Chapter 5

Relaxation behavior of rates in the phosphotransferase system

To understand the functioning of living cells, simplified views of the underlying biochemical networks are often of assistance. In this chapter we develop an approach that makes use of inherent timescale separation based on the zero-derivative principle, to construct simplified pictures of the behavior of the reaction rates in the phosphotransferase system during the slow, partially relaxed phase of its dynamics. We show that the rates in this network are partitioned into collectives; within each of these collectives, all rates assume the same magnitude during the slow phase, while rates belonging to different collectives assume different magnitudes. This relaxation behavior of the rates was observed upon a large variety of perturbations of the parameter set. These results suggest that this behavior is likely to be encountered for various environmental conditions as well as in other signal transduction pathways possessing structures similar to that of the phosphotransferase system.
5.1 Introduction

For systems biology, understanding of complex networks is essential. Understanding often requires simplification, but simplification without removing the essence [27]. Many simplification approaches that exist at the moment however tend to throw away information about the biological functionality that emerges in the nonlinear biochemical interactions in the cell. Here we report on the discovery that a new method based on the zero-derivative principle (ZDP) is able to provide simplified views of biochemical systems, while retaining essential information about their nonlinear behavior.

In the previous chapters we treated reduction of biochemical models with the primary aims of reducing the simulation times and of developing simple yet accurate kinetic expressions for reaction rates in terms of the concentrations of the reactants and the enzymes. In this chapter we shall focus on the interpretation and analysis of the simplified models and demonstrate how the simplified views of systems may provide additional insight in their biochemical behavior. In particular, we shall focus on the kinetic model of the phosphotransferase system (PTS) developed in [99] (and revamped in Appendix 3.A of this thesis) and on the simplified descriptions of this system based on the zero-derivative principle (ZDP) which were derived numerically in Chapter 3.

As described in Appendix 3.A, the PTS is a mixed signal transduction, metabolic, and transport pathway involved in transporting various sugars into enteric bacteria, in phosphorylating those sugars, and in signalling to the transport and gene-expression machinery that the sugar is available [26, 96]. The PTS model in [99] exhibits non-linear dynamics and it contains 13 state variables that represent the concentrations of the molecular components in the pathway. Consequently, its dynamics could be enormously complex. To elucidate this, we make the thought experiment that each of these state variables could assume only 10 different values each; then, the number of states that the full system could assume is \(10^{13}\), i.e., its dynamics would be tremendously complex. In reality, each component can take any of a continuum of values, i.e. the state can be any point in a 13-dimensional state space, in which the axes represent the concentrations of the 13 components. On the other hand, the interactions necessary for the biological function of the PTS, though possibly complex, might not need to be that complex: the PTS should transport, phosphorylate, and signal. The question we address in this chapter is whether the PTS exhibits maximal possible complexity, i.e., makes use of the entire 13-dimensional state space, or whether its behavior might be much less complex than that.

The approach we take here is based on simplified descriptions of PTS dynamics through the ZDP. In Chapter 3 we introduced the reduction of biochemical systems by implementing the ZDP, a method which may be seen as a more accurate extension of the quasi-steady-state approximation (QSSA). In the same chapter, we also employed the ZDP to calculate approximations to curves or manifolds towards which the trajectories of the model are attracted and on which the dynamics is slow, i.e., slow invariant manifolds (SIMs). We used such to reduce the computation times required for integration over the PTS.

A second potential asset of these approximations of the SIM is that they eluci-
date the essence of the dynamics of the PTS. In this chapter, we shall examine this
potential. We shall analyze the dynamics of the reaction rates in the PTS during the
partially relaxed phase, \textit{i.e.}, during the phase following the initial transient phase, by
evaluating the rate expressions on the numerical approximations of the SIM. We find
that along the SIM, the reactions have adapted their rates to each other.

In the next section we recapitulate the aspects of the calculation of the ZDP
manifolds for the PTS that are essential for the presentation here. In the subsequent
section, we describe the relaxation behavior of the rates in the PTS. Thereafter, we
describe the behavior of the rates for a variety of parameter sets. Finally, we discuss
our results.

5.2 One-dimensional ZDP manifolds for the PTS model with
13 state variables

In Chapter 3 we determined approximations to a SIM for a PTS model with 9 state
variables; this model was obtained by eliminating the 4 state variables representing
unphosphorylated proteins from the model in [99] with 13 state variables, using the 4
conservation relations, as described in Appendix 3.A. The reduction by these conser-
vation relations demonstrates that there is a first constraint on the complexity of the
behavior of the PTS: for as long as gene expression variation is absent, \textit{i.e.}, the total
protein concentrations are constant (as was assumed also in the original model), the
PTS does not move in a 13-dimensional space but in a 9-dimensional one.

In the same chapter, we also examined whether the behavior of the PTS might be
further confined to a subspace of the 9-dimensional space. To this end, we analyzed
the eigenvalues of the Jacobian at the steady state. These eigenvalues represent the
timescales present in the system close to the steady state: largely negative values
correspond to fast dynamics, negative values close to 0 correspond to slow dynamics,
while positive values correspond to unstable dynamics, \textit{i.e.}, a movement that is not
approaching the steady state. (In biological systems, all eigenvalues are typically
negative, \textit{i.e.}, the system is stable.) The information about the timescales at steady
state gives an indication of whether there exists a SIM and, in that case, which
dimension it has. If all eigenvalues are of the same order of magnitude, there is no
timescale separation and no SIM exists. If a few, say \(n_r\), of the eigenvalues are of
one order of magnitude while the rest of another—where the latter are more negative
than the former ones and hence correspond to the fast dynamics—then there exists an
\(n_r\)-dimensional SIM. As described in the remark in Section 3.2.2, there may also exist
several SIMs of different dimensions, those of lower dimension embedded in those
of higher dimension—in this case there are eigenvalues of more than two different
orders of magnitude. The comparison of the eigenvalues of the PTS model at steady
state in Section 3.5.1 showed a large gap between the two slowest eigenvalues. This
hence indicated that there exists a 1-dimensional SIM, \textit{i.e.}, a curve towards which
trajectories of the state of the system rapidly move. In other words, the behavior
of the PTS may be much simpler than a fairly arbitrary movement through its 9-
dimensional state space.

To compute an approximation to the 1-dimensional SIM, in order to investigate
the behavior on it, we then partitioned the state variables in the PTS model into a 1-dimensional slow component \( \bar{x} \), chosen to be the state variable \([\text{EIIA-P}]\), and an 8-dimensional fast component \( \bar{y} \), containing the remaining variables. For the formulation of the ZDP\(_0\) (which is equivalent to the QSSA) and ZDP\(_1\) conditions we put

\[
\frac{d\bar{y}}{dt} = 0 \quad \text{or} \quad \frac{d^2\bar{y}}{dt^2} = 0,
\]

respectively. These two conditions hence lead to two different algebraic equation systems consisting of 8 equations and 9 unknowns (i.e., the state variables). They define the 1-dimensional slow ZDP\(_0\) and ZDP\(_1\) manifolds, respectively, both of which approximations of the SIM. We employed the numerical algorithm presented in Chapter 4 to tabulate the ZDP\(_0\) and ZDP\(_1\) manifolds. This algorithm performs the tabulation over a grid consisting of a set of equidistant values of a parameterizing variable \(x\) which we chose as \(x = [\text{EIIA-P}]\), as this choice led to fast computations. In other words, the algorithm substituted each value of \([\text{EIIA-P}]\) on the grid into one of the equation systems in (5.1)—reducing the number of unknown variables to 8, i.e., the same as the number of equations—and for each grid point it computed values of the remaining 8 state variables that together solved the equation system (for the original parameter set given in Appendix 3.A) and the results were tabulated. In Figure 5.1 we display these manifolds together with the four free protein concentrations tabulated over the same manifolds using the conservation relations (3.20). For details on the computation of these two manifolds, see Section 3.5.1.

Due to physical constraints, the values of all state variables are bounded from below by 0 (since they represent concentrations) and from above by one of the total concentrations of the four proteins: \([\text{EI-P-Pyr}]\), \([\text{EI-P-HPr}]\), \([\text{EI}]\), and \([\text{EI-P}]\) have to be lower than \([\text{EI}]_{\text{tot}} = 5 \mu\text{M}\); \([\text{HPr}]\) and \([\text{HPr-P}]\) lower than \([\text{HPr}]_{\text{tot}} = 50 \mu\text{M}\); \([\text{HPr-P-EIIA}]\), \([\text{EIIA}]\), and \([\text{EIIA-P}]\) lower than \([\text{EIIA}]_{\text{tot}} = 40 \mu\text{M}\); and \([\text{EIIA-P-EIICB}]\), \([\text{EIICB-P-Glc}]\), \([\text{EIICB}]\), and \([\text{EIICB-P}]\) lower than \([\text{EIICB}]_{\text{tot}} = 10 \mu\text{M}\). On the part of the ZDP\(_1\) manifold where \([\text{EIIA-P}]\) is low—i.e., less than approximately 3 \(\mu\text{M}\)—\([\text{EIICB}]\), \([\text{EIICB-P-Glc}]\), and \([\text{EIIA-P-EIICB}]\) assume negative values while \([\text{EIICB}]\) assumes values above its upper bound \([\text{EIICB}]_{\text{tot}} = 10 \mu\text{M}\)—cf. Figure 5.1. Similarly, for large values of \([\text{EIIA-P}]\), i.e., where it is larger than approximately 35 \(\mu\text{M}\), \([\text{HPr}]\), \([\text{HPr-P-EIIA}]\), and \([\text{EIIA}]\) assume values below 0 while \([\text{HPr-P}]\) assumes values above its upper bound \([\text{HPr}]_{\text{tot}} = 50 \mu\text{M}\). These parts of the manifold have no biochemical interpretation; only trajectories with initial conditions outside the physically feasible domain are attracted to this part of the manifold while trajectories starting inside this domain will stay inside of it. Note, however, that \([\text{EIIA-P}]\) may well assume values lower than approximately 3 \(\mu\text{M}\) and higher than 35 \(\mu\text{M}\) before the trajectory reaches the SIM: it is constrained to values above between these bounds only during the slow phase.

5.3 Relaxation behavior of rates

To explore the behavior of the reaction rates in the PTS during the partially relaxed phase—i.e., the slow phase in which the dynamics evolves along the SIM—we
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Figure 5.1: One-dimensional ZDP\(_0\) and ZDP\(_1\) manifolds (dashed and solid lines, respectively) for the 9-dimensional PTS model plotted against the parameterizing variable \(x=[\text{EI}IA\cdot P]\) (eight upper panels) and the four total protein concentrations tabulated over the same manifolds and plotted against the same parameterizing variable (four bottom panels). In each plot, the steady state is indicated by a circle. Together, these 12 plots represent a SIM in the full model with 13 state variables. All concentrations are given in \(\mu\text{M}\).

tabulated the values of all ten rates, using the rate expressions in Table 3.2, over numerically calculated ZDP\(_0\) and ZDP\(_1\) manifolds obtained using \(x=\bar{x}=[\text{EI}IA\cdot P]\). In Figure 5.2 we show these rates plotted against the corresponding points on the ZDP\(_0\) and ZDP\(_1\) manifolds—these points are represented by the parameterizing variable \([\text{EI}IA\cdot P]\)—and we denote the resulting curves the ZDP\(_0\) and ZDP\(_1\) rate profiles, respectively. Evidently, the ZDP\(_0\) rate profiles suggest that the values of \(v_1\) through \(v_6\) are identical to each other during the slow phase, as are \(v_7\) through \(v_{10}\). This is to be expected, as ZDP\(_0\) sets the right-hand sides of all state equations except the one for the parameterizing variable equal to 0, and thus \(v_1=\ldots=v_6\) and \(v_7=\ldots=v_{10}\)—cf. (3.8) and the state equations of the PTS model given in Appendix 3.A. The ZDP\(_1\) rate profiles suggest a similar grouping on a major part of the calculated manifold with the important difference that \(v_5\) is now grouped in the latter rate collective on the major part of the manifold, including the vicinity of the steady state.

The difference in the results obtained by the ZDP\(_0\) and ZDP\(_1\) approaches raises the question whether any of them correctly describes the rate behavior in the partially relaxed phase. To investigate this issue, we calculated the rates along some trajectories and compared with the rate profiles; the rates along the trajectories were visualized through the curves \((v(t), [\text{EI}IA\cdot P](t))\) parameterized by time, which we de-
Figure 5.2: The ten reaction rates of the PTS model evaluated on (A) the ZDP\textsubscript{0} manifold and (B) the ZDP\textsubscript{1} manifold. On the x-axes, the value of the parameterizing variable [EIIA·P] represents the point of the manifold at which the rates were evaluated.

Note rate trajectories. In Figure 5.3, we display these rate trajectories together with the corresponding ZDP\textsubscript{0} and ZDP\textsubscript{1} rate profiles for two out of the ten rates. The rate trajectories approach the ZDP\textsubscript{1} rather than the ZDP\textsubscript{0} rate profiles, showing that ZDP\textsubscript{1} more accurately captures the behavior of the rates in the slow phase than ZDP\textsubscript{0} does. In particular, the fact that the slow dynamics of \(v_5\) is more accurately approximated by ZDP\textsubscript{1} than by ZDP\textsubscript{0} (Figure 5.3A) verifies the validity of grouping this rate in the latter collective, i.e., the constitution of the collectives of rates suggested by ZDP\textsubscript{1} was correct while that of ZDP\textsubscript{0} was wrong. Because of the better performance of ZDP\textsubscript{1} as compared to ZDP\textsubscript{0}, we will only use ZDP\textsubscript{1} for further analysis in this chapter.

The ZDP\textsubscript{1} results point to the following interesting phenomenon: starting at any initial condition in the state space, and following a short transient, the rates \(v_1, v_2, v_3, v_4,\) and \(v_6\) assume very similar magnitudes; the same holds for \(v_5, v_7, v_8, v_9,\) and \(v_{10};\) the magnitudes of the latter differ significantly from those of the former. In other words, the behavior of the rates in the PTS is much less complex than it could have been: their slow dynamics in the partially relaxed phase is restricted to approximately two main curves, i.e., a small part of the ‘rate space’, instead of being spread out on ten widely separated profiles, one for each rate.

Interestingly, the two collectives of reactions correspond to two different parts of the PTS network as seen from Figure 5.4. This suggests that the grouping pertains to partial equilibration between adjacent reactions and lack of such equilibration across the substances HPr·P, HPr·P·EIIA, and EIIA·P, i.e., the substances located on the boundary between the two network parts.
As a result of the behavior outlined above, only the concentrations of HPr-P, HPr-P-EIIA, and EIIA-P change significantly during the partially relaxed phase, as their production and consumption are governed by rates belonging to different collectives. The remaining state variables, instead, are produced and consumed by reactions with rates belonging to the same of the two collectives. Consequently, the latter variables change only to a minor extent during the partially relaxed phase and hence assume values close to their steady state levels already by the end of the fast transient.

The difference in the rates of change in the slow phase between the state variables on the boundary and the remaining ones is also reflected on the ZDP$_1$ manifolds. The boundary concentrations [HPr-P], [HPr-P-EIIA], and [EIIA-P] assume values on a wider range on the biochemically interpretable part of the ZDP$_1$ manifold than the remaining variables do: [EIIA-P], which is the parameterizing variable, assumes values between approximately 3 and 35 µM as discussed in the end of Section 5.2; further, as seen from Figure 5.1, [HPr-P] assumes values between approximately 15 and 50 µM on this domain and [HPr-P-EIIA] between approximately 30 and 0 µM.
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(recall that \([\text{EIIA-P}]\) is the parameterizing variable); \([\text{EI-P}]\), on the other hand, which is not on the boundary, assumes values on the small range between 0.8 and 1.3 \(\mu\text{M}\) and similarly holds for all the remaining state variables, \(i.e.,\) they are confined to relatively small intervals. Accordingly, the boundary variables change more extensively during the slow phase, as was also the conclusion from the difference in consumption and production rates discussed above.

5.4 Dependence on parameter values

We next asked whether there might be reduced complexity in the sense of minor dependence of the behavior on parameter values. In the (unlikely) simplest situation, the system’s behavior would be parameter independent. In the maximally complex case, every new parameter set would lead to an entirely different behavior.

5.4.1 Limited parameter dependence of the relaxation behavior

We first tabulated the values of the rates on the \(ZDP_1\) manifolds calculated for parameter sets that only differed from the original one in \(one\) of the four boundary concentrations \([\text{PEP}], [\text{Pyr}], [\text{Glc}], \text{and } [\text{Glc-P}]) by a multiplicative factor of 10 or 0.1, respectively. In all these eight cases we again used \(x = \bar{x} = [\text{EIIA-P}]\) for the formulation of the \(ZDP_1\) condition and for parametrization of the manifold; we verified the suitability of this choice by comparing the rate trajectories with the \(ZDP_1\) rate profiles (cf. the analogous analysis in the previous section) and in all cases the \(ZDP_1\) rate profiles proved to be highly accurate approximations (data not shown). The rate profiles obtained for the parameter set in which the concentration of external pyruvate was multiplied by a factor of ten (\(i.e., [\text{Pyr}] = 9000 \mu\text{M}\)) are shown in Figure 5.5A–B. In a neighborhood of the steady state, the rates \(v_1, \ldots, v_4\) appear to fall into one collective, \(v_8\) and \(v_9\) into another, while the profiles of the remaining rates are distinct from the other profiles (Figure 5.5A). However, on the part of the manifold where \([\text{EIIA-P}]\) is high, the grouping of the rates resembles the grouping obtained for the original parameter set in the sense that two main rate collectives are formed (\(i.e., v_1, v_2, v_3, v_4, \text{and } v_6\) constitute one collective and \(v_5, v_7, v_8, v_9, \text{and } v_{10}\) another one, see Figure 5.5B). In the remaining seven cases the original grouping into two parts was reproduced on a major part of the physically feasible range of \([\text{EIIA-P}]\), including the neighborhood of the steady state—one of these cases (\(i.e.,\) when \([\text{Glc}]\) is increased by a factor 10) is shown in Figure 5.5C.

We also calculated the \(ZDP_1\) rate profiles using parameter sets which differed from the original one in only one of the four total protein concentrations, also by a factor of 10 or 0.1. In the two parameter sets with \([\text{EI}]_{\text{tot}} = 50 \mu\text{M}\) (original value was 5 \(\mu\text{M}\)) and \([\text{EIICB}]_{\text{tot}} = 1 \mu\text{M}\) (original value was 10 \(\mu\text{M}\)), the same grouping as that for the original parameter set was achieved. The two cases with \([\text{HPr}]_{\text{tot}} = 500 \mu\text{M}\) (original value was 50 \(\mu\text{M}\)) and \([\text{EI}]_{\text{tot}} = 0.5 \mu\text{M}\) resulted in a grouping similar to that achieved above for \([\text{Pyr}] = 9000 \mu\text{M}\) (shown in Figure 5.5A-B): in a neighborhood of the steady state some of the rates were forming groups, in both cases of a constitution similar to that in Figure 5.5A), while on a large part of the manifold the two original collectives prevailed. For the parameter set with \([\text{HPr}]_{\text{tot}} = 5 \mu\text{M}\) a different pattern
was observed: on one part of the manifold which included the steady state, the two
collectives $v_1, \ldots, v_6$ and $v_7, \ldots, v_{10}$ were formed, while on the part of the manifold
where $\text{[EIIA}\cdot\text{P}]$ is high, the rate $v_5$ joined the opposite collective and hence again
the original grouping was achieved—this is shown in figure Figure 5.5D. The same
pattern was observed for $\text{[EIIA]}_{\text{tot}}=400 \mu M$ (with original value $40 \mu M$). For the
six parameter sets mentioned we used $x = \bar{x} = \text{[EIIA}\cdot\text{P}]$ and again we verified this
choice by comparing rate trajectories with the corresponding rate profiles. For the
case when $\text{[EICB]}=100 \mu M$, the two slowest eigenvalues of the Jacobian at steady
state were complex conjugates of each other and, effectively, no one-dimensional SIM
existed. When $\text{[EIIA]}_{\text{tot}}=4 \mu M$, the ratio between the two slowest eigenvalues was
only 1.4 and hence the one-dimensional SIM only weakly attracted the trajectories.
We also investigated the rates for the two parameter sets in which all four total enzyme
concentrations were either multiplied or divided by a factor of two. Both these cases
resulted in the original grouping. Here, again $x = \bar{x} = \text{[EIIA}\cdot\text{P}]$ was used and the
choice was verified.

For all the eighteen parameter sets investigated above, the resulting collectives
comprised connected parts of the network—the groupings obtained on the part of the
manifolds near the steady state for the parameter sets with $\text{[Pyr]}=9000 \mu M$ and with
$\text{[HPr]}_{\text{tot}}=5 \mu M$ are shown in Figure 5.6.

The fact that collectivization was observed for almost all parameter sets that were
investigated above suggests that the PTS may exhibit grouping of the rates under
a variety of environmental conditions, corresponding to changes in the parameter
values. The differences in the constitution of the rate collectives obtained for different
parameter sets—however with many similarities between the constitutions—show that
the collectivization of the PTS rates is not maximally simple, but relatively simple
with respect to the dependence of the parameters.

5.4.2 Relaxation behavior in signal transduction pathways in general

Signal transduction pathways consisting of coupled cycles of phosphorylation, acety-
lilation, or ubiquitination all exhibit network structures that are similar to that of the
PTS and hence they can be described by models with structures equivalent to that of
the PTS model (given in Appendix 3.A) but with other values of the parameters.
Therefore, analysis of the type outlined above for the same model structure but for ar-
bitrarily chosen parameter sets, which hence may differ vastly from those in the PTS,
gives information about whether the grouping behavior may be expected in other
signal transduction pathways. Two examples of the rates calculated on the ZDP$_1$
manifold for two arbitrarily chosen parameter sets (A and B, reported in Appendix
5.A) substantially different from that of the original model are shown in Figure 5.7.
Here we used $x = \bar{x} = z_8$ and $x = \bar{x} = z_7$ for the parameter sets A and B, respectively
(in the PTS $z_8=\text{[EIIA}\cdot\text{P}]$ and $z_7=\text{[HPr}\cdot\text{P]}$, see Appendix 3.A) and we verified that the
rate trajectories indeed approach the ZDP$_1$ rate profiles. In Figure 5.8 we display the
corresponding partitioning of the network for the same two cases. The same type of
analysis was also performed for several other parameter sets and in all cases grouping
of the rates was observed—but with different constitution of the collectives—and the
collectives constituted connected parts of the network (data not shown).
5.5 Discussion

The complexity of biochemical systems in living cells renders their dynamics unintuitive and their function difficult to fathom. It is therefore of interest to explore methods to construct simplified views of their dynamics—but importantly—without throwing away their essential complexity [27]. The PTS serves as a prime example of a model with complicated dynamics as it involves a large number of molecular components exhibiting nonlinear dynamics. This system is of great interest to study since it participates in advanced regulatory functions of many prokaryotic cells and it has the unique property that it integrates three major types of molecular processes, i.e. metabolism, transport and signalling. It is hence of interest to achieve a reduced—though not oversimplified—overall view of its dynamics. In this chapter we developed an approach based on the ZDP which provides such an overall view of the dynamics of the reaction rates in a model of the PTS [99].
We first employed both the zeroth and the first order ZDP, the former being equivalent to the QSSA, to determine the behavior of the rates in the slow, partially relaxed phase. These two approximation methods both yielded rate profiles partitioned into two groups, within which the levels of the rates were identical. The constitutions of the rate collectives were similar for the QSSA and ZDP1 cases but differed in the classification of one of the rates which was assigned to opposite collectives by the two methods. By comparing the rate profiles with the rates calculated along trajectories we showed that the ZDP1 yielded considerably more accurate results than the QSSA and hence only ZDP1 succeeded to reproduce the correct grouping of all rates. These results illustrate the limitations with QSSA, which oversimplifies the model and hence throws away important dynamic features. The ZDP1 approach, on the other hand, yields a correct simplified view of the behavior.

The reactions in the two collectives yielded by ZDP1 were clearly divided into two different parts of the PTS network (see Figure 5.4). This fact suggests that the grouping pertains to the communication between adjacent reactions; seemingly, the reactions within each collective communicate well on the fast timescale and, accordingly, the rates internally adjust already during the transient, i.e., they reach levels resembling steady state levels in view of the fast timescale. During the partially relaxed phase, then, the two network parts operate at these separate, internally adjusted levels while the communication between the two collectives, which occurs on the slow timescale, slowly brings the system towards the steady state in which all rates are equal.

Our analysis of the rates naturally describes the behavior of the model under
consideration and, due to the possibility of uncertainties in the model resulting from e.g. measurement errors or lacking information, the dynamics we observed may differ from the actual behavior in vivo. To investigate this issue further we analyzed the robustness of the collectivization to changes in parameter values by determining the rate profiles also for a variety of different parameter sets. Small alterations of the parameter set (i.e., changing one of the parameter values by a factor of ten, or four of the parameters by a factor two) all resulted in some variation in the grouping; the constitution of the collectives varied slightly. These results suggest that the grouping phenomenon is rather robust to errors in the parameter values and hence strengthens the probability of finding the phenomenon in vivo.

The sensitivity analysis above also indicates how the grouping may depend on natural environmental variations such as changes in the gene expression pattern, in metabolism, or in the availability of extracellular glucose. Interestingly, the constitution of the rate collectives seems to be rather robust to changes in the external metabolite concentrations [PEP], [Pyr], [Glc], and [Glc-P] but more sensitive to changes in total protein concentrations [EI]_{tot}, [HPr]_{tot}, [EIIA]_{tot}, and [EIIICB]_{tot}. In particular, when [EIIA]_{tot} was increased by a factor of ten or when [HPr]_{tot} was decreased by a factor of 0.1 (which may result e.g. from a differential expression of the corresponding genes), the rate of the fifth reaction (in which HPr·P and EIIA are forming a complex) was assigned to the opposite collective as compared to the original grouping (see Figure 5.5D) on a large part of the manifold including the steady state. The difference in constitution of the collectives upon variation of certain parameter values suggests that the PTS exhibits different operational modes and that the mode which it temporarily resides in depends on the environmental conditions; this differential behavior might play a role in the regulatory functions of the PTS.

We also altered the original parameter set to larger extents in order to investigate the likelihood of finding the grouping phenomenon also in other signal transduction pathways with the same structure as that of PTS, i.e., consisting of coupled cycles of phosphorylation, ubiquitination, or acetylation. In these cases we also observed grouping in all the cases that we analyzed, which affirms that the grouping might
be a more general phenomenon recurring in a variety of pathways. It would also be interesting to perform the rate analysis described in this chapter for models with similar network structures, such as models containing fewer or more coupled cycles, or for models with entirely different network structures in order to investigate whether the grouping phenomenon may appear also in such systems.

The behavior of the rates in the PTS that we have reported on in this chapter could have been partially unmasked also without the determination of a SIM; simple simulations of the model show that after an initial transient phase, certain rates assume the same magnitudes and subsequently they coherently approach their steady state values. Simulating the model for a large number of initial conditions would hence enable a characterization of the collectivization behavior. The power of the approach that we used, based on analysis of the SIM, is that it provides a general overview of the slow dynamics, and this without delving into details about the behavior of particular trajectories: any initial condition in the entire state space will result in a trajectory that initially approaches the SIM before approaching the steady state. An approach based on investigating time courses would become cumbersome in comparison with our SIM-based approach, yet providing a less general view, and would be particularly demanding when analyzing the behavior for a large variety of parameter sets.

With the progress in systems biology in recent decades it has become clear that cell function depends on the functioning of complex networks. Experimentally it has become possible to approach such networks and this has led to avalanches of data. To analyze these data, models are being constructed and analyzed. There is however
a time lag between the collection of data and the model construction and it will take a while until we have constructed mathematical models of equally high complexity as the data. Once the models have been produced, the more important challenge remains, \textit{i.e.} that of understanding the actual behavior of the network. For this it may be essential to achieve simplified views of the behavior of the network without losing the essence of its behavior. The ZDP approach developed here may be one of the methods that can be developed further to do this.

The analysis described in this chapter has shown that interesting biochemical information may be achieved by ZDP reduction and, accordingly, offers an example of the usefulness of ZDP in the biochemical context. It also opens up for further investigations into the reasons for this behavior and the biological consequences of the grouping on the functioning of the cells.
Appendix

5.A Parameter sets

Here we report two of the parameter sets used in Section 5.4.2. The units of the parameter values are arbitrary. For simplicity we use the same notation for the parameters as in the PTS model given in Appendix 3.A although these parameters concern signal transduction pathways in general and not the particular PTS pathway.

Parameter set A: \([\text{Glc}] = 1000, [\text{Glc-P}] = 50, [\text{Pyr}] = 87, [\text{PEP}] = 1, k_1f = 10, k_1r = 0.005, k_2f = 18, k_2r = 50, k_3f = 10, k_3r = 0.001, k_4f = 10, k_4r = 1, k_5f = 10, k_5r = 82, k_6f = 10, k_6r = 7, k_7f = 10, k_7r = 88, k_8f = 10, k_8r = 1, k_9f = 100, k_9r = 1, k_{10f} = 10, k_{10r} = 1, [\text{EI}]_{tot} = 1, [\text{HPr}]_{tot} = 1, [\text{EIIA}]_{tot} = 1, \text{ and } [\text{EIICB}]_{tot} = 1\)

Parameter set B: \([\text{Glc}] = 80, [\text{Glc-P}] = 50, [\text{Pyr}] = 70, [\text{PEP}] = 40, k_1f = 10, k_1r = 1, k_2f = 100, k_2r = 1, k_3f = 16, k_3r = 6, k_4f = 10, k_4r = 1, k_5f = 60, k_5r = 5, k_6f = 10, k_6r = 5, k_7f = 50, k_7r = 5, k_8f = 18, k_8r = 3, k_9f = 100, k_9r = 7, k_{10f} = 10, k_{10r} = 5, [\text{EI}]_{tot} = 10, [\text{HPr}]_{tot} = 30, [\text{EIIA}]_{tot} = 10, \text{ and } [\text{EIICB}]_{tot} = 10\).