**Boundary Effects**

This folder contains the core scripts and example outputs used for boundary effects evaluation described in the *supplementary Note 3c.*

The boundary effect analysis tests how altering the global flux boundary limits (±1,000, ±10,000, ±50,000) affects model-predicted flux ranges and sampling distributions.

All analyses were performed in MATLAB, and results were visualized directly within MATLAB.

**Contents**

* MATLAB scripts**:** Core functions for evaluating and visualizing boundary effects.
* Example data files**:** Representative sampling outputs (e.g., HSD.mat, NSD.mat within /03\_FSA\_bounds\_\* folders).
* Summary sheet**:** Summary sheet of flux saturation under different boundary limits.

**Notes**

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

To reproduce the full analysis, complete sampling outputs for each boundary condition (e.g., /03\_FSA\_bounds\_1000/, /03\_FSA\_bounds\_10000/, /03\_FSA\_bounds\_50000/) are 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\_01\_Evaluate\_Boundary\_Effects.m**
   1. Purpose:
      1. Evaluate the impact of varying boundary flux limits on model-predicted reaction ranges and sampled flux distributions.
   2. Input:
      1. model\_out\_cbra\_u.mat (contains NSD and HSD muscle-GEMs).
      2. Boundary conditions: ±1,000, ±10,000, ±50,000.
   3. Output:
      1. FVA results (out\_all.mat per boundary).
      2. FVA-bounded models (out\_all\_fvaBounded.mat).
      3. Flux sampling results (samples.mat for NSD and HSD).
2. **Script\_02\_plot\_boundaries.m**
   1. Purpose:
      1. Quantify and visualize how many reactions reach or approach boundary limits across NSD and HSD conditions.
   2. Input:
      1. Quantify and visualize how many reactions reach or approach boundary limits across NSD and HSD conditions.
   3. Output:
      1. Figure (boundary\_absolute, boundary\_relative)
      2. Summary table: Boundary\_Summary.csv