Rhodamine6G and Hœchst33342 narrow BmrA conformational spectrum for a more efficient use of ATP

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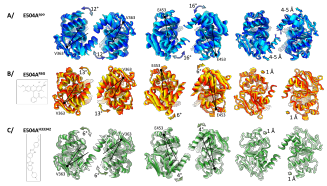

Multidrug ABC transporters harness the energy of ATP binding and hydrolysis to translocate substrates out of the cell and detoxify them. While this involves a well-accepted alternating access mechanism, molecular details of this interplay are still elusive. Rhodamine6G binding on a catalytic inactive mutant of the homodimeric multidrug ABC transporter BmrA triggers a cooperative binding of ATP on the two identical nucleotide-binding-sites, otherwise michaelian. We investigated this asymmetric behavior via a structural-enzymology approach, solving cryoEM structure of BmrA at defined ATP ratio along the enzymatic transition, highlighting the plasticity of BmrA as it undergoes the transition from inward to outward facing conformations. Analysis of continuous heterogeneity within cryoEM data and structural dynamics, reveals that Rhodamine6G narrows the conformational spectrum explored by the nucleotide-binding domains. We observe the same behavior for the other drug Hœchst33342. Following on these findings, the effect of drug-binding showed an ATPase stimulation and a maximal transport activity of the wild-type protein at the concentration-range where the cooperative transition occurs. Altogether, these findings provide a new description of the influence of drug binding on the ATP-binding sites through a change in conformational dynamics.