

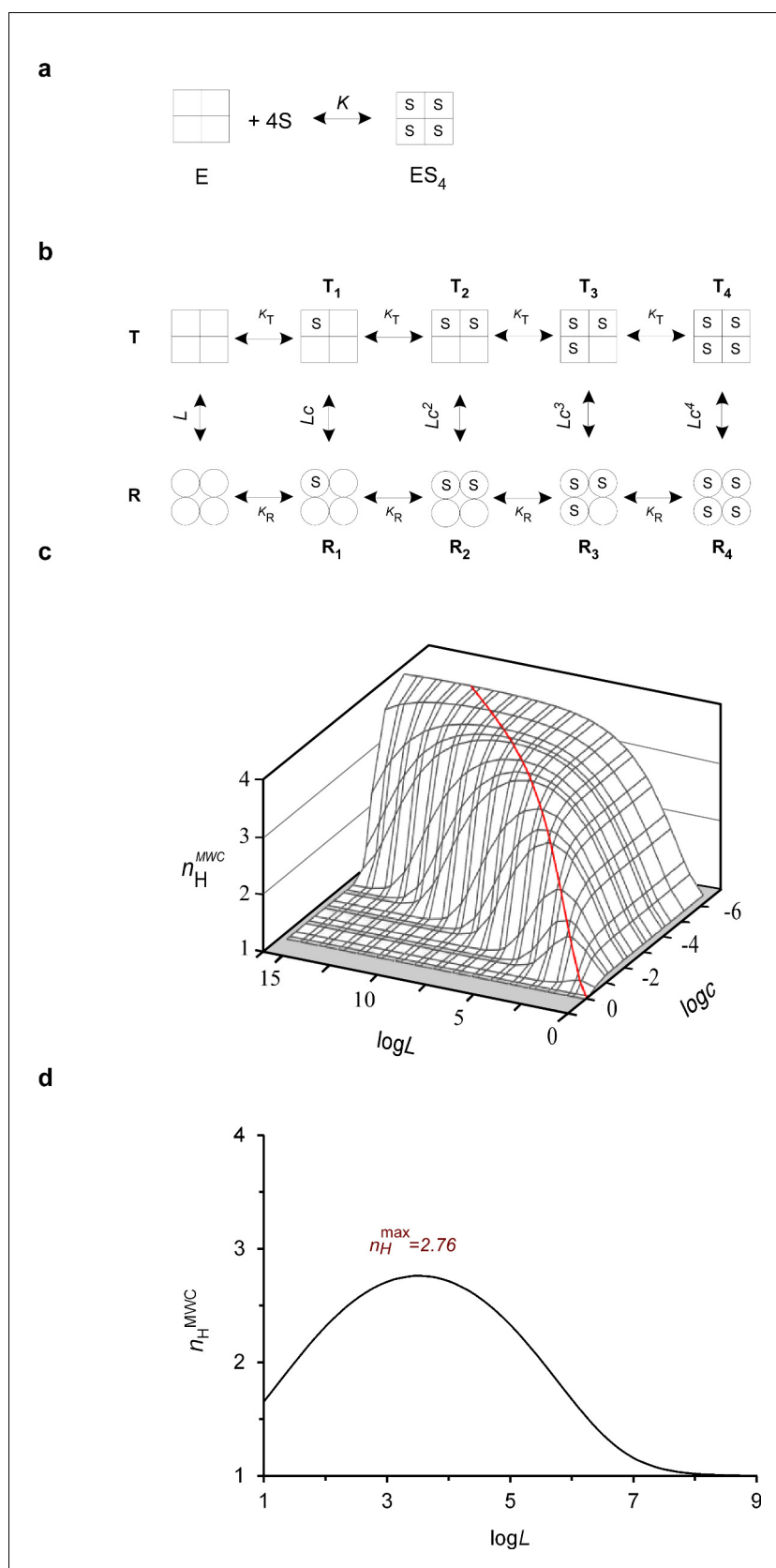


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## Figures and figure supplements

Evolutionary and functional insights into the mechanism underlying body-size-related adaptation of mammalian hemoglobin

**Olga Rapp and Ofer Yifrach**



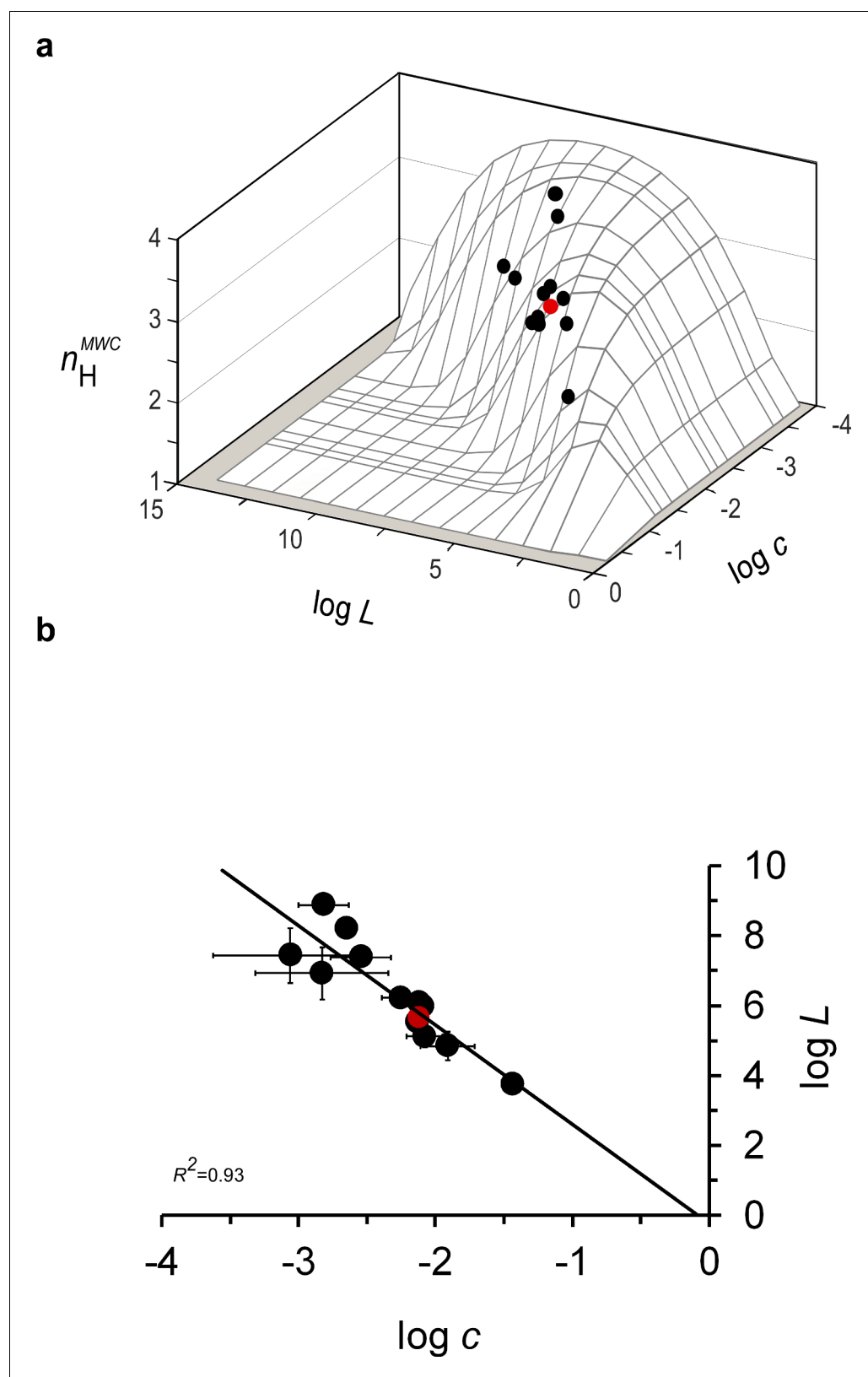
**Figure 1.** The biophysical Hill landscape of a tetrameric MWC protein. (a) Schematic representation of the all-or-none Hill binding mode by a tetrameric protein (E), with  $K$  referring to the substrate (S) dissociation equilibrium

Figure 1 continued on next page

*Figure 1 continued*

constant. **(b)** Schematic representation of the MWC model applied to a tetrameric allosteric protein. Square and round symbols represent the tense (**T**) and relaxed (**R**) subunit conformations, respectively.  $L$ ,  $K_T$  and  $K_R$  denote the T to R transition equilibrium constant in the absence of substrate and substrate affinity to the T and R conformations, respectively. The parameter  $c$  corresponds to the ratio of substrate affinity to the R and T conformations ( $=K_R/K_T$ ). **(c)** The theoretical three-dimensional Hill landscape describing the dependence of  $n_H^{MWC}$  at half-saturation on both the  $L$  and  $c$  parameters (calculated based on **Equation 5**). The red ridge line trajectory corresponds to parameter value pairs giving rise to maximum cooperativity. **(d)** Dependence of the Hill coefficient at half-saturation ( $n_H^{MWC}$ ) on the allosteric constant  $L$ , as determined according to **Equation 5**. The curve was plotted assuming a  $c$  value of  $\sim 0.01$ , typical for the ratio of oxygen affinity to the R and T conformations of human Hb. The saddle point of the bell-shaped curve corresponds to a Hill value of  $\sim 2.8$  (as observed for human Hb), fulfilling the  $L = c^{-2}$  criterion giving rise to maximum cooperativity.

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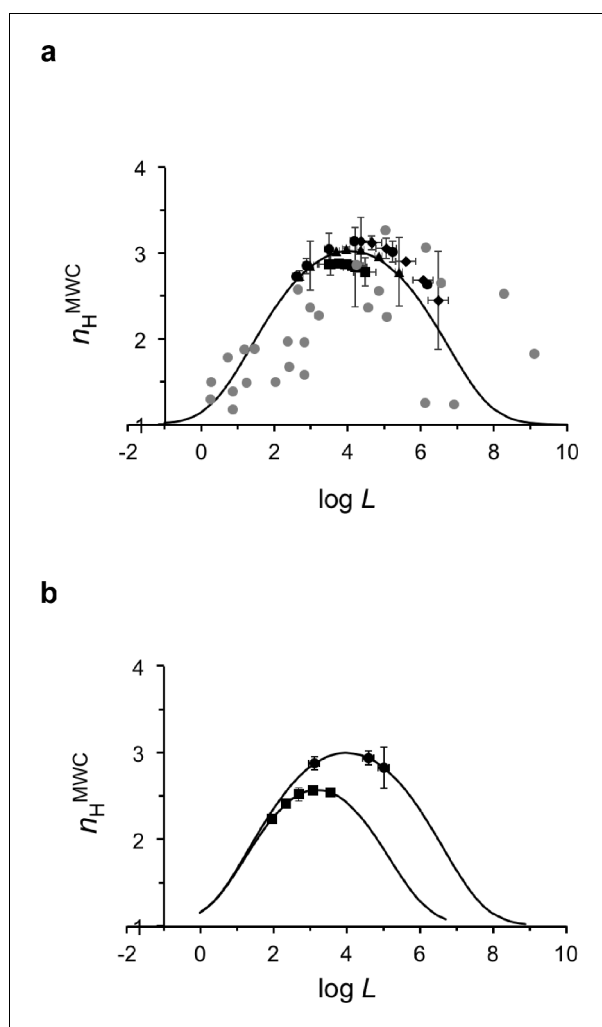


**Figure 2.** Mammalian hemoglobins exhibit maximum cooperativity  $n_H$  values. (a)  $n_H^{MWC}$  values of the different mammal Hbs from the evolutionary dataset, calculated based on derived values for the  $L$ ,  $K_T$  and  $K_R$  elementary MWC parameters, reside close to the ridge line of the three-dimensional biophysical Hill landscape. (b) Figure 2 continued on next page

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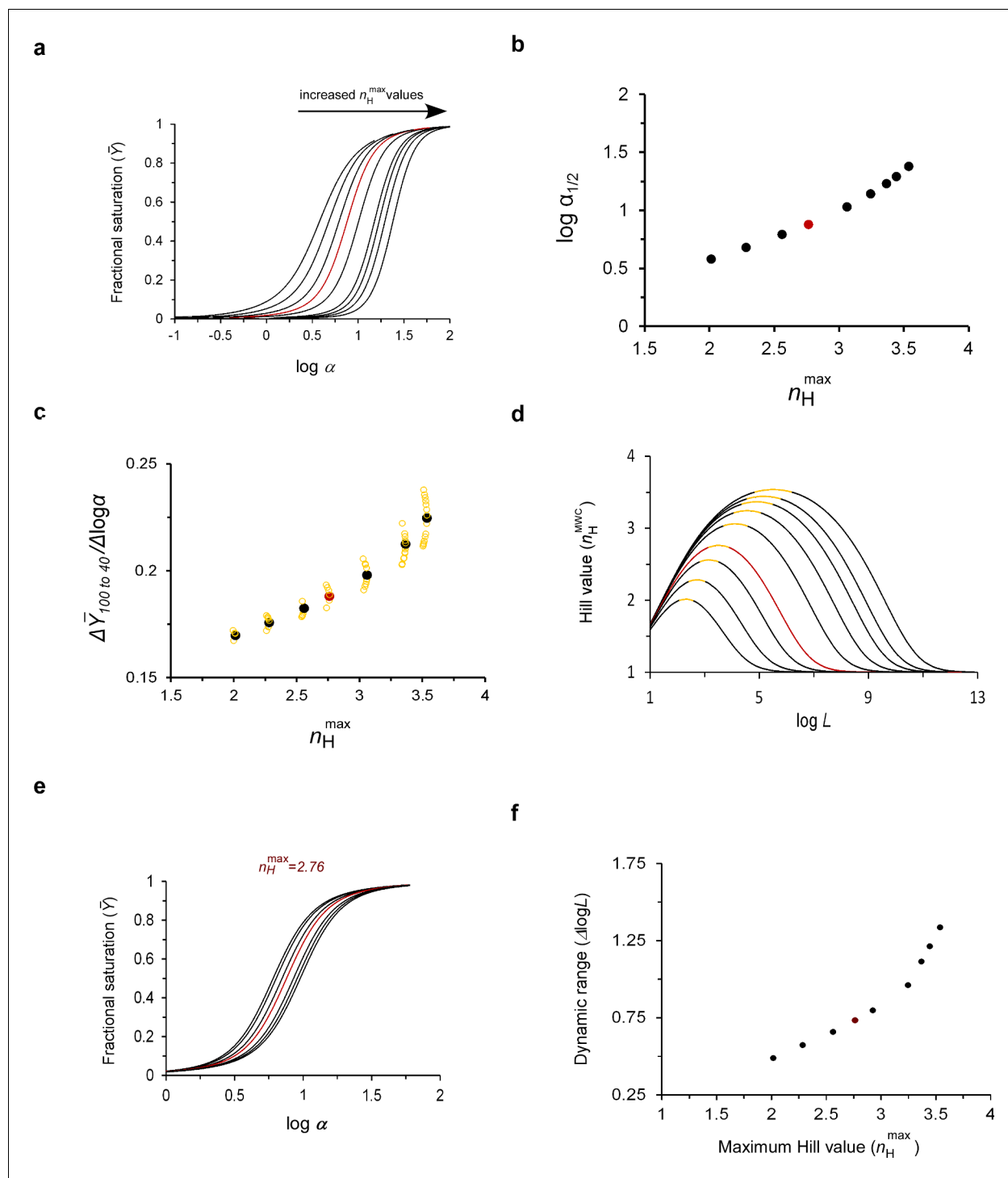
Correlation plot relating the logarithms of the relative affinity ( $c$ ) and conformational ratio ( $L$ ) of the T and R quaternary MWC states of different mammalian Hbs comprising the evolutionary dataset (**Supplementary file 1**). The solid curve corresponds to a linear regression with a  $R^2$  correlation coefficient of 0.93, a slope of -2.7 ( $\pm 0.3$ ) and an intercept at  $\log L$  of -0.2, very close to the 0 value expected based on the  $Lc^2=1$  criterion. In both panels, the red data point corresponds to the values for human Hb.

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**Figure 3.** A buffering of cooperativity physiological regime is observed for human hemoglobin around its maximum cooperativity value. (a) Mapping of the Hill coefficient and the logarithm of the apparent allosteric constant ( $L$ ) values for all human oxygen saturation curves of the pH, CO<sub>2</sub> and BPG physiological datasets (**Supplementary file 2**) onto the theoretical  $n_H$ - $\log L$  curve of human Hb. The  $n_H$  values for all physiological dataset curves were calculated using the derived apparent  $L$  value for each curve and assuming a common  $c$  value. The theoretical  $n_H$ - $\log L$  curve was calculated assuming a  $c$  value of 0.007 ( $\pm 0.001$ ), corresponding to the averaged  $c$  value of all human physiological datasets (**Supplementary file 2**). Black circles, squares and diamonds correspond to pH, CO<sub>2</sub> and BPG data points, respectively. Gray symbols represent pairs of  $\log L$ - $n_H$  values for Hb mutantS reported in the literature and assuming no change in  $c$  upon mutation (**Baldwin, 1976**). (b) Dependence of the Hill coefficient at half-saturation on the apparent allosteric constant for all oxygen saturation curves of the dog and bovine physiological datasets (squares and circles, respectively). For each species, the appropriate  $c$  value (**Supplementary file 1**) was used to calculate the theoretical curve, onto which the actual  $\log L$ - $n_H$  data points were mapped.

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**Figure 4.** The maximum cooperativity point determines MWC protein substrate unbinding sensitivity and the dynamic range of physiological adaptation. (a) Fractional saturation binding curves of a MWC protein as a function of the logarithm of the scaled concentration  $\alpha$ , with parameter values that give rise to systematically higher maximum cooperativity  $n_H$  values. The pairs of  $L$  and  $c$  values for all curves obey the maximum cooperativity criterion and are listed in **Table 1**. (b) Dependence of the logarithm of the scaled affinity ( $\log \alpha_{1/2}$ ) of the curves presented in (a) on the maximum cooperativity Hill value ( $n_H^{\max}$ ). (c) Dependence of the substrate unbinding sensitivity ( $\Delta \bar{Y}_{100 \rightarrow 40\%} / \Delta \log \alpha$ ) of a tetrameric MWC protein on  $n_H^{\max}$  (filled black symbols). The array of open yellow symbols decorating each maximum cooperativity point corresponds to changes in the  $\bar{Y}$  axis values due to changes in  $L$  that reside within the correspondingly encoded buffering of cooperativity regime (see text) (d) Dependence of  $n_H^{\text{MWC}}$  on the logarithm of the allosteric constant  $L$  around the different maximum cooperativity points indicated in (a) and **Table 1** (and assuming their different  $c$  values). For each curve, the portion highlighted in yellow corresponds to the  $\log L$  range fulfilling the 'buffering of cooperativity' regime, that is the  $L$

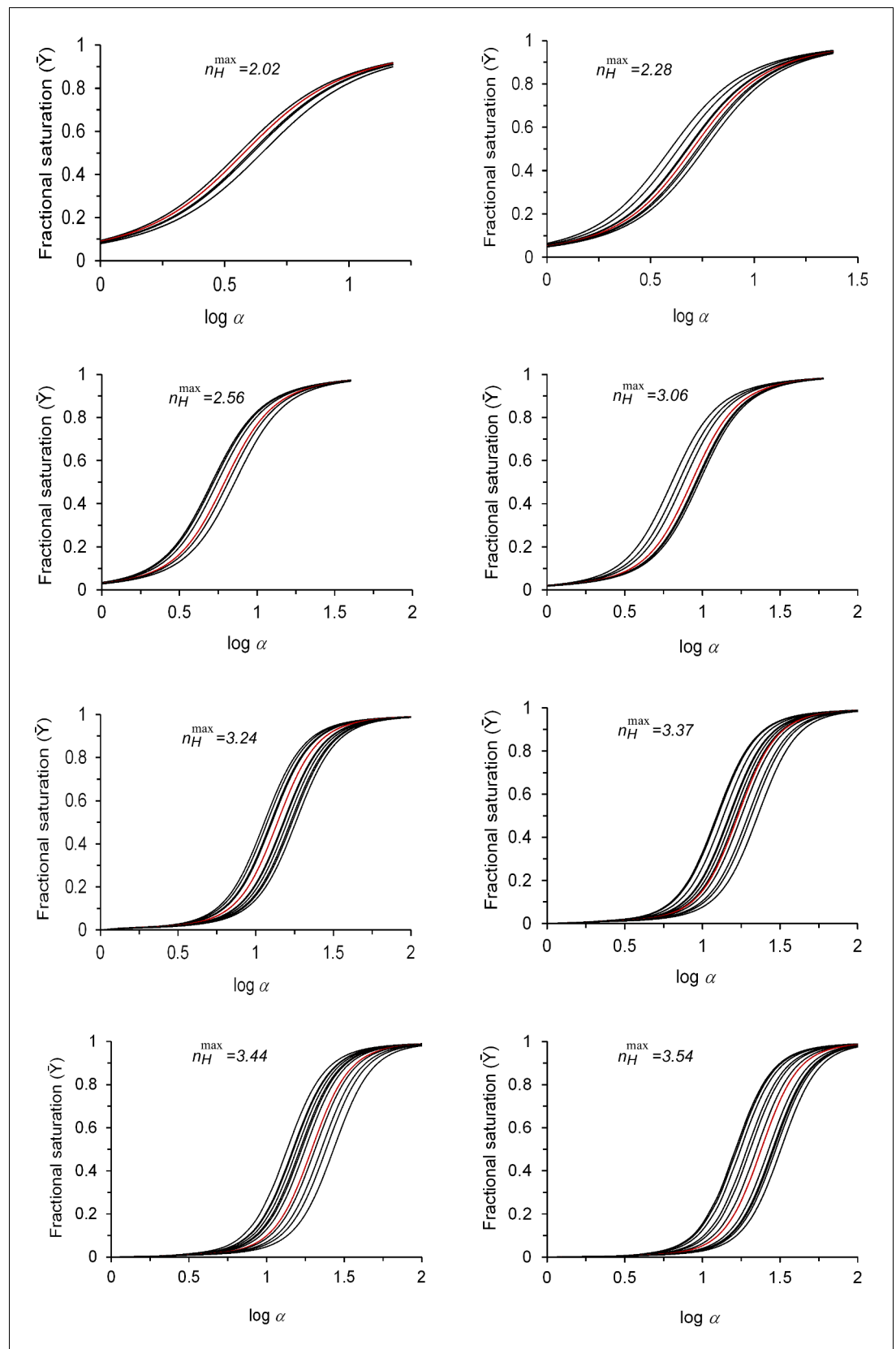
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range around the maximum Hill point that gives rise to fractional saturation curves all exhibiting less than 1% change in the maximum Hill coefficient value. Such a physiological dataset is observed for the maximum point of  $\sim 2.8$ , characteristic of human Hb in panel (e) (calculated using its appropriate  $c$  value; see **Supplementary file 1**). Similar data for different maximum cooperativity values are presented in **Figure 4—figure supplement 1**. In each physiological dataset, the curve indicated in red corresponds to the maximum cooperativity curve (f) Dependence of the broadness of the 'buffering of cooperativity' regime (the dynamic range), as defined above, on the maximum cooperativity Hill value. In all figure panels, the curves indicated in red were calculated using the experimental values for human Hb reported in the literature. The red data points in several of the panels represent approximated values of human Hb.

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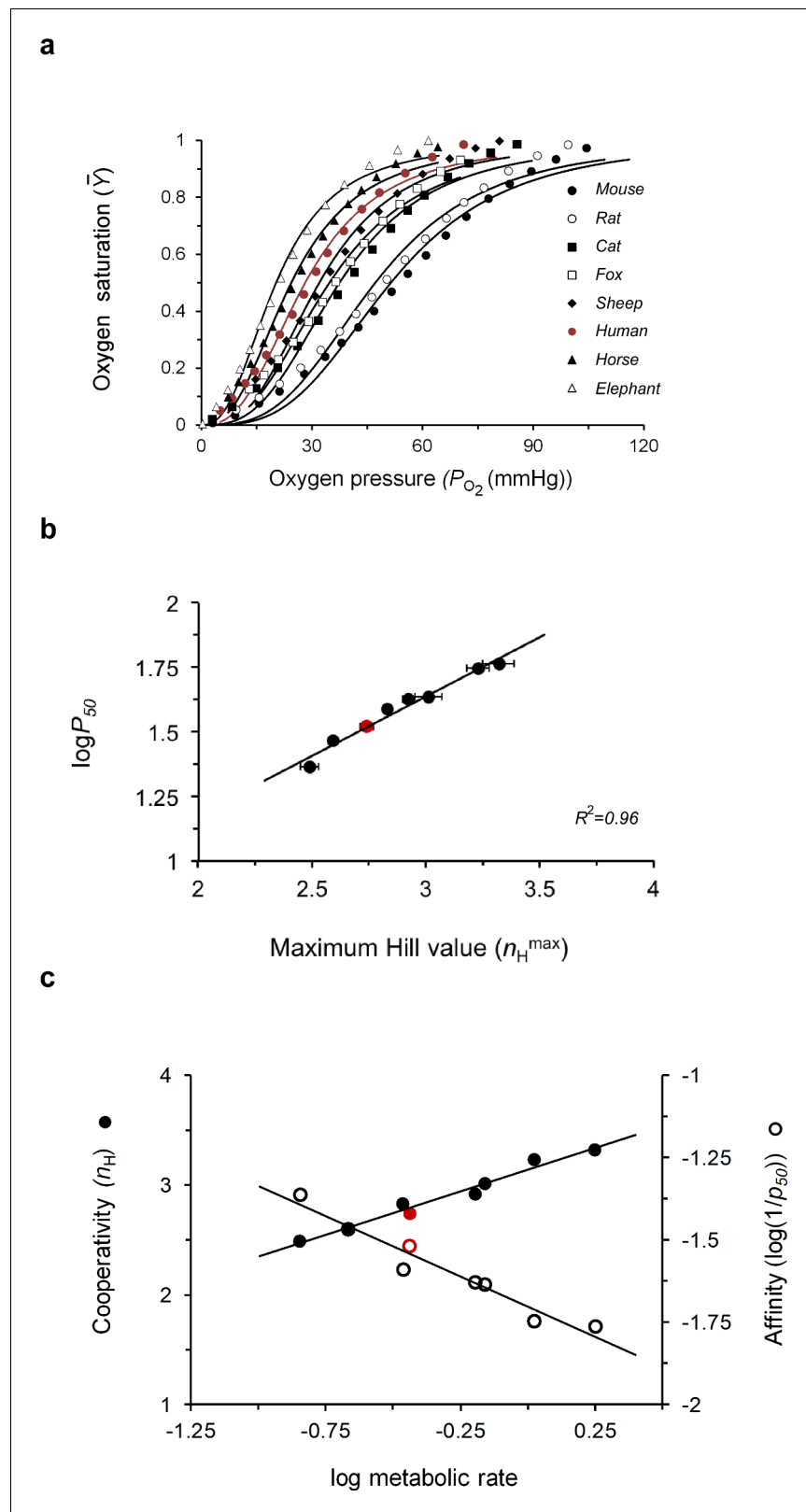


**Figure 4—figure supplement 1.** Simulated MWC-based physiological saturation data around different maximum cooperativity  $n_H^{\max}$  values. MWC-based simulated fractional saturation curves generated by changing the  $L$  value  
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around different maximum cooperativity points, as indicated above each panel. These values are identical to those used to generate the saturation curves presented in **Figure 4a**. For each dataset, the reference saturation curve of the maximum cooperativity point is indicated in red. For each maximum cooperativity point,  $L$  values were chosen such that the  $n_H$  value of any curve in the physiological dataset would not differ from the maximum  $n_H$  point by more than 1%, and would thus still be contained within the 'buffering of cooperativity' regime. The  $\log L$  range fulfilling this requirement is highlighted in yellow on the  $n_H$ - $\log L$  bell-shaped plots appropriate for each maximum cooperativity point, as described in the legend to **Figure 4d**.

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**Figure 5.** Evolutionary tuning of the maximum cooperativity and affinity points of mammalian hemoglobins underlies physiological adaptation. (a) Dependence of the fractional oxygen saturation of animal blood sample Hbs on partial oxygen pressure, as reported by *Schmidt-Nielsen and Larimer (1958)*, *Schmidt-Nielsen (1970)*. Figure 5 continued on next page

*Figure 5 continued*

Solid curves represent data fitting to the Hill equation (**Equation 1**).  $p_{50}$  and  $n_H$  values for oxygen binding to the different mammalian Hb datasets are reported in **Table 2**. (b) Correlation plot relating the maximum  $n_H$  and  $\log p_{50}$  values of the curves shown in a. (c) Dependence of the values for both cooperativity (filled symbols) and affinity (open symbols) properties of different animal Hbs on normalized metabolic rates in that animal (relative to body weight). A linear trend is observed in both cases, with  $R^2$  values of 0.97 and 0.9 for the cooperativity and affinity correlations, respectively. Values for animal metabolic rates were taken from **Schmidt-Neilsen and Larimer (1958)**. The red curve or data points presented in panels 5a-c correspond to the values for human Hb.

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