ligand treatment (Fig. 1)[4,5]. However, the cereblon modulators described to date do not appear to need any affinity for the free substrate, meaning that apparently 'unligandable' proteins can be degraded. In contrast, the linker-based molecules require a binding affinity for both the free ligase and the substrate protein but are free from the requirement for a specific degron.

The contribution of the protein surfaces for linker-based degraders has been examined in recent structural studies. The first structural description of a PROTAC-scaffolded ligase–substrate complex contained the E3 ligase subunit VHL combined with BRD4 and the degrader MZ1 (ref. [9]). The authors observed that the molecular linker in MZ1 had collapsed to enable a protein–protein interaction in the crystal and supported this observation with biophysical evidence demonstrating positive cooperativity in the formation of the complex. In contrast, Nowak et al.[2] did not find any evidence for positive cooperativity in the cereblon–BRD4 system, leading the authors to conclude that protein–protein interactions are not essential for efficient ubiquitin transfer. Extensive crystallographic, computational and biophysical studies on cereblon–BRD4 complexes revealed a set of lower energy binding sites between BRD4 and cereblon. The authors used these induced protein–protein interactions to develop degraders with minimal linkers, such as ZXH-3-26, that exploit negative interactions derived from sequence differences outside the ligand-binding site to achieve improved selectivity (Fig. 1).

The exploitation of sequence or structural differences outside of the ligand-binding site is a new trick for the toolbox, and this may open up further selective targeting among very highly conserved protein families. Although crystal structures of flexible complexes will always need to be interpreted with caution, the suite of computational and biophysical tools leveraged in recent works advances our understanding of the potential value in optimizing protein–protein interactions even in linker-based molecules. At the same time, extended molecular-glue molecules are appearing with ligand interactions beyond the degron, such as those reported for CC-885 and GSPT1 (ref. [4]). It seems that the differences between molecular-glue and PROTAC strategies may represent a continuum in which the distinctions will become increasingly blurred as the approaches evolve. There are predicted to be more than 600 E3 ligases in the proteome, and these new structural findings will help guide drug-discovery efforts redirecting both the well-established and as-yet unexplored E3 ligases. ❐

Philip P. Chamberlain
Celgene, San Diego, CA, USA.
e-mail: pchamberlain@celgene.com

Published online: 11 June 2018
https://doi.org/10.1038/s41589-018-0057-9

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Competing interests
The author is an employee and shareholder at Celgene.

TRANSPORTERS

Vitamin B_{12} import is all about timing

Single-molecule techniques combined with molecular dynamics simulations allowed visualization of a surprisingly high level of conformational homogeneity in the transport cycle of an ABC import system, BtuCD-F, and revealed an unexpected tight coupling of distinct conformational states responsible for vitamin B_{12} binding, transport and release.

Lutz Schmitt

Transport across membranes is essential for every cell[1]. In archaea and prokarya, members of the ATP-binding cassette (ABC) importer family catalyze the uptake of nutrients, vitamins and trace elements. Membrane transport proteins generally are inherently flexible and undergo substantial conformational changes to catalyze the translocation of their substrates across biological membranes. In this issue, Lewinson and co-workers examined the vitamin B_{12} import system BtuCD-F from *Escherichia coli* using a single-molecule approach, which elucidated the conformational dynamics during the transport cycle[2].

It is well established that ABC transporters rely on the energy released by ATP hydrolysis to shuttle their substrates across cellular membranes. However, little is known about the underlying dynamics and conformational changes that accompany such a transport cycle. ABC importers follow the 'two-site access' model[3], in which ATP binding and hydrolysis switch the accessibility of the transmembrane domain for the substrate from an inward-facing (accessible from the cytoplasm) to an outward-facing (accessible from the extracellular site) conformation. As a model system, the authors choose BtuCD-F, which structurally is an extremely well characterized vitamin B_{12} import system[4] that serves as a template for ABC importers. ABC importers are furthermore characterized by the presence of a substrate-binding protein (SBP) that captures the ligand within the extracellular space in archaea and Gram-positive bacteria, or in the periplasm in Gram-negative bacteria, and delivers it to the cognate transport unit. Numerous studies have shown that the SBP binds the substrate and subsequently docks to the transporter at the extracellular site[3]. This triggers ATP hydrolysis at the cytoplasmic site, inducing a conformational change, which allows import of the substrate. After import, the whole system resets into the initial state, enabling another round of substrate import.

The authors used an elegant set of experiments combining single-molecule FRET measurements and molecular dynamics (MD) simulations, allowing them to correlate motions of individual domains of the BtuCD-F transporter and conformational transitions, highlighting

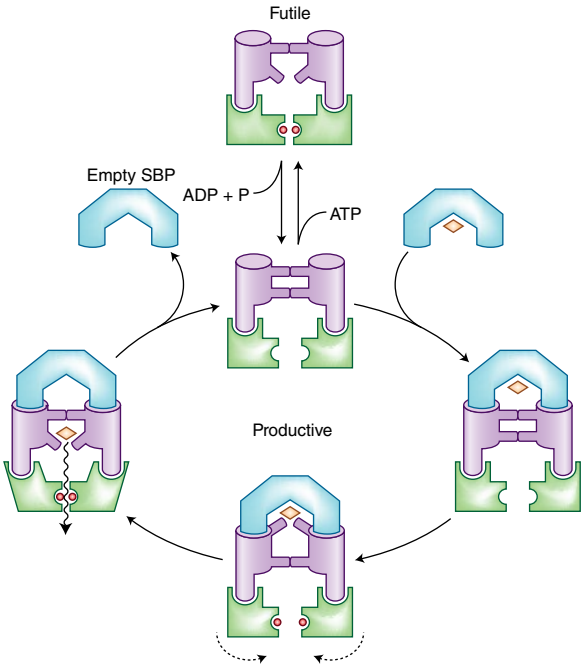

Fig. 1 | The transport cycle of BtuCD-F can be divided into a futile cycle in the absence of the SBP and a productive cycle in the presence of vitamin B_{12}-loaded SBP. In the futile mode, only the cytosolic gate (lower purple bar) open and closes. This motion is triggered by ATP-induced dimerization of the NBDs (green) and hydrolysis of ATP. In the productive mode, distinct conformations of the ABC transporter are populated in which the periplasmic and cytosolic gates (upper and lower purple bars) open in an ordered and exactly timed fashion. This is induced by docking of the SBP (blue) and subsequent ATP binding, which is followed by dimerization of the NBD. Overall, these events result in a 'squeezing motion' that expels the substrates from the TMDs into the cytosolic space. Credit: Katie Vicari/Springer Nature

distinct states within the transport cycle. For the single-molecule experiments, Cys residues for subsequent fluorophore labeling were engineered at sites that, based on already known structural information of the BtuCD-F system, are prone to conformational changes. Upon verification that the single Cys mutants were functional, MD simulations were performed. To extend the time scale of the simulations (typically in the nanosecond range) to match the time scale of the transport cycle in vivo, the authors employed a novel hybrid approach in which anisotropic network modeling was combined with stochastic Langevin dynamics. Here, trajectories covered the entire transport cycle and revealed the conformational changes. Surprisingly, this analysis revealed striking conformational homogeneity. These data revealed the sequence of events underlying the import of vitamin B_{12} (Fig. 1). In the proposed transport cycle, two modes need to be distinguished: (I) the transport-uncoupled and (II) the transport-coupled mode of action. The first one occurs in the absence of the SBP, BtuF and the substrate. As a consequence, ATP constantly binds and hydrolyzes. Due to this futile hydrolysis, the nucleotide-binding domains (NBD) fully open (in the apo or ADP-bound state) and close (ATP bound), and the cytoplasmic gate coupled to the movements of the NBD opens and closes, but the periplasmic site within the transmembrane domain (TMD) remains closed. In contrast, the transport-competent cycle is initiated in the presence of substrate-loaded BtuF. Docking to the TMD triggers ATP binding in the NBDs and their dimerization, which is transmitted back to the TMDs, resulting in an opening of the periplasmic gate. This opening and repositioning of helices in the TMD disturbs the ligand-binding site in BtuF, resulting in a release of the captured substrate. Such a dynamic system was not anticipated and is clearly different from the other membrane transport systems studied so far.

This study reveals for the first time an explanation as to how a transporter with low or no affinity for a ligand can grasp the ligand from a higher affinity binding site. The answer appears to be rather simple—steric clashes. After ligand transfer, BtuF induces closure of the periplasmic gate, and, in parallel, the cytoplasmic gate starts to gradually open, resulting in the release of vitamin B_{12} into the cytosol and inducing the full closure of the NBDs. This finally allows hydrolysis of ATP, resetting the entire system. Thus, the substrate is squeezed through the membrane, which the authors call a 'squeezing motion'. As this single-molecule data is not biased by ensemble averaging, it highlights a synchronization and tight coupling between ligand binding and transfer by various well-timed and defined conformational changes. This is in clear and striking contrast to the described conformational heterogeneity observed and described for ABC exporters (see, for example, ref. [5]) as well as members of the secondary transporter family (see, for example, ref. [6]). BtuCD-F is a founding member of the family of class II ABC importers, and the results determined in this study will likely hold for other import systems of this family. Whether these observations will hold for all ABC import systems, including class I and ECF-based systems[3], can only answered by additional investigations of such ABC import systems at the single-molecule level. ❐

Lutz Schmitt
Institute of Biochemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.
e-mail: lutz.schmitt@hhu.de

Published online: 18 June 2018
https://doi.org/10.1038/s41589-018-0087-3

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Competing interests

The author declares no competing interests.