Biochemical characterization and chemical inhibition of PfATP4-associated Na^+-ATPase activity in *Plasmodium falciparum* membranes

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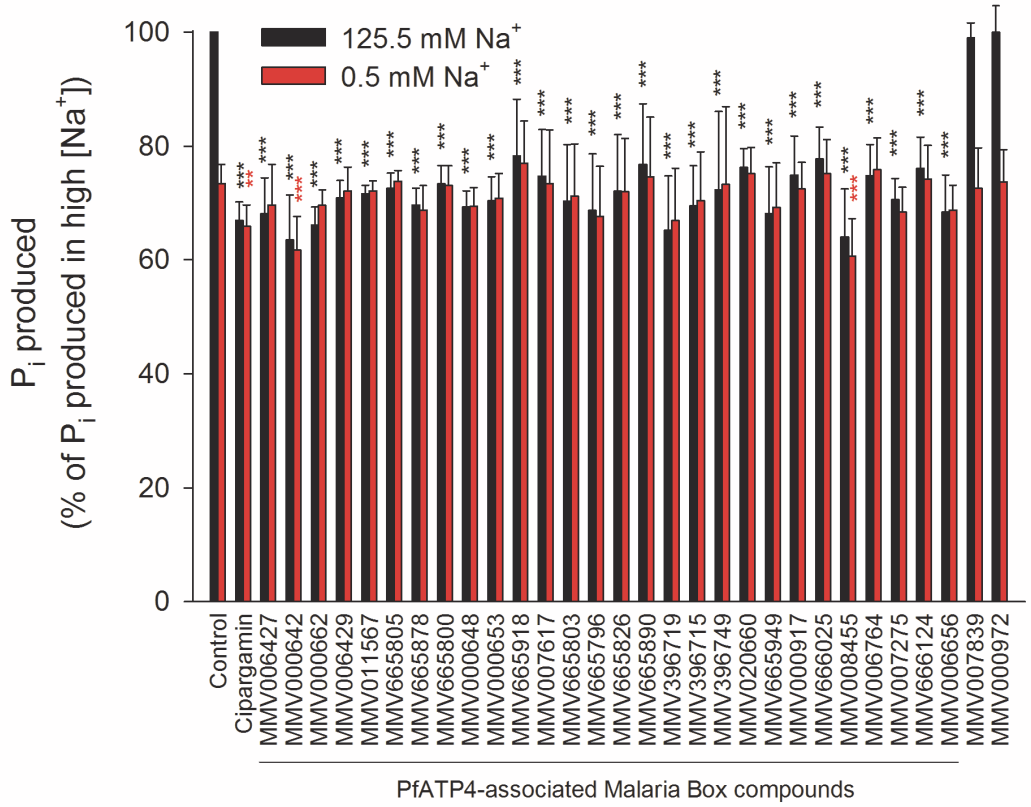
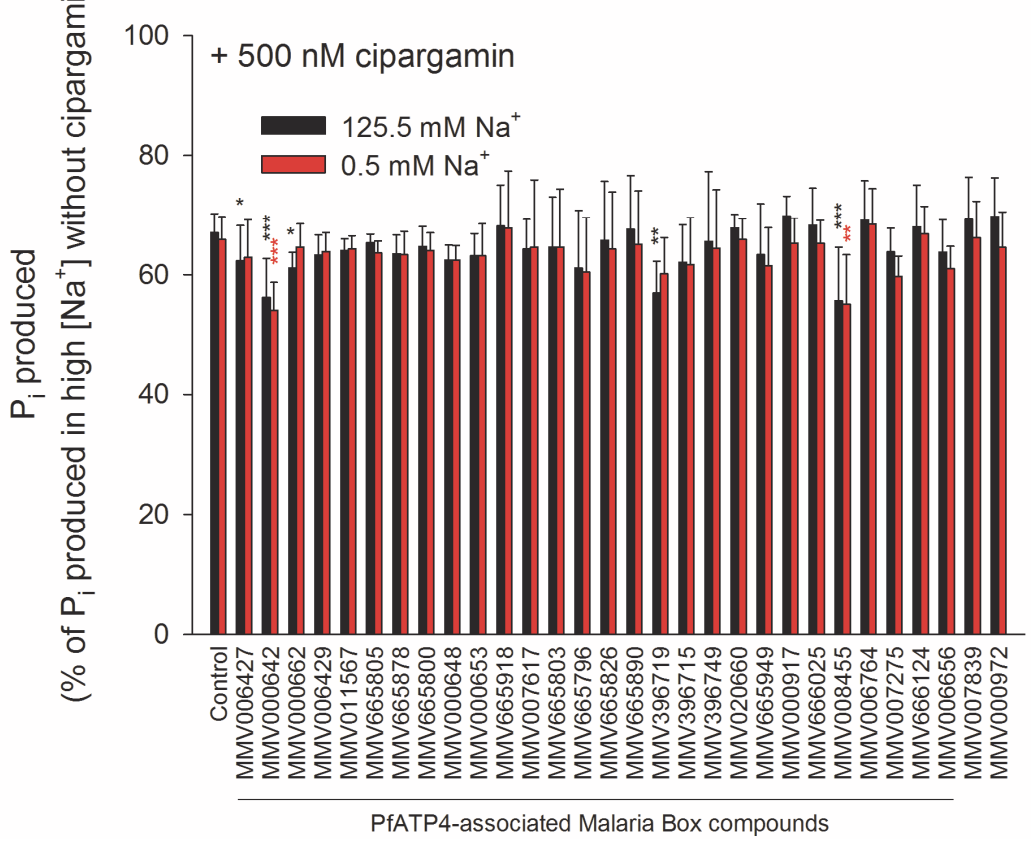
Supporting Information

Included material:

a


125.5 mM Na+
0.5 mM Na+
Pi produced
(% of Pi produced in high [Na+])
100
80
60
40
20
0
Control
Cipargamin
MMV006427
MMV000642
MMV000662
MMV006429
MMV011567
MMV665805
MMV665878
MMV665800
MMV000648
MMV000653
MMV665918
MMV007617
MMV665803
MMV665796
MMV665826
MMV665890
MMV396719
MMV396715
MMV396749
MMV020660
MMV665949
MMV000917
MMV666025
MMV008455
MMV006764
MMV007275
MMV666124
MMV006656
MMV007839
MMV000972
PfATP4-associated Malaria Box compounds

b


+ 500 nM cipargamin
125.5 mM Na+
0.5 mM Na+
Pi produced
(% of Pi produced in high [Na+] without cipargamin)
100
80
60
40
20
0
Control
MMV006427
MMV000642
MMV000662
MMV006429
MMV011567
MMV665805
MMV665878
MMV665800
MMV000648
MMV000653
MMV665918
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MMV008455
MMV006764
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MMV666124
MMV006656
MMV007839
MMV000972
PfATP4-associated Malaria Box compounds

Fig S1. The effects of various compounds on *P. falciparum* membrane ATPase activity under high-[Na^+] (125.5 mM) and low-[Na^+] (0.5 mM) conditions. Cipargamin was tested at a concentration of 500 nM; the other compounds (all from the Malaria Box, with 28 of them having PfATP4-associated activity and two of them, MMV007839 and MMV000972, having activity against an unrelated transport protein) were tested at a concentration of 4 μM. The compounds were tested for their effects individually (a) and in the presence of 500 nM cipargamin (b). In all cases the ATP concentration was 0.25 mM, the pH was 7.4, and the duration of the reaction was 15 min. The data were obtained with Dd2 parasites and are shown as the mean (+ S.D.) from three to five independent experiments, each performed on different days with different membrane preparations. The protein content of the membrane preparations was not quantified in these experiments; each reaction well contained membrane from 3×10^6 cells. For each compound, the (pre-normalised) data were tested for statistical significance compared to the Control (0.4% v/v DMSO (a) or 500 nM cipargamin only (b)) data for the same (high-[Na^+] or low-[Na^+]) condition using a one-way ANOVA (which yielded an F probability < 0.001 in each case) followed by a *post hoc* least significant difference test; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

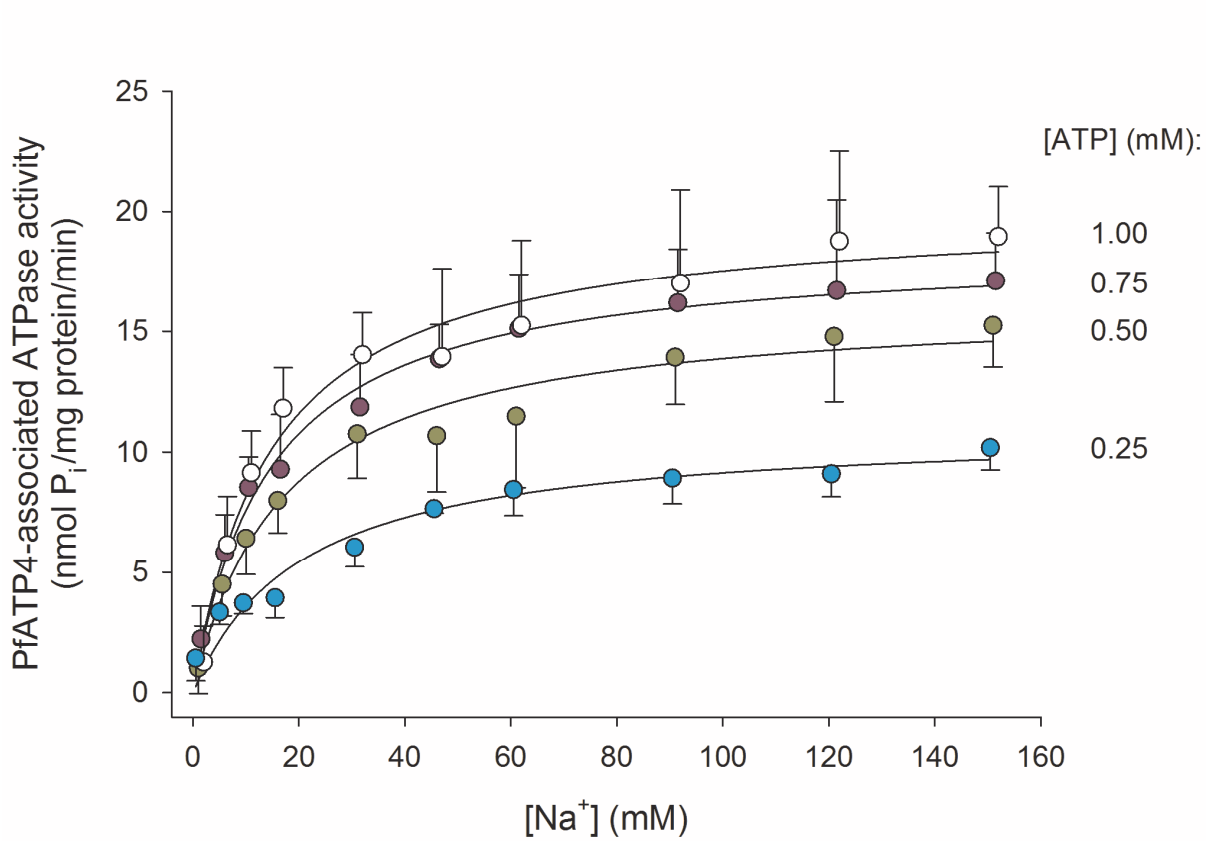

Fig S2. The effect of a range of ATP on the Na^+-dependence of PfATP4-associated ATPase activity. The data were obtained with Dd2 parasites and are shown as the mean (± S.D.) from five independent experiments, each performed on different days with different membrane preparations. For clarity, only upward error bars are shown for the data obtained with 1 mM and 0.75 mM ATP and only downward error bars are shown for the data obtained with 0.5 mM and 0.25 mM ATP. The addition of ATP to the reaction mixtures was found to give rise to minor effects on pH (see Fig 3 legend). The Michaelis-Menten equation (PfATP4-associated ATPase activity = $V_{max} \times [Na^+]/ ([Na^+] + K_m(Na^+))$) was fitted to the data obtained at each of the four ATP concentrations. The $K_m(Na^+)$ values (in mM; mean ± S.D.) estimated in this series of experiments at each of the different ATP concentrations were: 15.0 ± 4.0 with 1 mM ATP, 15.0 ± 5.7 with 0.75 mM ATP, 17.6 ± 3.6 with 0.5 mM ATP, and 20.6 ± 3.6 with 0.25 mM ATP. The $K_m(Na^+)$ obtained in the presence of 0.25 mM ATP differed significantly from that obtained in the presence of 1 mM ATP ($p = 0.03$, paired t-tests). All other comparisons did not yield statistically significant differences (i.e. $p > 0.05$). The V_{max} values (in nmol P_i per mg (total) protein per min; mean ± S.D.) were: 20.2 ± 3.5 with 1 mM ATP, 18.6 ± 2.9 with 0.75 mM ATP, 16.3 ± 2.3 with 0.5 mM ATP, and 11.0 ± 1.2 with 0.25 mM ATP. These values are all significantly different from one another ($p < 0.04$, paired t-tests).

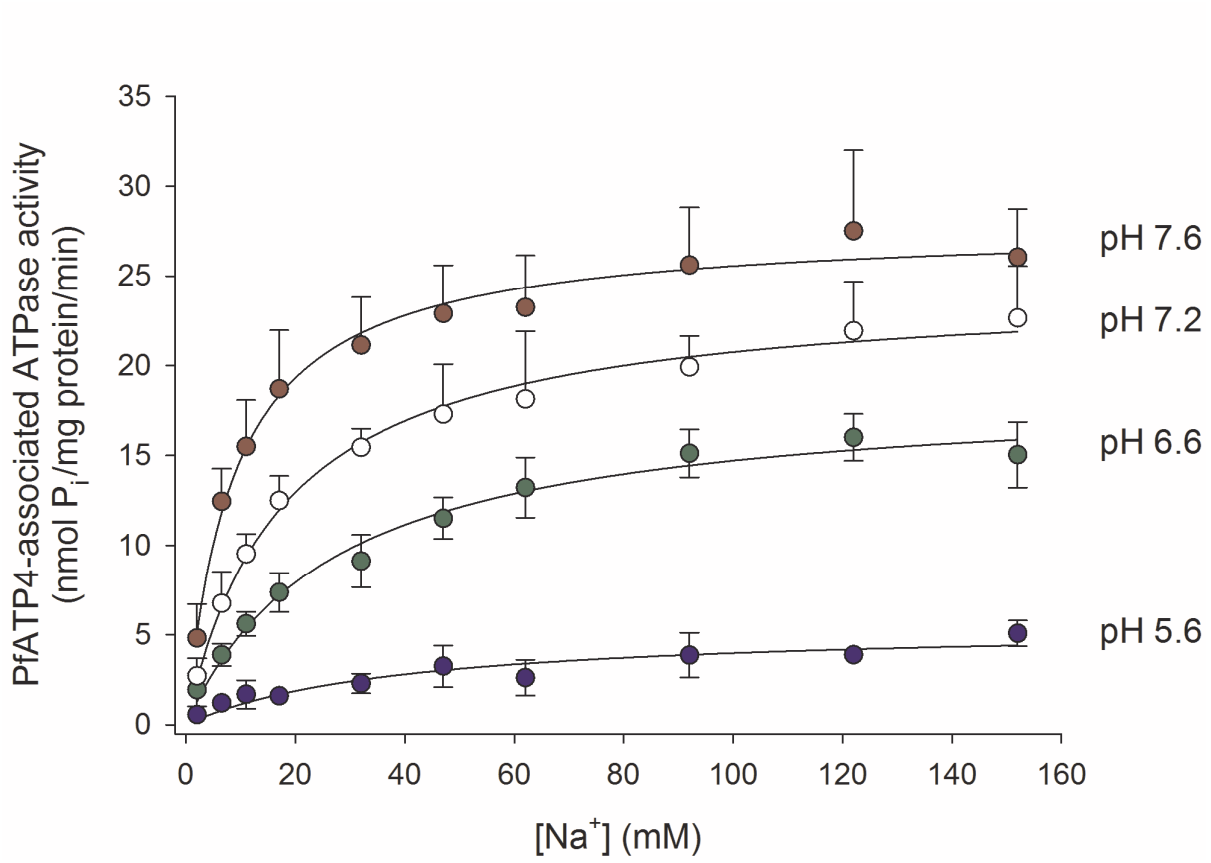

Fig S3. The effect of pH on the Na^+-dependence of PfATP4-associated ATPase activity. The data were obtained with Dd2 parasites and are shown as the mean (± S.D.) from three independent experiments, each performed on different days with different membrane preparations. Where not shown the error bars fall within the symbols. For clarity, only upward error bars are shown for the data obtained at pH 7.6 and 7.2. The Michaelis-Menten equation (PfATP4-associated ATPase activity = $V_{max} \times [Na^+]/ ([Na^+] + K_m(Na^+))$) was fitted to the data obtained at each of the four different pH values tested. The $K_m(Na^+)$ values (in mM; mean ± S.D.) estimated in this series of experiments at each of the different pH values were: 45 ± 19 at pH 5.6, 27.4 ± 4.5 at pH 6.6, 17.9 ± 3.3 at pH 7.2, and 8.9 ± 2.2 at pH 7.6. The difference in the estimated $K_m(Na^+)$ values attained statistical significance ($p = 0.007$, paired t-test) for the pH 7.6 versus pH 6.6 comparison. All other comparisons did not yield statistically significant differences (i.e. $p > 0.05$). The V_{max} values (in nmol P_i per mg (total) protein per min; mean ± S.D.) were: 5.7 ± 0.3 at pH 5.6, 18.7 ± 2.0 at pH 6.6, 24.5 ± 3.2 at pH 7.2, and 27.8 ± 3.4 at pH 7.6. These values are all significantly different from one another ($p < 0.05$, paired t-tests).