Figures and figure supplements

Human gut Bacteroides capture vitamin B₁₂ via cell surface-exposed lipoproteins

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**Figure 1.** BtuG homologs are exclusively found among the Bacteroidetes, facilitate the acquisition of cyanocobalamin in vitro and confer a fitness advantage in gnotobiotic mice. (A) Genetic loci encoding corrinoid transport components in *E. coli*, *B. thetaiotaomicron* and other Bacteroidetes. (B) BtuG2 (PDB 3DSM) adopts a seven-bladed β-propeller fold. (C) Growth curves for the *B. thetaiotaomicron* parent strain, btuG2 deletion strain or complemented strain grown in minimal media supplemented with methionine or vitamin B₁₂. Data are representative of three independent trials; error bars indicate ±SD from three technical replicates. (D) Gene expression ratios for *B. thetaiotaomicron* strains grown in minimal media with methionine and indicated concentrations of vitamin B₁₂. Expression of locus2 (BT1956) was normalized first to 16S rRNA and then to each strain’s expression in 0 nM vitamin B₁₂. Data are representative of two independent trials; error bars indicate ± SD from three biological replicates. (E, F) *B. thetaiotaomicron* strain ratios determined from gDNA extracted from fecal samples collected over time from gnotobiotic mice. (n.d., not detected; n = 4 mice/group; error bars indicate ± SD).

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Figure 1—figure supplement 1. A BtuG homolog is required for *B. thetaiotaomicron* fitness in gnotobiotic mice. *B. thetaiotaomicron* parent, Δ*btuG2* and complemented (Δ*btuG2 + btuG2*) strain ratios determined from gDNA extracted from fecal samples of gnotobiotic mice collected over time. (*n* = 5 mice; error bars indicate ± SD). The fitness defect of a *B. thetaiotaomicron* strain lacking *btuB2* (Figure 1F) is also complemented by expression of *btuB2* in single copy from a heterologous chromosomal locus (*Degnan et al., 2014a*). DOI: https://doi.org/10.7554/eLife.37138.004
Figure 2. BtuG2 is a cell surface-exposed lipoprotein that interacts with the outer membrane transporter BtuB2. (A) N-terminus of BtuG2 and sequence logo of 114 BtuG homologs reveal sequence signatures indicative of a surface-exposed lipoprotein, including a lipobox, adjacent cysteine residue and lipoprotein export signal (LES). (B) Protease degradation of BtuG2 on whole B. thetaiotaomicron cells suggests BtuG2 is surface-exposed. SusA is a periplasmic control. Data are representative of three independent trials. (C) B. thetaiotaomicron cells separated into membrane (M), cytoplasm/periplasm (C), and supernatant (S) fractions reveal that BtuG2 is predominantly associated with the membrane in parent cells, but predominantly associated with the supernatant in ΔbtuB2 cells. Data are representative of four independent trials. (D) In vivo pull-down of BtuG2 by TAP-tagged BtuB2 suggests an interaction with BtuB2, but not with an unrelated outer membrane β-barrel protein SusC. Data are representative of two independent trials.

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Figure 2—figure supplement 1. BtuG2 lipoprotein sequence signatures are required for protein production and can be functionally replaced with N-terminal sequences from the unrelated cell surface lipoprotein SusD. (A) Western blot of *B. thetaiotaomicron* whole cell lysates of parent strain and a panel of *btuG2* complemented strains probed with anti-BtuG2. Data are representative of three independent trials. (B) qRT-PCR of *B. thetaiotaomicron* strains grown in minimal media supplemented with methionine and either 37 nM or 0.37 nM vitamin B$_{12}$. Data are normalized first to 16S rRNA and then to expression of Δ*btuG2* + *btuG2* in 0.37 nM vitamin B$_{12}$. Data are representative of two independent trials; error bars indicate ± SD from three biological replicates. (C) Western blot of *B. thetaiotaomicron* whole cell lysates of a panel of *btuG2* complemented strains probed with anti-BtuG2. Data are representative of two independent trials. (D) Growth curves of *B. thetaiotaomicron* strains grown in minimal media supplemented with methionine or indicated concentrations of vitamin B$_{12}$. Data are representative of two independent trials; error bars indicate ± SD from three technical replicates; ss, signal sequence; LES, lipoprotein export signal.

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Figure 3. BtuG2 binds cyanocobalamin and its corrinoid precursor with femtomolar affinity. (A) SEC-MALS traces for recombinant BtuG2 incubated with vitamin B₁₂. BtuG2 (protein) absorbance is measured at 280 nm; vitamin B₁₂ absorbance is measured at 362 nm. Data are representative of three independent trials. (B) Kinetic rate constants and equilibrium dissociation constant for BtuG2 binding to dicyanocobinamide and cyanocobalamin determined by SPR. Data are representative of three independent trials; error represents ± SD from rate constants measured across three Biacore chip cells.

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<td>cyanocobalamin</td>
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<td>$(2.59 ± 0.96) × 10^4$</td>
<td>$(1.87 ± 0.76) × 10^{-13}$</td>
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Figure 3—figure supplement 1. Cyanocobalamin binding by diverse BtuG homologs. (A) A gene tree assembled from 114 BtuG homologs (Supplementary file 1) indicates the relative homology of three BtuG homologs from B. vulgatus, B. uniformis and B. coprophilus (BVU2056, BACUNI04578 and BACCOPRO02032, respectively; shown in red). (B) Purified proteins (and a no-protein control) were incubated with B\textsubscript{12}, filtered to remove free vitamin, and quantified for absorbance at 360 nm (corresponding to vitamin B\textsubscript{12} absorbance). BSA, bovine serum albumin; B\textsubscript{12}, no-protein control after B\textsubscript{12} incubation and filtering. *p<0.05 for samples compared against no-protein control (B\textsubscript{12}); error bars indicate ± SD from two technical replicates.

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Figure 3—figure supplement 2. Surface electrostatic profiles of the seven-bladed β-propeller proteins BtuG2, EUBREC_1955 and MSMAS_RS11935, and the globular enzyme acetylcholinesterase. (A) Model of surface electrostatic profile based on the crystal structure of BtuG2 (PDB 3DSM; left) reveals a largely positive electrostatic potential (blue) on the face of BtuG2 harboring the C-terminal 6xHis tag, and a largely negative electrostatic potential (red) on the opposing face, unlike the unrelated seven-bladed β-propellers EUBREC_1955 from the human gut anaerobe Eubacterium rectale (PDB 3S25; center left) and MSMAS_RS11935 from the methanogenic archeon Methanosarcina mazei (PDB 1L0Q; center right). Opposing electrostatic charge surfaces on enzymes like acetylcholinesterase (PDB 1GQR; right) are thought to aid in ligand orientation and binding during catalysis (Tan et al., 1993; Getzoff et al., 1983; Ripoll et al., 1993). (B) Structural similarity between BtuG2 (PDB 3DSM; gold) and the β-propeller domain of MSMAS_RS11935 (PDB 1L0Q; blue) (Jing et al., 2002). These domains are superimposed with a root mean square deviation of 1.75 Å.

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Figure 4. BtuG2 can function extracellularly as a corrinoid delivery protein and confers a fitness advantage to BtuG2 producer cells. (A) Schematic (left) and measured CFUs (right) from an experiment in which B. thetaiotaomicron recipient cultures (\(\Delta \)btuG2 or \(\Delta \)locus1,2,3) received donor supernatant from B. thetaiotaomicron donor strains (\(\Delta \)btuB2 or \(\Delta \)btuG2), with or without vitamin B\(_{12}\). Recipient cultures were plated for CFUs over time. Data are representative of four independent trials; *p<0.05 for black bars compared against red, yellow or blue bars; error bars indicate ± SD from two biological replicates. (B) B. thetaiotaomicron parent and \(\Delta \)btuG2 strains were co-cultured in minimal media supplemented with methionine or the indicated concentrations of vitamin B\(_{12}\) and incubated at 37 °C anaerobically either statically (solid lines) or shaking (dotted lines). Cells were passaged into fresh media daily and strain abundances were determined by qRT-PCR using barcode-specific primers. n.d., not detected; data are representative of two independent trials; error bars indicate ± SD from three technical replicates.

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**Figure 5.** BtuG2 mediates vitamin B₁₂ piracy from host intrinsic factor. (A) Schematic (left) and measured CFUs (right) from an experiment in which *B. thetaiotaomicron* recipient cultures (parent or ΔbtuG2) received recombinant human IF with or without vitamin B₁₂, or the filtrate from the last IF–B₁₂.

*Figure 5 continued on next page*
Figure 5 continued

wash. Recipient cultures were plated for CFUs over time. Data are representative of two independent trials; *p<0.05 for black bars compared against red, yellow or blue bars; error bars indicate ± SD from two biological replicates. (B) Schematic (left) and SEC-MALS traces at 362 nm absorbance (right) of recombinant human IF and/or recombinant BtuG2 incubated with vitamin B₁₂. 362 nm absorbance measures B₁₂-associated proteins and each trace represents one of the four conditions illustrated in the schematic. Data are representative of two independent trials. (C) Schematic (left) and CFUs (right) from an experiment in which B. thetaiotaomicron recipient cultures (ΔbtuG2) received recombinant human IF with or without vitamin B₁₂ or the filtrate from the last IF–B₁₂ wash, and donor supernatant from B. thetaiotaomicron strains (ΔbtuB2 or ΔbtuG2). Recipient cultures were plated for CFUs over time. Data are representative of two independent trials; *p<0.05 for black bars compared against red, yellow or blue bars; error bars indicate ± SD from two biological replicates.

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