# Deep Learning-Assisted Analysis of Non-Equilibrium smFRET Trajectories under Low-SNR Conditions

Xiao-Man Wang*, Long Chen* *and Yan-Wen Tan*
** State Key Laboratory of Surface Physics, Department of Physics, Fudan University, Shanghai 200433, China*
*** The University of Hong Kong, Department of Electrical and Computer Engineering, Hong Kong 999077, China*

## Introduction

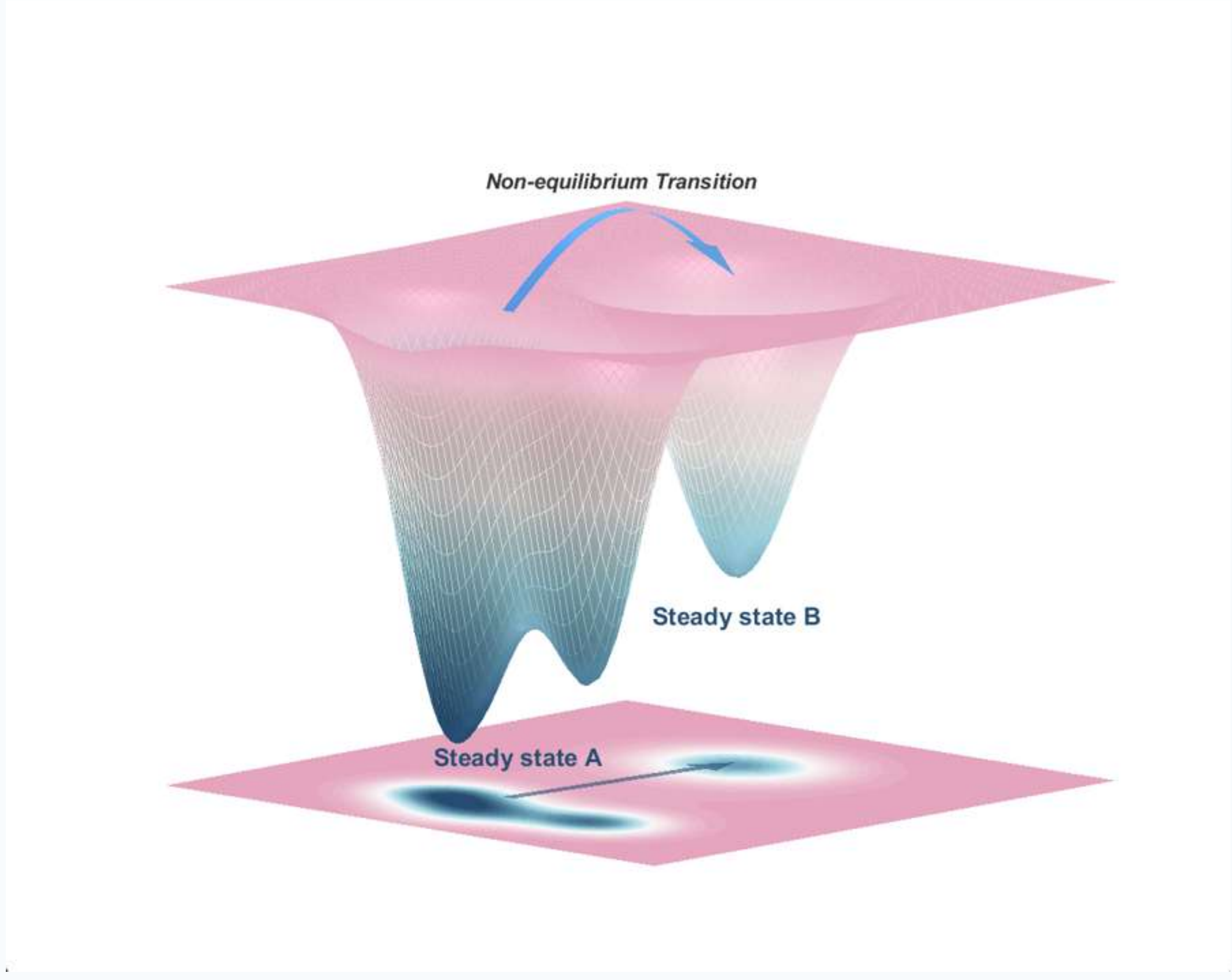

Figure : Illustration of the free energy landscape of biomolecules. Following external stimulation, they move from one steady-state status to another through non-equilibrium transition.

Single-molecule fluorescence resonance energy transfer (smFRET) is one of the key techniques for resolving conformational dynamics of biomolecules. Most studies in this field have focused on the dynamic conformational changes of biomolecules under a stable external environment. However, the response of many biomolecules to external stimuli, such as illumination at specific wavelengths, changes in ion concentration, or temperature variation, can drive their conformational dynamics from one steady state to another, resulting in a non-equilibrium transition.

Importantly, such non-equilibrium transitions do not necessarily occur instantaneously at the moment of external stimulation. Our project aims to analyze smFRET trajectories under external perturbations by discretizing or denoising noisy raw trajectories and identifying the position of the non-equilibrium transition point.

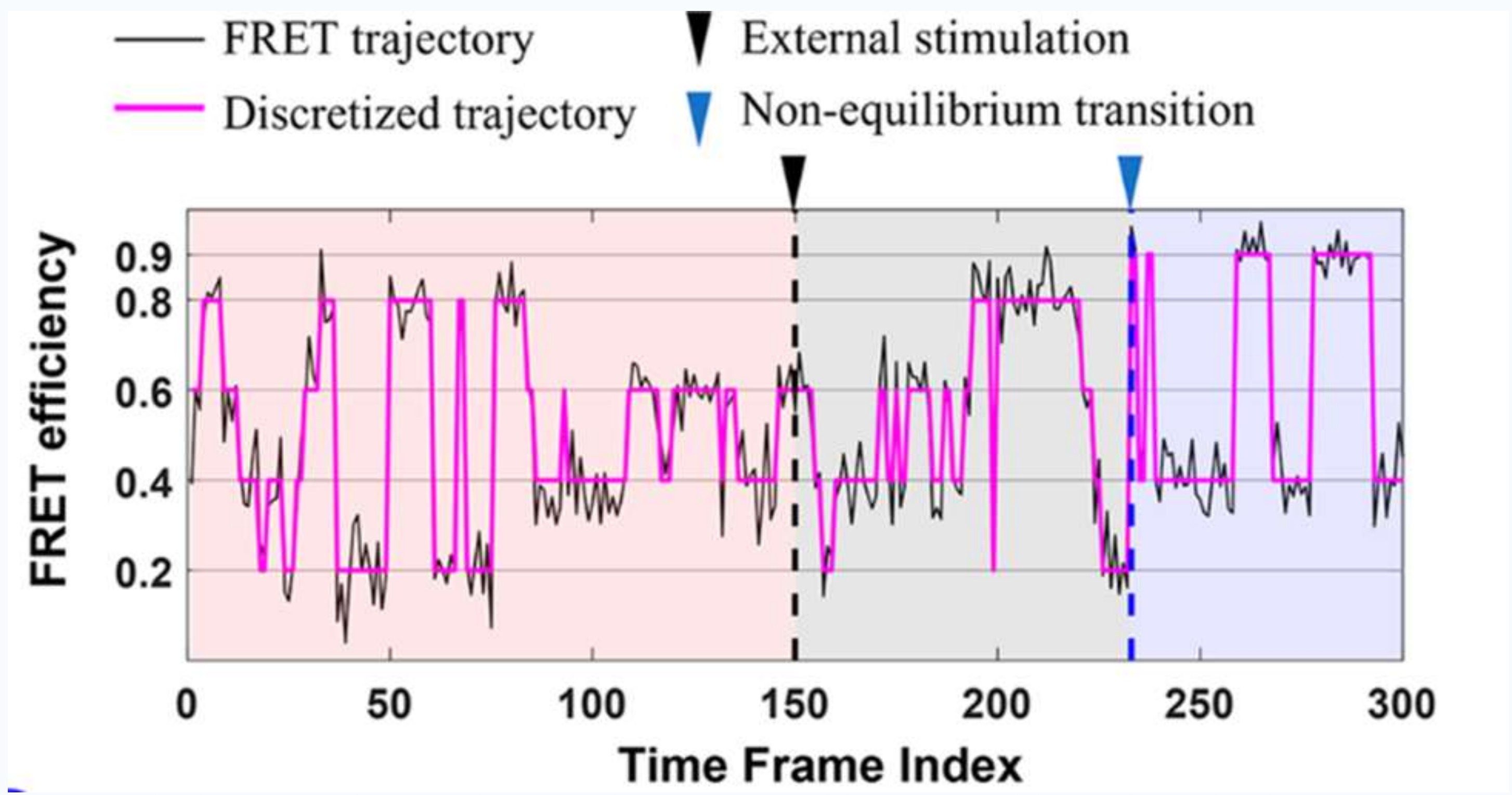

Figure: A typical FRET trajectory that contains a non-equilibrium transition, where the data is a mixture of contributions from two steady-state statuses. The black dashed line represents the position of external stimulation, while the blue dashed line indicates the position of the non-equilibrium transition.

## Classification and Evaluation Criteria

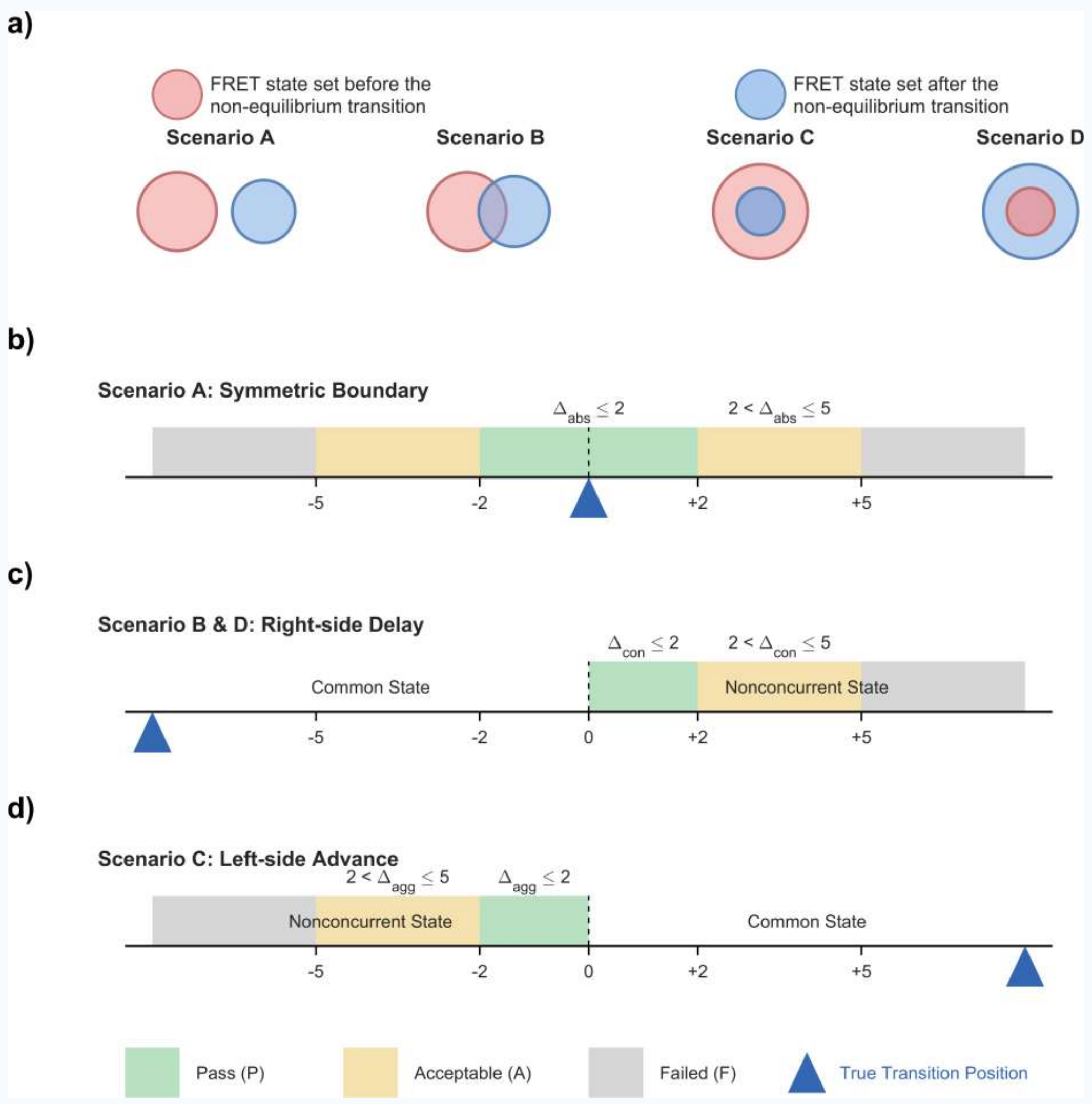

Figure: (a) Four scenarios of state compositions before and after the non-equilibrium transition, including the state sets before and after the non-equilibrium transition are completely nonconcurrent (scenario A); two state sets are partially overlapped (scenario B); the state set after (before) the non-equilibrium transition is a subset of that before (after) the non-equilibrium transition (scenarios C and D, respectively). (b)(c)(d)Grading criteria in outcomes in different scenarios.

## Results: Accuracy and Trajectory Error

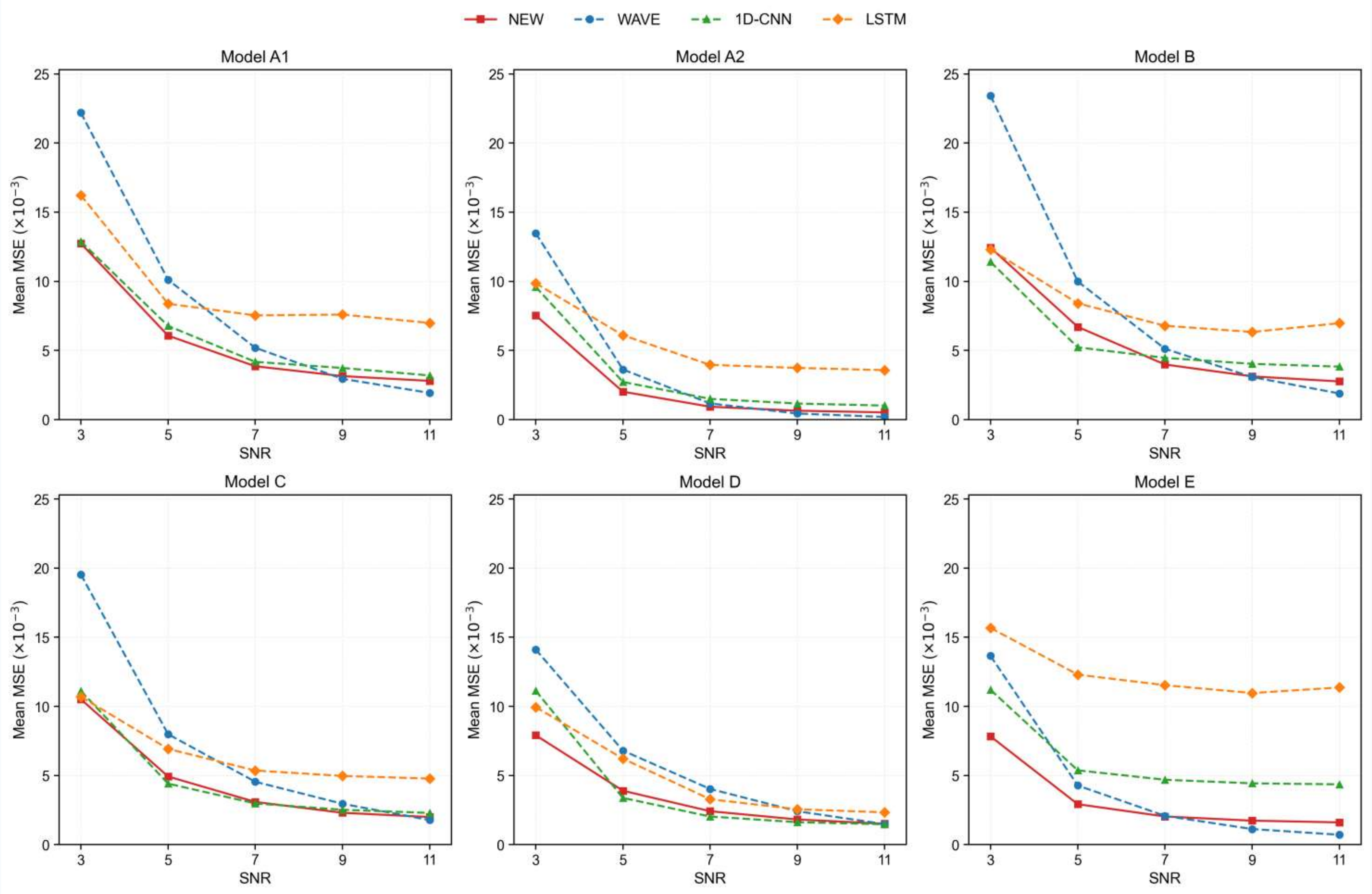

Figure: Comparison of the mean MSE between the discretized trajectories and the ideal trajectories across six kinetic models and five SNR levels. The NEW method achieves consistently low errors, particularly under low-to-medium SNR conditions, demonstrating improved robustness in discretizing noisy smFRET trajectories.

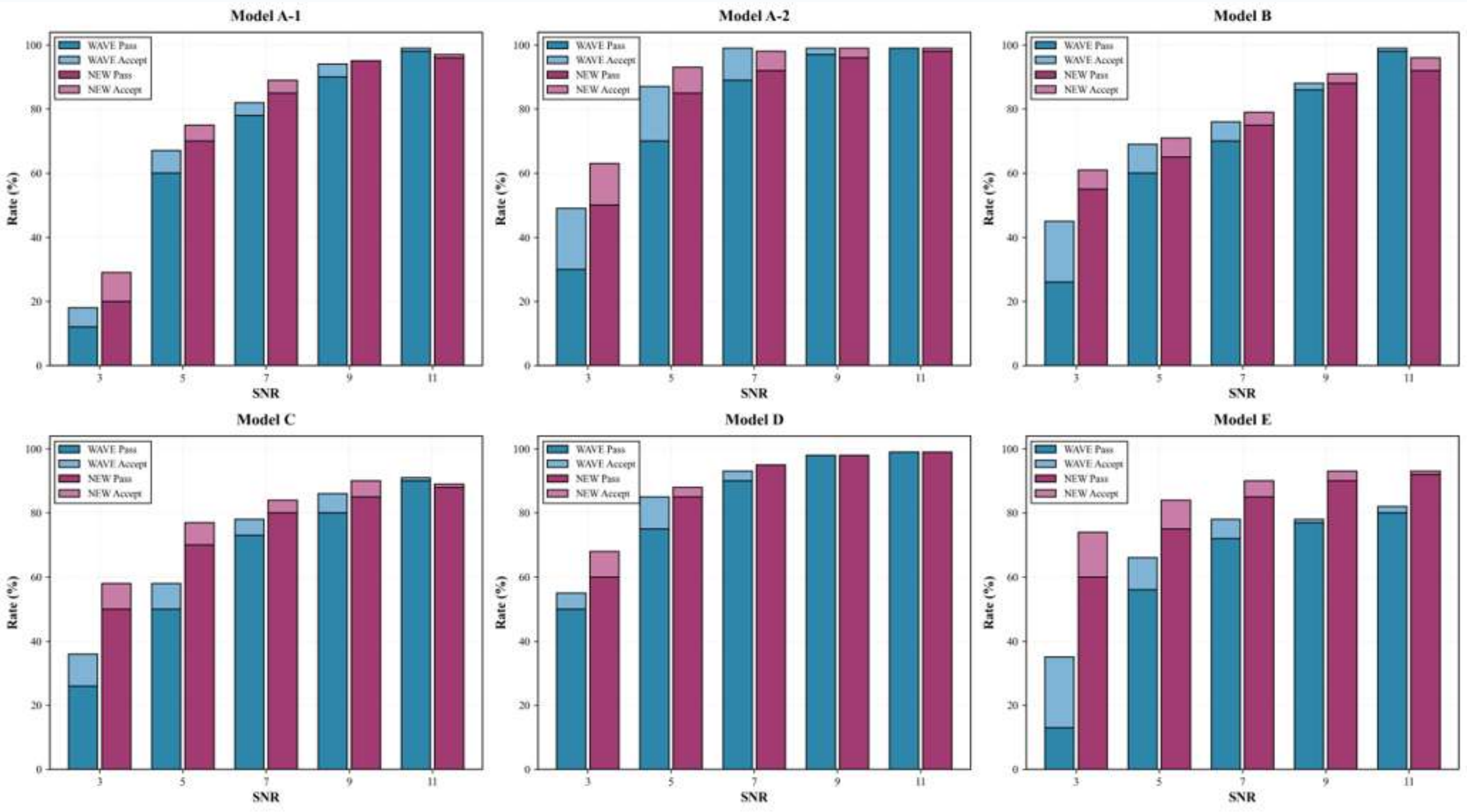

Figure: Comparison of non-equilibrium transition-point localization performance between the NEW method and WAVE across six kinetic models and five SNR levels. The dark segments represent the Pass rate, while the light segments represent the Accept rate. The NEW method achieves higher or comparable Pass + Accept rates under most conditions, especially at low SNR, indicating improved robustness in identifying transition points from noisy smFRET trajectories

## Conclusions

The NEW method demonstrates improved performance in both state identification and trajectory reconstruction. Compared with typical neural-network baselines such as 1D-CNN and LSTM, the NEW method achieves higher state-number identification accuracy and lower MSE between the discretized trajectories and the ideal trajectories, indicating more reliable recovery of noisy smFRET trajectories. In addition, the NEW method outperforms WAVE in non-equilibrium transition-point localization, especially under low-SNR conditions, showing stronger robustness in analyzing externally perturbed smFRET data.

Importantly, the NEW method can also be used in a modular manner. Even when transition-point localization is not required, its denoising and discretization components remain useful for steady-state smFRET trajectory analysis, providing accurate state recovery and improved trajectory reconstruction under noisy conditions.