ChemBam

Sensing genetic disorders with fluorescence – guide for teachers

AIMS

- For students to appreciate that many everyday items and fluorescent; teeth, highlighter pens, white clothing, bank notes, driving licenses, cheques, stamps, etc.

- For students to understand that 'fluorescent' materials absorb light, and re-emit light at a different wavelength (i.e. absorb UV light and emit visible light of a given colour)

- For students to understand that a sensor is a device that responds to an input, to give a measureable output. The fluorescence experiment uses riboflavin as a pH sensor; the brightness of the emission depends upon the pH of the solution. (This is because the emission depends upon which species is in solution, and there are several possible species across the pH range 1-14, due to the pKas of the various ring N-H bonds in the riboflavin molecule.)

- For students to understand that in the research lab, fluorescence sensors are being developed that respond to which DNA base is present in a sequence of DNA, so that we can detect SNPs, which can indicate disease.

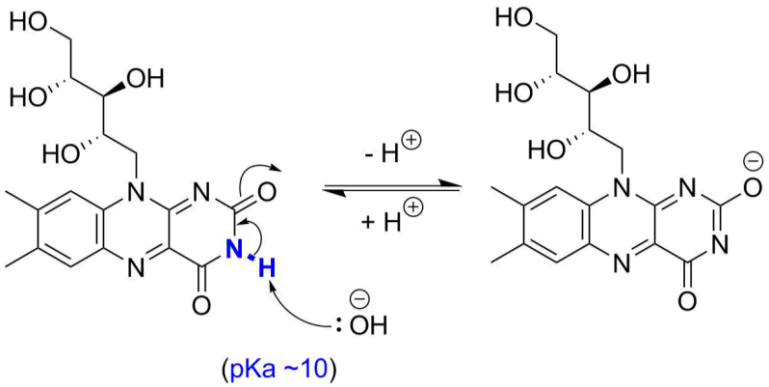
QUESTIONS – answer guide for teachers

1. The structures of organic fluorescent molecules all tend to have conjugated pi-electrons in them. Students should be able to recognise that all the molecules contain several double-bonds. This could be extended to point out that they all have alternating double-single-double-single bonds, like benzene, and this leads to the electrons being delocalised over a large part on the molecule. When a molecule is 'excited' by absorbing UV-Visible light, these electrons excite to a higher energy level, before relaxing back down and emitting light.

2. When a molecule is 'excited' by absorbing UV-Visible light, the electrons in the molecule excite to a higher energy level, before relaxing back down and emitting light, of a lower energy (a blue-shifted colour, a longer wavelength)

3. Optical brighteners are added to washing powders so that when we stand in the sun, which produces quite a lot of UV light, out white clothes fluoresce slightly, and they look 'bright white'…. They may not be overly clean, they just look clean!

4. Fluorescence is fast, the re-emission of light usually happens within 1 ns of the molecule absorbing light; hence when you switch off the UV light source, you can't see any fluorescence. (Note, 'glow in the dark' paints and ceiling stars are phosphorescent, which is a much slower process, and these emit light a long time after absorbing the excitation light)
5. At pH1/0 the solution is very dim yellow under UV, it is brightest at pH7, and then the fluorescence switches off completely at pH14. At pH 14 the N-H on the ring is deprotonated by the OH- base present, and forms a different molecule:



(pKa ~10)