

# Conformational dynamics of human dioxygenase ABH2 in the course of action on methylated DNA

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## Introduction

Methylation is a widely occurring chemical modification of nucleic acids. Methylation agents, both extracellular and intracellular, can attack vulnerable sites in DNA that can lead to cytotoxic or carcinogenic DNA damage. Methylation also plays a crucial role in signaling in the cell. Using S-adenosylmethionine (SAM) as the most common source of electrophilic methyl groups, various methyltransferases modify DNA, RNA or proteins, which regulates gene expression [1].

Methylation, both as a regulatory process and as a damage, implies the possibility of demethylation, return of DNA, RNA or protein to the original state. Some of the enzymes of the non-heme FeII-2-ketoglutarate-dependent dioxygenases family act as DNA repair enzymes, both in prokaryotes (AlkB [2]) and in eukaryotes (9 analogues of AlkB [2], TETs [3]). Common to all these enzymes are their function in the cell and their structure and chemical mechanism of catalysis.

## Alkylation damage and human AlkB homologues ABH2/3

To protect the genomic DNA from alkylating agents, a number of solution have evolved, including:

- removal of alkylated bases by BER glycosylases,
- repair methylguanine by suicidal O-6-methylguanine methyltransferase
- direct removal of the methyl group of oxidases family AlkB [2].

Although alkB gene in *E. coli* was discovered as early as 1984 [4], the characterization of this gene has taken more than two decades. AlkB gene encodes a protein belonging to the family of FeII/α-KG-dependent dioxygenases, which contains a wide variety of enzymes that catalyze the oxidation of various substrates oxygen molecule [5].

*E. coli* AlkB and its human homologues ABH2 and ABH3 deal with DNA alkylation, mostly with adenine and cytosine modifications (fig. 1). These enzyme perform DNA repair through direct dealkylation.

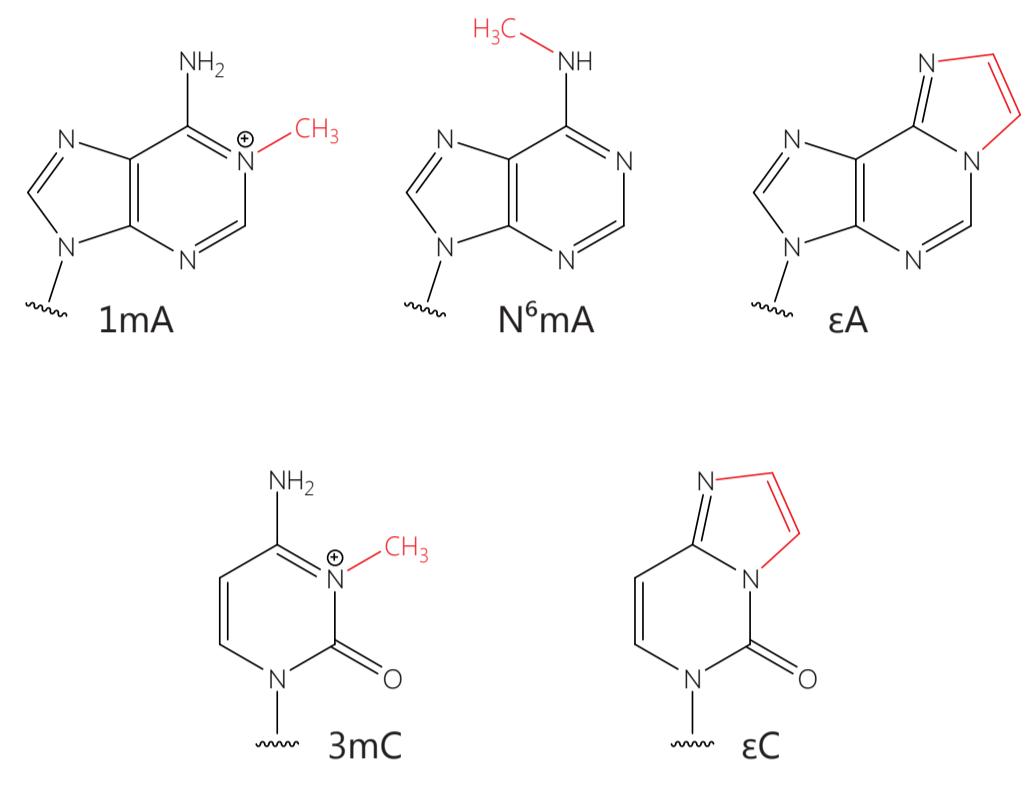


Figure 1. Major ABH2 and ABH3 substrates:  
1mA – 1-methyladenosine; N<sup>6</sup>mA – N<sup>6</sup>-methyladenosine;  
eA – 1,N<sup>7</sup>-ethenoadenosine; 3mC – 3-methylcytosine;  
εC – 3,N<sup>4</sup>-ethenocytosine.

The main difference in substrate specificity between ABH2 and ABH3 is relation to DNA state: ABH2 prefers double-stranded DNA and ABH3 prefers single-stranded.

## Structural basis of direct DNA dealkylation

Crystallographic studies have shown ABH2 to create extensive contacts with both DNA duplex chains (Fig. 2) [6]. ABH2 does not contain sites to search for specific lesions, instead, it appears that ABH2 detects damaged bases by checking base pairs for the stability of hydrogen bonds. Unlike AlkB, ABH2 contains a unique short hydrophobic beta-pin in close proximity to the active site, which provides preferential binding of ABH2 to double-stranded DNA (fig. 2).

Active site of ABH2 shares common architecture of AlkB homologues: metal ion is handled by two histidine and one aspartate residues, α-KG anion is bounded by arginine–arginine pair and damaged base is fixed with aromatic residue by π-stacking.

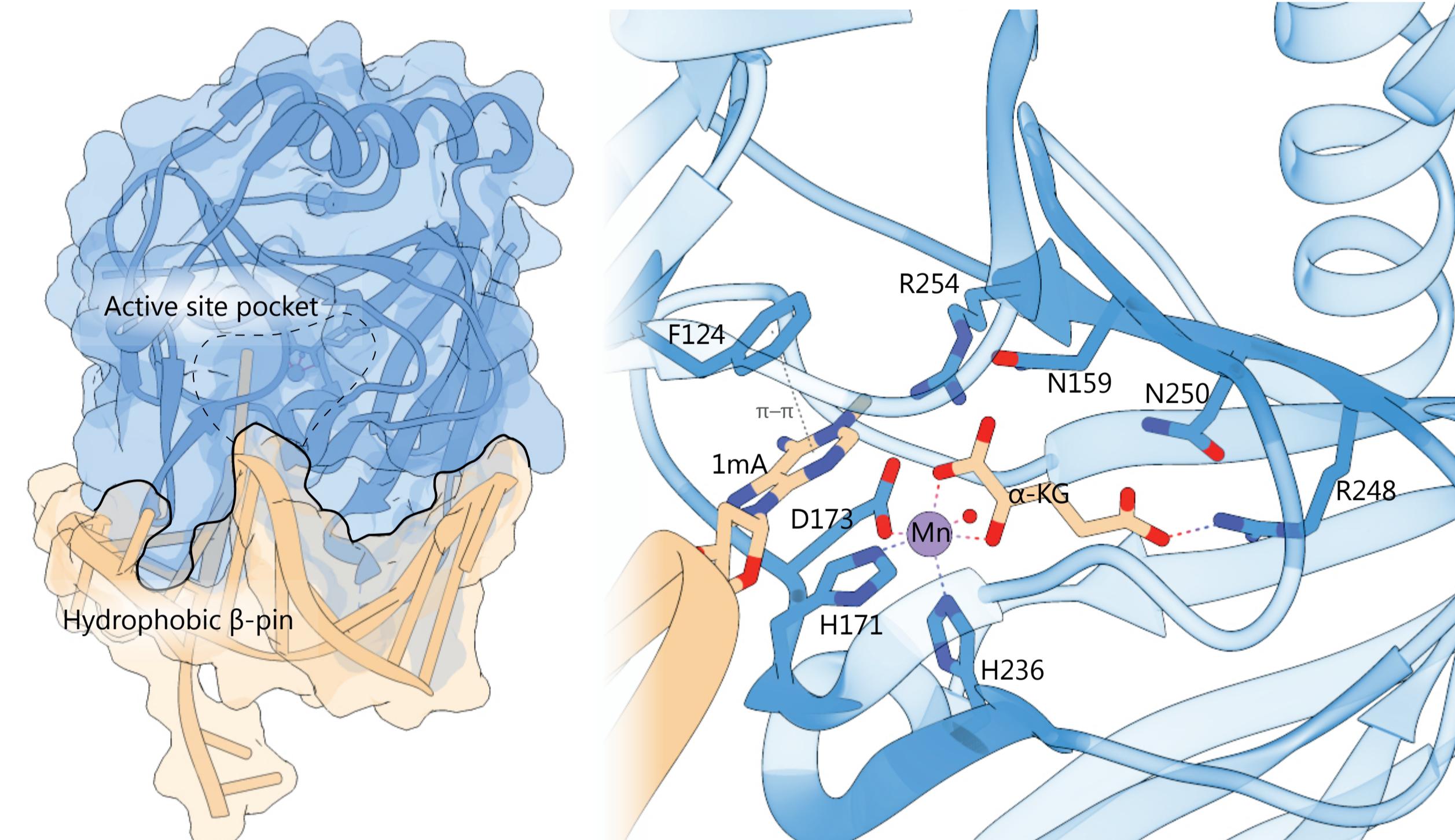


Figure 2. Crystal structure of human ABH2. PDB ID: 3BUC

A Overall structure. Active site region is highlighted with dashed line, protein–DNA interface is shown with thick line.

B Detailed view of catalytic site. H171-D173-H236 triade, α-KG and metal ion form the oxidative unit (works only if the ion is Fe<sup>2+</sup>). R248-R254 pair fixes α-KG anion.

Structural and kinetic data [6] of AlkB, ABH2 and, generally, non-heme dioxygenases action allow to predict their mechanism of action (fig. 3). Alkylated DNA oxydation goes through several steps: reduction of O<sub>2</sub> molecule, resulting in Fe<sup>IV</sup> complex, decarboxylating of α-KG and, finally, oxydation of alkyl group by Fe<sup>IV</sup> and regeneration of enzyme active site.

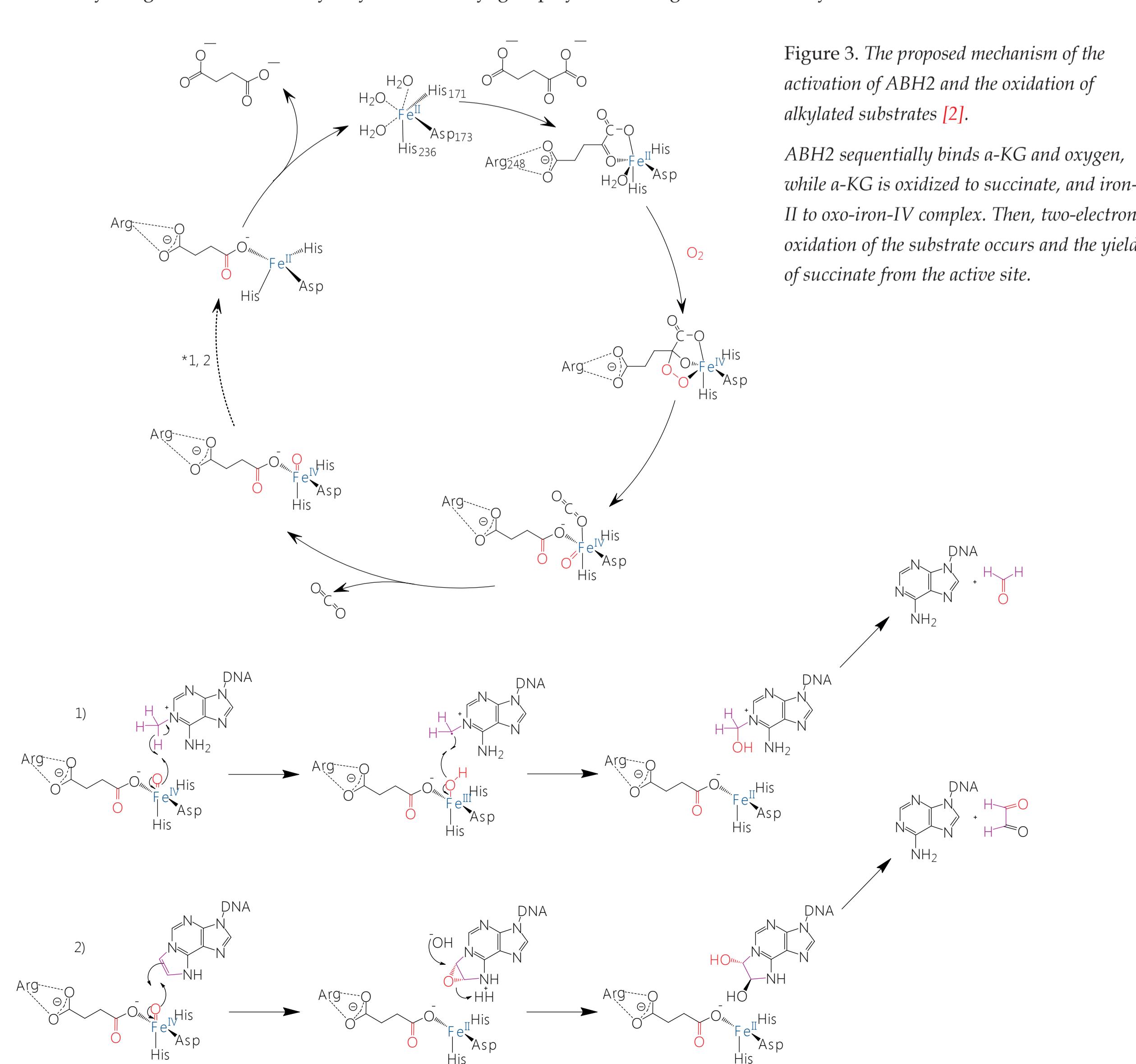


Figure 3. The proposed mechanism of the activation of ABH2 and the oxidation of alkylated substrates [2].

ABH2 sequentially binds α-KG and oxygen, while α-KG is oxidized to succinate, and iron II to oxo-iron-IV complex. Then, two-electron oxidation of the substrate occurs and the yield of succinate from the active site.

## Experiments and results

### Buffer tuning

Due to reach optimal reaction conditions, several buffers with different pH were screened. Optimal value was found to lay in range 7.4–7.6 (Fig. 4A).

To compensate low stability of Fe<sup>2+</sup> ions in aqueous solutions, 2 mM sodium ascorbate was added. Fe<sup>2+</sup>-ascorbate complex absorbs near UV light ( $\lambda_{\text{max}} \sim 270$  nm) so Trp excitation wavelength was changed from 290 to 300 nm to avoid buffer light absorption (Fig. 4B).

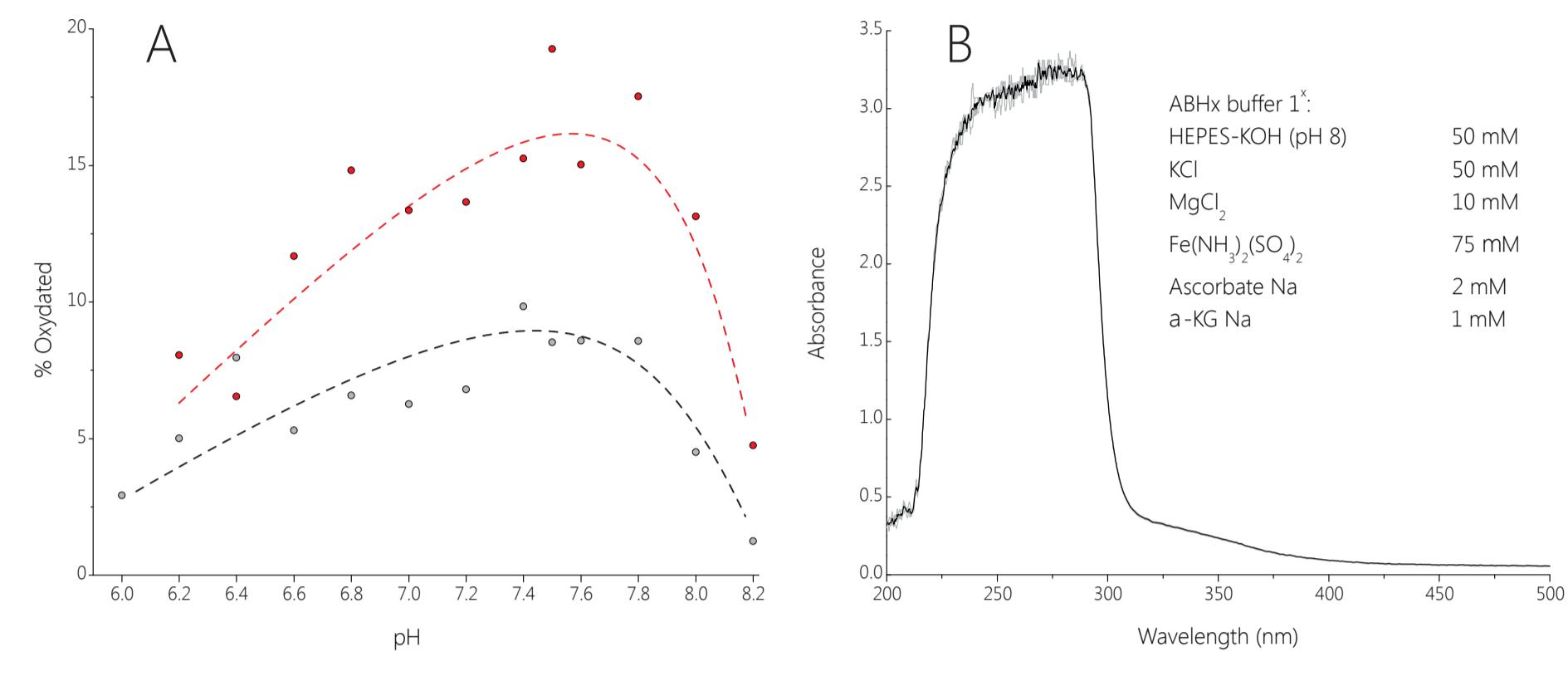


Figure 4. Buffer optimisation experiments  
A Demethylation level in reaction of 1 μM ABH2 and 1 μM DNA duplex containing 1mA at 30° and 10°  
(for details ask me :))  
B Reaction buffer absorbance spectrum.

### Kinetics of ABH2

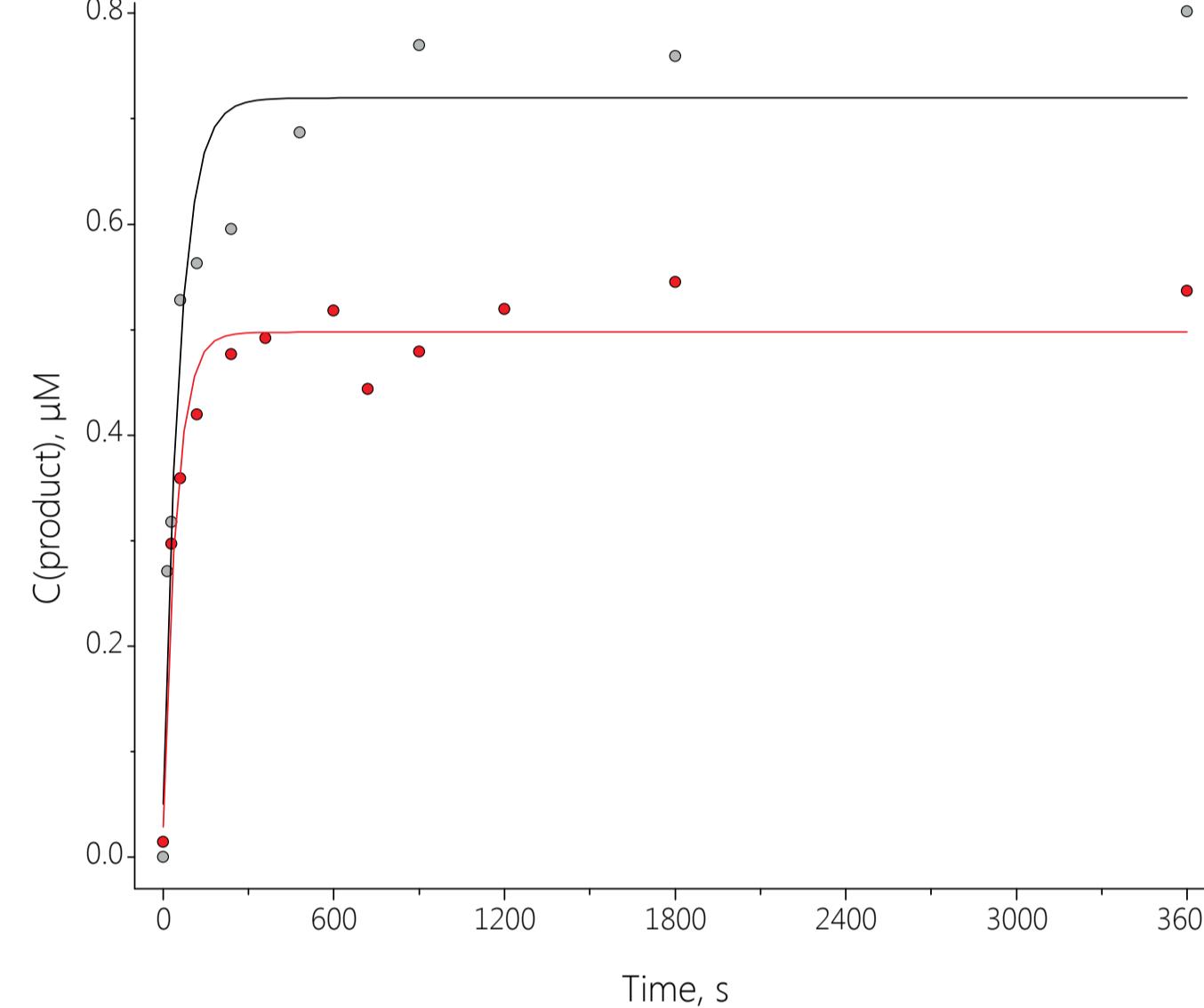


Figure 5. Polycrylamide gel electrophoresis assay.  
To estimate kinetic parameters, experiments with product separation by PAGE were made. 1 μM of model 1mA/T and εA/T duplexes were mixed with 1 μM and 5 μM ABH2 respectively. Product of the reaction was treated with DpnII endonuclease (active on A/T duplex and inactive 1mA/T and εA/T). Characteristic time of reaction is about 200 s.

$$\bullet 1mA/T + ABH2, k_{\text{obs}} \approx 0.022 \text{ s}^{-1}$$

$$\circ \varepsilon A/T + ABH2, k_{\text{obs}} \approx 0.018 \text{ s}^{-1}$$

### Stopped flow experiments

Conformational changes of enzyme and substrates usually could be investigated using stopped flow technique with protein/DNA fluorescence observing. Such an approach allows us to register phenomena in time range from 1 ms to 1000 s.

In case of ABH2, we register Trp residue fluorescence ( $\lambda_{\text{ex}} = 300$  nm) indicating conformational changes in protein and εA fluorescence ( $\lambda_{\text{ex}} = 316$  nm), which show changes in DNA. Unfortunately, we can study only early part of kinetic curves (up to 10 s) because of slow process of Fe<sup>2+</sup> oxidation – changes in fluorescence we mostly see in large times are provided by decrease in buffer absorbance and do not represent any information about protein/DNA conformation.

Two series of stopped flow experiments were made: ABH2 + 1mA/T (registering Trp fluorescence, fig. 6 A) and ABH2 + εA/T (registering εA fluorescence fig. 6 B). Early parts of curves were fitted with exponential function (see figure) yielding rate constants. Both these changes (in DNA and protein conformations) would be addressed to initial DNA binding and reaction complex formation.

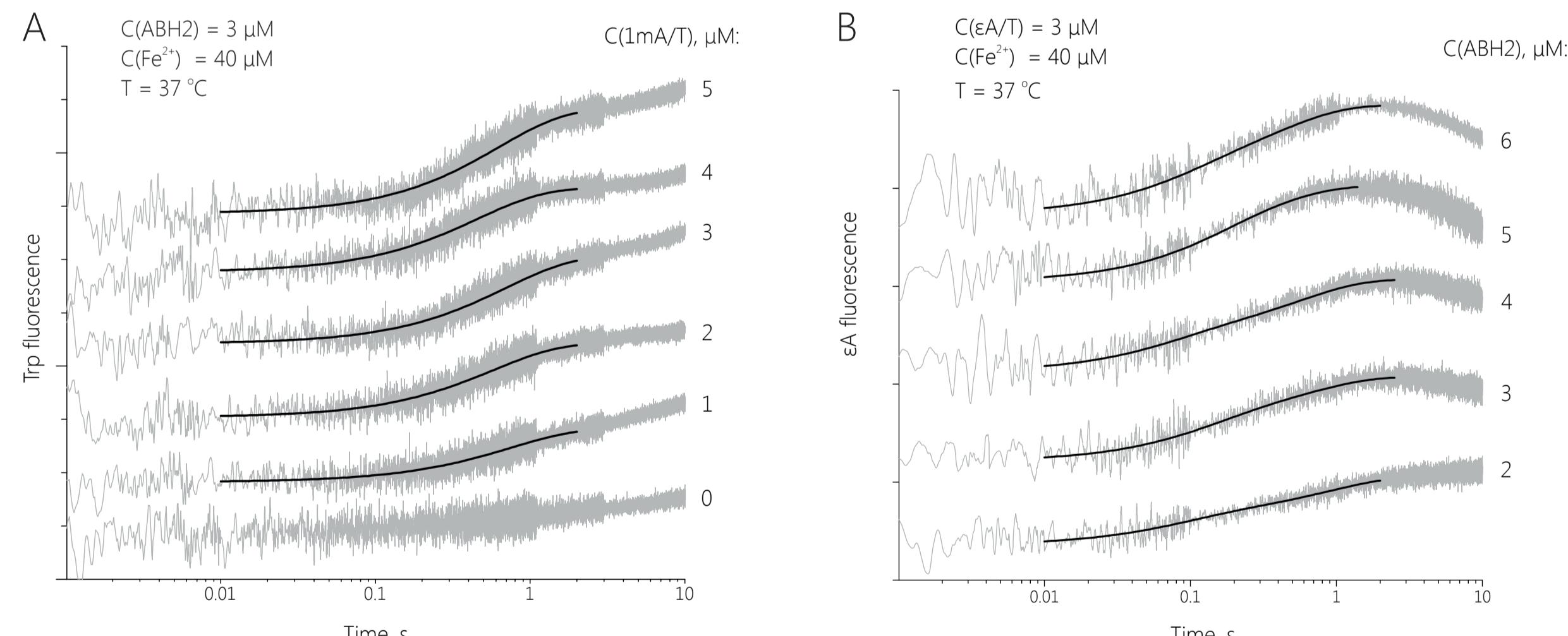


Figure 6. Concentration series of ABH2 + 1mA/T (A) and ABH2 + εA/T (B) reactions obtained by stopped-flow method. Experimental curves were fitted using Origin software; for A mono-exponential expression was used (1),  $k_1 = 1.62 \pm 0.01 \text{ s}^{-1}$ ; for B – double-exponential expression (2),  $k_1 = 1.62 \pm 0.05 \text{ s}^{-1}$ ,  $k_2 = 10.6 \pm 0.3 \text{ s}^{-1}$ .  
Reaction conditions: ABH2 and DNA solutions were incubated in reaction buffer (50 mM HEPES-KOH pH 7.5; 50 mM KCl; 10 mM MgCl<sub>2</sub>; 2 mM α-KG; 1 mM α-KG dysodium salt; 40 μM Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>) and then mixed in stopped-flow spectrometer SX.20 (Applied Photophysics). Optic parameters: A  $\lambda_{\text{ex}} = 300$  nm, filter WG 320 nm; B  $\lambda_{\text{ex}} = 316$  nm, filter LG 370 nm.

$$1) F = F_{\text{base}} + F_1 e^{-k_1 t}$$

$$2) F = F_{\text{base}} + F_1 e^{-k_1 t} + F_2 e^{-k_2 t}$$

## Conclusions

- It was shown, that stopped-flow approach could be applied to the studies of ABH2 kinetic mechanism.
- The conformational dynamics of ABH2 molecule was detected using intrinsic Trp fluorescence changes.
- The kinetic parameters for initial steps of ABH2 binding to DNA substrates containing mA and A were calculated.

## References

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