4.2-section-result

February 14, 2025

1 Section 4 - Avoiding data overinterpretation

1.1 Example 4.2

Application 4.2: In LiP-MS, one can overinterpret the statistical significance of proteolytic cut sites (that reflect potential changes in protein structure); we need to correct the p-values to reflect this.

- Run the following code cells in sequence and follow the instructions to test your knowledge at the end

1.1.1 Step 0 - Load libraries

[1]:
```
import pandas as pd
from statsmodels.stats.multitest import multipletests
import matplotlib.pyplot as plt
```

1.1.2 Step 1 - Load the data

- We will be using a new dataset for this analysis that includes information about the protein fragments detected during a LiP-MS experiment

[2]:
```
# "data10" is a pandas DataFrame object
data_path = "/home/jovyan/data-store/data/iplant/home/shared/NCEMS/
 ↪BPS-training-2025/"
data10 = pd.read_csv(data_path + "Ecoli_LiPMS_data.csv", usecols =␣
 ↪["Accession", "PeptidePValue1"])
```

1.1.3 Step 2 - Explore the data

[3]:
```
# print a quick summary of "data10"
data10.info()

# print the first 10 rows of "data10"
data10.head(10)
```

```
<class 'pandas.core.frame.DataFrame'>
RangeIndex: 41970 entries, 0 to 41969
Data columns (total 2 columns):
 #   Column          Non-Null Count  Dtype
---  ------          --------------  -----
 0   Accession       41970 non-null  object
 1   PeptidePValue1  41970 non-null  float64
dtypes: float64(1), object(1)
memory usage: 655.9+ KB
```

[3]:

```
  Accession  PeptidePValue1
0    P0A6Y8        7.740828
1    P0A6Y8        3.473137
2    P0A6Y8       14.137842
3    P0A6Y8        2.097467
4    P0A6Y8        1.451695
5    P0A6Y8        3.134283
6    P0A6Y8        3.354855
7    P0A6Y8        3.038093
8    P0A6Y8       -0.000000
9    P0A6Y8        7.027929
```

- Note that in the `pd.read_csv()` function call we have specified `usecols = ["Accession", "PeptidePValue1"]`, which causes only these two columns to be loaded; loading only the data you need can save memory and accelerate calculations
- We have a list of `Accession` codes alongside `PeptidePValue1` which represents the p-value
- Inspecting the p-values, we can see that they are not between 0 and 1 as we would expect; in this case, the input data set presents the *negative log base 10* of the p-values
- We will need to convert these -log10 values before carrying out the **false discovery rate** correction

1.1.4 Step 3 - Run the analysis

- We are now ready to run our analysis (after converting the p-values from -log10)

[4]:

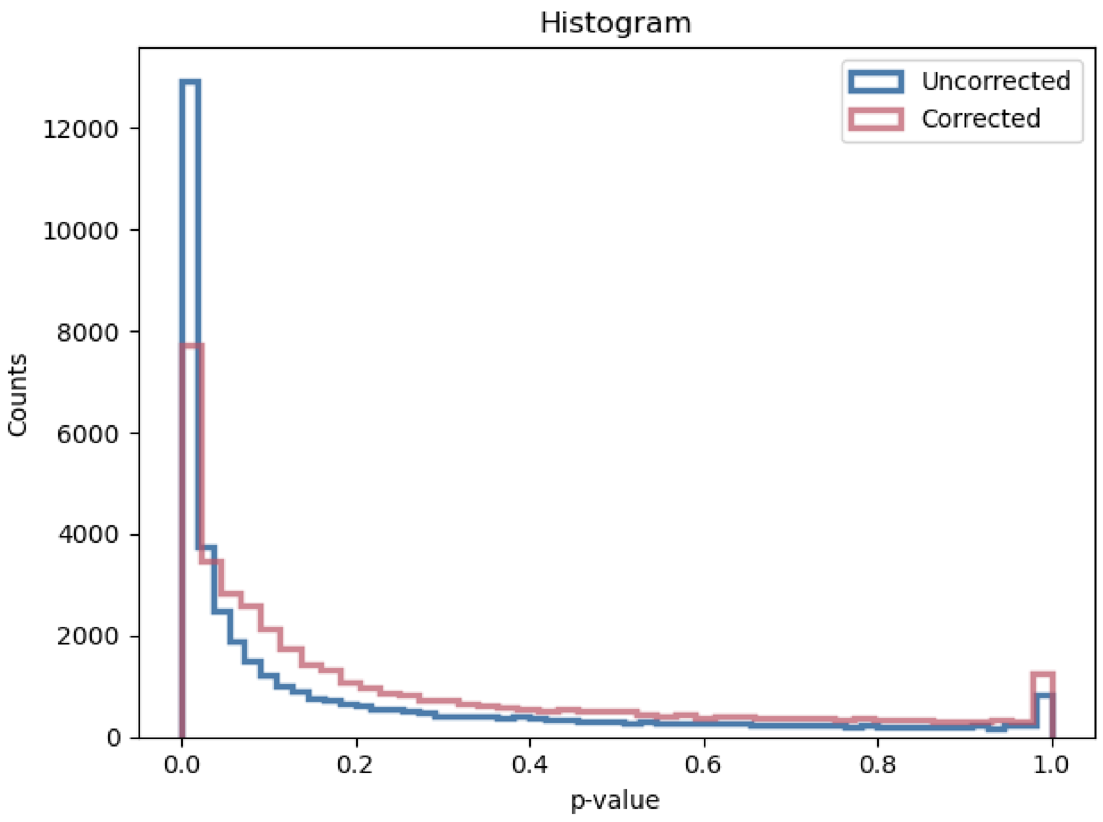
```
# define the significance level for our tests
alpha = 0.05

# convert p-values from -log10 and add these values as a new column
data10["PeptidePValue1_orig"] = 10 ** (-data10["PeptidePValue1"])
display(data10.head(10))

# apply the Benjamini-Hochberg procedure for FDR correction
_, pvals_corrected, _, _ = multipletests(data10['PeptidePValue1_orig'], alpha =␣
↪alpha, method = 'fdr_bh')

# add corrected p-values as a new column
data10['PeptidePValue1_adjust'] = pvals_corrected

# compute the proportion of uncorrected p-values < alpha
N_uncorr_acc = (data10['PeptidePValue1_orig'] < alpha).sum()
```

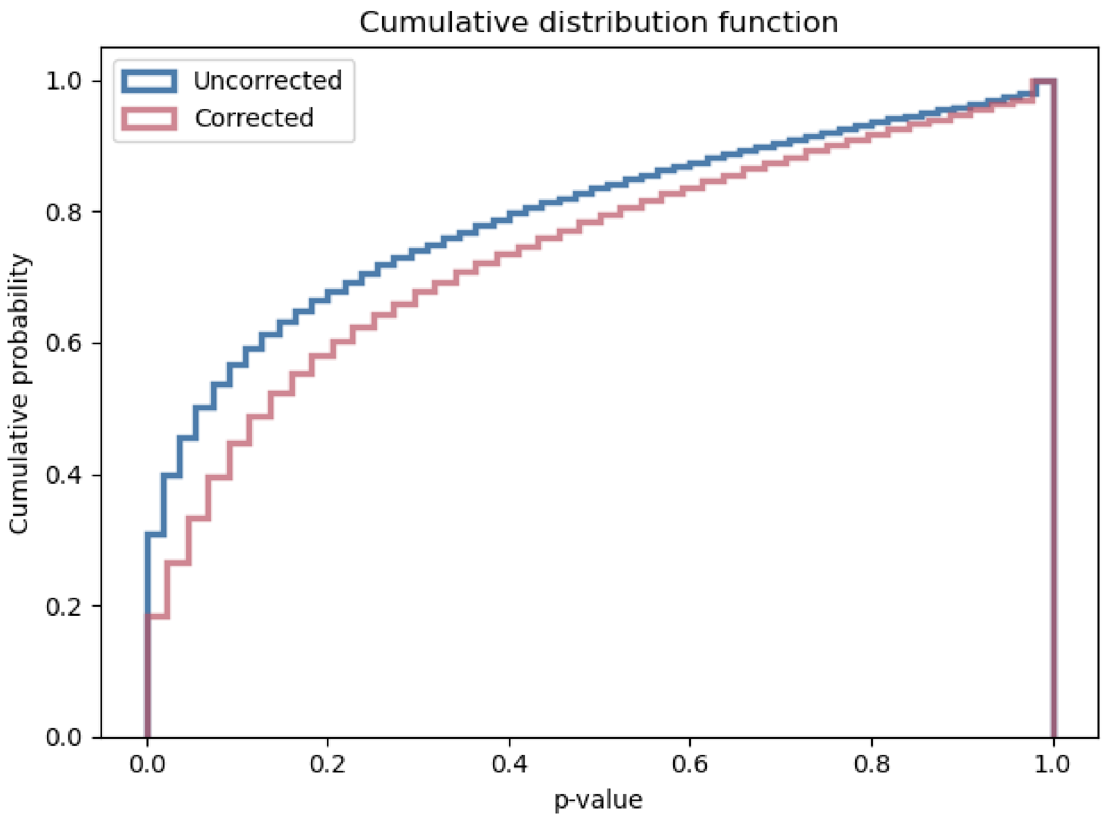
```python
N_corr_acc   = (data10['PeptidePValue1_adjust'] < alpha).sum()
print ("Using the uncorrected p-values, we would conclude", N_uncorr_acc,␣
 ↪"peptides are significantly different between the treated & untreated␣
 ↪samples")
print ("Using the corrected p-values, we would conclude", N_corr_acc, "peptides␣
 ↪are significantly different between the treated & untreated samples")

# make a plot of the distribution of p-values before & after the FDR correction
plt.clf()
plt.title("Histogram")
plt.hist(data10["PeptidePValue1_orig"], color = "#004488", alpha = 0.7, label =␣
 ↪"Uncorrected", histtype = "step", bins = "fd", linewidth=2.5) # here, alpha !
 ↪= significance level
plt.hist(data10["PeptidePValue1_adjust"], color = "#BB5566", alpha = 0.7, label␣
 ↪= "Corrected", histtype = "step", bins = "fd", linewidth=2.5)
plt.xlabel("p-value")
plt.ylabel("Counts")
plt.legend(loc = "best")
plt.tight_layout()
plt.show()

# make a plot of the cumulative distribution function of p-values before &␣
 ↪after the FDR correction
plt.clf()
plt.title("Cumulative distribution function")
plt.hist(data10["PeptidePValue1_orig"], color = "#004488", alpha = 0.7, label =␣
 ↪"Uncorrected", histtype = "step", bins = "fd", cumulative = True, density =␣
 ↪True, linewidth=2.5) # here, alpha != significance level
plt.hist(data10["PeptidePValue1_adjust"], color = "#BB5566", alpha = 0.7, label␣
 ↪= "Corrected", histtype = "step", bins = "fd", cumulative = True, density =␣
 ↪True, linewidth=2.5)
plt.xlabel("p-value")
plt.ylabel("Cumulative probability")
plt.legend(loc = "best")
plt.tight_layout()
plt.show()
```

```
  Accession  PeptidePValue1  PeptidePValue1_orig
0    P0A6Y8        7.740828         1.816236e-08
1    P0A6Y8        3.473137         3.364052e-04
2    P0A6Y8       14.137842         7.280451e-15
3    P0A6Y8        2.097467         7.989747e-03
4    P0A6Y8        1.451695         3.534310e-02
5    P0A6Y8        3.134283         7.340360e-04
6    P0A6Y8        3.354855         4.417176e-04
7    P0A6Y8        3.038093         9.160249e-04
8    P0A6Y8       -0.000000         1.000000e+00
```

```
9    P0A6Y8        7.027929        9.377163e-08
```

```
Using the uncorrected p-values, we would conclude 18656 peptides are
significantly different between the treated & untreated samples
Using the corrected p-values, we would conclude 11821 peptides are significantly
different between the treated & untreated samples
```





1.1.5 Step 4 - Interpret the results

- Use the QR code below to access a quiz question to test your knowledge about FDR correction

Quiz Link