

1. Give a clear statement of the problem.

Given a file containing 42 PrP (prion protein gene) sequences, I was tasked with find a strategy to predict the 2D secondary structure that best represents these sequences.

2. Give a brief description of the method of your procedure. This should be a few sentences describing the problem, solution, and results. It should be similar to an abstract in a scientific paper.

In order to effectively predict the 2D secondary structure of these 42 PrP sequences, I needed to first align the sequences with each other, find the consensus sequence and compute the covariation or mutual information of the sequences. I then calculate the structure based off of the alignment information I found. This calculation was performed using a dynamic programming strategy followed by a backtracking algorithm to translate the data into a dot-bracket form representation. This dot-bracket along with the consensus sequence that was found enabled me to utilize the Vienna package RNA plotting function to plot the 2D structure.

3. Present the procedure in detail, including any relevant mathematics. If the solution involves programming, give an overview in words of how the program operates. The presentation should contain sufficient detail so that a reader can follow each step without difficulty. *

#NOTE: before starting I used the MUSCLE multi-sequence aligner to obtain my input file based on the provided PRP.fasta file

procedure main:

```
    parse alignment file -> obtain sequences
    calculateConsensus(sequences) -> consensussequence, count_matrix (# nucleotide at i)
    convert count_matrix -> frequency_matrix
    calculateMutualInfo( frequency_matrix, sequences) -> obtain matrix M
    dynamic_program(M) -> obtain g matrix
    Backtrace(g) -> obtain pairs
    StructuredFromPairs(pairs) -> obtain dot-bracket
    #external program: plot dot-bracket to get 2D structure
```

procedure calculateConsensus:

```
    init count_matrix (5 nucleotide options x 42 columns)
    for each sequence , for each position, i:
        count_matrix[nucleotide at i in seq][i] += 1
    consensus_sequence = ""
    for each position in count_matrix:
        find nucleotide with max count and append to consensus_sequence
    return consensus_sequence, count_matrix
```

```

procedure convert(count_matrix):
    init frequency_matrix = count_matrix
    L = # of rows
    for each nucleotide, column (i) in count_matrix:
        frequency_matrix[nucleotide][i] /= L
    return frequency_matrix

procedure calculateMutualInfo( frequency_matrix, sequences)
    L = # of columns
    def combined_freq(i, j, n1, n2):
        count = 0
        for each sequence:
            if seq[i] = n1 and seq[j] == n2
                ++count
        return count

    def find_val1(i,j):
        answer = 0.0
        for each nucleotide, n1,n2:
            if n1n2 is a valid pair:
                answer += combined_freq(i,j,n1,n2)
        return answer

    def find_val2(i,j):
        answer = 0.0
        for each nucleotide n1,n2:
            f_i = frequency_matrix[n1][i]
            f_2 = frequency_matrix[n2][j]
            if n1n2 is valid f_ij = combined_freq(i,j,n1,n2)
            else f_ij = 0
            if none of the frequencies = 0:
                answer += log(f_ij / f_i*f_j)
        return answer

    for i in L:
        for j in L:
            if i != j:
                val1 = find_val1(i,j)
                val2 = find_val2(i,j)
                answer = val1 * val2
                if answer < 0 --> M[i][j] = 0.0
                else M[i][j] = answer

    return M

```

```

procedure dynamic_program(M):
    L = # of columns
    init D [L,L]

    def find_val4(i,j):
        answer = 0.0
        k = i+4
        while k <= (j-5)):
            answer = max(answer, D[i][k] + D[k+1][j])
            ++k
        return answer

    for i in M from L-1 ->0:
        for j from i+3 -> L-1:
            vall1 = D[i+1][j]
            value2 = D[i][j-1]
            value3 = D[i+1][j-1]+M[i][j]
            value4 = find_val4(i,j)
            D[i][j] = max of values1-4

    return D

```

##provided using the nussinov.py script

```

procedure Backtrace(g):
    """
    traceback - recursive back trace through g matrix
                fills pairs matrix defined in outer scope
    """
    nonlocal pairs

    if i < j:
        if g[i,j] == g[i+1, j]:
            traceback(i+1, j)
        elif g[i,j] == g[i, j-1]:
            traceback(i, j-1)
        elif g[i,j] == g[i+1, j-1] + M[i][j]:
            if abs(i-j) > 2:
                pairs += [i,j]
            traceback(i+1, j-1)
        else:
            for k in range(i+1, j):
                if g[i,j] == g[i, k] + g[k+1, j]:
                    traceback(i, k)

```

```

        traceback(k+1, j)
        break

L = g.shape[0]
pairs = []
traceback(0, L-1)

dot_bracket = np.zeros([L, L], dtype=int)
for i in range(0, len(pairs), 2):
    dot_bracket[pairs[i], pairs[i+1]] = 1

return pairs, dot_bracket

#provided by nussinov.py script
procedure StructuredFromPairs(pairs):
    struct = list('.' * L)
    for i in range(0, len(pairs), 2):
        struct[pairs[i]] = '('
        struct[pairs[i+1]] = ')'

    return ".join(struct)

```

- 4. If the solution involves programming code, output must be clearly labeled with each item having a descriptive title. Programming code must be documented with comments describing each major step in the program and all relevant variables. Source code should be submitted in a separate file. ***

Note: additional output appended to end of write up (pairs, Mutual information matrix and dynamic program matrix return)

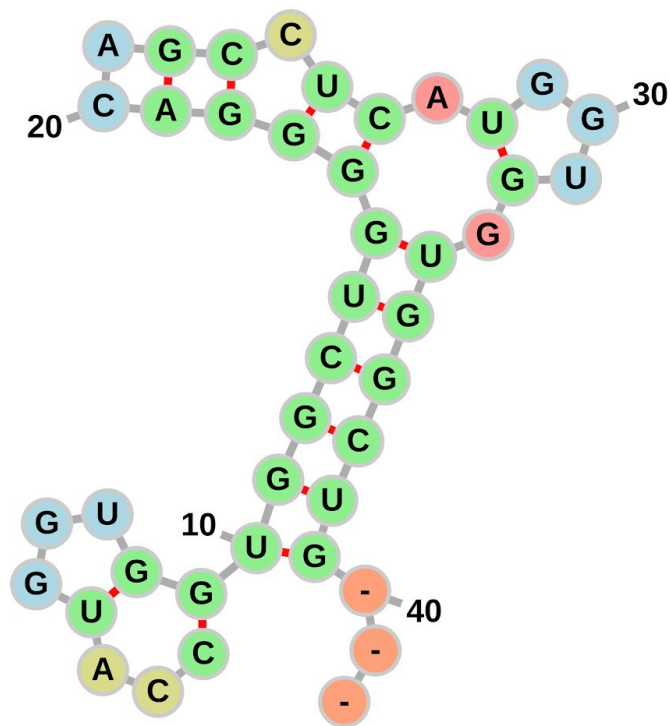
The consensus sequence is:

CCAUGGUGGUGGCUGGGGACAGCCUCAUGGUGGUGGCUG---

The dot-bracket format description of the consensus sequence is:

(..(...))((((((((((..)).)).(...).))))))...

Output from plot of 2d secondary structure:



5. Graphs must be titled and have each axis labeled clearly

No graphs for this assignment.

Appended Output

Pairs:

[0, 8, 3, 7, 9, 38, 10, 37, 11, 36, 12, 35, 13, 34, 14, 33, 15, 25, 16, 24, 17, 22, 18, 21, 27, 31]

Mutual Information Matrix

```
[[ 0.          3.8918203  54.10189853  0.          237.94069076
 236.33021061  25.38087662 241.29285848 239.59412208 17.43253549
 231.69047923 459.36357187  3.81177759  0.          236.33021061
 233.22956453 481.54428165 459.36357187 99.16967556  0.
 168.5565961 462.64634271  7.47533924  3.78645978 26.63636003
 0.          52.66474038  5.39589769 481.54428165 236.33021061
 29.48014348 236.33021061 234.76050651 36.49876588 234.76050651
 234.76050651 0.          0.          234.76050651 96.65934073
 0.          0.          ]
```

[3.8918203 0. 0. 0. 138.15911077
137.22399326 6.97271038 130.37850407 129.44693894 3.8918203
120.7691924 126.79624653 0. 0. 127.657052
135.42361812 126.79624653 126.79624653 23.35092179 3.8918203
0. 123.7744761 3.19867312 0. 11.67546089
0. 0. 0. 131.54011169 127.657052 0.
127.657052 131.54011169 7.7836406 126.79624653 131.54011169
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80.17592747 0. 0. 101.49464182 0.
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7.72884265 3.86442132 3.86442132 85.01726913 7.72884265
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0. 0. 101.49464182 0. 0.
23.18652794 134.43725504 3.86442132 3.86442132 0.
3.86442132]

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274.44798651 178.30714213 420.28719244 378.76442391 14.6019717
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270.84723623 264.03762098 490.78716853 243.98795322 0.
379.58253213 609.57196285 20.0180721 0. 30.44456311
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 462.59113318 541.67185375 514.63911371 318.63987506 57.59939006
 82.75887774 685.9104682 21.29238625 56.56807362 0.
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Dynamic Programming Matrix Return

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  1.85204488e+03 1.85204488e+03 1.85204488e+03 1.95162604e+03
  2.06614646e+03 2.52879280e+03 2.65960078e+03 3.02696284e+03
  3.15787258e+03 3.38201461e+03 3.38201461e+03 3.56134788e+03
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2.55419438e+03	2.64624590e+03	2.97861589e+03	3.00734560e+03
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[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
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0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
3.02916783e+02	5.88559576e+02	5.88559576e+02	5.88559576e+02
9.11218016e+02	1.01701995e+03	1.01701995e+03	1.14374670e+03
1.56963398e+03	1.90734350e+03	2.05405242e+03	2.33475273e+03

2.48242466e+03	2.48242466e+03	2.59519383e+03	2.59519383e+03
2.59519383e+03	2.89974878e+03	3.23099236e+03	3.23099236e+03
3.28647384e+03	3.31467382e+03	3.46234575e+03	3.50494761e+03
3.50494761e+03	3.50494761e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	6.08904488e+00	2.21142749e+02	4.61733760e+02
4.68515952e+02	4.71584572e+02	8.93016080e+02	1.14374670e+03
1.56963398e+03	1.90734350e+03	1.90734350e+03	2.33475273e+03
2.33475273e+03	2.33475273e+03	2.33475273e+03	2.33475273e+03
2.34153492e+03	2.77664349e+03	2.79036669e+03	2.79036669e+03
3.28647384e+03	3.31467382e+03	3.31467382e+03	3.31467382e+03
3.31467382e+03	3.31467382e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	2.21142749e+02	4.61733760e+02
4.65495527e+02	4.69257294e+02	8.35281789e+02	1.14374670e+03
1.41683157e+03	1.90734350e+03	1.90734350e+03	1.90734350e+03
1.90734350e+03	1.90734350e+03	2.16629175e+03	2.33475273e+03
2.33475273e+03	2.58513301e+03	2.79036669e+03	2.79036669e+03
3.04145833e+03	3.05809016e+03	3.05809016e+03	3.05809016e+03
3.05809016e+03	3.05809016e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	4.61733760e+02
4.61733760e+02	4.61733760e+02	8.35281789e+02	9.54240439e+02
1.34922776e+03	1.34922776e+03	1.34922776e+03	1.54243242e+03
1.54243242e+03	1.79520419e+03	2.14570958e+03	2.14570958e+03
2.14570958e+03	2.33154380e+03	2.33154380e+03	2.50744184e+03
2.75063765e+03	2.75142188e+03	2.75142188e+03	2.91638803e+03
2.92276476e+03	2.92276476e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
3.06861999e+00	4.12535716e+02	8.07555902e+02	8.07555902e+02

9.27174830e+02	1.24605101e+03	1.24605101e+03	1.24605101e+03
1.44701888e+03	1.74600614e+03	1.80287716e+03	1.89444229e+03
1.97036784e+03	2.27201861e+03	2.27511192e+03	2.44888037e+03
2.60095119e+03	2.60095119e+03	2.65504488e+03	2.91638803e+03
2.92207161e+03	2.92207161e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	4.12535716e+02	4.12535716e+02	4.12535716e+02
6.85910468e+02	7.31038126e+02	7.67561357e+02	9.67140134e+02
1.42975113e+03	1.54593937e+03	1.80287716e+03	1.89444229e+03
1.95912755e+03	2.27201861e+03	2.27201861e+03	2.29919391e+03
2.29919391e+03	2.36775625e+03	2.65504488e+03	2.91638803e+03
2.91638803e+03	2.91638803e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	5.20525317e+01	5.20525317e+01
6.85910468e+02	7.19621787e+02	7.23383555e+02	8.13810552e+02
1.42975113e+03	1.42975113e+03	1.68397582e+03	1.83164775e+03
1.83744255e+03	1.86981004e+03	2.17171411e+03	2.17171411e+03
2.19890836e+03	2.35314081e+03	2.65504488e+03	2.65504488e+03
2.65504488e+03	2.65504488e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	3.76176717e+00
6.85910468e+02	7.19621787e+02	7.19621787e+02	8.13810552e+02
8.13810552e+02	1.33347043e+03	1.68397582e+03	1.69075801e+03
1.69075801e+03	1.86981004e+03	1.86981004e+03	1.86981004e+03
2.19890836e+03	2.35314081e+03	2.35314081e+03	2.35314081e+03
2.35314081e+03	2.35314081e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00

0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
6.85910468e+02	6.85910468e+02	6.85910468e+02	6.85910468e+02
6.85910468e+02	1.33347043e+03	1.68397582e+03	1.68397582e+03
1.68397582e+03	1.86981004e+03	1.86981004e+03	1.86981004e+03
2.19890836e+03	2.24429411e+03	2.26258209e+03	2.26716705e+03
2.26716705e+03	2.26716705e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	4.27725939e+01	4.27725939e+01	4.27725939e+01
6.39462978e+02	1.33347043e+03	1.33347043e+03	1.33347043e+03
1.43897141e+03	1.43897141e+03	1.68192303e+03	1.68192303e+03
1.82957757e+03	1.89728938e+03	2.10775962e+03	2.10775962e+03
2.10775962e+03	2.10775962e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	2.24742128e+01	2.35223970e+01
6.39462978e+02	8.50411830e+02	8.50411830e+02	8.50411830e+02
1.16281194e+03	1.16281194e+03	1.19886443e+03	1.64132447e+03
1.64132447e+03	1.64132447e+03	1.79845314e+03	1.79845314e+03
1.79845314e+03	1.79845314e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	2.35223970e+01
2.76233672e+02	5.44025229e+02	5.44025229e+02	7.01078181e+02
7.86383005e+02	7.86383005e+02	1.02538388e+03	1.15925686e+03
1.18251256e+03	1.18251256e+03	1.43444775e+03	1.54088182e+03
1.54088182e+03	1.54088182e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
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0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00

0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
2.75190895e+02	2.75190895e+02	2.75190895e+02	5.00379822e+02
5.75081159e+02	5.75081159e+02	8.83023185e+02	1.15821408e+03
1.15821408e+03	1.15821408e+03	1.30769706e+03	1.30769706e+03
1.30769706e+03	1.30769706e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
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0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	3.06861999e+00	1.04346924e+02	4.27409228e+02
4.27409228e+02	5.35517331e+02	8.83023185e+02	8.83023185e+02
9.00920414e+02	1.03250617e+03	1.08438230e+03	1.10107576e+03
1.10107576e+03	1.10107576e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
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0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	5.32492367e+01	4.27409228e+02
4.27409228e+02	4.27409228e+02	8.83023185e+02	8.83023185e+02
8.83023185e+02	8.83023185e+02	1.08438230e+03	1.08438230e+03
1.08438230e+03	1.08438230e+03]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
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0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
0.00000000e+00	0.00000000e+00	0.00000000e+00	6.78219206e+00
6.78219206e+00	4.17324115e+02	4.21110575e+02	4.21110575e+02
7.12948035e+02	7.88000887e+02	7.88000887e+02	7.99479424e+02
7.99479424e+02	8.03265884e+02]		
[0.00000000e+00	0.00000000e+00	0.00000000e+00	0.00000000e+00
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