



**Figure 1. Principles and approaches of flux balance analysis (FBA).** The upper half of the figure illustrates the mathematical encoding of the structure of a metabolic network in the form of a stoichiometry matrix (S) and the activity of the network as a vector of fluxes (v). The lower half of the figure illustrates constraints-based optimisation approaches to predict flux distributions in the network by solving the linear set of equations  $S \cdot v = 0$ . The unconstrained n-dimensional solution space (shown in the left panel) can be reduced by adding upper and lower bounds for each flux and a set of solutions that lie within this constrained space can be found that also satisfy an optimisation objective (shown in the middle panel). To reduce this multiple set of feasible flux distributions, additional constraints derived from transcriptomic, proteomic and metabolomics data can be applied as well as secondary objective functions (shown in the right panel). This allows a smaller set of biologically-relevant flux distributions to be obtained.