Exploring the mechanistic process of vitamin B_{12} acquisition by human gut bacteria)

Translocation of vitamin B_{12} through the BtuB3G3 channel and the role of the surface-exposed lipoprotein BtuG3

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In Short

- Dynamics of two structurally different vitamin B_{12} uptake systems
- Structure and dynamics of BtuB3G3
- Vitamin B_{12} acquisition and transport by BtuB3G3
- BtuB3G3 dynamics in asymmetric membranes

Because of the energetically costly and intricate biosynthesis process of small molecule cofactors, microbes often consume essential small cofactors from their environment. BtuB is a TonB-dependent outer membrane transporter in Gram-negative bacteria and plays a vital role in actively transporting cyanocobalamin (CNCbl, vitamin B_{12}) and other essential nutrients. The human gut *Bacteroidetes* acquire vitamin B_{12} with the help of several B_{12} binding proteins, e.g., BtuB, BtuG, BtuH [1–3]. The previous study explored the CNCbl acquisition and translocation process using B_{12}-BtuG2 and BtuB2G2 crystal structures from locus two of *B. thetaiotaomicron* [2].

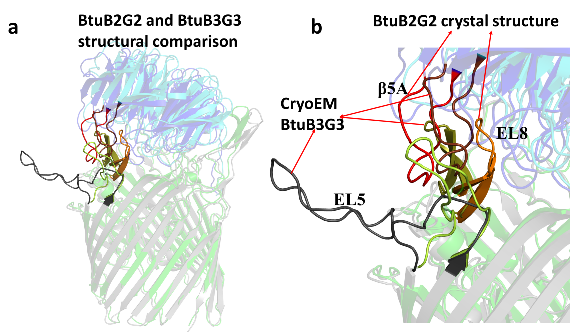

***Figure 1:** **a**, Structural comparison between the BtuB2G2 crystal structure and BtuB2G2 cryoEM structure without vitamin B_{12}. **b**, Noticeable differences between the EL5 and EL8 loop of BtuB2 and loop β5A of BtuG2 are depicted.*

Recently, a cyroEM structure of a B_{12}-bound BtuB3G3 protein complex was obtained from locus three of *B. thetaiotaomicron* and the BtuB3G3 structure is noticeably different from the BtuB2G2 one (see Fig. 1). The extracellular EL8 loop of BtuB3 remained outside of BtuB3G3 protein to make room for CNCbl and β5A of BtuG3 tightly bound to the vitamin B_{12} in the cyroEM structure of B_{12}-BtuB3G3. A PISA analysis also suggests a lower number of inter-molecular polar contacts between BtuG3 and BtuB3. The observed reduced number of polar contact between BtuB3 and BtuG3, along with the occupied vitamin B_{12} in the BtuG3 binding cavity, suggests that opening the ButG3 lid may be easier in the BtuB3G3 structure. In the present project year, we would like to explore the BtuG3 lid opening starting with the wild-type cryoEM structure as well as mutated ButB3G3 proteins by substituting strong hydrogen bond-forming amino acid residues with alanine residues, vitamin B_{12} acquisition into the BtuG3 cavity from a widely open-state structure to reproduce the cryoEM conformation, and further translocation of CNCbl from BtuG3 to BtuB3. Finally, the BtuB3G3 structural dynamics and vitamin B_{12} acquisition will be examined in an asymmetric lipooligosaccharide (LOS)-phospholipid bilayer membrane, i.e., LOS in the extracellular leaflet and a mixture of phospholipids in the inner leaflet.

Molecular dynamics (MD) simulations of B_{12}-BtuG2, B_{12}-BtuH, BtuGH, and BtuB2G2 were carried out using unbiased and enhanced sampling techniques. It was observed that the BtuG2 molecule can pull CNCbl from a more or less arbitrary position to its binding site by strong electrostatic and non-covalent interactions (see Fig. 2). The binding free energy, as calculated by metadynamics closely matches the experimentally determined dissociation constant. The unbiased and WTMtD MD simulations revealed that during the BtuG2 lid opening process, BtuG2 moves away from BtuB2 via a hinge loop and can open up to about 40°. This opening facilitates the acquisition of cobalamin-analogous molecules from the surrounding environment. Furthermore, we extensively examined the CNCbl, methylcobalamin (MeCbl), and adenosylcobalamin (AdoCbl) intake processes by the BtuG2 surface-exposed lipoprotein associated with the BtuB2 outer membrane protein, i.e., the BtuB2G2 protein complex. The process is similar to that of the SusCD and RagAB proteins recently examined by our research group [4–6]. Moreover, extensive MD simulations were performed to demonstrate the translocation process of the cobalamine analogous molecules from the active site cavity of BtuG2 to BtuB2. A newly determined crystal structure of the BtuH protein from *B. thetaiotaomi-*

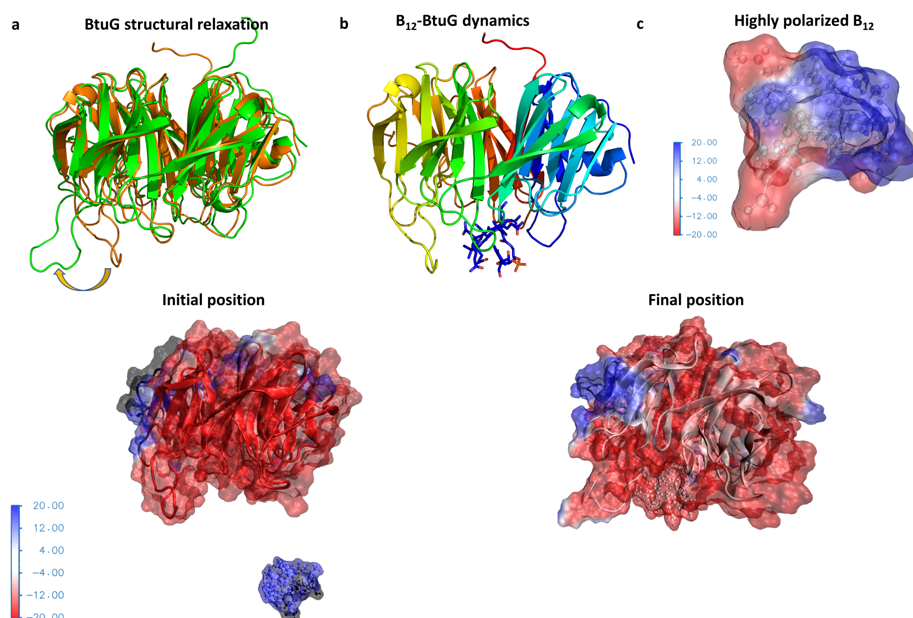

Figure 2: ***a****, Crystal (brown) and representative MD structures (green) of the BtuG protein extracted from a 1 μs-long run (starting structure from the CNCbl-BtuG2 crystal structure but without the CNCbl molecule).* ***b****, CNCbl-BtuG2 crystal structure after the end of a 1 μs-long run demonstrate a remarkable stability of the CNCbl-BtuG2 complex.* ***c****, Electrostatic potential map of the CNCbl molecule with a large dipole moment. The part of CNCbl, i.e., the cobalt (III)" is partially positive, whereas the phosphate part of the molecule is negatively charged.* ***d-e****, In the protein-ligand system, CNCbl is positively charged, whereas the protein system has a negative charge. Therefore, BtuG2 can easily attract CNCbl via electrostatic interactions.*

cron was studied concerning the stability of CNCbl in the binding cavity of BtuH. The MD results suggest that the stabilization of CNCbl results from its strong electrostatic interaction with Ca^{2+} and Na^{+} ions. Finally, we explored how CNCbl was transported from the BtuH to the BtuG binding pocket.

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More Information

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Project Partners

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DFG Subject Area

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