significantly modify the structural properties and organization of the protein. The crystal structure of TmPrxNtrC40S has been determined for the oxidized form and for the reduced form in presence of hydrogen sulfite, as well as two crystalline forms of the TmPrx module.

The full-length protein TmPrxNtr, which contains a FMN prosthetic group, displays nitroreductase, peroxidase and quinone, flavine, chromate and iron reductase activities using either NADH or NADPH as a donor. The mutation of the catalytic cysteine of the Prx module (TmPrxNtrC40S) does not alter the catalytic efficiency of the protein suggesting that the activity measured does not require the Prx module. Both modules TmPrx and TmNtr have peroxidase activity but the oxidized enzyme is not regenerated in the same way. NAD(P)H is the direct reductant for TmNtr while TmPrx is regenerated *in vitro* via glutaredoxins or thioredoxins. In addition TmPrx is able to reduce H_2O_2 and COOH whereas TMPrxNtr only reduces H_2O_2.

The TmPrxNtrC40S enzyme crystallizes as a dimer, the active form of the protein. The Ntr module of one monomer is in contact with the Ntr and Prx modules of the other one but no contact is observed between the two Prx modules. On the contrary, in the crystalline forms of the isolated domain, two TmPrx molecules are linked by an intermolecular disulfide bond. In TmPrxNtrC40S, the FMN prosthetic groups bind in deep pockets at the dimer interface and interact with elements of both monomers. An ion sulphate is positioned by hydrogen bonding to the catalytic site of the Prx modules. In the reduced TmPrxNtrC40S crystal, one sulphate is replaced by a bisulfite introducing structural modifications to the associated Prx module.

Altogether, the results indicate that the TmPrx and TmNtr modules apparently function independently, each possessing its own peroxidase activity, with the TmNtr module having diaphorase activity linked to the FMN moiety versus a broad range of substrates. This raises the question of their fusion which is specifically found in thermotogales.

Keywords: hybrid-flavoenzyme, reductase, X-ray

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Hpy188I-DNA structures - snapshots of the GIY-YIG nuclease mediated catalysis

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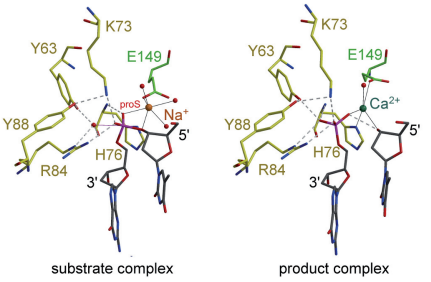
GIY-YIG nucleases are present in all kingdoms of life and have very diverse roles. Well characterized functions include transposon migration, flap cutting and Holliday junction resolution in eukaryotes and nucleotide excision repair in prokaryotes. Despite many roles of GIY-YIG nucleases, until very recently no GIY-YIG domain has ever been crystallized in complex with target DNA. Therefore, many questions about the catalytic mechanism of the GIY-YIG module were unresolved.

We have obtained Hpy188I crystal structures in ternary substrate and product complexes with DNA and metal ion in the active site [1]. Our structures suggest that GIY-YIG nucleases catalyze DNA hydrolysis by a single substitution reaction. They are consistent with a previous proposal that a tyrosine residue (which we expect to occur in the phenolate form) acts as a general base for the attacking water molecule. In contrast to the earlier hypotheses, our data identify the general base with the GIY and not the YIG tyrosine. A conserved glutamate residue (Glu149 provided in trans in Hpy188I) anchors a single metal cation in the active site. This metal ion contacts the phosphate proS oxygen atom and the leaving group 3'-oxygen atom, presumably to facilitate its departure. Taken together, our data reveal striking analogy in the absence of homology between GIY-YIG and ββα-Me nucleases. Our mechanistic conclusions agree with those for Eco29kI restriction endonuclease [2].



[1] M. Sokolowska, H. Czapinska, M. Bochtler, *Nucleic Acids Res.* **2011,** *39*, 1554-1564. [2] A.N. Mak, A.R. Lambert, B.L. Stoddard, *Structure* **2010**, *18*, 1321-1331.

Keywords: GIY-YIG nuclease, mechanism, restriction enzyme

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Structural variability of type II restriction endonucleases

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Type II restriction endonucleases are very diverse in sequence and have therefore long been considered as phylogenetically unrelated. This picture changed when crystal structures revealed that the frequently used enzymes all belong to the PD-(D/E)XK class of nucleases, which use a two metal ion mechanism to catalyze DNA hydrolysis. We have systematically studied restriction endonucleases that cleave DNA with unusual staggers. This property turns out to "select" for structurally novel enzymes. Almost simultaneously with Prof. Barry Stoddard, we have solved the first structures of ββα-Me and GIY-YIG restriction endonucleases, in our case with Hpy99I and Hpy188I as the model enzymes [1], [2], [3]. The structures reveal interesting mechanistic analogies between the new groups of restriction endonucleases and "classic" PD-(D/E)XK enzymes. Surprisingly, the metal ion B, which promotes the departure of the 3'-OH leaving group, and is "optional" in at least some of the PD-(D/E)XK nucleases is conserved in both ββα-Me and GIY-YIG nucleases. In contrast, the metal ion A, which is obligatory in PD-(D/E)XK enzymes and activates the nucleophilic water molecule is absent from both new nuclease families. The attacking water molecule is instead activated by a histidine or a tyrosine residue (we presume the latter to be present in the phenolate form).

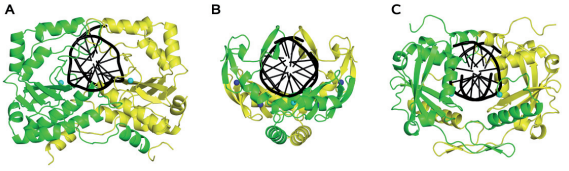

Fig. 1: PD-(D/E)XK endonuclease ThaI (**A**), ββα-Me endonuclease Hpy99I (without N-terminal domain) (**B**) and GIY-YIG nuclease Hpy188I (**C**). All three enzymes are functional dimers and recognize palindromic (symmetric) or pseudopalindromic (approximately symmetric) target sites.