1 (20 pts.). Conformational changes involved in ribozyme function can be studied using single-molecule fluorescence spectroscopy. The ribozyme can undergo a reversible self-catalyzed cleavage reaction. The cleaved form of the ribozyme can shift between two conformations, docked and undocked.

You prepare a construct in which the two strands of RNA are labeled with two fluorophores, a donor and an acceptor. You find that this construct exhibits high FRET in the ligated and cleaved/docked states, but low FRET in the undocked state.

![Graph showing high and low FRET states](image)

Looking at changes in FRET with single-molecule resolution, you find that there are long-lived sojourns in the high-FRET state that are interrupted by bursts of rapid transitions between high- and low-FRET states. The lifetimes for the long-lived and short-lived high-FRET states are 50 s and 0.32 s, respectively. The lifetime of the low-FRET state is 0.29 s. Each burst of low-FRET sojourns contains on average, 16 sojourns in the low-FRET state.

Based on these data, calculate the rate constants for cleavage, ligation, undocking, and docking.

2. You are studying the interaction of single-stranded DNA with fabricated nanopores in wafers of silicon nitride. You measure the conductance of ions through the nanopores at a constant voltage, and find that in the absence of DNA, the nanopores pass a current of 200 pA. In the presence of single-stranded DNA, however, the current transiently decreases to 75 pA.

A (5 pts.). Will the lifetime in the high-conductance state be dependent on the DNA concentration? Will the lifetime in the low-conductance state be dependent on the DNA concentration? Explain.
B1 (3 pts.). The transient decrease in conductance might be due either to
blockade of the pore from the DNA-containing side or to translocation of the
DNA from one side to the other. What do each of these hypotheses predict about
how the blocked time will depend on the length of the DNA? Explain. Are these
hypotheses mutually exclusive?

B2 (2 pts.). Propose an independent test of the hypothesis that the DNA is
translocated from one side of the nanopore to the other.

C (5 pts.). You discover that the mean conductance of the low conductance state
depends on the base composition of the DNA for homopolymeric DNAs. In
addition, you discover that the standard deviation of the conductance also depends
on the base composition. Propose an explanation for these observations.

D (5 pts.). Nanopores of this type have been proposed as a basis for a high-
throughput DNA sequencing technique. How might the observed dependence of
both the mean and the standard deviation of the conductance on base composition
be useful in sequencing applications?