

## Supplementary text 2

### *Interpretation of the logarithmic form of the Richards curve as titration of chemical potential*

Chemical potential  $\mu$  by definition is

$$\mu = \mu^\circ + \ln(a) \quad (S1)$$

where  $\mu^\circ$  is standard chemical potential,  $a$  is relative thermodynamic activity:

$$a = c\gamma \quad (S2)$$

where  $c$  is molar concentration and  $\gamma$  is activity coefficient.

The chemical potential of a given antibody species is then described, after replacing  $a$  in (S1) with  $\gamma c$  from (S2)

$$\mu_{Ab} = \mu_{Ab}^\circ + \ln(c_{Ab}\gamma_{Ab}) \quad (S3)$$

$$\mu_{Ab} = \mu_{Ab}^\circ + \ln(c_{Ab}) + \ln(\gamma_{Ab}) \quad (S4)$$

Omitting the standard term and raising both sides to the exponent, we obtain the proportionality

$$e^{\mu_{Ab}} \sim e^{\ln(c_{Ab}) + \ln(\gamma_{Ab})} \quad (S5)$$

We assume that the fluorescence intensity signals are a measure of the absolute thermodynamic activity of antibodies,  $\lambda_{Ab}$

$$FI_{Ab} \sim \lambda_{Ab} = e^{\mu_{Ab}} \quad (S6)$$

Therefore, we suggest that in the logarithmic form of Richards function with  $k=1$

$$\ln(FI_{Ab}) = \ln(A) + \ln\left((1 + (d - 1)e^{-(x-x_i)})^{\frac{1}{1-d}}\right) \quad (S7)$$

$\ln(A)$  is the logarithm of fluorescent intensity corresponding to the detection antibody with molar concentration  $\ln(c_{Ab})$  in S5, and the rest of the equation corresponds to logarithm of activity coefficient of antibody  $\ln(\gamma_{Ab})$ . However, we also propose that this second part of the equation also comprises activity coefficients and molar concentrations.

Distributing the negative sign in the exponent in S7, we get

$$\ln(FI_{Ab}) = \ln(A) + \ln\left((1 + (d - 1)e^{x_i - x})^{\frac{1}{1-d}}\right) \quad (S8)$$

$$\text{then assuming } 1/(d-1) = \gamma_{Ag}^\infty \quad (S9)$$

where  $\gamma_{Ag}^\infty$  is constant for a given interaction at infinite Ab dilution, we obtain

$$\ln(FI_{Ab}) = \ln(A) + \ln\left((1 + \frac{1}{\gamma_{Ag}^\infty} e^{x_i - x})^{-\gamma_{Ag}^\infty}\right) \quad (S10)$$

By substituting  $e^x = [Ag]$  and  $e^{x_i} = [Ag]_i$

$$\ln(FI_{Ab}) = \ln(A) + \ln\left((1 + \frac{1}{\gamma_{Ag}^\infty} \frac{[Ag]_i}{[Ag]})^{-\gamma_{Ag}^\infty}\right) \quad (S11)$$

Comparing S4 and S11, the proposed activity coefficient of the measured antibody (highlighted in yellow) changes as a function of Ag concentration at inflection point  $[Ag]_i$ , concentration of antigen  $[Ag]$ , and antigen activity coefficient  $\gamma_{Ag}^\infty$  at infinite antibody dilution.

Overall, chemical potentials of the interaction partners are interdependent and are determined by the affinity distribution of antibodies in our measurement system.