

FUNCTIONAL AND PHARMACOLOGICAL IMPORTANCE OF THE
COMPOSITE ATP BINDING SITE 1 IN CFTR CHLORIDE CHANNELS

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ABSTRACT

The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride ion channel whose defects cause the deadly genetic disease cystic fibrosis (CF). Like other ATP binding cassette (ABC) proteins, CFTR encompasses two cytoplasmic nucleotide binding domains (NBDs). Upon ATP binding, the two NBDs can coalesce into a head-to-tail dimer with ATP buried at two interfacial composite sites (sites 1 and 2). Although evidence suggests that gating of CFTR is mainly controlled by site 2, the role of site 1 remains less understood. I have used pyrophosphate as a probe or adopted a ligand exchange protocol to investigate ATP binding status in site 1 in real time. With these novel approaches, I have identified a “partial” NBD dimer state mediated by an ATP molecule tightly bound in site 1. A molecular model of CFTR gating was then established with opening and closing of CFTR coupled to the formation and partial separation of the NBD dimer. Moreover, I discovered several mutations that enhance ATP binding in site 1 and demonstrated that the activity of CF-associated mutant channels, $\Delta F508$ - and G551D-CFTR, can be significantly improved by these mutations, thus providing evidence that site 1 is a potential target for developing pharmaceutical reagents to treat patients with CF.

