Real-time cell based assays: Exploring LigandTracer beyond living mammalian cells

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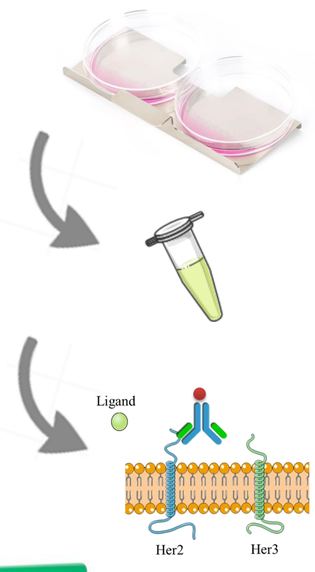
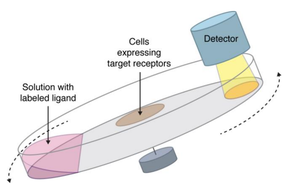
During drug development it is crucial to analyze the **binding properties**, such as the **affinity** and the **kinetics**, of new leads. The LigandTracer® Green (Ridgeview Instruments AB, Sweden) technology allows us to measure the binding of a drug or a protein to a receptor expressed on **living** mammalian cells in **real-time**. Recently, we extended the applications of LigandTracer Green for measuring interactions of ligands to **bacterial** surface proteins and we demonstrated that it is possible to conduct real-time measurements with living cells at **different temperatures**.

Methods

Mammalian cells

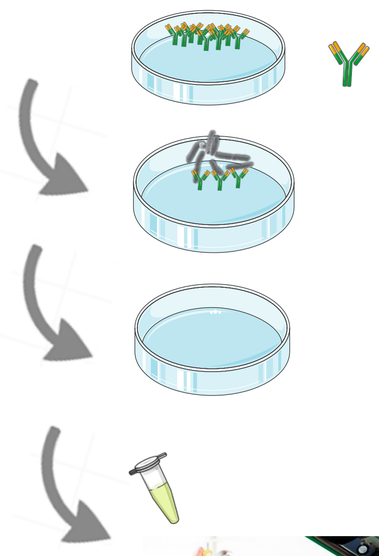
1. Adherent HER2 expressing human ovarian carcinoma cells (SKOV3) were seeded in a local area of a Petri dish and allowed to attach at 37 °C.
2. The dish was placed in LigandTracer Green and a fluorescently labeled anti-HER2 Ab (TexasRed-Pertuzumab) was added to the dish.
3. The increase in fluorescent signal at the cell area was measured over time at different temperature, and subtracted with the signal from a cell free area.





Bacterial cells

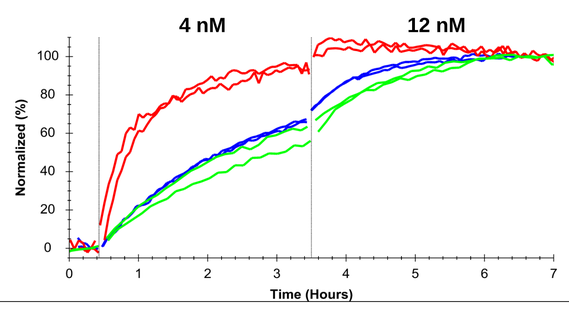
1. Antibodies against surface proteins of bacteria were adsorbed to a polystyrene petri dish for 3 h at room temperature.
2. The antibody solution was removed, the dish was washed and bacteria (OD = 1-2) was incubated for 1h at 37ºC.
3. The bacteria solution was removed and the dish was washed and blocked with 1 % BSA for 30 min to prevent unspecific binding during the interaction measurement.
4. The binding of fluorescent antibodies to the living bacteria was investigated with LigandTracer Green over time.

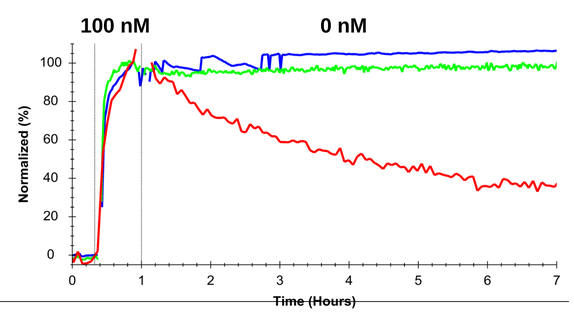


Results

Temperature influence on antibody interactions in cancer cells

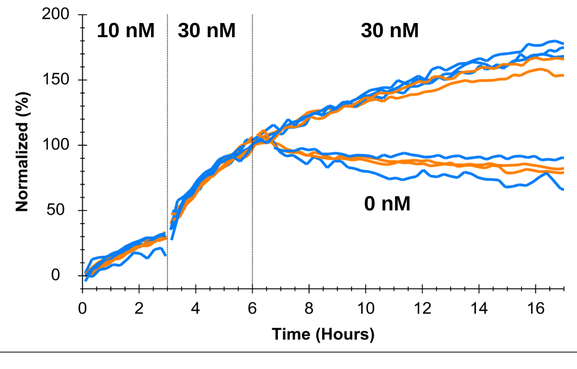
SKOV3 cells were incubated with TexasRed-pertuzumab (4 and 12 nM to follow the association rate, or a short 100 nM incubation followed by 0 nM to follow the dissociation rate). Measurements were conducted at 15ºC (blue), 21ºC (green) and 37ºC (red), which showed that the interaction was similar at 15ºC and 21ºC, but with a significantly faster association and dissociation rate at 37ºC.

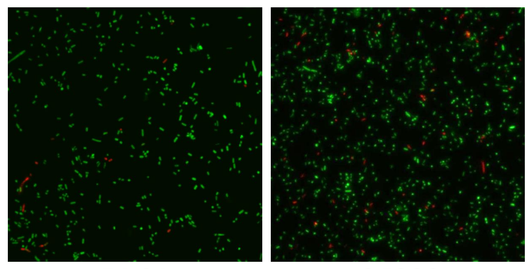




Real-time interactions measurements with living *E. coli*

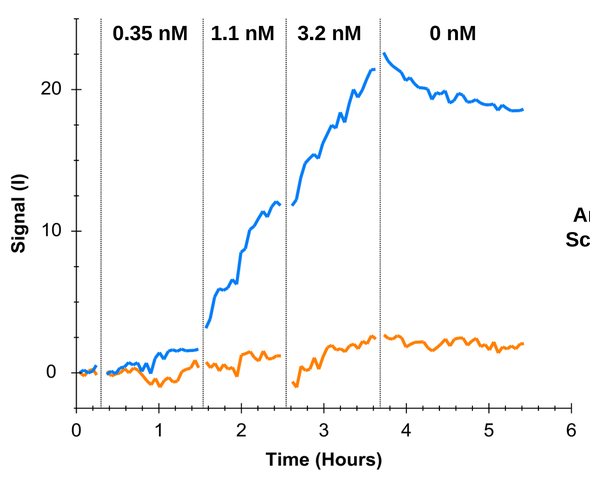
The *E. coli* strains CJ236 (blue) and BL21 (orange) were attached to a Petri dish using an adsorbed anti-*E. coli* Ab and the association and dissociation of the FITC-labeled Ab99 to the bacteria was followed for at least 17 h. The data was highly reproducible and there was no clear sign of bacteria detachment over time. The viability was above 90 % after 16 h, as quantified by confocal images using a Live (green)/Dead (red) viability kit.

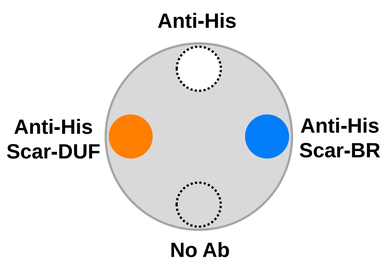


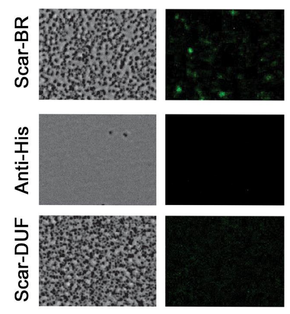


Specificity measurements with *S. carnosus*

An anti-His Ab was adsorbed to a Petri dish and two *S. carnosus* strains (Scar-DUF: displaying a His-tag fused with a Domain with an Unknown Function (DUF); Scar-BR: displaying a His-tag, DUF and a pneumococcal Binding Region (BR)) were bound to the adsorbed antibody. The polyclonal affinity-purified FITC-labeled anti-BR antibodies clearly bound to Scar-BR (blue), but not to a bacteria free anti-His area (used for reference) or to Scar-DUF (orange). The white and green channel images were taken after the binding experiment using a phase-like contrast microscope, demonstrating FITC-staining of the immobilized Scar-BR, but not of Scar-DUF or the anti-His antibody control.







Conclusions

In summary, we demonstrate that the LigandTracer technology can be used to monitor molecular interactions in various **temperature** conditions and with a **living bacterial environment**. This method can therefore be used in the development of new drugs as well as in the **optimization** of biosimilars against specific targets on mammalian and bacterial cells.

References

Encarnação, J.C., Barta, P., Fornstedt, T., Andersson, K. (2017) Impact of assay temperature on antibody binding characteristics in living cells: A case study. Biomedical Reports, 7(5): 400-406

Encarnação, J.C., Schulte, T., Adnane, A., Björkelund, H., Andersson, K. (2018) Detecting ligand interactions in real-time on living bacterial cells. *Applied Microbiology and Biotechnology*, 102(9): 4193–4201

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