Unravelling the druggability of DNA helicases through enzyme kinetics and mathematical modelling

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Introduction

- DNA helicase inhibitors have the potential to impact several therapeutic areas, including oncology, viral and microbial diseases.
- Despite their promise and extensive interest from the drug discovery industry, there are no approved drugs targeting helicases.
- To discover and successfully characterise new helicase inhibitors, effective high throughput enzyme assays are required. Critically, suitable mechanistic models of enzyme catalysis and function need to be developed. To date, there is limited evidence describing the enzymatic characterization of DNA helicase.
- **We present a detailed description of the enzyme kinetics of a bacterial DNA helicase, using a differential equation-based model and a FRET-based assay approach.**

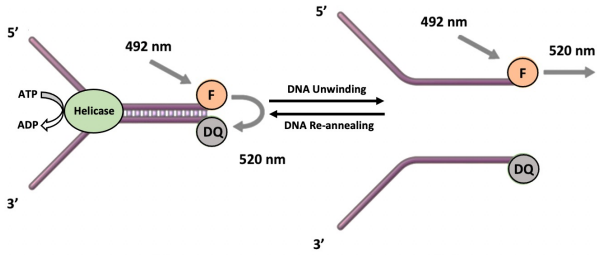
Methods

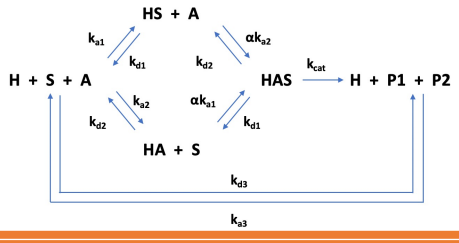
- ***FRET assay:***

Substrates = ATP; DNA forked duplex (tagged with a fluorophore (F) and a non-emitting dark quencher (DQ)).

- ***Kinetic model:***

Ordinary differential equations were built in Origin C to detail the double-substrate reaction, considering a monomeric helicase forming a ternary complex with ATP and DNA, in which the initial DNA duplex can be spontaneously reformed.





H = Helicase
S = Substrate DNA
A = ATP
P1 = Product 1 (Oligo-F)
P2 = Product 2 (Oligo-DQ)
α = Cooperativity
(We are not considering the products of the ATP in this model: P3 = ADP and P4 = Pi)

Results & Discussion

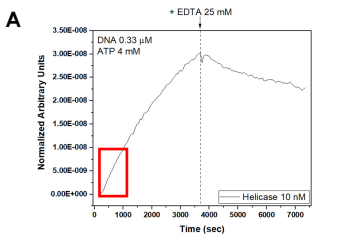
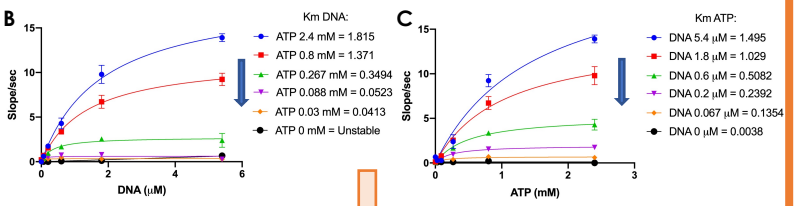


Figure A. Time-course measurement of the helicase reaction. The enzyme activity increased until the addition of EDTA to stop the reaction. After this point, the signal decreases due to the substrate slowly reforming from the product. In the red box: linearity of initial rate, considered for the analysis of the double substrate titration (see Figures B and C).

Double Substrate Titration. Figure B. ATP dependency of the helicase in presence of different DNA concentrations. **Figure C.** DNA dependency of the helicase in presence of different ATP concentrations. These results suggest the existence of an **ANTI-COOPERATIVITY** mechanism. A more precise way to obtain the Km for DNA and ATP is represented by progress curves (see Figure D).



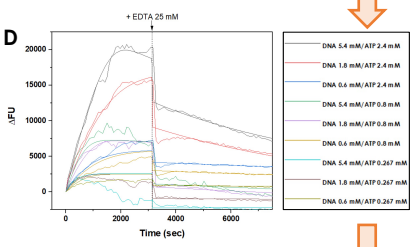


Figure D. Progress curves representing the enzyme activity in presence of different DNA and ATP combinations. The fit performed with the model created on Origin C describes well the data. Kinetic parameters deriving from the analysis are:
Km DNA = 31.9 ± 0.7 nM
Km ATP = 12.9 ± 0.2 µM
α = 54.6 ± 14.6

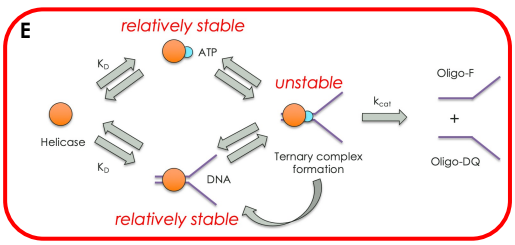


Figure E. The formation of the ternary complex (helicase + ATP + DNA) does not stabilise the complex itself, since a high k_{cat} drives the reaction to proceed rapidly towards product formation.

We measured the Km of ATP to be in the region of 10-100 µM at low DNA concentrations. Given previous HTS have been run at low DNA concentrations with high ATP concentrations, the conditions may not have been ideal to identify ATP competitive inhibitors.

Conclusions

- DNA helicase catalytic mechanisms require a rationalisation that can be achieved with specific mathematical models.
- The description of such a kinetic enzyme mechanism will allow us to better understand inhibitory mechanisms to create more reliable helicase-targeting drugs.