Chemistry 184
Biological Chemistry Laboratory
Spring 2007
Altman, Elrad, Kool, Zare

Rules

Always ask if:

- you are unsure of how to use lab equipment
- have questions regarding a protocol
- do not understand something

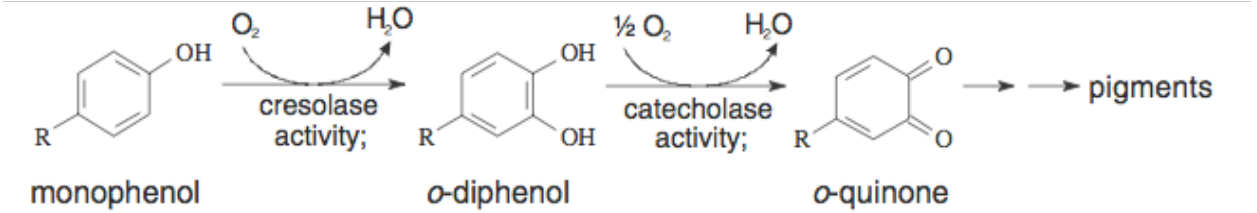
Share your information with other students

Logistics

- **Laboratory**: Tuesday and Thursday 1:15 – 5:00 PM, Clark W250
- **Lecture or Mini-Lecture**: See schedule, Clark E205
- **Website**: http://coursework.stanford.edu
- **Required Textbook:**
  - Rodney F. Boyer, Biochemistry Laboratory: Modern Theory and Techniques, Benjamin Cummings, 2006.
  - Roaring Springs #77649 lab notebook with carbon copies
- **Grades**: 67% lab notebook/lab performance and 33% Final Exam
- **Missing Lab:** Email me (delrad@stanford.edu) and your lab partner ASAP.
- **Lab rotations**

Enriching and Characterizing the Enzymatic Activity of Mushroom Tyrosinase

- Copper oxygenase; involved in the first steps of melanin, which is a polymer, synthesis.
- Involved in catalyzed oxidation of phenolic substrates to quinones, which are yellowish in color.



hydroxylation of monophenol

oxidation of diphenol

Enriching and Characterizing the Enzymatic Activity of Mushroom Tyrosinase

- Select tissue
- Grind tissue
- Enrich using a DEAE (anion exchanger) column
- Enrich using a Hydroxyapatite column
- Characterize the enzymatic activity

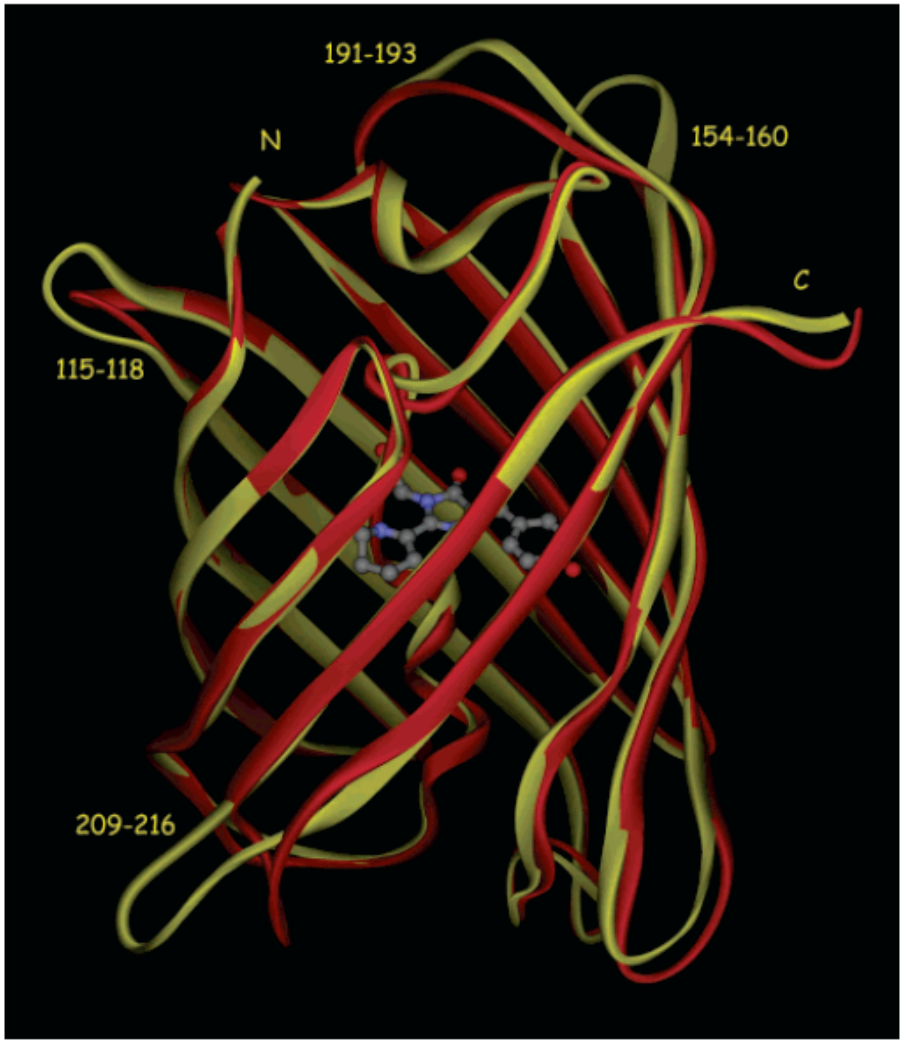
Get picture of real system.

Enriching and Characterizing the Enzymatic Activity of Mushroom Tyrosinase

- A tactile and visual “wet” lab experience
- The LP system is the opposite of a black box
- Each group will choose a tissue type
- Each group will each choose different characteristics of the enzymes to study
- Each group will choose different inhibitors
- At the end of the end of the quarter we will collect all the data and discuss

Site Directed Mutagenesis of ZsYellow

Matz et al., (1999)



Site Directed Mutagenesis of ZsYellow

Start with an *E.coli* strain expressing HIS-tagged ZsYellow (HIS-ZsYellow)

Generate a strain expressing HIS-tagged ZsYellow with a mutation of your choice

Purify HIS-ZsYellow and your mutant protein using a Nickel Column

Analyze the absorbance, emission, and excitation spectra of HIS-ZsYellow and your mutant protein

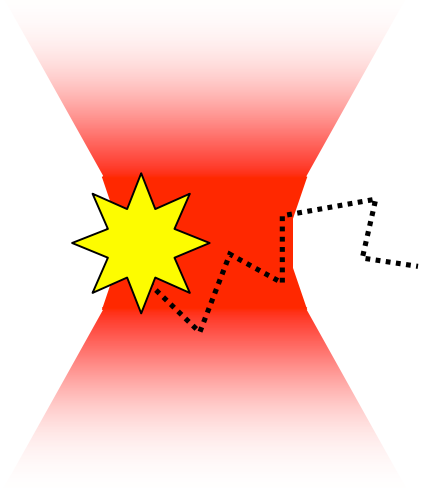
Calculate the molar absorption co-efficient and quantum yield of both proteins

Analyze the effect of pH, urea, and/or temperature on the stability and fluorescence characteristics of both proteins

Site Directed Mutagenesis of ZsYellow

- Gain an understanding of the protein sequence / structure / function dynamic and how it is studied
- Apply protein tagging technology
- Gain molecular biology experience
- Learn about spectrophotometric techniques and their use in biological chemistry and protein biochemistry
- Learn about fluorescent proteins
- Your mutant protein will be used for the microscopy lab

Building a single molecule detection device and measuring the diffusion constant of zsYellow

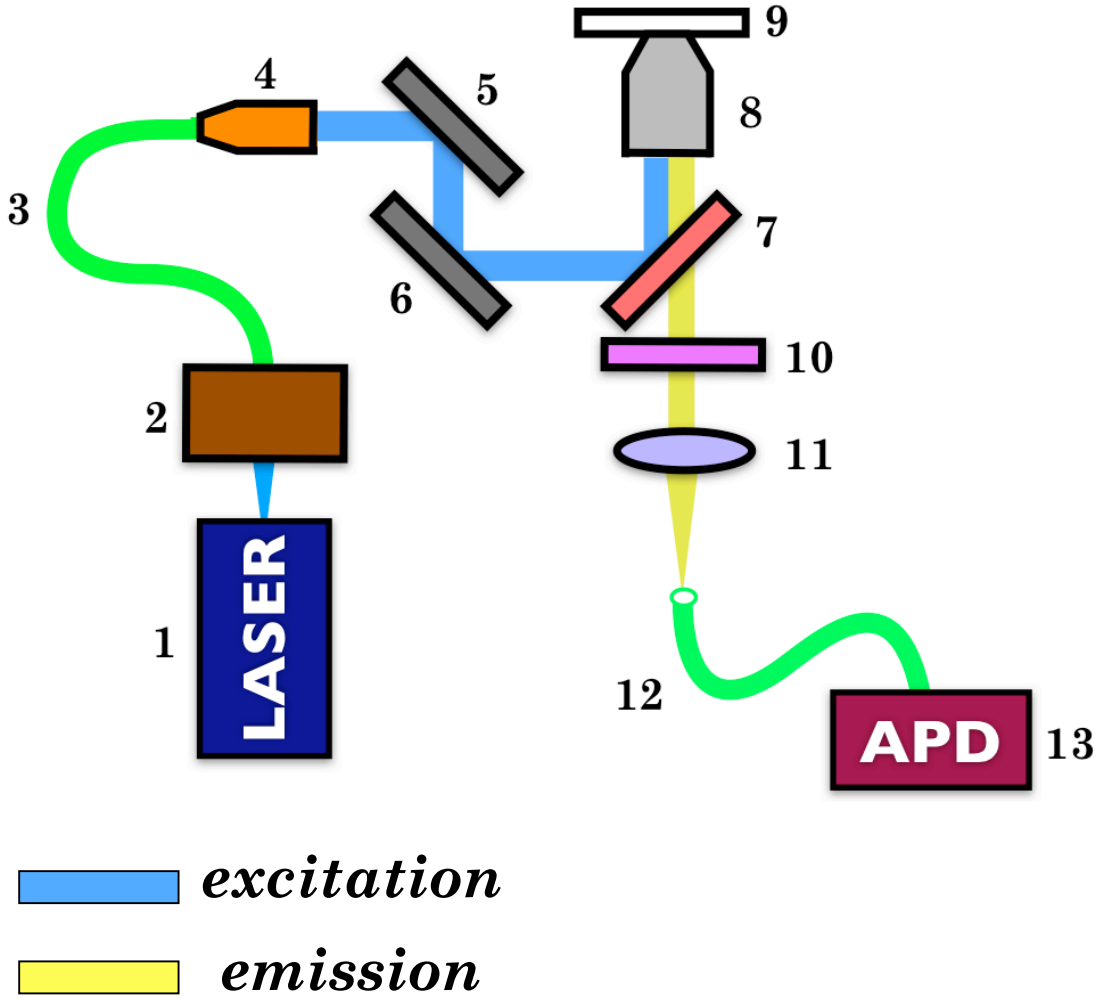


Goal: To build a device where a fluorophore is detected only when it passes through the confocal volume.

At low fluorophore densities: single molecules passing through the confocal volume will result in isolated photon bursts. You can "see" single molecules.

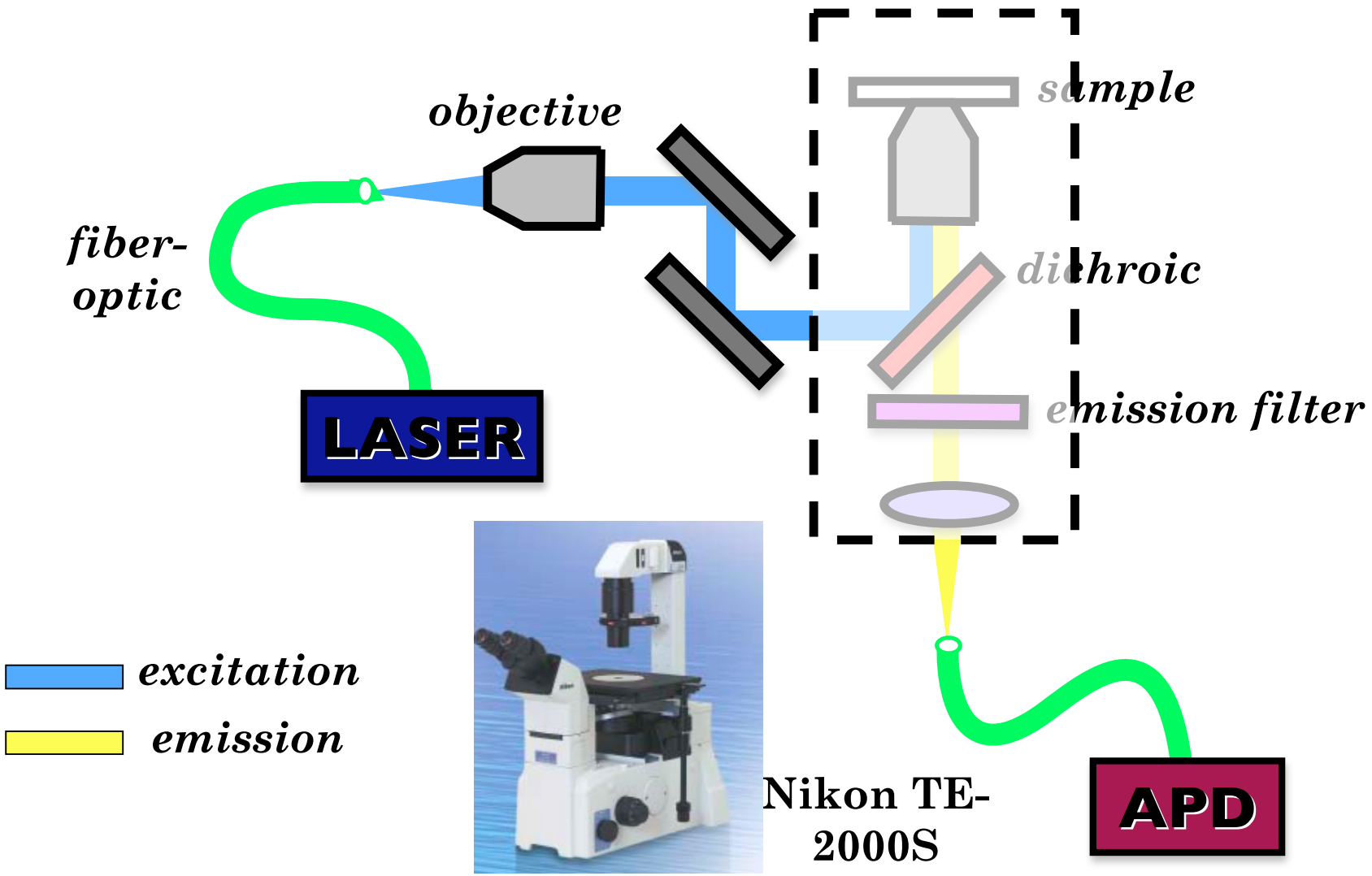
At higher fluorophore densities: fluctuations in the fluorescence intensity signal can be used to calculate a diffusion constant. (fluorescence correlation spectroscopy - FCS)

Building a single molecule detection device



1. Laser
2. (splitter)
3. Fiber optic
4. Collimator
5. Mirror
6. Mirror
7. Dichroic
8. Objective
9. Sample
10. Emission filter
11. Lens
12. Fiber optic
13. Photodiode

Building a single molecule detection device



Building a single molecule detection device

- Have a chance to “see” single molecules
- Learn why we as biological chemists might want to “see” single molecules
- Gain a familiarity with single-molecule fluorescence
  - Instrumentation
  - Data acquisition
  - Data analysis
- Learn about other uses of single-molecule fluorescence (lectures)
- Learn about microscopy and optics
- Have a chance to “build” something

Any Questions?