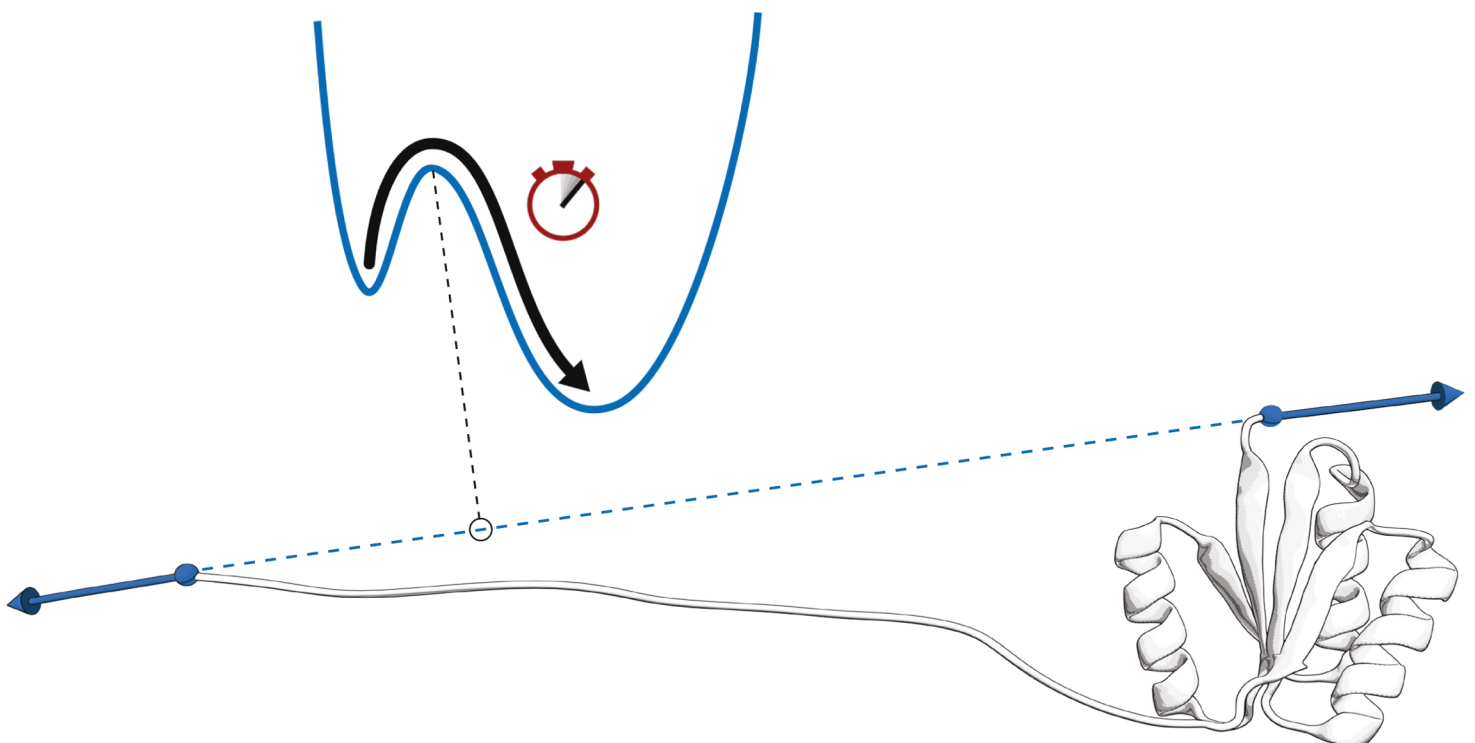


ALEXANDER MEHLICH

TRANSITION PATHS OF PROTEIN-FOLDING
PROBED WITH OPTICAL TWEEZERS

Establishing transition path analysis techniques in single-molecule
force spectroscopy based on simulation and experiments performed
on natural and artificial proteins

DISSERTATION AT THE PHYSICS DEPARTMENT E22
TECHNISCHE UNIVERSITÄT MÜNCHEN



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Berichte aus der Biophysik

Alexander Mehlich

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probed with optical tweezers**

Establishing transition path analysis techniques in single-molecule force spectroscopy based on simulation and experiments performed on natural and artificial proteins

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All that is gold does not glitter,
not all those who wander are lost;
the old that is strong does not wither,
deep roots are not reached by the frost.

— J.R.R. Tolkien

Exponential dependencies are nature's ace in the hole
to make miracles become reality.

— A. M.

ABSTRACT

Proteins are essential for life. To be able to perform their tasks, proteins need to fold into their functional form. From a physicist's perspective, nature solves this 'folding problem' by providing a multidimensional energy landscape which efficiently guides a loose peptide towards a distinct three-dimensional structure which is solely predefined by its unique amino acid sequence. A powerful method to directly study the folding mechanics of proteins is single-molecule force spectroscopy, which is used in this thesis. With a variety of sophisticated analysis tools it is possible to derive transition state positions and barrier heights up to entire one-dimensional projections of the folding energy landscapes of proteins from single-molecule trajectories. Recent technological advances to improve temporal and spatial resolution have opened doors towards directly accessing protein folding transition paths. The establishment of appropriate transition path analysis techniques and their correct interpretation was one of the main objectives of this work.

To this end, transition path analysis techniques were introduced and thoroughly tested using two-dimensional Brownian dynamics simulations. Using this, a very high inter-dependence between the friction involved in protein diffusion and the friction inherent to the detection system was revealed. Owing to this inter-dependence, previously described methods which aim to localise transition states from transition path ensembles or reconstruct barrier heights from committers typically fail to directly provide correct results. Conversely, the strong friction-dependence of detected transition path ensembles have enabled the introduction of a new, merely diffusion-based method of internal protein friction determination. This new method was successfully applied to experimental data of the three-helix bundle protein R15 and revealed the lowest internal friction directly reported from optical tweezers experiments.

The second main objective of this work was the comparison between the folding mechanics of natural and artificial proteins. One artificial protein, a labile re-designed version of the Ferredoxin-like fold, was found to be a relatively simple two-state folder. By contrast, another *de novo* designed protein, the Rossmann fold, was revealed to have an extremely rough energy landscape as opposed to the naturally occurring 'ideal' two-state folder R15. This high energy landscape roughness of the Rossmann fold was characterized by misfolds, multi-pathway folding, a greater effective roughness of $\epsilon_{\text{rms}} = 2.4 k_B T$ with respect to R15, a local roughness of up to $10 k_B T$, two orders of magnitude longer transition path time averages $\langle \tau_{\text{TP}} \rangle$ and, hence, slower folding. Various mutants showed the enforced key lock mechanism of the C-terminal α -helix and its potential bending around position P86 to be the main cause of the folding problems of the Rossmann fold.

The results presented in this work pave the way to a more detailed understanding of protein folding mechanics from a transition path perspective.

ZUSAMMENFASSUNG

Proteine sind lebenswichtig. Um ihre biologischen Aufgaben erfüllen zu können, müssen Proteine ihre funktionsfähige Form durch die sogenannte Faltung annehmen. Aus physikalischer Sicht löst die Natur dieses 'Faltungsproblem' mit Hilfe einer mehrdimensionalen Energielandschaft, in der ein loses Peptid in seine dreidimensionale Struktur überführt wird, die ausschließlich durch seine einzigartige Aminosäuresequenz vorbestimmt ist. Eine sehr mächtige Methode zur direkten Untersuchung der Faltungsmechanik von Proteinen ist die Einzelmolekül-Kraftspektroskopie, die auch in dieser Arbeit zum Einsatz kommt. Mit einer Vielzahl anspruchsvoller Methoden zur Auswertung von Einzelmolekül-Messungen wurde es möglich, die Position und Energiebarrierenhöhe von Übergangszuständen bis hin zu vollständigen Projektionen eindimensionaler Energielandschaften der Proteinfaltung zu bestimmen. Die jüngsten technologischen Fortschritte in Bezug auf die zeitliche und räumliche Auflösung haben nun auch die Türen zur direkten Detektion von Übergangspfaden der Proteinfaltung geöffnet. Die Einführung passender Analysemethoden von Übergangspfaden und deren korrekte Interpretation stellen wesentliche Ziele dieser Arbeit dar.

Um dies zu erreichen, wurden Analysemethoden von Übergangspfaden eingeführt und im Rahmen von zweidimensionalen Brownschen Bewegungssimulationen umfassend getestet. Auf diese Art wurde eine starke Abhängigkeit zwischen der Reibung der eigentlichen Proteinfaltung und der Reibung im Messsystem festgestellt. Aufgrund dieser Abhängigkeit scheitern Methoden, die auf der Grundlage von Ensembles aus Übergangspfaden die Position von Übergangszuständen feststellen oder die Höhe von Energiebarrieren rekonstruieren sollen, oft daran, auf direkte Weise korrekte Ergebnisse zu liefern. Im Gegensatz dazu konnte die starke Reibungsabhängigkeit der Ensembles aus Übergangspfaden dazu genutzt werden, eine neue, ausschließlich auf Diffusion basierte Methode zur Bestimmung der inneren Proteinreibung zu entwickeln. Diese neue Methode wurde erfolgreich am dreifachen Helixbündel-Protein R15 angewandt und offenbarte dabei die bisher geringste Reibung, die direkt von Messungen mit optischen Fallen berichtet wurden.

Ein weiteres wichtiges Ziel dieser Arbeit ist es, die Faltungsmechanik von natürlichen und künstlichen Proteinen miteinander zu vergleichen. Ein künstliches Protein, welches ein explizit mechanisch labiles Design des Ferredoxin-Faltungsmotivs repräsentiert, wies eine 'einfache' Faltung auf. Ein weiteres *de novo* Protein, das sogenannte Rossmann-Faltungsmotiv, wies hingegen eine extrem raue Energielandschaft auf, die im völligen Gegensatz zu der 'idealen' Zweizustandsfaltung des natürlichen Proteins R15 steht. Zusammengefasst zeichnet sich die starke Rauheit des Rossmann-Proteins durch eindeutige Hinweise auf Fehlfaltungen, mehrere Faltungspfade, eine gegenüber R15 um $\epsilon_{\text{rms}} = 2.4 \text{ k}_\text{B} \text{T}$ erhöhte effektive Reibung, eine lokale Rauheit von bis zu $10 \text{ k}_\text{B} \text{T}$ und um zwei Größenordnungen längere mittlere Übergangspfadzeiten $\langle \tau_{\text{TP}} \rangle$

und damit eine entsprechend langsamere Faltung, aus. Verschiedene Mutanten weisen darauf hin, dass der verstärkte Verriegelungsmechanismus der C-terminalen α -Helix und deren Verkrümmung im Bereich der Prolinposition P86 für die Probleme bei der Faltung des Rossmann Proteins verantwortlich sein könnten.

Die Ergebnisse dieser Arbeit ebnen den Weg in Richtung eines besseren Verständnisses der Proteinfaltungsmechanik sowohl im Allgemeinen als auch im Besonderen aus der Sicht von Übergangspfaden.

PUBLICATIONS

While the focus of this thesis lies on unpublished scientific results, a few of the concepts and figures which emerged along with this work can be found in the following publications:

- RINGER P, WEIßL A, COST A.-L., FREIKAMP A, SABASS B, MEHLICH A, TRAMIER M, RIEF M, GRASHOFF C (2017) Multiplexing molecular tension sensors reveals piconewton force gradient across talin-1. *Nature Methods*
- FREIKAMP A, MEHLICH A, KLINGNER C, GRASHOFF C (2016) Investigating piconewton forces in cells by FRET-based molecular force microscopy. *Journal of Structural Biology*
- AUSTEN K¹, RINGER P¹, MEHLICH A¹, CHORSTEK-GRASHOFF A, KLUGER C, KLINGNER C, SABASS B, ZENT R, RIEF M, GRASHOFF C (2015) Extra-cellular rigidity sensing by talin isoform-specific mechanical linkages. *Nature Cell Biology*
- MEHLICH A¹, AUSTEN K¹, RINGER P¹, RIEF M, GRASHOFF C (2015) Evaluation of molecular tension sensors using single-molecule force spectroscopy and live cell FRET imaging. *Nature Protocol Exchange*²
- FANG J, MEHLICH A, KOGA N, HUANG J, KOGA R, GAO X, HU C, JIN C, RIEF M, KAST J, BAKER D, LI H (2013) Forced protein unfolding leads to highly elastic and tough protein hydrogels. *Nature Communications*
- VON HANSEN Y, MEHLICH A, PELZ B, RIEF M, NETZ R (2012) Auto- and cross-power spectral analysis of dual trap optical tweezer experiments using Bayesian inference. *Review of Scientific Instruments*

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² Non-peer reviewed.

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ACRONYMS

AFM	Atomic Force Microscope
BM(PEG)₂	1,8-Bismaleimido-diethyleneglycol
BSA	Bovine Serum Albumin
DTDP	2,2'-Dithiodipyridine
DTT	Dithiothreitol
DNA	Deoxyribonucleic Acid
FRET	Förster Resonance Energy Transfer
FWHM	Full Width at Half Maximum
GFP	Green Fluorescent Protein

GODCAT Glucose/Glucose-Oxidase/Catalase oxygen scavenging system

HMM Hidden Markov model

MD Molecular dynamics

NMR Nuclear Magnetic Resonance

PBS Phosphate Buffered Saline

PCR Polymerase Chain Reaction

PSF Point Spread Function

RT Room Temperature

SEC Size Exclusion Chromatography

TCEP Tris(2-carboxyethyl)phosphine

TPT Transition Path Theory

TST Transition State Theory

WHAM Weighted Histogram Analysis Method

WLC Worm-like Chain

eWLC extensible Worm-like Chain

YFP Yellow Fluorescent Protein