Transmembrane helix 6b links proton- and metal-release pathways and drives conformational change in an Nramp-family transition metal transporter

Aaron T. Bozzi, Anne L. McCabe, Benjamin C. Barnett and Rachelle Gaudet

Supplemental Information

List of material included:

Figure S1

Figure S2

Figure S3

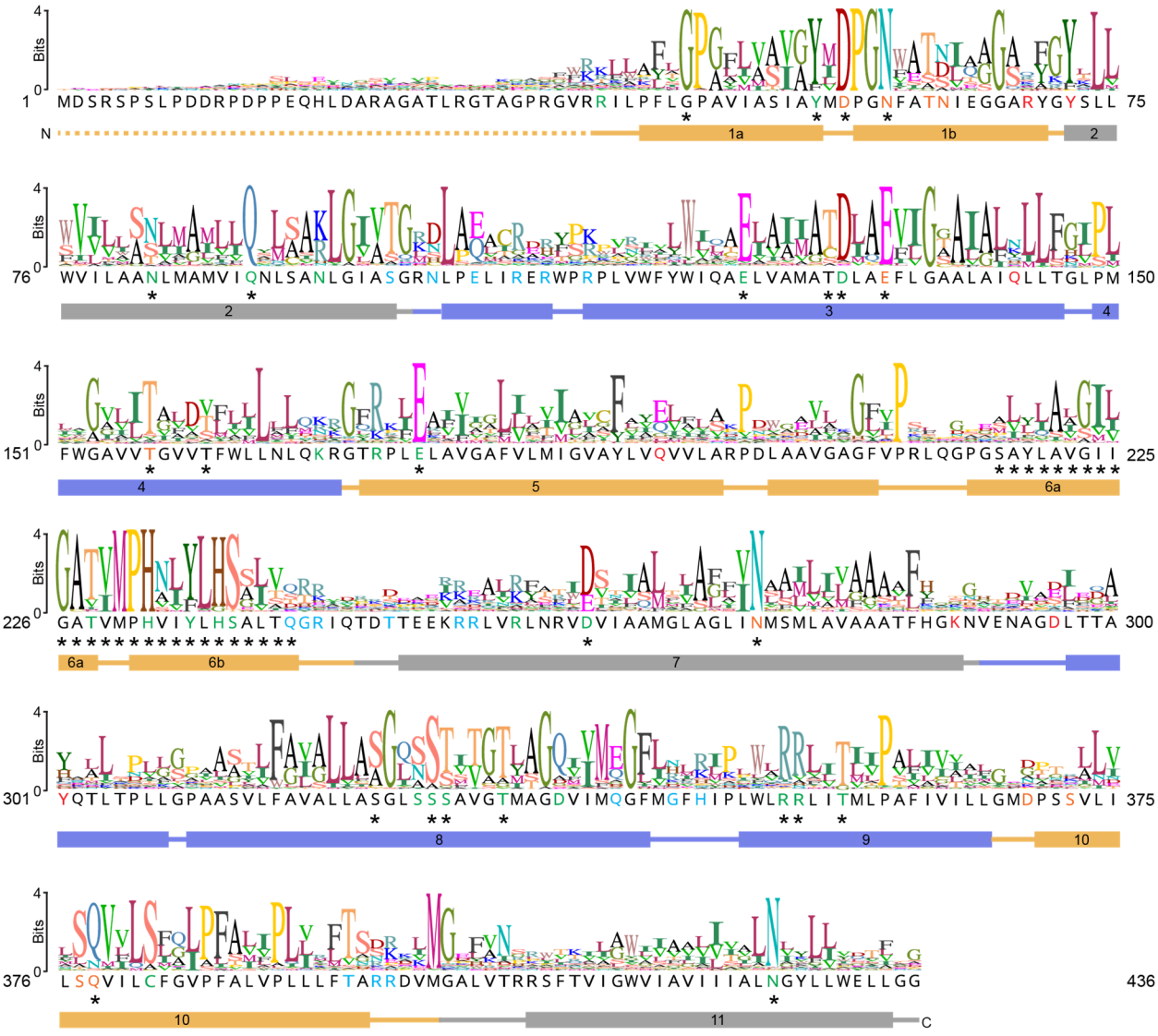

Figure S1. Secondary structure of DraNramp and sequence logo from Nramp alignment.

The primary sequence of DraNramp is annotated with the secondary structure seen in PDB # 6BU5 and 6C3I. The sequence logo was generated using Geneious version 9.1 (Biomatters) from an alignment of 6878 sequences containing the canonical Nramp TM1 "DPGN" and TM6 "MPH" motifs (21). Conserved hydrophilic positions (defined as Ser + Thr + Tyr + Asn + Gln +Asp + Glu + His + Lys + Arg > 80%) are colored in DraNramp's sequence consistent with the location in the structure as depicted in Figure 1. * indicates residues for which the effect of mutagenesis on transport ability was determined.

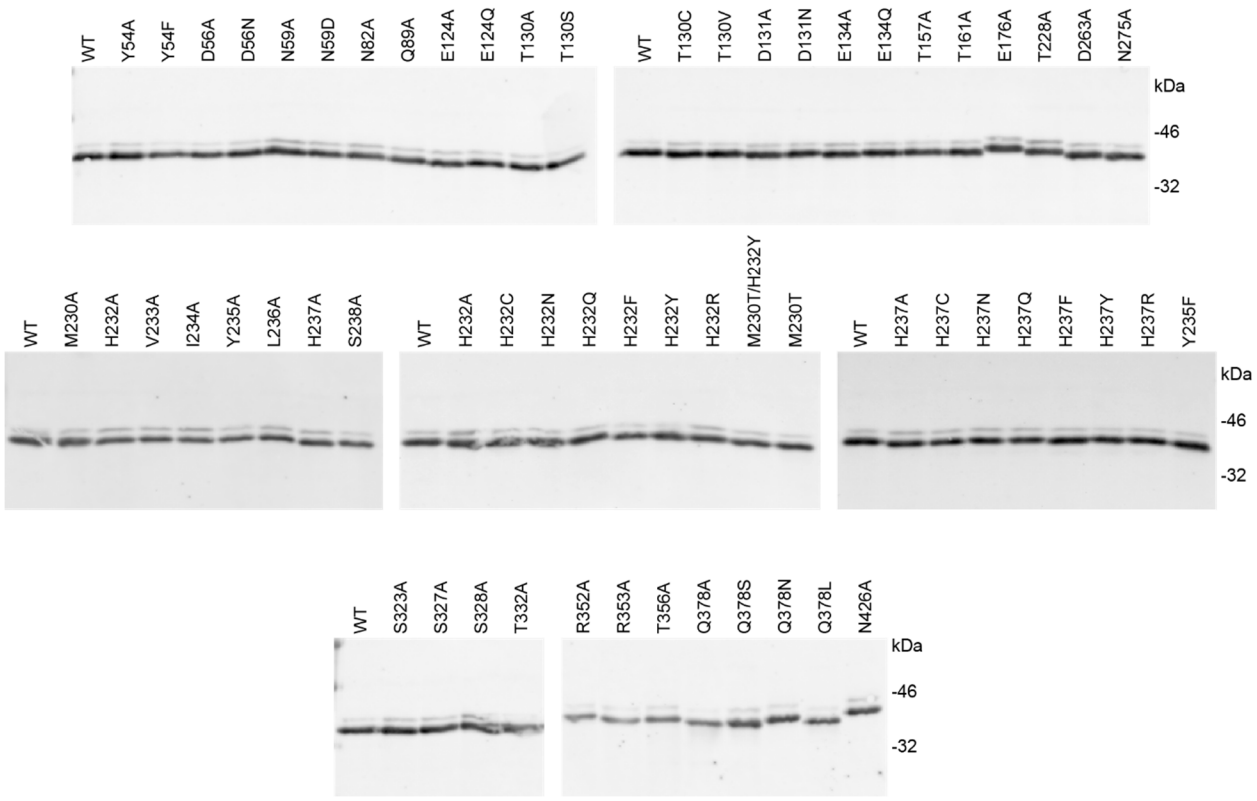

Figure S2. Expression of DraNramp mutants in C41(DE3) *E. coli*.

Western blots for the N-terminal 8xHis-tag show that all Nramp point mutants discussed in this paper expressed in *E. coli*, most at a level similar to WT.

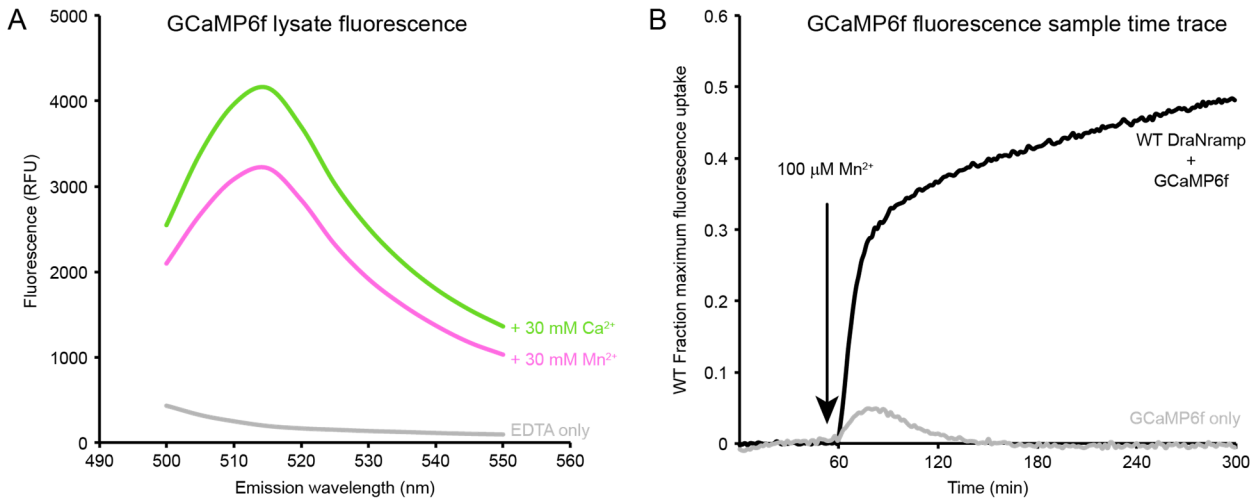

Figure S3. Validation of GCaMP6f as a Mn^{2+}-sensitive fluorescent reporter.

(A) C41(DE3) cells expressing GCaMP6f were lysed, EDTA was added to sequester native Ca^{2+}, the lysate was buffered to pH 7.3, and excess additional Ca^{2+} (green) or Mn^{2+} (pink) was added before measuring fluorescence at an excitation wavelength of 470 nm. The gray trace corresponds to no added metal. (B) Sample time traces comparing relative fluorescence increases observed for C41(DE3) cells co-expressing WT DraNramp and GCaMP6f (black) and expressing GCaMP6f only (EV control; grey). The maximum fluorescence was determined by adding 10 mM Ca^{2+} to separate aliquots of cells.