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Application Note

Characterization of a high-affinity anti-EPO antibody by switching speed measurements

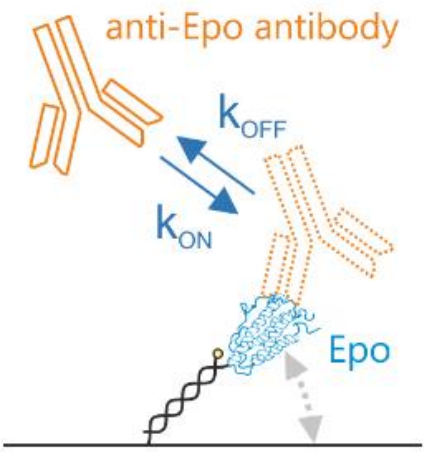
Keywords: Kinetics I antibody I (high)affinity I association and dissociation rates k_{on}, k_{off}

Background

Erythropoietin (EPO) is a glycoprotein that upregulates the production of red blood cells. Recombinantly produced EPO is used as a drug to treat anemia and also as an illegal doping agent in sports. Understanding the interaction of antibodies with EPO is important for the diagnosis of a potentially fatal immune response of patients to exogenous EPO as well as for the development of assays for the in-vitro detection of EPO.

Results

Recombinant EPO (37 kDa) from CHO cells was coupled to 48bp DNA nanolevers using the DBS amine reactive coupling kit. A switchSENSE sizing measurement gave a protein diameter of 4.6 nm, in perfect agreement with the prediction of the hydrodynamic diameter from atomic structure (PDB: 1BUY), confirming that EPO was attached to the nanolever as a monomer at a 1:1 stoichiometry. Real-time association experiments were performed with a monoclonal mouse IgG1 anti-EPO antibody at concentrations from 30 pM to 1 nM. A decrease in the switching speed can be observed during the association of the antibody, as it slows the motion of the EPO-modified nanolever (Figure 1). Association kinetics were analyzed globally with a single exponential model, $k_{ON} = 7.0 \pm 0.1 \times 10^6\ M^{-1}s^{-1}$. Despite the fast on-rate, mass transport limitations were not observed. Additionally, linear fits were made to evaluate the possibility of quickly estimating the magnitude of the signal change at the time of analyte injection. The slopes of linear fits are found to



correlate linearly with the Ab concentration (Figure 2), enabling a concentration measurement in the pM range. The dissociation of the Ab from a saturated sensor surface was followed overnight, revealing a very slow off-rate of $k_{OFF} = 7.0 \pm 1.8 \times 10^{-6}\ s^{-1}$. The dissociation constant is $K_D = k_{OFF}/k_{ON} = 1.0 \pm 0.2$ pM.

Conclusions

The kinetic rate constants as well as the dissociation constant were successfully determined for a high-affinity monoclonal antibody against EPO. A quick quantification of the Ab concentration is possible by analyzing the initial signal change after Ab injection. Sub-pM concentrations of the antibody could be detected in less than 20 min.

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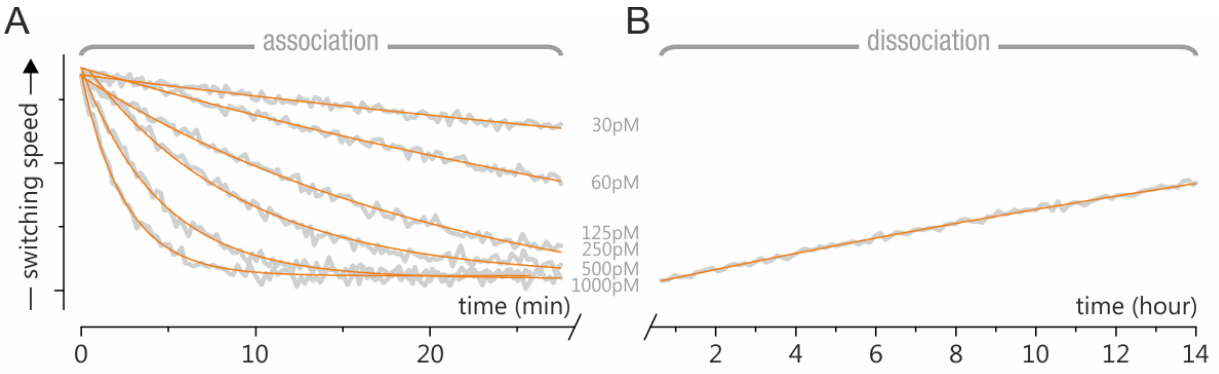

Figure 1 | **Binding kinetics:** Real-time association and dissociation kinetics of a monoclonal mouse IgG1 to EPO modified nanolevers. Here, the switching speed is evaluated as the been Dynamic Response parameter during the first 10 µs of the upward switching motion. Single exponential fit lines from a global analysis are orange, straight blue lines are linear fits. k_{ON} = 7.0 ± 0.1 x 10^6 $M^{-1}s^{-1}$, k_{OFF} = 7.0 ± 1.8 x 10^{-6} s^{-1}, K_D = 1.0 ± 0.2 pM.

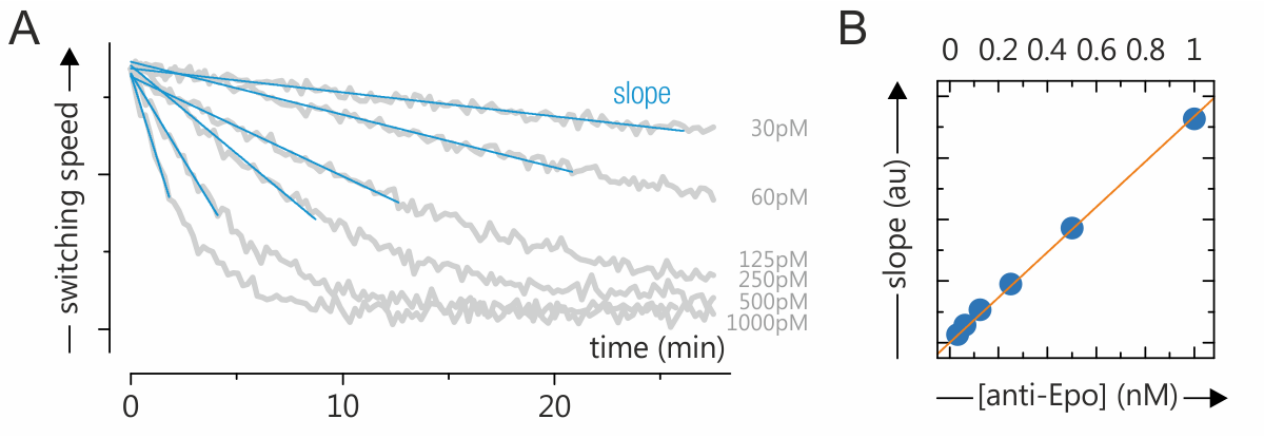

Figure 2 |**Concentration measurement:** Concentration calibration. The slopes from the linear fits in Fingure 1 depend linearly on the antibody concentration (R^2=0.993).

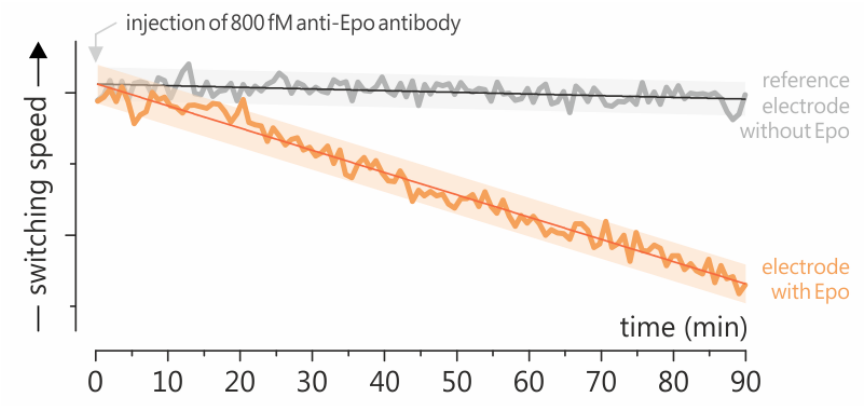

Figure 3 | **Limit of detection**

Real-time detection of very low antibody concentrations. The responses of an EPO-modified electrode versus a negative control with bare DNA nanolevers are shown for the injection of a 800 fM antibody solution.

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