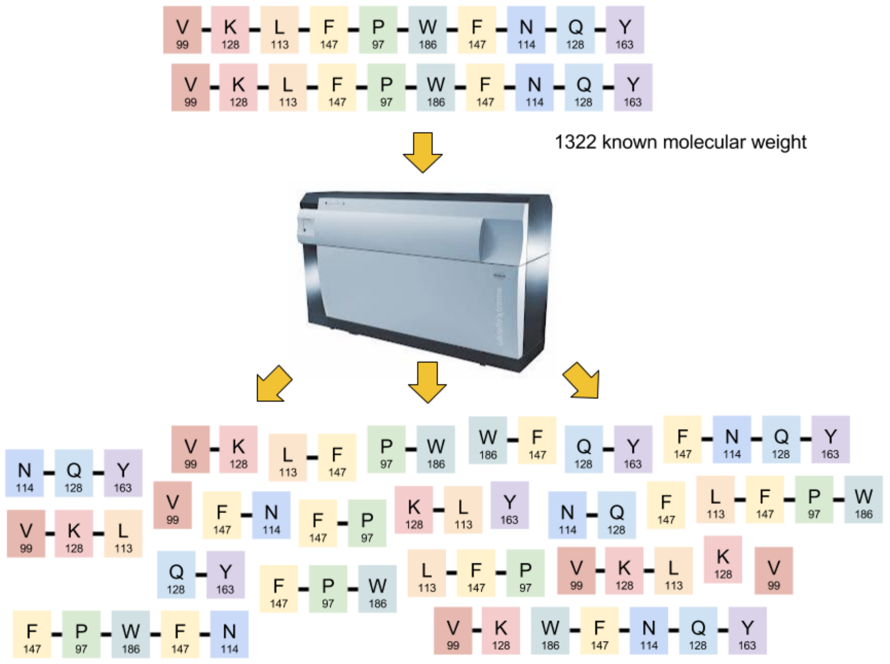
Determining a Peptide's Sequence

- From last time we learned that we can't always use DNA to resolve peptide/protein sequences
- What else can we do?
  - Extract and purify a pure sample of the peptide/protein
  - Try to resolve the peptide sequence by analyzing this sample
- Today's approach
  - Randomly fracture the peptide
  - Assemble an answer from the peices

Molecular Weights are the Puzzle Peices


1322 known molecular weight
V 99 - K 128 - L 113 - F 147 - P 97 - W 186 - F 147 - N 114 - Q 128 - Y 163

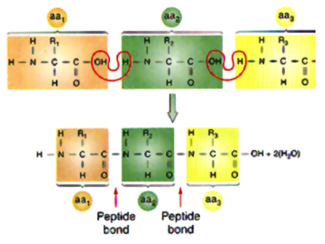
Structure of a Peptide Chain

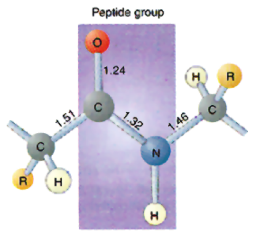
- Peptides are chains of amino acids that are joined by *peptide* bonds
- These bonds reduce the weight of each amino acid by one H_2O molecule
- The result is called a *residue*
- A Mass Spectrograph can precisely measure the molecular weight (and charge and abundance) of any peptide chain
- Since the molecular weight of each of the possible 20 residues is known precisely, one can ask the question, which combination of residues would give a particular weight?
- The problem is ambiguous for the entire molecule
  - Consider all permulations of 'PIT':

    ```
    'PIT', 'PTI', 'ITP', 'IPT', 'TPI', and 'TIP' all weigh the same
    ```

  - But they differ in their 2-peptide fragments:

    ```
    'PIT' breaks into 'PI' and 'IT', while
    'PTI' breaks into 'PT' and 'TI'
    ```





An Simplified Peptide Weight table

- The actual molecular weight of an amino acid is a real number. This acounts for the relative abundances of atomic isotopes
- Today, we will use a simplified version that assumes only integer molecular weights
- Example:
  - Molecular weight of Glycine Amino Acid

$$W(C_2H_5NO_2) = 12 \times 2 + 5 \times 1 + 14 + 16 \times 2 = 75$$

  - Molecular wieght of Glycine Residue (Minus the H_2O lost forming the peptide bond)

$$W(C_2H_5NO_2 - H_2O) = 57$$

- We can repeat this for all 20 Amino Acids to get a integer molecular weight table, which I call *Daltons*

Table Definitions

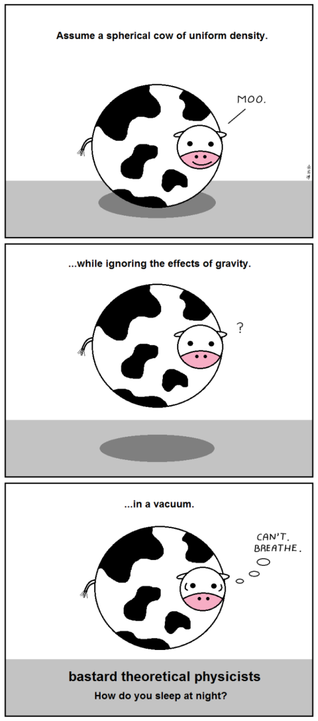
```
AminoAcid = {
    'A': 'Alanine', 'C': 'Cysteine', 'D': 'Aspartic acid', 'E': 'Glutamic acid',
    'F': 'Phenylalanine', 'G': 'Glycine', 'H': 'Histidine', 'I': 'Isoleucine',
    'K': 'Lysine', 'L': 'Leucine', 'M': 'Methionine', 'N': 'Asparagine',
    'P': 'Proline', 'Q': 'Glutamine', 'R': 'Arginine', 'S': 'Serine',
    'T': 'Theronine', 'V': 'Valine', 'W': 'Tryptophan', 'Y': 'Tyrosine',
    '*': 'STOP'
}

AminoAbbrv = {
    'A': 'Ala', 'C': 'Cys', 'D': 'Asp', 'E': 'Glu',
    'F': 'Phe', 'G': 'Gly', 'H': 'His', 'I': 'Ile',
    'K': 'Lys', 'L': 'Leu', 'M': 'Met', 'N': 'Asn',
    'P': 'Pro', 'Q': 'Gln', 'R': 'Arg', 'S': 'Ser',
    'T': 'Thr', 'V': 'Val', 'W': 'Trp', 'Y': 'Tyr',
    '*': 'STP'
}

# Here's a new dictionary!
Daltons = {
    'A':  71, 'C': 103, 'D': 115, 'E': 129,
    'F': 147, 'G':  57, 'H': 137, 'I': 113,
    'K': 128, 'L': 113, 'M': 131, 'N': 114,
    'P':  97, 'Q': 128, 'R': 156, 'S':  87,
    'T': 101, 'V':  99, 'W': 186, 'Y': 163
}
```

Some Issues with our Table

- We can't distinguish between Leucine (L) and Isoleucine (I). They both weight 113 d
- Nor can we distinguish Lysine (K) and Glutamine (Q), which weigh 128 d
- For long peptide chains >50, our errors can build up
- In reality, peptides can loose or gain one or more small molecules from their side chains and fractured peptide bonds
    - Gain Hydrogen ions (H, +1 Dalton)
    - Lose Water (H_2O, -18 Daltons)
    - Lose Ammonia (NH_3, -17 Daltons)
- This leads to measurements that vary around the ideal sums we assume
- Regardless of these caveats, let's keep going

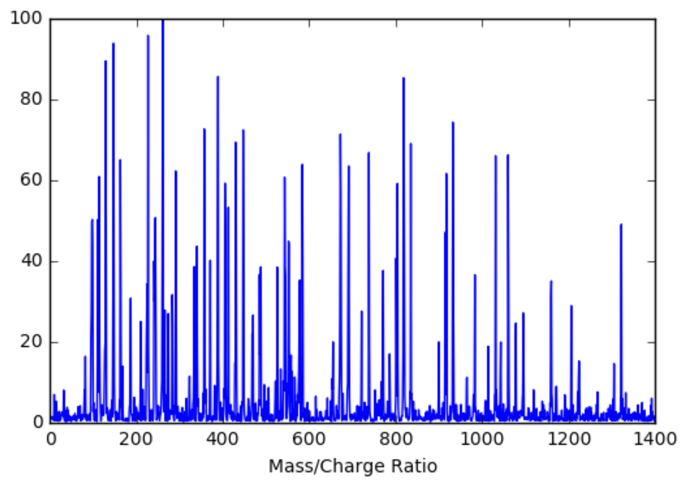

Assume a spherical cow of uniform density.
MOO.
...while ignoring the effects of gravity.
?
...in a vacuum.
CAN'T. BREATHE.
bastard theoretical physicists
How do you sleep at night?

The total molecular weight of our target

```
TyrocidineB1 = "VKLFPWFNQY"

# The weight of Tyrocidine B1
print sum([Daltons[res] for res in TyrocidineB1])
```

```
1322
```



- Generally, we will assume that the peptide's total molecular weight is known
- We will use it as a terminating condition for many of our algorithms that attempt to reconstruct the measured set of weights

Ideally, what Weights should we get?

- We will make the optimistic assumption that we will fracture our given petide chain into all of its constituent parts
- For a 10 peptide chain
  - 10 single peptides
  - 9, 2-peptide chains
  - 8, 3-peptide chains
  - 7, 4-peptide chains
  - 6, 5-peptide chains
  - 5, 6-peptide chains
  - 4, 7-peptide chains
  - 3, 8-peptide chains
  - 2, 9-peptide chains
  - 1, 10-peptide chain
- This gives an upper bound of $\binom{11}{2} = 55$ molecular weights
- In reality both the peptide chains and their weights may not be unique
- The collection of all possible sub-peptide molecular weights from a peptide is called the peptide's *Theoretical Spectrum*

Code for computing a Theoretical Spectrum

```
def TheoreticalSpectrum(peptide):
    # Generate every possible fragment of a peptide
    spectrum = set()
    for fragLength in xrange(1,len(peptide)+1):
        for start in xrange(0,len(peptide)-fragLength+1):
            seq = peptide[start:start+fragLength]
            spectrum.add(sum([Daltons[res] for res in seq]))
    return sorted(spectrum)

print TyrocidineB1
spectrum = TheoreticalSpectrum(TyrocidineB1)
print len(spectrum)
print spectrum
```

```
VKLFPWFNQY
51
[97, 99, 113, 114, 128, 147, 163, 186, 227, 241, 242, 244, 260, 261, 283, 291, 333, 340, 357, 388, 389, 405, 430, 447, 485, 487, 543, 544, 552, 575, 5
4, 671, 672, 690, 691, 738, 770, 804, 818, 819, 835, 917, 932, 982, 1031, 1060, 1095, 1159, 1223, 1322]
```

- Why are we using a set rather than a list? Notice that we end up returning a list.

Fragments and their Spectrums

```
peptide = TyrocidineB1
fragList = []
for fragLength in xrange(1,len(peptide)+1):
    for start in xrange(0,len(peptide)-fragLength+1):
        seq = peptide[start:start+fragLength]
        fragList.append((sum([Daltons[res] for res in seq]), seq))

print peptide
print len(fragList)
N = 0
lastWeight = 0
for weight, frag in sorted(fragList):
    print "%12s: %4d%s" % (frag, weight, "*" if (weight == lastWeight) else " "),
    N += 1
    if (N % 5 == 0):
        print
    lastWeight = weight
```

```
VKLFPWFNQY
55
           P:   97             V:   99             L:  113             N:  114             K:  128
           Q:  128*            F:  147             F:  147*            Y:  163             W:  186
          VK:  227            KL:  241            NQ:  242            FP:  244            LF:  260
          FN:  261            PW:  283            QY:  291            WF:  333           VKL:  340
         LFP:  357           KLF:  388           FNQ:  389           NQY:  405           FPW:  430
         PWF:  430*          WFN:  447          KLFP:  485          VKLF:  487          LFPW:  543
        PWFN:  544          FNQY:  552          WFNQ:  575          FPWF:  577         VKLFP:  584
       KLFPW:  671         PWFNQ:  672         LFPWF:  690         FPWFN:  691         WFNQY:  738
      VKLFPW:  770        LFPWFN:  804        KLFPWF:  818        FPWFNQ:  819        PWFNQY:  835
     VKLFPWF:  917       KLFPWFN:  932       LFPWFNQ:  932*      FPWFNQY:  982      VKLFPWFN: 1031
    KLFPWFNQ: 1060      LFPWFNQY: 1095     VKLFPWFNQ: 1159     KLFPWFNQY: 1223    VKLFPWFNQY: 1322
```

Let's try a smaller example

```
peptide = 'PLAY'
spectrum = TheoreticalSpectrum(peptide)
print len(spectrum), spectrum

fragList = []
for fragLength in xrange(1,len(peptide)+1):
    for start in xrange(0,len(peptide)-fragLength+1):
        seq = peptide[start:start+fragLength]
        fragList.append((sum([Daltons[res] for res in seq]), seq))

print len(fragList)
N = 0
lastWeight = 0
for weight, frag in sorted(fragList):
    print "%12s: %4d%s" % (frag, weight, "*" if (weight == lastWeight) else " "),
    N += 1
    if (N % 5 == 0):
        print
    lastWeight = weight
```

```
10 [71, 97, 113, 163, 184, 210, 234, 281, 347, 444]
10
           A:   71            P:   97            L:  113            Y:  163           LA:  184
          PL:  210           AY:  234          PLA:  281          LAY:  347         PLAY:  444
```

Can we Invert the Process of creating a Spectrum?

- In essence, the problem of inferring a peptide chain from the set of mass values reported by a Mass Spectrometer is the inverse of the code we just wrote

  Easy Problem: Peptide Sequence → Spectrum
  Hard Problem: Peptide Sequence ← Spectrum

- Why is computing a spectrum from a peptide sequence easy? $O(N^2)$?
- Why is computing a peptide sequence from a specturm hard? $O(?)$

"I'm trying to back it up, but I can't find reverse

How might you approach this problem?

- Can you think of a Brute-Force way of solving this problem?
- Here's one:

  1. For every peptide sequence with the target peptide's molecular weight
  2. Compute the sequence's Theoretical Spectrum
  3. If it matches the one given, report this peptide as a possible solution

- Which step in this algorithm is the hard part?

A Brute-Force Attempt

```
def PossiblePeptide(spectrum, prefix=''):
    """ A brute force method of generating all peptide sequences that add up to our target weight from the given spectrum """
    global peptideList
    if (len(prefix) == 0):
        peptideList = []
    current = sum([Daltons[res] for res in prefix])
    target = max(spectrum)  # our target
    if (current == target):
        peptideList.append(prefix)
    elif (current < target):
        for residue in Daltons.iterkeys():
            PossiblePeptide(spectrum, prefix+residue)

def TestPeptides(candidateList, target):
    filteredList = []
    for peptide in candidateList:
        candidateSpectrum = TheoreticalSpectrum(peptide)
        if (candidateSpectrum == target):
            filteredList.append(peptide)
    return filteredList

spectrum = TheoreticalSpectrum('PLAY')
%time PossiblePeptide(spectrum)
print len(peptideList), "candidates", "PLAY" in peptideList
%time matches = TestPeptides(peptideList, spectrum)
print matches, "PLAY" in matches
```

```
CPU times: user 3.57 s, sys: 3 ms, total: 3.58 s
Wall time: 3.57 s
3687 candidates True
CPU times: user 73 ms, sys: 0 ns, total: 73 ms
Wall time: 73.2 ms
['PIAY', 'PLAY', 'YAIP', 'YALP'] True
```

Impressions?

- Not so bad for a first attempt, but how will it perform for longer peptides?
- We are getting the expected answer as well as answers with the indistinguishable amino acids substituted
- We are also getting the sequence reversed? Is this a surprise?
- We could code around this, but for today we'll just include the reversed peptide chain as a possible answer

Could we do better?

- The brute force method does not make good use of the spectrum it is given
- It only ever considers the largest value from this table
- How might we make use of the other values?

Improving on Brute Force

- We could extend our prefix using *only* residues that appear in our spectrum
- The weight of every new prefix that we consider should also be in our spectrum

Actual fragments: `P L A Y PL LA AY PLA LAY PLAY`

Growing and Checking prefixes:

```
A             I             L             P             Y

AI   = LA     IA   = LA     LA   = LA     PI   = PL     YA   = AY
AIP  = PLA    IAP  = PLA    LAP  = PLA    PIA  = PLA    YAI  = LAY
AIPY = PLAY   IAPY = PLAY   LAPY = PLAY   PIAY = PLAY   YAIP = PLAY
AIY  = LAY    IAY  = LAY    LAY  = LAY                  YAL  = LAY
AIYP = PLAY   IAYP = PLAY   LAYP = PLAY                 YALP = PLAY

AL   = LA     IP   = PL     LP   = PL     PL   = PL
ALP  = PLA    IPA  = PLA    LPA  = PLA    PLA  = PLA
ALPY = PLAY   IPAY = PLAY   LPAY = PLAY   PLAY = PLAY
ALY  = LAY
ALYP = PLAY

AY   = AY
AYI  = LAY
AYIP = PLAY
AYL  = LAY
AYLP = PLAY
```

Only a Small Change to the Code

```
def ImprovedPossiblePeptide(spectrum, prefix=''):
    global peptideList
    if (len(prefix) == 0):
        peptideList = []
    current = sum([Daltons[res] for res in prefix])
    target = max(spectrum)
    if (current == target):
        peptideList.append(prefix)
    elif (current < target):
        for residue in Daltons.iterkeys():
            # make sure that this residue appears in our spectrum
            if (Daltons[residue] not in spectrum):
                continue
            # make sure that adding this residue to the sequence we have so far appears in our spectrum
            extend = prefix + residue
            if (sum([Daltons[res] for res in extend]) not in spectrum):
                continue
            ImprovedPossiblePeptide(spectrum, extend)

spectrum = TheoreticalSpectrum('PLAY')
%time ImprovedPossiblePeptide(spectrum)
print len(peptideList), "PLAY" in peptideList
print peptideList
%time matches = TestPeptides(peptideList, spectrum)
print matches, "PLAY" in matches
```

```
CPU times: user 1 ms, sys: 0 ns, total: 1 ms
Wall time: 761 µs
16 True
['AIPY', 'AIYP', 'ALPY', 'ALYP', 'AYIP', 'AYLP', 'IAPY', 'IAYP', 'IPAY', 'LAPY', 'LAYP', 'LPAY', 'PIAY', 'PLAY', 'YAIP', 'YALP']
CPU times: user 0 ns, sys: 0 ns, total: 0 ns
Wall time: 404 µs
['PIAY', 'PLAY', 'YAIP', 'YALP'] True
```

Impact of a small change

- Provides a HUGE performace difference
- Yet another example of Branch-and-Bound
- We improved both the enumeration and verification phases, but the difference was much more significant in the enumeration step

```
for peptide in peptideList:
    print peptide,
```

```
AIPY AIYP ALPY ALYP AYIP AYLP IAPY IAYP IPAY LAPY LAYP LPAY PIAY PLAY YAIP YALP
```

```
TheoreticalSpectrum('PLAY')
```

```
[71, 97, 113, 163, 184, 210, 234, 281, 347, 444]
```

```
TheoreticalSpectrum('LAPY')
```

```
[71, 97, 113, 163, 168, 184, 260, 281, 331, 444]
```

```
print sum([Daltons[res] for res in 'AP'])  # Suffix of 'LAP' prefix
print sum([Daltons[res] for res in 'APY']) # Suffix of 'LAPY'
print sum([Daltons[res] for res in 'PY'])  # Suffix of 'LAPY'
```

```
168
331
260
```

- There are still differences in the spectrums, yet every prefix was in the spectrum when we added it. What are we missing?
- Suffixes!

We can do Even Better

- All *suffixes* of each prefix that we consider should also be in our spectrum

```
def UltimatePossiblePeptide(spectrum, prefix=''):
    global peptideList
    if (len(prefix) == 0):
        peptideList = []
    current = sum([Daltons[res] for res in prefix])
    target = max(spectrum)
    if (current == target):
        peptideList.append(prefix)
    elif (current < target):
        for residue in Daltons.iterkeys():
            extend = prefix + residue
            # test every new suffix created by adding this new reside
            # Note: this includes the residue itself as the length 1 suffix
            suffix = [extend[i:] for i in xrange(len(extend))]
            for fragment in suffix:
                if (sum([Daltons[res] for res in fragment]) not in spectrum):
                    break
            else:
                UltimatePossiblePeptide(spectrum, extend)

spectrum = TheoreticalSpectrum('PLAY')
%time UltimatePossiblePeptide(spectrum)
print len(peptideList), peptideList, "PLAY" in peptideList
%time matches = TestPeptides(peptideList, spectrum)
print matches, "PLAY" in matches
```

```
CPU times: user 3 ms, sys: 0 ns, total: 3 ms
Wall time: 2.44 ms
4 ['PIAY', 'PLAY', 'YAIP', 'YALP'] True
CPU times: user 0 ns, sys: 0 ns, total: 0 ns
Wall time: 147 µs
['PIAY', 'PLAY', 'YAIP', 'YALP'] True
```

- A little slower, but our list is pruned significantly
- All of theses have identical spectrums

Now let's return to our *real* peptide

```
spectrum = TheoreticalSpectrum(TyrocidineB1)
%time UltimatePossiblePeptide(spectrum)
print len(peptideList)
print TyrocidineB1 in peptideList
%time matches = TestPeptides(peptideList, spectrum)
print len(matches)
print TyrocidineB1 in matches
```

```
CPU times: user 66 ms, sys: 8 ms, total: 74 ms
Wall time: 63.9 ms
16
True
CPU times: user 1e+03 µs, sys: 0 ns, total: 1e+03 µs
Wall time: 1.55 ms
16
True
```

```
print TyrocidineB1
for i, peptide in enumerate(peptideList):
    print peptide,
    if (i % 4 == 3):
        print
```

```
VKLFPWFNQY
VKIFPWFNKY VKIFPWFNQY VKLFPWFNKY VKLFPWFNQY
VQIFPWFNKY VQIFPWFNQY VQLFPWFNKY VQLFPWFNQY
YKNFWPFIKV YKNFWPFIQV YKNFWPFLKV YKNFWPFLQV
YQNFWPFIKV YQNFWPFIQV YQNFWPFLKV YQNFWPFLQV
```

All of these peptides give also give us our desired spectrum

Great, but our assumptions are a little Naïve

- In reality, Mass Spectometers don't report the Theoretical Spectrum of a peptide
- Instead they report a measured or *Experimental Spectrum*
- This spectrum might *miss* some fragments
- It might also report *false* fragments
    - From Contaminants
    - New peptides formed by unintended reactions between fragments
- The result is that some of the masses that appear may be misleading, and some that we want might be missing
- We need to develop algorithms for reporting candidate protein sequences that are robust to noise

Example experimental spectrum for Tyrocidine B1

97, 99, 113, **114**, 128, 147, 163,
186, **200**, 227, 241, 242, 244, 260,
261, 283, 291, 333, 340, 357, **388**,
389, 405, 430, 447, **457**, **485**, 487,
543, 544, 552, 575, 577, 584, **659**,
671, 672, 690, 691, **731**, 738, 770,
804, 818, 819, 835, **906**, 917, 932,
982, 1031, **1060**, 1095, 1159, **1223**, 1322

False Masses: present in the experimental spectrum, but not in the theoretical spectrum

Missing Masses: present in the theoretical spectrum, but not in the experimental spectrum

Example experimental spectrum for Tyrocidine B1

97,	99,	113,		128,	147,	163,
186,	**200**,	227,	241,	242,	244,	260,
261,	283,	291,	333,	340,	357,	
	405,	430,	447,	**457**,		487,
543,	544,	552,	575,	577,	584,	**659**,
671,	672,	690,	691,	**731**,	738,	770,
804,	818,	819,	835,	**906**,	917,	932,
982,	1031,		1095,	1159,		1322

False Masses: We don't know which these are

Missing Masses: And these values don't appear

An aside: Faking an Experimental Spectrum

```
# generate a synthetic experimental spectrum with 10% Error
import itertools
import random
random.seed(1961)

spectrum = TheoreticalSpectrum(TyrocidineB1)

# Pick around ~10% at random to remove
missingMass = random.sample(spectrum[:-1], 6)  # keep largest mass
print "Missing Masses = ", missingMass

# Add back another ~10% of false, but actual, peptide masses
falseMass = []
for i in xrange(5):
    fragment = ''.join(random.sample(Daltons.keys(), random.randint(2,len(TyrocidineB1)-2)))
    weight = sum([Daltons[residue] for residue in fragment])
    falseMass.append(weight)
print "False Masses = ", falseMass

experimentalSpectrum = sorted(set([mass for mass in spectrum if mass not in missingMass] + falseMass))
```

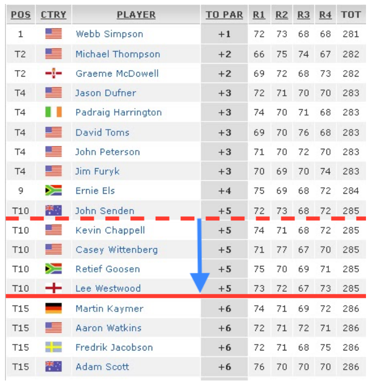
```
Missing Masses =  [1159, 114, 691, 186, 819, 357]
False Masses =  [457, 200, 731, 906, 659]
```

```
print experimentalSpectrum
```

```
[97, 99, 113, 128, 147, 163, 200, 227, 241, 242, 244, 260, 261, 283, 291, 333, 340, 388, 389, 405, 430, 447, 457, 485, 487, 543, 544, 552, 575, 577, 584, 65
9, 671, 672, 690, 731, 738, 770, 804, 818, 835, 906, 917, 932, 982, 1031, 1060, 1095, 1223, 1322]
```

A Golf Tournament Analogy

- After the first couple of rounds of a major golf tournament a *cut* is made of all golfers who are so far back from the leader that it is deemed they are unlikely to ever finish in the money
- These *cut* golfers are removed from further consideration
- This choice is *heuristic*
  - It is possible that a player just below the cut could have two exceptional rounds, but that is considered unlikely
- What is the equivalent of a score in our peptide finding problem?
  - The number of matching masses in the candidate peptide's Theoretical Spectrum and the Experimental Spectrum
  - Normalized score, why?
  - len(intersection of candidate and experimental spectrums) / len(union of candidate and experimental spectrums)
  - ***Jaccard Index*** for sets
- In our peptide *golf game* a round will be considered a one peptide extension of a active set of *player* peptides
- We will do cuts on every round, keeping to top 5% of finishers or the top 5 players, which ever is more
- Why 5%? It is arbitrary, but on each round we will extend the current set of players by one of 20 amino acids, thus increasing the number of peptides by a factor of 20, so reducing by 5% leaves the poolsize realtively stable.



POS	CTRY	PLAYER	TO PAR	R1	R2	R3	R4	TOT
1	🇺🇸	Webb Simpson	+1	72	73	68	68	281
T2	🇺🇸	Michael Thompson	+2	66	75	74	67	282
T2	🇬🇧	Graeme McDowell	+2	69	72	68	73	282
T4	🇺🇸	Jason Dufner	+3	72	71	70	70	283
T4	🇮🇪	Padraig Harrington	+3	74	70	71	68	283
T4	🇺🇸	David Toms	+3	69	70	76	68	283
T4	🇺🇸	John Peterson	+3	71	70	72	70	283
T4	🇺🇸	Jim Furyk	+3	70	69	70	74	283
9	🇿🇦	Ernie Els	+4	75	69	68	72	284
T10	🇦🇺	John Senden	+5	72	73	68	72	285
T10	🇺🇸	Kevin Chappell	+5	74	71	68	72	285
T10	🇺🇸	Casey Wittenberg	+5	71	77	67	70	285
T10	🇿🇦	Retief Goosen	+5	75	70	69	71	285
T10	🏴󠁧󠁢󠁥󠁮󠁧󠁿	Lee Westwood	+5	73	72	67	73	285
T15	🇩🇪	Martin Kaymer	+6	74	71	69	72	286
T15	🇺🇸	Aaron Watkins	+6	72	71	72	71	286
T15	🇸🇪	Fredrik Jacobson	+6	72	71	68	75	286
T15	🇦🇺	Adam Scott	+6	76	70	70	70	286

An Implementation

```
def LeaderboardFindPeptide(noisySpectrum, cutThreshold=0.05):
    # Golf Tournament Heuristic
    spectrum = set(noisySpectrum)
    target = max(noisySpectrum)
    players = [''.join(peptide) for peptide in itertools.product(Daltons.keys(), repeat=2)]
    round = 1
    currentLeader = [0.0, '']
    while True:
        print "%8d Players in round %d [%5.4f]" % (len(players), round, currentLeader[0])
        leaderboard = []
        for prefix in players:
            testSpectrum = set(TheoreticalSpectrum(prefix))
            totalWeight = max(testSpectrum)
            score = len(spectrum & testSpectrum)/float(len(spectrum | testSpectrum))
            if (score > currentLeader[0]):
                currentLeader = [score, prefix]
            elif (score == currentLeader[0]):
                currentLeader += [prefix]
            if (totalWeight < target):
                leaderboard.append((score, prefix))
        remaining = len(leaderboard)
        if (remaining == 0):
            print "Done, no sequences can be extended"
            break
        leaderboard.sort(reverse=True)
        # Prune the larger of the top 5% or the top 5 players
        cut = leaderboard[max(min(5,remaining-1),int(remaining*cutThreshold))][0]
        players = [p+r for s, p in leaderboard if s >= cut for r in Daltons.iterkeys()]
        round += 1
    return currentLeader

spectrum = TheoreticalSpectrum(TyrocidineB1)
experimentalSpectrum = [mass for mass in spectrum if mass not in missingMass] + falseMass
%time winners = LeaderboardFindPeptide(experimentalSpectrum)
print winners
print len(winners) - 1, "Candidate residues with", winners[0], 'matches'
print TyrocidineB1, TyrocidineB1 in winners
```

```
     400 Players in round 1 [0.0000]
     480 Players in round 2 [0.0600]
    1280 Players in round 3 [0.1200]
    1560 Players in round 4 [0.2000]
    2000 Players in round 5 [0.2745]
    2600 Players in round 6 [0.3654]
    3320 Players in round 7 [0.4615]
    3520 Players in round 8 [0.5556]
    3840 Players in round 9 [0.6545]
    2400 Players in round 10 [0.8036]
     160 Players in round 11 [0.8036]
Done, no sequences can be extended
CPU times: user 1.52 s, sys: 55 ms, total: 1.58 s
Wall time: 1.51 s
[0.8035714285714286, 'YQNFWPFLQV', 'YQNFWPFLKV', 'YQNFWPFIQV', 'YQNFWPFIKV', 'YKNFWPFLQV', 'YKNFWPFLKV', 'YKNFWPFIQV', 'YKNFWPFIKV', 'VQLFPWFNQY', 'VQLFPWFN
KY', 'VQIFPWFNQY', 'VQIFPWFNKY', 'VKLFPWFNQY', 'VKLFPWFNKY', 'VKIFPWFNQY', 'VKIFPWFNKY']
16 Candidate residues with 0.803571428571 matches
VKLFPWFNQY True
```

Next Time

- This method works well, but it relys on heuristcs, and thus might miss the best answer
- Our methods are still make a lot of simplfying assumptions
- Relying only exact matches might mislead us
- We will continue to explore ways of assembling peptide sequences from a given experimental spectrum