Research Opportunities in the Poutsma Group
(fun with mass spectrometers)
Mass Spectrometers

LCQ-DECA ion trap with Shimadzu UPLC

LTQ-ETD with Eksigent nano-HPLC

TSQ-quantum ultra (QQQ)
On-going Projects

• Gas-phase proteomics research *(using the rules)*
  • collaborative studies with Biology Dept.
  • *E. coli* infection project with Professor Williamson

• Gas-phase ion structure *(understanding/improving the rules)*
  • custom solid-phase synthesis of peptides
  • mass spectrometer fragmentation studies
  • H/D exchange of peptides and fragments
  • Infrared multiphoton dissociation (IRMPD)

• Gas-phase thermochemistry *(the basis for the rules)*
  • effects of systematic substitutions on amino acid thermochemistry
  • thermochemistry of small peptides
Proteomics

- proteomics is the study of proteins
- one might wish to determine the identity and concentration of all the proteins expressed by an organism
- or one can do a differential study of targeted proteins
MS-based peptide sequencing

YGGWL⁺

select parent record mass (595.6)
collision induced dissociation
product ions

[YGGW-CO]⁺ (436.2)
WL⁺ (375.2)
GWL⁺ (318.2)
YGGW⁺ (432.2)

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YGGW⁺ (432.2)

protein identification
peptide identification
fingerprint matching
Actual real-life samples

*M. Smegmatis* infection study

- Many novel bacteriophage viruses can infect the soil bacterium *M. smegmatis*.
- *M. Smegmatis* is a non-pathogenic model system for *M. tuberculosis*.
- We freeze *M. Smegmatic* cells at varying time points after infection by novel pages that were discovered in the PhageLab Freshman Biology Lab experience.
- We lyse the cells, harvest the proteins, and digest with trypsin.
- We perform shotgun proteomics experiments aim to identify all of the proteins being expressed by the bacterium and the virus at varying time points.
- By determining which viral proteins are expressed at different times after infection, we can begin to classify sequenced proteins of unknown function as regulatory (early time points) or structural (late time points).
- In addition to the information gleaned from the viral proteins, we can also begin to look for bacterial responses to infection.
**Preliminary Results**

*E. coli* sample: 15 minutes after infection with T7 phage

>3800 proteins, >20,000 peptides

<table>
<thead>
<tr>
<th>time</th>
<th># proteins</th>
<th>representative proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>21</td>
<td>DNA primase/helicase, DNA-directed DNA polymerase, peptidoglycan hydrolase gp 16</td>
</tr>
<tr>
<td>30 min</td>
<td>27</td>
<td>endonuclease I, portar protein</td>
</tr>
<tr>
<td>45 min</td>
<td>30</td>
<td>bacterial RNS polymerase inhibitor, exonuclease inhibitor of dGTPase, terminase (large and small subunits)</td>
</tr>
<tr>
<td>60 min</td>
<td>26</td>
<td>capsid assembly scaffolding protein, nucleotide kinase, tail tubular protein</td>
</tr>
</tbody>
</table>
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Selective Cleavages (improving the rules)

peptides normally fragment randomly along peptide backbone

some residues such as proline produce selective cleavages that can confound searching algorithms
Hydrogen-deuterium Exchange (understanding the rules)

the rate at which a gas-phase ion substitutes H for D gives an indication of the availability of exchangeable hydrogens, and thus an indirect indication of structure.

we can measure rates for H/D exchange in our ion trap mass spectrometer.
IRMPD: Vibrational Spectroscopy
(understanding the rules)

- CLIO (Orsay) and FELIX (Nijmegen) FEL’s
- infrared action spectroscopy of ions
- gives direct information of structure
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measuring the fragment intensities of competitive cleavage of proton-bound dimer ions gives us acid-base properties for peptides
Pro-Asp: kinetic method plot

The graph is used to determine the basicity of prolylaspartic acid dipeptide.
Computational Studies

• We use computational chemistry to support and guide our experimental work.

• Collaborator: Prof. Jennifer Poutsma (ODU)

Pro-Gly

Pro-Ala