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Luminescence resonance energy transfer spectroscopy of ATP-binding cassette proteins

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ABSTRACT

The ATP-binding cassette (ABC) superfamily includes regulatory and transport proteins. Most human ABC exporters pump substrates out of cells using energy from ATP hydrolysis. Although major advances have been made toward understanding the molecular mechanism of ABC exporters, there are still many issues unresolved. During the last few years, luminescence resonance energy transfer has been used to detect conformational changes in real time, with atomic resolution, in isolated ABC nucleotide binding domains (NBDs) and full-length ABC exporters. NBDs are particularly interesting because they provide the power stroke for substrate transport. Luminescence resonance energy transfer (LRET) is a spectroscopic technique that can provide dynamic information with atomic-resolution of protein conformational changes under physiological conditions. Using LRET, it has been shown that NBD dimerization, a critical step in ABC proteins catalytic cycle, requires binding of ATP to two nucleotide binding sites. However, hydrolysis at just one of the sites can drive dissociation of the NBD dimer. It was also found that the NBDs of the bacterial ABC exporter MsbA reconstituted in a lipid bilayer membrane and studied at 37°C never separate as much as suggested by crystal structures. This observation stresses the importance of performing structural/functional studies of ABC exporters under physiologic conditions.

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