

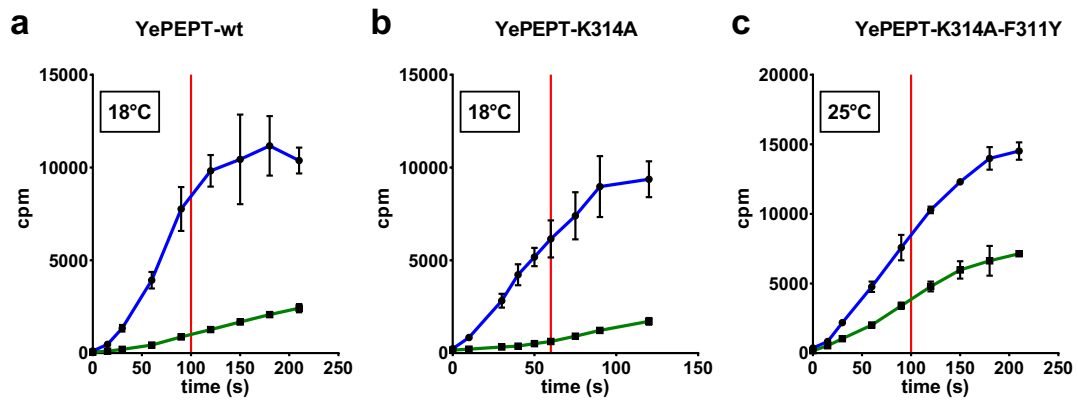
# Supplementary Information for:

## Engineering and functional characterization of a proton-driven $\beta$ -lactam antibiotic translocation module for bionanotechnological applications

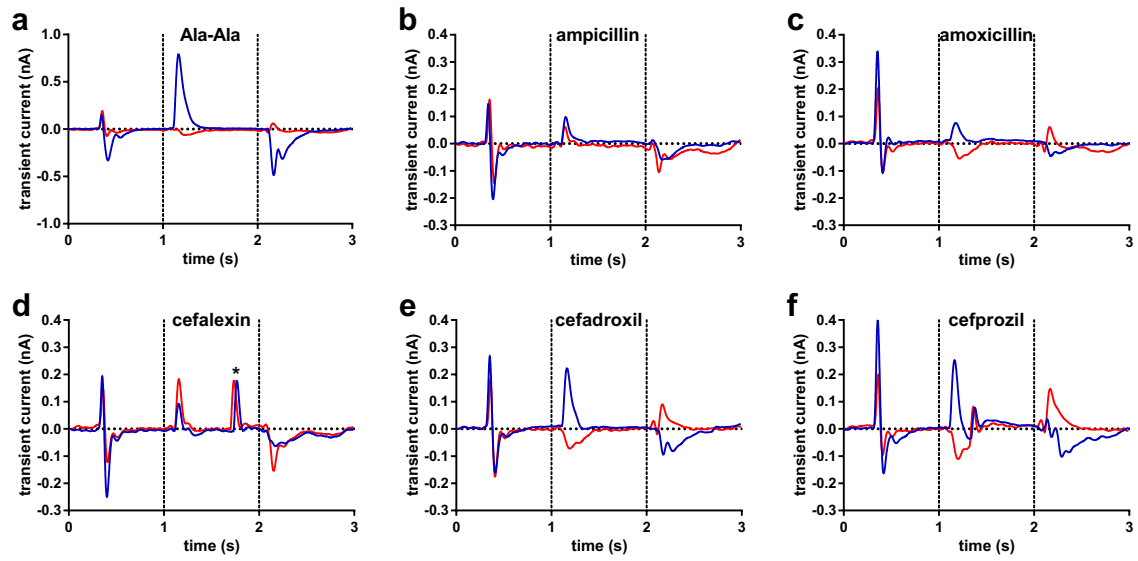
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**Supplementary Fig. S1** Representative time course analysis of [<sup>3</sup>H]Ala-Ala uptake in *E. coli* cells transformed with different YePEPT variants (blue) or empty vector (green). Total signal of [<sup>3</sup>H]Ala-Ala uptake by (a) YePEPT<sup>WT</sup>, (b) YePEPT<sup>K314A</sup> and (c) YePEPT<sup>K314A-F311Y</sup> at different time points and the indicated temperatures. Data points represent counts per minute (cpm) ± SEM of at least triplicates. The selected duration for uptake experiments of each YePEPT variant are indicated by a red, vertical line.



**Supplementary Fig. S2** Representative electrophysiology traces for **a** Ala-Ala, **b** ampicillin, **c** amoxicillin, **d** cefalexin, **e** cefadroxil and **f** cefprozil of proteoliposomes containing YePEPT<sup>K314A-F311Y</sup> (blue) or empty control liposomes (red). In **d** a second peak (\*) appeared consistently in all measurements, including when using control liposomes. Concentration of all compounds was 5 mM.

**Supplementary Table S1** Isoelectric points (pI) and net charges of compounds used in the SSM-based electrophysiology transport assay.

Compound	Isoelectric point (pI)	Net charge at pH = 6.7
Ala-Ala	6.21	-0.02
Ampicillin	5.38	-0.23
Amoxicillin	5.38	-0.23
Cefalexin	5.49	-0.23
Cefadroxil	5.48	-0.23
Cefprozil	5.52	-0.23

All values were calculated using the Isoelectric Point plugin of the MarvinView software (ChemAxon Ltd.).