



Masterarbeit am Institut für Mathematik der Freien Universität Berlin

Computing the minimal rebinding effect for nonreversible processes

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Abstract

The aim of this thesis is to investigate the rebinding effect, which occurs when projecting a Markov process onto a finite state space and may spoil the Markovianity. Under the assumption of a fuzzy clustering in terms of membership functions $\chi = XA$, being a linear combination of Schur vectors, a minimal bound for the rebinding effect included in a given system is computed as the solution of an optimization problem.

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Introduction

Markov processes are memoryless stochastic processes with applications in many different kinds of areas. They are employed to describe molecular systems like protein folding or ligand-binding processes. Such processes act on very large state spaces and additionally require simulations on rather long time-scales in order to observe rare conformational changes. Consequently, a reduction of dimension is aimed at, which can be realized by a projection onto a smaller state space. The reduced model should represent the correct long-time behaviour of the process, while being less complex. The existence of metastable sets can be exploited to create such a “Markov State Model”. A well-established solution is the fuzzy clustering algorithm PCCA+, which identifies metastable sets with the aid of membership functions $\chi = XA$, being a linear combination of eigenvectors.

When projecting a process onto a finite state space, it can lose its Markov property, more precisely it can include short-time memory effects. Such memory effects were observed in the context of ligand-binding-systems, where in certain configurations significantly increased binding affinities were detected. They are explained by an additional memory caused by the projection: short time after a ligand unbound from its target, it is assumed to be still nearby and thus rebinds with a high probability. Consequently, this short-time memory is denoted as **rebinding effect**. This memory effect is strongly related to the **overlap** of the membership functions χ determining the clustering. Hence, knowing them makes it easy to compute the actual rebinding effect caused by this projection. However, in many cases the original process and the membership functions are not known. For instance, a finite process can be constructed as the solution of a differential equation and just be interpreted as the projection of a larger process. In order to identify possible memory effects included in that system, it is favorable to estimate the rebinding effect. This can be achieved by solving an optimization problem, revealing a minimal bound:

“Given a clustered system, how much rebinding is included **at least**?”

The computation of the minimal rebinding effect included in a given kinetics has been accomplished in 2014 by Weber and Fackelley[48] for reversible processes. In this thesis, the formulation of the corresponding optimization problem is extended onto non-reversible processes. This is achieved by employing GenPCCA, a recent modification of PCCA+ by Weber and Fackelley[46] from 2017, which is based on Schur vectors instead of eigenvectors and includes non-reversible processes. This generalization is of particular interest since many real-world processes are non-reversible.

A significant application of the presented topic lies in the area of computational drug design. In order to treat diseases, ligands are designed such that they bind to pathogenic target molecules. Improving the binding affinity is one important goal in drug design. For a precise prediction of the binding behaviour, it is important to consider possible rebinding events, since they can influence the binding affinity.

The main topic and structure of this thesis comply with Weber and Fackeldey[48], though with the addition of considering non-reversible processes as proposed by Weber and Fackeldey[46]. The mathematical foundations presented in the first two chapters are inspired by the book “Metastability and Markov State Models in Molecular Dynamics” by Schütte and Sarich[37]. Furthermore, the dissertations of Huisinga[17], Weber[44], Sarich[30], Nielsen[25] and the habilitation of Weber[45] have been particularly useful for the deeper understanding of the mathematical concepts behind metastability, clustering and transfer operators.

Thesis Structure

Chapter 1 - Markov State Models

We give a short overview about Markov processes and how their evolution in time can be described by transition functions and transfer operators. We show how such a continuous operator can be projected onto a finite-dimensional space with the aid of a Galerkin discretization. Finally, we analyze the discretization error and the possible loss of the Markov property which can occur by this projection.

Chapter 2 - Dominant Structures

In order to create a suitable Markov State Model preserving the long-time behaviour of the original process, we introduce the concept of metastability. We define metastable sets mathematically and explain their relevance for molecular systems. We reveal their relation to the spectrum of the transfer operator and show that the “best” metastable decomposition is achieved in terms of fuzzy membership functions, which may be overlapping. Finally, we extend this well-established clustering method to non-reversible processes by employing the Schur decomposition.

Chapter 3 - Rebinding Effect

In this chapter, we characterize the rebinding effect as a memory effect which occurs in the context of receptor-ligand systems, a special case of molecular systems. We apply the methods presented in the first chapters in order to rigorously describe a molecular system respectively its projection onto a finite subspace. Finally, we compute a minimal bound for the rebinding effect as the solution of an optimization problem, for reversible as well as for non-reversible systems.

Chapter 4 - Illustrative Examples

The results from chapter 3 are verified by means of some illustrative examples. At first, the minimal rebinding effect is computed for some clusterings of a reversible system in order to evaluate the quality of this estimation. Afterwards, the same is done for a non-reversible system and compared to the outcome of the reversible case. As the rebinding effect is characterized within receptor-ligand systems, such a system is presented. In order to demonstrate the appearance of this effect in different contexts as well, we explain it in a system describing the chemical reaction of formic acid dimer.

1 Markov State Models

In order to be able to describe dynamical systems having a nondeterministic behaviour, we introduce Markov processes. They are memoryless stochastic processes which are commonly used to model different kinds of real-world processes, []. Recently they have been applied a lot in the research area of modelling biomolecular systems, []. As those systems are enormous large, it is very difficult to perform simulations on feasible time scales. That is why a reduction of complexity is needed, []. The originally large process is projected onto a suitable subspace, while maintaining the most relevant dynamical properties of the process. We present such a reduced model, called “Markov State Model”.

In order to adequately define a Markov State Model, we need at first some basic definitions and properties of stochastic processes, especially Markov processes, and how their evolution in time can be described using the transfer operator. The actual dimension reduction of the process is realized by a Galerkin projection applied to the transfer operator. By that action, states of the original process are clustered conveniently.

1.1 Markov Process

We introduce Markov processes, which are a special type of stochastic processes and a generalization of the well-known Markov chains. Markov chains were defined as a memoryless process acting on a finite state space and evolving in discrete time, a behaviour that can be described by a stochastic matrix. For general Markov processes, both these properties can be continuous and thus, we need some more extensive formulations and tools in order to describe such processes respectively their time-evolution rigorously.

Transition function

We will denote by $E := (E, \Sigma)$ a *measurable space*, that is a set E with some σ -algebra Σ defined on it. The triple $\Omega := (\Omega, \mathcal{A}, \mathbb{P})$ will be a *probability space*, that is a measurable space with a probability measure \mathbb{P} defined on it; for detailed information about these basic measure theoretic notations, see Bogachev[2, chapter 1].

A *random variable* $X : \Omega \rightarrow E$ is a *measurable function* from a probability space Ω into a measurable space E , meaning that preimages of measurable sets in E are measurable in Ω :

$$A \in \Sigma \Rightarrow X^{-1}(A) \in \mathcal{A}.$$

Then the probability measure \mathbb{P} of Ω induces a canonical probability measure on E , by

$$\mu(A) := \mathbb{P}(X \in A) := \mathbb{P}(X^{-1}(A))$$

for all $A \in \Sigma$, called the *distribution* of X , see Øksendal[26, Section 2.1].

Definition 1.1. (Stochastic Process)

A family $(X_t)_{t \in \mathbb{T}}$ of random variables $X_t : \Omega \rightarrow E$ on some index set \mathbb{T} is called a *stochastic process* on a state space E .

In the following, we consider stochastic process on real state spaces $E \subset \mathbb{R}^d, d \in \mathbb{N}$, equipped with the Borel- σ -algebra $\Sigma = \mathcal{B}(E)$. In order to introduce Markov processes as a special type of stochastic processes, we need a tool to describe the time evolution or propagation of a process. This can be done using the transition function which describes the propagation of the distribution functions of a stochastic process.

Definition 1.2. (Transition function)

A function $p : \mathbb{T} \times E \times \Sigma \rightarrow [0, 1]$ is a *transition function* if it fulfills the following properties:

- i) $x \mapsto p(t, x, A)$ is measurable on E for all $t \in \mathbb{T}$ and $A \in \Sigma$,
- ii) $A \mapsto p(t, x, A)$ is a probability measure for all $t \in \mathbb{T}$ and $x \in E$,
- iii) $p(0, x, E \setminus x) = 0$ for all $x \in E$,
- iv) the Chapman-Kolmogorov equation

$$p(t + s, x, A) = \int_E p(t, x, dz) p(s, z, A). \quad (1.1)$$

holds for all $t, s \in \mathbb{T}, x \in E$ and $A \in \Sigma$.

In this definition, the first three properties ensure that we get reasonable results and that the process can only be in one state at the same time. From the Chapman-Kolmogorov equation (1.1), it follows that the transition function $p(t, x, A)$ can be considered as the probability to get into a certain subset A in a time interval t starting from a point x . That means that we can describe the time evolution of a stochastic process by a transition function. In particular, the transition matrix of a Markov chain is a special case of the transition function since it fulfills the above properties.

Markov Process

With the aid of a transition function, we are able to define Markov processes.

Definition 1.3. (Markov Process)

A stochastic process $(X_t)_{t \in \mathbb{T}}$ on a state space E is a *Markov process* if its transition function fulfills the equation

$$p(t, x, A) = \mathbb{P}(X_{t+s} \in A \mid X_s = x). \quad (1.2)$$

for all $s, t \in \mathbb{T}, x \in E$ and $A \in \Sigma$. If that probability is independent from s , then the Markov process is called *time-homogeneous*.

We are especially interested in time-homogeneous processes, which will be presumed from now on. As we can see from the definition, all possible transition probabilities are given and hence, the time evolution of a Markov process is completely described by its transition function. Thus a Markov process is uniquely determined by its transition function and an initial distribution μ . It is a process that has “no memory” in the sense that only the last known state of the process has an influence on the future of the process, as we can see on the right side of (1.2).

Indeed, there is a one-to-one relation between transition functions and Markov processes, i.e. every homogeneous Markov process defines a transition function and vice versa, see Meyn and Tweedie[24, Section 3.4]. The beginning of a Markov process X_t with the transition function p fulfills

$$\mathbb{P}_\mu(X_0 \in A, X_t \in B) = \int_A p(t, x, B) \mu(dx) \quad (1.3)$$

for any $A, B \in \Sigma$, where \mathbb{P}_μ indicates that $X_0 \sim \mu$, or equivalently $\mu(A) = \mathbb{P}(X_0 \in A)$.

The transition function for a Markov process plays the same role as the transition matrix for a Markov chain; it propagates its distributions. If for the transition function we choose $t = 1$ and transitions into one-elementic subsets, then the transition function corresponds to the 1-step transition matrix $[p_{ij}] = P \in \mathbb{R}^{n \times n}$ of a Markov chain. Having introduced the notion of Markov processes, we can define some important properties and give some examples. ?

Invariant Measure

Definition 1.4. (Invariant measure)

Let $(X_t)_{t \in \mathbb{T}}$ be a Markov process. The probability measure μ is *invariant* with respect to $(X_t)_{t \in \mathbb{T}}$ if for all $t \in \mathbb{T}$ and $A \in \Sigma$ we have

$$\int_E p(t, x, A) \mu(dx) = \mu(A).$$

In other words, a measure is invariant with respect to a Markov process if the probability to **be** in any subset of the state space is the same as the probability to **get** into that subset by the evolution of the Markov process for any fixed transition time.

Ergodicity

The long-time behaviour of stochastic processes can be described using ergodicity.

Definition 1.5. (ergodic process)

Let $(X_t)_{t \in \mathbb{T}}$ be a Markov process with invariant probability measure μ . Then $(X_t)_{t \in \mathbb{T}}$ is *ergodic* with respect to μ if for all functions $u : E \rightarrow \mathbb{R}$ with $\int_E |u| \mu(dx) < \infty$ we have

$$\lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T u(X_t) dt = \int_E u(x) \mu(dx).$$

for almost all initial values $X_0 = x_0$.

In that sense, a Markov process is ergodic if its time average is the same as its average over the probability space. In an ergodic process, the state of the process after a long time is nearly independent of its initial state.

Reversibility

Reversibility describes the invariance of a process with respect to time-reversal. In the next section, we will see that an operator describing a reversible process yields some very favorable properties, which makes it easy to analyze such a process.

Definition 1.6. (reversible process)

Let $(X_t)_{t \in \mathbb{T}}$ be a Markov process with invariant probability measure μ . Then $(X_t)_{t \in \mathbb{T}}$ is *reversible* with respect to μ if

$$\int_A p(t, x, B) \mu(dx) = \int_B p(t, x, A) \mu(dx)$$

for all $t \in \mathbb{T}$ and $A, B \in \Sigma$. If μ is unique, then X_t is simply called *reversible*.

For a reversible process, the probability to get from any subset A to another subset B in a fixed time is the same as the probability for the reverse transition in the same time span. Thus this definition implies that the process keeps the same probability law even if its movement is considered backwards in time.

If the stochastic transition function is absolutely continuous with respect to μ , then reversibility corresponds to $p(t, x, y) = p(t, y, x)$ for all $t \in \mathbb{T}$ and μ -almost all $x, y \in E$.

Example: Markov Chain

Let $(X_t)_{t \in \mathbb{T}}$ be a Markov chain on discrete time $\mathbb{T} = \mathbb{N}$ and finite state space $E = \{1, \dots, n\}$. Since we consider 1-step transitions, the associated transition function is given by $p(x, y) := p(1, x, y)$ and corresponds to the entries of the *transition matrix* $P \in \mathbb{R}^{n \times n}$, that is

$$P_{xy} = p(x, y) = \mathbb{P}(X_1 = y \mid X_0 = x).$$

The propagation of a probability distribution $v_0 \in \mathbb{R}^n$ in the state space can be written as $v_1^T = v_0^T P$, where v_0^T denotes the transposed vector of v_0 . The invariant measure is given by the stationary distribution $\pi \in \mathbb{R}^n$, a normalized positive vector satisfying $\pi^T = \pi^T P$. If P is irreducible, such an eigenvector exists due to Perron-Frobenius theorem[16, P7.3.5] and in this case, the corresponding eigenvalue 1 is simple.

Reversibility of a Markov chain can be characterized by the *detailed balance condition*

$$\pi_i \cdot \mathbb{P}(X_1 = j \mid X_0 = i) = \pi_j \cdot \mathbb{P}(X_1 = i \mid X_0 = j) \quad \forall i, j \in E.$$

A more compact way to write this equation uses the diagonal matrix $D = \text{diag}(\pi_1, \dots, \pi_n)$. Then a Markov chain is reversible if and only if its transition matrix P fulfills

$$DP = P^T D. \tag{1.4}$$

This notation will be useful later in order to measure the degree of nonreversibility of a process.

1.2 Transfer Operator

With the previously defined transition function, we have a tool to describe the propagation of **distributions** of stochastic processes. Now we introduce an operator that propagates **probability densities** of Markov processes. Before defining such an operator, we have to specify the space of functions the operator is acting on.

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resp. sets?

L^r -Spaces

It seems natural to define such a density propagating operator as acting on $L^1(\mu)$, the Banach space that includes all probability densities with respect to μ . However it is sometimes advantageous to restrict the analysis to $L^2(\mu)$, since this can lead to a self-adjoint operator. As there are different motivations for the choice of a suitable space, we define an operator which acts on $L^r(\mu)$ -spaces, i.e. spaces of r -integrable functions.

Definition 1.7. (L^r -Spaces)

Let (E, Σ, μ) a measure space. Then we define the corresponding L^r -spaces as equivalence classes of measurable functions

$$L^r(E, \Sigma, \mu) = \{f : E \rightarrow \mathbb{R} \mid \int_E |f(x)|^r \mu(dx) < \infty\}$$

for $1 \leq r < \infty$ and

$$L^\infty(E, \Sigma, \mu) = \{f : E \rightarrow \mathbb{R} \mid \text{ess sup}_{x \in E} |f(x)|^r \mu(dx) < \infty\},$$

with the corresponding norms $\|\cdot\|_r$ and $\|\cdot\|_\infty$.

In these equivalence classes, two functions f, g are identified if $f = g$ μ -almost everywhere, see Werner[50, section I.1]. If it is clear from the context, which measure space (E, Σ, μ) is in consideration, we just write shortly $L^r(\mu) := L^r(E, \Sigma, \mu)$. Due to Hölders inequality, we have $L^r(\mu) \subset L^s(\mu)$ for all $1 \leq s \leq r \leq \infty$. All L^r -spaces are Banach spaces, though $L^2(\mu)$ is the only one which can be equipped with a canonical scalar product and thereby becomes a Hilbert space, see Werner[50, section V.1]. For $f, g \in L^2(\mu)$, the scalar product is defined as

\mathbb{C} ?

$$\langle f, g \rangle_\mu := \int_E f(x) \overline{g(x)} \mu(dx).$$

Now let ν_0 be the density function of a given start distribution. Then the density function of a subset $A \in \Sigma$ at time t is given in terms of the transition function by

$$\nu_t(A) = \int_E \nu_0 p(t, x, A) \mu(dx).$$

On the other hand, the density ν_t is given by

$$\nu_t(A) = \int_A \nu_t(x) \mu(dx).$$

Forward and Backward Transfer Operator

The two above equations result in the following intuitive definition of a transfer operator which should “propagate” probability densities according to a given Markov process. But instead of limiting us to density functions, we define the transfer operator as acting on any r -integrable function.

Definition 1.8. (Propagator or Forward Transfer Operator)

Let $p : \mathbb{T} \times E \times \Sigma \rightarrow [0, 1]$ be the transition function of a Markov Process $(X_t)_{t \in \mathbb{T}}$ and μ be an invariant measure of $(X_t)_{t \in \mathbb{T}}$. The semigroup of *propagators* or *forward transfer operators* $\mathcal{T}^t : L^r(\mu) \rightarrow L^r(\mu)$ with $t \in \mathbb{T}$ and $1 \leq r \leq \infty$ is defined via

$$\int_A \mathcal{T}^t \nu(y) \mu(dy) = \int_E \nu(x) p(t, x, A) \mu(dx) \quad (1.5)$$

for all $A \in \Sigma$ and $\nu \in L^r(\mu)$.

The propagator is well-defined on the Banach spaces $L^r(\mu)$, $1 \leq r \leq \infty$, see [17]. We will list already some properties of this operator which will be useful in the following chapters. \mathcal{T}^t is a *Markov operator*, i.e. it conserves the norm, $\|\mathcal{T}^t \nu\|_1 = \|\nu\|_1$, and is positive, $\mathcal{T}^t \nu \geq 0$ for $\nu \geq 0$. $\mathcal{T}^t \nu_0$ describes the transport of the function ν_0 in time t by the underlying dynamics given by the process X_t and weighted with respect to μ via

$$\nu_0 \mapsto \nu_t = \mathcal{T}^t \nu_0.$$

Since μ is invariant, we see immediately that the characteristic function $\mathbb{1} := \mathbb{1}_E$ of the entire state space is invariant under the action of \mathcal{T}^t , that is

$$\mathcal{T}^t \mathbb{1} = \mathbb{1}.$$

It means that \mathcal{T}^t has the eigenvalue 1 which corresponds to its eigenfunction $\mathbb{1}$.

Definition 1.9. (Backwards Transfer Operator¹)

The *backwards transfer operator* $\mathcal{U}^t : L^r(\mu) \rightarrow L^r(\mu)$ with $t \in \mathbb{T}$ and $1 \leq r \leq \infty$ is defined by

$$\mathcal{U}^t f(x) = \int_E f(y) p(t, x, dy). \quad (1.6)$$

We have again 1 as eigenvalue to the eigenfunction $\mathbb{1}$, that is for all $t \in \mathbb{T}$ we have

$$\mathcal{U}^t \mathbb{1} = \mathbb{1}.$$

The operator \mathcal{U}^t is *adjoint* to \mathcal{T}^t , denoted by $(\mathcal{T}^t)^* = \mathcal{U}^t$, that is they are related via the duality bracket, namely for all $f \in L^p(\mu)$, $g \in L^q(\mu)$ with $\frac{1}{p} + \frac{1}{q} = 1$, we have

$$\langle \mathcal{T}^t f, g \rangle_\mu = \langle f, \mathcal{U}^t g \rangle_\mu.$$

¹This nomenclature is motivated by the fact that for some models the forward transfer operator is related to the forward Kolmogorov relation, while the backward transfer operator is related to the backward Kolmogorov relation.

We again remark that both forward as well as backward operator can be defined on arbitrary $L^r(\mu)$ -spaces. But the previous equation shows us that either the choice $p = q = 2$ or the choice $p = 1, q = \infty$ or conversely, make sense, in order to obtain this useful adjointness/duality-relation of the two operators.

If we compare the equations (1.5) and (1.6), the notion of “forward” and “backwards” becomes clear. For the forward case, the state average with respect to f is taken over all initial states x which are propagated forward in time. In the backward case, we take the state average over all final states y .

If the state space is finite and the corresponding process reversible, then we can see the relation of the forward and backward operator still better. Then the forward operator corresponds to the transition matrix, propagating probability distributions, while the backward operator corresponds to the transposed transition matrix, propagating subsets. ?

Spectrum of Transfer operator

Later in this thesis, we will be interested in examining the spectrum of the transfer operator of a given Markov process. The following theorems give us an important insight about the spectrum and its relation to the reversibility of the process.

Definition 1.10. (Self-adjoint Operator)

An operator \mathcal{T} on $L^2(\mu)$ is called *self-adjoint* if for all $f, g \in L^2(\mu)$ we have

$$\langle f, \mathcal{T}g \rangle_\mu = \langle \mathcal{T}f, g \rangle_\mu.$$

Theorem 1.11. (Werner[50, theorem VI.1.2, theorem VI.1.3, lemma VI.3.1])

Let X be a Banach space and $\mathcal{T} : X \rightarrow X$ a linear continuous operator. Then we have

$$|\lambda| \leq \|\mathcal{T}\| \text{ for all } \lambda \in \sigma(\mathcal{T}).$$

If X is a Hilbert space, then

$$i) \sigma(\mathcal{T}^*) = \{\bar{\lambda} \mid \lambda \in \sigma(\mathcal{T})\},$$

$$ii) \text{ if } \mathcal{T} \text{ is self-adjoint, i.e. if } \mathcal{T}^* = \mathcal{T}, \text{ then } \sigma(\mathcal{T}) \subset \mathbb{R},$$

$$iii) \text{ if } \mathcal{T} \text{ is self-adjoint, then each two eigenfunctions corresponding to different eigenvalues are orthogonal.}$$

Since we know that the operator norm of any transfer operator \mathcal{T} is 1, it follows immediately from theorem 1.11 that its spectrum $\sigma(\mathcal{T})$ is contained in the unit circle of the complex plane, that is we have $|\lambda| \leq 1$ for all $\lambda \in \sigma(\mathcal{T}) \subset \mathbb{C}$.

Theorem 1.12. (Huisinga[17, proposition 1.1])

Let $\mathcal{T}^t : L^2(\mu) \subset L^1(\mu) \rightarrow L^2(\mu)$ be the propagator corresponding to the Markov process $(X_t)_{t \in \mathbb{T}}$. Then \mathcal{T}^t is self-adjoint with respect to the scalar product $\langle \cdot, \cdot \rangle_\mu$ in $L^2(\mu)$ if and only if $(X_t)_{t \in \mathbb{T}}$ is reversible.

Thus, the transfer operator of a reversible process has a spectrum $\sigma(\mathcal{T}) \subset [-1, 1]$. Furthermore, theorem 1.11 guarantees us that the spectrum of a self-adjoint operator is equal to the spectrum of its adjoint. Thus, if we are given a reversible process, it doesn't matter if we examine the spectrum of the forward or the backward transfer operator.

Infinitesimal Generator

For $\mathbb{T} = \mathbb{R}$ the Chapman-Kolmogorov property (1.1) of the transition functions makes the family $\{\mathcal{T}^t\}_{t \in \mathbb{R}}$ of transfer operators a continuous *semigroup* due to

$$\mathcal{T}^{t+s} = \mathcal{T}^t \mathcal{T}^s.$$

This leads to the following definition of the (time-independent) infinitesimal generator.

Definition 1.13. (Infinitesimal Generator)

For the semigroup of propagators or forward transfer operators $\mathcal{T}^t : L^r(\mu) \rightarrow L^r(\mu)$ with $t \in \mathbb{T}$ and $1 \leq r \leq \infty$ we define $\mathcal{D}(L)$ as the set of all $f \in L^r(\mu)$ s.t. the strong limit

$$\mathcal{Q}f = \lim_{t \rightarrow 0} \frac{\mathcal{T}^t f - f}{t}$$

exists. Then the operator $\mathcal{Q} : \mathcal{D}(L) \rightarrow L^r(\mu)$ is called the *infinitesimal generator* corresponding to the semigroup \mathcal{T}^t .

The infinitesimal generator is an operator which describes the behaviour of a Markov process in infinitesimal time. That becomes clear by the relation

$$\mathcal{T}^t = \exp(t\mathcal{Q})$$

in $L^2(\mu)$. We say that \mathcal{Q} “generates” the semigroup of transfer operators $\{\mathcal{T}_t\}_{t \in \mathbb{R}}$ since the whole semi-group of transfer operators can be derived from it.

If the corresponding Markov process is reversible, then \mathcal{Q} is self-adjoint in $L^2(\mu)$ and consequentially the spectrum is contained in $(-\infty, 0]$. Therefore the dominant eigenvalues $1 = \lambda_1, \dots, \lambda_n$ of the propagator \mathcal{T}^t are related to the dominant eigenvalues $0 = \xi_1, \dots, \xi_n$ of the generator \mathcal{Q} via

$$\lambda_k = \exp(t\xi_k)$$

for all $1 \leq k \leq n$ and the associated eigenfunctions are identical. Thus the invariant measure $\mathbb{1}$ of \mathcal{T}^t satisfies

$$\mathcal{Q}\mathbb{1} = 0.$$

1.3 Galerkin Projection

So far we considered Markov processes on very large, possibly continuous, state spaces. For many applications, simulations of a given process are needed in order to obtain informations about the corresponding system. But computations on **large** state spaces require an enormous amount of computation power and time. On larger state spaces, the computation effort increases exponentially fast, see “curse of dimension”. Therefore

we are interested in reducing the number of states in order to make computations on reasonable time-scales feasible. Such a reducing can for instance be done by “grouping similar states together”. In this section, we develop a mathematical concept which enables us to create such a reduced model.

Galerkin Projection

The first step in order to create a finite process is to determine a convenient finite state space $D \subset L^2(\mu)$. For this purpose, we choose a partition of unity as a basis, which is a generalization of a set of characteristic functions. That more general idea gives us more flexibility for later applications. The relevance of the choice of a partition of unity for the projection will be clarified in section 2.3.

Definition 1.14. (Partition of Unity)

A family of measurable functions $\{\chi_1, \dots, \chi_n\} : E \rightarrow [0, 1]$ in $L^2(\mu)$ is called a *partition of unity* if the following two conditions are fulfilled:

- i) The χ_i are non-negative and linear independent.
- ii) $\sum_{i=1}^n \chi_i(x) = 1$ for all $x \in E$.

Definition 1.15. (Galerkin Projection)

Let $\{\chi_1, \dots, \chi_n\}$ be a partition of unity, $D = \text{span}\{\chi_1, \dots, \chi_n\}$ the associated finite-dimensional ansatz space and $\hat{S} \in \mathbb{R}^{n \times n}$ with $\hat{S}_{kj} = \langle \chi_k, \chi_j \rangle_\mu$. The *Galerkin projection* onto D is defined by $G : L^2(\mu) \rightarrow D$ via

$$Gf = \sum_{k,j=1}^n \hat{S}^{-1}(k, j) \langle \chi_k, f \rangle_\mu \chi_j. \quad (1.7)$$

The matrix \hat{S} is invertible since it is the Gramian matrix of linear independent functions. In the easy case that the $\{\chi_1, \dots, \chi_n\}$ are the characteristic functions $\{\mathbb{1}_{A_1}, \dots, \mathbb{1}_{A_n}\}$ belonging to a full partition $\{A_1, \dots, A_n\}$, equation (1.7) becomes

$$Gf = \sum_{k=1}^n \frac{1}{\mu(A_k)} \langle \chi_k, f \rangle_\mu \chi_k,$$

since the χ_i are orthogonal which means that $\chi_k \chi_j = 1$ if $j = k$ and 0 otherwise. A Galerkin projection can be applied on the transfer operator of a Markov process as well.

Definition 1.16. (Projected Transfer Operator)

Let $\mathcal{P} := \mathcal{P}^t$ be the transfer operator of a Markov process on a state space E with unique invariant measure μ , $\{\chi_1, \dots, \chi_n\}$ be a partition of unity and G the Galerkin projection onto the associated subspace D . Then an operator of the form

$$G\mathcal{P}G : L^2(\mu) \rightarrow D$$

is called *projected transfer operator* and we abbreviate it by $G(\mathcal{P})$.

Matrix Representation

Since we are interested in transitions inside of the projected space, we want to propagate n -dimensional vectors by the projected transfer operator. For this reason, we consider the projection of the restricted transfer operator $G\mathcal{P}|_D : D \rightarrow D$, which will be denoted by $G(\mathcal{P})$ as well.

We recall that every linear map between finite-dimensional vector spaces can be represented by a matrix which is determined by chosen bases. Accordingly, we can write the projected transfer operator as a $n \times n$ -matrix in the following useful way.

Theorem 1.17. (Sarich [30])

Let \mathcal{P} be the transfer operator of a Markov process, $\{\chi_1, \dots, \chi_n\}$ a partition of unity and $G(\mathcal{P})$ the Galerkin projection of the transfer operator onto the associated subspace. Then $G(\mathcal{P})$ has a matrix representation

$$P_c = TS^{-1},$$

where

$$S_{kj} = \frac{\hat{S}(k, j)}{\langle \chi_k, \mathbb{1} \rangle_\mu} = \frac{\langle \chi_k, \chi_j \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu} \quad (1.8)$$

and

$$T_{kj} = \frac{\langle \chi_j, \mathcal{P}\chi_k \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu}.$$

Proof. Remember that P_c is a (left) matrix representation of $G(\mathcal{P})$ with respect to a basis $\{\psi_1, \dots, \psi_n\}$ of D if for any function $f : D \rightarrow D$ with

$$f = \sum_{i=1}^n \alpha_i \psi_i \quad \text{and} \quad G(\mathcal{P})f = \sum_{i=1}^n \beta_i \psi_i \quad (1.9)$$

it holds that

$$(\alpha_1, \dots, \alpha_n)P_c = (\beta_1, \dots, \beta_n). \quad (1.10)$$

We assume that (1.9) is true and we aim to show (1.10). For that purpose, we choose a basis $\{\psi_1, \dots, \psi_n\}$ of D with

$$\psi_k = \frac{\chi_k}{\langle \chi_k, \mathbb{1} \rangle_\mu}. \quad (1.11)$$

As $G(\mathcal{P})$ is a linear map, we have $G(\mathcal{P})f = \sum \alpha_i G(\mathcal{P})\psi_i$. We exploit this fact, as well as

the definitions of the Galerkin projection and the basis to compute

$$\begin{aligned}
G(\mathcal{P})f &= \sum_{k=1}^n \alpha_k G(\mathcal{P})\psi_k \\
&\stackrel{(1.7)}{=} \sum_{k,l,j=1}^n \alpha_k \hat{S}^{-1}(j,l) \langle \chi_j, \mathcal{P}\psi_k \rangle_\mu \chi_l \\
&\stackrel{(1.11)}{=} \sum_{k,l,j=1}^n \alpha_k \hat{S}^{-1}(j,l) \langle \chi_j, \mathcal{P}\psi_k \rangle_\mu \langle \chi_l, \mathbb{1} \rangle_\mu \psi_l \\
&\stackrel{(1.9)}{=} \sum_{l=1}^n \beta_l \psi_l.
\end{aligned}$$

Comparing the coefficients of the last two equations, we can express β_l as

$$\begin{aligned}
\beta_l &= \sum_{k,j=1}^n \alpha_k \hat{S}^{-1}(j,l) \langle \chi_l, \mathbb{1} \rangle_\mu \langle \chi_j, \mathcal{P}\psi_k \rangle_\mu \\
&= \sum_{k=1}^n \alpha_k \underbrace{\sum_{j=1}^n \hat{S}^{-1}(j,l) \langle \chi_l, \mathbb{1} \rangle_\mu \frac{\langle \chi_j, \mathcal{P}\chi_k \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu}}_{\stackrel{!}{=}(P_c)_{kl}}. \tag{1.12}
\end{aligned}$$

The underbraced term **should** be equal to $(P_c)_{kl}$ because we wish that (1.10) is fulfilled. Thus, we compute the (k,l) -th entry of $P_c = TS^{-1}$, employing the fact that $(S^{-1})_{jl} = (\hat{S}^{-1})_{jl} \langle \chi_l, \mathbb{1} \rangle$, as

notation!

$$\begin{aligned}
(TS^{-1})_{kl} &= \sum_{j=1}^n T_{kj} (S^{-1})_{jl} \\
&= \sum_{j=1}^n \frac{\langle \chi_j, \mathcal{P}\chi_k \rangle}{\langle \chi_k, \mathbb{1} \rangle} (\hat{S}^{-1})_{jl} \langle \chi_l, \mathbb{1} \rangle
\end{aligned}$$

and discover that it is equal to the underbraced term in (1.12). Hence, (1.10) is true and therefore P_c is the requested matrix representation of $G(\mathcal{P})$. \square

Theorem 1.18. *The matrices S and T from theorem 1.17 are stochastic.*

Proof. In order to be stochastic, each row must sum up to 1. We exploit the partition of unity property $\sum_j \chi_j = 1$ for all j and the aforementioned properties $\mathcal{P}\mathbb{1} = \mathbb{1}$ and $\mathcal{P}^*\mathbb{1} = \mathbb{1}$ of a transfer operator respectively its adjoint:

$$\begin{aligned}
\sum_{j=1}^n S_{kj} &= \frac{\langle \chi_k, \sum_j \chi_j \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu} = \frac{\langle \chi_k, \mathbb{1} \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu} = 1, \\
\sum_{j=1}^n T_{kj} &= \frac{\langle \sum_j \chi_j, \mathcal{P}\chi_k \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu} = \frac{\langle \mathbb{1}, \mathcal{P}\chi_k \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu} = \frac{\langle \mathcal{P}^*\mathbb{1}, \chi_k \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu} = 1.
\end{aligned}$$

Non-negativity follows from the non-negativity of the $\{\chi_1, \dots, \chi_n\}$. \square

Since S and T are both stochastic matrices, they have the constant vector $\mathbb{1}_D$ as right eigenvector to the eigenvalue 1. It implies that the same holds for P_c , that is its rows sum up to 1 and thus the product TS^{-1} is **at least pseudostochastic**. But nonnegativity is not assured since inverting S can provoke negative entries. The non-negativity depends on the choice of the partition of unity. As we will see, there are examples such that TS^{-1} is a stochastic matrix.

Theorem 1.19. *The matrix representation P_c from theorem 1.17 has the left eigenvector $\hat{\mu} \in D$ with the entries*

$$\hat{\mu}_j = \langle \mathbb{1}, \chi_j \rangle_\mu = \int_E \chi_j(x) \mu(dx).$$

Proof. We observe that $\hat{\mu}^T S = \hat{\mu}^T$ and $\hat{\mu}^T T = \hat{\mu}^T$ since

$$(\hat{\mu}^T S)_j = \sum_{k=1}^n \langle \mathbb{1}, \chi_k \rangle_\mu \frac{\langle \chi_k, \chi_j \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu} = \langle \mathbb{1}, \chi_j \rangle_\mu = \hat{\mu}_j$$

and

$$(\hat{\mu}^T T)_j = \sum_{k=1}^n \langle \chi_j, \mathcal{P}\chi_k \rangle_\mu = \langle \chi_j, \mathcal{P}\mathbb{1} \rangle_\mu = \langle \chi_j, \mathbb{1} \rangle_\mu = \hat{\mu}_j.$$

We can deduce that $\hat{\mu}^T P_c = \hat{\mu}^T TS^{-1} = \hat{\mu}^T S^{-1} = \hat{\mu}^T SS^{-1} = \hat{\mu}^T$. \square

Remark 1.20. We remark that in theorem 1.17 we stated the matrix representation of the projection of a **forward** transfer operator. For a backward operator, the proof works almost simultaneously and yields the matrix representation $P_c = S^{-1}T$, where $T = \frac{\langle \chi, \mathcal{P}\chi \rangle_\mu}{\langle \chi, \mathbb{1} \rangle_\mu}$. In that case, P_c is a right matrix representation and propagates sets instead of probability distributions.

This matrix decomposition will be of particular interest for our later investigations.

Example: Full Partition Discretization

Let A_1, \dots, A_n be a partition of the state space E , i.e. they are pairwise disjoint sets such that $\cup A_i = E$. We consider the family of the corresponding characteristic functions

$$\chi_i(x) = \mathbb{1}_{A_i}(x).$$

Since they are orthogonal, the matrix S is the identity matrix and therefore, the matrix representation of the Galerkin projection is $P_c = T$. We can compute it by combining

$$\langle \mathcal{P}\mathbb{1}_{A_i}, \mathbb{1}_{A_j} \rangle_\mu = \int_{A_j} (\mathcal{P}\mathbb{1}_{A_i})(x) \mu(dx) \stackrel{(1.5)}{=} \int_{A_i} p(t, x, A_j) \mu(dx) \stackrel{(1.3)}{=} \mathbb{P}_\mu(X_t \in A_j, X_0 \in A_i)$$

and

$$\langle \mathbb{1}_{A_i}, \mathbb{1} \rangle_\mu = \int_E \mathbb{1}_{A_i}(x) \mu(dx) = \mu(A_i) = \mathbb{P}_\mu(X_0 \in A_i).$$

The entries of the resulting matrix representation are given by

$$T_{ij} = \frac{\langle \mathcal{P}\mathbb{1}_{A_i}, \mathbb{1}_{A_j} \rangle_\mu}{\langle \mathbb{1}_{A_i}, \mathbb{1} \rangle_\mu} = \mathbb{P}_\mu(X_t \in A_j \mid X_0 \in A_i).$$

Thus, P_c represents a Markov chain whose state space consists of the partition sets A_i , e.g. each A_i is a “macro state” of the projected process. The stationary distribution of this Markov chain P_c is just the projection of the invariant measure μ onto D .

For a full partition discretization, the matrix S is a diagonal matrix. If we choose a partition of unity that is *close* to a full partition, i.e. we choose *almost characteristic functions*, then the matrix S is not diagonal, but close to that. We will later see the consequences of that fact regarding to the examination of the rebinding effect.

Properties of Galerkin Projection

As the matrix representation of a projected transfer operator is in general **not** a stochastic matrix, and stochastic matrices are in a one-to-one relation with Markov chains, we can immediately deduce that the process can lose its Markovianity by projecting it onto a subspace. This possible loss of Markovianity is certainly a really undesirable effect. But before examining that later in section 1.4, let us now first analyze further properties of the matrix representation P_c .

what about $S^{-1}T$?

We already know that the matrices S and T from Theorem 1.17 are stochastic matrices. This leads to some good properties of P_c :

- The eigenvalue $\lambda = 1$ of P_c has the associated right-eigenvector $e = (1, \dots, 1)^T$ and left-eigenvector $\hat{\mu}^T$ from theorem 1.19.
- If \mathcal{P} is self-adjoint in $L^2(\mu)$, then $G(\mathcal{P})$ as well. Then the matrices S and T are self-adjoint with respect to the discrete scalar product

P_c self-adj.?

$$\langle Av, w \rangle = \langle v, Aw \rangle$$

$$\langle u, v \rangle_{\hat{\mu}} = \sum_{i=1}^n u_i v_i \hat{\mu}_i.$$

Since self-adjointness of the operator is equivalent to reversibility of the corresponding process, see theorem 1.12, detailed balance equation (e.g. $\hat{\mu}_k T_{kl} = \hat{\mu}_l T_{lk}$ for all $k, l = 1, \dots, n$) is fulfilled for both S and T .

eigenv. of S, T real

- If the transfer operator has a simple and dominant eigenvalue 1 and the continuous part of the spectrum is bounded away from the discrete part, then the process is irreducible and aperiodic which is inherited by the matrix T . In particular T has the simple and dominant eigenvalue $\lambda = 1$ which is the only eigenvalue with $|\lambda| = 1$ and the discrete invariant density $\hat{\mu}$ is the unique invariant density of T .
- As seen in the last example, a full-partition projection yields the transition matrix $P_c = T$ of a Markov chain describing transitions between the partition sets.

The discretization inherits many important properties of the original process.

Interpretation

?

The matrix T is defined in terms of \mathcal{P} , while S is defined only by the partition of unity χ_i . Thus, the matrix T represents the propagation of the process and is also called “coupling matrix” as it describes how the clustered subsets are interacting.

If the partition of unity consists of characteristic functions, i.e. the χ_i are orthogonal, then the behaviour of the clustered process is completely determined by T . The larger the deviation from the χ_i to being orthogonal, the larger the deviation of the clustered process from T . This argumentation will be elaborated in chapter 2.

Projected infinitesimal generator

The Galerkin projection of an infinitesimal generator yields a similar matrix representation as the transfer operator. It can also be written as the product of two stochastic matrices, one of them being the inverted mass matrix of the partition of unity functions.

Theorem 1.21. (Schütte and Sarich[37])

Let $\mathcal{Q} : L^2(\mu) \rightarrow L^2(\mu)$ be a generator of a semigroup of transfer operators with unique invariant measure μ and satisfying $\mathcal{Q}\mathbb{1} = 0$. Let χ be a partition of unity with a projection G onto the associated subspace spanned by χ . Then the projected generator $G(\mathcal{Q})$ has the matrix representation $Q_c = RS^{-1}$ with the stochastic mass matrix S from (1.8) and

$$R(k, j) = \frac{\langle \mathcal{Q}\chi_k, \chi_j \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu}$$

The eigenvalue problem of Q is equivalent to the generalized eigenvalue problem $Ru = \Lambda Su$. For both Q and R the largest eigenvalue is $\lambda = 0$. The associated right eigenvector is $e = (1, \dots, 1)^T$, the associated left eigenvector is $\hat{\mu}^T$ from theorem 1.19.

The proof is similar to theorem 1.17 and equivalently, the matrix representation of the infinitesimal generator corresponding to the backward transfer operator is given by $Q_c = S^{-1}R$ with $R = \frac{\langle \chi_k, \mathcal{Q}\chi_j \rangle_\mu}{\langle \chi_k, \mathbb{1} \rangle_\mu}$.

There are obviously many possible Galerkin projections of a given transfer operator. We showed the example of a full-partition discretization, which results in a very easy matrix representation. But as arbitrary partitions of unity χ_1, \dots, χ_n are allowed for a Galerkin projection, there will be more variational results. In chapter 2 we are going to see which choice of χ results in a good discretization in the sense that it represents the correct long-time behaviour of the process in terms of so called *metastability*.

1.4 Recrossing Effect

In this section, we examine the so called recrossing effect which is one of the main topics of this thesis. This effect occurs when projecting a process onto a smaller state space and may spoil the Markov Property of the process.

We describe it by means of an easy example. Additionally, we have to face the problem that a projected transfer operator does **not** necessarily propagates as the original process.

We explain the relevance of this iteration error without going into further details, since in the following chapters we will be able to employ a projection where this error vanishes.

Initial Situation

Assume we are given a Markov process $(X_t)_{t \in \mathbb{T}}$ on a continuous or very large state space E , described by the transfer operator $\mathcal{P} := \mathcal{P}(\tau)$. In order to get a discrete process out of it, we project the time onto \mathbb{N} and the state space onto a finite set $\{1, \dots, n\}$. Discretizing the time can be done naturally without problems since for every lag-time $\tau > 0$, the process $(X_{k\tau})_{k \in \mathbb{N}}$ is again Markovian.

why?

However, the state-space discretization has to be observed a bit more elaborated. We do this on the example of a full partition discretization. We consider the operator $G(\mathcal{P}^k) := G\mathcal{P}^k G$, that is we first propagate the process and project it afterwards. Then for all k -multiples of τ , we assign the current state of the original process X_t to the projected process \tilde{X}_k :

$$\tilde{X}_k = i \Leftrightarrow X_{k\tau} \in A_i.$$

The process \tilde{X}_k describes the *snapshot dynamics* of X_t with lag time τ between the partition sets A_1, \dots, A_n . The so defined process is not necessarily Markovian, since $(G(\mathcal{P}^k))_k$ is in general **not** a semigroup, [].

?

Recrossing in a Double Well Potential

Let X_t be the Markov process corresponding to the double-well potential $V(x) = (x^2 - 1)^2$. We consider a full-partition of the state space into two sets A and B around the local minima of the energy landscape, as shown in figure 1.1. We are interested if the induced process \tilde{X}_k inherits the Markovianity of X_t or if it contains any memory effects.

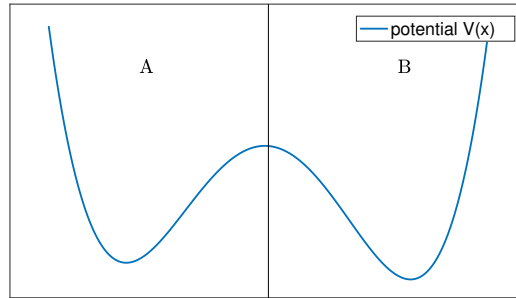


Figure 1.1: Full-partition of a double-well potential

For a small lag-time $\tau = 0.1$ we compute the probability of \tilde{X}_k to make a transition from B to A in one time-step. We compare it to the probability of the same transition with the **additional** information of having been in A one time-step before/earlier. If the process was Markovian, then this additional information about the past should make no difference and consequently, both probabilities should be equal. We compute them in

terms of the original process X_t by

$$\mathbb{P}_\mu[X_{(k+1)\tau} \in A \mid X_{k\tau} \in B] \text{ and } \mathbb{P}_\mu[X_{(k+1)\tau} \in A \mid X_{k\tau} \in B, X_{(k-1)\tau} \in A].$$

Using the density functions v_B and v_{BA} , we get

$$\mathbb{P}_\mu[X_{(k+1)\tau} \in A \mid X_{k\tau} \in B] = \int_A v_B^\tau(x) dx = \dots, \quad (1.13)$$

$$\mathbb{P}_\mu[X_{(k+1)\tau} \in A \mid X_{k\tau} \in B, X_{(k-1)\tau} \in A] = \int_A v_{BA}^\tau(x) dx = \dots \quad (1.14)$$

We see that for such a short lag-time τ , the process \tilde{X}_k is **not** independent of the past and hence **not** a Markov process. Equation (1.13) describes the probability to get from B to A , where “being in B ” could mean everything from “close to the transition region” to “far away from the transition region”. This probability is averaged over **all** possible starting points in B , since the spatial arrangement inside of B is not included in this model. We compare it to (1.14), where being in A shortly before being in B **increases** the probability to return to A again. This behaviour can be interpreted such that for a short time after a **transition**, the process is likely to be still inside of the **transition region**. In our example, the transition region is the area close to the maximum of potential energy. Thus, there still is an increased probability to return to the previous state, because of the spatial situation shortly after the transition.

This issue is called the *recrossing effect*, since additional memory leads to an increased probability to “recross” the energy barrier shortly after a transition. On the other hand, if we choose a large lag-time, e.g. $\tau = 100$, then the past transition from A to B in (1.14) took place a long time ago. In that case we do not know if the process is still in the critical transition region; during that long lag-time it could also have been gone anywhere else. That means that the memory effect included in \tilde{X}_k gets smaller for larger lag-times and consequently can be considered as a “short-time memory”.

Comparison to Markov State Model

After having observed the recrossing effect as a memory effect when projecting the time-series of a given continuous process onto a finite subspace, we want to compare that result to the corresponding *Markov State Model*.

So far, we considered the process \tilde{X}_k belonging to the operator $G(\mathcal{P}^k)$, that is the projection of a time-series of the original process. Now, let $(\hat{X}_k)_{k \in \mathbb{N}}$ be the Markov chain that is described by the transition matrix P_c , i.e. the matrix representation of the discretized transfer operator $G(\mathcal{P}) := GPG$.

A desirable behaviour of this model would be that \hat{X}_k and \tilde{X}_k have the same trajectory when started on the same initial distributions \hat{X}_0 and \tilde{X}_0 . It will turn out that this is normally not the case. This question is visualized in diagram 1.2. Does it make a difference if we project the propagated process or if we propagate the projected process?

In general, this diagram is **not** commuting and hence, in general we have

$$(P_c)^k \neq (P^k)_c.$$

$$\begin{array}{ccc}
\mathcal{P}(\tau) & \xrightarrow{\tau \rightarrow \tau k} & (\mathcal{P}(\tau))^k \\
\downarrow & & \downarrow \\
P_c(\tau) & \xrightarrow{\tau \rightarrow \tau k} & (P_c(\tau))^k
\end{array}$$

Figure 1.2: Projecting and propagating a transfer operator.

For the example of a full-partition discretization, we know from section 1.3 that the resulting Markov State Model is a Markov chain. Thus, the non-Markovian process \tilde{X}_k is modelled by a Markov chain \hat{X}_k . That is also where the term Markov State Model comes from.

Discretization Error

The maximal possible error between the distributions of \tilde{X}_k and \hat{X}_k after k time-steps is given by

$$E(k) = \|G(\mathcal{P}^k) - (G(\mathcal{P}))^k\|.$$

which norm?

Theorem 1.22. *Let \mathcal{P} be a transfer operator with the dominant spectrum $1 = \lambda_0 > \lambda_1 \geq \dots \geq \lambda_n$. Then the projection error can be bounded from above in terms of the second-largest eigenvalue by*

$$\|(G(\mathcal{P}))^k - \Pi_0\| \leq \lambda_1^k,$$

where Π_0 is the orthogonal projection of

For a proof, see Schütte and Sarich[37, p.72]. In the next chapter we demonstrate the deep relation between the spectrum of the transfer operator and the long-time behaviour of the process. We will see how to choose a convenient basis for a MSM such that the iteration error vanishes, i.e. the long-time behaviour of the original process is preserved. ?

Conclusion

We have to distinguish between two kind of “errors” that can occur:

- Rebinding Events: Projection can include some kind of memory effect
- Iteration Error: Deviation of $G(\mathcal{P}^k)$ and $(G(\mathcal{P}))^k$

2 Dominant Structures

With the Galerkin discretization, we introduced a method to reduce the dimension of a Markov process by projecting it onto a smaller state space. However we don't know yet how to choose a partition of unity such that this projection yields a reasonable Markov State Model, in the sense that important properties of the original process are maintained. Usually, the long-time behaviour of a process is of particular interest. It is often determined by a so called metastable behaviour. We will see why it makes sense to project a process onto its metastable sets and, in order to detect them, analyze their relation to the dominant spectrum of the transfer operator. We will also see that the optimal metastable decomposition is not sharp/crisp but soft/fuzzy.

Additionally, with the aid of the Schur decomposition we introduce a rather new approach to create a Markov State Model. In contrast to the spectral approach, it includes nonreversible processes and is even able to identify further structures.

2.1 Metastability

There exist several definitions of metastability. Shortly said, metastability is the property of a process to act on particular regions such that transitions between these regions are rare events while the durations of stay inside of each of them is rather long. Some possible characterizations of that behaviour are based on large hitting times or small exit rates, see Schütte and Sarich[37, chapter 3], where a good overview of the most common definitions can be found.

Mathematical concept of metastability

In order to describe the concept of metastability, it is a good way to start with so called *stable* or *invariant subsets*. A measurable subset $A \subset E$ of the state space of a Markov process X_t is called stable or invariant if it cannot be left, i.e. if $\mathbb{P}(X_t \in A \mid X_0 \in A) = 1$ for all t . Analogously, we can define a *metastable* or *almost invariant subset* as a subset in which the process will stay for a very long time before exiting it into any other subset, that is $\mathbb{P}(X_{t_f} \in A \mid X_0 \in A) \approx 1$ for a convenient timescale t_f . Thus, a full partition A_1, \dots, A_n of the state space E is called *metastable* if

$$\sum_{k=1}^n \mathbb{P}_\mu(X_{t_f} \in A_k \mid X_0 \in A_k) \approx n. \quad (2.1)$$

Then each of the sets A_k is almost invariant with respect to timescale t_f ; the probability to stay in one of the partition sets being started there is almost 1, while the probability to change between any two different partition sets is almost 0. Such a partition is also called a *metastable decomposition*.

Obviously, being “close to 1” or “close to n ” are rather vague statements. However, that lack of concreteness will be eliminated later, since we will only be interested in the “best” metastable decomposition. That means that we want to obtain a decomposition where the probability to stay inside of each metastable set is as close as possible to 1, resulting in the sum (2.1) being as close as possible to n . Likewise, the choice of the timescale t_f is not specified in general and will depend on the particular system in consideration. Hence, the only parameter in (2.1) that has to be determined is the number n of subsets we are looking for.

Metastability in molecular systems

Metastability is a very important concept for stochastic processes corresponding to molecular systems. Such processes describe the movement of atoms or molecules in space and have the characteristic behaviour to oscillate or fluctuate around equilibrium positions on the smallest time scales (about one femtosecond). In contrast to these fast oscillations, the process often stays inside of a certain region, called *conformation*, for a long time before switching to another region (nano- or millisecond time scale). Since transitions between conformations are relatively rare events, they can be identified as metastable sets if we choose a convenient timescale. Such a behaviour is depicted in figure 2.1 on the example of the dihedral angle of a molecule, taken from Weber[45].

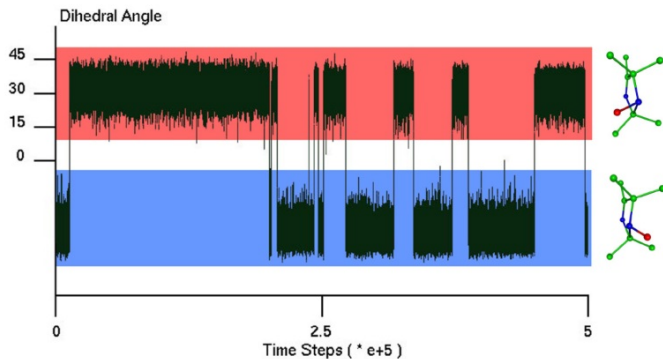


Figure 2.1: Example of a molecule with two (metastable) conformations. The dihedral angle of the molecule can take values between $+45^\circ$ and -45° . There are two regions, highlighted red and blue, where the process stays for a rather long time and oscillates **inside**. Transition between these regions don’t occur often. Thus, these two conformations can be identified as metastable sets.

As transitions between metastable sets are rare events, long-time simulations of a process are required in order to get informations about conformational changes. However long-time simulations of such large systems are not feasible in reasonable time even with the best computers nowadays, see Anton[38] or its successor Anton2[39].

Hence, in order to be able to compute some long-time simulations of a given molecular system, a reduction of complexity is needed. This can be achieved by a clustering of the state space via a Galerkin projection as depicted in section 1.3. Different states will be

clustered appropriately such that we get a process on a smaller state space.

This point of view also motivates the following terminology. A state in the original state space is called a *micro state*, as it is a state considered on the microscopic or atomistic level. In order to get a smaller state space, micro states are grouped together and such a cluster is called a *macro state*, since we are now considering the process on a macroscopic level (cannot distinguish between smaller states/atoms anymore).

For instance, the spatial coordinates of a single atom could be considered as a micro state, while the corresponding macro state is a cluster of several atoms. If we are working on this smaller state space, we cannot distinguish anymore between the single atoms of the cluster (forget information).

Clustering into metastable sets

The question **how** to cluster a given process such that it maintains the long-time behaviour of the original process can be answered easily with the following intuitive approach: As the long-time behaviour of a process is described by the metastability of the system, we choose the metastable sets as clustering sets. More clearly, we create a new process where each macro state corresponds to one of the metastable sets. In order to represent the correct long-time behaviour of the original process, the transition probabilities of the clustered process/reduced model should correspond to the transition rates between the metastable sets.

As metastability is determined on long timescales, the projected process maintains the long-time behaviour of the original process, but forgets about short-time transitions, i.e. transitions inside of a conformation/metastable set.

Since there is not one unique metastable decomposition of the state space, we need to find a decomposition which is in some sense “the best”; then we can use it to create a reduced model. In the next sections we will see how to find such a decomposition.

Most importantly, the clustered process will have the desired property of a reduced complexity since the model acts on a smaller state space. Therefore, the computation effort for long-time simulations is definitely decreased. Furthermore, we get a better overview of the system, since it is always easier to consider a process on a few states in comparison to a process on a very large or even continuous state space. However, it has to be guaranteed that the clustered process represents the **correct** long-time behaviour of the process. How this can be ensured will be explained in section 2.3.

2.2 Spectral Approach

In this section, we demonstrate the strong relation of the metastability of a Markov process to the spectrum of the associated transfer operator. More precisely, the existence of metastable sets implies the existence of dominant eigenvalues of the transfer operator and vice versa. The idea to detect metastable sets via dominant eigenvalues has been first proposed by Dellnitz and Junge[9] and transferred to molecular dynamics by Schütte et al[34, 35].

Existence of dominant eigenvalues

We consider the transfer operator $\mathcal{P} := \mathcal{P}(\tau)$ of a Markov process for some fixed lag-time τ in the Hilbert space $L^2(\mu)$. We are interested in *dominant eigenvalues* of \mathcal{P} , that is large eigenvalues which are close to 1 and separated from the rest of the spectrum. The *discrete spectrum* $\sigma_{\text{discr}}(\mathcal{P})$ is the set consisting of all eigenvalues $\lambda \in \sigma(\mathcal{P})$ that are isolated and of finite multiplicity. The *essential spectral radius* $r_{\text{ess}}(\mathcal{P})$ is defined as

$$r_{\text{ess}}(\mathcal{P}) = \inf\{r \geq 0 \mid \lambda \in \sigma(\mathcal{P}) \text{ with } |\lambda| > r \text{ implies } \lambda \in \sigma_{\text{discr}}(\mathcal{P})\}.$$

The existence of dominant eigenvalues requires that the continuous part of the spectrum is bounded away from the dominant elements of the discrete spectrum. To ensure that the process we are considering actually possesses metastable sets, we need to pose some conditions on the spectrum of the transfer operator:

C1 The essential spectral radius of \mathcal{P} is less than one, i.e. $r_{\text{ess}} < 1$.

C2 The eigenvalue $\lambda = 1$ of \mathcal{P} is simple and dominant, i.e.

$$\eta \in \sigma(\mathcal{P}) \text{ with } |\eta| = 1 \text{ implies } \eta = 1.$$

We will not go into further details for which processes the two above conditions are fulfilled; some criteria can be found in Huisinga[17, chapter 4]. Since we are interested in a metastable behaviour, we assume that the processes under investigation satisfy these conditions. We need condition **C1** to ensure that the continuous part of the spectrum is bounded away from the discrete eigenvalues. Otherwise they would not be dominant anymore and the process would be rather rapidly mixing than having any metastable sets. Condition **C2** however is important because the state space of a transfer operator with more than one eigenvalue of absolute value 1 can be decomposed into invariant sets, that is subsets which cannot be left. However, such a case is not interesting for us. Instead, we want to know more about **almost** invariant sets and their critical transition regions.

The transfer operator $\mathcal{P} : L^2(\mu) \rightarrow L^2(\mu)$ of a reversible process satisfying the properties **C1** and **C2** is self-adjoint by theorem 1.12 and has a spectrum of the form

$$\sigma(\mathcal{P}) \subset [a, b] \cup \{\lambda_n\} \cup \dots \cup \{\lambda_2\} \cup \{1\}$$

with $-1 < a \leq b < \lambda_n \leq \dots < \lambda_1 = 1$ and isolated, not necessarily simple eigenvalues of finite multiplicity that are counted according to multiplicity.

Relation of dominant spectrum to metastability

In order to find out about the quality of an arbitrary decomposition, we present upper and lower bounds for the metastability of the decomposition in terms of dominant eigenvalues and eigenvectors of the transfer operator. We will denote by *metastability of a decomposition* \mathcal{D} the sum of the metastability of its subsets.

Theorem 2.1. (Huisinga and Schmidt[18, Theorem 2.4])

Let \mathcal{P} be the transfer operator of a reversible process satisfying **C1** and **C2**. Let $\lambda_1, \dots, \lambda_n$ denote its isolated eigenvalues and v_1, \dots, v_n the corresponding eigenfunctions, normalized to $\|v_i\|_2 = 1$. Let Q be the orthogonal projection of $L^2(\mu)$ onto $\text{span}\{\mathbb{1}_{A_1}, \dots, \mathbb{1}_{A_n}\}$. The metastability of an arbitrary decomposition $\mathcal{D} = \{A_1, \dots, A_n\}$ of the state space E can be bounded from above by

$$p(A_1, A_1) + \dots + p(A_n, A_n) \leq 1 + \lambda_2 + \dots + \lambda_n,$$

while it is bounded from below by

$$1 + \rho_2 \lambda_2 + \dots + \rho_n \lambda_n + c \leq p(A_1, A_1) + \dots + p(A_n, A_n),$$

where $\rho_j = \|Qv_j\|_{L^2(\mu)}^2 = \langle Qv_j, Qv_j \rangle \in [0, 1]$ and $c = a(1 - \rho_1) \dots (1 - \rho_n)$.

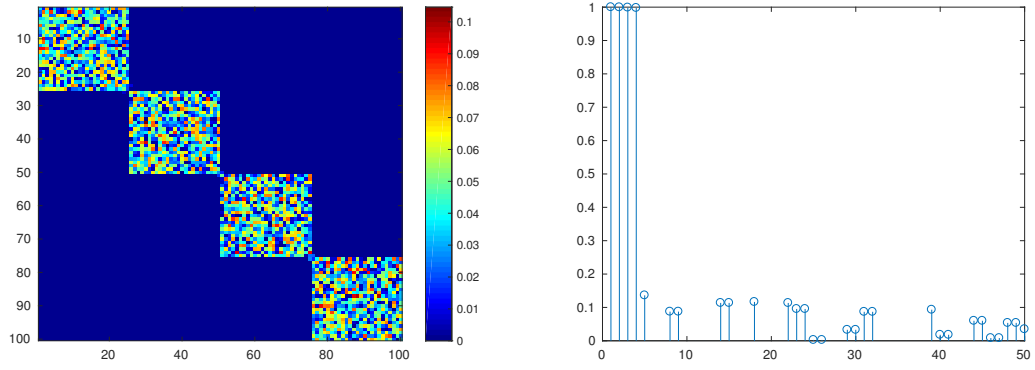
Theorem 2.1 reveals the strong relation between metastable sets and the dominant eigenvalues of the transfer operator. It allows us to evaluate the quality of a decomposition by comparing the lower and the upper bound of metastability. The upper bound shows that eigenvalues far away from 1 worsen the metastability of a decomposition. The lower bound is close to the upper bound if the dominant eigenfunctions v_2, \dots, v_n are almost constant on the metastable subsets A_1, \dots, A_n , implying $\rho_j \approx 1$ and $c \approx 0$. Moreover, Huisinga and Schmidt[18] show that the lower and upper bounds from theorem 2.1 are sharp and asymptotically exact.

That provokes the question if there exists an **optimal** decomposition with the highest possible metastability. By all means, this theorem indicates that the number of metastable sets should be determined by the number of dominant eigenvalues.

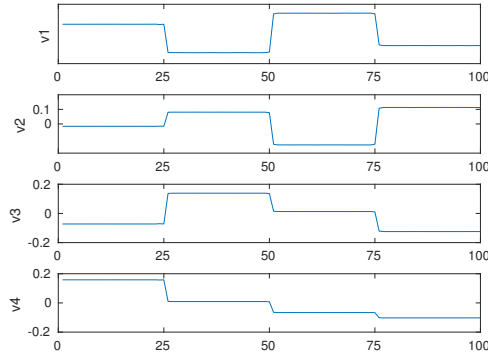
maybe ill-conditioned

Deuffhard et al[10] proposed an algorithm to identify the metastable sets of a Markov chain by exploiting the sign structure of the dominant eigenvectors of the transition matrix.

In figure 2.2, we get a good overview of the relation between the eigenfunctions of the transfer operator and the metastable sets of the corresponding process. We have a potential/energy landscape with 4 energy minima, that is 4 regions where the process could be *trapped* such that it is hard to get outside again. The transition matrix shows this metastable behaviour since we can see 4 regions which large probabilities to stay inside and very small probabilities to go to a different region. Furthermore we see that the process has 4 dominant eigenvalues. Three of them have a change of sign, inducing a metastable decomposition.



(a) Transition matrix P on 100 states with 4 visible metastable sets (b) Spectrum of P with 4 dominant eigenvalues



(c) Dominant eigenvectors of P with change of signs corresponding to the transition regions

Figure 2.2: Relation of the transition matrix to its spectrum with regard to metastability

This relation can be explained as follows. A process consisting of n invariant sets $\{A_1, \dots, A_n\}$ has the n -fold eigenvalue 1 and the corresponding eigenvectors $\mathbb{1}_{A_i}$ are constant on the invariant sets. A metastable process consists of almost invariant sets and thus, can be interpreted as the perturbation of a process with invariant sets, by introducing small transitions between the invariant sets. Accordingly, the eigenvalues and eigenvectors are perturbed. This results in one eigenvalue 1 and $n - 1$ eigenvalues close to 1, having eigenvectors that are almost constant on the almost invariant sets. A detailed perturbation analysis can be found in Deuffhard et al[10].

Disadvantages

The spectral approach is an appropriate method to cluster a process with respect to metastability, though it bears some disadvantages. The result is only applicable on reversible processes, because real eigenvalues are only guaranteed if the transfer operator is self-adjoint. Particularly, the previous procedure to compute a metastable decomposition

does not take into consideration the existence of transition regions. This can lead to an iteration error, i.e. the clustered process does not represent the correct long-time behaviour.

This section has been presented mainly with the aim to emphasize the strong relation of the spectrum of the transfer operator to the metastability of the system. However, this approach does **not** represent the state of the art. In the next section, we deduce an enhanced method, resulting in a **soft** clustering instead of a full partition decomposition.

2.3 Fuzzy Clustering

The above considerations result in a metastable full decomposition of the state space, assigning each state to **exactly** one of the partition sets. In fact, there exist better solutions, considering the fact that states in transition regions are adjacent to several conformations and therefore cannot be uniquely assigned to one of them. We introduce a more general method, allowing some “overlap” of the conformations.

Set-based vs. Function-based Approach

The intuitive approach to decompose the state space of a process is to determine a certain number of metastable sets which form a full partition, such that each state belongs to exactly one of the metastable sets. The problem with that approach is that likewise each state in a transition region has to be assigned to one of these partition sets. Though why would you assign a state in a transition region to one particular adjacent metastable set and not to another one? Such a strict assignment is obviously not an accurate description of the actual behaviour of the process.

Therefore this *set-based* clustering method has been replaced by a *function-based* method. That means that the states of the process are assigned with certain “degrees” to the conformations. This approach is justified by the existence of transition regions. A state inside of a transition region (around local energy maximum) can enter into different adjacent conformations with similar probabilities. Therefore, instead of assigning it to a single conformation, we define that it should belong to each of these adjacent conformations with a certain degree. In that sense, the conformations may be “overlapping”.

Membership Functions

Assume we are given a transfer operator having n dominant eigenvalues. Hence, we want to create a Markov State Model on n states, corresponding to the metastable sets of the process. We follow the approach of Weber[44] to define macro states as *overlapping partial densities*. They can be identified by membership functions that assign degrees of membership to the micro states.

Definition 2.2. (Membership Function)

The functions $\chi_1, \dots, \chi_n : E \rightarrow [0, 1]$ are called *membership functions* if they fulfill

- $\chi_j(x) \geq 0 \ \forall i \in E$ and $\forall j \in \{1, \dots, n\}$ (positivity),
- $\sum_{j=1}^n \chi_j(x) = 1 \ \forall x \in E$ (partition of unity).

The value $\chi_j(x)$ is the *degree of membership* of state x to macro state χ_j .

The example of a full-partition discretization corresponds to the choice of characteristic functions $\{\mathbb{1}_{A_1}, \dots, \mathbb{1}_{A_n}\}$ as membership functions. Each micro state is uniquely assigned to one of the partition sets, without any overlap. Therefore such a clustering is called *crisp* or *hard*, whereas the general, possibly overlapping, membership functions result in a *fuzzy* or *soft* clustering. As there are many possible membership functions, we need to find a choice that yields a reasonable metastable decomposition.

Usually they are chosen to be **close** to a characteristic function, also called *almost characteristic function*, as depicted in 2.3. This is a reasonable choice, since it puts the emphasis of a conformation on a certain region by assigning a high degree of membership, though likewise includes the adjacent transition regions by a low degree of membership. Thus, they fulfill the following two desired conditions:

- There should be a **soft** assignment inside of a transition region, in order to respect the ambiguous membership of transition states.
- The clustering should be **crisp** enough to distinguish the conformations.

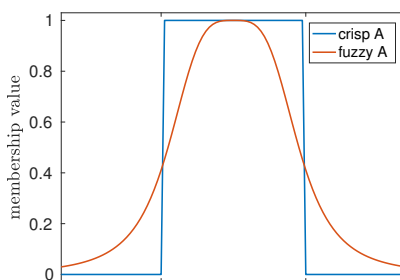
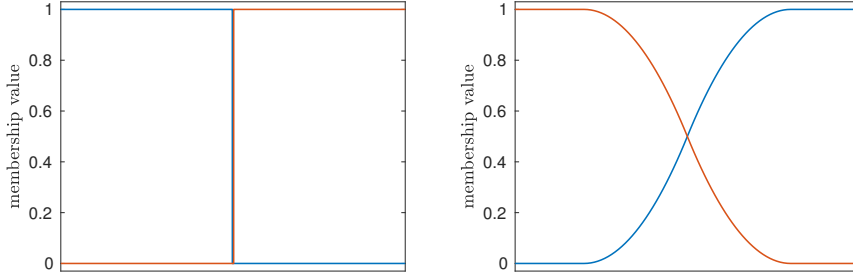


Figure 2.3: The crisp set A represented by a characteristic function is approximated by a fuzzy set represented by an “almost characteristic function”.

These requirements are clarified in figure 2.4 at the recurring example of a double-well potential, i.e. a system consisting of two conformations with one transition region between them. A crisp clustering does not consider the transition region, while a “very fuzzy” choice of membership functions does not represent the conformations.

The degrees of membership are no actual probabilities, yet they can be interpreted as such. Consider for instance the energy maximum of a symmetric double well potential. This transition state tends with the same probability to the left and to the right well. Therefore it seems plausible to assign it with the same degree of membership to both conformations. On the other hand, a state in the middle of a well cannot immediately jump into the other well, therefore this transition probability is 0 and the state can be assigned with degree of membership 1 to the conformation corresponding to its well.

For each conformation $j \in \{1, \dots, n\}$, there is a membership function χ_j which determines the portion of the partial density with respect to the total density function. They



(a) Characteristic functions resulting in a hard clustering. (b) Almost characteristic functions resulting in a soft clustering.

Figure 2.4: Possible membership functions: From hard to fuzzy clustering.

form a partition of unity, in order to sum up to the total density. In the following, the χ_j will also be denoted as the conformation j they represent.

In order to represent the conformations as almost invariant sets, almost characteristic functions should be almost eigenfunctions of the transfer operator:

$$\mathcal{P}\chi_j \approx \chi_j \quad \forall j = 1, \dots, n.$$

Matrix Representation of Projection

Having determined the shape of the membership functions, we can apply the Galerkin projection as defined in section 1.3. If we choose almost characteristic functions representing the (fuzzy) conformations, then the resulting Markov State Model consists of the metastable sets of the original process. We remember the matrix representation $P_c = S^{-1}T$ of a clustered process. The trace of the coupling matrix T is referred to as *metastability* of the conformations $\{\chi_1, \dots, \chi_n\}$.

We recall that a full-partition decomposition yields a matrix S being equal to the identity matrix, justified by the orthogonality of the characteristic functions. With the motivation to choose almost characteristic functions as membership functions, the χ_i are still close to being orthogonal and therefore, S should still be close to the identity matrix, at least diagonal dominant.

Thus, we have the coupling matrix T , representing the dynamical behaviour of the process. If S is close to the identity matrix, then the dynamics of the projected process is completely determined by T . In contrast, if S deviates from identity, then it rather influences the dynamics.

What is the meaning of S ? The entries are defined by the scalar products of the membership functions. Thus, the *crispness* of a clustering can be measured by the matrix S . Nonoverlapping membership functions yield an overlap matrix S equal to the unit matrix. Overlapping membership functions result in a matrix with non-zero outer diagonal elements. The higher the outer diagonal elements, the higher the overlap. Therefore the diagonal of S can be seen as a “measure of crispness” of the χ_1, \dots, χ_n .

Each macro state yields a statistical weight

$$w_j = \langle \chi_j, \mathbb{1} \rangle_\mu = \int_E \chi_j(x) \mu(dx),$$

describing the “portion” of a membership function to the total density function. We remark that the statistical weight vector $w = (w_1, \dots, w_n)$ coincides with the left eigenvector of the matrix representation P_c of the clustered process, see theorem 1.19. The diagonal matrix $D = \text{diag}(w_1, \dots, w_n)$ consists of the statistical weights of the membership functions. Then $S = D^{-1} \langle \chi, \chi \rangle_\mu$ and $T = D^{-1} \langle \chi, \mathcal{P}\chi \rangle_\mu$, compare theorem 1.17.

Perron Cluster Analysis

The term *Perron Cluster Analysis* denotes the objective of clustering a Markov process into metastable sets using the *Perron eigenvalues* respective *Perron eigenfunctions*, being eigenvalues close to 1 and the corresponding eigenfunctions. Perron Cluster Analysis respectively its algorithmic implementation PCCA (“Perron Cluster Cluster Analysis”) has been developed by Deuffhard et al[10], employing the sign structure of the dominant eigenvalues of the transition matrix. This has been improved by Deuffhard and Weber[11] who transformed the system of eigenvectors into a system of membership functions resulting in a fuzzy clustering of the state space of the original process; their algorithm is called PCCA+ (“Robust Perron Cluster Analysis”). Originally, PCCA+ was formulated only for discrete Markov chains, but Weber[45] extended it even on continuous processes.

Let $\mathcal{P} := \mathcal{P}(\tau)$ on $L^2(\mu)$ be the transfer operator describing a **reversible** process, that is \mathcal{P} is μ -self-adjoint, see theorem 1.12. We consider the set of dominant eigenvalues $\{\lambda_1, \dots, \lambda_n\}$ with the corresponding set of eigenfunctions $\mathcal{X} = \{\mathcal{X}_1, \dots, \mathcal{X}_n\}$. They fulfill the eigenvalue problem $\mathcal{P}\mathcal{X} = \mathcal{X}\Lambda$ of the transfer operator \mathcal{P} , where $\Lambda = \text{diag}(\lambda_1, \dots, \lambda_n)$. The set of membership functions $\chi = \{\chi_1, \dots, \chi_n\}$ can be built as a linear combination $\mathcal{X}\mathcal{A}$ of the dominant eigenfunctions, that is

$$\chi_j(x) = \sum_{i=1}^n \mathcal{A}_{ij} \mathcal{X}_i(x), \quad j = 1, \dots, n. \quad (2.2)$$

Here, $\mathcal{A} = \{\mathcal{A}_{ij}\}_{i,j=1,\dots,n} \in \mathbb{R}^{n \times n}$ is a real matrix which has to be chosen in such a way that the resulting membership functions χ fulfill the positivity and partition of unity constraints. As there are infinitely many such transformations \mathcal{A} of the eigenfunctions, we have to determine one that satisfies some optimality condition. The algorithm PCCA+ computes the transformation matrix \mathcal{A} as the solution of a **convex** maximization problem, see Weber[44]. With the resulting membership functions, the Galerkin projection is

$$P_c(\tau) = G(\mathcal{P}(\tau)) = (\langle \chi, \chi \rangle_\mu)^{-1} (\langle \chi, \mathcal{P}(\tau)\chi \rangle_\mu). \quad (2.3)$$

Weber[45] shows that for any linear combination of the eigenfunctions $\chi = \mathcal{X}\mathcal{A}$, the discretization error of the Galerkin projection vanishes. Hence diagram 1.2 commutes, implying that propagating and projecting of a transfer operator are commutative actions. In particular, such membership functions preserve the Markov property.

Theorem 2.3. (Weber [45, Theorem 2])

Let $\mathcal{P} := \mathcal{P}(\tau)$ be a μ -self-adjoint transfer operator with a set $\mathcal{X} = \{\mathcal{X}_1, \dots, \mathcal{X}_n\}$ of normalized eigenfunctions s.t. $\mathcal{P}\mathcal{X} = \mathcal{X}\Lambda$, where $\Lambda = \text{diag}(\lambda_1, \dots, \lambda_n)$ is the eigenvalue matrix. Let $\chi = \mathcal{X}A$ be a set of functions that is a linear combination of the eigenfunctions \mathcal{X} with a regular $n \times n$ -transformation matrix A as defined in (2.2). Then the iteration error for the Galerkin discretization $P_c = G(\mathcal{P})$ vanishes.

Proof. The Galerkin projection of the transfer operator \mathcal{P} is computed by

$$\begin{aligned} G(\mathcal{P}) &\stackrel{(2.3)}{=} (\langle \chi, \chi \rangle_\mu)^{-1} (\langle \chi, \mathcal{P}\chi \rangle_\mu) \\ &\stackrel{(2.2)}{=} (A^T \langle \mathcal{X}, \mathcal{X} \rangle_\mu A)^{-1} (A^T \langle \mathcal{X}, \mathcal{P}\mathcal{X} \rangle_\mu A) \\ &= (A^T \langle \mathcal{X}, \mathcal{X} \rangle_\mu A)^{-1} (A^T \langle \mathcal{X}, \mathcal{X} \rangle_\mu \Lambda A) \\ &= (A^T A)^{-1} (A^T \Lambda A) \\ &= A^{-1} \Lambda A. \end{aligned}$$

In particular, after k time-steps we get

$$(G(\mathcal{P}))^k = (A^{-1} \Lambda A)^k = A^{-1} \Lambda^k A = G(\mathcal{P}^k).$$

The last two lines are obtained by inserting the eigenvalue problem $\mathcal{P}\mathcal{X} = \mathcal{X}\Lambda$ and employing the μ -orthogonality of the eigenfunctions, by theorem 1.11, and therefore $\langle \mathcal{X}, \mathcal{X} \rangle_\mu = \mathcal{I}$, being the identity operator. \square

orthogonal by s
metrization trick?

In this proof, it is essential that the process is reversible. If the process is non-reversible, then the transfer operator is not self-adjoint and possibly possesses complex eigenvalues, leading to complex-valued eigenfunctions, which can not be transformed into meaningful membership functions. Thus, in order to tackle non-reversible processes as well, an enhanced method is presented in section 2.4.

Even though the algorithm of PCCA+ is valid for continuous processes defined by a transfer operator \mathcal{P} , for real applications a discretization of \mathcal{P} to a matrix P is necessary:

$$\mathcal{P} \rightarrow P \rightarrow P_c.$$

One approach for that are direct sampling methods, counting transitions between subsets. However, since long-time simulations are required in order to obtain valuable informations about transition between metastable sets, they are not the best choice. Another option are adaptive sampling methods, for instance Voronoi tessellation, see Weber[45]. Having a discrete matrix P , it is easy to compute the eigenvalues and eigenvectors in order to apply PCCA+ to get a clustered matrix P_c . In this thesis, we circumvent this first discretization step, by directly examining finite matrices P . ?

Objective Function: Crispness of Membership Functions

The matrices S and T from the matrix representation P_c can be expressed as

$$\begin{aligned} T &= D^{-1} \langle \chi, \mathcal{P}\chi \rangle_\mu = D^{-1} A^T \Lambda A \quad \text{and} \\ S &= D^{-1} \langle \chi, \chi \rangle_\mu = D^{-1} A^T A. \end{aligned} \tag{2.4}$$

Different objective functions are possible, some are proposed in Weber[44, Chapter 3.4]. Originally, one objective was to maximize metastability by maximizing $\text{trace}(T)$. In the context of stochastic matrices, a high trace corresponds to a high determinant, since $\text{trace}(T)$ is bounded by above from n and $\det(T)$ by 1. This upper bound is achieved only for the case of the identity matrix, thus for a “strong diagonal”. Trying to increase the trace to being close to n is the same as increasing the determinant to being close to 1. Since the trace is not multiplicative, we resort to the determinant to calculate the following relation:

$$\begin{aligned}\det(T) &= \det(S) \det(A^{-1} \Lambda A) \\ &= \det(S) \det(\Lambda) \\ &= \det(S) \prod_{i=1}^n \lambda_i.\end{aligned}$$

In order to obtain a high metastability of the system, both factors need to be high. The term $\det(\Lambda)$ is high if the dominant eigenvalues λ_i are as close as possible to 1, whereas $\det(S)$ is maximized if the linear combination $\chi = \mathcal{X}\mathcal{A}$ is as **crisp** as possible. That means that they are as orthogonal as possible, having only few overlap. Since S is a stochastic matrix as well, maximizing its determinant is equivalent to maximizing its trace.

Thus the choice of maximizing $\text{trace}(S)$ as objective function for PCCA+ is plausible, since it provides a clustering with high metastability, while the metastable sets are well distinguishable because of the crispness. This was proposed by Röblitz[28]. Moreover, trace is a **convex** function, which is a necessary criterion for the objective function of PCCA+:

$$\max_{A \in \mathbb{R}^{n \times n}} \text{trace}(S) \text{ such that } \chi = XA \geq 0 \text{ and } \sum_j \chi_{ij} = 1. \quad (2.5)$$

Example

2.4 Schur Decomposition

All previous projections of processes onto metastable sets were based on the assumption of **reversibility**. Unfortunately, many real-world processes are **not** reversible. In that case the corresponding transfer operator is not self-adjoint and therefore we have to deal with possibly complex eigenvalues and eigenfunctions/eigenvectors, which is not compatible with PCCA+. This problem can be circumvented by considering the real Schur decomposition of the transition matrix instead of its spectral decomposition. This yields an invariant subspace of real Schur vectors on which we can apply PCCA+. This approach is feasible, since the real Schur vectors span the same subspace as the corresponding complex Schur vectors and those span the same subspace as the corresponding eigenvectors.

Beyond enabling us to analyze non-reversible processes, this new approach yields several more advantages, like allowing us to identify a broader class of structures, including dominant cycles or sinks.

see ...
?

NESS processes

The method we present in this section is only employable for finite processes. Therefore we consider in the following an irreducible and aperiodic (i.e. ergodic) Markov chain on the finite state space $E = \{1, \dots, N\}$ given by the transition matrix P . By irreducibility and aperiodicity, the process possesses a unique invariant measure π being positive everywhere. Then π is the normalized eigenvector of P for the unique eigenvalue $\lambda_1 = 1$.

μ ?

Definition 2.4. (NESS process)

A Markov process is called *nonequilibrium steady state (NESS)* process if it is nonreversible, but still has a steady state, given by an invariant measure π , such that the process is ergodic with respect to π .

As a NESS process is nonreversible, there are regions where the detailed balance equation is not fulfilled, i.e. there is an effective probability flow $p(\tau, A, B) - p(\tau, B, A) \neq 0$ between some subsets $A, B \subset E$ of the state space.

Definition 2.5. (Flow Matrix)

The probability flow associated to a Markov process is given by the flow matrix

$$F = DP,$$

where P is the transition matrix of the process and D the diagonal matrix $D = \text{diag}(\pi_1, \dots, \pi_N)$ with the entries of the invariant measure π .

So the (steady state) probability flow from state i to j is given by $F_{ij} = \mu_i P_{ij}$. If the process is reversible, the flow matrix F is symmetric due to detailed balance. For a NESS process, F is not symmetric since there are states $i, j \in E$ with $F_{ij} \neq F_{ji}$.

Schur Decomposition

We have the same objective as in the previous section: given a Markov process acting on a large state space E , a projection onto a smaller state space $\{1, \dots, n\}$ is aimed at, where each cluster represents one metastable set of the process. For this purpose, we introduce a matrix decomposition generalizing the spectral decomposition.

Definition 2.6. (Schur Decomposition)

Let $P \in \mathbb{R}^{N \times N}$ be a transition matrix. Then it can be written as

$$X^{-1}PX = \Lambda, \quad (2.6)$$

where X is a unitary matrix and Λ is an upper triangular matrix, having $\lambda_1, \dots, \lambda_N$ as diagonal entries, which is called a *Schur decomposition* of P . If $X = [v_1 \mid \dots \mid v_N]$ is a column partitioning of X , then the v_i are referred to as *Schur vectors*.

The existence of such a matrix X is shown in Golub and van Loan[16, Theorem 7.1.3]. Since Λ is similar to P , both matrices have the same eigenvalues. Since Λ is triangular, they correspond to the diagonal entries $\lambda_1, \dots, \lambda_N$ of Λ . The Schur vectors v_k satisfy

$$Pv_k = \lambda_k v_k + \sum_{i=1}^{k-1} n_{ik} v_i, \quad k = 1, \dots, N,$$

and therefore span an invariant subspace given by

$$S_k = \text{span}\{v_1, \dots, v_k\}.$$

Moreover, if we choose a matrix $X_k = [v_1 \mid \dots \mid v_k]$, then $\sigma(X_k^{-1}PX_k) = \{\lambda_1, \dots, \lambda_k\}$. The eigenvalues λ_i in (2.6) can be arbitrarily ordered by an appropriate choice of X . Thus each subset of k eigenvalues induces at least one k -dimensional invariant subspace.

For our purpose, decomposition (2.6) is not sufficient, since it can include complex Schur vectors. As P is a real matrix, its non-real eigenvalues come in complex conjugate pairs. This fact can be utilized to build a *real Schur decomposition*, where both X and Λ are real matrices. Though this alternative decomposition does not yield a triangular matrix, but only a *quasi-triangular* one, allowing 2×2 -blocks on its diagonal.

Theorem 2.7. (Real Schur Decomposition)

If $P \in \mathbb{R}^{N \times N}$, then there exists an orthogonal matrix $X_s \in \mathbb{R}^{N \times N}$ such that

$$X_s^{-1}PX_s = \Lambda_s,$$

where Λ_s is block-triangular with 1×1 and 2×2 -blocks on its diagonal. The 1×1 -blocks contain the real eigenvalues of P and the eigenvalues of the 2×2 -blocks are the complex eigenvalues of P .

A proof can be found in Golub and van Loan[16, Theorem 7.4.1]. Each real matrix can be decomposed into such an upper quasi-triangular matrix. Thus the real Schur decomposition can be considered as an “eigenvalue-revealing” decomposition; the real and the imaginary part of the complex eigenvalues are easily obtained from the 2×2 -blocks.

Block-diagonal structure

We want to make use of the real Schur decomposition to analyze transition matrices. Depending on the shape of the quasi-triangular matrix Λ_s , we can not only find out informations about the eigenvalues of P , but also on the reversibility of the corresponding process. A case study is presented in Weber[46] and shortly summarized in the following.

By definition, the reversibility of a process is determined by the flow matrix $F = DP$. According to detailed balance, a process is reversible if and only if this matrix is symmetric. However, nonreversibility of P can as well be identified by its Schur decomposition Λ_s .

A Schur decomposition is block-diagonal with eigenvalues and 2×2 -blocks on its diagonal. The meaning of an 1×1 -block as an eigenvalue is clear, so we are just going to analyze the shape of such an 2×2 -block and its influence on reversibility and eigenvalues of P . Assume we are given the Schur decomposition

$$\Lambda_s = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \boxed{\lambda_2 \quad \epsilon} \\ 0 & -\delta & \lambda_3 \end{pmatrix} \quad (2.7)$$

with $\epsilon, \delta \geq 0$. We examine the properties of this matrix (eigenvalues, reversibility) by inserting different values for ϵ and δ in the 2×2 -block.

Reversible If $\epsilon = \delta = 0$ and furthermore $\lambda_2 \neq \lambda_3$, then Λ_s is a diagonal matrix and P is reversible. In this case, the eigenvalues are identical to the Schur values, $\Lambda_e = \Lambda_s$, and the eigenvectors are identical to the Schur vectors, $X_e = X_s$.

Reversibility of P is equivalent to the symmetry of Λ_s (+ geometric and algebraic multiplicities should correspond; no double eigenvalues).

Nonreversible with real eigenvalues If we add an additional upper diagonal element $\epsilon > 0$, then we make Λ_s asymmetric. The eigenvalues of P correspond to the entries $\lambda_1, \dots, \lambda_n$ on the diagonal of Λ_s . The eigenvectors of P are real, but **not** π -orthogonal anymore. In contrast to that, the Schur decomposition still leads to π -orthogonal Schur vectors X_s .

Non-reversibility of P can be seen by the fact that the Schur matrix Λ_s is not symmetric. ?

Nonreversible and not diagonalizable If $\lambda_2 = \lambda_3$ with $\epsilon > 0$ and $\delta = 0$, then P has an “incomplete” 2×2 -block, meaning that it has an eigenvalue whose geometrical multiplicity does not correspond to its algebraic multiplicity. Therefore, P does not have an eigendecomposition and is not diagonalizable.

Since P is not diagonalizable, it is non-reversible.

While the eigendecomposition does **not exist**, the Schur decomposition still exists.

Nonreversible with complex eigenvalues If $\lambda_2 = \lambda_3$ and additionally $\epsilon, \gamma > 0$, then we have a “complete” 2×2 -block, encoding the existence of two **complex eigenvalues**.

The complex-valued spectrum implies the non-reversibility of P . However, the Schur decomposition still yields **real** Schur vectors.

Summarized, there are several problems that can appear when dealing with the eigendecomposition of non-reversible processes. The existence of an eigendecomposition is not always given (not diagonalizable matrix). Moreover it can happen that the eigenvectors are not π -orthogonal or contain complex entries, which is problematic for the use of PCCA+. However, we can circumvent these issues by using the real Schur vectors instead.

GenPCCA: Clustering in terms of Schur vectors

The aim is to cluster a given process P into metastable sets based on membership functions $\chi = XA$. However, the application of PCCA+ requires certain conditions to be fulfilled in order to yield a meaningful projection, which is in general not the case for eigenvectors of a non-reversible process. A solution replacing them by Schur vectors has been proposed by Fackeldey and Weber[13]. Their approach is a generalization of PCCA+ and therefore called Gen-PCCA (“Generalized-Perron Cluster Cluster Analysis”).

Looking back to the proof of theorem 2.3, we identify two conditions implying a correct projection, i.e. a projection without discretization error. The matrix $X \in \mathbb{R}^{N \times n}$ has to span an invariant subspace by

$$PX = X\Lambda \quad (2.8)$$

for $\Lambda \in \mathbb{R}^{n \times n}$. Furthermore, the orthogonality relation

$$X^T D_\eta X = I, \quad (2.9)$$

has to be satisfied with respect to some initial distribution η .

These properties are in general not fulfilled for the eigendecomposition of a nonreversible process, as we can see from a comparison with the previous case study. For a non-reversible process, the eigenvectors can be complex or non-orthogonal. Moreover, it is not even guaranteed that P is diagonalizable.

However, real Schur vectors for a stochastic matrix P can always be constructed such that they satisfy both criteria. In order to do so, the following symmetrization trick can be applied. If \tilde{X} are n Schur vectors of $\tilde{P} = D^{0.5}PD^{-0.5}$, then we get

$$\begin{aligned} \tilde{P}\tilde{X} &= \tilde{X}\Lambda \\ \Leftrightarrow D^{0.5}PD^{-0.5}\tilde{X} &= \tilde{X}\Lambda \\ \Leftrightarrow PD^{-0.5}\tilde{X} &= D^{-0.5}\tilde{X}\Lambda \\ \Leftrightarrow PX &= X\Lambda, \text{ with } X = D^{-0.5}\tilde{X}. \end{aligned}$$

Since Schur vectors of a symmetric matrix are always orthogonal, the same holds for its multiplication with a diagonal matrix. Thus, we are guaranteed that X fulfill conditions (2.8) and (2.9). A further advantage in comparison to the previous section is, that by this trick, orthogonality can be achieved for **any** initial distribution η . However, since we

assume NESS processes, having a stationary distribution π , we can as usual just employ orthogonality with respect to π .

By this procedure, we obtained a set of orthogonal vectors X spanning an invariant subspace and we can apply PCCA+ to them in order to obtain an optimal transformation matrix A . Gen-PCCA can be summarized as follows:

- i) Compute a real Schur decomposition (\tilde{X}, Λ_s) of the symmetrized matrix $\tilde{P} = D^{0.5} P D^{-0.5}$. Then $X = D^{-0.5} \tilde{X}$ fulfills the conditions (2.8) and (2.9).
- ii) Sort the Schur values and the 2×2 -blocks by using SRSchur by Brandts[4] such that they are in a descending order of their absolute value. Pick the dominant Schur values.
- iii) Determine the submatrix $X(:, 1 : n)$ of n dominant Schur vectors and apply PCCA+ in order to determine the membership functions χ .

Metastable Cycles

Besides metastable sets, nonreversible processes can also contain metastable/dominant cycles. They are different dominant structures representing a cyclic behaviour of the process. Kalpazidou[19], Djurdevac Conrad et al[12].

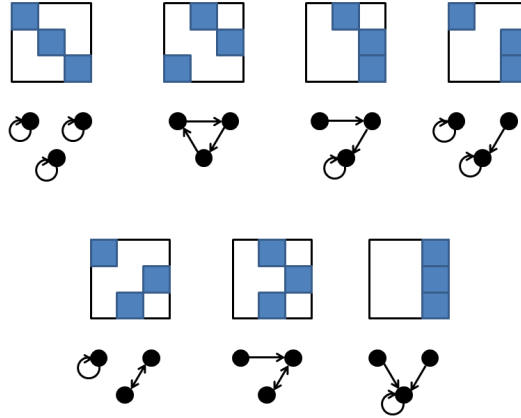


Figure 2.5: Possible structures that can be revealed by GenPCCA.

GenPCCA+ is not only able to identify metastable sets, but also cycles or mixtures of both structures, as depicted in figure 2.5. They can be detected since we consider the dominant eigenvalues and Schur blocks with respect to the absolute value. Dominant cycles correspond to large eigenvalues $|\lambda| \approx 1$. sinks?

Conclusion

Even though the Schur decomposition is known for a long time, being described by Schur[33] in 1909, the approach to utilize it for identifying metastable sets is relatively new. There is a huge amount of scientific papers using eigenfunctions or eigenvectors to cluster a process into metastable sets. The entire framework of Markov State Models is built on the spectral analysis of the transfer operator respectively transition matrix; at the beginning in terms of hard sets, nowadays in terms of fuzzy sets. Since the Schur decomposition is a generalization of the spectral decomposition, it seems to be an appropriate enhancement of this well-known clustering method. Its main advantages are:

- Schur decomposition **always** exists,
- includes reversible as well as **non-reversible** processes,
- orthogonality with respect to **any** initial distribution η ,
- detects not only metastable sets, but also **dominant cycles** and **sinks(?)**,
- Schur decomposition is **well-conditioned**.

In contrast, the disadvantages are not too dramatic:

- Schur decomposition is not unique,
- Does not exist for a continuous transfer operator.

The main point in employing the Schur decomposition was to extend PCCA+ to non-reversible processes, which is already a strong improvement. Beyond that, this approach brings several further advantages along, being more flexible than the original PCCA+ and allowing to identify a broader class of structures. Though, these different structures are not of particular interest in this thesis.

3 Rebinding Effect in a Given Kinetics

In this chapter, we examine receptor-ligand-systems, a special type of molecular systems, consisting of so called receptors and ligands. These molecules can interact in such a way that under certain conditions they can bind to each other and afterwards dissociate again. Such a system is originally Markovian and can be described by a transfer operator. However, by projecting this operator onto a finite-dimensional state space, the Markov property of the process may be spoiled.

We explain this memory effect, the so called rebinding effect, and examine its influence on the stability of a receptor-ligand-system. We show how this effect can be measured with tools known from chapter 1. In particular, we present a lower bound for the rebinding effect included in a given system as the solution of an optimization problem.

This chapter highly complies with Weber and Fackeldey[48]. Additionally, we introduce some fundamental definitions and notations from biochemistry, which are necessary for the understanding of receptor-ligand-systems. The importance of such systems is illustrated by an outlook of its application in drug design.

3.1 Receptor-Ligand System

We present a particular molecular system, the *receptor-ligand-system*, and model it mathematically using a differential equation. We discuss the so called rebinding effect and set it in relation to the recrossing effect known from section 1.4. As a motivation, we explain its relevance in the application of drug design.

Molecular Dynamics vs Molecular Kinetics

A molecular system consists of molecules, that are atoms which are connected by *covalent bonds*. The motion inside of such a system can be characterized in different ways. The term *molecular dynamics* denotes the analysis of a **single** trajectory and is mainly employed in the context of simulations. It means that one initial configuration of the system is fixed and its evolution in time is observed. One example is depicted in figure 2.1. It represents **one** trajectory of a stochastic system. This can give an insight about the structure of the system, like identifying possible metastabilities. However, it is not representative in the sense that a second simulation could yield a completely different trajectory. In contrast to that, in *molecular kinetics*, an **ensemble** of trajectories is considered. Accordingly, it is formulated in terms of densities, concentrations or transition rates. However, these quantities are related to the molecular dynamics approach as they represent an average of many single realizations of a process. ?

Receptors and Ligands

references

In biochemistry, a *receptor* is a molecule, often a protein, that is usually located on the surface of a cell and can receive signals from outside the cell. A molecule that has the ability to *bind* or *associate* to a receptor is called a *ligand*. Each receptor will only bind with ligands of a particular structure, which is often referred to as the “key-lock principle”. Both receptor and ligand need to have specific complementary geometric shapes that fit exactly into one another, as exemplarily depicted in figure 3.1.

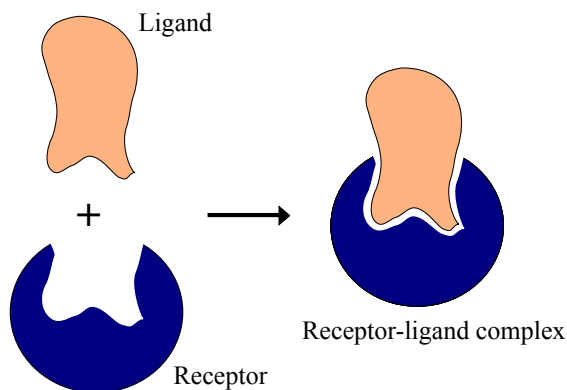


Figure 3.1: Ligand (“key”) binds to a receptor (“lock”). Their shapes fit together.

Such a binding between a receptor and a ligand can *activate* (“unlock”) the receptor by producing some kind of a chemical signal and thereby provoke a physiological response. For instance, that could be a conformational change in a protein, caused by a hormone binding to it. However, instead of engaging in the actual physiological consequences of a binding, we focus on the **act** of binding events.

The action of binding is typically reversible¹ through *dissociation* of the involved receptor and ligand. Ligand binding is a *chemical equilibrium* process, which means that the reaction rates of the binding and dissociating events are equal, once this equilibrium is reached. From then on, the concentrations of the reactants (ligands) and the products (complexes) are constant. It is a *dynamic equilibrium*, since reactions take place, even though no net change in the concentrations can be observed.

The binding behaviour of a simple receptor-ligand system is formalized as follows. A ligand (L) can bind to a receptor (R) and form a receptor-ligand complex (LR) which can dissociate again into its original components. This process can be represented by a reaction equation



Being a process in chemical equilibrium, the law of mass action states that the ratio between the concentration of reactants and products is constant. The corresponding

¹We remark that in this context, *reversible* means that a ligand can bind and unbind to a receptor, i.e. the reaction can run forward and backward. In contrary to the mathematical reversible, which means that a process behaves **equally** when running backwards in time.

dissociation constant k_d is given by

$$k_d = \frac{[L] \cdot [R]}{[LR]},$$

where $[L]$ is the concentration of unbound ligands, $[R]$ is the concentration of unoccupied receptors and $[LR]$ is the concentration of receptor-ligand complexes. This constant is used to describe the *binding affinity* between a ligand and a receptor, that is how strongly/tightly the ligand can bind to his particular receptor. If the dissociation constant is small, then there are relatively many complexes in comparison to unbound molecules, and for this reason, the binding affinity between the ligand and the receptor is high. The *association constant* k_a is just the inverse of the dissociation constant

$$k_a = \frac{[LR]}{[L] \cdot [R]}.$$

There are different factors which can influence the binding affinity of a process. It depends on the nature of the constituent molecules, like their shape, size and possible charge. The binding affinity of a particular ligand-protein interaction can also change significantly with solution conditions (e.g., temperature, pH and salt concentration).

In general, high-affinity binding results in a higher degree of occupancy for the ligand at its receptor binding site than is the case for low-affinity binding; the residence time (lifetime of the receptor-ligand complex) does not correlate. ?

Mathematical Model of Receptor-Ligand-System

Starting from the reaction equation (3.1), we can deduce that the ligand can be found in two different macro states: “unbound” (L) or “bound” (LR). Then the probabilities of the ligand to be in one of these states can be described by the probability vector $x^T = \frac{1}{s}([L], [LR])$, where $s = [L] + [LR] = \text{const.}$ is the normalization constant. This leads to an ordinary differential equation

$$\dot{x}^T = x^T Q_c.$$

The matrix Q_c consists of the rates of reaction,

$$Q_c = \begin{pmatrix} -k_a[R] & k_a[R] \\ k_d & -k_d \end{pmatrix},$$

where k_a and k_d are the association and dissociation constants. It corresponds to the transition rate matrix of a Markov chain, that means it describes a **memoryless** process. We will later see that this mathematical description of a receptor-ligand-system is not accurate, since in fact, such a process **will** have some kind of memory.

The two possible macro states for the easiest case of a ligand-binding-system consisting of one receptor and one ligand are depicted in figure 3.1. We notice that the spatial arrangement of the receptor and the ligand in the unbound case is **not** included in the above model. Therefore, we cannot distinguish if, at a given time, the receptor and the ligand are close to each other or not.

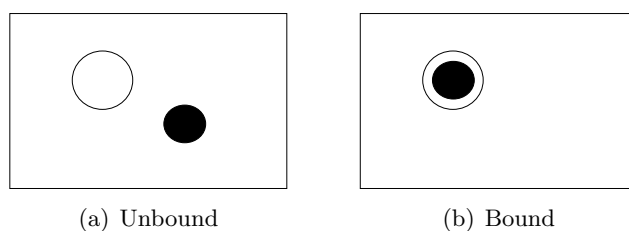


Figure 3.2: Two possible macro states of a ligand-binding system

Rebinding Effect

In fact, a stochastic process describing a receptor-ligand system is **not** necessarily Markovian. That is due to the spatial arrangement of the system after the dissociating of a receptor-ligand-complex took place. Shortly after such a dissociating, it is more likely that the corresponding receptor and ligand will bind again, since they are still close to each other. Such a binding shortly after being dissociated is called a **rebinding**. The memory effect which thereby occurs is called **rebinding effect**. On large timescales, this effect will vanish since the favorable spatial situation is not necessarily given anymore and the system will be rather mixed again. Thus, Markovianity can be spoiled by the rebinding effect. It is depicted in figure 3.1.

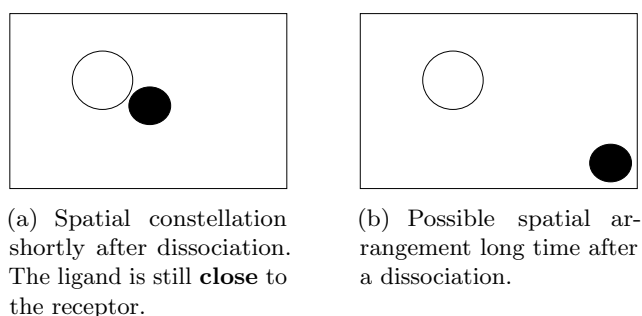


Figure 3.3: Rebinding Effect. Two configurations of a system consisting of one receptor and one ligand, represented by the same macro state (“unbound”).

The rebinding effect and its occurrence in natural science has been described and analyzed by several authors[15, 43]. In chemistry, it has been discussed in the context of clustered receptors and clustered ligands[6]. A mathematical description of the rebinding effect has been realized by Weber et al[47, 48].

The rebinding effect has been discussed to increase the binding affinity of a process.

Rebinding Effect vs Recrossing Effect

The characterization of the rebinding effect reminds us of the recrossing effect, as described in section 1.4. There, we considered the projection of a possibly continuous process onto a finite state space. This projected process (MSM) was described by a

transition matrix, thus being a Markov chain, even though the process actually contained a (short-time) memory. The same phenomena occurs with the rebinding effect. We have a process which is **modelled** by a Markov chain, even though the process actually **has** a memory. We can interpret the two states of the ligand-binding-system as macro states resulting from the projection of a process on a larger state space (for instance, including more informations about the spatial situation of the receptor and the ligand). In this case, the rebinding effect originates from the loss of information caused by the projection and therefore, qualitatively corresponds to the recrossing effect.

it. error vs reb. eff.

Thus, the rebinding effect coincides with the recrossing effect in the special context of a receptor-ligand-system. The different nomenclatures are justified by the use in their original context. While the term recrossing effect denotes the act of **recrossing** an energy barrier, the term rebinding effect denotes the **rebinding** of two molecules in a receptor-ligand-system. Such a system can also be represented by a potential energy function, having energy minima around the bound (closest possible distance) and unbound state (farthest possible distance).

In chapter 2, we learned that a fuzzy clustering should be chosen instead of a hard one, in order to yield a valid projection. Therefore, we are going to include the spatial situation of the system by introducing degrees of membership, which can be interpreted as “intermediate” states such as “almost bound”. In this sense, an unbound state with a high degree of membership to the bound state can be interpreted as a ligand being **close** to a receptor, for instance shortly after dissociating.

In the next sections, we are going to quantify this effect by embedding the molecular system and its projection into the mathematical framework established in the first two chapters.

Application: Drug Design

The term *drug design* denotes the development of new medications based on the knowledge of a biological target, playing the role of the receptor. Drug design is basically about designing a molecule which is complementary in shape and charge to the biomolecular target and therefore will bind to it, see Strømgaard et al[41]. More precisely, drug design describes the design of ligands, that is molecules that will bind tightly to the given target, see Tollenaere[42]. In general, we can distinguish between the following two most common functionalities of drugs.

- **Activators** are able to activate, or even deactivate, a receptor and result in a strong biological response. An example for such a drug is morphine, which acts directly to the central nervous system, mimics the actions of endorphins and thereby reduces pain.
- **Inhibitors** bind to a receptor without activating it. Though, as they “block” the binding sites of receptors, they prevent possibly disease causing particles to bind. A well-known example are protease inhibitors, a class of antiviral drugs that are widely used to treat HIV and hepatitis C.

Independently of the fact whether a drug activates or inhibits receptors, a high binding affinity is required in order to be an efficient drug. The central dogma of receptor pharmacology (“occupation theory”) is that a drug effect is directly proportional to the number of receptors that are occupied. Furthermore, a drug effect ceases as a drug-receptor complex dissociates. Thus, a low binding affinity needs to be compensated by a higher concentration of ligands. Though, high concentrations should be avoided, because of possible side effects. Accordingly, the most fundamental goal in drug design is to predict whether a given molecule will bind to a target and if so how strongly.

3.2 Molecular Kinetics as a Projection

We will deduce a rigorous description of molecular systems, in particular receptor-ligand-systems, basically by embedding the mathematical concepts from chapter 1 into an adequate physical context. When considering such systems, we can distinguish between two points of view: we will see how we can get from the *microscopic* or *atomistic* to a *macroscopic* scale by a projection.

Micro States

A micro state of a molecular system with N atoms can be represented in a $6N$ -dimensional *phase space* $\Gamma = \Omega \times \mathbb{R}^{3N}$, consisting of the *configuration space* $\Omega = \mathbb{R}^{3N}$ and the *momentum space* \mathbb{R}^{3N} . In the following, we consider systems in *thermodynamical equilibrium*. One possible model is given by the *Boltzmann distribution* $\pi : \Omega \times \mathbb{R}^{3N} \rightarrow \mathbb{R}$, a probability distribution assigning to each micro state a probability depending on its energy and temperature, see McQuarrie[23]. It can be expressed as

$$\pi(q, p) = \frac{1}{Z} \exp(-\beta H(q, p)), \quad (3.2)$$

where $\beta = 1/(k_B T)$ is the inverse of the temperature T multiplied with the Boltzmann constant k_B and $Z = \int_{\Gamma} \exp(-\beta H(q, p)) d(q, p)$ is the normalization factor. The Hamilton function denoted by H is given by $H(q, p) = K(p) + V(q)$, the sum of the kinetic energy $K(p)$ and the potential energy $V(q)$. Thus, the Boltzmann distribution π can be decomposed into $\pi = \pi_p \pi_q$,

$$\pi(q, p) = \underbrace{\frac{1}{Z_p} \exp(-\beta K(p))}_{\pi_p} \cdot \underbrace{\frac{1}{Z_q} \exp(-\beta V(q))}_{\pi_q},$$

where $\pi_p : \mathbb{R}^{3N} \rightarrow \mathbb{R}$ is the probability density function of the kinetic part in the momentum space \mathbb{R}^{3N} and $\pi_q : \Omega \rightarrow \mathbb{R}$ is the probability density function of the potential part in the configuration space Ω . As we are interested in examining conformations, that are objects in configuration space, we will restrict ourselves to Ω , see Huisinga[17]:

“A conformation $C \subset \Omega$ will be identified with the particular metastable sub-ensemble $\mu_{C \times \mathbb{R}^{3N}}$ corresponding to the particular subset $C \times \mathbb{R}^{3N} \subset \Gamma$. Hence, for every position $q \in C$, the conformation contains all states with $q \in \Omega$ and arbitrary $p \in \mathbb{R}^{3N}$.”

In this sense, conformations contain no information on momenta and are determined in configuration space only. We consider a reduced model with the *reduced density* $\pi_q = \int_{\mathbb{R}^{3N}} \pi(q, p) \, dp$.

Macro States via Membership Functions

As the configuration space of an average molecular system is very large, we aim to reveal the underlying discrete Markov State Model by clustering a collection of the micro states having the same or similar values in one observable. Such a collection of micro states will be called a *macro state*. For instance, that could be the states “bound” or “unbound” for a simple receptor-ligand system.

We apply the function-based fuzzy clustering method presented in section 2.3. We define macro states as **overlapping** partial densities, which can be identified as membership functions $\chi_1, \dots, \chi_n : \Omega \rightarrow [0, 1]$. They form a partition of unity, that is

$$\sum_{i=1}^n \chi_i(q) = 1.$$

By grouping micro states, the corresponding macro states yield statistical weights

$$w_i = \langle \chi_i, \mathbb{1} \rangle_\pi := \int_{\Omega} \chi_i(q) \pi_q(q) \, dq, \quad (3.3)$$

corresponding to the “probability for the system to **be** in conformation χ_i ”.

Transfer Operator

Each micro state $(q, p) \in \Gamma$ determines a *probability density function* $\Psi^{-\tau}(\cdot \mid (q, p))$ describing the possible evolutions of the system in configuration space Ω in time τ , having started at the initial state (q, p) . Weber[45] defines a transfer operator $\mathcal{P}(\tau) : L_{\pi_q}^{1,2}(\Omega) \rightarrow L_{\pi_q}^{1,2}(\Omega)$ acting on membership functions via

$$\mathcal{P}(\tau)f(q) = \int_{\mathbb{R}^{3N}} \left(\int_{\Omega} f(\tilde{q}) \Psi^{-\tau}(\tilde{q} \mid (q, p)) \, d\tilde{q} \right) \pi_p(p) \, dp. \quad (3.4)$$

In this definition, the density function $\Psi^{-\tau}(\cdot \mid (q, p))$ can be interpreted as a transition function as defined in section 1.1. We notice that this operator corresponds to a **backward** transfer operator as introduced in section 1.2.

It is a *generalized* transfer operator in the sense that it includes deterministic as well as stochastic dynamical models. In order to describe deterministic dynamics, the density function $\Psi^{-\tau}$ has to be chosen as a Dirac delta function, since an initial state $(q(0), p(0))$ determines exactly the future states in configuration space.

It is important to remark that the transfer operator $\mathcal{P}(\tau)$ also defines a projected Markov operator $\overline{\mathcal{P}}(\tau)$ acting in configuration space Ω , see Weber[45], by

$$\overline{\mathcal{P}}(\tau) = \pi_q \circ \mathcal{P}(\tau) \circ (\pi_q)^{-1}, \quad (3.5)$$

which propagates density functions and thus corresponds to a **forward** transfer operator. The previous equation shows that the space of membership functions is connected to the space of density functions by multiplication with π_q . We will keep that relation in mind, though just use \mathcal{P} in the following.

As $\mathcal{P}(\tau)$ in (3.4) propagates **membership functions**, stationarity is characterized by the equation $e = \mathcal{P}(\tau)e$ for the constant function $e = 1$ in Ω . For the Markov operator $\overline{\mathcal{P}}(\tau)$ in (3.5) propagating **densities**, stationarity can be characterized by $\pi = \overline{\mathcal{P}}(\tau)\pi$, where π is the Boltzmann density. These two operators are adjoint operators. This can also be seen by the fact that a discretization of $\mathcal{P}(\tau)$ results in a matrix P_c , while a discretization of $\overline{\mathcal{P}}(\tau)$ will result in the transposed matrix P_c^T .

Markov State Model for reversible Processes

For now, we consider a reversible process. In that case, the corresponding transfer operator $\mathcal{P} := \mathcal{P}(\tau)$ is **self-adjoint** with respect to π_q and thus has a real spectrum with $\sigma(\mathcal{P}) \subset [-1, 1]$. In order to apply the spectral approach from section 2.2, we assume that the **discrete spectrum** of the transfer operator \mathcal{P} has n **dominant eigenvalues** $1 = \lambda_1 \geq \lambda_2 \geq \dots \geq \lambda_n$ which are all close to 1 and bounded away from the essential spectrum. The corresponding dominant eigenfunctions are denoted by $\mathcal{X} = \{\mathcal{X}_1, \dots, \mathcal{X}_n\}$ and therefore the eigenvalue problem is given by $\mathcal{P}\mathcal{X} = \mathcal{X}\Lambda$, with the eigenvalue matrix $\Lambda = \text{diag}(\lambda_1, \dots, \lambda_n)$.

As we know from chapter 2, the number of metastable sets of a Markov process is determined by the number of dominant eigenvalues; thus we are going to create a Markov State Model on n states. The state space of this model should consist of the conformations of the molecular system in consideration and its transition behaviour should be described by a $n \times n$ -transition matrix $P_c := P_c(\tau)$. In order to obtain this discrete matrix from the continuous operator \mathcal{P} , we need at first to determine the size and shape of the membership functions χ_i . As described in section 2.3, this can be done by computing a linear combination of the dominant eigenfunctions via

$$\chi_j(q) = \sum_{i=1}^N A_{ij} \mathcal{X}_i(q), \quad j = 1, \dots, n, \quad (3.6)$$

where $A = \{A_{ij}\}_{i,j=1,\dots,n}$ is the solution of PCCA+ (convex maximization problem). As a linear combination of (orthogonal) eigenfunctions, the membership functions χ_i might have an overlap; they are not orthogonal, but span the same subspace as the \mathcal{X} .

A Markov State Model is created by applying the Galerkin discretization

$$P_c(\tau) = G(\mathcal{P}(\tau)) = (\langle \chi, \chi \rangle_\pi)^{-1} (\langle \chi, \mathcal{P}(\tau) \chi \rangle_\pi). \quad (3.7)$$

Theorem 2.3 implies that there is no discretization error under this projection, that is we have $G(\mathcal{P}^k) = (P_c)^k$. In particular, Markovianity is preserved. We can use the matrix representation $P_c(\tau) = S^{-1}T$ from theorem 1.17. Then S and T are stochastic matrices with

$$\begin{aligned} T &= D^{-1} \langle \chi, \mathcal{P}(\tau) \chi \rangle_\pi = D^{-1} A^T \Lambda A \quad \text{and} \\ S &= D^{-1} \langle \chi, \chi \rangle_\pi = D^{-1} A^T A, \end{aligned} \tag{3.8}$$

where $D = \text{diag}(w_1, \dots, w_n)$ is the diagonal matrix of statistical weights from (3.3).

Measuring the Rebinding Effect

We remember the interpretation of the matrix representation $P_c = S^{-1}T$ of the Markov State Model from section 2.3. The stochastic matrix T determines the long-time behaviour of the clustered process, yet the Markov State Model differs from T by

$$SP_c(\tau) = T.$$

This “deviation” of the Markov State Model $P_c(\tau)$ from the stochastic matrix T can be measured by the matrix S . If S is equal to the identity matrix, then the Markov State Model is solely determined by T . If S is close to the identity matrix, then $P_c(\tau)$ is close to T and not strongly influenced by the overlap matrix S . The more the matrix S differs from the identity matrix, the more the Markov State Model $P_c(\tau)$ differs from the transition matrix T . This is due to the recrossing/rebinding events. The larger this deviation, the larger the occurring memory effects. Thus, the rebinding effect, introduced in section 3.1 as a **memory effect** provoked by a projection, can be measured by the matrix S .

These observations correspond to our previous results from the examination of the recrossing effect in section 1.4 and the qualitative description of the rebinding effect in section 3.1. The more **overlap** occurred in the membership functions, the more the matrix S deviates from being the identity matrix (higher outer diagonal elements) and thereby includes more memory effects. ?

Thus, the rebinding effect can be measured by the trace of the matrix S , the sum of its diagonal elements. It can lie between 0 (very much overlap) and n (diagonal matrix). This approach has been introduced by Weber and Fackeldey[48] and will be used in the next chapter to detect the minimal rebinding effect included in a system.

Since PCCA+ maximizes the crispness of S , we can assume that it induces a small rebinding effect.

wrong?

Infinitesimal Generator to transition rate matrix

Often it is more convenient to analyze transition rate matrices instead of transition matrices. Thus, we consider the infinitesimal generator \mathcal{Q} , which is defined from $\mathcal{P}(\tau)$ via

$$\mathcal{Q} = \lim_{\tau \rightarrow 0} \frac{\mathcal{P}(\tau) - \mathcal{I}}{\tau}.$$

Moreover, these two operators are related by

Chapman

$$\mathcal{P}(\tau) = \exp(\tau \mathcal{Q}).$$

Weber[45] shows that such an infinitesimal generator exists for a discretization in terms of membership functions. Since the eigenfunctions of \mathcal{Q} and \mathcal{P} are the same and their

eigenvalues are related via $\exp(\xi_i) = \lambda_i$, we can apply the same Galerkin Projection for the infinitesimal generator as for the transfer operator. We get a $n \times n$ -rate matrix

$$Q_c = A^{-1}\Xi A = (\langle \chi, \chi \rangle_\pi)^{-1}(\langle \chi, Q\chi \rangle_\pi), \quad (3.9)$$

where Ξ is the diagonal matrix consisting of the n leading eigenvalues $0 = \xi_1 > \xi_2 \geq \dots \geq \xi_n$ of Q and A is the transformation matrix of (3.6), which analogously transforms the eigenfunctions of Q into membership functions of the macro states. The clustered matrix Q_c can be interpreted as a transition rate matrix.

3.3 Minimizing the Rebinding Effect

So far, we were mainly concerned to compute the projection of a large (continuous) process and, of particular interest, to analyze how such a projection introduces memory effects in the clustered process. However, in most of the cases, we don't know the continuous transfer operator describing a system. Instead, we are often given a finite matrix, for instance stemming from experimental data. In either case, such a matrix can be **interpreted** as a projection, since it is basically a model for an originally continuous process (movement of molecules in \mathbb{R}^3).

Assume we are in the situation that we only know the projected process. Nevertheless, we would like to know how much rebinding is included in that system, originating from the (unknown) projection. Since we don't know on which membership functions the projection is based on, we can only compute an estimation for that. Considering all possible membership functions, how much rebinding is **at least** included in the system?

We showed that the overlap matrix S from (3.8) provides a measure for the quantity of the rebinding effect. In particular, being close to the identity matrix implies a low rebinding, while high outer diagonal elements of S result in a high rebinding effect. However, we don't know yet the actual influence of this effect on the system. From its qualitative description, we assume that it slows down the process, in the sense that conformational changes occur less frequently. In order to verify that, we set the rebinding effect in relation to the stability of the system. Afterwards we formulate an optimization problem in order to deduce a lower bound for the rebinding effect.

For the sake of simplicity, we assume in the further course that the transition rates can be measured experimentally. Accordingly we examine the given transition rate matrix Q_c of a process.

Influence of Rebinding to Stability

If the eigenvalues $\xi_i \in (-\infty, 0]$ of Q_c are close to 0, then the macro states are very stable in the sense that the probability to stay inside of such a state is close to 1. The trace of Q_c corresponds to the sum of the dominant eigenvalues of Q . Thus, we can measure the *stability* of the molecular system by the quantity $F := -\text{trace}(Q_c) \in (0, \infty)$. If F is close to 0, then the system is very stable, while it is less stable for a high value of F . We want to set the stability F in relation to the measure of the rebinding effect, the matrix S .

Lemma 3.1. *Let Q_c be the projected infinitesimal generator of a process and $P_c(\tau)$ the corresponding projected transfer operator with the matrix representation $P_c(\tau) = S^{-1}T$, then the quantity $F := -\text{trace}(Q_c)$ can be measured by*

$$F = \tau^{-1}(\log(\det(S)) - \log(\det(T))), \quad (3.10)$$

if we assume that T is diagonal dominant (metastable).

Proof. We use the trace formula [1, p. 208] for matrices $\exp(\text{trace}(A)) = \det(\exp(A))$, the fact that Q_c “generates” $P_c(\tau)$, theorem 1.17 and multiplicativity of determinants to obtain

$$\exp(\tau Q_c) = P_c(\tau)$$

$$\begin{aligned} F &= -\text{trace}(Q_c) \\ &= -\tau^{-1} \log(\exp(\text{trace}(\tau Q_c))) \\ &= -\tau^{-1} \log(\det(\exp(\tau Q_c))) \\ &= -\tau^{-1} \log(\det(P_c(\tau))) \\ &= \tau^{-1}(\log(\det(S)) - \log(\det(T))). \end{aligned}$$

This expression is well-defined. By positive definiteness of the Gram matrix, the determinant of S is in $(0, 1]$. The diagonal dominance of T is a natural property for a metastable process and ensures us a determinant of T in $(0, 1]$. \square

Interpretation: Relevance of the Rebinding Effect

Before interpreting the result of lemma 3.1, we recall the **meaning** of the stochastic matrices S and T . The coupling matrix T describes the stochastic movement of the process and encodes the metastable behaviour of the system. Large diagonal elements result in a strong metastability and a slow process, while higher outer diagonal elements lead to faster transitions between the metastable sets. On the other hand, the overlap matrix S merely includes informations about the crispness of the membership functions, implying the magnitude of the rebinding effect.

Lemma 3.1 shows that **both** determinants of S and T influence the stability of the system, though in opposite directions. If $\det(T)$ is close to 1, then F is low and consequently the process is rather stable. If $\det(T)$ is close to 0, then the process is rather unstable, since F is high. These relations are expected and correspond to the observations from section 2.3; a high determinant of T leads to a high metastability of the system and thus describes a slower process, while a low determinant implies higher outer diagonal elements of T and thus, makes the process faster.

If $\det(S)$ is close to 1, then the first term in (3.10) vanishes and hence, S barely contributes to the stability, which is instead mainly determined by T . On the other hand, if $\det(S)$ is close to 0, the system becomes more stable. That means that a higher overlap of the membership functions, and thus a **strong rebinding effect**, leads to a more stable process. This relation is not obvious at first sight, yet corresponds to the qualitative description of the rebinding effect from section 3.1.

Why is this result counter-intuitive? At first sight, it sounds plausible to equalize the stability of a system to its slowness. A slow system (strong metastability) implies a stable

system. However, a stable system does **not** necessarily imply a slow system. Instead, a fast system (weak metastability) can obtain a certain stability by the rebinding effect. Why that? The fast system has many transitions between its metastable sets. However, because of a strong rebinding, the quitting of a metastable set can with high probability be followed by a fast returning to the metastable set. Thus, the rapidness of the process can to a certain extent be compensated by the rebinding effect.

Concluding, we can differentiate between two kinds of systems with a high stability:

- $\det(T)$ high: The system has a high metastability and well-separated metastable sets. Therefore, transitions between the metastable sets are rare (slow process).
- $\det(S)$ low: This leads to a high rebinding effect, making the process more stable. Transitions out of a metastable set can be compensated by a fast transition back. In particular, an originally rapidly mixing process, $\det(T) \ll 1$, can be stabilized by the rebinding effect.

A stable system is naturally reached by a strongly metastable matrix T , though can likewise be obtained for a weaker metastable matrix T , if a lot of rebinding is included.

Lower bound for the rebinding effect

What is the meaning of this influence of S to the stability of the system? The rebinding effect causes rebinding events and thereby increases the stability. Thus, in order to determine the stability of a given system, it is of interest to know how much rebinding is included. We compute a lower bound to find out how much rebinding there is **at least**.

Let us first of all remember how S is determined. A transfer operator \mathcal{P} is projected onto a finite-dimensional state space via membership functions χ_i . These membership functions are computed as a linear combination of the eigenfunctions of \mathcal{P} with a regular matrix A . Thus, the choice of the matrix A determines S , respectively the size of its determinant. In order to estimate the rebinding effect included in a system, we take into consideration all possible, *feasible*, matrices A .

We formulate an optimization problem to reveal which choice of A results in the **lowest** rebinding effect, measured by an *optimal matrix* S_{opt} . This problem is equivalent to finding the largest possible determinant of S .

Optimization Problem: Maximizing determinant of S

Since Q_c has the same eigenvalues as Q , the eigenvalue problem of Q_c is given by

$$Q_c X = X \Xi, \quad (3.11)$$

where the first column of X corresponds to the first eigenvector $X_1 := (1, \dots, 1)^T$. By (3.9), we see that A^{-1} is an eigenvector matrix of Q_c as well. Therefore the columns of A^{-1} consist of multiples of the eigenvectors X_i , yielding

$$A^{-1} = \begin{pmatrix} 1 & & & \\ \vdots & \alpha_2 X_2 & \cdots & \alpha_3 X_3 \\ 1 & & & \end{pmatrix}$$

with $\alpha_2, \dots, \alpha_n \in \mathbb{R}$. We know from lemma 3.1 that a $\det(S)$ close to 1 results in a low rebinding effect. Thus, in order to find a lower bound for the rebinding effect, we try to maximize $\det(S)$, or equivalently minimize $|\det(S) - 1|$, since S is a stochastic matrix having 1 as largest possible determinant. Then the *objective function* of the optimization problem is given by

$$\min_{\alpha_1, \dots, \alpha_n \in \mathbb{R}} |\det(S) - 1|, \quad (3.12)$$

where we have to include several *side constraints*. As the inverse matrix A^{-1} consists of linear combinations of eigenvectors X_i , we have to consider

$$\alpha_1 = 1 \quad \text{and} \quad A_{ij}^{-1} = \alpha_j X_{ij} \quad \forall i, j.$$

Furthermore, S is a stochastic matrix, see theorem 1.18, and its structure is given in terms of the linear transformation matrix A , so we have two further constraints

$$S = D^{-1} A^T A \quad \text{and} \quad S_{ij} \geq 0 \quad \forall i, j.$$

A *feasible solution* of this optimization problem is a matrix S fulfilling all side constraints, but not necessarily being an optimum.

Interpretation

In section 3.2, we explained how the matrix S comprises the *overlap* of the membership functions. For this reason, any feasible solution of optimization problem (3.12) will be called a *real overlap matrix* S_{real} , while an actual optimum will be called an *optimal overlap matrix* S_{opt} . Clearly, we get $\det(S_{\text{real}}) \leq \det(S_{\text{opt}}) \leq 1$.

The real occurring rebinding effect is high if the determinant of S_{real} is low. Thus, a small determinant of S_{opt} implies a high rebinding effect, while a large determinant of S_{opt} gives us only few information about the actual quantity of the rebinding effect, it could be either large or small. Unfortunately, a reversible process Q_c yields a trivial solution of optimization problem (3.12) and therefore, provides us with no information, as the following theorem shows.

Theorem 3.2. (Weber and Fackeldey[48, Theorem 1])

Let $Q_c \in \mathbb{R}^{n \times n}$ be a reversible matrix that stems from a clustering with positive definite overlap matrix S . Then there exists a matrix $A \in \mathbb{R}^{n \times n}$ in optimization problem (3.12) such that $\det(S_{\text{opt}}) = 1$.

Proof. It is enough to show that for a given reversible Q_c , we can find a matrix A fulfilling all constraints such that $S = D^{-1} A^T A$ is equal to the identity matrix I .

Assume that there is a regular matrix B , such that $Q_c = B^{-1} \Xi B$.

Since Q_c is reversible, we have $DQ_c = Q_c^T D$, by detailed balance (1.4), and thus

$$DB^{-1} \Xi B = B^T \Xi^T B^{-T} D.$$

With $C := B^{-T} D$, we get

$$Q_c = D^{-1} B^T \Xi B^{-T} D = C^{-1} \Xi C.$$

...

Have a real positive matrix $M = \text{diag}(m_1, \dots, m_n)$ and therefore a real positive diagonal matrix $\widetilde{M} = \text{diag}(\sqrt{m_1}, \dots, \sqrt{m_n})$.

$$S = D^{-1}B^T B = \dots$$

Let $A := \widetilde{M}^{-1}B$. Show: A fulfills the constraints of (3.12), in order that S is a feasible matrix. Then

$$\begin{aligned} S &= D^{-1}A^T A = D^{-1}B^T \widetilde{M}^{-1} \widetilde{M}^{-1} B \\ &= D^{-1}B^T M^{-1} B \\ &= C^{-1}M^{-1} B \\ &= B^{-1} M M^{-1} B = I. \end{aligned}$$

Since all constraints of (3.12) are fulfilled, S is a feasible matrix with $\det(S) = 1$. \square

This theorem does **not** imply that a reversible process has no rebinding effect. It just means that for **every** reversible projected process, it is possible to find a transformation matrix A such that the system includes no rebinding.

In particular, only systems with at least 3 states are of interest to examine, since Q_c is reversible for $n = 2$.

Linear Optimization Problem: Maximizing trace of S

Now we present a different formulation of optimization problem (3.12). The objective function is slightly changed and thereby the problem is turned into a *linear optimization problem*. As a special case of the class of convex optimization problems, they have the nice property that any local optimum is also a global optimum.

We still want to **minimize** the rebinding effect, i.e. we want to give a lower bound for it. The closer the matrix S is to the identity matrix, the smaller is the rebinding effect. A matrix is close to the identity matrix, if its determinant is close to 1 or (equivalently) if its trace is close to n . So in order to compute the minimal rebinding effect, we can either maximize the determinant (get it as close as possible to 1) or maximize the trace of S (get it as close as possible to n), as the following theorem shows.

Theorem 3.3. *In optimization problem (3.12), we have $\det(S) \leq 1$ and $\text{trace}(S) \leq n$ with equality if and only if S is the identity/unit matrix.*

Proof. \square

So instead of maximizing $\det(S)$, we can also maximize $\text{trace}(S)$. In order to do so, let us first make some further observations about the eigenvectors of Q_c . We already found out that A^{-1} is a right eigenvector matrix of Q_c , with vectors being linear combinations of the eigenvectors X_i . Similarly, the matrix A is a *left* eigenvector matrix of Q_c , with row vectors being linear combinations of the eigenvectors Y_i . That fact can be expressed as

$$A = \tilde{U}Y^T = \begin{pmatrix} \tilde{\alpha}_1 Y_1 \\ \vdots \\ \tilde{\alpha}_n Y_n \end{pmatrix},$$

where each Y_i is a left eigenvector of Q_c (row vector) and the $\tilde{\alpha}_i \in \mathbb{R}$ are again some optimization parameters. The first eigenvector Y_1 corresponds to the leading eigenvalue $\xi_1 = 0$ and is thus the stationary density of the process. The first row of A consists of the statistical weights of the clusters and therefore we have again $\tilde{\alpha}_1 = 1$. With these notations we can write the new objective function as

$$\begin{aligned}\text{trace}(S) &= \text{trace}(D^{-1}A^T A) \\ &= \text{trace}(D^{-1}Y\tilde{U}^2Y^T) \\ &= \sum_{i=1}^n \sum_{k=1}^n \tilde{\alpha}_k^2 \frac{y_{ik}^2}{y_{k1}}.\end{aligned}$$

The side constraints remain the same, i.e. $S_{ij} = \dots \geq 0$. Let $\beta = (\beta_1, \dots, \beta_n)$ with $\beta_i = \tilde{\alpha}_i^2$. Then the linear optimization problem of maximizing $\text{trace}(S)$ is given by

$$\max_{\beta} \sum_{k=1}^n \beta_k \left(\sum_{i=1}^n \frac{y_{ik}^2}{y_{k1}} \right), \quad (3.13)$$

fulfilling the side constraints

$$\beta_i \geq 0, \quad \beta_1 = 1$$

and

$$\sum_{k=1}^n \beta_k y_{ik} y_{jk} \geq 0.$$

At first sight, this second formulation of the optimization problem might seem a bit more complex/confusing since we introduced several new matrices and variables. But in fact, the only change is that we maximize now the trace instead of the determinant (trace is easier to compute as it is just a sum). This formulation is better since, we have a *linear* program, which makes it easier to solve. And we have fewer constraints than before, because we merged some of the constraints into the objective function.

Let $B = \text{diag}(\beta_1, \dots, \beta_n)$. Then a solution β of (3.13) yields an optimal matrix $S_{\text{opt}} = D^{-1}BY^T$ resulting in the smallest possible rebinding effect.

Conclusion

A nontrivial rebinding effect can be estimated only if the kinetics Q_c of a system is nonreversible, since by theorem 3.2 a reversible system always permits a feasible transformation matrix A such that $S = D^{-1}A^T A$ yields $\det(S) = 1$.

3.4 Approach for non-reversible Processes

Employing the ideas presented in section 2.4, we extend the problem of computing the minimal rebinding effect of a system to non-reversible processes.

Schur Decomposition

The transfer operator \mathcal{P} from (3.4) describing a **non-reversible** process is **not** self-adjoint and hence, real eigenvalues are not guaranteed. In that case, problems as depicted in section 2.4 like complex eigenvectors can occur and consequently, we have to employ the Schur decomposition instead of the eigendecomposition in order to obtain a set of **real** vectors X spanning an invariant subspace. However, we cannot compute a real Schur decomposition for a continuous operator. Therefore, we need to discretize the transfer operator \mathcal{P} to a matrix $P \in \mathbb{R}^{N \times N}$ at first. Then we can determine a subset $X \in \mathbb{R}^{N \times n}$ of real Schur vectors in order to compute the membership vectors as a linear combination $\chi = XA$ with a transformation matrix A , for instance obtained by Gen-PCCA.

As shown in section 2.4, a reasonable result is achieved by employing Schur vectors that are orthogonal with respect to **any** initial distribution η . However, we choose orthogonality with respect to the stationary distribution μ .

Galerkin Projection

The clustered transition matrix is given by

$$P_c = S^{-1}T = \langle \chi, \chi \rangle_\mu^{-1} \langle \chi, P\chi \rangle_\mu.$$

As demonstrated in the proof of theorem 2.3, it can as well be represented by

$$P_c = A^{-1}\Lambda_s A,$$

where Λ_s is the Schur matrix with the dominant eigenvalues and blocks. The corresponding transition rate matrix Q_c can be computed by $Q_c = \frac{1}{\tau} \log(P_c)$ or equivalently by

$$Q_c = A^{-1}\Xi_s A, \tag{3.14}$$

where $\Xi_s = \frac{1}{\tau} \log(\Lambda_s)$ is the Schur decomposition of the Q_c .

Rebinding Effect

Again, we are interested in the rebinding effect included in a clustered system Q_c . If we know the utilized membership functions χ or the transformation matrix A , then we can measure the **real** rebinding effect which is encoded in the overlap matrix $S = D^{-1}A^T A$.

However, if we want to estimate the **minimal** rebinding effect included in a system Q_c , then it is not sufficient to solve optimization problem (3.12). It was based on the clustering with eigenvectors, assuming an originally reversible system, while the actual clustering is based on Schur vectors. In (3.14), the invariant subspace Ξ_s is not necessarily diagonal, but may have a block-diagonal shape.

Consequently, the columns of the transformation matrix A are not multiples of the eigenvectors of Q_c , but of Schur vectors. Thus, optimization problem (3.12) has to be modified such that it is formulated in terms of Schur vectors:

$$Q_c X = X \Xi_s. \quad (3.15)$$

Apart from that, the optimization problem coincides with (3.12); we aim at minimizing the rebinding effect by maximizing $\det(S)$. The side constraints guarantee the necessary structure of A , leading to a stochastic matrix S . Though the columns of A correspond to multiple of Schur vectors X from (3.15) now:

$$\begin{aligned} & \min_{\alpha_1, \dots, \alpha_n \in \mathbb{R}} |\det(S) - 1|, \\ & \alpha_1 = 1 \quad \text{and} \quad A_{ij}^{-1} = \alpha_j X_{ij} \quad \forall i, j. \\ & S = D^{-1} A^T A \quad \text{and} \quad S_{ij} \geq 0 \quad \forall i, j. \end{aligned} \quad (3.16)$$

Comparison

In section 3.3, we examined originally **reversible** processes Q , clustered onto a subspace Q_c . In that case, the clustered process could be either reversible or non-reversible (not to confuse with a full decomposition clustering where reversibility is inherited). The reversible (clustered) case is rather uninteresting as it yields the trivial solution as minimal rebinding effect. However, also non-reversible clustered matrices can be examined via the eigen-decomposition, since the eigenvalues are real and thus, no complex entries in the eigenvectors are possible.

In contrast to that, an originally **non-reversible** process is clustered in terms of Schur vectors. Then, the initial point for the optimization problem is not given by the eigenvalue problem (3.11), but in terms of the Schur decomposition (3.15), with Ξ_s possibly having outer diagonal elements.

The advantages of employing the Schur decomposition instead of the spectral decomposition have already been emphasized in section 2.4. Also in this context, it is advantageous, since it generalizes the optimization problem, including reversible as well as non-reversible original systems.

Motivation

In which cases would it be interesting to compute the minimal rebinding effect? We can use it as an estimation (though bad!) for the real rebinding effect. If we know the clustering, the real rebinding effect is calculated fast/easily. Thus: interesting for systems which are clustered (or can be interpreted as a projection), where we don't know the original process or the clustering (χ, A) .

However, in that case it is not adequate to solve the previous optimization problem (3.12). If the original process was non-reversible, then the clustering has to be interpreted

as based on Schur vectors instead of eigenvectors.

Thus, we can compute the minimal rebinding effect for **any** system, independent of the reversibility/non-reversibility of the original process.

Nevertheless, this estimation/bound may be good or not. But at least: applicable!

The quality of this estimation will be evaluated in the next chapter by means of an exemplary reversible process, which is perturbed to non-reversibility.

4 Illustrative Examples

With the aim of consolidating and illustrating the results from chapter 3, we apply them on some easy examples. At first, we examine a **reversible** process and demonstrate the relation of the minimal rebinding effect to the degree of non-reversibility of the clustered system. Afterwards, we repeatedly analyze the first example, now introducing small perturbations to make it **non-reversible**, in order to observe possible consequences for the rebinding effect. Since the rebinding effect was introduced by its occurrence in ligand-binding processes, we present an easy **bivalent binding system** and investigate the included rebinding effect. As a further application, we analyze a system describing the movement of **electron densities** in the chemical reaction of a molecule. This example illustrates that the rebinding effect plays a role in many different kind of systems. It is not solely restricted to binding processes and consequently, for each process one has to interpret the meaning of this effect.

In the following examples, the minimal rebinding effect is computed by solving optimization problem (3.12) implemented with the help of Optimization Toolbox in Matlab. Schur

4.1 Rebinding in a reversible System

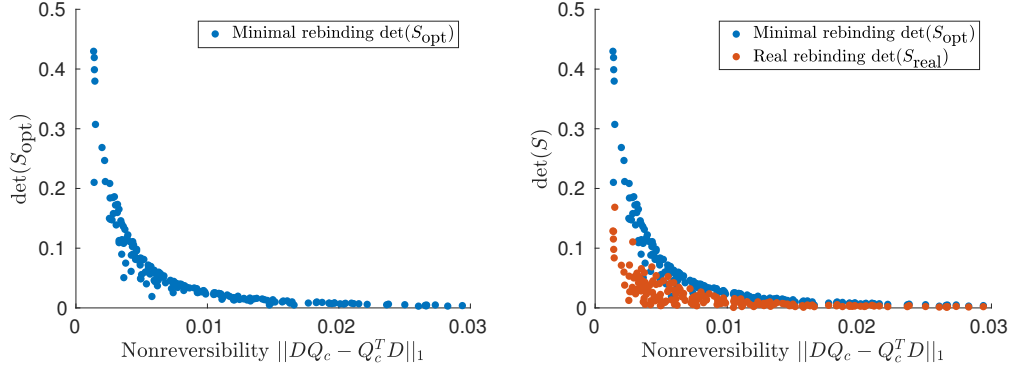
The clustering Q_c of a **reversible** process Q can be **non-reversible**. Hence, we are interested to compare the minimal rebinding effect included in a clustered system with its degree of non-reversibility. Furthermore, we want to compare the **minimal** rebinding effect with the **real** rebinding effect stemming from the clustering.

Different clusterings of a system

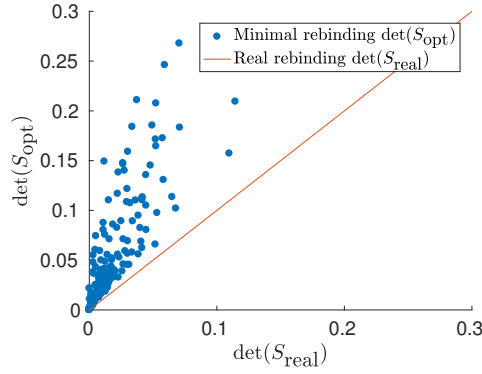
Let a **reversible** metastable process be given by the transition matrix

$$P = \begin{pmatrix} 0.9876 & 0.0011 & 0.0011 & 0.0051 & 0.0051 \\ 0.0033 & 0.4973 & 0.4949 & 0.0036 & 0.0009 \\ 0.0033 & 0.4949 & 0.4973 & 0.0009 & 0.0036 \\ 0.0076 & 0.0018 & 0.0004 & 0.4969 & 0.4932 \\ 0.0076 & 0.0004 & 0.0018 & 0.4932 & 0.4969 \end{pmatrix}. \quad (4.1)$$

This matrix stems from Weber[46] and obviously describes a system on three metastable sets, having the dominant eigenvalues $\sigma(P) = \{1, 0.99, 0.98\}$. Thus, we are interested to examine different clusterings on a three-dimensional state space. For that aim, we employ several transformation matrices $A \in \mathbb{R}^{3 \times 3}$, turning the dominant eigenvectors $X \in \mathbb{R}^{5 \times 3}$ into membership functions $\chi \in \mathbb{R}^{5 \times 3}$. In order that χ fulfills the partition of unity and non-negativity properties, the set of feasible matrices F_A has to meet certain conditions, see Weber[44, Chapter 3.4]. Accordingly, we generate 200 feasible transformation matrices A and examine the rebinding effect caused by the projection.



(a) The minimal rebinding effect compared to (b) The minimal and the real rebinding effect the degree of non-reversibility of the clustered compared to the degree of non-reversibility of system Q_c .



(c) The minimal rebinding effect $\det(S_{\text{opt}})$ compared to the real rebinding effect $\det(S_{\text{real}})$ included in Q_c .

Figure 4.1: The system described by the transition matrix P is clustered with 200 randomly generated feasible transformation matrices A .

The results of this example are presented in figure 4.1 and can be interpreted as follows. In a), we see that the minimal rebinding effect correlates with the degree of non-reversibility $\|DQ_c - Q_c^T D\|_1$ of the clustered system, where $D = \text{diag}(\pi_1, \dots, \pi_n)$ is the diagonal matrix consisting of the entries of the stationary distribution. The higher this degree of non-reversibility, the higher the minimal rebinding effect. This correlation also implies that for highly non-reversibly system, the minimal rebinding effect is a better estimation for the real rebinding effect, as represented in b). The last picture shows that in general, $\det(S_{\text{opt}})$ can be a rather good or a rather bad estimation for the real rebinding effect. The less rebinding is included in the system, the worse this estimation gets.

4.2 Rebinding in a non-reversible System

In order to compare the rebinding effect in the clustering of a **non-reversible** system to the reversible case, we further examine the example from section 4.1. We modify it slightly by introducing small **perturbations** in the eigendecomposition of the reversible process, leading to non-reversibility, as proposed by Weber[46]. The outcome shall be a Schur decomposition given by

$$\Lambda_s = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 0.99 & \epsilon & 0 & 0 \\ 0 & -\gamma & 0.98 + \delta & 0 & 0 \\ 0 & 0 & 0 & 0.005 & 0 \\ 0 & 0 & 0 & 0 & 0.001 \end{pmatrix}. \quad (4.2)$$

We compute the corresponding transition rate matrix by $P = X\Lambda_s X^{-1}$ with the Schur vectors X being equal to the eigenvectors from (4.1). If Λ_s is a diagonal matrix, then this equation represents the eigenvalue problem of a reversible process P . By introducing non-zero values for ϵ, γ and δ , the system gets non-reversible.

Why is this example interesting? We examine different systems/matrices, having the same Schur vectors and rather similar Schur decompositions. However, these small changes in the Schur decomposition lead to different results when it comes to compute the rebinding effect.

Accordingly to the example from section 4.1, we compute the real rebinding effect, when projecting the process onto a three-dimensional subspace represented by the transition rate matrix Q_c and compare it to the minimal rebinding effect included in that subspace. Starting from the projected process Q_c , we use the Schur vectors X from $Q_c X = X\Xi_s$, where Ξ_s is the Schur decomposition with **sorted** Schur values. Since Ξ_s has a 2×2 block on its diagonal, we cannot utilize optimization problem (3.12) to compute the minimal rebinding effect. Instead, we employ the same approach based on Schur vectors as described in section 3.4.

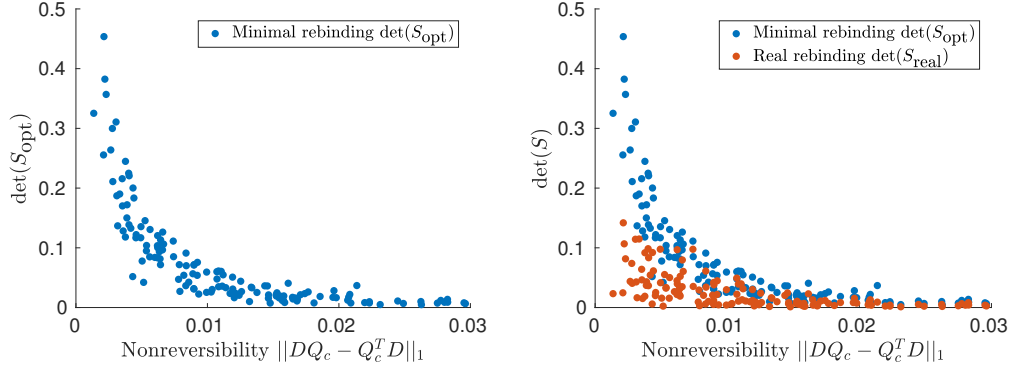
P is reversible

P is reversible if we set $\epsilon = \gamma = \delta = 0$ in the Schur decomposition (4.2). In that case, the Schur vectors are also eigenvectors of P and the system is equal to the one from section 4.1. Therefore, we should obtain the same results when solving the optimization problem based on Schur vectors instead of eigenvectors. In order to verify that, we compute again 200 clusterings with random feasible transformation matrices. The result, depicted in appendix A.1 coincides with the result from section 4.1.

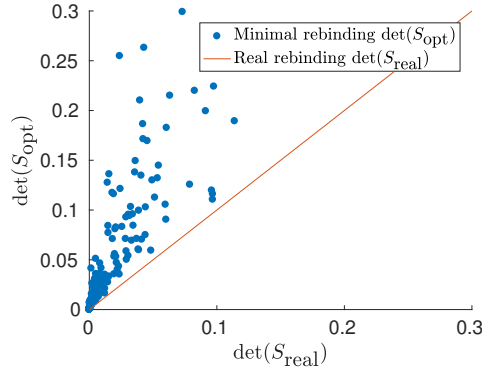
P is nonreversible with real eigenvalues

If we set $\epsilon = 0.004$, the matrix P becomes nonreversible, but still has real eigenvalues, since ϵ is on the upper triangle of Λ_s , while all eigenvalues are distinct (same algebraic and geometric dimension).

$$P = \begin{pmatrix} 0.9886 & 0.0011 & 0.0011 & 0.0046 & 0.0046 \\ 0.0001 & 0.4973 & 0.4949 & 0.0052 & 0.0025 \\ 0.0001 & 0.4949 & 0.4973 & 0.0025 & 0.0052 \\ 0.0086 & 0.0018 & 0.0004 & 0.4964 & 0.4928 \\ 0.0086 & 0.0004 & 0.0018 & 0.4928 & 0.4964 \end{pmatrix}.$$



(a) The minimal rebinding effect compared to (b) The minimal and the real rebinding effect the degree of non-reversibility of the clustered compared to the degree of non-reversibility of system Q_c .



(c) The minimal rebinding effect $\det(S_{opt})$ compared to the real rebinding effect $\det(S_{real})$ included in Q_c .

Figure 4.2: The system described by the transition matrix P is clustered with 200 randomly generated feasible transformation matrices A for the values $\epsilon = 0.004$, $\delta = 0$.

Need: 3 dominant Schur vectors X . Generate some feasible matrices A . Compute real rebinding $\det(S_{real}) = \det(D^{-1}A^T A)$.

Assumption: since Schur vectors X are the same as for the reversible case (matrix P constructed in terms of this set of real, orthogonal vectors), the set of feasible matrices remains the same and thus, the computed real rebinding effect should also remain the same.

However, the **minimal** rebinding could change, since the optimization problem required the clustered Schur vectors as input. They are not necessarily the same as in the reversible case. Maybe this estimation gets better or worse than it was the case for the reversible process?

Have given the process as $P_c = A^{-1}\Lambda_s A$, hence the columns of A are multiples of the dominant Schur vectors.

All other things to compute remain the same. $S = D^{-1}A^T A$ and minimized rebinding effect for $\det(S)$ maximized.

In order to compute Schur Decomposition of the clustered process Q_c , we need to come back to the trick presented in section 2.4 to employ the symmetrized matrix $D^{1/2}PD^{-1/2}$. Thus we need at first to compute the stationary distribution of the clustered process.

P is non-diagonalizable

The Schur decomposition has an upper diagonal element, while the eigenvalue 0.99 occurs algebraically twice.

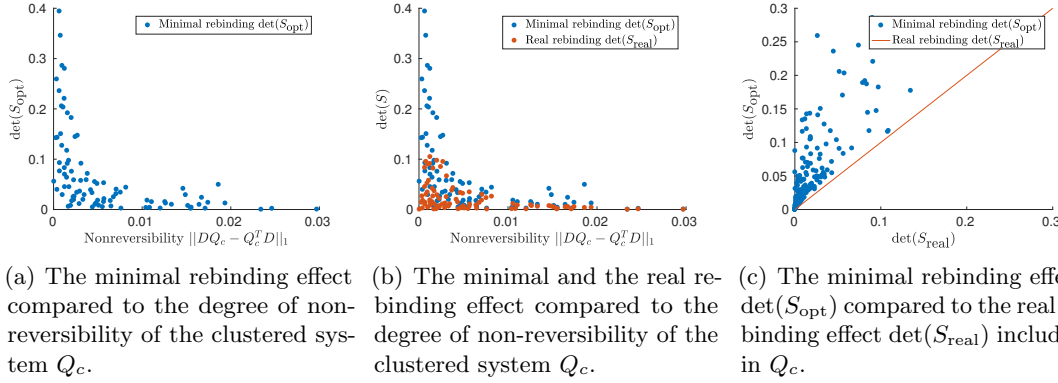


Figure 4.3: The system described by the transition matrix P is clustered with 200 randomly generated feasible transformation matrices A for the values $\epsilon = 0.004$, $\delta = 0.01$.

P is non-reversible with complex eigenvalues

Complex eigenvalues always occur in pairs and are indicated by a complete 2×2 -block in the Schur decomposition. For $\epsilon = 0.004$, $\delta = 0.01$ and $\gamma = 10^{-15}$, we get two complex eigenvalues $0.99 + 2.3 \cdot 10^{-9}i$ and $0.99 - 2.3 \cdot 10^{-9}i$.

Conclusion

It makes sense that the solution of optimization problem bla using/based on Schur vectors is a **worse** estimation for the real rebinding effect than the solution based on eigenvectors. When computing the minimal rebinding effect in terms of Schur vectors, we do not specify if the original process has been reversible or non-reversible. Hence, this extended approach includes **more** possible solutions.

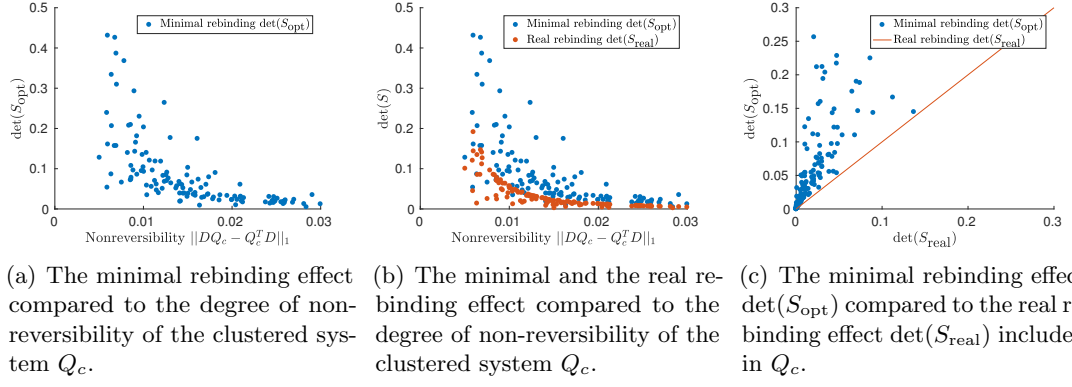


Figure 4.4: The system described by the transition matrix P is clustered with 200 randomly generated feasible transformation matrices A for the values $\epsilon = 0.002$, $\gamma = 0.01$.

4.3 Bivalent Binding Process

After having analyzed a purely theoretical example in order to visualize the quality of the estimation for the rebinding effect, we continue to examine a **binding process**, being the original motivation for the investigations of the rebinding effect.

Multivalent System

In section 3.1, we introduced the rebinding effect as a memory effect included in a system of receptors and ligands, without any connection of the ligands. However, one can distinguish between **monovalent** and **multivalent** binding processes. Whenever the receptor molecules are spatially preorganized, the corresponding binding process is denoted as multivalent. We can imagine that as several receptors being connected by a linker.

Especially the bivalent or polyvalent case often is observed in nature. These systems are of significant interest for pharmaceutical and technical applications. If the ligands are linked to each other in an appropriate way to match the preorganized receptor molecules and, thus, are also presented multivalently, then extremely high binding affinities are often observed, which is conjectured to originate by the rebinding effect. This is clarified

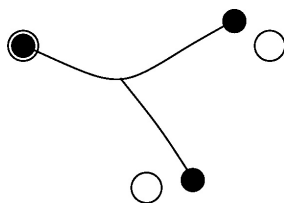


Figure 4.5: Trivalent System.

in figure 4.5, representing a trivalent system. If one of the connected ligands dissociated from its receptor, after being in the completely bound state (triple bound), then the probability of the ligand to be still close to its receptor and to rebind, is high. We can imagine that this rebinding effect is even higher than it would have been the case in a monovalent system, since the ligand is kept at its place by its two connected ligands.

The strength of this “adherence” depends also from the flexibility of the linker. They can be either rigid/stiff or more flexible. A rigid linker holds the connected ligand more strongly at its place than a flexible linker. This relation is shown by Weber et al[47].

The mathematical modelling of a monovalent system is well understood. Furthermore, if defined on the two macro states “unbound” and “bound”, the computation of the minimal rebinding effect in such a system yields only the trivial solution, by theorem 3.2.

Hence, as the the easiest multivalent case, we consider a **bivalent** process. Such a system can be described by three macro states: “unbound”, “singly bound” and “doubly bound”, depicted in figure 4.6. This model can be represented by the reversible reactions

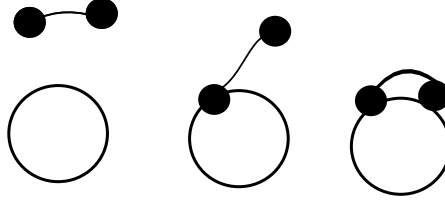


Figure 4.6: Possible macro states of a bivalent system.



resulting in a transition rate matrix

$$Q_c = \begin{pmatrix} -(k_{01} + k_{02})[RR] & k_{01}[RR] & k_{02}[RR] \\ k_{10} & -(k_{10} + k_{12}) & k_{12} \\ k_{20} & k_{21} & -(k_{20} + k_{21}) \end{pmatrix},$$

depending on the concentrations of the bivalent receptor molecules $[RR]$. This matrix is constructed in the same fashion as explained in section 3.1 for the monovalent case and as well describes changes of concentrations by the ordinary differential equation

$$\dot{x}^T = x^T Q_c.$$

By this equation, changes of concentrations $x^T = ([LL], [L(LR)R], [(LRLR)])$ in this system can be observed/described.

We can either measure or determine some plausible/feasible association/dissociation constants. Remark: the last reaction equation in (4.3) should have **very** small association and dissociation constants, since transitions from the unbound state directly to the bound state or vice versa are not realistic; if they happen then extremely rarely. However, we don't set them 0, in order to avoid a sparse transition rate matrix Q_c .

Rebinding Effect

We are in the situation that we are given a process which can be **interpreted** as a projection, while we do **not** know the original process and therefore cannot compute the actual rebinding effect. However, we assume that by the unknown projection, there is some rebinding effect included in Q_c . Assuming that it is clustered in terms of overlapping membership functions $\chi = XA$, we again solve optimization problem (3.12) to derive the minimal rebinding effect as an estimation.

Need: eigenvectors/Schur vectors of Q_c in order to compute the minimal rebinding effect included in this system. It does not play a role if the original system was reversible or non-reversible, since we use Schur vectors to solve this problem.

When is Q_c reversible or non-reversible?

We consider it at first as an artificial system, by inserting convenient association and dissociation constants in order to obtain general informations about bivalent systems. Afterwards we examine a real system.

Artificial Binding Process

First try: choice as in Weber and Fackeldey[48].

- For the reaction “unbound” \leftrightarrow “singly bound”: very high association k_{01} , low dissociation k_{10}
- For the reaction “singly bound” \leftrightarrow “doubly bound”: high association k_{12} , very low dissociation k_{21}
- For the reaction “unbound” \leftrightarrow “doubly bound”: very low association k_{02} , very low dissociation k_{20}

These parameters describe a system with desirable properties, since we want to obtain a high occupation of the receptors. Unbound ligands have a strong preference to the single bound state, while dissociations do not happen often. From the singly bound state to the doubly bound state happens still quite often, while dissociating not. Going directly from unbound to doubly bound and vice versa can almost be neglected.

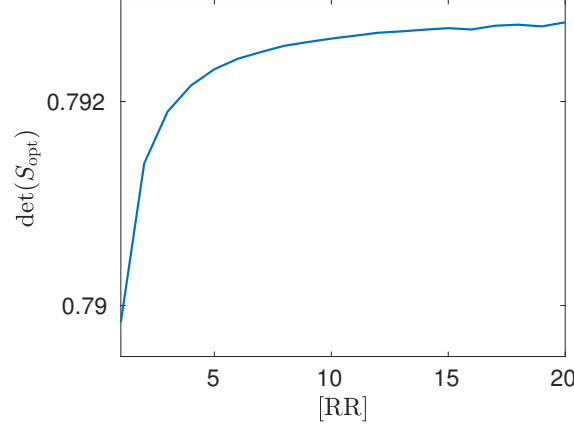


Figure 4.7: The minimal rebinding effect of Q_c depending on the concentration $[RR]$ of receptor molecules.

In figure 4.3, we see that with increasing concentration of receptor molecules, the minimal rebinding effect decreases. Even though the resulting difference of $\det(S_{opt})$ is not very large, this tendency is unambiguous. That makes sense: in a system with a large concentration of receptors, a ligand dissociating from a receptor is more likely to be already close to another receptor. If they bind, rebinding is prevented.

included in model?

Even though this result qualitatively makes sense, it **should not**. The model (4.3) cannot distinguish if a dissociated ligand rebinds to its receptor or if it binds to a close receptor.

Accordingly to Weber and Fackeldey[48], this decreasing rebinding effect represented in figure 4.3 can be explained by a decrease in the transition regions between the binding events caused by the increased receptor concentration.

For **low** receptor concentrations the result is plausible; bindings shortly after a dissociation are likely to be a **rebinding**, since there are no other receptors nearby. However, in realistic models the receptor concentration is higher.

We need better models in order to correctly include rebinding effects. How?

The presented model does not include enough informations/states to distinguish between a rebinding and a normal binding, since a dissociated ligand possesses no memory about the previous binding. How could that be included? Some approaches

- considering larger systems, i.e. systems with more informations/ more states than just “bound”, “singly bound”, “doubly bound”, making it possible to distinguish between rebinding and other bindings
- switching from the molecular kinetic to the molecular dynamic point of view: informations about the rebinding effect could be detected by simulations; i.e. generate a trajectory and analyze it for rebinding events

Finally one further outlook would be to compute the rebinding effect for time-dependent systems as well[14]. Consequent enhancement of PCCA+. Recently improved to non-reversible processes. New extension: including time-dependent systems by coherent sets (metastable sets with time) [49].

Real Binding Process

Something with low receptor-concentrations.

Picture

4.4 Analysis of a Chemical Reaction

Since each clustered matrix can be written as $S^{-1}T$, with the familiar meaning of S as overlap matrix and T as coupling matrix, we can deduce that **each** clustered system possesses some kind of (more or less) rebinding effect (not only restricted to receptor-ligand systems). The meaning of this effect has to be interpreted for each system individually. We present a different system (chemical/physical), describing the movement of electron densities of a given molecule.

Formic acid is a molecule consisting of one carbon atom C , two oxygen atoms O and two hydrogen atoms H . In hydrocarbons and in the vapor phase, it consists of hydrogen-bonded dimers rather than individual molecules.

ref

In such a system, reactions between the individual molecules can be take place. An H atom which is attached to an O atom/nucleus moves to the O atom of another molecule and vice versa. These reactions are possible because of the (double) proton tunneling, see Schild[32, Chapter 4]. According to these reactions, changes in the electron density can be observed.

ref

Why is this electron density $\pi(t)$ time-dependent?

pericyclic
metastable sets
coherent sets

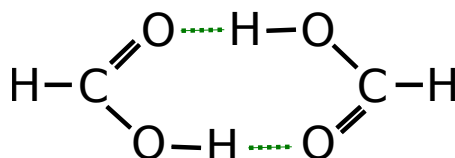


Figure 4.8: Electron densities are measured in a system of formic acid dimers.

Such a system can be described by a Markov chain, where the transition matrix P consists of the (time-dependent) electron densities. Each of the different time-steps yields its own transition matrix. Then P can be constructed of these matrices such that it is reversible.

As P is compound of several transition matrices, it possesses many dominant eigenvalues. However, for each of the individual transition matrices, 4 metastable regions can be identified. Clustering P into 4 metastable sets using PCCA+ yields the following transition rate matrix:

$$Q_c = \begin{pmatrix} -2.0040 & 1.6859 & 0.1490 & 0.1690 \\ 1.6192 & -2.0010 & 0.1724 & 0.2095 \\ 0.1451 & 0.1747 & -1.9548 & 1.6350 \\ 0.1632 & 0.2106 & 1.6217 & -1.9955 \end{pmatrix}.$$

Since it has been clustered with PCCA+, we assume that this system includes/contains **no** large rebinding effect (objective of PCCA+: make membership functions as crisp as possible). Applying optimization problem (3.12) to this matrix, we find out that the minimal rebinding effect included in this system is given by

$$\det(S_{\text{opt}}) = 1,$$

and thus the optimal overlap matrix is the identity matrix.

Unfortunately, this bound gives us no information about how much rebinding is obtained by the clustering. This bound can be explained by the reversibility of the clustered system, $\|DQ_c - Q_c^T D\|_1 = 0$, and theorem 3.2.

However, from observing the given membership functions χ in figure 4.9, we notice that they are not very crisp, but rather overlapping.

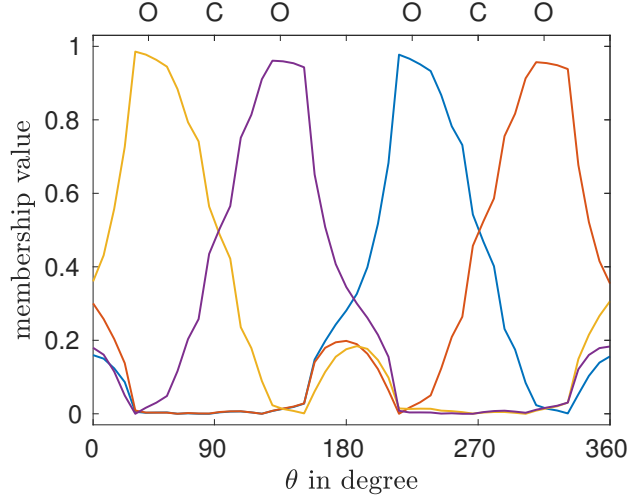


Figure 4.9: Membership functions created by PCCA+

Knowing the membership functions χ and the stationary distribution π of the original process, we can compute the **real** rebinding effect by

$$S_{\text{real}} = D^{-1} \langle \chi, \chi \rangle_{\pi}$$

and thereby obtain

$$\det(S_{\text{real}}) = 0.2925.$$

We have already seen before, that the minimal rebinding $\det(S_{\text{opt}})$ is not always a good estimation for the real rebinding and in particular is useless if the clustered process is reversible.

However, it is astonishing that in this clustered system, there is actually so much real rebinding included. That was unexpected, since clustering with PCCA+ aims for rather crisp membership functions which would result in few rebinding.

Thus, even though the estimation $\det(S_{\text{opt}})$ was bad in that example, we can interpret the meaning of the actual rather high rebinding caused by the clustering.

In that example, movements of electron densities around a given molecule have been considered. The existence of the 4 metastable conformations located around the *O*-molecules is plausible, since hydrogen is attracted to *O*. However, these conformations are strongly overlapping. That can be explained by the rebinding effect. A hydrogen atom which is about to leave its *O*-atom and wandering to the next, possesses still a large probability to “go back”/return to its original connected *O*.

Conclusion and Outlook

Summary We have seen how we can get from a continuous stochastic process to a finite process defined on its metastable sets. The relevance of metastability and the differences between reversible and nonreversible processes have been highlighted, in order to solve an optimization problem for both kind of processes in a given molecular system.

The rebinding effect included in a receptor-ligand system stems from the projection onto a finite state space. Its relevance is justified by its influence to the stability of this system. That means that a high rebinding effect increases the binding affinity of a process, which is relevant for applications like (computational) drug design where it is important to predict the exact binding affinity of ligands.

Role of overlap matrix We want to highlight the role of the matrix S , from theorem 1.17, which is used to measure the rebinding effect. In chapter 1, it has been introduced mathematically as a part of the matrix representation of a clustered Markov process. It basically consists of the scalar products of the different membership functions representing the macro states. In chapter 2, we have seen its relation to the overlap of the membership functions, that is S can be interpreted as an “overlap matrix”, containing information about the degree of fuzzyness of the clustering. In chapter 3, we stated a lower bound for the rebinding effect in terms of the matrix S . We have seen that a high rebinding effect, and thus strongly overlapping membership functions (information encoded in S), result in a more stable system. Thus, S influences the metastability of a given system.

Rebinding Effect \leftrightarrow Overlap of Membership Functions (=Degree of fuzzyness) \leftrightarrow Stability of System \leftrightarrow Degree of Nonreversibility?

Role of Nonreversibility In a reversible system, the minimal rebinding effect is always given by $\det(S_{\text{opt}}) = 1$, meaning that there is no rebinding. Such a clustering in metastable sets is hard and not fuzzy and therefore not recommended. As described in chapter 2, some overlap in the clustering is good.

In a nonreversible system however, the minimal rebinding effect is higher, meaning that the clustering includes some overlap. The higher the nonreversibility of a system, the higher the minimal rebinding effect and thus, the higher the overlap of the membership functions.

Outlook

This thesis combines two research topics that are highly discussed recently. The general field of molecular design, in particular applications in drug design like the rebinding effect, as well as the analysis of nonreversible processes are ongoing research topics with

Molecular Design Recently: reduction of dimension using metastable decompositions (PCCA+) helped to perform simulations which would have been impossible on the larger (cont.) state space.

Outlook: But still it is only possible to simulate on ...timescales.. longer timescales like several seconds, like needed for protein folding etc, are still infeasible, but with increasing computing power of supercomputers become more and more realistic.

(Noe Weber..)

Rebinding Effect In order to design drug molecules, it is important to know the exact binding affinity of a given system (set of ligands). Therefore the knowledge of the occurring rebinding effect can help to improve this design process/..

Multivalence The kinetics and design of multivalent processes is a current research project of the “Computational Molecular Design” group at ZIB.

Nonreversible Processes The study of reversible processes is very advanced/well-established, based on eigendecomposition. The idea to apply a Schur Decomposition instead has been proposed by Röblitz and further promoted by Weber. This generalized approach includes the special case of non-reversible processes and thus could become the generalized approach to analyze stochastic processes.

In general, the research of non-reversible processes is rather at the beginning. Using Schur Decomposition could yield many results for those processes.

Many processes occurring in real life are nonreversible (which ones?).

Time-Dependent Processes: Coherent Sets

“Rebinding Effect” for other processes The Rebinding Effect, or Recrossing Effect, can occur if we project a process. Thus, rebinding or recrossing events can happen in all other kind of processes. What is the role of it for these processes? For this reason, we brought up the topic of Raman spectroscopy. examples

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Berlin, den 4. September 2017

Susanne Röhl