

# Can you trust your model?

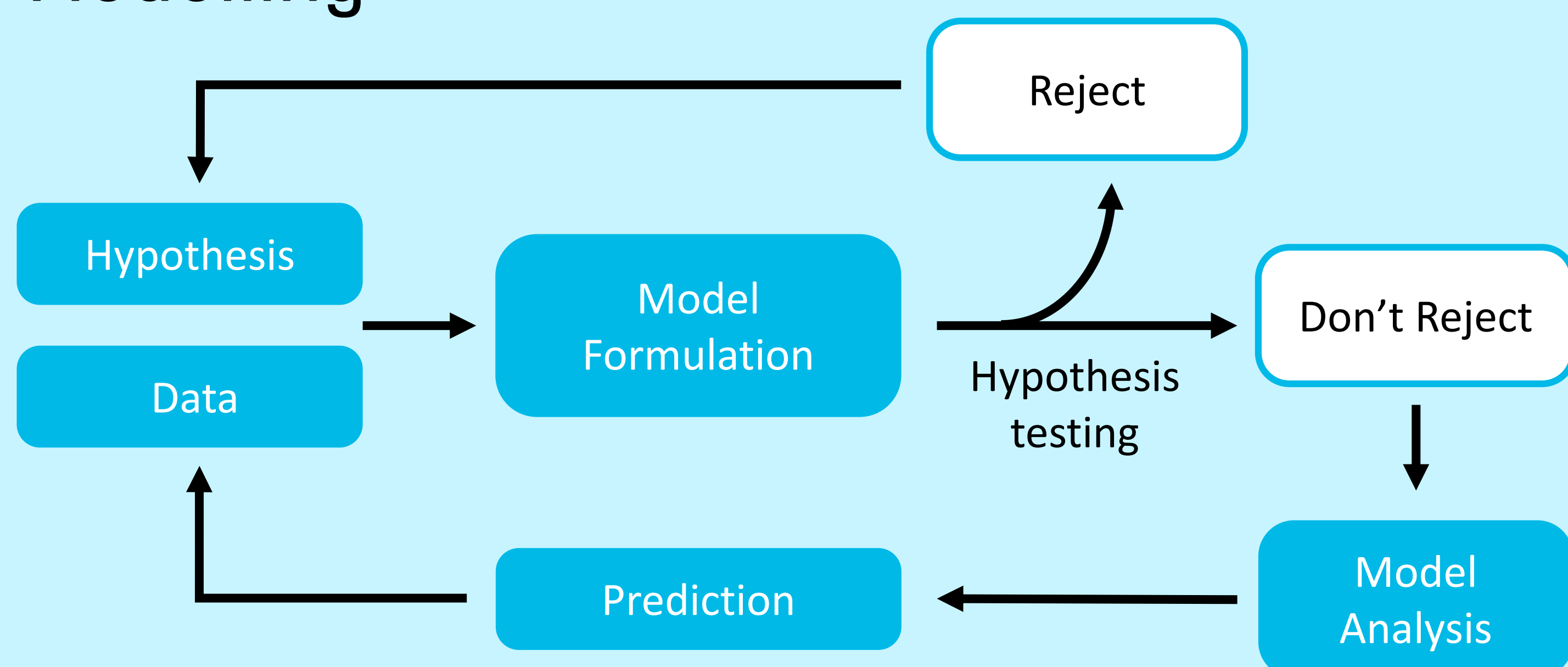
## A showcase study of validation in $^{13}\text{C}$ metabolic flux analysis

Nicolas Sundqvist, William Lövfors, Gunnar Cedersund

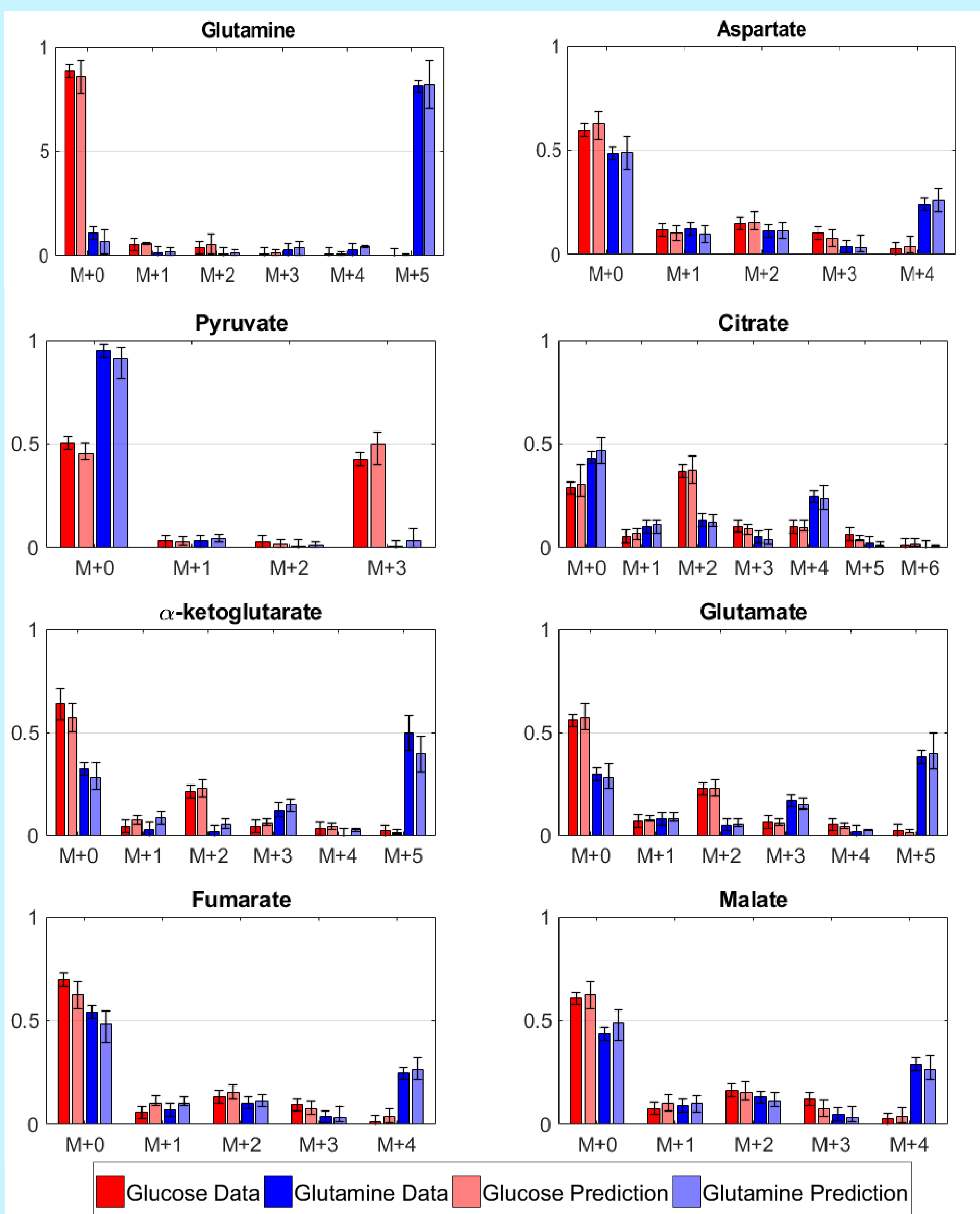
### Introduction

One of the key parameters in understanding complex metabolic networks are the metabolic fluxes, which are the rates of conversion of metabolic intermediates. Currently, one of the main approaches for determining these fluxes is metabolic flux analysis (MFA), in which isotope-labelled compounds, commonly  $^{13}\text{C}$ , are introduced into the system and measured. Mathematical models are then used to calculate a prediction of the systems flux configuration, based on the distribution of these labels. However, the current paradigm of MFA lack established methods for validating that a model can accurately predict quantities for which there are no experimental data. In this study a new approach by which such validation can be done was developed and tested for MFA models of the human metabolism.

### Modelling

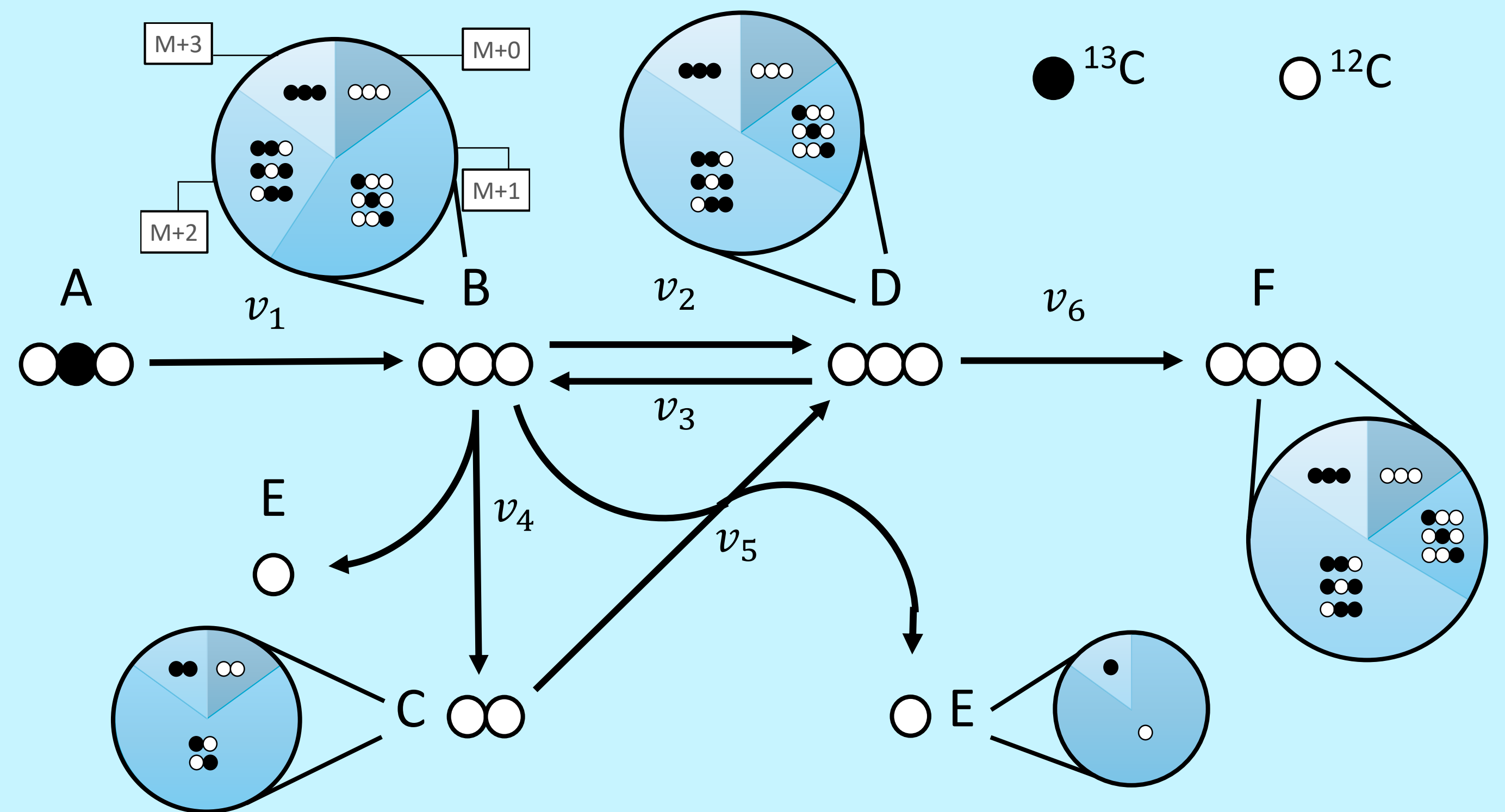


### Results



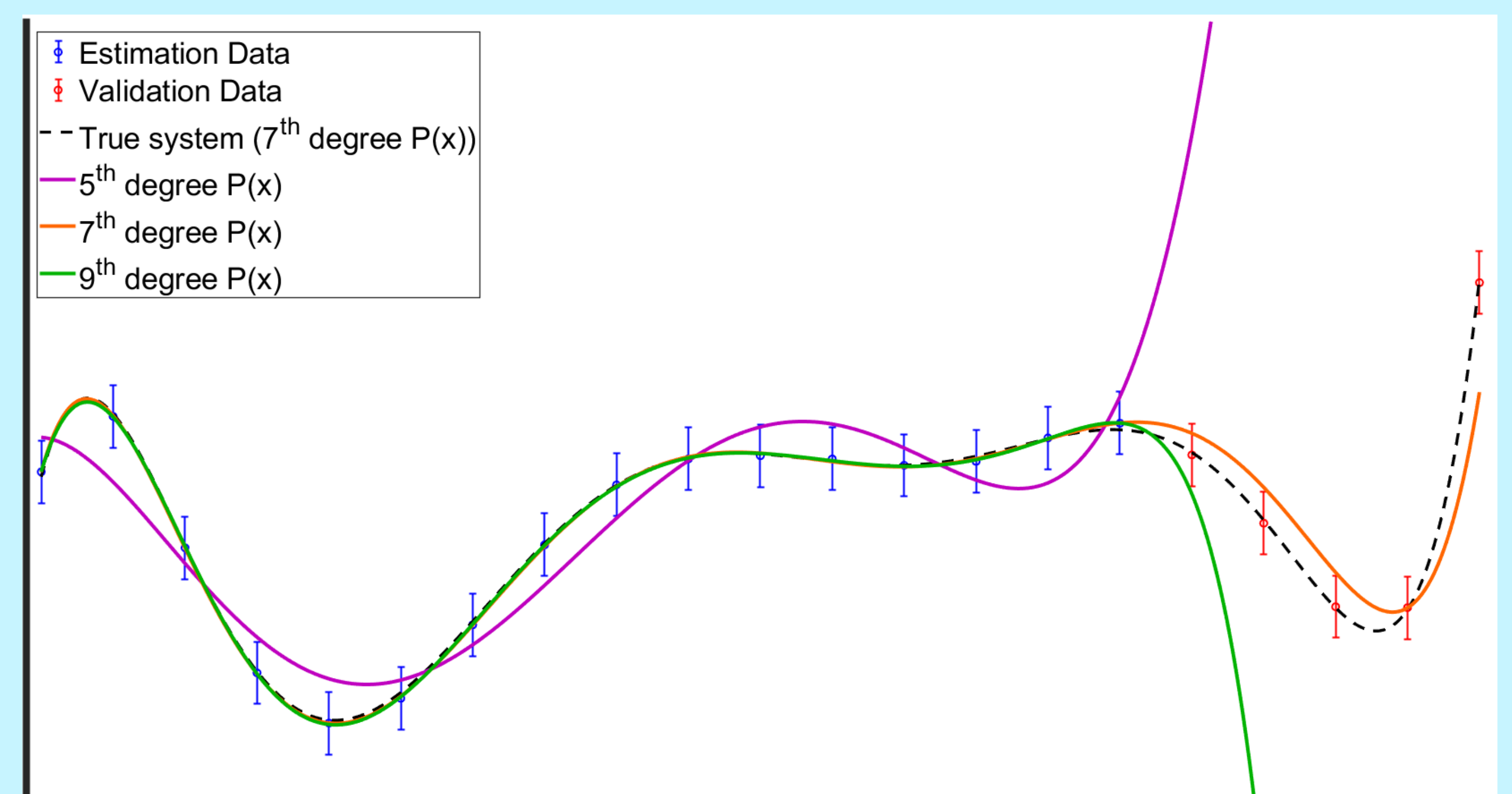
The model predictions for six metabolites in the central human metabolism. The prediction of each respective metabolite were made with the model trained on the remaining eight metabolites. Two parallel isotope tracers were used, Glucose (red) and Glutamine (blue).

### Metabolic Flux Analysis



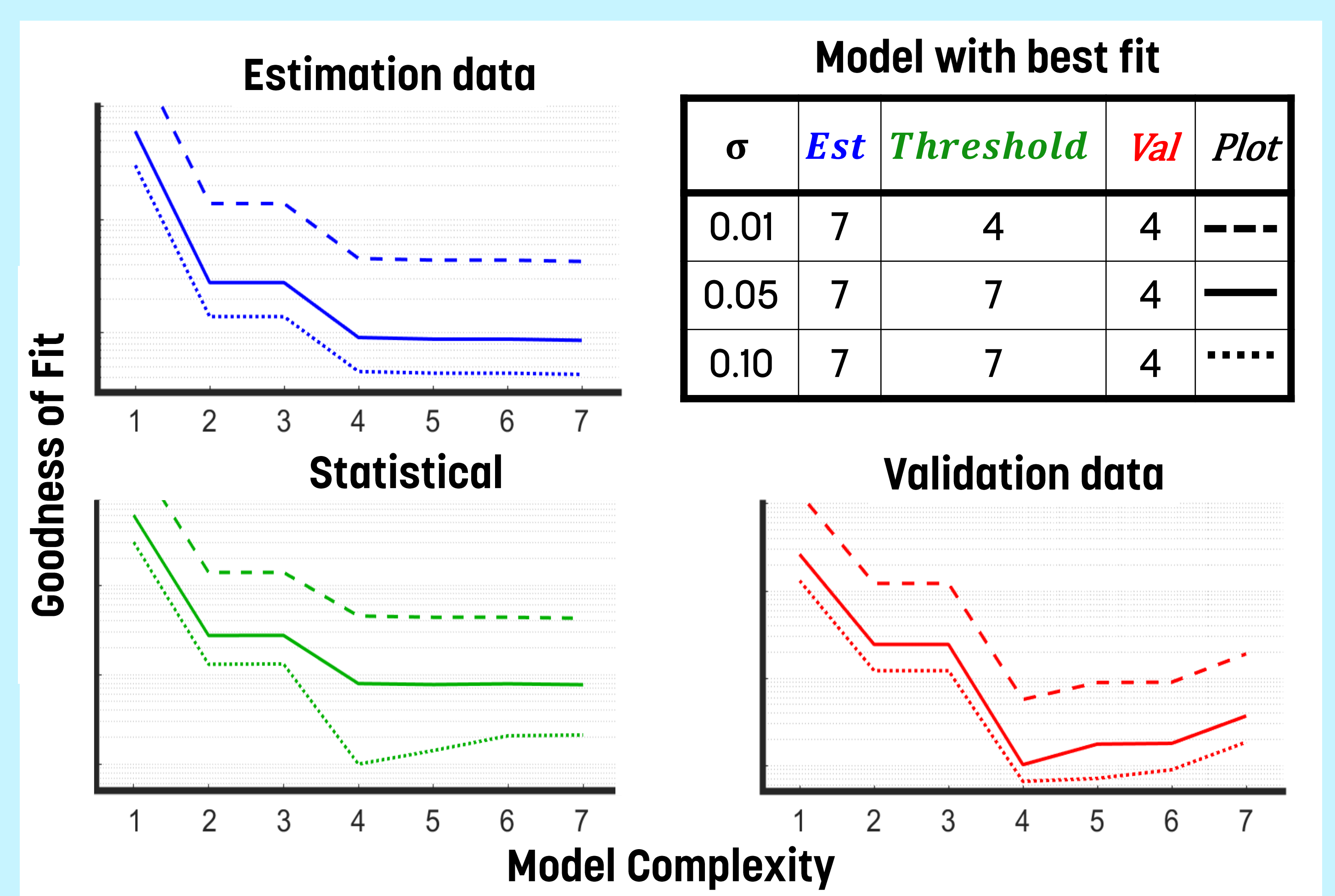
A schematic illustration of a metabolic network consisting of metabolites A-F. A  $^{13}\text{C}$  isotopic label is introduced in A and will distribute itself across metabolites B-F. This Distribution is fully and uniquely determined by the administered label and the metabolic fluxes  $V_1-V_6$ .

### Model Validation



|  | 5 <sup>th</sup> Degree $P(x)$ | 7 <sup>th</sup> Degree $P(x)$ | 9 <sup>th</sup> Degree $P(x)$ |
|--|-------------------------------|-------------------------------|-------------------------------|
| Acceptable fit with overestimated $\sigma$ | YES                           | YES                           | YES                           |
| Acceptable fit with true $\sigma$          | NO                            | YES                           | YES                           |
| Describes validation data                  | NO                            | YES                           | NO                            |

A theoretical example that emphasizes the importance of model validation. The data is generated from a normal random distribution with a true  $\sigma = 0.1$ , but the illustrated error bars correspond to an overestimated  $\sigma = 0.4$ .



A summary of different approaches for finding the model that best describes the system. Seven  $^{13}\text{C}$  MFA models of different complexity were evaluated on their ability to describe in silico generated data, generated from model 4. The table, in the top right panel, shows which model is considered the best description for the different methods and different values of  $\sigma$ .