

Vysens Application Note (AN) #2

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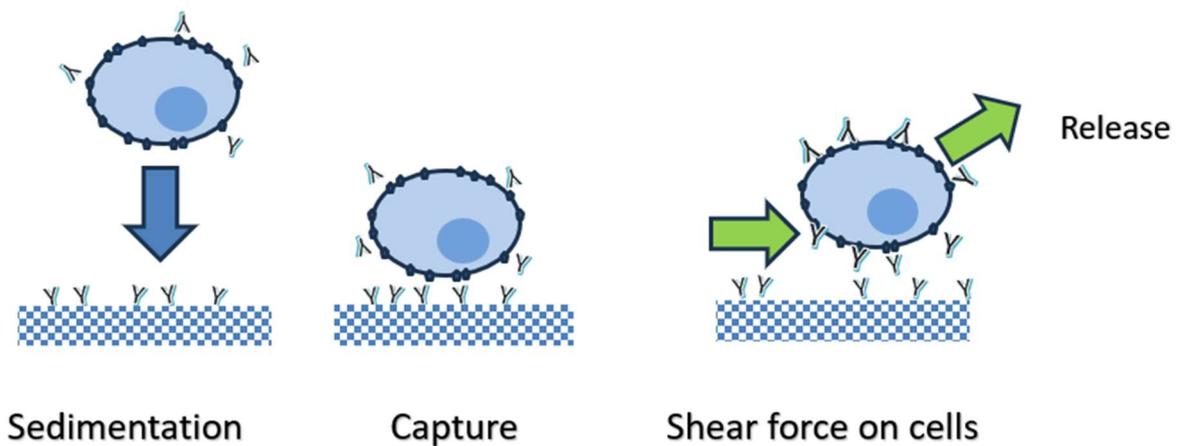
Software Version: StrepGui + CellVysion Analysis Software

1. Title

Measurement of the opsonization effect with a competing antibody using a LNCaP–anti-Her2 interaction.

2. Objective of This Measurement

This application note describes the measurement of LNCaP–anti-Her2 interaction under increasing shear conditions using the Vysens CellVysion system with a continuous ligand density gradient. Theoretically the equilibrium dissociation constant (K_D) determines the degree of coverage of the cells. Prior to the measurement, LNCaPs were opsonized with anti-Her2 antibodies in increasing concentrations



3. Biological System

The interaction between a human prostate cancer cell line and a surface-immobilized antibody was examined after cells were pre-incubated with the same antibody in various concentrations.

Cell type

LNCaP (human prostate cancer cell line)

Target / ligand

Anti-Her2-Biotin antibody, pre-incubation with anti-Her2 antibody

Material origin

LNCaP cells were purchased from ATCC.

Relevant characteristics

The LNCaP cell line has been reported to express approximately 30,000 Her2 antigens per cell (literature data).

4. Experimental Setup

The experiment was performed using a streptavidin-functionalized SPR sensor surface on which a continuous ligand density gradient was generated.

Sensor surface

SPCV SAHC200M SPR sensor prism (CytoVysion compatible, Xantec Bioanalytics)

Surface coating

200 nm streptavidin-derivatized linear polycarboxylate hydrogel (medium density)

Functionalization

A continuous anti-Her2-Biotin ligand density gradient was generated on the streptavidin-coated surface.

Pre-incubation of cells

LNCaPs were pre-incubated with anti-Her2 in different concentrations: 64-32-16-8-4-2-1-0 pM.

Measurement conditions

- Running buffer: PBS + 1% BSA + 0.25% EDTA (0.5M v/v) + 0.01% Tween-20
- Cell concentration: $1,5 \times 10^6$ cells/mL, without opsonization: 3×10^6 cells/mL
- Sedimentation phase: 10 minutes
- Automatically increasing shear flow applied after sedimentation
- Temperature: ambient

5. Measurement Procedure

LNCaPs were pre-incubated with anti-Her2 in different concentrations: 64-32-16-8-4-2-1-0 pM for at least 30 minutes.

The sensor was inserted into the CellVysion system and primed using running buffer and MilliQ water. The optimal SPR angle was set prior to calibration.

Calibration was performed by injecting a 945 RU calibration solution, allowing pixel intensities to be converted to RU values. The calibration run was recorded using ThorCam software.

Subsequently, a continuous anti-Her2-Biotin gradient was generated by injecting 90 μL of a 5 $\mu\text{g}/\text{mL}$ antibody solution into the cuvette. The gradient formation was recorded for approximately 10 minutes.

After rinsing the system with running buffer, 50 μL of LNCaP cell suspension was injected, starting with the highest concentration of anti-Her2 opsonization. A sedimentation phase of 10 minutes allowed cells to settle onto the surface. Following sedimentation, an automatically increasing shear flow was applied.

The entire run was recorded using the ThorCam software and analyzed using the CellVysion Analysis Software. The tipping point was identified based on pixel position in the SPR image (using ImageJ software).

After this run, sensor was rinsed with running buffer, and the next LNCaP cell suspension was injected. This procedure was repeated with decreasing opsonization concentration.

6. Data Output

The measurement produced multiple data outputs describing calibration, gradient formation, and cell retention under shear.

Output types

- Reflectivity versus time during calibration
- Ligand density gradient profile (RU per pixel)
- SPR image showing cell distribution after shear
- Ligand density value at the identified tipping point (RU)

Single measurement (n = 1)

- Gradient generation curve, Figure 2.
- Final ligand density per pixel, Figure 3.

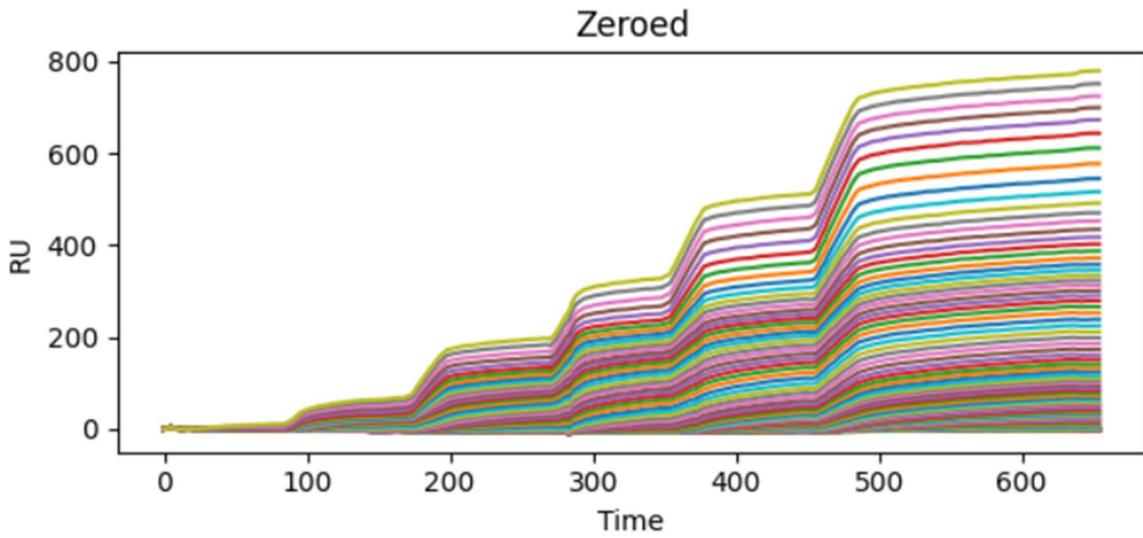


Figure 2. Anti-Her2-Biotin continuous ligand density gradient generation. Process of gradient generation plotted in time.

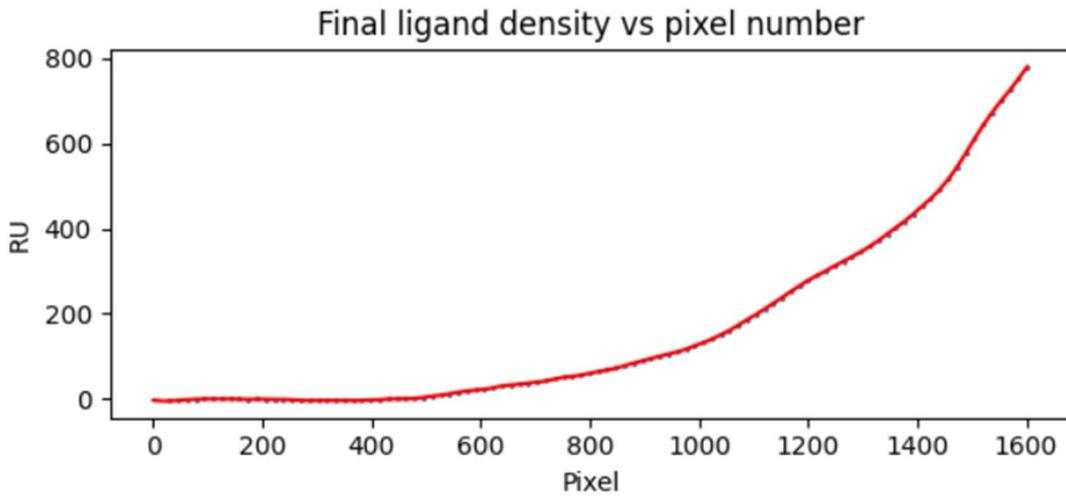


Figure 3. Anti-Her2-Biotin continuous ligand density gradient generation. Final ligand density on the sensor surface plotted per pixel.

2. SPR images with tipping point locations for each corresponding anti-Her2 opsonization concentrations

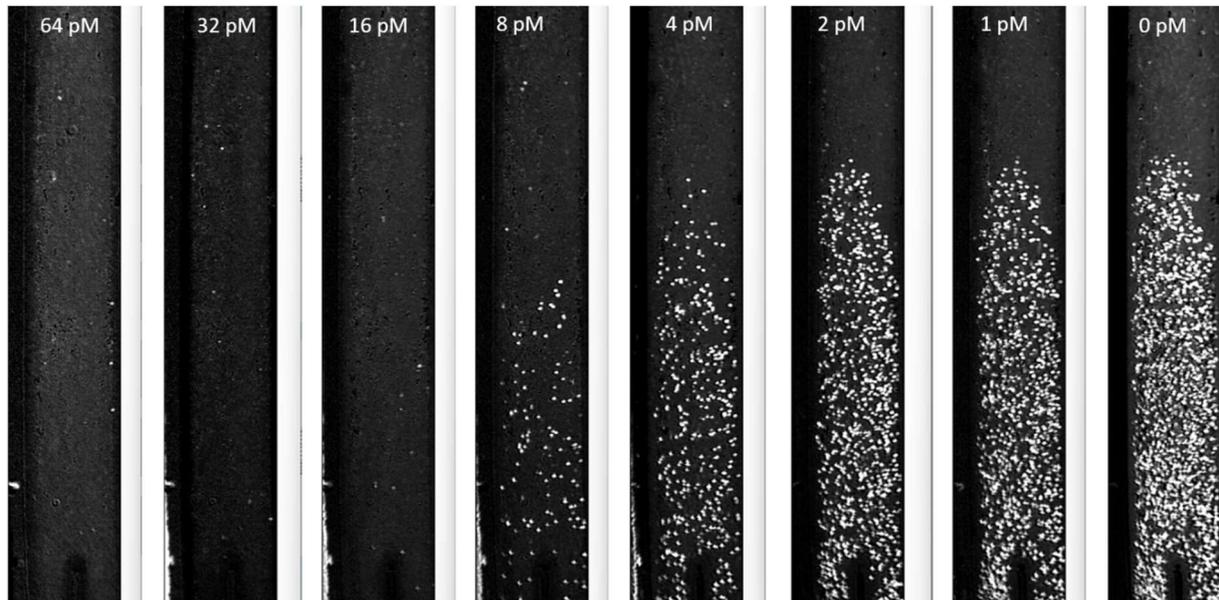


Figure 3. Cell Avidity of LNCaPs on an anti-Her2-Biotin ligand density gradient. LNCaPs were opsonized with anti-Her2 in different concentrations. Tipping points are visualized in the SPR images where LNCaPs remain bound under flow.

7. Technical Observations

Opsonization/incubation of the cell suspension with competing antibodies will reduce the number of free receptors per cell. As a result, the tipping point will shift caused by this avidity change. Generally the equilibrium dissociation constant is at the concentration where 50% of the receptors are covered by the antibodies.

During gradient generation, a continuous variation in ligand density was observed across the sensor surface. Ligand density varied along the pixel axis as recorded in the gradient profile. (fig. 2.)

After cell injection and sedimentation, cells were visible on the surface in the SPR images. During increased shear, unbound or loosely bound cells detached from the sensor surface.

A transition point was observed at a specific pixel position where LNCaPs remained bound under flow. For each anti-Her2 opsonization concentration a different tipping point was observed.

8. Limitations

The measurement requires viable cell suspension and a functional streptavidin-coated SPR surface.

A continuous gradient must be successfully generated prior to cell injection.

The measurement was performed under defined shear conditions only.

The data presented represents a single measurement without replicates.

9. References

Vysens Application Guide – CellVysion Gradient Measurements
Vysens Handbook – SPR Imaging and RU Calibration