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## 'Updated' mechanism of multidrug ABC transporters from *Bacillus subtilis*

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 Family of ubiquitous transporters, importers + exporters: ~ 25 000 / 71 659 (Transporter DataBase) Ren et al (2007) NAR
 Modular Structure : 4 core domains ( + 1 substrate binding protein for importers).

## Structure of the homo-dimer of 2 NBDs : the transient 'sandwich' dimer

lipoproteins

C E D D LolD subunit

Out

Mb

In

Conserved motifs :

Walker A & B: ATP
H & Q: ATP
ABC signature
=> ATP too

The inactive mutant traps 2 ATP molecules at the interface of the 2 NBDs Smith (2002) Mol Cell





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# Are these open conformations physiologically relevant?

Aller et al. (2009) Science







Open apo EcMsbA

apo P-gp

#### NEWS AND VIEWS

JUNE 2009 NATURE BIOTECHNOLOGY

#### Structure of a multidrug transporter

Michael M Gottesman, Suresh V Ambudkar & Di Xia

... "Thus, the structure may represent a crystallization artifact or a nonfunctional conformation that has only very transient existence".

## Overexpression of BmrA and purification

 Purification in a single step (Ct-polyhistidine-tagged)
 ~2-3 mg of BmrA/Liter

 Inside-out membrane vesicles: BmrA ~ 50% of mb proteins





Steinfels et al. (2002) BBA

### Behavior of BmrA in membrane or in detergent : 2 different conformations ? Membrane DDM purified









The Vi-inhibited BmrA is much less sensitive to limited digestion by trypsin than BmrA in the resting state. ==> BmrA switches between two very different conformations in mb or in detergent

## Are the two BmrA conformations really 'stable'?

## Rigid body motion ?





interaction between TMD (ICD) & NBD similar in open or closed state





## The H/D exchange technique

### • Different types of hydrogen in proteins





H : Do not exchange

- H : Too fast to be monitored
- **H** : Best candidates for deuterium exchange;

exchange rate depends on structure and accessibility







## Portfolio of different peptides



WT Apo

E504A (ATPase inactive) + ATP/Mg

## Few examples : Walker A motif





#### % H/D Exchange 80 → Open 60 ---- Closed 40 20 15 60 300 600 1800 3600 Time (s) 372-383 Protection by ATP Walker A

## ABC signature peptide





#### % H/D Exchange



## Walker B motif







## Peptide from ICD1 (cis with its own NBD)







% H/D Exchange



## Peptide from ICD2 (trans with the other NBD)





## 2nd Peptide from ICD2 (trans with the other NBD)







Highly exchangeable, especially for ICDs Different from 3D structure Poorly exchangeable OK with 3D structure

Greater flexibility of ICDs Reorientation of the NBDs and/or unfolding

### Conclusions from H/D exchange

Similar behavior of BmrA in membrane and purified in DDM

BmrA switches between two conformations :

Resting state : open conformation (high H/D exchange)
ATP-bound state : closed conformation (low H/D exchange)

In the resting state, the ICDs and in particular ICD2, are highly flexible

ICDs disengaged from the NBD
different orientation and/or unfolding of the NBD
≠ 3D structure of EcMsbA (rigid body motion)

==> Can this flexibility help to recognize many substrates ?

Mehmood et al (2012) PNAS

## Is this flexibility of the ICDs restricted to BmrA?



Heterodimer with asymmetric NBDs (MRP, CFTR, TAP1/TAP2...)



BmrC/BmrD model based on the TM heterodimer (Seeger's group)

## Multidrug ABC transporters need to...



..even in the resting state

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