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Novel Data Analysis Methods in Multi-Channel and Multi-State Binding Experiments

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Abstract

Single-Molecule studies are advanced microscopy techniques to view biomolecules, such as proteins and DNA. Traditionally, this is done using fluorescently labeled molecules. To extract this information, advanced, customizable data analysis tools must be created. The first goal is to create a method to readily extract information from multiple molecules. To extract this information, advanced, customizable data analysis tools must be created. The second goal is to extend pattern recognition of binding events to multi-state and multi-channel binding patterns. The KERA 3.0 suite is designed to extend idealization software to extract this information from previously extractable data. This allows researchers to study protein complexes, their inhibitors, and their mechanisms, more holistically and more efficiently.

Introduction

KERA: Theory

KERA is based on the Microsoft Excel Macro created by Dr. Elizabeth Boehm and Eric Boehm1,2.

- The goal: To classify and count all binding events found within a set of traces
- Binding order matters (ABa ≠ BAa)
- Complex events may be grouped together or separately depending on length
- Use of Regular Expressions (regexp function) allows for limitless flexibility
- Once an event classification is defined, every event matching the description is catalogued and analyzed by the code in a single, comprehensive table

KERA: Applications

Complex Systems:
- Allows any number of channels, fluorophore colors, or states in the binding model
- Useful in discriminating between simultaneous-binding events (top right: whether A or B dissociates first is recorded to classify which binding scenario occurred)1
- Accepts non-binary state models produced by ebFRET (trajectory at right) or QuB

Kinetics Data
- Records detailed kinetics data of each portion of complex events
- Category-specific dwell-time histograms
- Customizable binning strategies2
- Fitting toolbox handles single and double exponentials, or more complex functions as desired
- Logarithmic binning is also supported

Right: off time data (Δtu) for simple bindings of protein A, with both logarithmic and default binning

Background

Binding Experiments
- With TIRFM (Total Internal Reflection Fluorescence Microscopy), fluorescent molecules are visible only at slide surface
- Non-tagged binding partners are tethered to the slide
- Immobilization and low background from bulk solution allows single-molecule tracking and colocalization
- Multiple channels used to image distinct colors of fluorophore (different biomolecules) concurrently in each location
- Interaction of binding partners may exhibit cooperative, inhibitory, or order-specific behavior
- Processing and analyzing the large volume of data produced can be a daunting task

Trace Idealization
- The fluorescence trajectories of molecules may be idealized (converted into dwell times in discrete states)
- A model may simply translate “On” and “Off” periods into 1% and 0% (a binary state model) or assume more possible levels of fluorescence
- ebFRET, designed for uses in FRET applications, can also be used to idealize traces into multi-state models3
- Challenges for trace idealization include background noise, missing states, uncertain on times, and photo bleaching
- A method of normalization which is robust under these conditions is being tested for ebFRET as part of this work

Results and Future Work

Results
- KERA is an accessible, user-friendly MATLAB suite which can analyze binding experiment data in a highly versatile way
- Improves user interface, data input methods, and output data
- Multi-channel and multi-state analysis extends capabilities of the original KERA
- Analysis gives dwell time, off time, frequency, and exhaustive event list for all classes of events
- Time data may be automatically fit to an exponential histogram and plotted

Future Work
- User-defined classes will allow users to examine interesting patterns in any variety of forms
- Create regex and reverse regex translators to increase user-friendliness
- Allows for flexibility in defining “default state” within trajectories

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References

Below: Gaussian fit from TIRFM slide movie analysis program suite. Above: Possible idealized trace of two tagged molecules.