1.1-section-result

February 14, 2025

1 Section 1 - Quantifying the association of a feature with an outcome

- We frequently want to know if a **feature** is **associated** with an **outcome**. For example, we might want to determine:
  - Is the presence of a specific cell stress condition (feature) associated with the formation of stress granules (outcome)?
  - Is the amino acid sequence of a protein (feature) associated with its propensity to misfold (outcome)?
- This type of analysis is invaluable for checking for correlations between features and outcomes in complex data
- In this section we will explore using contingency tables, odds ratios, and Fisher's Exact Test to test for association

1.1 Example 1.1

Application 1.1: We hypothesize that *E. coli* proteins that contain native entanglements are more likely to misfold than proteins without entanglements.

- To proceed, we need information about which proteins in *E. coli* contain entanglements and which proteins in *E. coli* misfold.

1.1.1 What is an entanglement? How can we tell if a protein is entangled?

- Entanglements are a structural motif in proteins formed by two segments: a loop (closed by a native contact) and a thread (**Figures 1.1.1** & **1.1.2**).

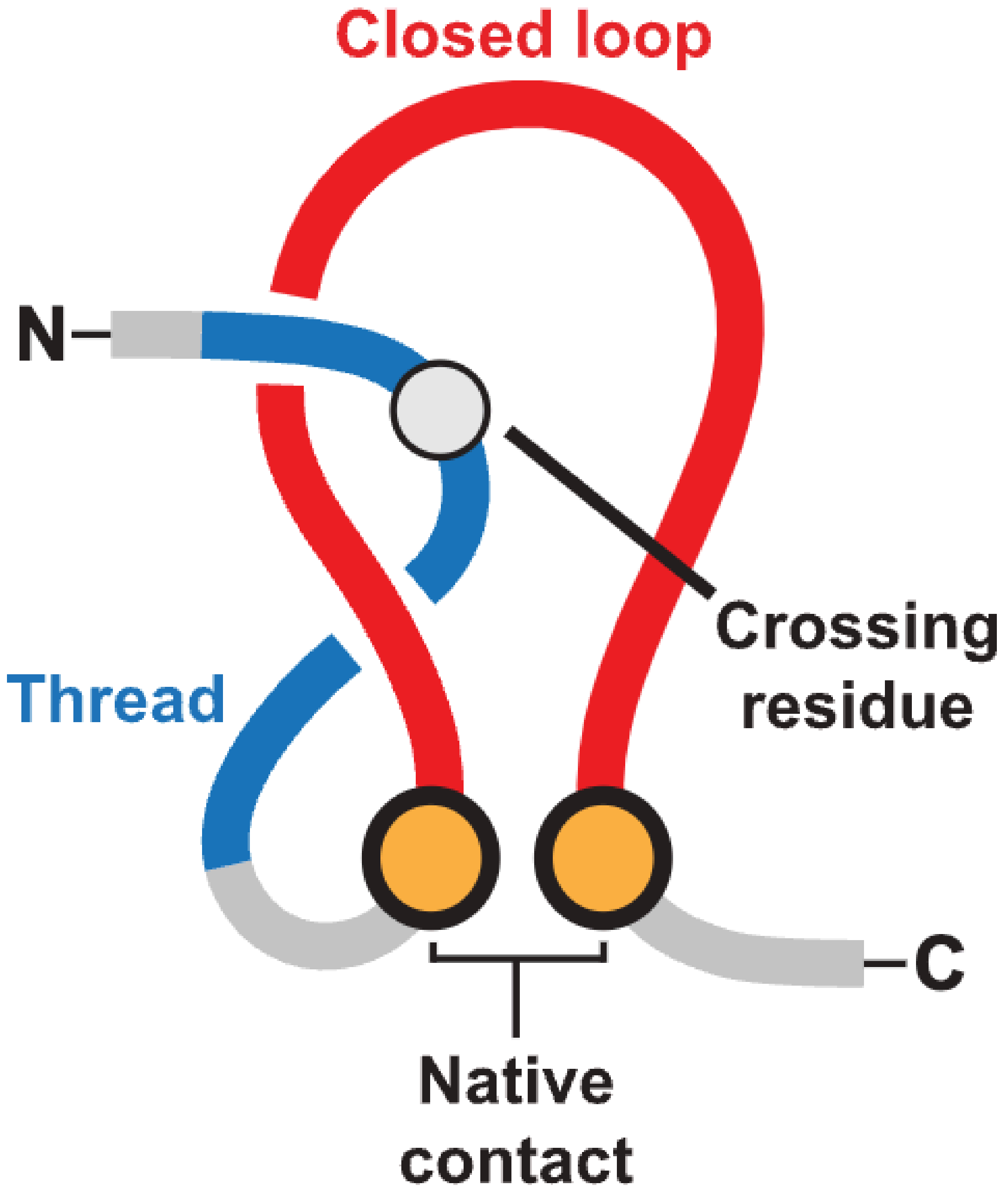


Figure 1.1.1. *General structure of a non-covalent lasso entanglement. The threading segment (blue) passes through a loop (red) that is closed by a native contact (yellow). J. Mol. Biol. 436 (2024) 168487.*

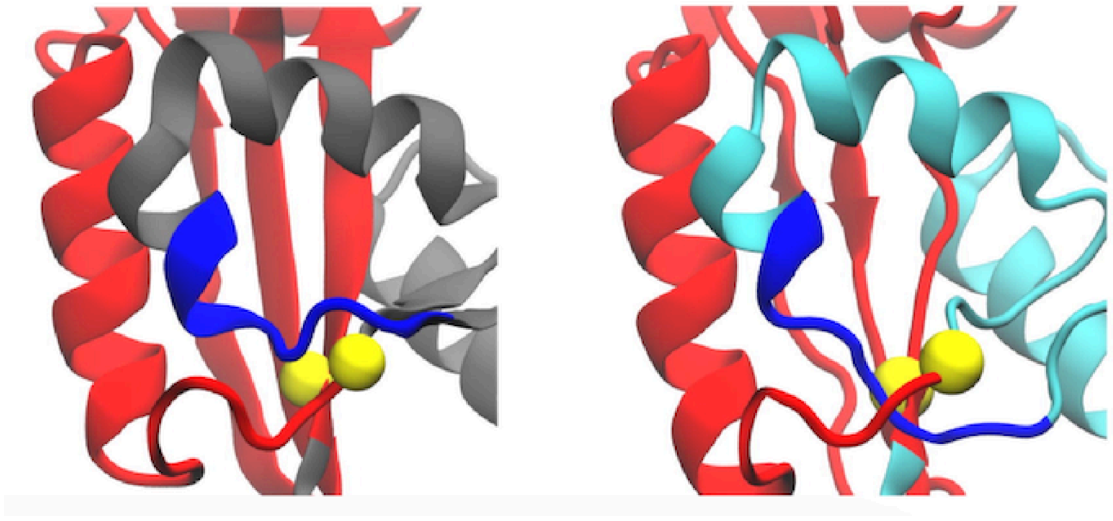


Figure 1.1.2. *3D structures of oligoribonuclease without (left) and with (right) an entanglement. The threading segment (blue) passes through a loop (red) that is closed by a native contact (yellow). J. Mol. Biol. 436 (2024) 168487.*

- Some proteins contain entanglements in their native state (*i.e.*, native entanglements)
- Some proteins can gain or lose entanglements during misfolding
- For the current hypothesis, we are concerned with native entanglements; this information can be obtained by analyzing either experimental structures or predicted structures
- Our analysis will use data on entanglements computed from experimental structures of *E. coli* proteins

1.1.2 How can we tell if a protein misfolds?

- The structural proteomics technique *limited proteolysis mass spectrometry* (LiP-MS) profiles changes in protein structures across the proteome in response to perturbations (**Figure 1.1.3**)

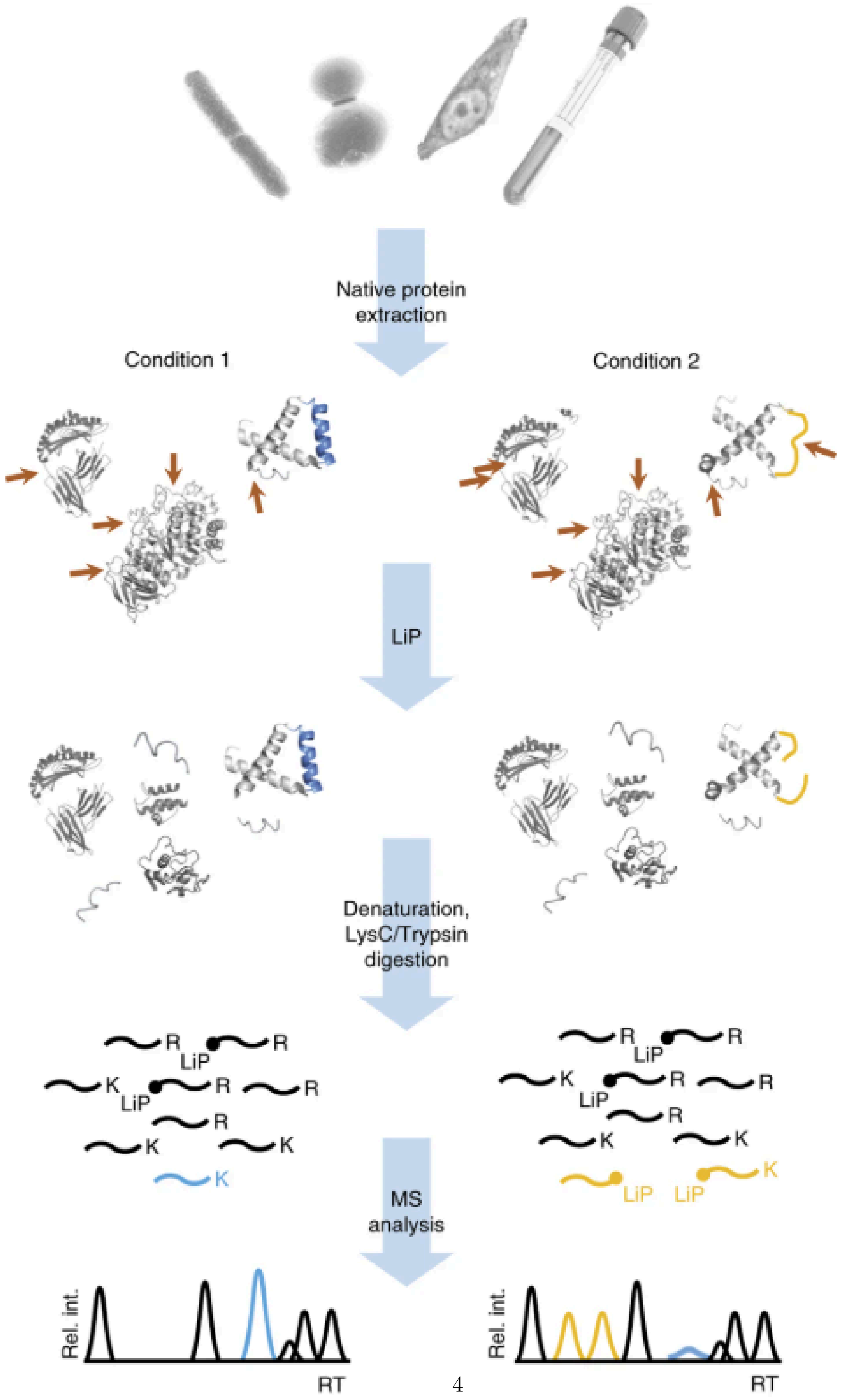

Native protein extraction
Condition 1
Condition 2
LiP
Denaturation, LysC/Trypsin digestion
R
LiP
K
MS analysis
Rel. int.
RT

Figure 1.1.3. *Schematic of a LiP-MS experiment. When studying misfolding, one sample will be treated with guanidinium chloride to induce unfolding before a dilution jump is used to stimulate refolding; the other sample is not treated with guanidinium chloride, preserving protein native states. Nature Protocols volume 12, p. 2391–2410 (2017).*

- LiP-MS compares differences in protein structures between two samples
- In the case at hand, a protein is considered to misfold if there is a significant change in its limited proteolysis digestion pattern between a guanidinium chloride-unfolded/refolded and an untreated sample containing natively folded protein
- We will use LiP-MS data from *E. coli* to match the *E. coli* entanglement data

1.1.3 We have our data - what now?

- Now that we have identified relevant data to test our hypothesis, let's dive into some code.

1.2 Testing our hypothesis in Python

1.2.1 Step 0 - Load libraries

- We first need to make sure we have access to all of the functions etc. that we need for this analysis - let's load some libraries

[1]:
```
import numpy as np
import pandas as pd
from scipy.stats import fisher_exact
import matplotlib.pyplot as plt
```

1.2.2 Step 1 - Load the data

- After loading the libraries, we now need to load the data into memory

[2]:
```
# "data1" is a pandas DataFrame object
data_path = "/home/jovyan/data-store/data/iplant/home/shared/NCEMS/
 ↪BPS-training-2025/"
data1     = pd.read_csv(data_path + "NativeEntanglements_and_SigCuts_EXP_buffC.
 ↪csv")
```

1.2.3 Step 2 - Explore the data

- Let's explore the data quickly to get a better understanding of what we need to do

[3]:
```
# first, print a quick summary
print ("Create a quick summary of the DataFrame:\n")
data1.info()

# second, print the first 10 rows of data1
print ("\nPrint the first 10 rows of the DataFrame:\n")
display(data1.head(10))
```

```
# third, count the number of unique gene identifiers in column "gene" of data1
print ("\nThe number of unique genes is:", len(data1["gene"].unique()))
```

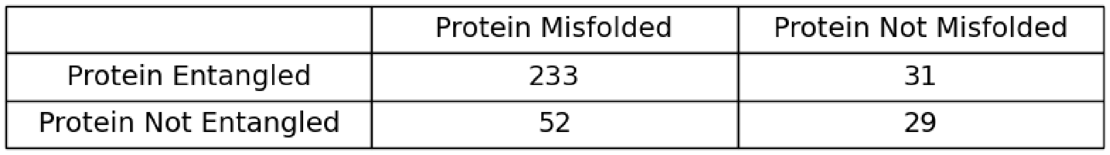
```
Create a quick summary of the DataFrame:

<class 'pandas.core.frame.DataFrame'>
RangeIndex: 345 entries, 0 to 344
Data columns (total 4 columns):
 #   Column         Non-Null Count  Dtype 
---  ------         --------------  ----- 
 0   buff           345 non-null    object
 1   gene           345 non-null    object
 2   NativeEnt      345 non-null    bool  
 3   NonRefoldable  345 non-null    bool  
dtypes: bool(2), object(2)
memory usage: 6.2+ KB

Print the first 10 rows of the DataFrame:


  buff    gene  NativeEnt  NonRefoldable
0    C  P00350       True           True
1    C  P00370       True           True
2    C  P00448       True           True
3    C  P00509       True           True
4    C  P00561       True          False
5    C  P00579      False          False
6    C  P00864       True           True
7    C  P00934       True           True
8    C  P00954      False           True
9    C  P00957       True           True


The number of unique genes is: 345
```

- We can see from this summary that this data file contains 4 columns:
  - `buff`: the buffer condition for the experiment
  - `gene`: the unique gene identifier (there are no duplicates!)
  - `NativeEnt`: `True` if the protein has a native entanglement, `False` if it does not
  - `NonRefoldable`: `True` if the protein *did not* refold in LiP-MS experiment (i.e., misfolded), `False` if it *did* refold
- Now that we have a better understanding of the data, we are ready to run our analysis.

1.2.4 Step 3 - Run the analysis

[4]:
```python
# compute the values of {a, b, c, d} and construct the contingency table
a = len(data1[(data1["NativeEnt"] == True ) & (data1["NonRefoldable"] == True␣
  ↪)])
b = len(data1[(data1["NativeEnt"] == True ) & (data1["NonRefoldable"] ==␣
  ↪False)])
c = len(data1[(data1["NativeEnt"] == False) & (data1["NonRefoldable"] == True␣
  ↪)])
d = len(data1[(data1["NativeEnt"] == False) & (data1["NonRefoldable"] ==␣
  ↪False)])

# put values into a new format to enable a nice print statement & analysis
contingency_table = pd.DataFrame({"Protein Misfolded"    : [a, c],
                                  "Protein Not Misfolded": [b, d]},
                                 index = ["Protein Entangled", "Protein Not␣
  ↪Entangled"])

# print the output
print ("This is our contingency table:\n")

# create a table from our contingency_table using matplotlib
plt.clf()
fig, ax    = plt.subplots(figsize = (5, 2))
ax.axis("tight")
ax.axis("off")
cell_text  = contingency_table.reset_index().values.tolist()
col_labels = [""] + contingency_table.columns.tolist()
table      = ax.table(cellText=cell_text, colLabels=col_labels, loc="center",␣
  ↪cellLoc="center")
table.auto_set_font_size(False)
table.set_fontsize(14)
table.scale(2, 2)
plt.show()
```

```
This is our contingency table:


<Figure size 640x480 with 0 Axes>
```



	Protein Misfolded	Protein Not Misfolded
Protein Entangled	233	31
Protein Not Entangled	52	29

- We can complete this analysis by computing the odds ratio and *p*-value

[5]:
```
# use the fisher_exact function from scipy.stats to compute the odds ratio and␣
↪p-value
odds_ratio, fisher_p_value = fisher_exact(contingency_table, alternative =␣
↪"two-sided")

# print the results of this analysis
print ("The odds ratio is:", "%.2f" %odds_ratio)
print ("The p-value is   :", "%.2e" %fisher_p_value)
```

```
The odds ratio is: 4.19
The p-value is   : 2.93e-06
```

1.2.5 Step 4 - Interpret the results

- The odds ratio of 4.19 indicates that there is a **positive association** between entanglement and misfolding
- In other words, entanglement and misfolding tend to co-occur in the same protein, supporting our hypothesis
  - Odds ratios > 1 indicate positive association
  - Odds ratios $= 1$ indicate no association
  - Odds ratios < 1 indicate negative association
- We can also say that the **odds** of an entangled protein misfolding are 4.19 times greater than the odds of a non-entangled protein misfolding
- The *p*-value is «0.05, which is a common threshold for significance; in this instance, we conclude the result is significant