Supplemental material for:

Insights into the structure-function relationships of pneumococcal cell wall lysozymes: LytC and Cpl-1*

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Running head*: pneumococcal cell wall lysozymes*

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SUPPLEMENTAL FIGURE 1. **Self-association equilibria of LytC and Cpl-1: regulation by choline.** *A*, elution profiles of LytC and Cpl-1 in Pi buffer in the absence (curves *a* and *c*) and in the presence (curves *b* and *d*) of 140 mM choline (initial protein concentration ~9 μM). Plus (+) and minus (-) labels refer to data with and without choline. *B,* Sedimentation equilibrium profile of LytC at 60 mM choline. Circles are the experimental data and the solid line is the best fitting function for a single solute with a M_w value corresponding to the monomer. The upper panel (*C*) shows the fit residuals. *D*, Dependence of the apparent weight average molecular mass of LytC (circles; 4 ºC) and Cpl-1 (triangles; 25 ºC) on choline concentration (M_0 is the monomer molecular mass). Measurements were performed in Pi buffer and choline concentrations are in molar units.

SUPPLEMENTAL FIGURE 2. **Temperature CD transition curves of LytC and Cpl-1**. *A* and *B* depict, respectively, the thermal denaturation curves of LytC and Cpl-1, monitored by following the ellipticity changes at 225 nm in the absence and in the presence of 140 mM choline (right scale in *A*). Measurements were carried out in Pi buffer; plus (+) and minus (-) labels refer to data with and without choline.

SUPPLEMENTAL FIGURE 3. **Influence of heating rate on the DSC transitions of Cpl-1**. *A* and *B* depict, respectively, the heat capacity profiles of Cpl-1 recorded in the absence and in the presence of 140 mM choline. Heating rates are indicated in the figure labels. Symbols correspond to the experimental curves and solid lines are the best fits of the curves calculated with parameters summarized in Supplemental Table 2. For clarity, one data point out of four is plotted on average, although full data sets were used for fitting.

Supplemental TABLE 1

LytC denaturation parameters in the absence and in the presence of choline

The experimental curves were analyzed using of the three-state $N \leftrightarrow D \rightarrow I$ model for each transition in the absence and in the presence of 19 mM choline and the two-state irreversible model $N \rightarrow I$ at higher choline concentrations. Data monitored at 20.4 ºC·h^{-1}.

Parameters[c]	*Phosphate*[a]	*19 mM choline*[a]		*60 mM choline*[b]		*140 mM choline*[b]	
	CM	CBM	CM	CBM	CM	CBM	CM
E_{app} $(kcal \cdot mol^{-1})$	61 ± 30	359 ± 16	103 ± 8	266 ± 2	76 ± 2	325 ± 9	94 ± 4
T^* (℃)	48 ± 9	40.8 ± 0.7	46.7 ± 0.3				
T_m	-	-	-	45.8 ± 0.1	46.2 ± 0.1	49.9 ± 0.1	49.2 ± 0.1
ΔH^{vH}_D $(kcal \cdot mol^{-1})$	79 ± 4	245± 1	86 ± 2				
T_D (ºC)	45.5 ± 0.6	39.2 ± 0.1	43.2 ± 0.1				
ΔH_t $(kcal \cdot mol^{-1})$	60 ± 1	156± 2	93 ± 2	188± 3	78 ± 3	152± 4	110 ± 10

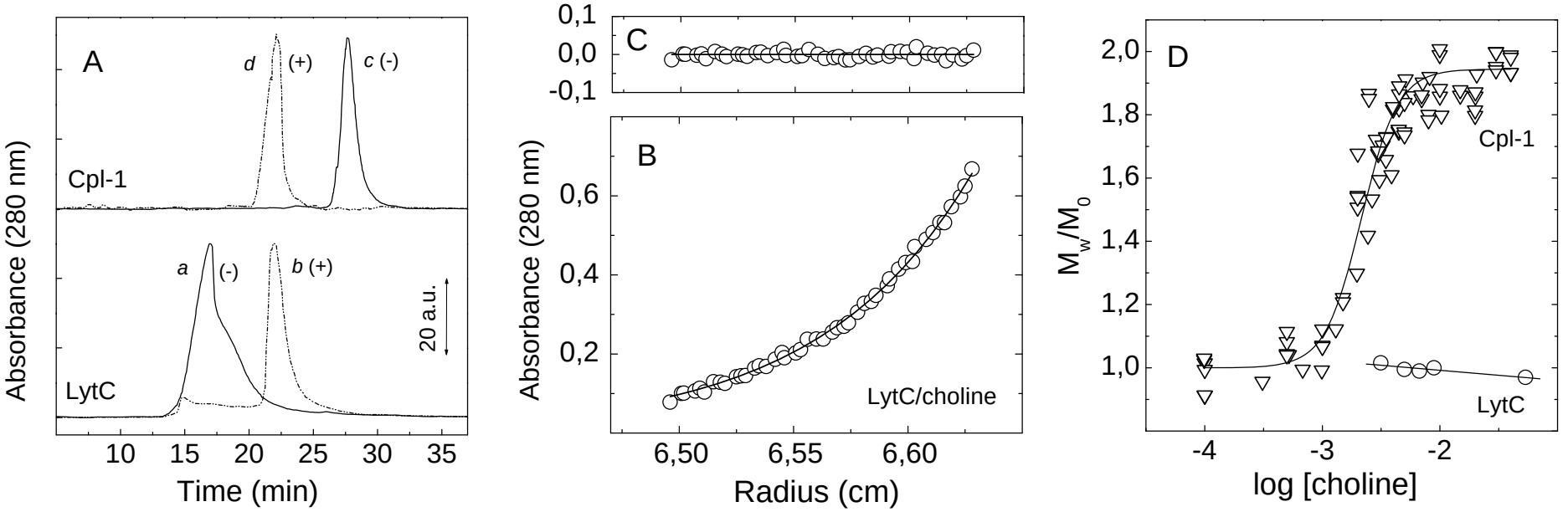
[a]Three-state model. [b]Two-state model. [c]The parameters for the three-state ($N \leftrightarrow D \rightarrow I$) model are: E_{app}, the apparent activation energy for the rate limiting step; T^*, the temperature where the rate constant k_{app}=1; ΔH^{vH}_D and T_D, the van t'Hoff enthalpy change and the transition temperature for the equilibrium step ($N \leftrightarrow D$) and ΔH_t, the total enthalpy change from N to I. The fitting parameters for the two-state $N \rightarrow I$ model are E_{app}, ΔH_t and T_m, the temperature of the maximum in the endotherm.

Supplemental TABLE 2
Denaturation parameters of Cpl-1 lysozyme in the absence and in the presence of 140 mM choline

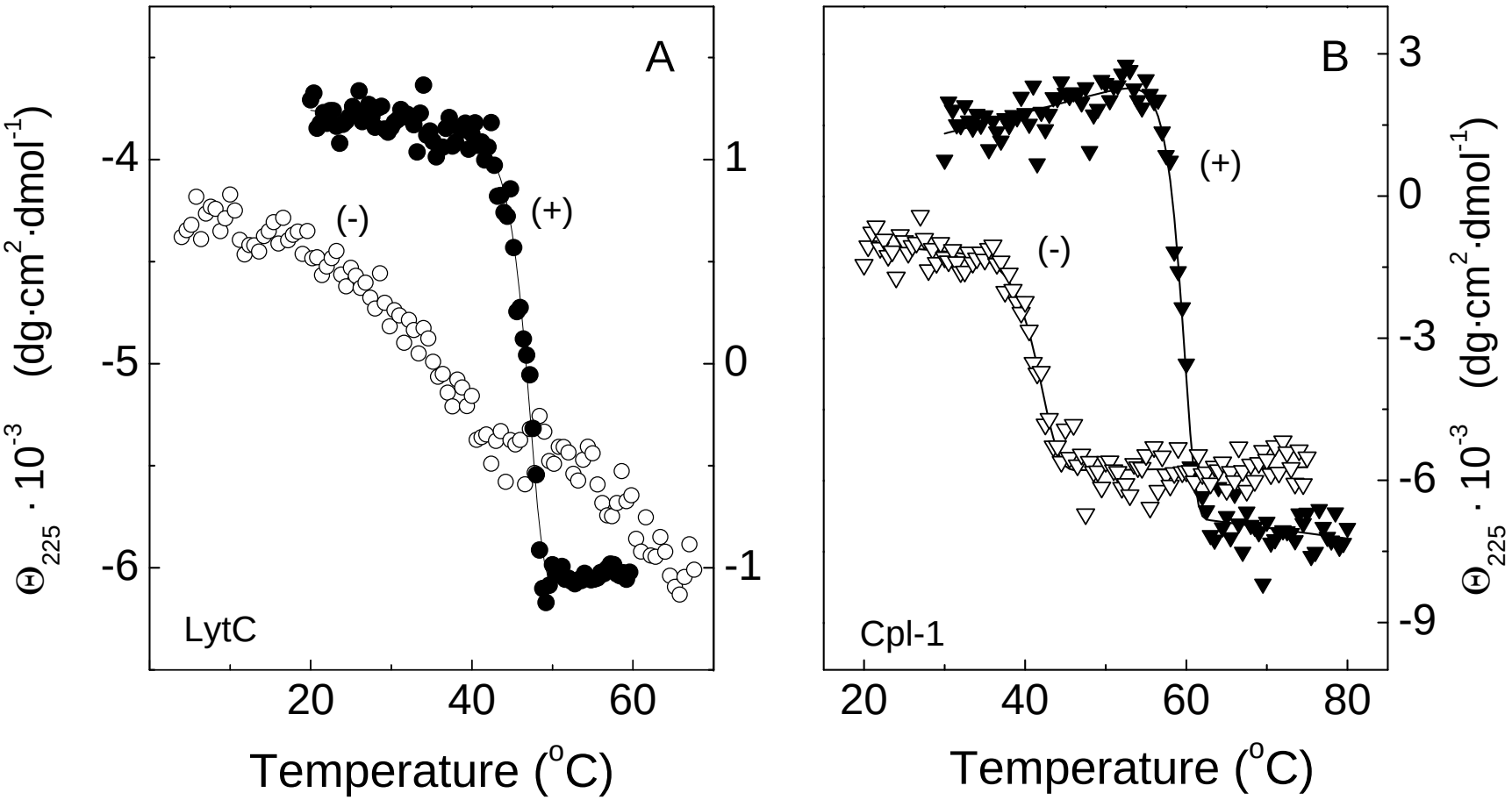
The experimental curves were analyzed using the three-state $N \leftrightarrow D \rightarrow I$ model for each transition and were monitored at 20.4 ºC·h^{-1}.

Parameters[a]	*P_i buffer*		*140 mM choline*	
	CBM	CM	CBM	CM
E_{app} (*kcal·mol^{-1}*)	137 ± 6	79 ± 9	120 ± 10	132 ± 8
T^* (*ºC*)	40.7 ± 0.4	50 ± 1	59.7 ± 0.3	51.1 ± 0.3
ΔH^{vH}_D (*kcal·mol^{-1}*)	230 ± 10	78 ± 3	230 ± 8	112 ± 2
T_D (*ºC*)	42.1 ± 0.2	47.5 ± 0.1	61.6 ± 0.2	54.0 ± 0.1
ΔH_t (*kcal·mol^{-1}*)	123 ± 5	39 ± 3	119 ± 3	96 ± 3

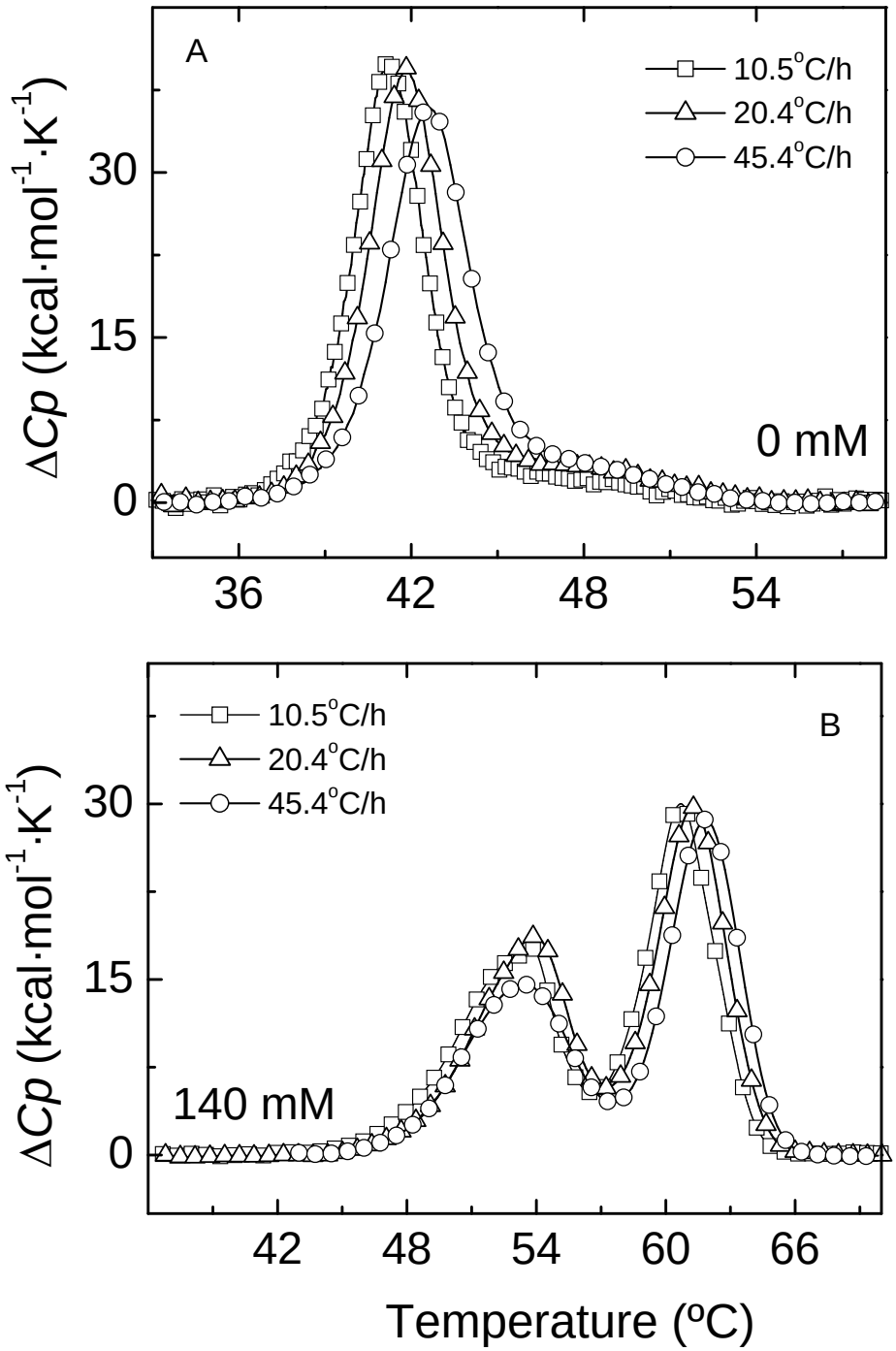
[a]Average values of 4-7 independent measurements at different protein concentrations and temperature scan-rates. The parameters have the same meaning as in Supplemental Table 1.



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3