**SUPPORTING INFORMATION**

# Formation of an Unfolding Intermediate State of Soluble Chloride Intracellular Channel Protein CLIC1 at Acidic pH

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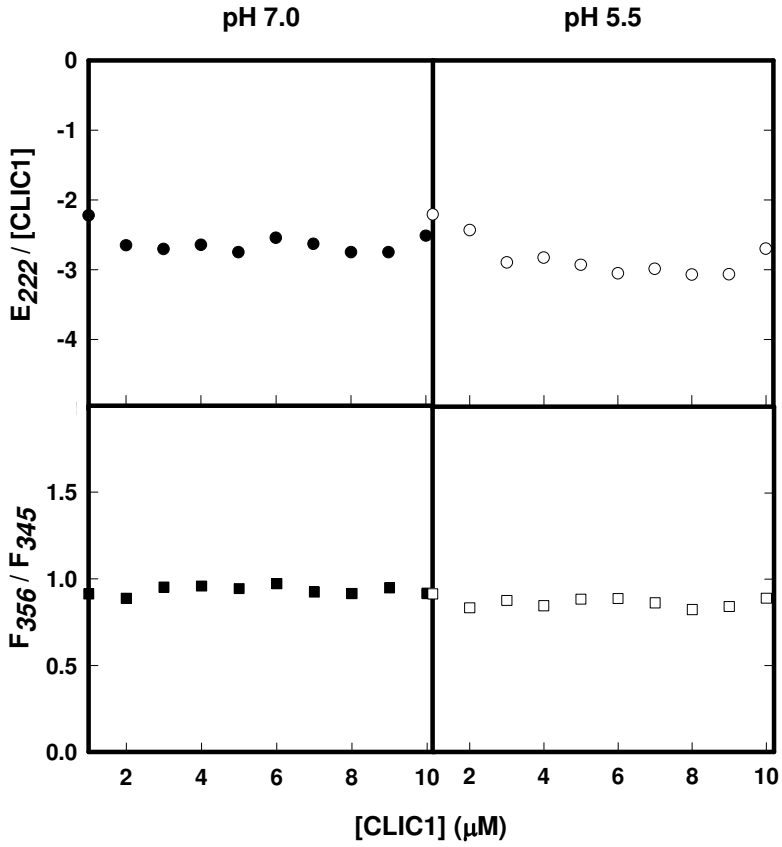


**Figure S1. Protein concentration dependence study**

The CLIC1 concentration dependence of the midpoint of the equilibrium unfolding transition at both pH 7.0 (closed) and pH 5.5 (open). The study was conducted at 20°C using both $E_{222}$ (●) and the ratio of fluorescence $F_{356}$ / $F_{345}$ (■) as probes. The concentration of CLIC1 ranged from 1 μM to 10 μM. The buffer used was a 50 mM $Na_2HPO_4$ containing 0.02% $NaN_3$; 1 mM DTT and 4.8 M urea at pH 7.0 or 3.5 M urea at pH 5.5.

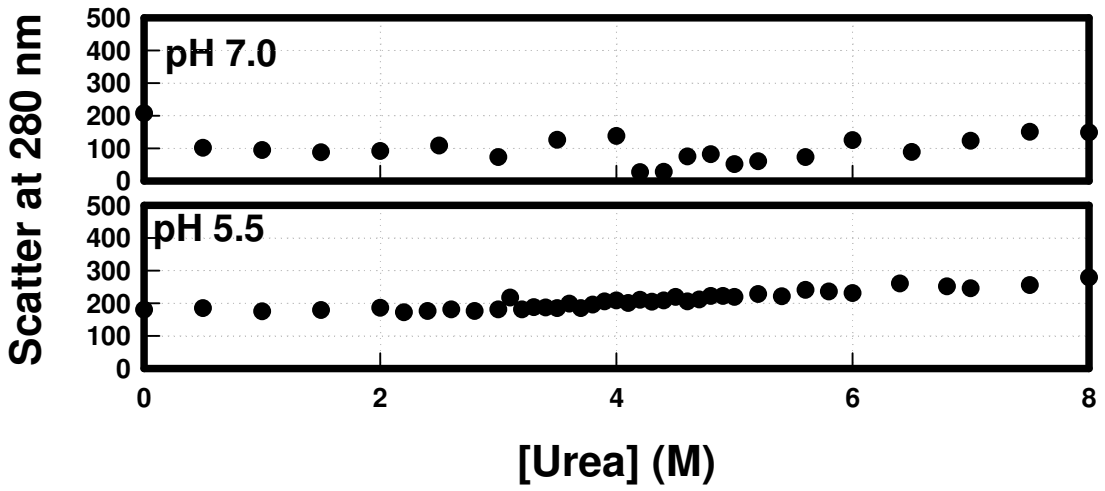


**Figure S2. Raleigh scatter to detect aggregation**

The plots show the scatter produced by 2 μM CLIC1 at 280 nm when excited at 280 nm at pH 7 and pH 5.5 in the presence of increasing concentrations of urea. The plots do not show a significant increase in scatter at any point along the unfolding transition which implies the absence of aggregation.

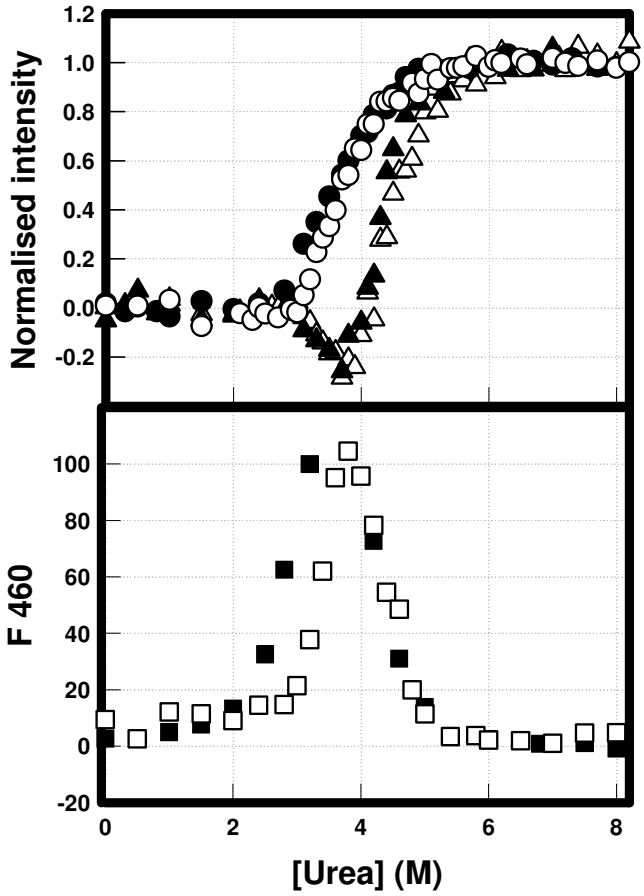


**Figure S3. Comparison of unfolding at pH 7, 37°C and pH 5.5, 20°C**

Comparison of the urea-induced equilibrium unfolding of 2 μM CLIC1 at pH 7.0, 37°C (closed) and pH 5.5, 20°C (open). The unfolding was monitored using $E_{222}$ (●/○), $\lambda_{max}$ (▲/△) and ANS binding (■/□) as probes.