**Electronic Supplementary Information**

# *Interplay of Ion Specificity, pH and Buffers: Insights from Electrophoretic Mobility and pH Measurements of Lysozyme Solutions*

Francesca Cugia,[‡] Maura Monduzzi,[‡] Barry W. Ninham,[†] and Andrea Salis[‡,*]

[‡]*Department of Chemical and Geological Sciences, University of Cagliari-CSGI and CNBS, Cittadella Universitaria, S.S. 554 bivio Sestu, 09042- Monserrato (CA), Italy.*

[†]*Research School of Physical Sciences and Engineering, Australian National University, Canberra, 0200 Australia.*

## Materials and Methods

**Chemicals.** Lysozyme from hen egg white (70,000 units/mg; 62971), sodium chloride (≥ 99%; S3014), sodium thiocyanate (≥ 98%; 251410) and sodium phosphate dibasic (≥ 99.5%; S0876), potassium thiocyanate (≥ 99%; P2713), sodium hydroxide (≥ 97%; 221465) and cacodylic acid (≥ 98%; C0125) were purchased from Sigma-Aldrich. Sodium hydrogen carbonate (≥99% ;35061), sodium phosphate monobasic (≥ 98%; 401101) and potassium hydroxide (≥ 86%; 314019) were purchased from J.T. Baker. Sodium nitrate (≥ 99%; 205960010) was purchased from Acros Organics. Potassium nitrate (≥ 99.5%; 203180010), potassium thiocyanate (≥ 99%; 197120010) and Tris (hydroxymethyl)aminomethane (≥ 99.8%; 1610719) were from Biorad. Citric acid (≥ 99.5%; 27490) was from Fluka. Potassium chloride (≥ 99%; 12636) was purchased from Riedel-de Haen.

**Instrumental details.** Electrophoretic mobility ($\mu_E$) measurements of lysozyme-buffer and lysozyme-buffer-salt solutions were carried out through electrophoretic light scattering (laser Doppler velocimetry) technique by means of a Zetasizer nano series (Malvern Instruments).

pH measurements, of the same samples used for the determination of $\mu_E$, were performed by using a XS-PC 650 (Eutech instruments) pH-meter. The electrode was calibrated by a 3 point calibration through standard buffers at pH 4; 7; and 9.

**Specific ion effects on $\mu_E$ and pH.** In order to study the effect of salt addition to the electrophoretic mobility and the pH of lysozyme-buffer solutions the following procedure was carried out. Commercially available Lysozyme powder was used without any additional purification step. Lysozyme solutions (1 mg/mL) in 10 mM buffers - Tris (hydroxymethyl)aminomethane, cacodylate, hydrogen carbonate, phosphate and citrate – were all set to the same pH=7.15 by using a small volume of a concentrated base/acid solution. Then different amounts of sodium salts (NaCl, $NaNO_3$, NaSCN) or potassium salts (KCl, $KNO_3$, and KSCN) were added to 100 mL of a lysozyme-buffer solution obtaining a salt concentration range: 1 mM - 200 mM. After each salt addition the pH was measured. Then a small volume of the resulting solution was put in a scattering cell for the measurement of electrophoretic mobility. Each value of mobility is the average of at least 6 measurements, obtained by mediating 20 simple readings, for each salt concentration. Standard deviations were calculated and displayed as error bars.