Temperature Dependent Binding Kinetics of Protein-Protein Interactions Using OpenSPR

Summary

- A protein-protein interaction was analyzed on the OpenSPR™ instrument at two different temperatures: 30°C and 10°C
- Kinetic analysis was used to determine the kinetics and affinity constant of the interaction
- The off rate was found to decrease by 40% going from 30°C to 10°C

Overview

OpenSPR™ is a powerful instrument providing indepth label-free binding kinetics for a variety of different molecular interactions. One common application of surface plasmon resonance is to investigate the effect of temperature on the binding kinetics and affinity of an interaction, which can also be used to determine thermodynamic parameters. In this application note, we demonstrate how OpenSPR™ is used to analyze the effect of temperature of the kinetics and affinity of a protein-protein interaction by using the Nicoya Temperature Control Add-On.

Materials and Equipment

- OpenSPR™ Instrument + Temperature Control Unit
- OpenSPR Streptavidin Sensor Chip
- TraceDrawer Kinetic Analysis Software
- Biotin Tagged Ligand (protein, MW
- 10kDa)
- Analyte (protein, MW 33kDa)
- Tris Running Buffer with 0.1% BSA and 0.05% Tween 20
- Regeneration Buffer: 10mM Glycine HCl

Procedure

OpenSPR Experiment

1. Following the start-up procedure found in the OpenSPR manual, setup the OpenSPR instrument and load a Streptavidin Sensor Chip.
2. Run non-specific control tests by injecting the analyte at the working concentrations given below.
3. Immobilize biotin tagged ligand protein by injecting 100µL at a concentration of 1µM and a flow rate of 20µl/min for a 5 minute incubation period.
4. Set the instrument temperature to 30°C and allow the signal to stabilize for 5 minutes.
5. Inject the analyte protein at a concentration of 1µM at 20µL/min for an association time of 300 seconds and a dissociation time of 900 seconds.
6. Regenerate the surface with an injection of regeneration buffer at a speed of 150ul/min.
7. Repeat steps 5 and 6 with analyte concentrations of 333nM, 111nM, and 37nM.
8. Load a new sensor chip in the instrument, set the temperature to 10°C and repeat steps 3-8.

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Results and Discussion

Results from the protein-protein interaction at the two different temperature are shown in Figure 1 (30°C) and Figure 2 (10°C). The curves look similar, but upon analysis in TraceDrawer, differences in the kinetics and affinity constant can be identified. A one to one binding model was fit to the data, and the optimized model has been overlaid in each figure. The fits are excellent, and no non-specific binding was observed. Table 1 shows the on rate, off rate, and affinity constant of the interaction for the two temperatures tested. From this analysis, the on rates are similar while the off rate is reduced by 40% in the experiment run at 10°C, causing the affinity to decrease from 13.1nM to 8.04nM. A decrease in the off rate is expected at lower temperatures, possibly due to a decrease in the random thermal motion of the molecules. To confirm these results, another experiment was run at a single concentration on the same chip at 30°C and 10°C. The results are shown in Figure 3, with the responses normalized to the same maximum, which confirms the findings from the two independent experiments as the off rate is clearly higher for the higher temperature. These results were confirmed with independent tests run on a Biacore™ instrument.

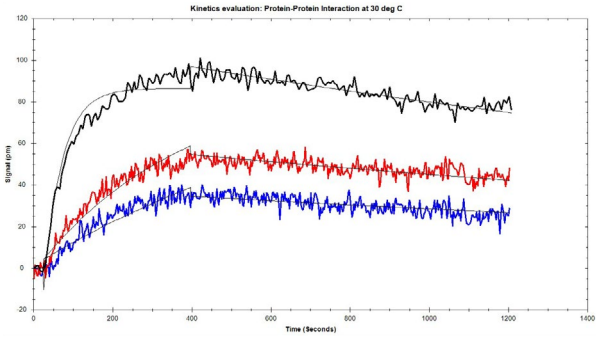

Figure 1. Protein-protein interaction measured at 30°C at 1μM, 111nM, and 37nM (black, red, and blue)

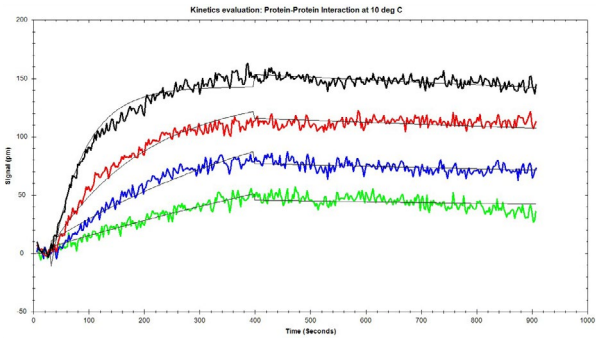

Figure 2. Protein-protein interaction measured at 10°C at 1μM, 333nM, 111nM, and 37nM (black, red, blue, and green)

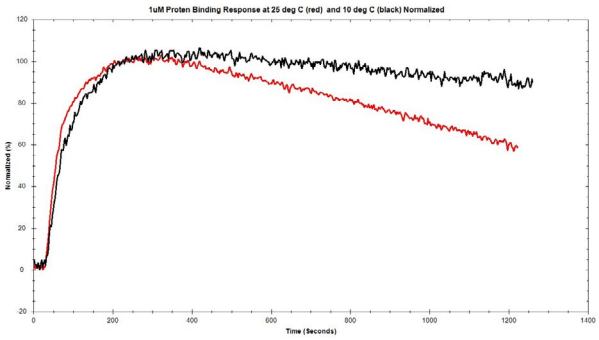

Figure 3. Direct comparison of protein-protein interaction at 1μM concentration at 30°C (red) and 10°C (black).

Conclusions and Summary

This study demonstrates how OpenSPR can be used to examine the impact of temperature on the kinetics and affinity of biomolecular interactions, a powerful capability providing new insight into complex biochemical processes.

	30°C
k_{on} [1/M*s]	5.40e-3 s-1 (+/- 6.95e-7)
k_{off} [1/s]	4.27e4 M-1*s-1 (+/- 8.11e1)
K_D [nM]	126 nM (+/- 0.26)

Table 1. Kinetic and affinity constants