**NADH-dependent reaction analysis**

This folder contains the core scripts and representative outputs used for comprehensive analysis of NAD(H)-dependent reaction fluxes across FBA, pFBA, and flux sampling (FVA-sampling) approaches under NSD and HSD conditions. All analyses were performed in MATLAB and R.

All analyses were performed using MATLAB, and visualization or overlap analyses were completed in both MATLAB and R.

**Contents**

* MATLAB scripts:  
  Perform NADH production/consumption quantification, reaction-level Z-tests, and data preparation for visualization.
* R scripts:  
  Integrate FBA, pFBA, and sampling results to identify consistent NAD(H) reaction changes, perform Venn/UpSet, compartment, subsystem, and gene–subsystem network analyses, and generate final visualization plots.

**Notes**

Due to file size constraints, the sampling result files (HSD.mat, NSD.mat) are not included. Only representative .mat files and summary tables are provided for illustration.

For access to the full dataset or additional information, please contact: [sunjin\_moon@hms.harvard.edu](mailto:sunjin_moon@hms.harvard.edu)

**Summary of scripts**

1. **script\_01\_check\_nadh\_production\_consumption.m**
   1. Purpose:  
      Quantify and compare NADH production and consumption across reactions under NSD and HSD conditions.
   2. Input:
      1. NSD.mat, C\_sampling/HSD.mat (flux sampling results)
      2. Model structure containing reactions, metabolites, and stoichiometric matrix
   3. Output:
      1. NADH\_Production\_Consumption\_Analysis.xlsx — reaction, compartment, and overall NADH flux summaries
      2. NADH\_Production\_Consumption\_BarGraph.pdf/png
      3. Console summary of net NADH changes and compartment balance verification
2. **script\_02\_z\_test\_sampling\_nad.m**
   1. Purpose:  
      Perform reaction-level Z-tests on NAD(H)-dependent reactions to identify statistically significant flux changes between NSD and HSD muscle-GEM sampling results.
   2. Input:
      1. C\_sampling/NSD.mat, HSD.mat (sampling flux distributions)
      2. NADH\_Production\_Consumption\_Analysis.xlsx (reaction list of NAD(H)-dependent reactions)
   3. Output:
      1. Z\_test\_NAD\_reactions\_Bonferroni.xlsx — full Z-test results with p-values, adjusted p-values, and log2FC
      2. Z\_test\_NAD\_reactions\_sorted\_by\_Zscore.xlsx and Z\_test\_NAD\_reactions\_sorted\_by\_log2FC.xlsx
      3. Z\_test\_NAD\_reactions\_significant\_only.xlsx — significant reactions (p\_adj < 0.05)
      4. Z\_test\_NAD\_reactions\_for\_R\_visualization.xlsx — formatted dataset for R-based plotting
      5. Summary of significant NAD(H) flux changes between NSD and HSD
3. **script\_03\_nad\_analysis\_all.R**
   1. Purpose:  
      Perform comprehensive analysis of all NAD-associated reactions across FBA, pFBA, and flux sampling results to identify consistent increases or decreases under HSD versus NSD. The script integrates Venn, UpSet, compartment, and subsystem analyses, and generates gene–subsystem networks for commonly altered NAD reactions.
   2. Input:
      1. venn\_diagram\_data\_th0\_forNAD.xlsx (binary matrix of reaction changes across methods)
      2. Reaction-level annotations including subsystems, compartments, and associated genes
   3. Output:
      1. Overlap analyses:
         1. NAD\_All\_Venn\_Increased\_Reactions.png/.svg, NAD\_All\_Venn\_Decreased\_Reactions.png/.svg
         2. NAD\_All\_Venn\_Overlap\_Summary.csv
         3. NAD\_All\_Reactions\_Analysis\_Data.csv
      2. Compartment/subsystem summaries:
         1. NAD\_Compartment\_Analysis\_All.csv, NAD\_Compartment\_Changes\_Pie.png/.pdf/.svg
         2. Common\_NAD\_Subsystem\_Summary.csv, Common\_NAD\_Compartment\_Summary.csv
      3. Network analyses:
         1. NAD\_Network\_Increased.pdf/.svg, NAD\_Network\_Decreased.pdf/.svg
         2. NAD\_Gene\_Data\_Increased.csv, NAD\_Gene\_Data\_Decreased.csv
4. **script\_04\_z\_test\_NAD\_vis\_dot.R**
   1. Purpose:  
      Generate dot plot visualizations of NAD(H)-dependent reaction flux changes (HSD vs NSD) using Z-test statistics, highlighting significance, magnitude, and overlap across analytical methods.
   2. Input:
      1. Z\_test\_NAD\_reactions\_for\_R\_visualization.xlsx (Z-test results)
      2. NAD\_All\_Common\_Decreased\_Reactions.csv and NAD\_All\_Common\_Increased\_Reactions.csv (common reaction lists from all three methods)
   3. Output:
      1. dotplot\_NAD\_reactions\_all.pdf/png/svg — all NAD(H) reactions with color-coded significance and size-scaled flux differences
      2. dotplot\_NAD\_reactions\_significant\_log2FC\_gt\_0.5.pdf/png/svg — filtered plot showing significant reactions (|log2FC| > 0.5, p\_adj < 0.05)
      3. NAD\_reactions\_dotplot\_ready.xlsx — processed data for plotting
      4. NAD\_reactions\_detailed\_log2FC\_gt\_0.5.xlsx — summary tables of all and filtered reactions with statistics and annotations