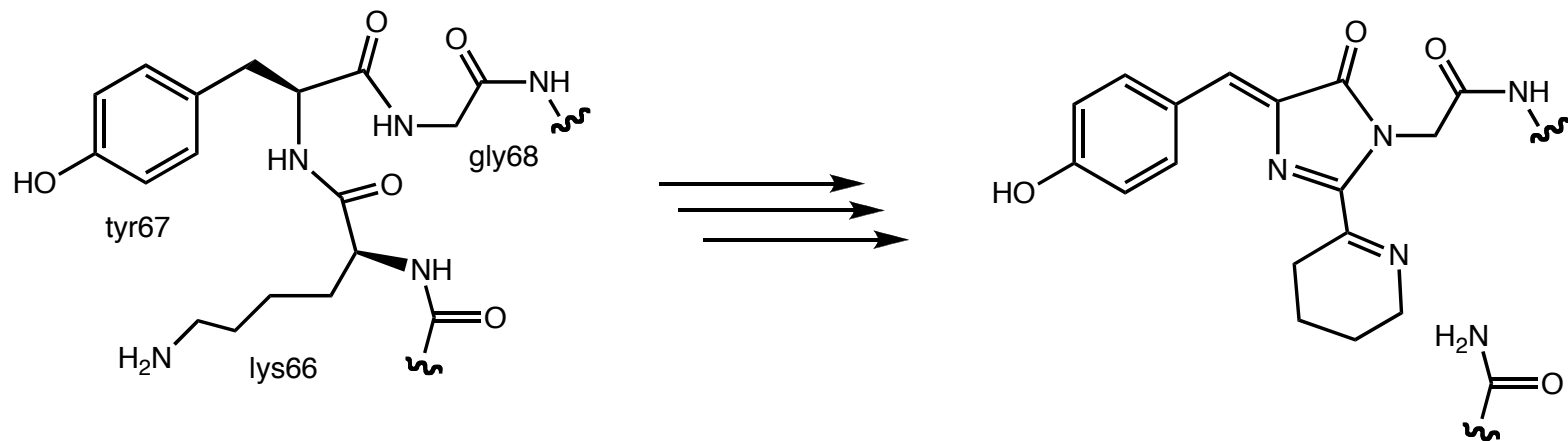
- **sequence substantially different than GFP but same fold**

The chromophore



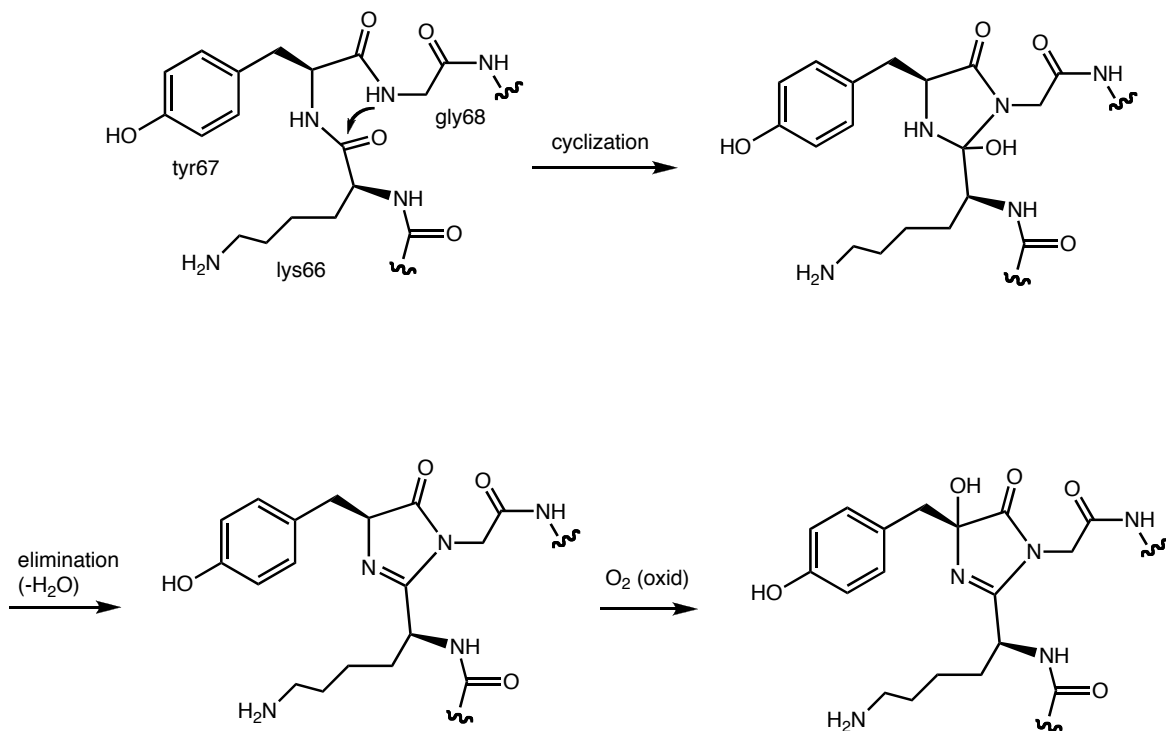
- **conjugated pi-system**
- **more orbitals involved than GFP**

Mechanism for formation

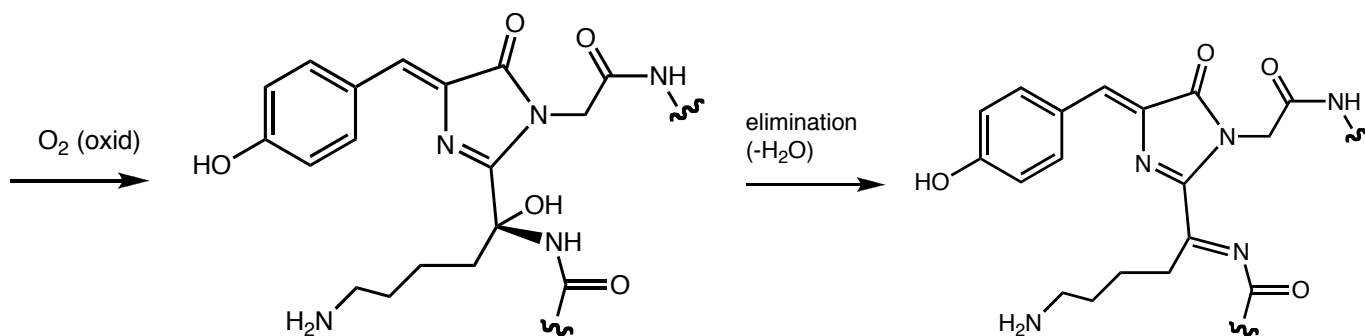
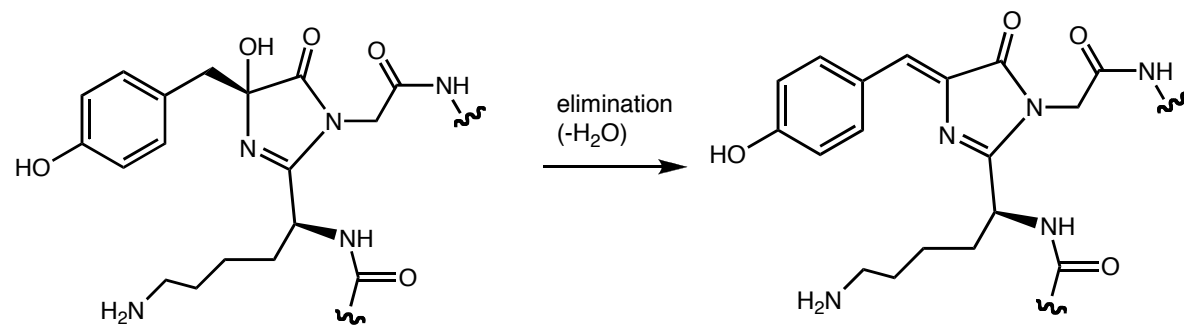


- self-catalyzed reactions (no other enzymes / reagents (exc. O₂) involved)

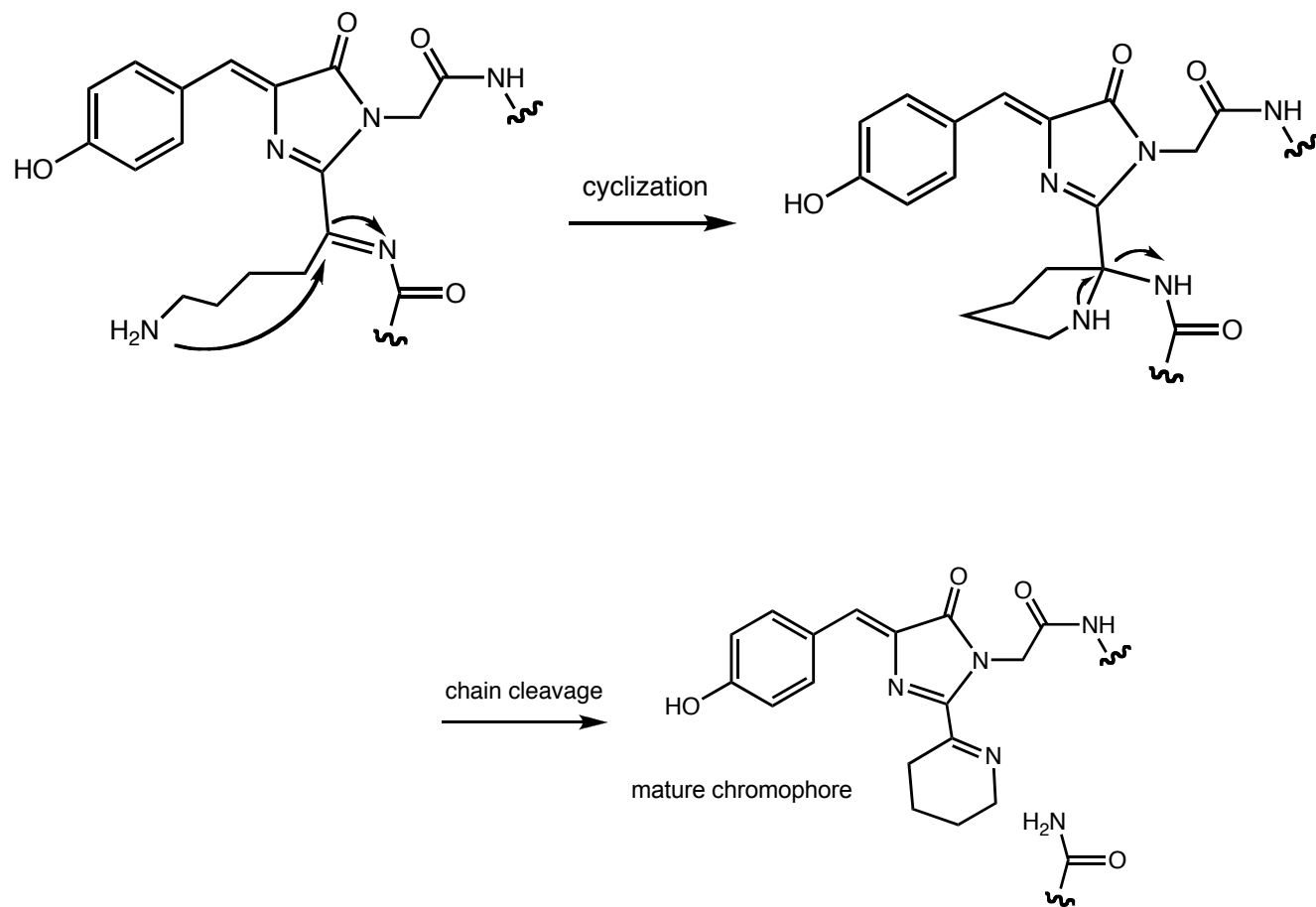
Mechanism for formation



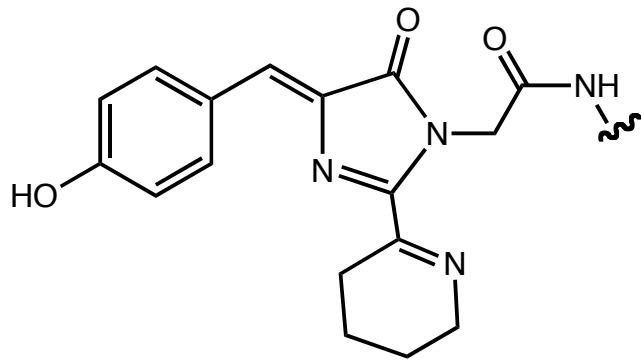
Mechanism for formation



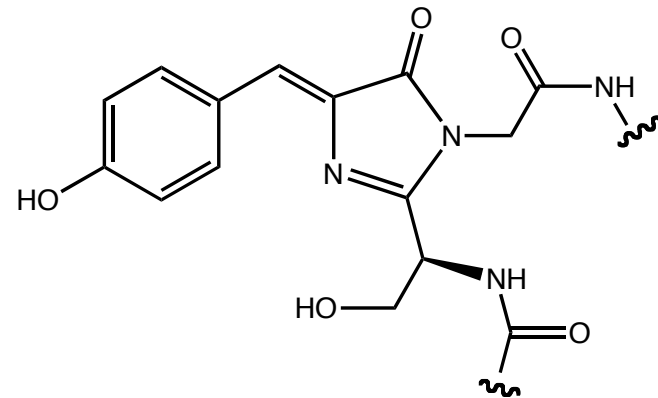
Mechanism for formation



Similarity to green fluorescent protein (GFP)



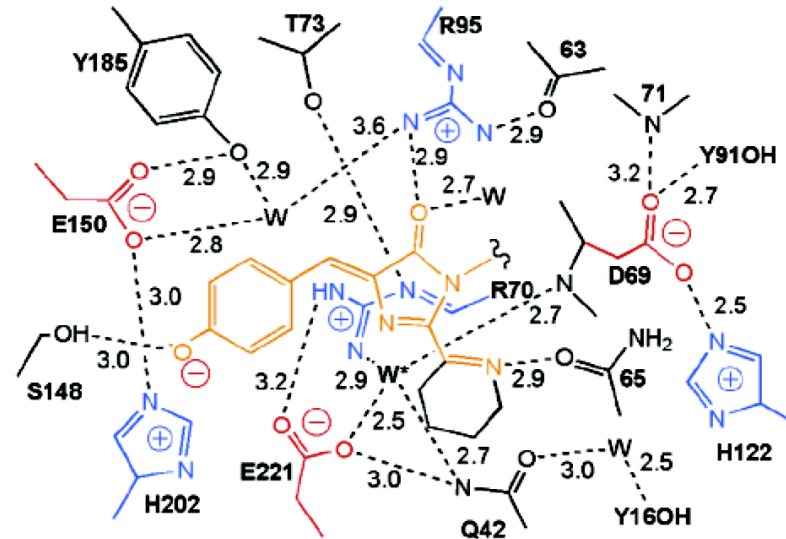
ZSY



GFP

- additional orbitals in ZSY chromophore structure (red-shifted)
- protein surroundings can make a difference also

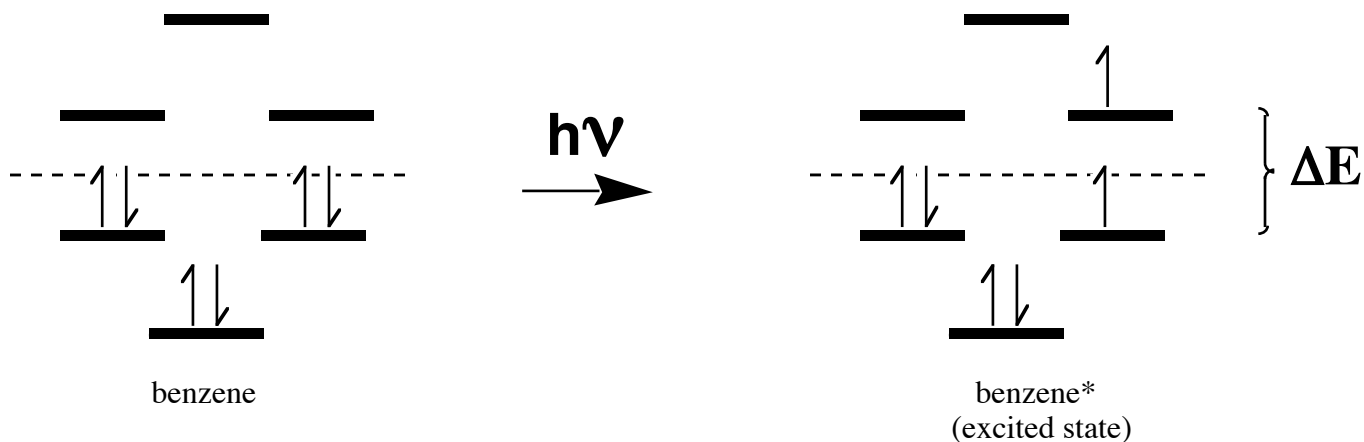
The Zsyellow protein environment around the chromophore



wild-type zFP538

- different sidechains can make a difference sterically, electronically
- changes in amino acids can indirectly affect chromophore also

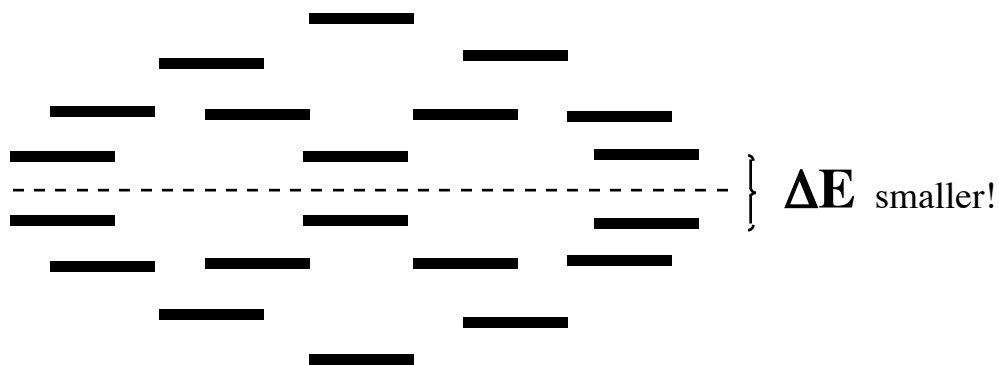
Why do conjugated molecules absorb visible light?



- light can excite electrons from lower energy molecular orbitals to higher

Why do conjugated molecules absorb visible light?

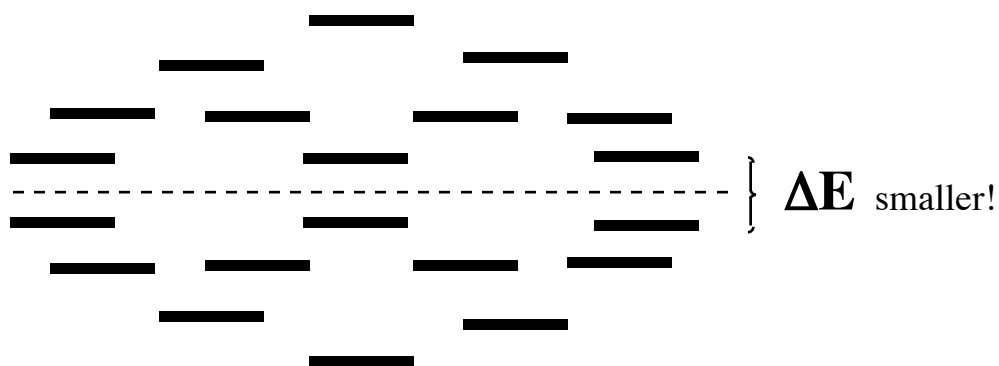
The closer together the energy levels,
the less energy it takes to excite the electrons



The more orbitals involved, the smaller the HOMO-LUMO gap is

Why do conjugated molecules absorb visible light?

The closer together the energy levels,
the less energy it takes to excite the electrons



from

$$E = h\nu$$

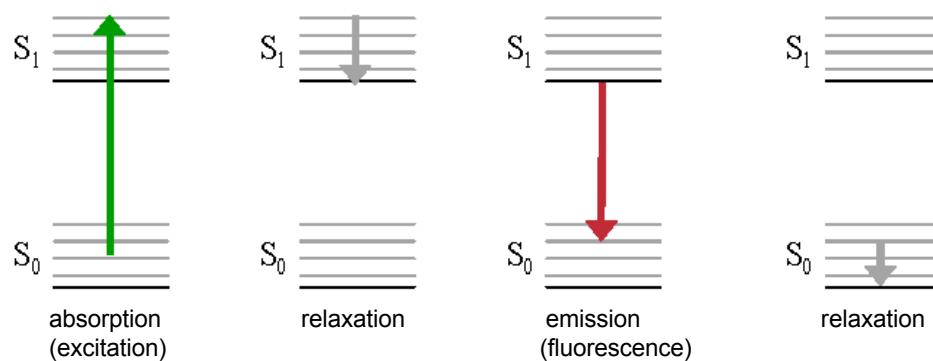
and

$$\nu = c/\lambda$$

We see that the longer the wavelength,
the lower the energy of the light

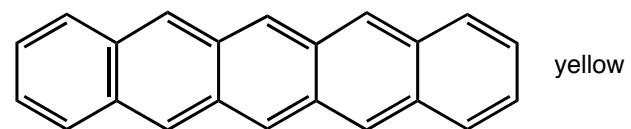
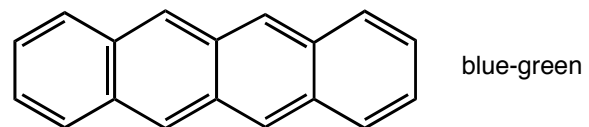
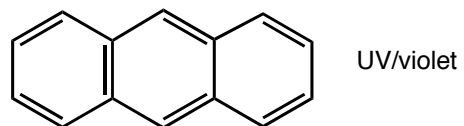
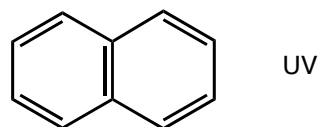
**Therefore, small organic molecules absorb in the UV,
large conjugated molecules absorb in the visible
(v. large ones in the infrared)**

Some notes on what makes a molecule fluorescent



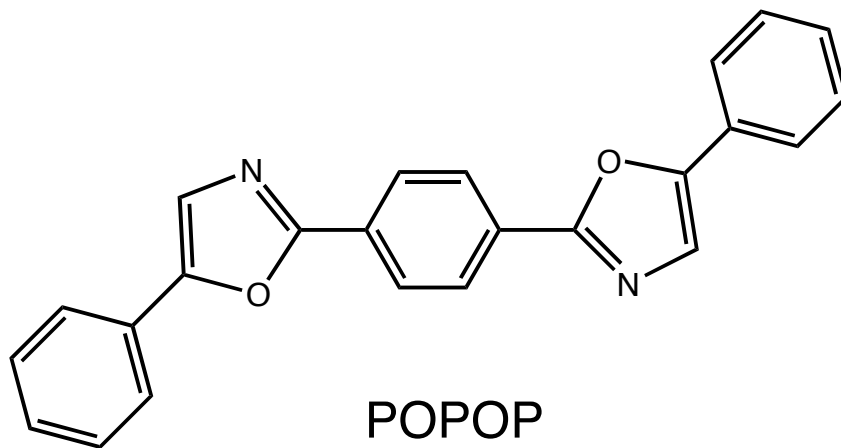
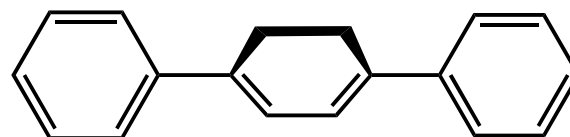
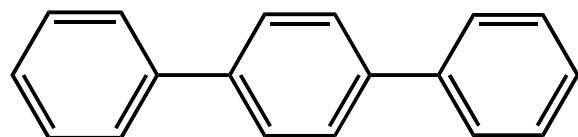
- light absorption, loss of small amount of energy (as heat)
- re-emission of photon, shifted to lower energy (redder)

Some notes on what makes a molecule fluorescent



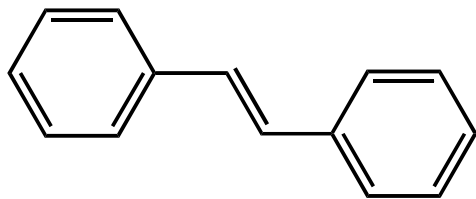
- **if interested in visible fluorescence (~400-700nm), then need several conjugated bonds**

Some notes on what makes a molecule fluorescent

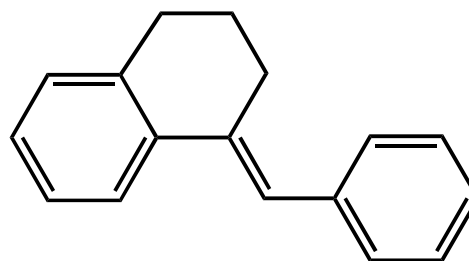


- **planarity important for efficient conjugation**

Some notes on what makes a molecule fluorescent



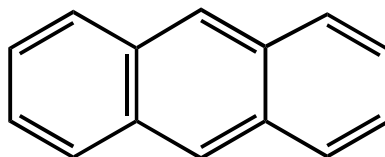
low quantum yield



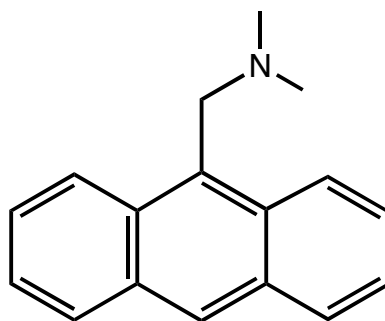
higher quantum yield

- **rigid enforcement of planarity**
(avoid radiationless decay via internal rotations)

Some notes on what makes a molecule fluorescent



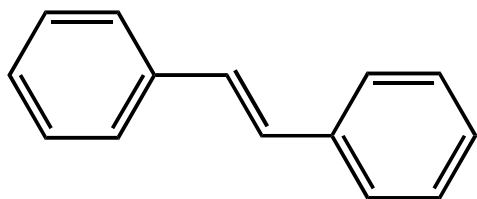
High quantum yield



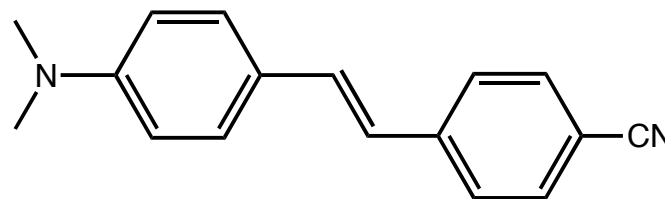
low quantum yield

- for efficient fluorescence (high quantum yield), need avoidance of strong electron acceptors / donors or heavy atoms

Some notes on what makes a molecule fluorescent



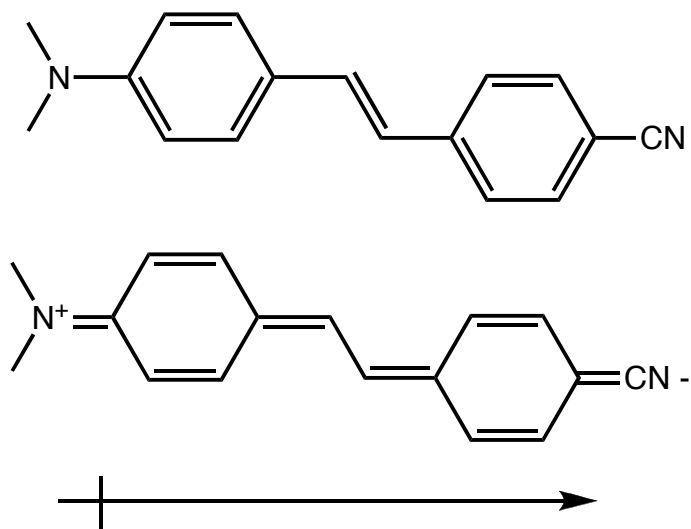
environmentally insensitive



sensitive to local environment

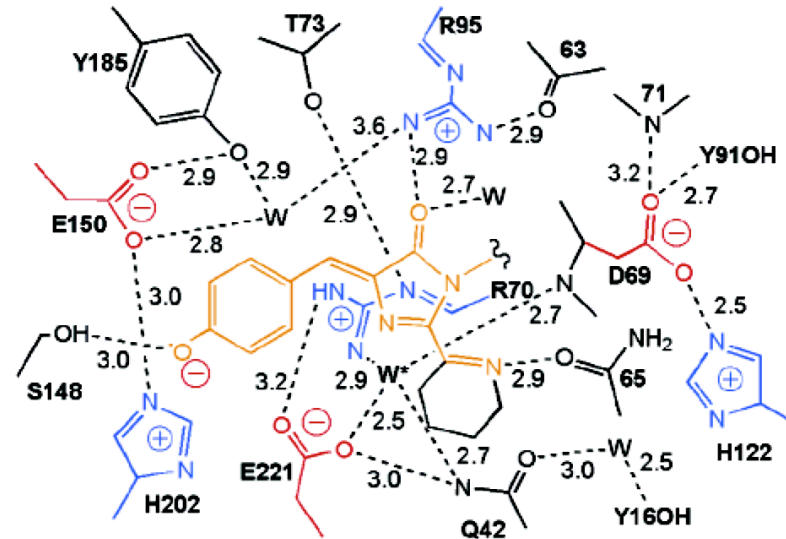
- **strong dipoles in fluorescent molecules can lead to environmental sensitivity (e.g. changes with solvent polarity)**

Some notes on what makes a molecule fluorescent



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