

# Unravelling the dihydrofolate reductase catalytic reaction with nanopore test-tube

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The reaction catalyzed by dihydrofolate reductase (DHFR) is an archetype reaction that have been used to link protein dynamics and catalytic function. Here we show that single DHFR molecules can be trapped inside the ClyA nanopore and the catalytic reaction can be observed in real-time by nanopore currents.

Our data showed that DHFR adopts multiple conformations with different affinities for the substrates. Intriguingly, in the presence of only one substrate or when substrate analogues are used the enzyme could only bind with one configuration. When both substrates are present, the tertiary complex formed multiple times prior reaction, and enzymes were observed to cycles between multiple configurations.

This work provides the first single-molecule characterization of an enzymatic reaction during several turnovers, and contributes to understand the fundamental link between of enzyme dynamics related to the catalysis.