

Quantifying protein-protein interactions in real-time using LigandTracer®

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Introduction

Real-time quantification of biomolecular interactions is receiving increasing attention within biological research¹. We show that information about on- and off-rates and affinity of protein-protein interactions can be provided using LigandTracer®, an instrument originally developed for use with cellular systems^{2,3}.

Experimental

LigandTracer detects the interaction between a target protein attached to magnetic beads and a radiolabelled ligand in solution. The reagents are put in an empty petri dish which in turn is placed on an inclined, rotating support with an external magnet anchoring the magnetic beads (fig 1). Each round, the bead area will pass a time-resolved detector which is mounted over the elevated part of the dish. If there is an interaction, the detected label will be seen as a radiation intensity peak. By following peak height over time, a real-time binding trace is created³.

Results

The binding trace of radiolabelled HSA interacting with mouse monoclonal antibody is found in figure 2. Three traces are shown, the differential signal and its two components (bead area signal and empty area signal). Specificity was proven by comparing the differential signal from a radiolabelled irrelevant protein with the signal from HSA.

The method has been tested using several molecular systems of known characteristics (table 1), including a radiolabelled therapeutic antibody (Trastuzumab) binding to the growth factor receptor HER2 (fig 3). Dose-dependent and specific signals reproducing the known characteristics were recorded (fig 3 and 4).

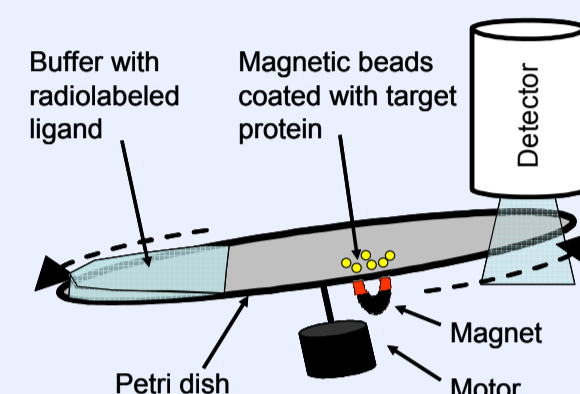


Figure 1. Schematic of LigandTracer technology

Table 1

Instrument:

LigandTracer Grey (Ridgeview Instruments AB, Uppsala, Sweden, www.ligandtracer.com)

Magnetic beads:

Tosyl activated Dynabeads®

Molecular interactions:

- Mouse monoclonal Ab – HSA*
- HER2 – Trastuzumab*
- Mouse monoclonal Ab – β -2-microglobulin*
- Rabbit polyclonal Ab – β -2-microglobulin*
- Goat polyclonal Ab – GST*

Ab = antibody, * = Labeled with ¹²⁵I

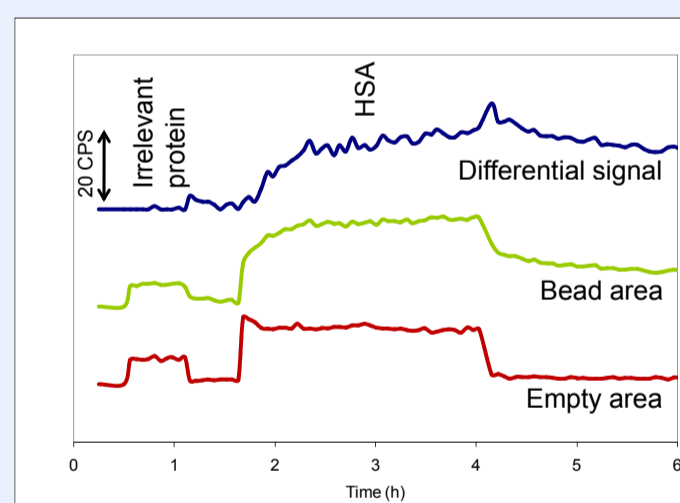


Figure 2. Radiolabelled HSA and an irrelevant protein interacting with an anti-HSA antibody. The binding-traces from bead area, empty area and the difference bead-empty are shown.

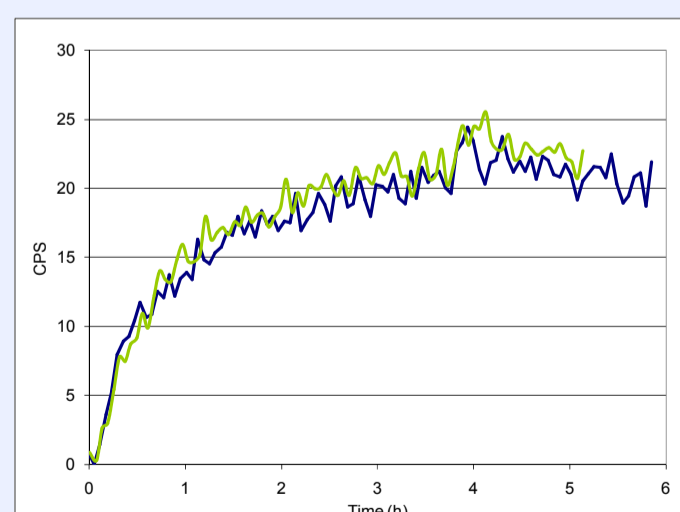


Figure 4. Repeatability illustrated using two binding-traces from radiolabelled β -2-microglobulin binding to a rabbit polyclonal anti- β -2-microglobulin antibody.

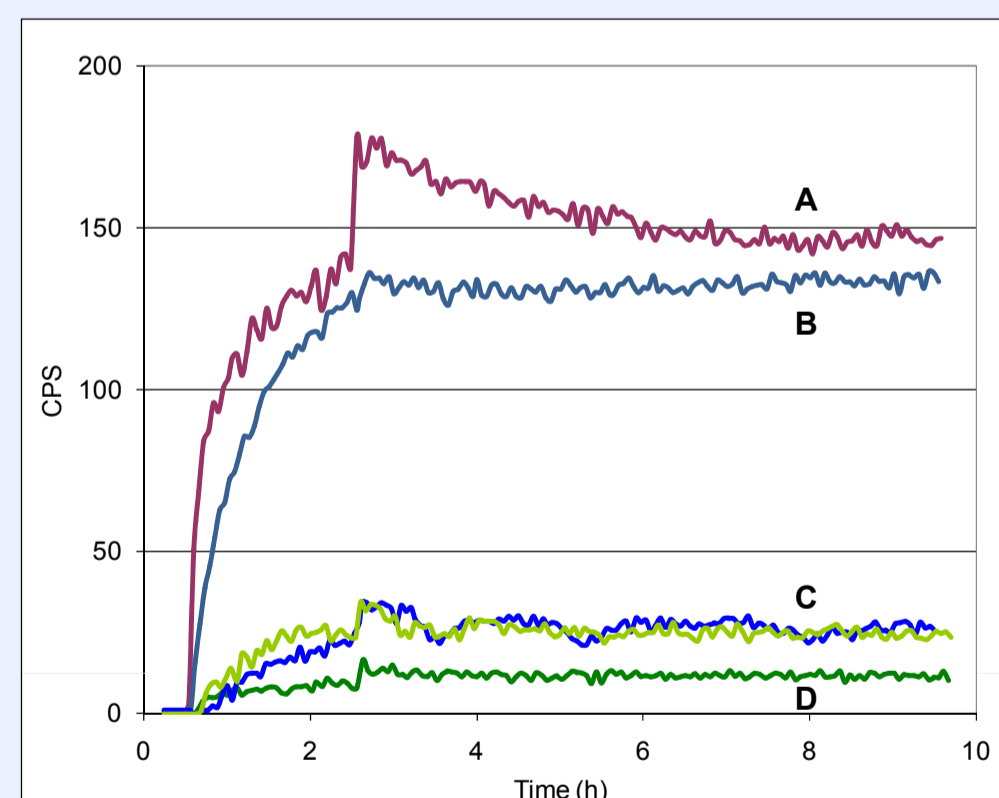


Figure 3. Binding traces for Trastuzumab binding to HER2-coated beads. Amount of magnetic beads and concentration Trastuzumab were varied in the following manner:

	A	B	C	D
Amount of magnets	high	high	medium	low
Concentration Trastuzumab	50 nM	5 nM	15 nM	5 nM

Conclusion

In summary, real-time binding data has been created with several molecular systems, showing that magnetic beads can be used to carry ligands in LigandTracer. The method has potential to improve the basis for decisions in several scientific areas, including the development of radiopharmaceuticals and PET agents.

References

1. J Mol Recognit. 2007;20(5):300-66.
2. Appl.Radiat.Isot.2006: 64:32-37
3. Appl.Radiat.Isot. 2006: 64:901-905

www.ligandtracer.com