ATP-binding cassette (ABC) transporters constitute one of the largest membrane protein families, and prevail in all domains of life. These proteins actively transport their substrates across the lipid bilayer, and are directly related to human disease and multidrug resistance.

In E. coli, the inner membrane ABC transporter BtuCD interacts with the periplasmic binding protein BtuF to form the vitamin B12 transport complex, BtuCD–BtuF. The conventional model assumes that BtuF binds the substrate and then associates with BtuCD. Consequently, BtuCD–BtuF complex must dissociate and re-associate in a cyclic manner to resume vitamin B12 transport into the cytoplasm. However, in vitro, BtuCD–BtuF complex is extremely stable ($K_D = 1.16 \times 10^{-13}$ M). Hence we ask how the complex disassembles.

We aim to first assemble BtuCD–BtuF complex in vitro, and then to dismantle it. As was shown in vitro, either vitamin B12 or ATP reduces the affinity between BtuF and BtuCD. Moreover, when both ligands are present, there is no complex formation. For this reason, the substrate and the nucleotide are the prime candidates for BtuCD–BtuF complex disassembly. Another possibility is that a different molecule of BtuF (pre-loaded with vitamin B$_{12}$) is necessary for disassembly of the present transport complex and assembly of the next one. Also, we should take other factors – yet to be identified – into consideration.

In any case, we expect to gain insights as to how the vitamin B$_{12}$ transport complex falls apart; and this will further our understanding of complex recycling.