A Mass Spectrometry Simulation for Biochemistry Education

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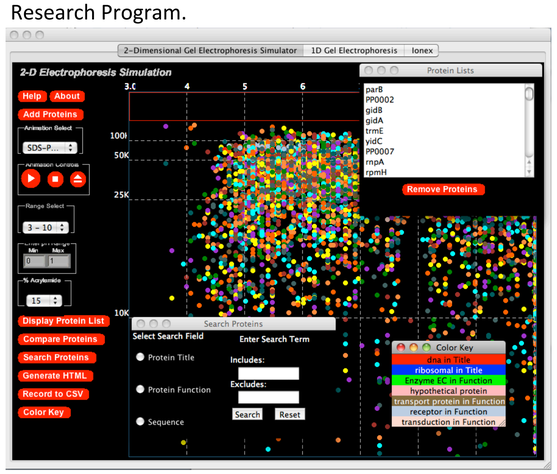
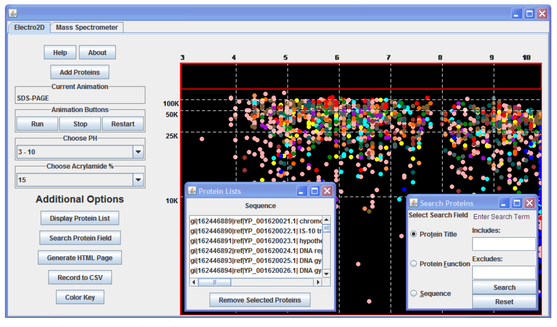
Program #579.2 Abstract # 2342

The separations simulations team at RIT has been developing interactive simulations of biological separation processes for over 10 years. The JBioFramework collection of interactive simulations is now platform independent and runs equally well on all common operating systems. The updated 2D electrophoresis simulation utilizes multiple file types and Internet resources, and a new simulation has been added - tandem mass spectrometry. The tandem MS simulation accepts data from the 2D electrophoresis simulation, allowing a student to select a protein from the gel and select a protease to digest it. Users can click on a peptide fragment on the first Mass Spectrometer readout, then see the b- and y- fragments that give the peptide sequence in the second Mass Spectrometer readout. Support for this work has been provided by the RIT Colleges of Science and Computing and Information Science and by the Merck/AAAS Undergraduate Science Research Program.



The original JBioFramework 2D Electrophoresis simulation on a Macintosh computer.

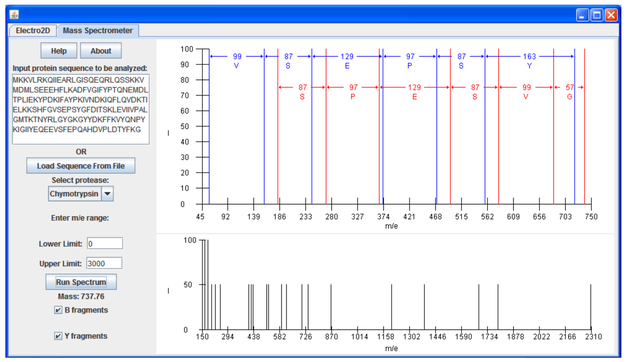
The original 2D Electrophoresis simulation was updated to be platform independent, compatible with commonly used modern file types for proteome files, and to capitalize on expanded online resources.



Current JBioFramework on a Windows computer.

After updating the 2D Electrophoresis simulation, a new simulation for Tandem Mass Spectrometry was added to JBioFramework. This new simulation (E2D-TMS) is designed to teach the concept of peptide sequencing and protein identification. The complete program can be downloaded for free at https://sourceforge.net/projects/jbf/.

The Tandem Mass Spectrometry Simulation:

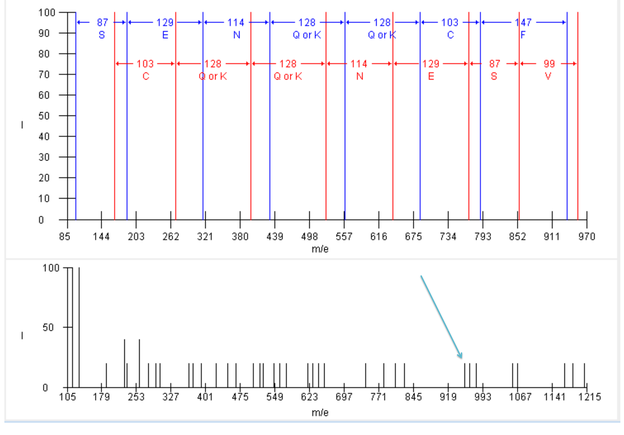


The JBioFramework Tandem Mass Spectrometry Simulation on a Windows Computer.

The tandem mass spectrometry simulation receives input in the form of a peptide sequence from user entry, a FASTA format file, or from the 2D Electrophoresis simulation. Once entered, the user can select from four proteases to digest the peptide sequence:

Trypsin	• Cuts after (C-terminal side) an Arginine(R) or Lysine(K), except when either is followed by a Proline(P).
Chymotrypsin	• Cuts after Tyrosin(Y), Tryptophan(W), or Phenylalanine(F).
Proteinase K	• Cuts after Alanine(A), Phenylalanine(F), Isoleucine(I), Leucine(L), Valine(V), Tryptophan(W), or Tyrosine(Y).
Thermolysin	• Cuts before (N-terminal side) Isoleucine(I), Leucine(L), Methionine(M), or Valine(V).

Once the user clicks the Run Spectrum button the simulation uses the logic of the protease to divide the entered peptide sequence into fragments and displays them in the lower output graph. Clicking on one of these fragment peaks allows its sequence to be read through b- and y- fragmentation in the upper graph.



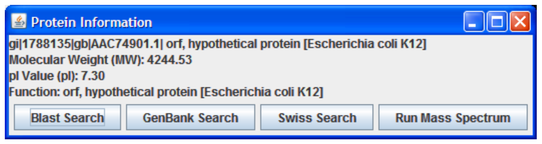
Output and tandem output graphs for peptide sequencing.

Creating a Familiar Format:

The Tandem Mass Spectrometer simulation interface is designed to resemble the output layout from a real instrument. The y-axis of the initial output graph is a calculated intensity, I, that accurately represents the relative frequencies of each peptide fragment. The x-axis of both graphs plots the mass over charge ratios of each peptide fragment.

An Integrated Approach:

The 2D Electrophoresis simulation is integrated with the Tandem Mass Spectrometry simulation in that it allows a user to select a protein dot from the gel canvas and send its peptide sequence to the tandem MS simulation.

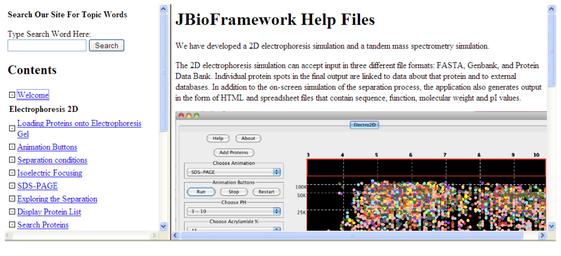


The dialog box that appears when a user clicks on a protein dot in the 2D Electrophoresis simulation.

Students can explore the technique of using tandem mass spectrometry to create the b- and y- fragments used to read the sequence of a fragment of a protein through the mass difference between subsequent peaks. They may then perform online database searches for that deduced sequence to identify the unknown protein.

Additional Resources:

Extensive help files were created and are available through the program to help students learn about the concepts at work behind the laboratory techniques of 2D Electrophoresis and Tandem Mass Spectrometry. Additionally, problem sets are in development.

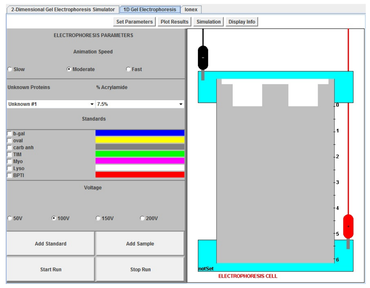


The Welcome screen of the JBioFramework Help Files.

The E2D-TMS application is freely available at https://sourceforge.net/projects/jbf/.

Future Goals

- Update 1D Electrophoresis



Original 1D Electrophoresis simulation

1D Electrophoresis needs to be converted from Java AWT and absolute positioning to Java Swing and relative positioning in much the same way the 2D Electrophoresis simulation was, so that students everywhere will see the same GUI.

- Add chemical drawings of peptide sequences to the tandem mass spectrometer simulation.

An example peptide, drawn by Java computer code.

Allow students to click on a peptide fragment peak, sequence it, and then request a visual drawing of that peptide sequence including backbone and R-group side chains.

- Integrate Chromatography Simulations into the software package.

Develop additional simulations for Ion Exchange Chromatography and Reversed Phase HPLC. Allow interactions between the simulations as seen in the integration between the 2D Electrophoresis simulation and the Tandem Mass Spectrometry simulation.

- Use 2DE-TMS as a teaching tool

Paul Craig has used the 2D Electrophoresis simulation in his proteomics, biochemistry and separations classes at RIT. Problem sets for the 2DE-TMS simulation are available for use in the classroom. As additional simulations are developed, problem sets for in-class use will be made available.

Acknowledgements

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