Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting information

Thermodynamic study of the interaction between hen egg white lysozyme and Ce(IV)-Keggin polyoxotungstate as artificial protease

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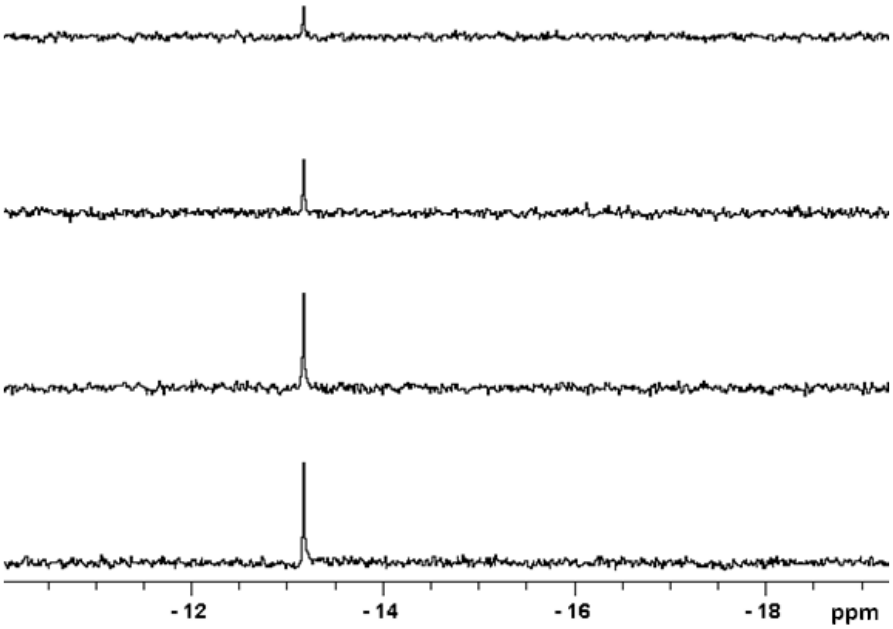

Figure S1. ^{31}P-NMR spectra of of **1** during the hydrolysis of HEWL at pH 4.5. From bottom to top: (1) in the absence of HEWL after mixing; (2) in the absence of HEWL after incubation; (3) in the presence of HEWL after mixing; (4) in the presence of HEWL after incubation.

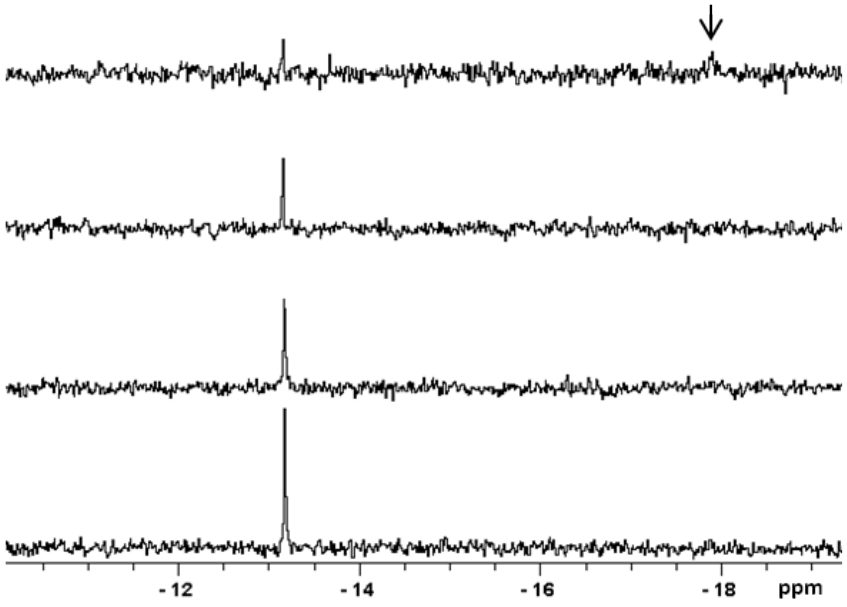

Figure S2. ^{31}P-NMR spectra of of **1** during the hydrolysis of HEWL at pH 7.4. From bottom to top: (1) in the absence of HEWL after mixing; (2) in the absence of HEWL after incubation; (3) in the presence of HEWL after mixing; (4) in the presence of HEWL after incubation. The arrow indicates the appearance of Ce(III)-Keggin.

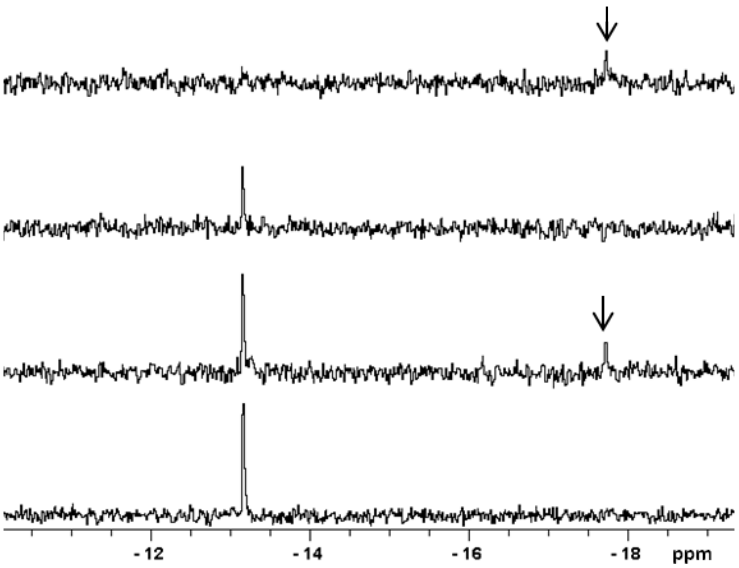

Figure S3. ^{31}P-NMR spectra of of **1** during the hydrolysis of HEWL at pH 9.0. From bottom to top: (1) in the absence of HEWL after mixing; (2) in the absence of HEWL after incubation; (3) in the presence of HEWL after mixing; (4) in the presence of HEWL after incubation. The arrows indicate the appearance of Ce(III)-Keggin.

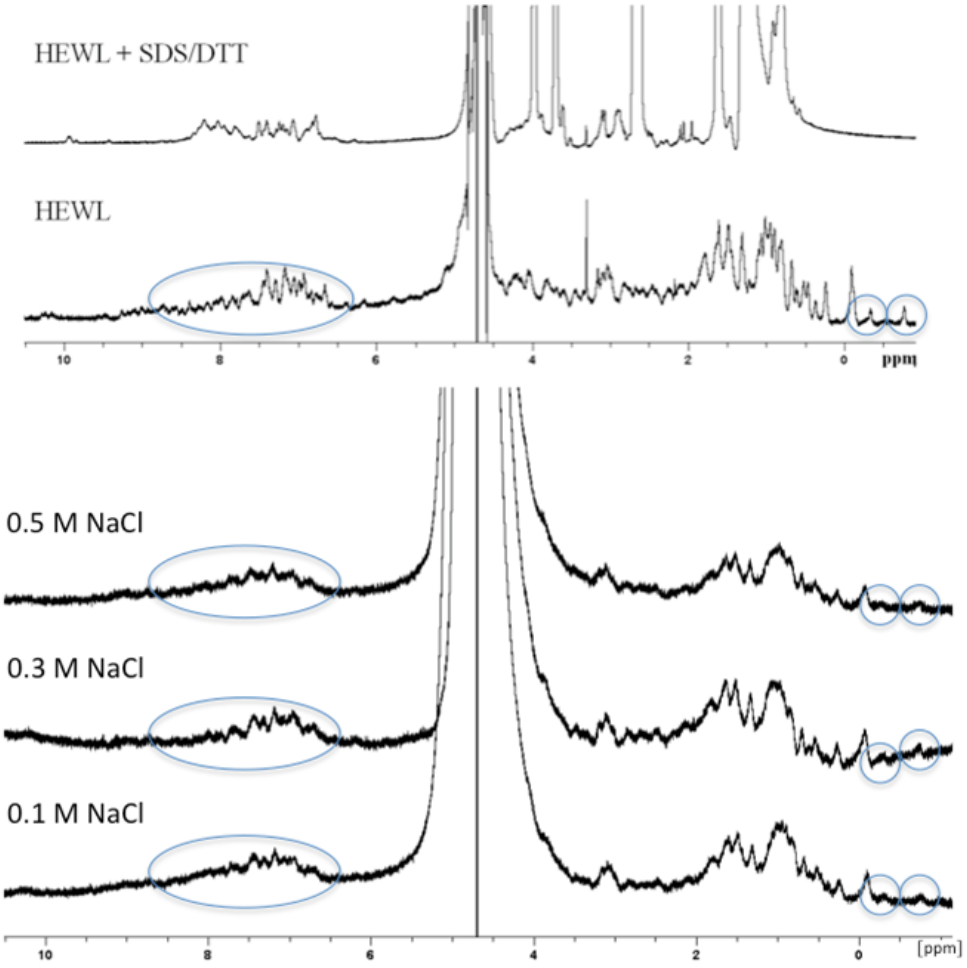

Figure S4. ^{1}H NMR spectra of HEWL in phosphate buffer (pH 7.4, 10mM) in the absence and presence of SDS/DTT (top) and in phosphate buffer (pH 7.4, 100mM) with added NaCl (0.1 to 0.5 M - bottom). Features of a folded protein conformation (spread between 6 and 10 ppm, peaks < 0 ppm – circled in blue) are lost in the presence of SDS/DTT. In the presence of different NaCl concentrations, these features are observed, indicating that HEWL remains in a folded conformation.

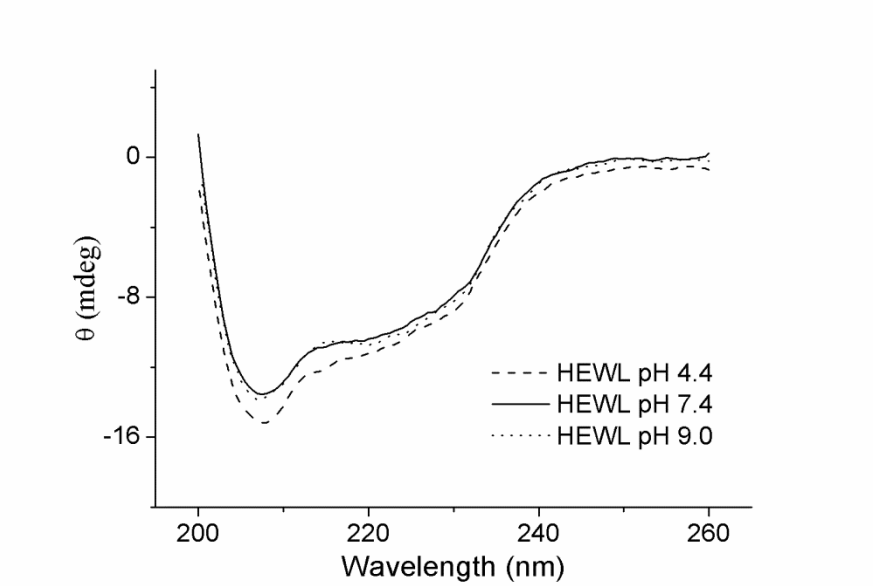

Figure S5. CD spectra of HEWL in acetate (pH 4.4), phosphate (pH 7.4) and Tris-Cl (pH 9.0) buffer, as indicated. Only small changes in the secondary structure content are observed.

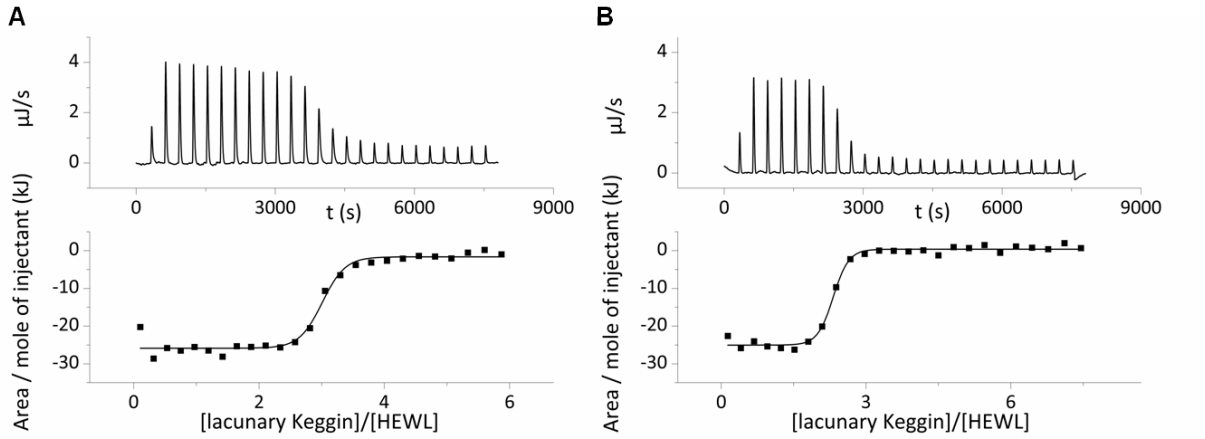

Figure S6. Thermogram (represented with exotherms up) and isotherm corresponding to lacunary Keggin binding to HEWL. Aliquots of a solution of **1** (A:420 / B:328 µM) were added to A:20 / B:12 µM of HEWL at 25 (**A**) or 37 (**B**) °C in 10 mM phosphate buffer (pH 7.4). Each peak in the top panel represents a single injection of lacunary Keggin in the protein solution. In the bottom panel, a plot of the amount of heat liberated per mole of injectant as a function of the molar ratio of HEWL to lacunary Keggin is represented. A nonlinear least-square fit to an independent binding site model is shown by the solid line.

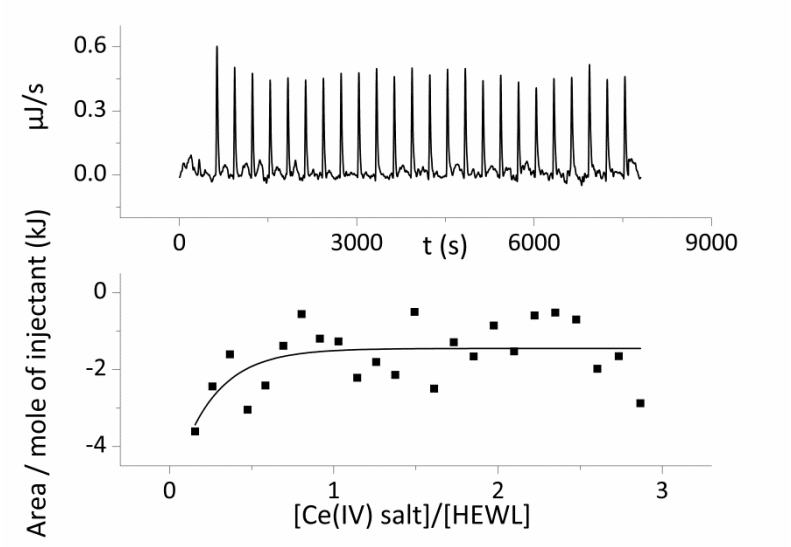

Figure S7. Thermogram (represented with exotherms up) and isotherm corresponding to Ce salt binding to HEWL. Aliquots of a solution of $(NH_4)_4Ce(SO_4)_4 \cdot 4H_2O$ (400 µM) were added to 39 µM of HEWL at 25 °C in 10 mM phosphate buffer (pH 7.4). Each peak in the top panel represents a single injection of $(NH_4)_4Ce(SO_4)_4 \cdot 4H_2O$ in the protein solution. In the bottom panel, a plot of the amount of heat liberated per mole of injectant as a function of the molar ratio of HEWL to $(NH_4)_4Ce(SO_4)_4 \cdot 4H_2O$ is represented. A nonlinear least-square fit to an independent binding site model is shown by the solid line.

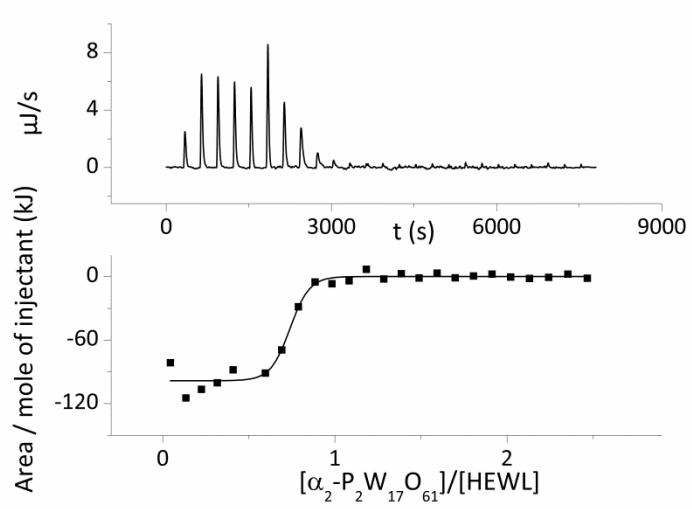

Figure S8. Thermogram (represented with exotherms up) and isotherm corresponding to α_2-Wells Dawson binding to HEWL. Aliquots of a solution of α_2-$P_2W_{17}O_{61}$ (203 µM) were added to 23 µM of HEWL at 25 °C in 10 mM phosphate buffer (pH 7.4). Each peak in the top panel represents a single injection of α_2-$P_2W_{17}O_{61}$ in the protein solution. In the bottom panel, a plot of the amount of heat liberated per mole of injectant as a function of the molar ratio of HEWL to α_2-$P_2W_{17}O_{61}$ is represented. A nonlinear least-square fit to an independent binding site model is shown by the solid line.

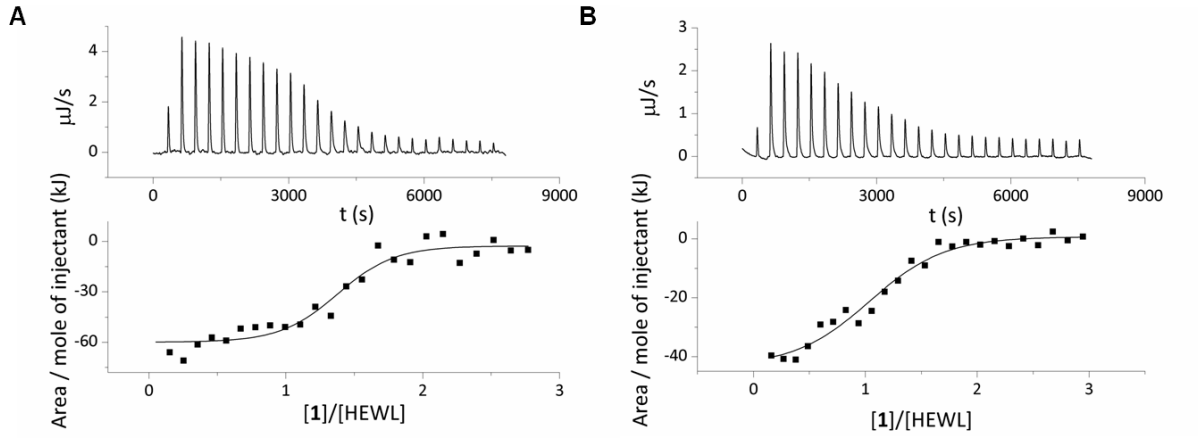

Figure S9. Thermogram (represented with exotherms up) and isotherm corresponding to **1** binding to HEWL. Aliquots of a solution of **1** (A:211 / B:209) were added to A:21 / B:20 µM of HEWL at 37 °C in phosphate buffer (pH 7.4 – 100 mM), with addition of 0.1 M NaCl (**A**) and 0.5 M NaCl (**B**). Each peak in the top panel represents a single injection of **1** in the protein solution. In the bottom panel, a plot of the amount of heat liberated per mole of injectant as a function of the molar ratio of HEWL to **1** is represented. A nonlinear least-square fit to an independent binding site model is shown by the solid line.

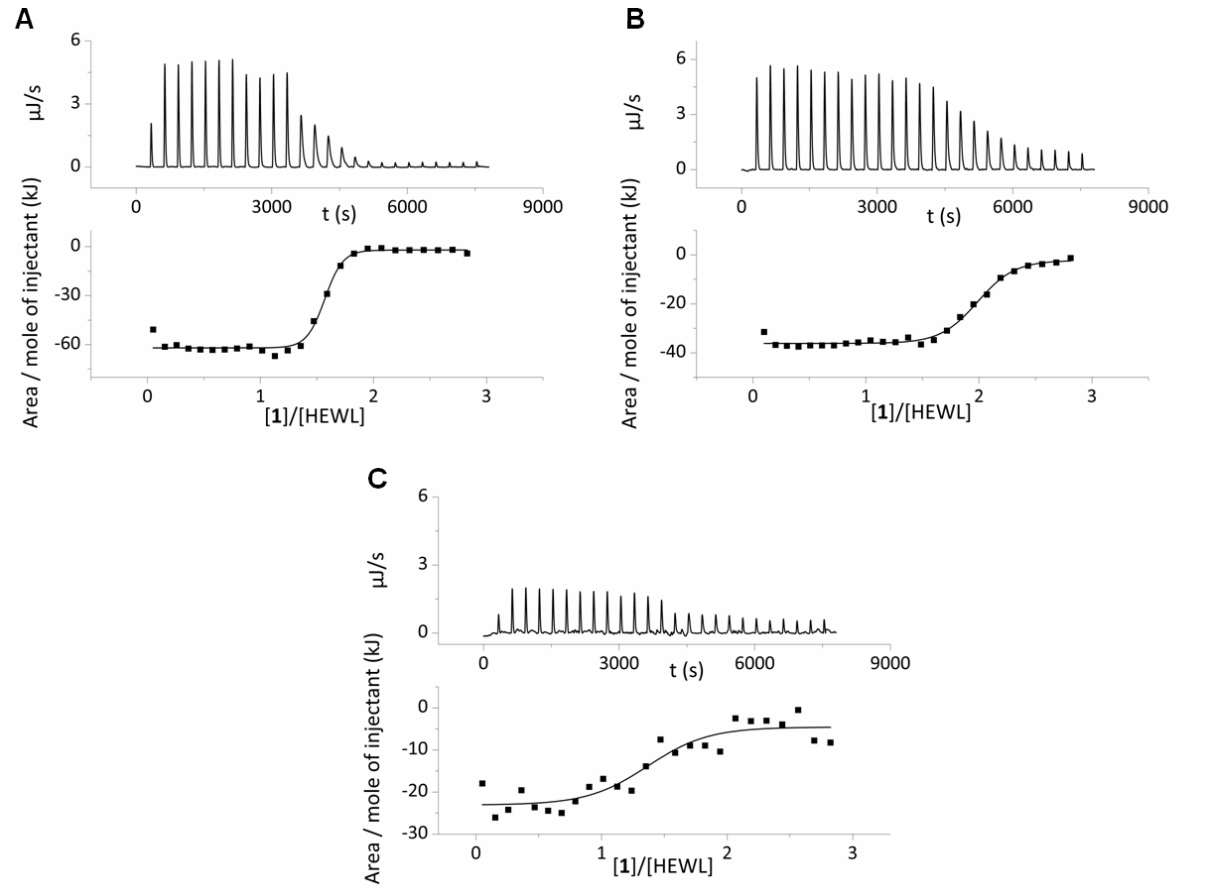

Figure S10. Thermogram (represented with exotherms up) and isotherm corresponding to **1** binding to HEWL. Aliquots of a solution of **1** (A:224 / B:419 / C: 238 µM) were added to A:22 / B:43 / C:24 µM of HEWL at 37 °C in acetate (**A** – pH 4.4 – 10 mM), phosphate (**B** – pH 7.4 – 10 mM) and Tris-Cl (**C** – pH 9.2 – 100 mM) buffer. Each peak in the top panel represents a single injection of **1** in the protein solution. In the bottom panel, a plot of the amount of heat liberated per mole of injectant as a function of the molar ratio of HEWL to **1** is represented. A nonlinear least-square fit to an independent binding site model is shown by the solid line.

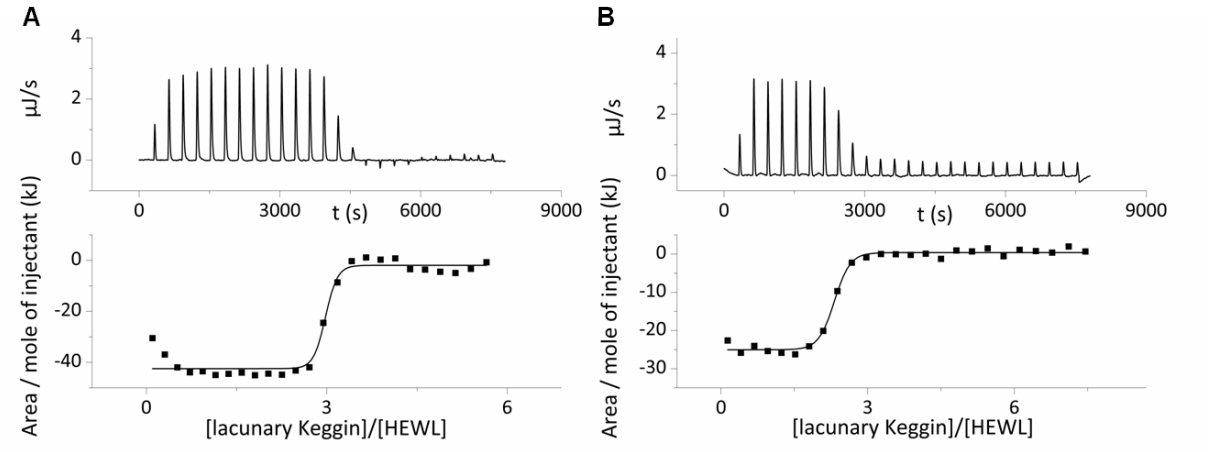

Figure S11. Thermogram (represented with exotherms up) and isotherm corresponding to lacunary Keggin binding to HEWL. Aliquots of a solution of lacunary Keggin (A:224 / B:328 µM) were added to A:11 / B:12 of HEWL at 37 °C in acetate (**A** – pH 4.4 – 10 mM) and phosphate (**B** – pH 7.4 – 10 mM) buffer. Each peak in the top panel represents a single injection of lacunary Keggin in the protein solution. In the bottom panel, a plot of the amount of heat liberated per mole of injectant as a function of the molar ratio of HEWL to lacunary Keggin is represented. A nonlinear least-square fit to an independent binding site model is shown by the solid line.

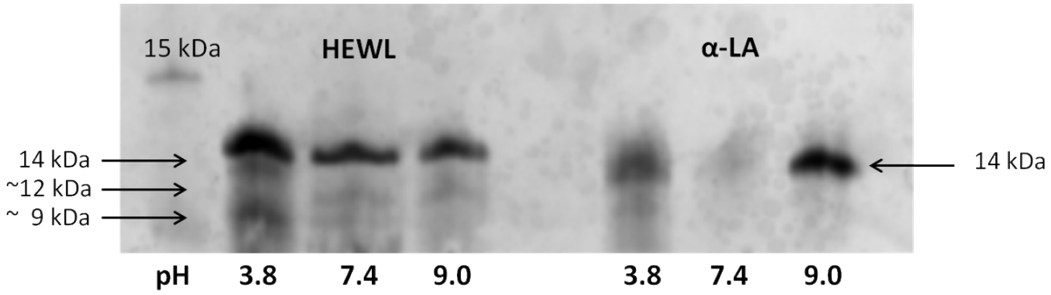

Figure S12. SDS-PAGE gel of HEWL and α-LA hydrolysis in presence of **1** at pH 3.8, 7.4 and 9.0 after incubation at 37 °C during 7 days.