**Sensitivity** **analysis**

This folder contains the core scripts and example outputs used for the **s**ensitivity analysis described in the *Methods (Sensitivity analysis)* section of the main text.

The sensitivity analysis evaluates how perturbations in individual reaction fluxes affect the overall glycolytic output flux (e.g., pyruvate consumption).

All analyses were performed using MATLAB, and results were visualized in both MATLAB and R.

**Contents:**

* MATLAB scripts: Core functions and scripts for performing the sensitivity analysis
* R scripts: Visualization of sensitivity coefficients across perturbation levels
* Example data files: Representative sampling outputs (e.g., HSD\_baseline\_MAR04375.mat, HSD\_perturbed\_MAR04375\_95.mat)
* Summary sheet: Comparative summary of sensitivity coefficients from different perturbation magnitudes

**Notes**

Due to file size constraints, only example .mat files are provided.

To perform complete analysis, a full set of sampling output files (e.g., HSD\_baseline\_MAR04379.mat, etc.) is required.

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\_0\_perturb\_rxns.m**
   1. Purpose:
      1. Perform FVA-based sampling and create baseline/perturbed flux datasets for target glycolytic reactions.
   2. Input:
      1. model\_out\_cbra\_u.mat, enzyme list, perturbation factors, sampling parameters.
   3. Output:
      1. Individual .mat files for baseline and perturbed conditions (HSD\_baseline\_\*.mat, HSD\_perturbed\_\*.mat), plus summary files (analysis\_summary\_sampling.mat, condition\_list\_sampling.xlsx).
2. **script\_1\_Check\_pyruvate\_Con\_rxns.m**
   1. Purpose:
      1. Check pyruvate-consuming reactions and quantify flux changes upon perturbation.
   2. Input:
      1. analysis\_summary\_sampling.mat, condition .mat files from script 0, model\_out\_cbra\_u.mat.
   3. Output:
      1. pyruvate\_c\_consumer\_flux.xlsx (flux comparison summary) and pyruvate\_c\_reactions\_full\_data\_v3.mat (detailed data).
3. **script\_2\_sensitivity\_analysis.m**
   1. Purpose:
      1. Compute sensitivity coefficients
   2. Input:
      1. pyruvate\_c\_reactions\_full\_data\_v3.mat (from script 1).
   3. Output:
      1. pyruvate\_consumption\_sensitivity\_results\_v3.xlsx with four sheets (Detailed\_Summary, Raw\_Sample\_Data, Individual\_Reactions, Summary) containing sensitivities, system responses, and flux statistics.
4. **script\_3\_sensitivity\_visualize.R**
   1. Purpose:
      1. Visualize sensitivity results
   2. Input:
      1. pyruvate\_consumption\_sensitivity\_results\_v3.xlsx and related annotation .tsv files. From script 2.
   3. Output:
      1. plots (.png, .pdf, .svg) and summary statistics showing top sensitive enzymes, flux changes, and subsystem effects.
5. **script\_04\_compare\_all.m**
   1. **Purpose:**
      1. Compare sensitivity results across multiple perturbation levels (50–95%).
   2. **Input:**
      1. pyruvate\_consumption\_sensitivity\_results.xlsx from each perturbation folder (p50, p70, p80, p90, p95).
   3. **Output:**
      1. Aggregated sensitivity tables and multi-panel figures (1\_perturbation\_verification\_pyruvate, 2b/2c\_sensitivity\_by\_perturbation\_pyruvate, 33\_combined\_sensitivity\_plot)