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Application Note

Real-time measurement of Ni^{2+}-ion chelation kinetics

Key words: NTA, His-tagged proteins, Chelation, Ions

Background

Nitrilotriacetic acid (NTA) is a common chelating agent, which forms coordination compounds with metal ions (chelates) such as Ni^{2+}, Cu^{2+} and Co^{2+}. It is primarily used for the purification and immobilization of His-tagged proteins, which are captured by NTA-Ni^{2+} chelates bound to solid supports. The loading of NTA with Ni^{2+}-ions is an essential step for the activity of the chelate. Thus, we determined the association rate of Ni^{2+} to NTA and investigated the stability of the chelate against a competitive chelator, EDTA. Such information is required for research applications involving His-tag capture and industrial protein purification methodologies.

Methods and Results

Using a standard switchSENSE chip with 48 bp DNA, the binding of Ni^{2+} to NTA was measured by Fluorescence Proximity Sensing. Complementary trisNTA-modified DNA from the Dynamic Biosensors NTA kit was used to functionalize the surface with NTA groups.

When chelated by the NTA groups, the bound Ni^{2+}-ions quench the dye fluorescence by approximately -15% due to the Fluorescence Proximity effect. Binding kinetics follow a single-exponential behavior (Fig. 1) with an association rate of k_{ON} = 1.2 ± 0.1 x 10^4 $M^{-1}s^{-1}$.

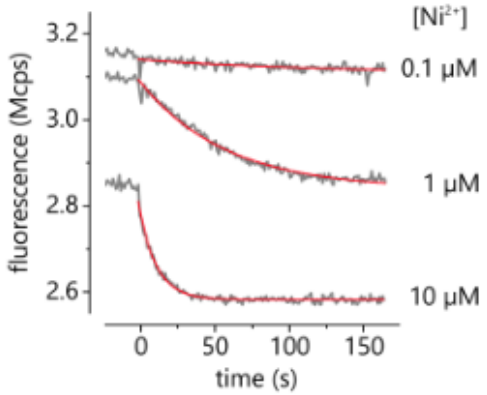

Figure 1 I Real-time measurement of binding of Ni^{2+}-ions to NTA measured by Fluorescence Proximity Sensing. Red lines are single exponential fits with an association rate of k_{ON} = 1.2 ± 0.1 x 10^4 $M^{-1}s^{-1}$.

Ni^{2+}-ions are stably bound to NTA for many hours. To remove Ni^{2+} from NTA, high concentrations of a competitive chelator like EDTA are necessary. Only when using EDTA concentrations higher than 1 mM the dissociation of Ni^{2+} could be observed (Fig. 2) and even at an EDTA concentration of 10 mM the dissociation rate was relatively low: k_{OFF} = 6.2 ± 0.4 x 10^{-3} s^{-1}.

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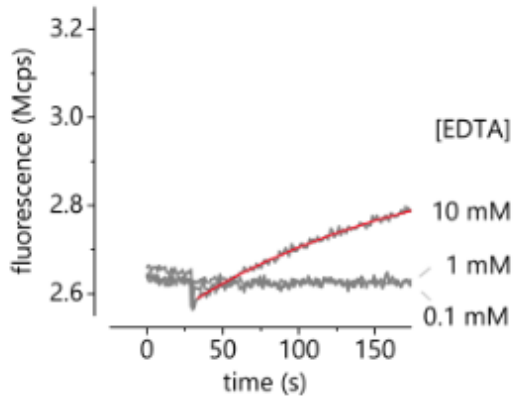

Figure 2 I Real-time measurement of the dissociation of Ni^{2+}-ions from NTA via competition with EDTA. The red line is a single exponential fit. The dissociation rate at 10 mM EDTA is $k_{OFF} = 6.2 \pm 0.4 \times 10^{-3}\ s^{-1}$.

Conclusions

The binding kinetics of Ni^{2+}-ions to NTA could be measured using the Fluorescence Proximity Sensing measurement mode. For complete saturation of an NTA-surface within 1 minute, a concentration of at least 10 µM Ni^{2+} is required. A low background concentration of e.g. 100 µM EDTA does not affect the chelated ions. To wash Ni^{2+} from NTA, EDTA concentrations of at least 10 mM must be used.

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