1. Objectives
   • Elementary flux modes (EFMs) describe the topology of metabolic networks.
   • Fig. 1 shows that the robustness of a network is not fully described by the number of EFMs in the original network.
   • Therefore, we introduced robustness measures based on the relative number of EFMs remaining after single, double and multiple knockouts of enzymes.

2. The basic measures for single knockouts
   \[ R_1 = \frac{\sum_{i=1}^{r} z^{(i)}}{r \cdot z} \]
   \[ z^{(i)}: \text{Number of elementary flux modes remaining after knockout} \]
   \[ R_2 = \min \{ R_1^{(1)}, R_1^{(2)}, \ldots, R_1^{(n)} \} \]
   \[ R_3 = \frac{\sum_{k=1}^{n} R_3^{(k)}}{n} \]
   \[ R_3^{(k)}: \text{Robustness concerning essential product } P_k \]

3. Multiple knockouts
   • We generalized the robustness measures to multiple knockouts.
   • The calculation then changes as follows:
   \[ R_1 = \frac{\sum_{l=1}^{n} c^{(l)}}{c \cdot z} \]
   \[ c^{(l)}: \text{Number of elementary flux modes remaining after knockout} \]

4. Application to metabolisms of human hepatocytes, erythrocytes and E. coli
   • We applied our robustness measures to the amino acid synthesis network of human hepatocytes and E. coli, as well as the central metabolism of human erythrocytes [2].
   • The amino acid synthesis models are based on data from KEGG, EcoCyc and HumanCyc. We also did literature search (e.g. [3]) to consider compartmentation in the hepatocyte. The erythrocyte model was taken from [4].
   • We further compiled two subsystems of the E. coli amino acid synthesis, comprising the amino acids that are non-essential for humans and the essential amino acids, respectively.
   • The elementary flux modes are calculated by METATOOL 5.0 [5].

5. Results
   • Table 1: The calculated robustnesses for the metabolic networks described above

<table>
<thead>
<tr>
<th>Metabolic network</th>
<th>R_1(1)</th>
<th>R_1(2)</th>
<th>R_1(3)</th>
<th>R_1(4)</th>
<th>R_1(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (non-essential amino acids)</td>
<td>0.776</td>
<td>0.602</td>
<td>0.468</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. coli (essential amino acids)</td>
<td>0.654</td>
<td>0.430</td>
<td>0.285</td>
<td>0.191</td>
<td>0.129</td>
</tr>
<tr>
<td>Human erythrocyte</td>
<td>0.659</td>
<td>0.443</td>
<td>0.305</td>
<td>0.258</td>
<td>0.172</td>
</tr>
<tr>
<td>Human hepatocyte</td>
<td>0.463</td>
<td>0.223</td>
<td>0.112</td>
<td>0.058</td>
<td>0.031</td>
</tr>
</tbody>
</table>

6. Conclusions
   • Our extended robustness framework takes into account single, double and multiple knockouts.
   • The robustness for the human erythrocyte model is clearly lower than for the E. coli network and the human hepatocyte.
   • Human hepatocytes and E. coli are very adaptable to various conditions (more robust) while human erythrocyte lives under homeostatic conditions.
   • The robustness of the human hepatocyte model is even higher than that of the E. coli subsystem comprising those amino acids that are non-essential for humans. This is remarkable because the hepatocyte is compartmented and hence comprises carriers that are bottlenecks in the system.

7. References