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**Supporting Information for**  
**“Cation effects on haemoglobin aggregation: balance of**  
**chemisorption against physisorption of ions”**  
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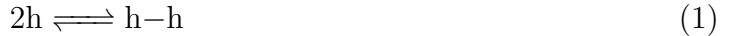
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## 15 I. SUPPORTING INFORMATION

### 16 A. Aggregation Equilibria and the Second Virial Coefficient

17 Turbidity experiments monitor the aggregation of protein clusters (aggregation of haemoglobin  
18 tetramers [1]). Transmittance  $\mathcal{T} = T/T_0$  falls when aggregation is promoted. That is tur-  
19 bidance (absorbance)  $S = -\ln \mathcal{T}$  rises, proportional to the concentration  $c_{\text{agg}}$  of aggregates.

20 If we assume aggregation is dominated by association of 2 haemoglobin tetramers then  
21 the aggregation process is



22 (h represents one haemoglobin tetramer) with aggregation equilibrium constant

$$K_{\text{agg}} = \frac{\gamma_{\text{agg}} c_{\text{agg}}}{\gamma_1^2 c_1^2} \quad (2)$$

23 Here  $c_1$  is the concentration of single haemoglobin tetramer and  $c_{\text{agg}}$  is the concentration of  
24 aggregates.  $\gamma_1$  and  $\gamma_{\text{agg}}$  are the activity coefficients of the single tetramer and the aggregates,  
25 representing additional nonideal interactions. For simplicity here we take  $\gamma_1$  and  $\gamma_{\text{agg}} = 1$ ,  
26 with protein-protein interactions represented via the aggregation constant  $K_{\text{agg}}$ .

27 The second virial coefficient  $b$  characterises 2-body interactions (Eq.19 in the main  
28 manuscript),

$$b = 2\pi \int_0^\infty (L + 2R)^2 dL (1 - \exp[-G(L)/kT]) \quad (3)$$

29  $G(L)$  is the interaction free energy between two tetramers.  $b$  is evaluated here with respect to  
30 the separation distance  $L$  between protein surfaces, rather than the distance between protein  
31 centres  $r = L + 2R$ , assuming hard sphere contact between tetramers. For a 2-member  
32 cluster, the aggregation constant is determined directly from second virial coefficient [2, 3],

$$K_{\text{agg}} = -b \quad (4)$$

33 Additional corrections can be added to account for excluded volume [2, 3] or metastable  
34 aggregation [4].

35 The total protein (tetramer) concentration is

$$c_p = c_1 + 2c_{\text{agg}} \quad (5)$$

36 Combining with the equilibrium condition Eq. (2) (and taking ideal activity coefficients),  
 37 we obtain an expression for the concentration of single tetramers in terms of  $c_p$  and  $b$ ,

$$c_1 = \frac{1 - \sqrt{1 - 8bc_p}}{4b} \quad (6)$$

38 We simplify this expression with the expansion  $\sqrt{1+x} \approx 1 + x/2 - x^2/8$ ,

$$c_1 \approx c_p (1 + 2bc_p) \quad (7)$$

39 Aggregation occurs when the interaction  $G(L)$  is attractive, in which case  $b < 0$ . That is,  
 40 we obtain a finite  $c_1 < c_p$ . It follows from Eq. (5) that the concentration of aggregates is

$$c_{\text{agg}} \approx -bc_p^2 \quad (8)$$

41 That is, the concentration of aggregates is directly proportional to the second virial co-  
 42 efficient. Of course, a more complex dependence on the second virial coefficient could  
 43 be obtained by retaining the nonideal activity coefficient  $\gamma_1$ , which also depends on  $b$  via  
 44  $\gamma_1 = \exp(2bc_p)$  [5].

## 45 B. Turbidity and the Second Virial Coefficient

46 The turbidance (absorbance)  $S = -\ln \mathcal{T}$  is proportional to the concentration of absorb-  
 47 ing species, assuming standard Beers-Lambert absorbance. We suppose that only single  
 48 haemoglobin tetramers and aggregates of tetramers absorb significantly, with extinction  
 49 coefficients  $k_1$  and  $k_{\text{agg}}$  respectively. If  $l$  is the length of the turbimetry cell, then

$$S = -\ln \mathcal{T} = l [k_1 c_1 + k_{\text{agg}} c_{\text{agg}}] \quad (9)$$

50 Taking concentrations from Eq. (7) and Eq. (8), we obtain

$$S = -\ln \mathcal{T} = lc_p [k_1 - bc_p (k_{\text{agg}} - 2k_1)] \quad (10)$$

51 We expect absorbance to be stronger for the larger aggregates,  $k_{\text{agg}} > k_1$  (in fact if  $\mathcal{T}$  falls  
 52 between 0 and 100%, then  $k_{\text{agg}} > 2k_1$ ). We simplify analysis by assuming  $k_{\text{agg}}$  is much  
 53 greater than  $k_1$  such that  $k_1$  can be dropped, leaving

$$S = -\ln \mathcal{T} \approx -lbc_p^2 \quad (11)$$

54 That is, turbidance ( $-\ln \mathcal{T}$ ) is proportional to the second virial coefficient.

55 By taking  $b_{\text{Cs}}$  as a reference (assuming no binding of  $\text{Cs}^+$  to carboxylate sites), we can  
56 determine the best-fitting binding constants for  $\text{Li}^+$  and  $\text{K}^+$  from the ratio

$$\frac{\ln \mathcal{T}_M}{\ln \mathcal{T}_{\text{Cs}}} = \frac{b_M}{b_{\text{Cs}}} \quad (12)$$

57 which is Eq. 20 in the main manuscript.

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