NOV 13, 2022

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Downstream Analysis of ebFRET Data

This protocol is published without a DOI.

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COMMENTS 0

ABSTRACT

This protocol follows from the "Using ebFRET for Hidden Markov Modeling" protocol and is the fourth and final step towards analyzing your single-molecule FRET traces using Hidden Markov Modeling. In this protocol, I describe some of the questions that you may ask of the data generated by ebFRET and list some of the programs that I have made to do the calculations required to answer these questions.

PROTOCOL CITATION

Clark Fritsch 2022. Downstream Analysis of ebFRET Data. **protocols.io** https://protocols.io/view/downstream-analysis-of-ebfret-data-ci8juhun

CREATED

Nov 13, 2022

LAST MODIFIED

Nov 13, 2022

PROTOCOL INTEGER ID

72683

1 After creating your "2stateHMM_practice_Analysis.dat", as described in the "Using ebFRET for Hidden Markov Modeling" protocol, you can then calculate / retrieve various types of information from your data.

2 The downstream analysis that I have done so far for the ebFRET data can be divided into the following questions:

1. What is the distribution of FRET values for each state that was identified by ebFRET?

ebFRET_HMM_State_Histograms.R

2. What is the idealized FRET efficiency for each FRET state that was identified by ebFRET?

FRET_Determination_ebFRET_v1.R

3. What is the actual FRET efficiency (non-idealized FRET) for each FRET state that was identified by ebFRET?

FRET_Determination_ebFRET(Actual FRET, Not Viterbi Mean).R

4. What is the lifetime of each FRET state that was identified by ebFRET? Outputs a file that can be used in MEMLET to determine rate constants for each state.

Rate_Determination_ebFRET_v2.R

3 Each of the programs included above were made for simple ad-hoc analysis of the data outputted from ebFRET and will likely need to be modified slightly depending on the number of states that you had ebFRET fit to your data.