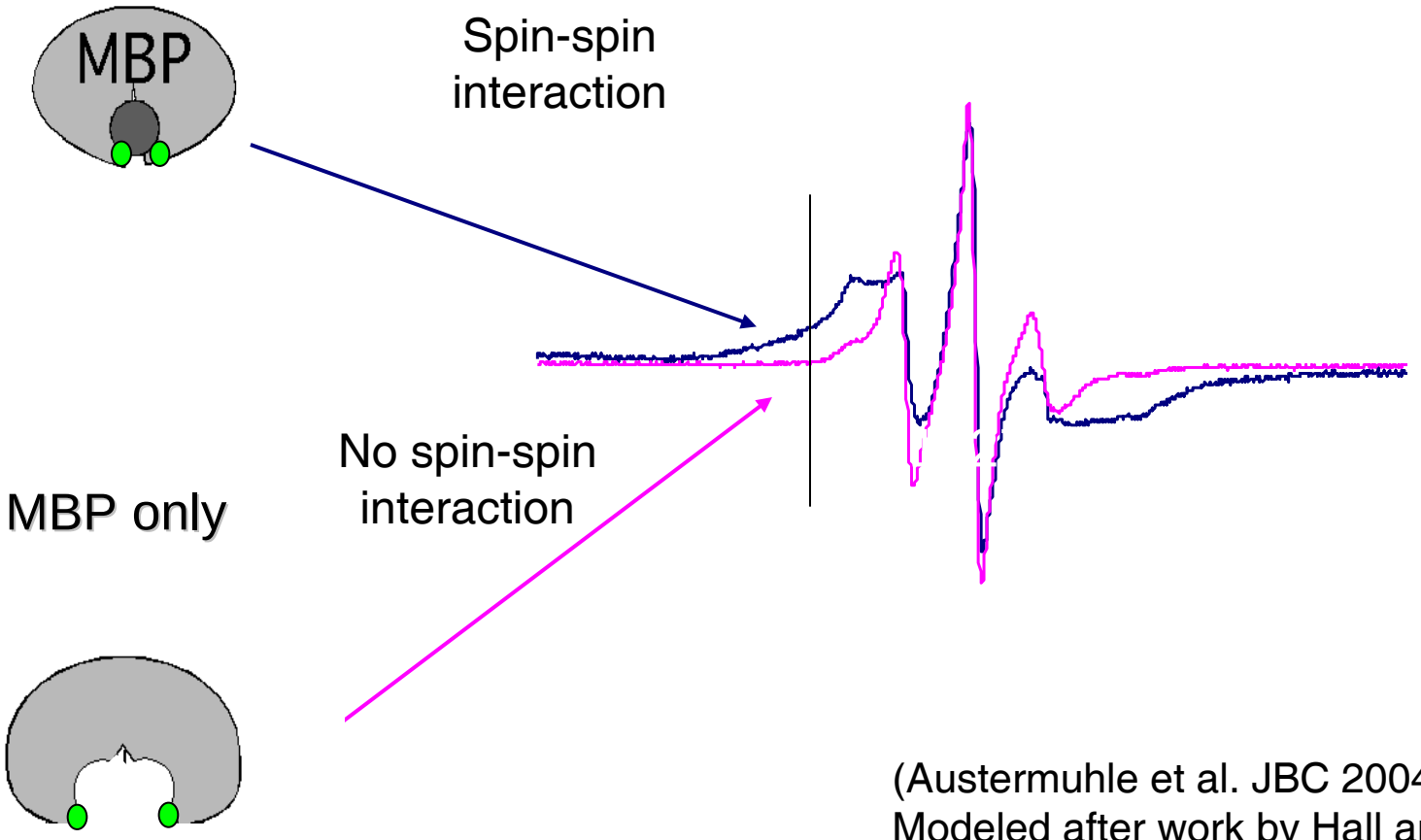
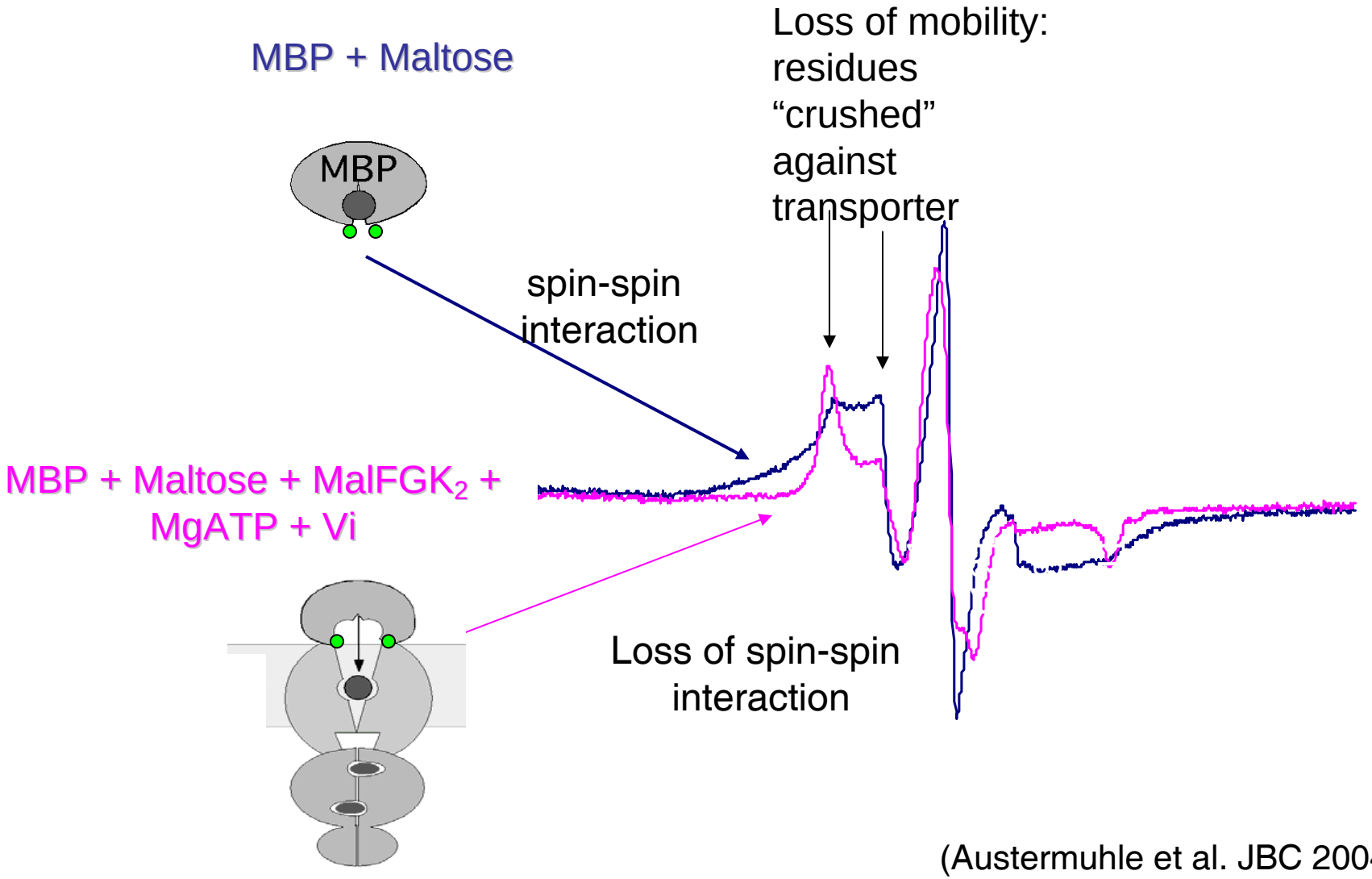
Use EPR to probe the conformation of MBP

MBP + Maltose



(Austermuhle et al. JBC 2004)
Modeled after work by Hall and NIkaido JBC 1997

MBP is open in the vanadate-trapped transition state



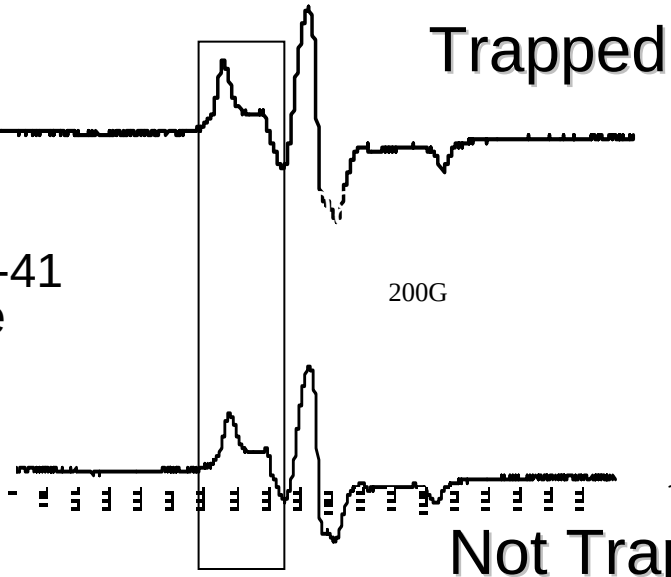
(Austermuhle et al. JBC 2004)

ATP binding is sufficient to drive major conformational change in transport complex

(Austermuhle et al. JBC 2004)

MBP 211-41
+ maltose
+FGK_2
+MgATP
+V_i

Trapped!

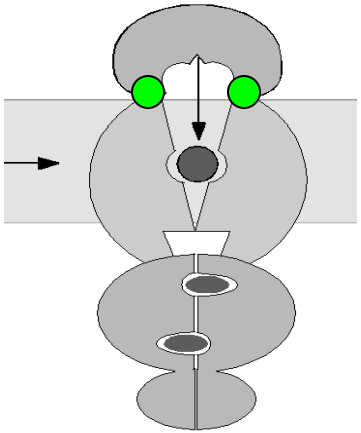


MBP 211-41
+ maltose
+FGK_2
+ATP
+EDTA

Not Trapped!

200G

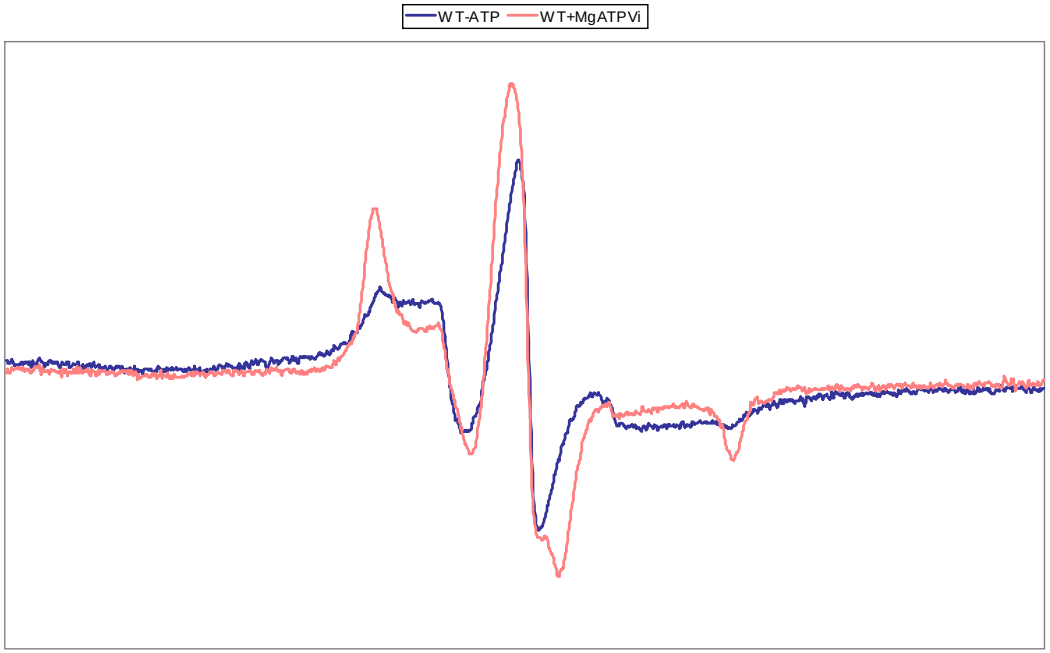
Open MBP
(No spin interaction)



Rather, binding of MBP and ATP (in absence of Mg) shifts equilibrium between two conformations

A third way to make this complex:

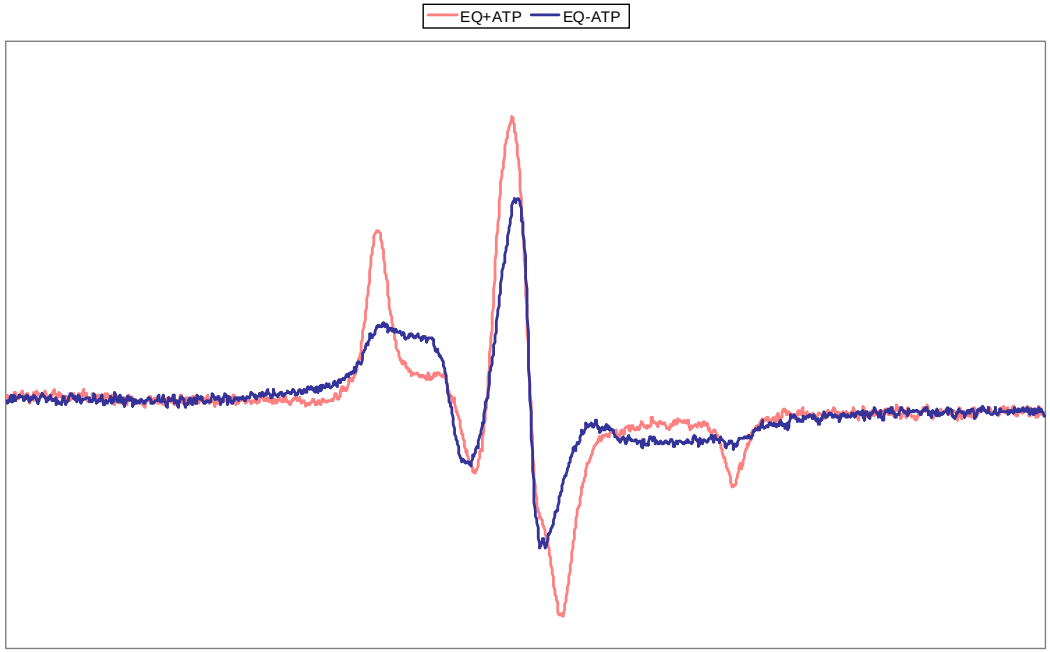
Removal of Key Catalytic Residue: Glu159Gln (E159Q) & Addition of ATP+/-Mg



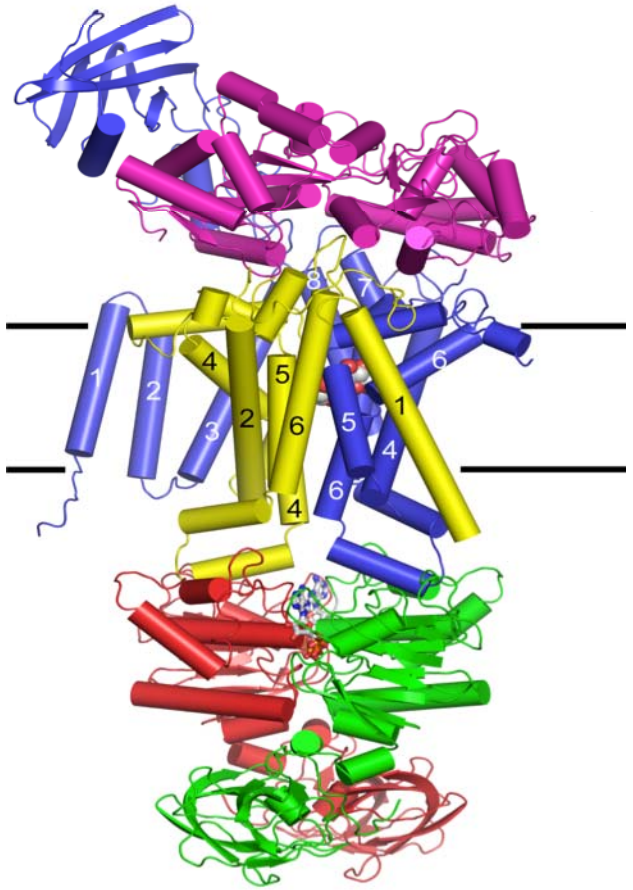
Fourth way:
Use of non-hydrolyzable analogues of ATP:

MgATP γ S

MgAMP-PNP

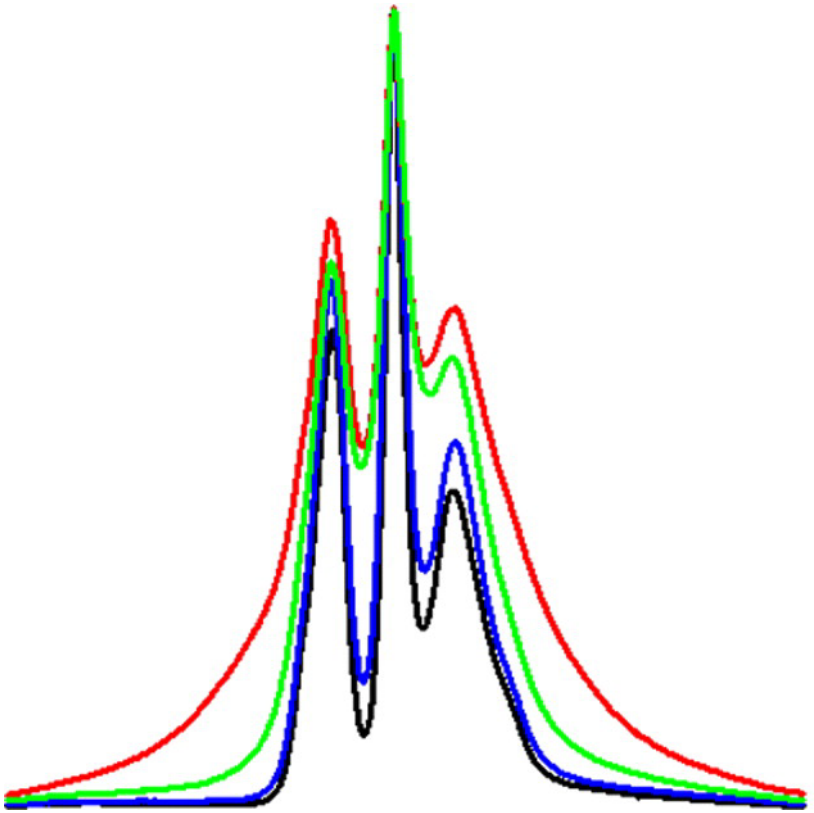


Structure of the maltose transporter



Oldham et al. Nature. 2007 450(7169):515-21.

Electron Paramagnetic Resonance (EPR) provides evidence of three different conformational states for the maltose transporter with increasing distance between ATP-binding domains



Orelle C et al. PNAS 2008;105:12837-12842