APPENDIX

Hypothesis: k_1, k_1', k_3, and k_3' are pH-independent.

At (a) constant pH_{cyt} 7.5 the K_M value increases upon a change from pH_{vac} 7.5 to pH_{vac} 5.5 whereas at (b) constant pH_{cyt} 5.5 it drops.

(a) $K_M^{7.5cyt/7.5vac} < K_M^{7.5cyt/5.5vac}$

$$\Rightarrow \frac{k_1' k_3 + k_3\left(k_1 \cdot 10^{-7.5}\right)^{c1} + k_1'\left(k_3' \cdot 10^{-7.5}\right)^{c1}}{k_3 + \left(k_1 \cdot 10^{-7.5}\right)^{c1} + \left(k_3' \cdot 10^{-7.5}\right)^{c1}} < \frac{k_1' k_3 + k_3\left(k_1 \cdot 10^{-7.5}\right)^{c2} + k_1'\left(k_3' \cdot 10^{-5.5}\right)^{c2}}{k_3 + \left(k_1 \cdot 10^{-7.5}\right)^{c2} + \left(k_3' \cdot 10^{-5.5}\right)^{c2}}$$

$$\Rightarrow k_1' k_3 (k_1 \cdot 10^{-7.5})^{c2} + (k_3)^2 (k_1 \cdot 10^{-7.5})^{c1} + k_3 (k_1 \cdot 10^{-7.5})^{c1} (k_3' \cdot 10^{-5.5})^{c2} + k_1' (k_3' \cdot 10^{-7.5})^{c1} (k_1 \cdot 10^{-7.5})^{c2} < k_1' k_3 (k_1 \cdot 10^{-7.5})^{c1} + (k_3)^2 (k_1 \cdot 10^{-7.5})^{c2} + k_3 (k_1 \cdot 10^{-7.5})^{c2} (k_3' \cdot 10^{-7.5})^{c1} + k_1' (k_3' \cdot 10^{-5.5})^{c2} (k_1 \cdot 10^{-7.5})^{c1}$$

$$\Rightarrow \left(k_3 - k_1'\right)\left[k_3\left(k_1 \cdot 10^{-7.5}\right)^{c1} - k_3\left(k_1 \cdot 10^{-7.5}\right)^{c2} + \left(k_1 \cdot 10^{-7.5}\right)^{c1}\left(k_3' \cdot 10^{-5.5}\right)^{c2} - \left(k_1 \cdot 10^{-7.5}\right)^{c2}\left(k_3' \cdot 10^{-7.5}\right)^{c1}\right] < 0$$

$$\Rightarrow \left(k_3 - k_1'\right)\left(k_1 \cdot 10^{-7.5}\right)^{c1} \cdot \left[k_3\left(1 - \frac{\left(k_1 \cdot 10^{-7.5}\right)^{c2}}{\left(k_1 \cdot 10^{-7.5}\right)^{c1}}\right) + \left(k_3' \cdot 10^{-5.5}\right)^{c2}\left(1 - \frac{\left(k_1 \cdot 10^{-7.5}\right)^{c2}\left(k_3' \cdot 10^{-7.5}\right)^{c1}}{\left(k_1 \cdot 10^{-7.5}\right)^{c1}\left(k_3' \cdot 10^{-5.5}\right)^{c2}}\right)\right] < 0$$

$$\Rightarrow \left(k_3 - k_1'\right)\left(k_1 \cdot 10^{-7.5}\right)^{c1} \cdot \left[k_3\left(1 - \left(k_1 \cdot 10^{-7.5}\right)^{-(c1-c2)}\right) + \left(k_3' \cdot 10^{-5.5}\right)^{c2}\left(1 - \frac{1}{100^{c2}}\left(\frac{k_3'}{k_1}\right)^{(c1-c2)}\right)\right] < 0 \quad \text{(Eq. A1)}$$

Similarly, it follows for (b) $K_M^{5.5cyt/7.5vac} > K_M^{5.5cyt/5.5vac}$

$$\Rightarrow \left(k_3 - k_1'\right)\left(k_1 \cdot 10^{-5.5}\right)^{c1} \cdot \left[k_3\left(1 - \left(k_1 \cdot 10^{-5.5}\right)^{-(c1-c2)}\right) + \left(k_3' \cdot 10^{-5.5}\right)^{c2}\left(1 - \frac{1}{100^{c1}}\left(\frac{k_3'}{k_1}\right)^{(c1-c2)}\right)\right] > 0 \quad \text{(Eq. A2)}$$

Our experiments show that $c1 > c2$. The pump cycle has a preference for pumping from the cytosol into the vacuolar lumen. Therefore, $k'_3 < k_1$ is a reasonable estimation. Consequently, it holds for the terms in square brackets in Eqs. A1 and A2: […] > 0. Thus, the equations can only be fulfilled if

(a) $k_1' > k_3$

(b) $k_1' < k_3$

This, however, is a contradiction to the assumption that all four rate-constants are pH-independent.

Conclusion: The hypothesis is wrong. Thus, at least one of the constants k_1, k_1', k_3, and/or k_3' must be pH-dependent. For instance, k_1' may decrease upon a drop of pH_{cyt} (and/or k_1 increases) while k_3 increases upon an increase of pH_{vac} (and/or k_3' decreases). This would mean that an increasing proton concentration would favor the binding of protons to the protein and full dissociation might not be achieved in each pump cycle.