Supplementary File S3 to the manuscript "Common mechanism of activated catalysis in P-loop fold nucleoside triphosphatases – united in diversity"

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Local molecular electric field in the catalytic sites of P-loop NTPases

In structures of P-loop NTPases that are shown in Fig. 1-7, the positive charged groups that stabilize the triphosphate chain, namely the Lys^{WA} residue, the backbone **HN** groups, the stimulatory moiety(ies), the auxiliary residues, and the positively charged N-terminus of the first α-helix are opposed by acidic residues that interact with W_{cat} either directly or via water bridges. Together with Asp^{WB}, these catalytic acidic residues form negatively charged clusters. Fig. SF3_1 below shows, for diverse P-loop NTPases, these positively and negatively charged clusters, which should produce a strong local electric field.
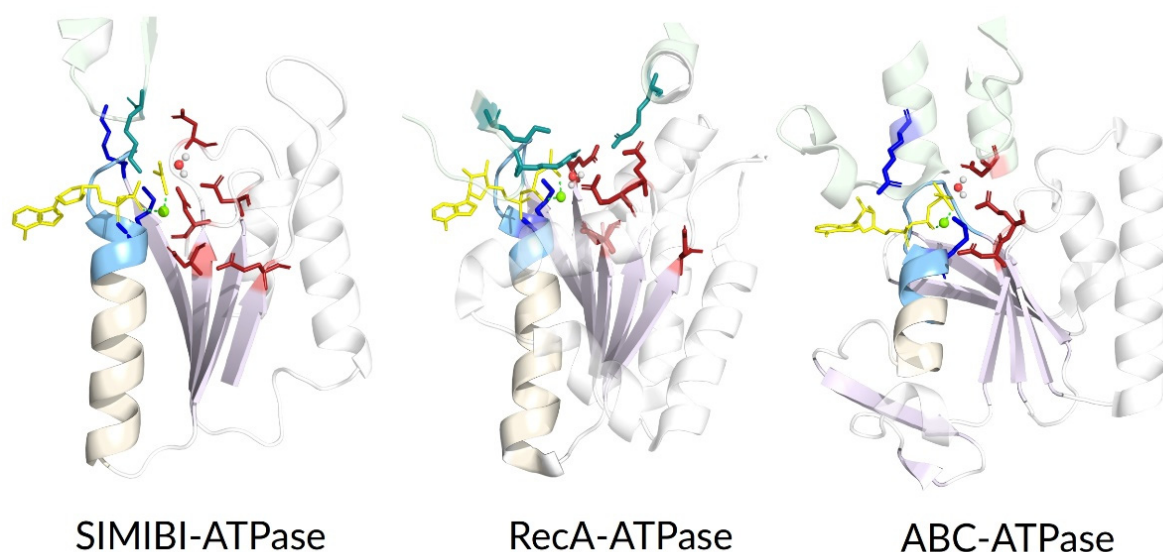

SIMIBI-ATPase   RecA-ATPase   ABC-ATPase

Figure S3. Uneven distribution of charged residues around nucleotide binding pockets in different classes of P-loop NTPases. Nucleotide analogs are shown in yellow, negatively charged residues are shown in red, positively charged residues are shown in blue and teal, the α1 helix is shown in beige with its N-terminus and the P-loop shown in light blue.
A, SIMIBI class, light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein chlL) (PDB ID 2YNM, [1]); **B**, RecA class, circadian clock protein kinase KaiC (PDB ID 4TL7, [2]); **C**, ABC-ATPase class, MBP-maltose transporter complex (PDB ID 3PUW,[3]).

Hence, the electrostatic potential at the catalytic sites of diverse P-loop NTPases is distributed unevenly, which has already been noted for particular enzymes of this family, see, e.g., [4]. The strength of the local electric field can be roughly estimated using the Coulomb's equation as ~ 10^8 V/m under the very modest assumptions that (i) the resulting electric charge difference at a distance of 10 Å (between the acid residues and the P-loop) corresponds to one elementary charge and (ii) the effective dielectric permittivity of the catalytic pocket is about 10 [5,6]. Such an electric field strength, albeit large, is compatible with those measured in the catalytic pockets of other enzymes [7].

Notably, in those cases where the stimulator is positively charged, its positive charge not only secures the bonding with particular oxygen atom(s) of triphosphate and increases the positive charge on P^G, but additionally polarizes the whole catalytic pocket.

Consequently, the local electric field is directed approximately from the P-loop to Asp^{WB}, which should drive a proton from W_{cat} to Asp^{WB}. Hence, the catalytic pocket is strongly polarized in P-loop NTPases, which also can contribute to catalysis, as discussed below.

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