1. Model description and experimental setup.

The mathematical model presented in [1] describes the concentration dynamics of molecular compounds \( \mathbf{x} \) involved in the interplay of the hormone erythropoietin (Epo) and its corresponding membrane receptor (EpoR). Epo is an important factor in the differentiation of blood cells. The dynamics of the molecular compounds are formulated as ordinary differential equations (ODE) \( \frac{d}{dt}\mathbf{x}(t, \theta) = \mathbf{f}(\mathbf{x}(t, \theta), \theta) \) and a mapping of the compounds to experimentally accessible quantities by a function \( \mathbf{y}(t, \theta) = \mathbf{g}(\mathbf{x}(t, \theta), \theta) \) is used. The model comprises six dynamical variables and ten unknown parameters including one nuisance parameter. The dynamics are given by the ODE system

\[
\begin{align*}
\frac{d}{dt}x_1 &= -k_{on} \cdot x_1 \cdot x_2 + k_{on} \cdot k_D \cdot x_3 + k_{ex} \cdot x_4 \\
\frac{d}{dt}x_2 &= -k_{on} \cdot x_1 \cdot x_2 + k_{on} \cdot k_D \cdot x_3 + k_t \cdot B_{max} - k_t \cdot x_2 + k_{ex} \cdot x_4 \\
\frac{d}{dt}x_3 &= +k_{on} \cdot x_1 \cdot x_2 - k_{on} \cdot k_D \cdot x_3 - k_e \cdot x_3 \\
\frac{d}{dt}x_4 &= +k_e \cdot x_3 - k_{ex} \cdot x_4 - k_{di} \cdot x_4 - k_{de} \cdot x_4 \\
\frac{d}{dt}x_5 &= +k_{di} \cdot x_4 \\
\frac{d}{dt}x_6 &= +k_{de} \cdot x_4 
\end{align*}
\]

that describes the time evolution of the concentration of molecular compounds. The modelled compounds are: Epo in the extracellular medium (\( x_1, \text{Epo} \)), Epo receptor on the cell membrane (\( x_2, \text{EpoR} \)), occupied receptor complex on the cell membrane (\( x_3, \text{Epo_EpoR} \)), internalized receptor complex (\( x_4, \text{Epo_EpoR_i} \)), degraded Epo inside the cell (\( x_5, \text{dEpo_i} \)) and degraded Epo in the extracellular medium (\( x_6, \text{dEpo_e} \)). It is assumed that two compounds have non zero initial value defined as unknown parameters: \( x_1(t = 0) = Epo_0 \) and \( x_2(t = 0) = B_{max} \). The molecular
interaction that are considered are: ligand-independent EpoR endocytosis ($k_t$), association of Epo and EpoR ($k_{on}$), dissociation of Epo and EpoR ($k_{off} = k_{on} \cdot k_D$) with dissociation constant for Epo-EpoR ($k_D$), ligand-induced EpoR endocytosis ($k_e$), recycling of Epo and EpoR ($k_{ex}$), degradation of ligand-EpoR complex that remaining intracellular ($k_{di}$) and is secreted extracellular ($k_{de}$). The experimentally accessible quantities are given by

\[
\begin{align*}
y_1 &= \text{scale} \cdot (x_1 + x_6) \\
y_2 &= \text{scale} \cdot x_3 \\
y_3 &= \text{scale} \cdot (x_4 + x_5)
\end{align*}
\]

and describe measurements of radioactively labeled Epo in different compartments: in the extracellular medium ($y_1$, Epo\textsubscript{ext}), on the cell membrane ($y_2$, Epo\textsubscript{mem}) and inside the cell ($y_3$, Epo\textsubscript{int}), see table S1 for data. The mathematical model and the experimental data is available online from the original publication [1] in standard formats.

For the analysis two experimental setups are distinguished. The initial experimental setup includes only measurements of $y_1$, $y_2$, and results in non-identifiability, see posterior profiles in figure S1. Based on the results of the profiling approach additional experiments were suggested, see in [2] for details. The target of the experimental design was to resolve non-identifiabilities. Additional measurements of $y_3$ and independent direct measurements of $B_{max}$, $k_D$ and Epo\textsubscript{0} were included in the estimation procedure. The posterior profiles computed for the extended setup confirm that the non-identifiabilities were resolved, see in figure S7. The MAP parameter values for the extended setup are given in table S2.

For MCMC sampling both initial and extended setup have been analysed by the SIM and MMALA algorithms. The SIM algorithm uses a scaled identity matrix for generating proposals. To improve efficiency the MMALA algorithm that takes into account the local geometry of the posterior PDF [3]. (i) For the initial setup the results of the SIM algorithm are displayed in figures S1–S3. They show that the SIM algorithm produced correlated results and did not converge yet. (ii) For the initial setup the results of the MMALA algorithm are displayed in figure S4–S6. The MMALA algorithm converged. (ii) For the extended setup the results of the SIM algorithm are displayed in figures S7–S9. They show that the SIM algorithm still produced correlated results and did not converge yet. (iv) For the extended setup the results of the MMALA algorithm are displayed in figure S10–S12. The MMALA algorithm converged.

2. Implementation of the numerical methods.

The ODE system was solved by the CVODES algorithm [4]. For numerical optimisation the trust-region method LSQNONLIN from MATLAB was used yielding the MAP estimates [5]. For efficiency it takes into account local gradient and curvature information. For the calculation of the posterior profiles as shown in figures S1, S4, S7 and S10 an algorithm described in [6] can be used.

Both MMALA and LSQNONLIN rely on the accuracy of first order sensitivities $d\vec{y}/d\theta$. For ODE systems finite difference should not be used to calculate sensitivities [7]. Therefore the sensitivity equations [8] are solved simultaneously by the ODE
solver CVODES. For the implementation of the MMALA algorithm, the simplified version was used that is computationally more efficient [3].

For the MCMC sampling, it is important to ensure that the Markov process starts in a high density region of $P(\theta|y)$ either by performing a burn-in or by searching for the MAP point, the latter option was used here. To monitor convergence of the Markov chain the generated samples can be checked for independence, e.g. by computing their auto-correlation function (ACF), see figures S2, S5, S8 and S11. If the samples are correlated thinning can be used to increase independence. Correlation in the samples can be visualised directly by the Markov chains, see figures S3, S6, S9 and S12. Each sampling run was aimed at obtaining $10^4$ independent samples after applying thinning. For the SIM algorithm the 1/100 thinning still did not result in independent samples. The SIM algorithm turned out to be impractical both for the initial and extended setup. In contrast, the MMALA algorithm was much more efficient. A thinning of 1/100 and 1/10 for the initial and respectively the extended setup was sufficient to obtain $10^4$ independent samples.

For the propagation of the parameter uncertainties contained in the posterior PDF to the prediction of the dynamics of unobserved molecular compounds, all trajectories corresponding to the samples shown in figure S12 or S13 were evaluated. For each time point of the resulting set of curves a density estimate [9] was computed using the MATLAB function KSDENSITY.

For the calculations MATLAB on a 2.1 GHz quad-core processor was used. The calculation of the profiles took $\approx 5$ minutes for the initial setup and $\approx 1$ minute of computation time for the extended setup. The generation of $10^6$ MCMC samples using the MMALA algorithm took $\approx 18.5$ hours for the initial setup and $\approx 20$ minutes of computation time for the extended setup.

References


Table S1. Experimental data: The experimentally accessible quantities are time-course measurements of radioactively labeled Epo collected in triplicates and units of counts per minute (cpm) over time in minutes (min) in different compartments: in the extracellular medium ($y_1$, Epo$_{ext}$), on the cell membrane ($y_2$, Epo$_{mem}$) and inside the cell ($y_3$, Epo$_{int}$).

<table>
<thead>
<tr>
<th>time / min</th>
<th>Epo$_{ext}$ / cpm</th>
<th>Epo$_{mem}$ / cpm</th>
<th>Epo$_{int}$ / cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>321194 ±19461</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>277333 ±4984</td>
<td>44698 ±1731</td>
<td>9125 ±274</td>
</tr>
<tr>
<td>20</td>
<td>249910 ±3957</td>
<td>44904 ±1123</td>
<td>50698 ±346</td>
</tr>
<tr>
<td>60</td>
<td>231849 ±12291</td>
<td>27209 ±2161</td>
<td>83887 ±2311</td>
</tr>
<tr>
<td>120</td>
<td>222047 ±2145</td>
<td>23076 ±1624</td>
<td>102060 ±4124</td>
</tr>
<tr>
<td>180</td>
<td>235203 ±7075</td>
<td>13680 ±1057</td>
<td>97952 ±1493</td>
</tr>
<tr>
<td>240</td>
<td>251188 ±6437</td>
<td>10024 ±1523</td>
<td>82699 ±4096</td>
</tr>
<tr>
<td>300</td>
<td>260945 ±5290</td>
<td>5447 ±436</td>
<td>65967 ±3741</td>
</tr>
</tbody>
</table>

Table S2. MAP estimates of the model parameters: The estimated values $\hat{\theta}$ were obtained by maximising the unnormalised posterior PDF by using the optimisation algorithm LSQNONLIN [5] from MATLAB for the extended experimental setup.

<table>
<thead>
<tr>
<th>parameter</th>
<th>log$_{10}(\hat{\theta})$</th>
<th>unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_{max}$</td>
<td>+2.7126</td>
<td>pM</td>
</tr>
<tr>
<td>$Epo_0$</td>
<td>+3.3075</td>
<td>pM</td>
</tr>
<tr>
<td>$k_D$</td>
<td>+2.2148</td>
<td>pM</td>
</tr>
<tr>
<td>$k_{de}$</td>
<td>-1.7850</td>
<td>1/min</td>
</tr>
<tr>
<td>$k_{dt}$</td>
<td>-2.4977</td>
<td>1/min</td>
</tr>
<tr>
<td>$k_e$</td>
<td>-1.1259</td>
<td>1/min</td>
</tr>
<tr>
<td>$k_{ex}$</td>
<td>-2.0027</td>
<td>1/min</td>
</tr>
<tr>
<td>$k_{on}$</td>
<td>-3.9790</td>
<td>1/(pM-min)</td>
</tr>
<tr>
<td>$k_t$</td>
<td>-1.4823</td>
<td>1/min</td>
</tr>
<tr>
<td>scale</td>
<td>+2.2311</td>
<td>cpm/pM</td>
</tr>
</tbody>
</table>


Figure S1. Results of the posterior profiling and MCMC sampling using the SIM algorithm for the initial setup: In contrast to the results obtained by the simplified MMALA algorithm shown in figure S4, the SIM algorithm converges too slowly for parameters affected by non-identifiability. The sampling results are of bad quality. $10^6$ MCMC samples were generated and a thinning of 1/100 was used. The auto-correlation function of the samples still shows high correlation, see figure S2. This indicates that the process did not converge yet. The Markov chains for each parameter are displayed in figure S3.
Figure S2. ACF of the Markov chain using the SIM algorithm for the initial setup: The figure displays the auto-correlation function (ACF) of the generated MCMC samples for each parameter along the horizontal axis. A thinning of 1/100 was used to increase independence of the samples. A rapidly decaying ACF indicates independence of the samples and convergence of the Markov chain. The sample are still highly correlated.
Figure S3. Markov chains for the initial setup using the SIM algorithm: The figure displays the generated samples for each parameter after thinning along the horizontal axis. For parameters affected by non-identifiability, i.e. parameters in the upper and lower row, the Markov process did not converge after $10^4$ samples were generated. In contrast to the results obtained by the simplified MMALA algorithm the SIM algorithm converges too slowly.
Figure S4. Results of the posterior profiling and MCMC sampling using the MMALA algorithm for the initial setup: For most parameters the results of profiling and MCMC sampling are similar though not identical. For parameter $k_t$ substantial difference are observed. $10^6$ MCMC samples were generated. A thinning of 1/100 was. The independence of samples was checked by their auto-correlation function, see figure S5. This indicates that the process did converge. The Markov chains for each parameter are displayed in figure S6.
Figure S5. ACF of the Markov chain using the MMALA algorithm for the initial setup: The figure displays the auto-correlation function (ACF) of the generated MCMC samples for each parameter along the horizontal axis. A thinning of 1/100 was used to increase independence of the samples. A rapidly decaying ACF indicates independence of the samples and convergence of the Markov chain. For parameter \( k_{on} \), some dependency remained. However, the ACF drops to a value close to zero within a lag of 50 which is much smaller than the number of MCMC samples generated.
Figure S6. Markov chains using the MMALA algorithm for the initial setup: The figure displays the generated samples for each parameter after thinning along the horizontal axis.
Figure S7. Results of the posterior profiling and MCMC sampling using the SIM algorithm for the extended setup: In contrast to the results obtained by the simplified MMALA algorithm shown in figure S10, the SIM algorithm converges too slowly. Nevertheless, the agreement of profiles and MCMC samples is already acceptable. $10^6$ MCMC samples were generated and a thinning of 1/100 was used. The auto-correlation function of the samples still shows high correlation, see figure S8. The Markov chains for each parameter are displayed in figure S9 and indicate that the process did not converge yet. The dashed red lines indicates the threshold $\Delta_{\alpha}$ that can be used to assess likelihood based confidence intervals.
Figure S8. ACF of the Markov chain using the SIM algorithm for the extended setup: The figure displays the auto-correlation function (ACF) of the generated MCMC samples for each parameter along the horizontal axis. A thinning of 1/100 was used to increase independence of the samples. A rapidly decaying ACF indicates independence of the samples and convergence of the Markov chain. However the sample are still highly correlated.
Figure S9. Markov chains for the extended setup using the SIM algorithm: The figure displays the generated samples for each parameter after thinning along the horizontal axis. For some parameters the Markov process did not converge after $10^4$ samples were generated. In contrast to the results obtained by the simplified MMALA algorithm the SIM algorithm converges too slowly.
Figure S10. Results of the posterior profiling and MCMC sampling using the MMALA algorithm for the extended setup: the results of profiling and MCMC sampling are in good agreement. $10^5$ MCMC samples were generated. A thinning of 1/10 was used. The independence of samples was checked by their auto-correlation function, see figure S11. This indicates that the process did converge. The Markov chains for each parameter are displayed in figure S12. The dashed red lines indicates the threshold $\Delta_\alpha$ that can be used to assess likelihood based confidence intervals.
Figure S11. ACF of the Markov chain using the MMALA algorithm for the extended setup: The figure displays the auto-correlation function (ACF) of the generated MCMC samples for each parameter along the horizontal axis. A thinning of 1/10 was used to increase independence of the samples. A rapidly decaying ACF indicates independence of the samples and convergence of the Markov chain.
Figure S12. Markov chains using the MMALA algorithm for the extended setup. The figure displays the generated samples for each parameter after thinning along the horizontal axis.
Figure S13. Dependency structure of posterior samples: The results of the MCMC sampling allow for considering the full high dimensional posterior distribution. For example, non-linear relationship between parameter $k_{di}$ and $k_{de}$ are taken into account.