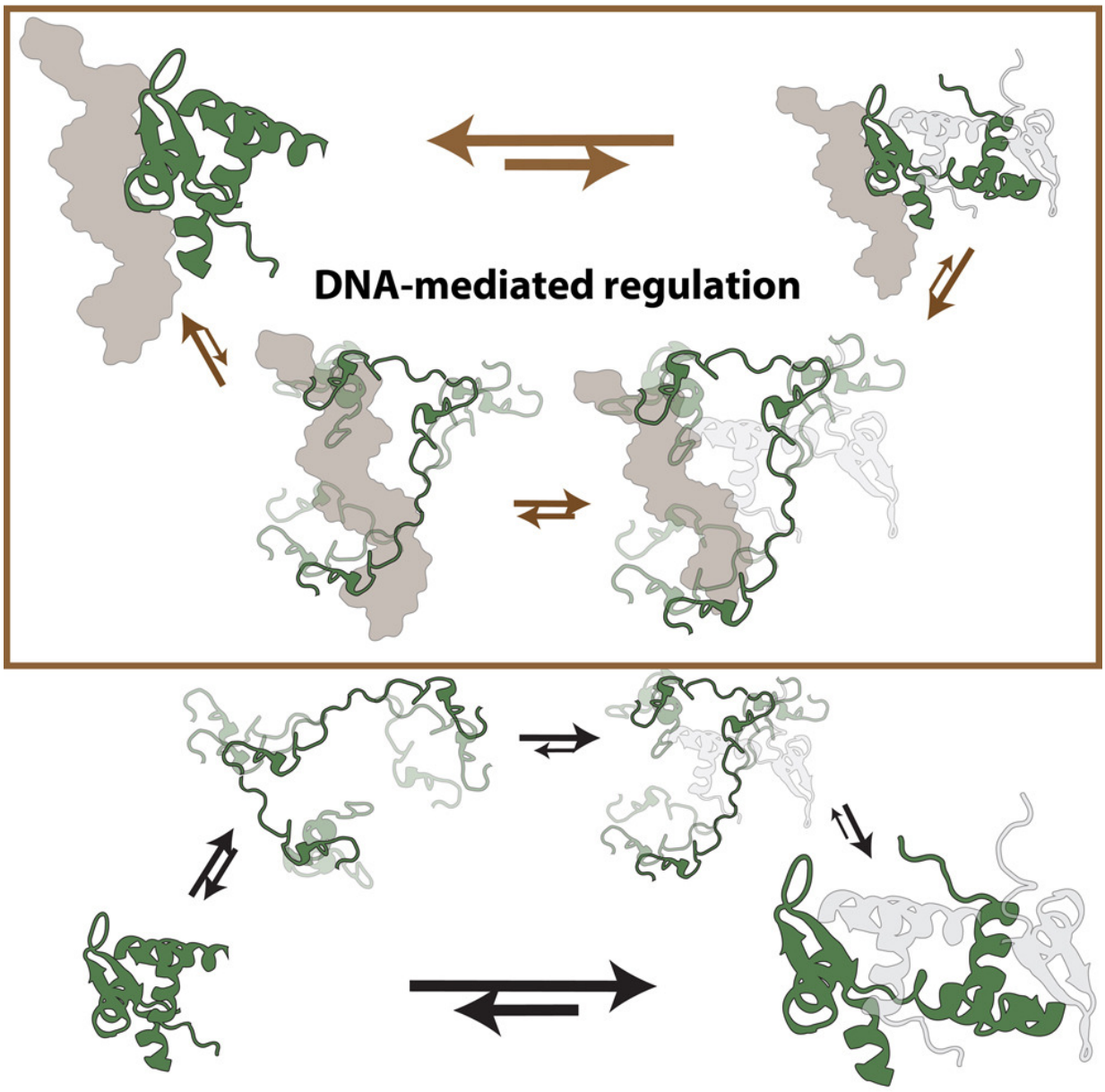
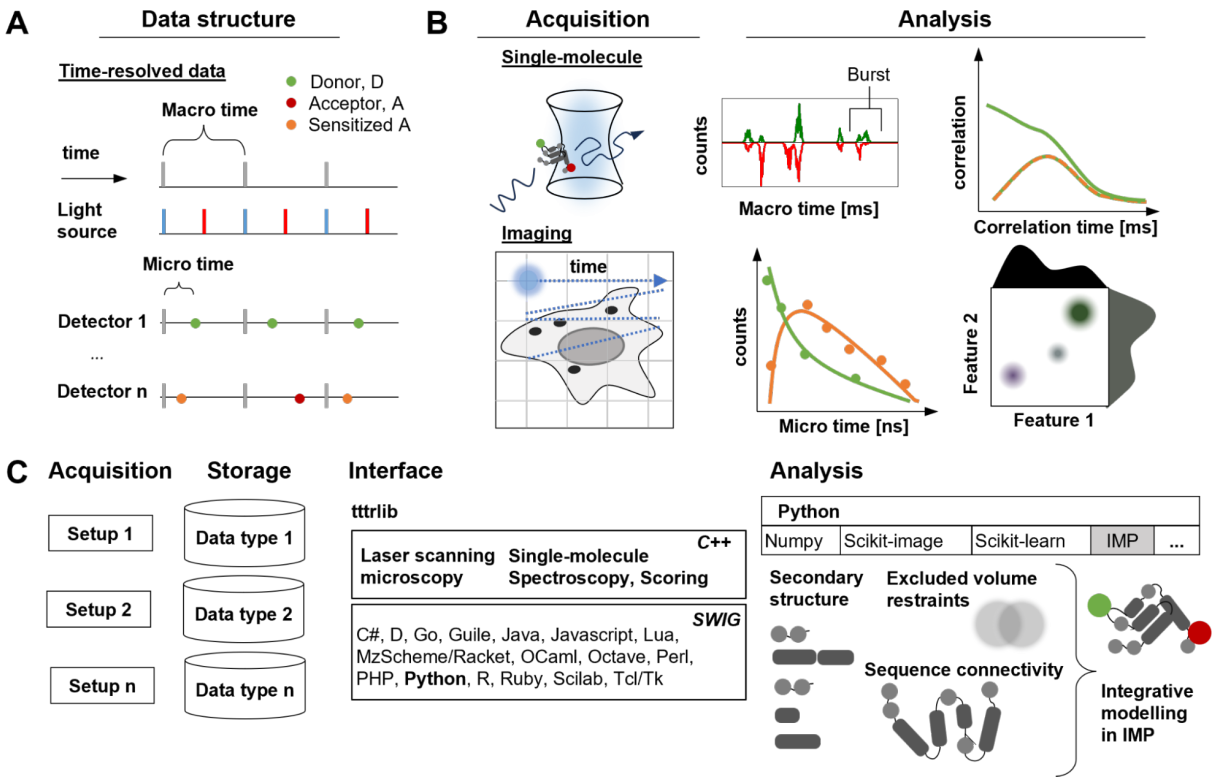


Highlights

•The FKH domain of FoxP1 is structurally dynamic in monomer and dimeric conditions
•The dimerization of the FKH domain stabilizes the intramolecular dynamics
•The presence of DNA destabilizes the dimeric FKH domain and promotes a disordered state
•The presence of DNA favors the monomeric state of the FKH domain

Summary

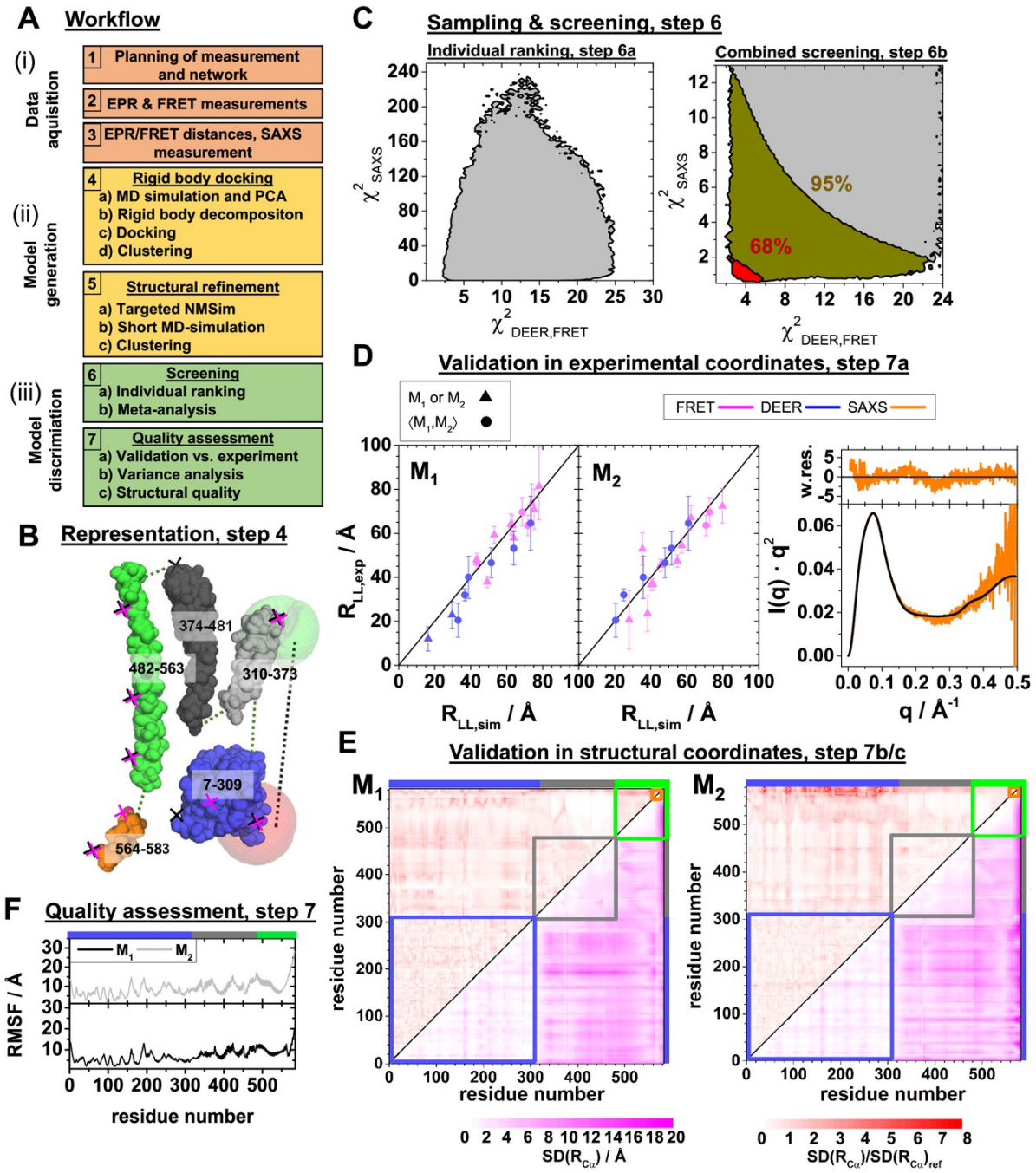
Transcription factors (TFs) regulate gene expression by binding to specific DNA sequences and gating access to genes. Even when the binding of TFs and their cofactors to DNA is reversible, indicating a reversible control of gene expression, there is little knowledge about the molecular effect DNA has on TFs. Using single-molecule multiparameter fluorescence spectroscopy, molecular dynamics simulations, and biochemical assays, we find that the monomeric form of the forkhead (FKH) domain of the human FoxP1 behaves as a disordered protein and increases its folded population when it dimerizes. Notably, DNA binding promotes a disordered FKH dimer bound to DNA, negatively controlling the stability of the dimeric FoxP1:DNA complex. The DNA-mediated reversible regulation on FKH dimers suggests that FoxP1-dependent gene suppression is unstable, and it must require the presence of other dimerization domains or cofactors to revert the negative impact exerted by the DNA.



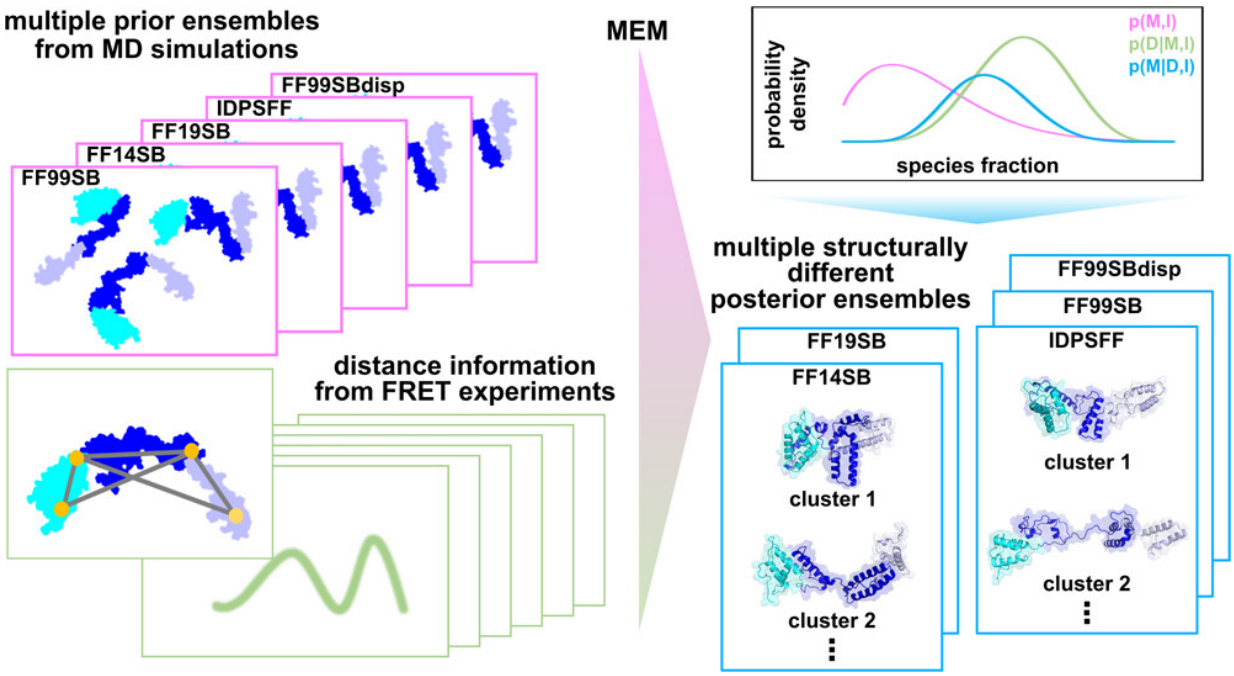
We introduce software for reading, writing and processing fluorescence single-molecule and image spectroscopy data and developing analysis pipelines to unify various spectroscopic analysis tools. Our software can be used for processing multiple experiment types, e.g., for time-resolved single-molecule spectroscopy, laser scanning microscopy, fluorescence correlation spectroscopy, and image correlation spectroscopy. The software is file format agnostic, processes and outputs multiple time-resolved data formats. Our software eliminates the need for data conversion and mitigates data archiving issues.

Availability and implementation

tttrlib is available via pip (https://pypi.org/project/tttrlib/) and bioconda while the open-source code is available via GitHub (https://github.com/fluorescence-tools/tttrlib). Presented examples and additional documentation demonstrating how to implement *in vitro* and live-cell image spectroscopy analysis are available at https://docs.peulen.xyz/tttrlib and https://zenodo.org/records/14002224.



Guanylate binding proteins (GBPs) are soluble dynamin-like proteins that undergo a conformational transition for GTP-controlled oligomerization and disrupt membranes of intracellular parasites to exert their function as part of the innate immune system of mammalian cells. We apply neutron spin echo, X-ray scattering, fluorescence, and EPR spectroscopy as techniques for integrative dynamic structural biology to study the structural basis and mechanism of conformational transitions in the human GBP1 (hGBP1). We mapped hGBP1's essential dynamics from nanoseconds to milliseconds by motional spectra of sub-domains. We find a GTP-independent flexibility of the C-terminal effector domain in the µs-regime and resolve structures of two distinct conformers essential for an opening of hGBP1 like a pocket knife and for oligomerization. Our results on hGBP1's conformational heterogeneity and dynamics (intrinsic flexibility) deepen our molecular understanding relevant for its reversible oligomerization, GTP-triggered association of the GTPase-domains and assembly-dependent GTP-hydrolysis.

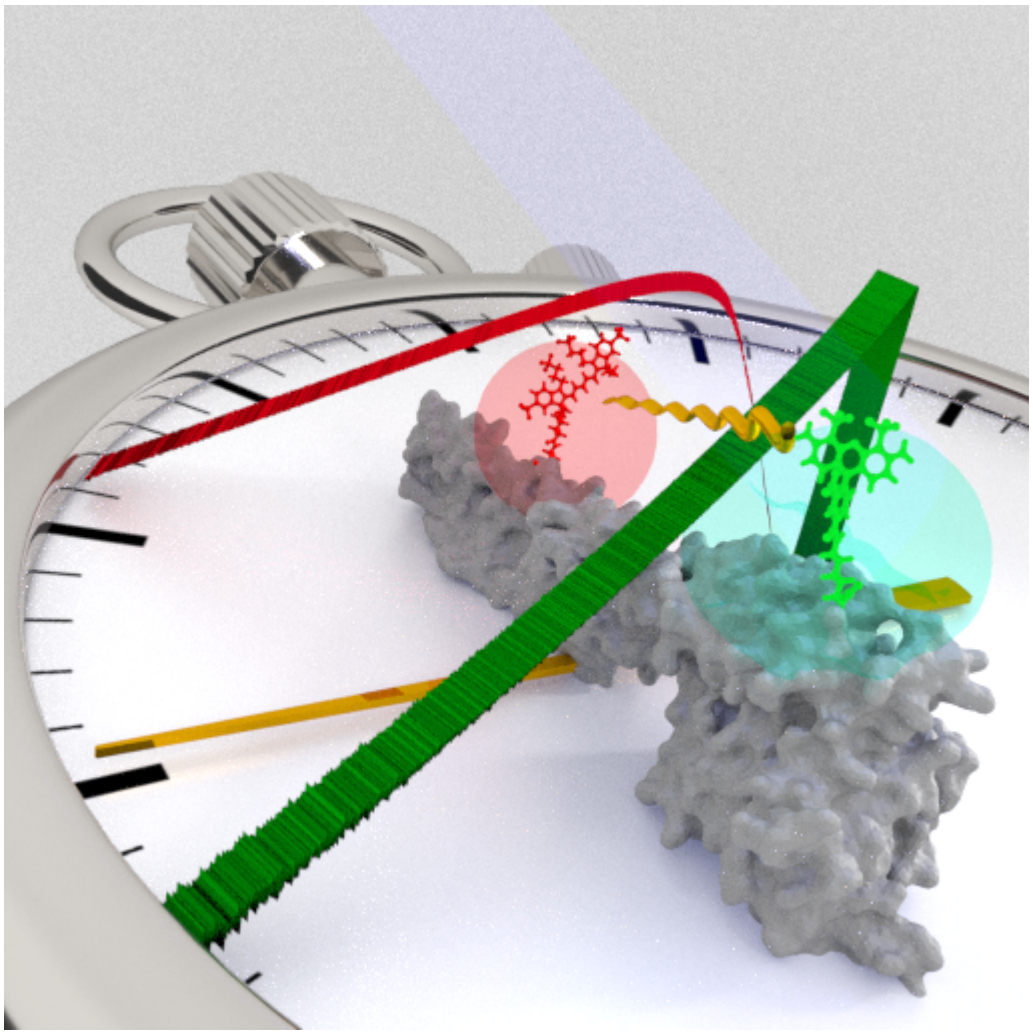


Integrative modeling computes a model based on varied types of input information, be it from experiments or prior models. Often, a type of input information will be best handled by a specific modeling software package. In such a case, we desire to integrate our integrative modeling software package, Integrative Modeling Platform (IMP), with software specialized to the computational demands of the modeling problem at hand. After several attempts, however, we have concluded that even in collaboration with the software's developers, integration is either impractical or impossible. The reasons for the intractability of integration include software incompatibilities, differing modeling logic, the costs of collaboration, and academic incentives. In the integrative modeling software ecosystem,

several large modeling packages exist with often redundant tools. We reason, therefore, that the other development groups have similarly concluded that the benefit of integration does not justify the cost. As a result, modelers are often restricted to the set of tools within a single software package. The inability to integrate tools from distinct software negatively impacts the quality of the models and the efficiency of the modeling. As the complexity of modeling problems grows, we seek to galvanize developers and modelers to consider the long-term benefit that software interoperability yields. In this article, we formulate a demonstrative set of software standards for implementing a model search using tools from independent software packages and discuss our efforts to integrate IMP and the crystallography suite Phenix within the Bayesian modeling framework.

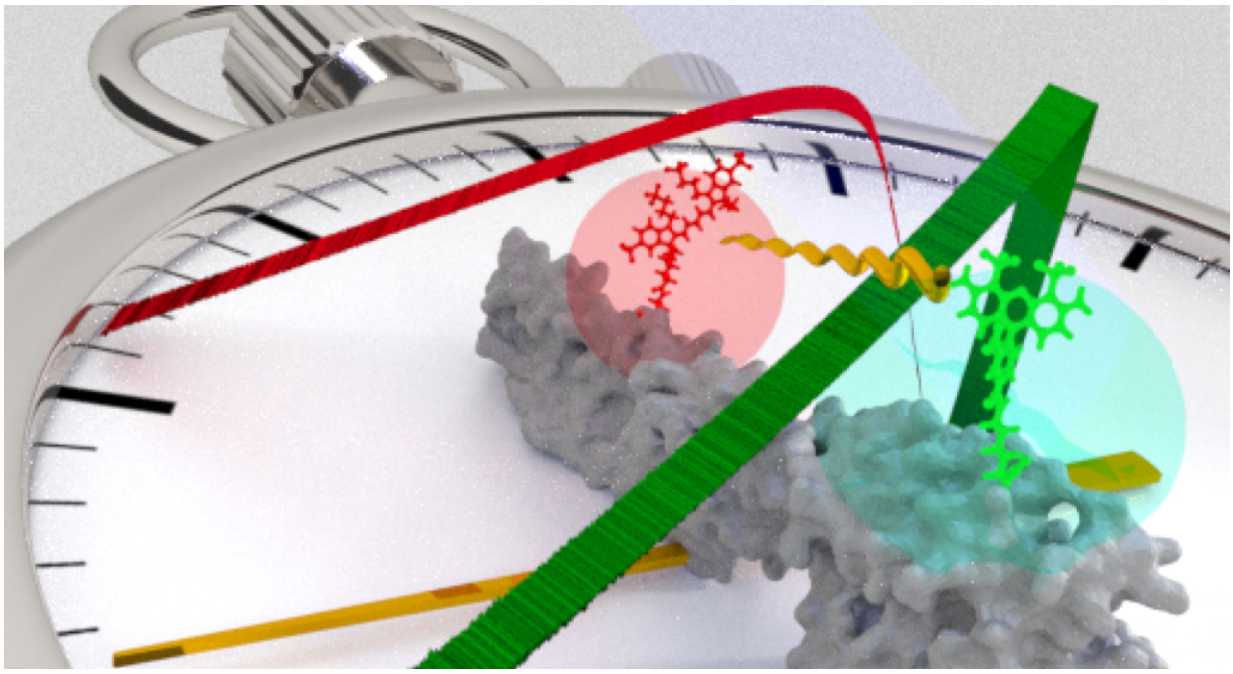
I (Thomas Peulen) am a PostDoc at the Cordes lab (TU-Dortmund) working on advanced spectroscopic method to unravel biomolecular mechanisms by Bayesian methods and integrative modelling focusing on time-resolved microscopy and spectroscopy data. Before joining the Cordes lab, I was PostDoc at the Heinze lab at the Rudolf Virchow Centre for integrative microscopy and a postdoctoral researcher UCSF at Prof. Andrej Šali laboratory and obtained a Ph.D. in Chemistry at Heinrich Heine University (Düsseldorf, Germany.

The goal are rigorous mechanistic insights into the structure and dynamics of macromolecular complexes ideally in living systems, with emphasis on how the interaction between proteins, DNA and carbohydrates determine their biologically relevant function.



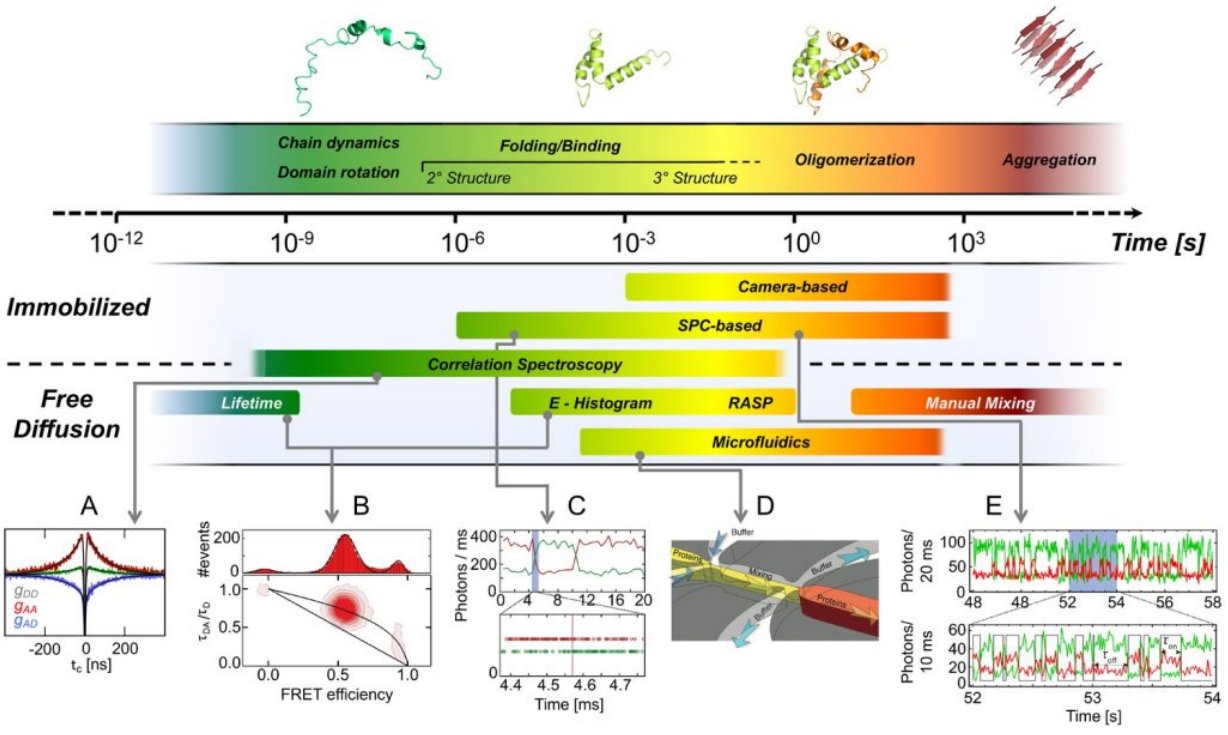
In my research I use experimental, theoretical, and computational methods from molecular biophysics, statistical mechanics, stochastic modelling, molecular modelling and scientific and statistical computing to understand how molecules work.

https://orcid.org/0000-0001-8478-9755



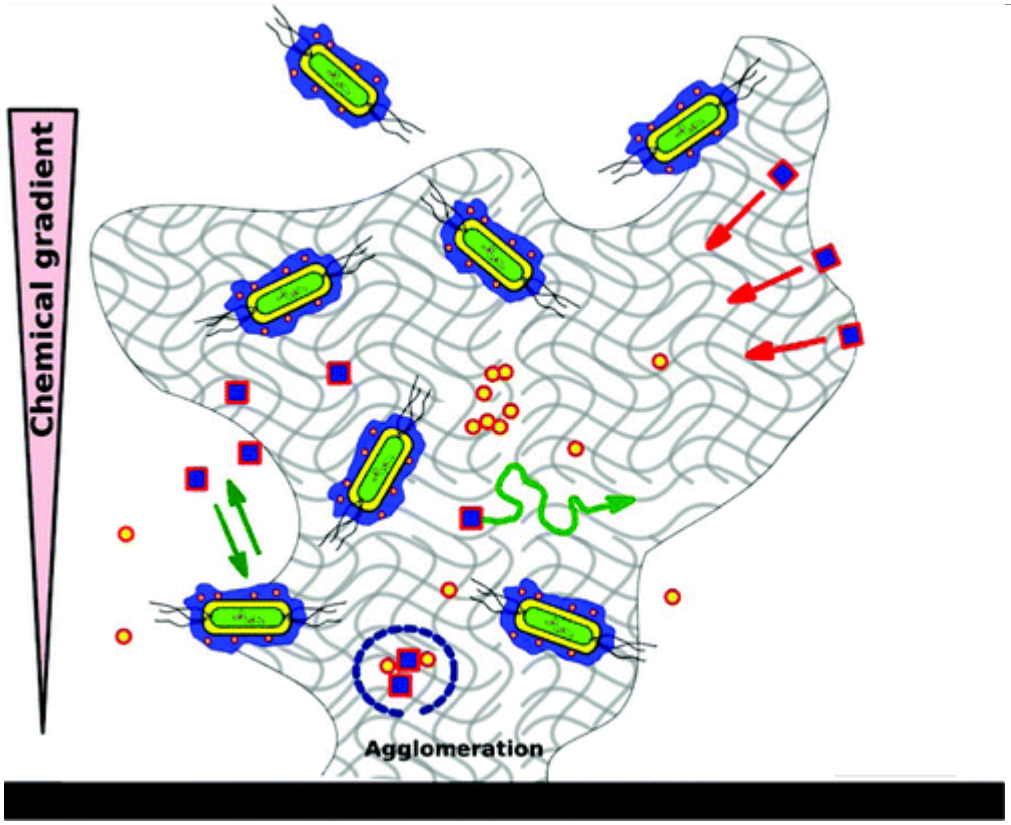
Spectroscopy

Applications, theoretical and methodological developments in fluorescence spectroscopy focused on time-resolved fluorescence applied to biomolecules (Single-molecule FRET, image spectroscopy, time-resolved spectroscopy, fluorescence correlation spectroscopy, ...).



Structure & dynamics

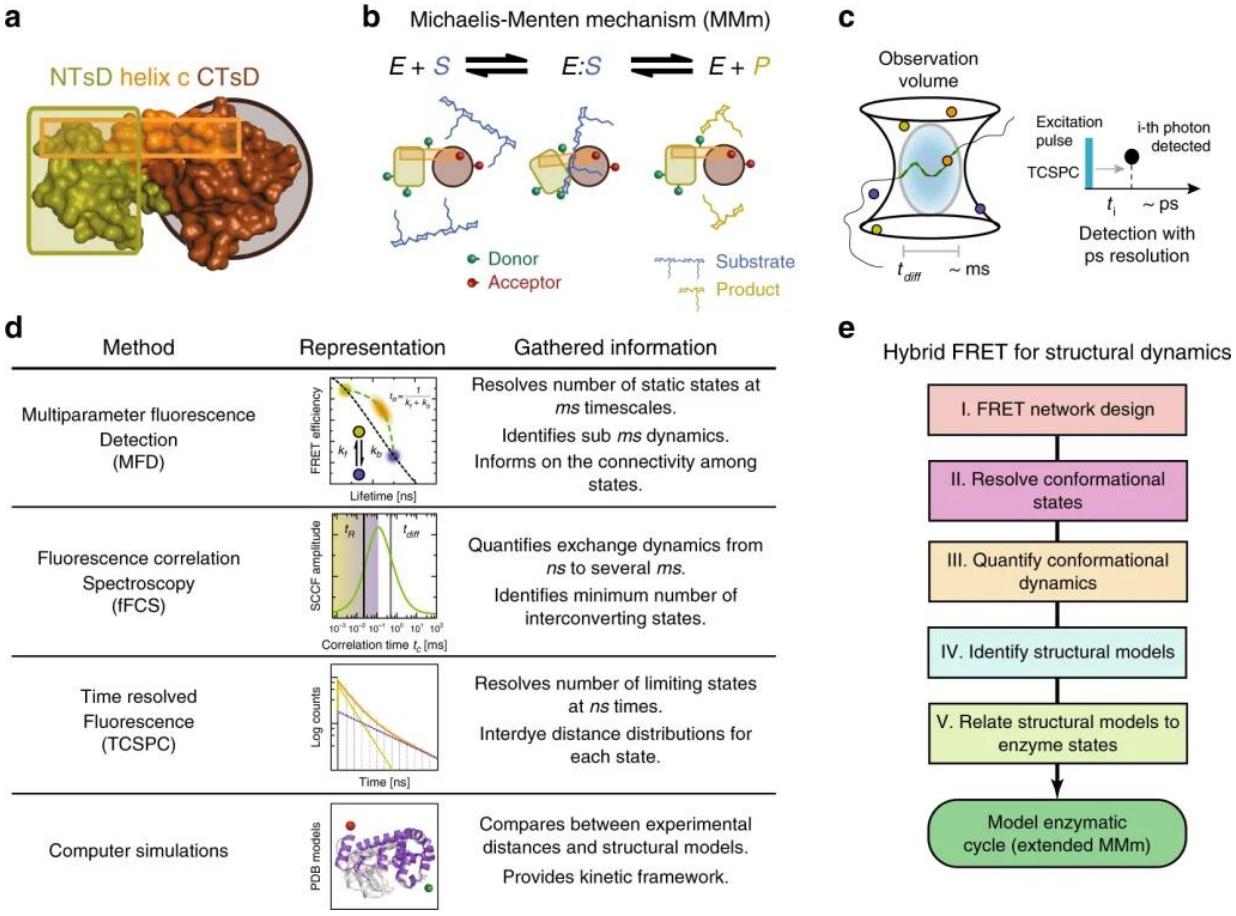
Experimental studies of biomolecular structure and dynamics for unraveling molecular mechanisms.



Live-cell

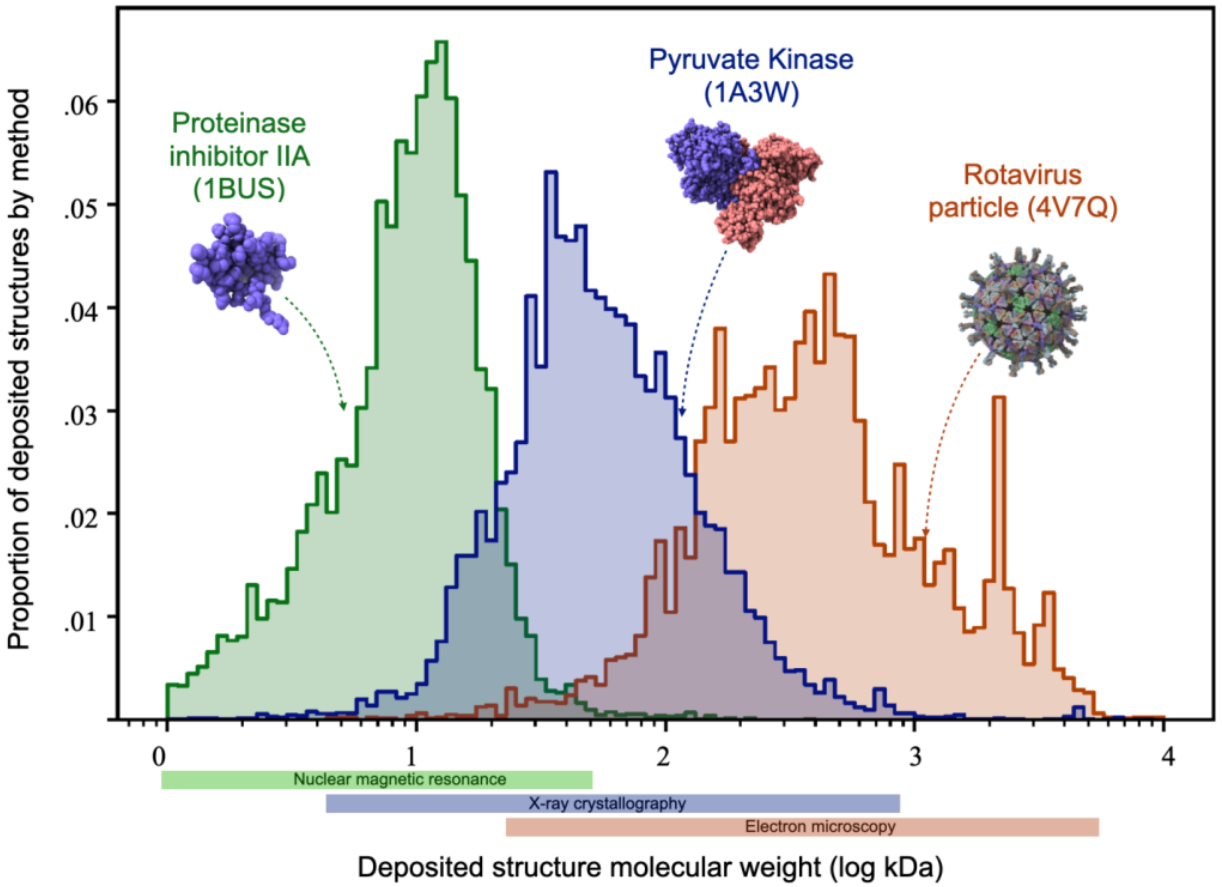
Live-cell microscopy and spectroscopy to bridge high-resolution in-vitro information with processes in living systems.

- Biofilms
- Intracellular pathogens
- GPCRs



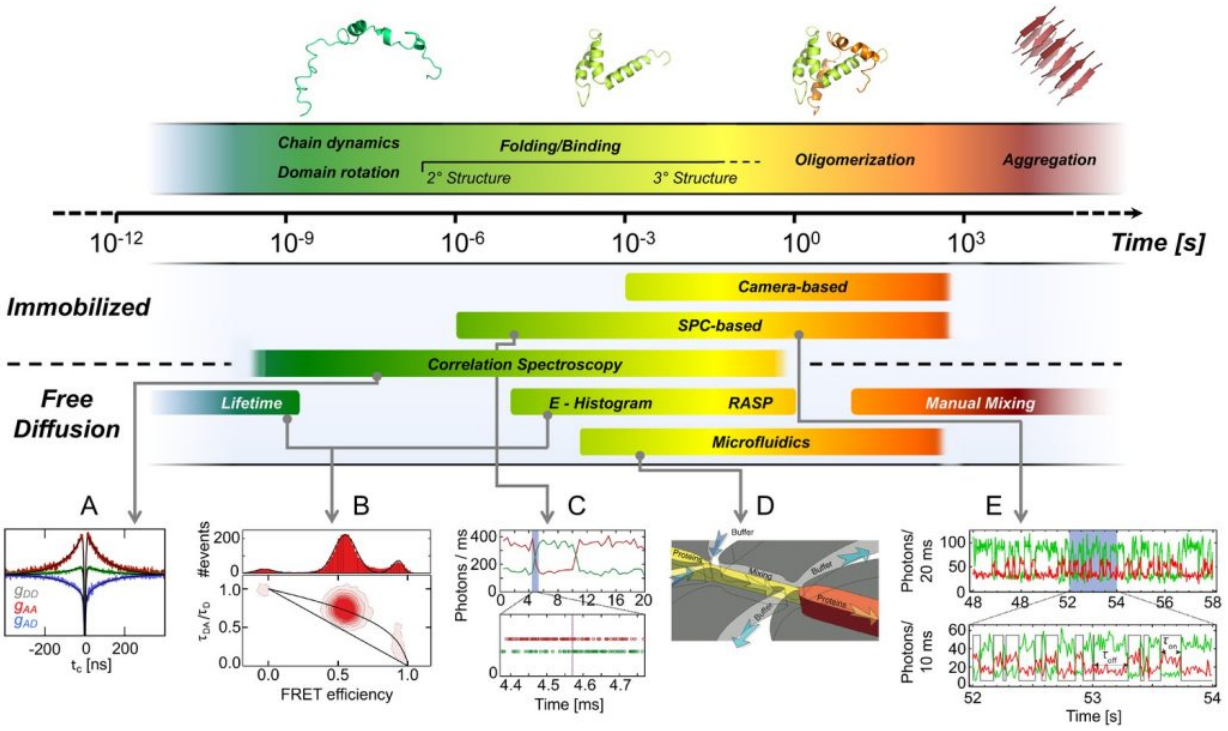
Integrative modeling

High-precision FRET erlaubt eine quantitative Charakterisierung der Strukturen heterogener Biomoleküle. Es können gleichzeitig die Strukturen mehrerer Zustände und die Austauschdynamik (ns- bis s-Zeitbereich) zwischen ihnen untersucht werden.



Integrative modeling computes a model based on varied types of input information, be it from experiments or prior models. Often, a type of input information will be best handled by a specific modeling software package. In such a case, we desire to integrate our integrative modeling software package, Integrative Modeling Platform (IMP), with software specialized to the computational demands of the modeling problem at hand. After several attempts, however, we have concluded that even in collaboration with the software's developers, integration is either impractical or impossible. The reasons for the intractability of integration include software incompatibilities, differing modeling logic, the costs of collaboration, and academic incentives. In the integrative modeling software ecosystem, several large modeling packages exist with often redundant tools. We reason, therefore, that the other development groups have similarly concluded that the benefit of integration does not justify the cost. As a result, modelers are often restricted to the set of tools within a single software package. The inability to integrate tools from distinct software negatively impacts the quality of the models and the efficiency of the modeling. As the complexity of modeling problems grows, we seek to galvanize developers and modelers to consider the long-term benefit that software interoperability yields. In this article, we formulate a demonstrative set of software standards for implementing a model search using tools from independent software packages and discuss our efforts to integrate IMP and the

crystallography suite Phenix within the Bayesian modeling framework.



Single-molecule FRET (smFRET) has become a mainstream technique for studying biomolecular structural dynamics. The rapid and wide adoption of smFRET experiments by an ever-increasing number of groups has generated significant progress in sample preparation, measurement procedures, data analysis, algorithms and documentation. Several labs that employ smFRET approaches have joined forces to inform the smFRET community about streamlining how to perform experiments and analyze results for obtaining quantitative information on biomolecular structure and dynamics. The recent efforts include blind tests to assess the accuracy and the precision of smFRET experiments among different labs using various procedures. These multi-lab studies have led to the development of smFRET procedures and documentation, which are important when submitting entries into the archiving system for integrative structure models, PDB-Dev. This position paper describes the current ‘state of the art’ from different perspectives, points to unresolved methodological issues for quantitative structural studies, provides a set of ‘soft recommendations’ about which an emerging consensus exists, and lists openly available resources for newcomers and seasoned practitioners. To make further progress, we strongly encourage ‘open science’ practices.