

Welcome to the Contemporary Science Center!

The CSC thanks the following:



Amphora

Amphora Discovery



The Museum of Life and Science



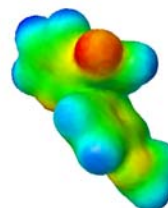
Student by student, we're changing the face of science.



Chemistry Field Study – Drug Development

Agenda:

- I. Drug Discovery
 - I. Why do it?
 - II. How do you do it?
 - III. How does Amphora Discovery do it?
 - I. Fluorescent tagging
 - II. Electrophoretic separation
 - IV. Analyzing screening results
 - I. Perform screen
 - II. Choose the best compounds from the data developed
- II. Drug Design
 - I. Compare SARs of successful compounds
 - II. Design a new compound exploiting those structures
 - III. Build your structure using MOPAC
 - IV. Report on your structure to the group



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Definitions

- Target: Enzyme of interest involved in disease progression or termination
- Compound: Chemical entity. At Amphora our chemists tweaked them to improve potency and to decrease side effects and toxicity
- IC50: The molar concentration of an compound, which produces 50% of the maximum possible inhibitory response for a certain target.

Movement in an electric field

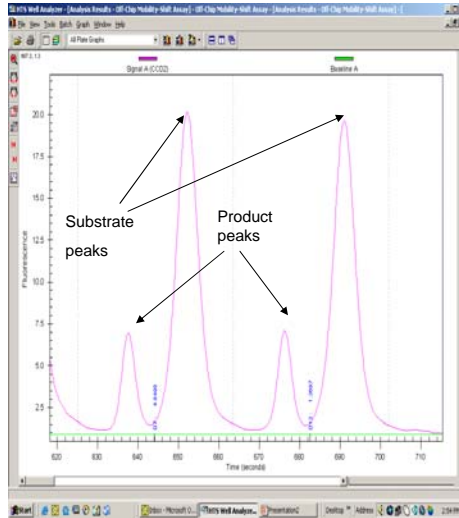
•Separation by electrophoresis is based on differences in solute velocity in an electric field. The velocity of an ion is a function of its electrophoretic mobility and the applied voltage. The mobility for an ion in a particular medium is constant and characteristic of that ion. The ions mobility is a result of two factors:

- The ion is attracted to the electrode of opposite charge, pulling it through the medium.
- At the same time however frictional forces try to prevent the ion moving. The balance of these determines the actual overall mobility.

A small ion will have less frictional drag and hence move through the medium faster than a large one. Similarly a multiply charged ion will experience more attraction to the electrode and also move through the medium faster.

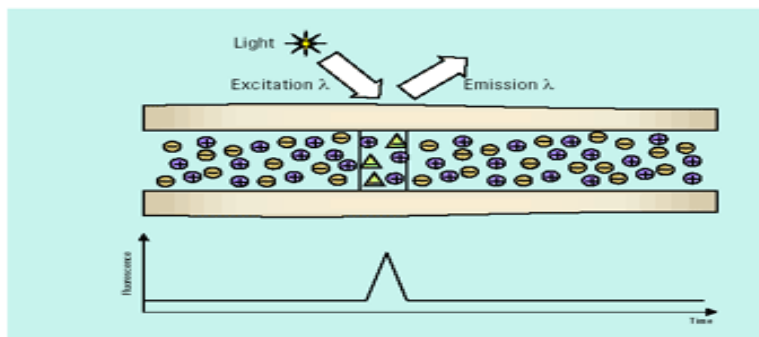
It is the difference in velocities that is responsible for the separating effect in electrophoresis.

- In our graphs, the product peak passes through the window of detection in our chips first because the negatively charged product molecules migrate towards the positively charged electrodes more quickly than do the neutrally charged substrate molecules



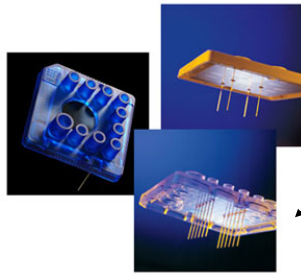
A common approach is fluorescence. Before analysis the molecules of interest are chemically labeled with a fluorophore, and then separation is performed as normal. A light source which excites the fluorophore is used as a source of radiation, and as the analytes move past the detection window the fluorophores excite and emit radiation at a different wavelength. This can then be detected, once again in a quantitative fashion. This form of detection is very useful for analytes present at low concentrations as a powerful fluorophore can be chosen, increasing the limit at which they can be detected. Also useful powerful lasers to excite the fluorophore can lead to more gains in sensitivity.

Fluorescence is illustrated in the following figure.

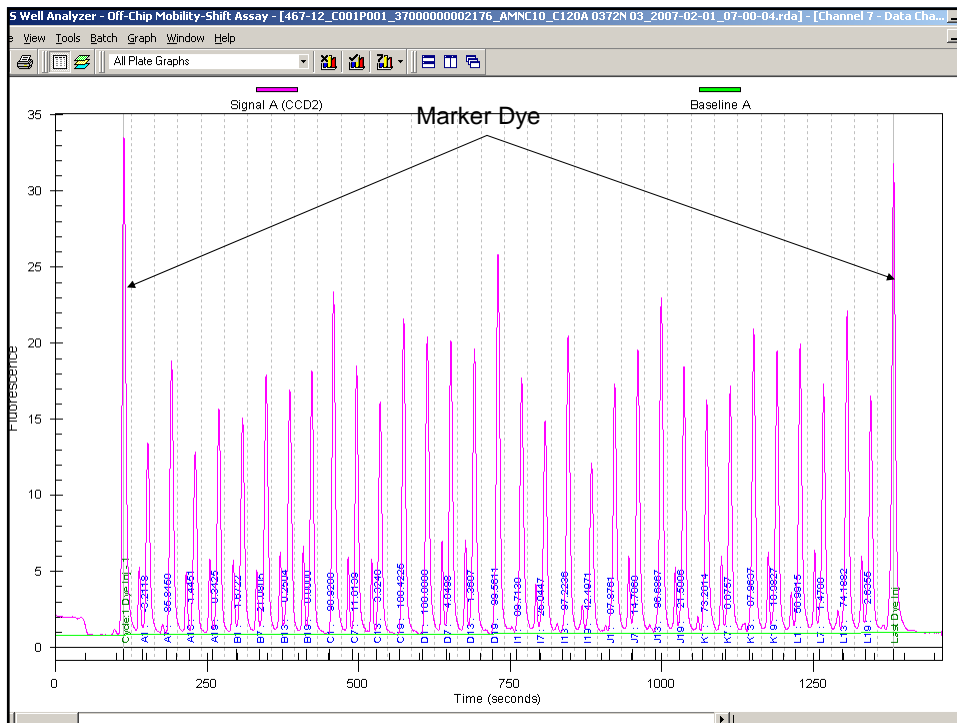


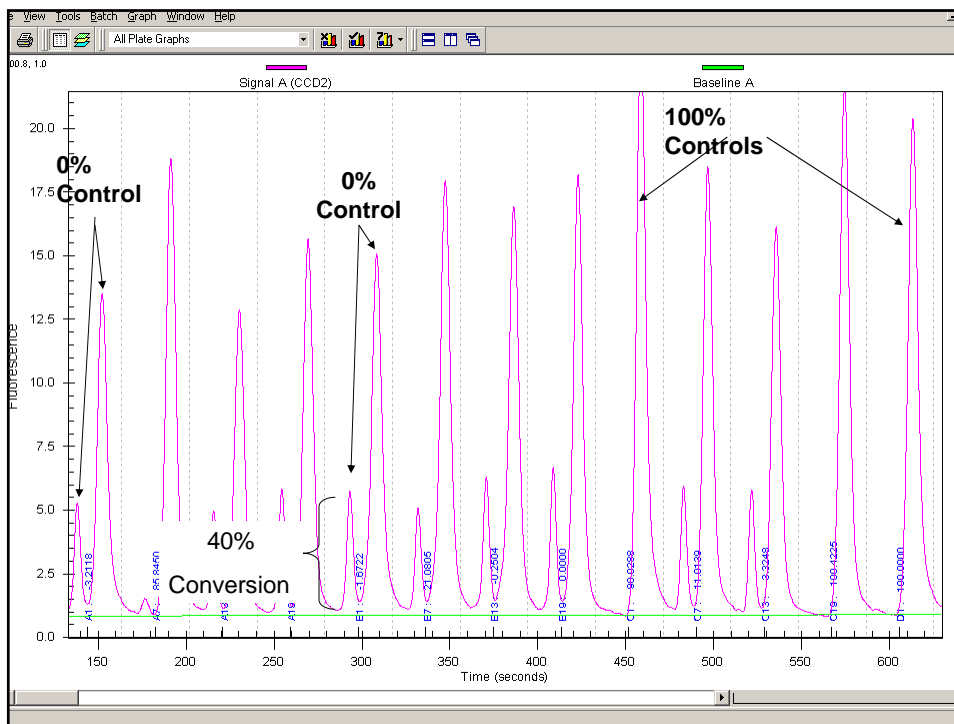
AT AMPHORA

With our system, the target enzyme is incubated with fluorescently labeled substrate and test compounds in a standardized reaction mixture in 384 well plates. Upon termination (completion) of the reaction, samples are introduced onto microfluidic chips. The samples migrate through channels in the chips and product and substrate are separated based on the difference in their charge and mass (electrophoretic mobility shift). Enzyme activity is measured by direct comparison of the fluorescence in the product and substrate peaks. This readout allows for very precise determination of enzyme activity based on changes in the product/substrate ratio.

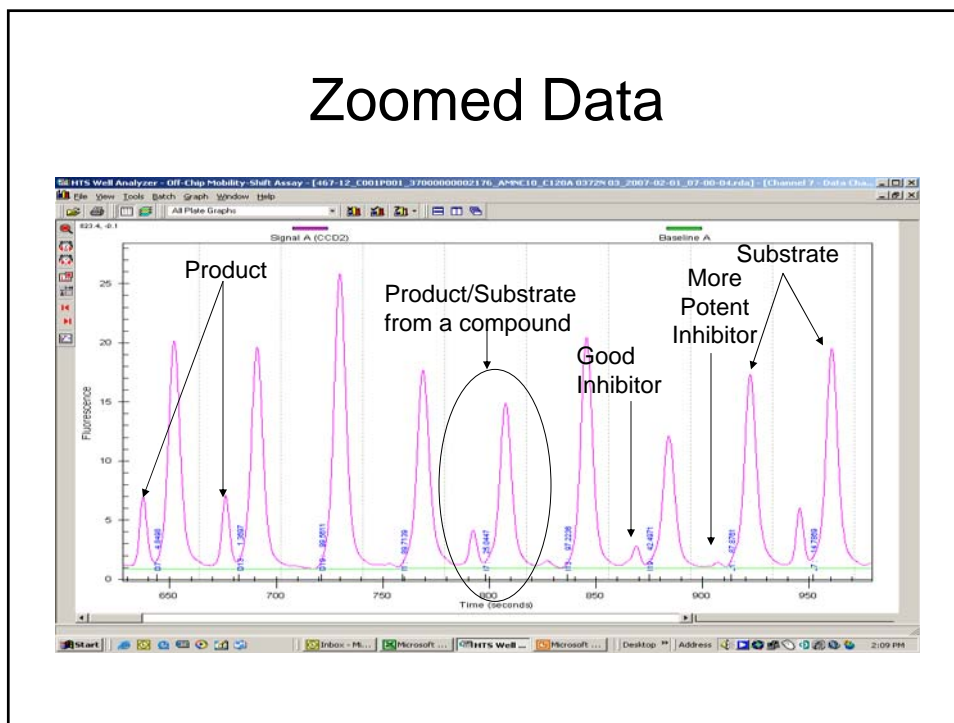


We use the 12-sipper chip!





Zoomed Data



The Experiment

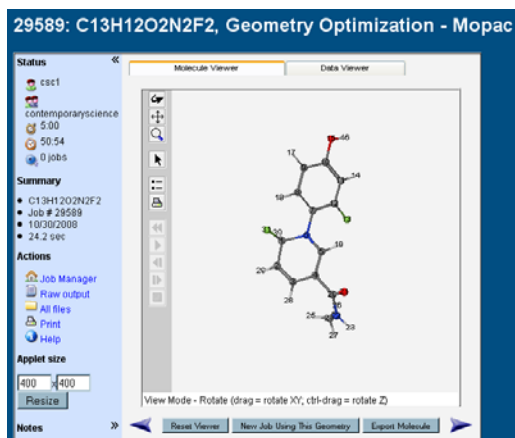
- Each reaction needs three components:
 1. Enzyme (target)
 2. Substrate
 3. Inhibitor (compound)
- Add each to the wells on the plate according to the protocol
- Incubate for 30 minutes
- Read fluorescent intensity
 - High fluorescence means high conversion

Structure-Activity Relationship (SAR)

- What about structures E and G that make them more potent?
- Look for common shapes, protrusions, elements, etc.
- Prepare an argument for your guesses!
- View the 3-D versions of the structures using ViewerLite.

Using WebMO/MOPAC

- Login
- Draw your structure
- Clean your structure
- Run your job using the instructions on the sheet



Structure Analysis

- Compare yours to E/G
 - Polarity?
 - Geometry (shape/orientation)
 - Element composition
 - Charges distributed in a similar manner?