Rational design of catalytic antibody A.17

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Diisopropyl fluorophosphatase (DFP-ase) of *Loligo vulgaris* catalyzes the hydrolysis of many organophosphorus toxins, including paraoxon. It has the simplest active site of all known paraoxonases. Though its activity is rather small.

Antibodies may provide a handy structure for insertion of a small active site of DFP-ase. Catalytic antibodies (abzymes) which are able to hydrolyze organophosphorus compounds may be used for therapy against toxic nerve agents. They are stable in blood, poorly immunogenic and usable for *in vitro* tests.

Rational design of an abzyme containing DFP-ase active site may help to get a powerful drug to deactivate chemical warfare agents or pesticides. Detailed notion of reaction mechanism is required for reaction modelling and *in silico* selection of abzyme structures. However the mechanism of DFP-ase-mediated hydrolysis is still discussed.

We aimed to analyze the first stage of DFP-ase-mediated paraoxon hydrolysis and to insert the active site of DFP-ase into the structure of abzyme A17 in order to obtain mutant structures with conserved reaction mechanism.

Semiempirical QM/MM modelling of the first stage of paraoxon hydrolysis showed an associative reaction mechanism, which was consistent with previous hypothesis based on the results of molecular docking [1]. Repeated modelling with more accurate theory level (DFTB) revealed the possibility of an alternative dissociative mechanism of the first stage of the reaction. In order to choose between two reaction mechanisms the reaction energy profile was build for both of them by a series of Umbrella sampling simulations.

Several positions in A.17 which have the same C-α or C-β geometry with DFP-ase active site and the minimal crossing with other backbone atoms were detected. 9000 mutant structures of A.17 were ranked by the active site geometry preservation, conformational stability and number of mutations. The modelling of the paraoxon hydrolysis was carried out for the best mutant structures. 5 mutants were selected for further *in vitro* tests to investigate their paraoxonase activity.

1. M.-M.Blum, F.Löhr, A.Richardt , H.Rüterjans, J.C.-H.Chen (2006) Binding of a Designed Substrate Analogue to Diisopropyl Fluorophosphatase: Implications for the Phosphotriesterase Mechanism, *Journal of the American Chemical Society*, **128:** 12750–12757.