# Development of a protein-protein interaction inhibition assay using Biacore 8K

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## Introduction

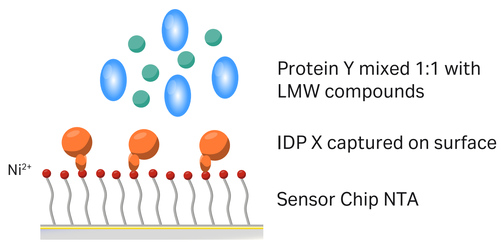
Small molecule inhibition of protein-protein interaction (PPI) is a hot research area in drug discovery and the number of interesting drug targets are increasing by the day. However, PPI's comprise a lot of challenges, such as identifying how a specific protein-protein interaction should be inhibited. The interaction area is usually large and potential inhibitors tend to be larger and more lipophilic than the average fragment. Thus, there are often problems with specificity and interactions are generally weak. Consequently, PPI research depend largely on technically advanced instrumentation and intuitive and dedicated software to allow for efficient screening and confident selection of candidates.

Here we present a study to identify suitable candidates for inhibition of the interaction between Intrinsic Disordered Protein (IDP) X and protein Y. Early interaction data showed that the preparation of IDP X was impure. Purification was performed using size-exclusion chromatography on ÄKTA™ pure chromatography system. Biacore™ 8K was used to efficiently identify the active fraction and to optimize assay conditions, followed by the setup and run of a PPI screening experiment in less than one day.


Biacore 8K

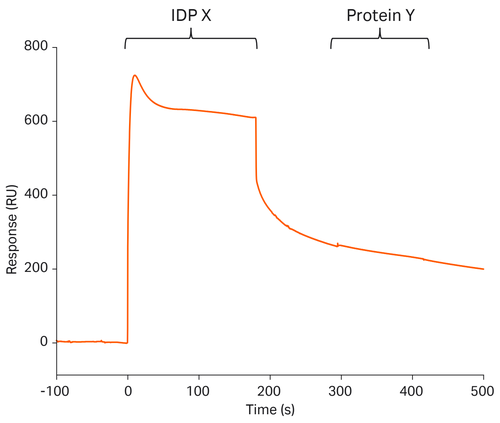
## Assay setup

IDP X was captured on Sensor Chip NTA. Protein Y was mixed 1:1 with different LMW compounds and injected over IDP X to check for inhibition of protein-protein binding. Protein Y mixed 1:1 with buffer was used as positive control.



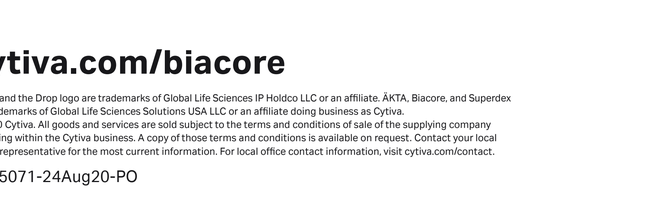

## Initial test of protein activity

An initial experiment to test the activity of the two proteins was performed. IDP X was captured on Sensor Chip NTA and protein Y was injected as analyte. The results showed that the preparation of IDP X was impure and very likely contained a substantial amount of nonrelated material, such as fragments and/or aggregates. In addition, capture stability was low and no analyte response could be seen. To be able to perform a screening, it was concluded that additional purification of IDP X was needed.




## Purification using ÄKTA pure

Size-exclusion chromatography was performed on ÄKTA pure 25 using a Superdex™ 200 Increase 10/300 column. The multiple peaks in the chromatogram suggests that the protein sample contains a mixture of full size and aggregated as well as degraded IDP X. Five fractions (11, 12, 14, 17, 18) were selected for further optimization on Biacore 8K.





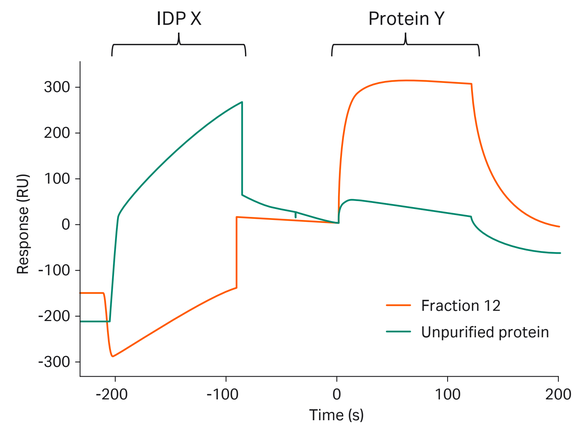

ÄKTA pure 25

## Rapid optimization of assay conditions using Biacore 8K

Using only two analysis cycles in less than 1 h, the eight-channel parallel configuration of Biacore 8K was utilized to assess optimal running conditions for the PPI screening experiment.

The first analysis cycle was used to determine which of the collected fractions contained active IDP X. This was done by capturing eight different fractions on Sensor Chip NTA (one fraction per channel) followed by an injection of protein Y. The second analysis cycle was used to assess optimal dilution factor by diluting the selected fraction to eight different concentrations and capture each concentration on Sensor Chip NTA (one concentration per channel).

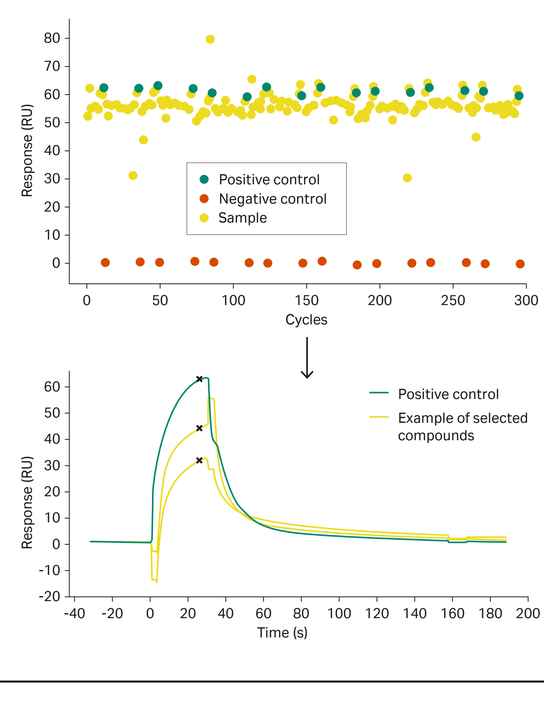
Results showed that fraction 12, which was diluted eight-fold, gave the highest capture stability in relation to analyte response level.




| Capture solution | IDP X Capture level (RU) | Protein Y Binding_late (RU) | Ratio protein Y/ IDP X |
|---|---|---|---|
| IDP X fraction 12 | 162.5 | 306.1 | 1.9 |
| IDP X unpurified | 250.4 | 15.4 | 0.1 |

## Rapid, high-quality PPI screening using Biacore 8K

The PPI screening experiment was performed using the optimized protein conditions. IDP X was coupled to Sensor Chip NTA. Protein Y was mixed 1:1 with compound and then injected over IDP X. 352 compounds were screened in 3.5 h, which is eight times faster than the corresponding experiment on a singe-needle instrument. Data was adjusted using blank subtraction and adjustment for positive control. No fixed cutoff was used to evaluate. Instead, compounds that was found to generate responses considerably lower than the positive control were selected as potential inhibitors of the protein-protein interaction. Selected compounds were subsequently verified with characterization experiments using SPR and orthogonal techniques.




## References

1. Data file: Biacore 8K and Biacore 8K+. Cytiva, CY11892-12Mar20-DF
2. Data file: ÄKTA pure 25. Cytiva, CY11416-12Mar20-DF
3. Data file: Superdex 200 Increase. Cytiva, KA6435291119DF/29045269 AD

## Conclusion

- The parallel configuration of Biacore 8K was utilized to efficiently scout for optimal screening conditions
- Screening of 352 PPI compounds was completed in only 3.5 h using Biacore 8K
- Promising candidates were identified and moved on for further characterization and development
- The combination of ÄKTA pure 25 and Biacore 8K generated a highly pure and active protein preparation, which resulted in high-quality data in a complex application

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