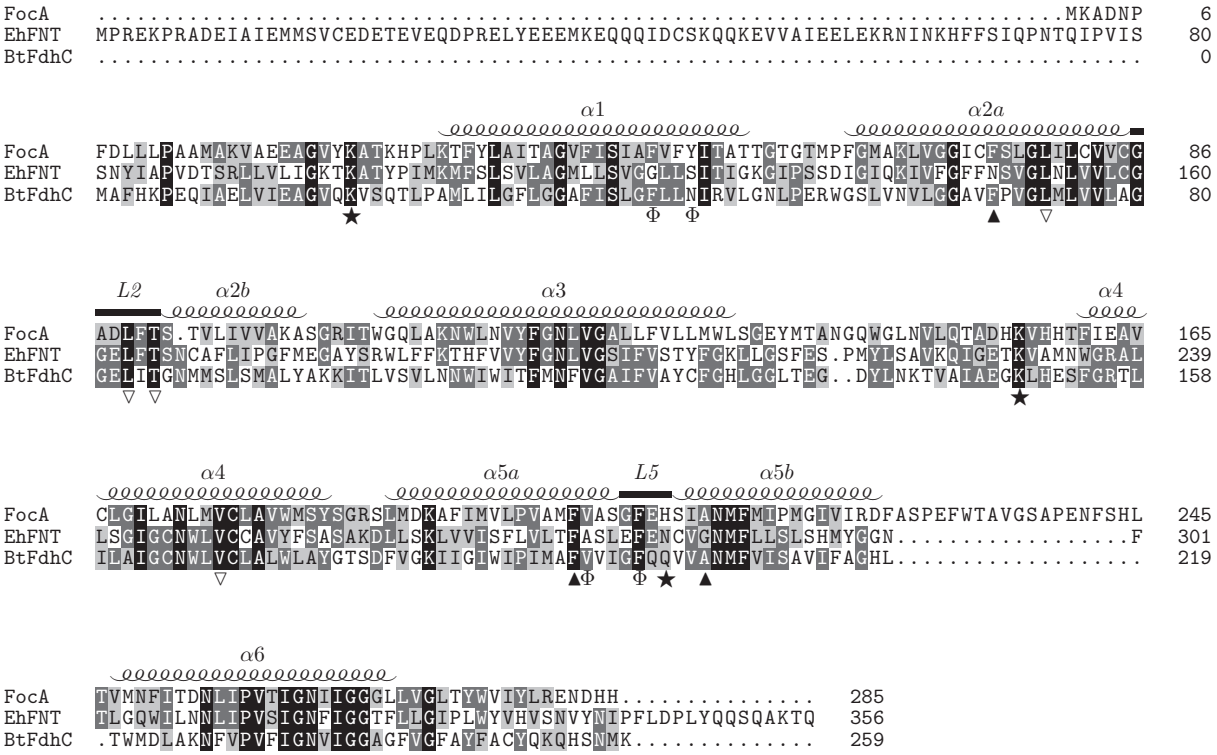
Formate-nitrite transporters carrying nonprotonatable amide amino acids as natural replacements of a central histidine maintain pH-dependent transport

Folknand Helmstetter, Philipp Arnold, Bastian Höger, Lea Madlen Petersen, and Eric Beitz



	FocA	EhFNT	BtFdhC	
FocA	—	50.0	48.2	
EhFNT	29.3	—	48.2	% simil.
BtFdhC	28.4	30.8	—	
	% identity			

Figure S1. Sequence alignment of FocA, EhFNT, and BtFdhC. The locations of the transmembrane domains were taken from chain A of the FocA structure PDB #3q7k and are shown above the sequences; L2 and L5 indicate helix-breaking loop regions. The residues forming the lipophilic constrictions sites are labelled by triangles (outer constriction: black; inner constriction: open). Residues denoting the Φ/K selectivity filter are marked with an "Φ". The central histidine (His-209 in FocA), and the alternative asparagine (Asn-283 in EhFNT) or glutamine (Gln-202 in BtFdhC), as well as the conserved lysine residues in the vestibules are labelled by stars.

Formate-nitrite transporters carrying nonprotonatable amide amino acids as natural replacements of a central histidine maintain pH-dependent transport

Folknand Helmstetter, Philipp Arnold, Bastian Höger, Lea Madlen Petersen, and Eric Beitz

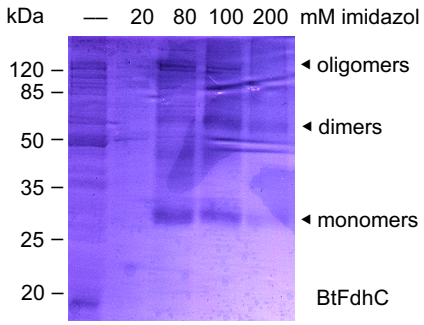


Figure S2. Ni^{2+}-affinity purification of cell-free produced and Brij78-solubilized BtFdhC. Similar to EhFNT, the BtFdhC elution fractions contain partially SDS-resistant homodimers and oligomers.

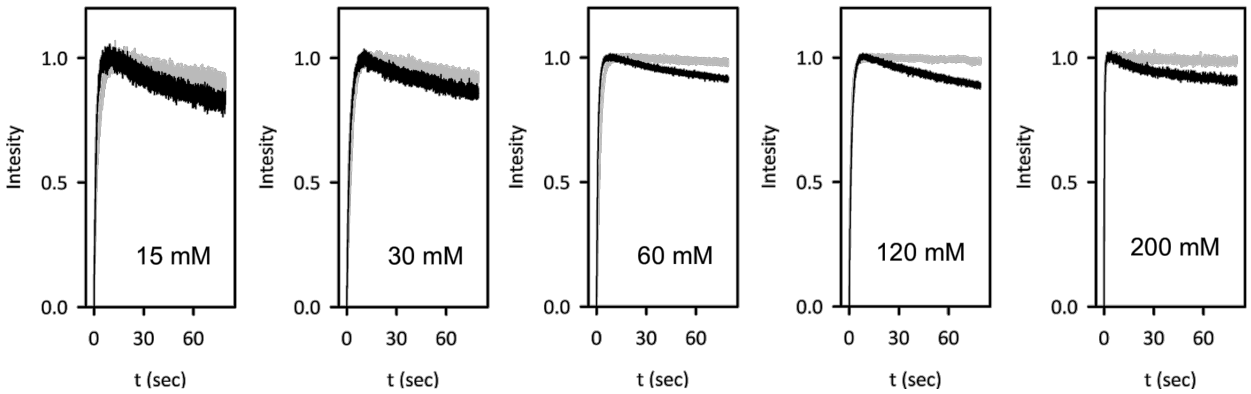


Figure S3. EhFNT substrate affinity. Transport of acetate at 15–200 mM concentrations was monitored by light scattering at pH 6.8 in both, the external and internal proteoliposome buffer. The rates from the second, acetate uptake phase were determined by exponential fitting, and were plotted in Fig. 5D of the main paper.

Formate-nitrite transporters carrying nonprotonatable amide amino acids as natural replacements of a central histidine maintain pH-dependent transport

Folknand Helmstetter, Philipp Arnold, Bastian Höger, Lea Madlen Petersen, and Eric Beitz

Figure S4. Optimization of the coding sequence for EhFNT. The original sequence is shown in the top line, changes for optimization towards the *E. coli* and yeast codon usage are indicated in lower case below, and the resulting, unchanged protein sequence is depicted in the bottom line (blue).