

The characterization of the interaction between a ligand and a cell-surface receptor includes several different measurements. Affinity measurements reveal the strength of the interaction. This application note shows how the affinity of an interaction can be measured using rotating RIA.

Experimental

Cancer cells (A431, U343, or SKOV-3) were seeded in a local part of a cell dish, as indicated in Figure 1. The seeded cells were allowed to attach firmly to the dish surface for at least 12 hours. The dish was placed on an inclined, rotating support and a radiation detector was mounted over the elevated part. Cell culture medium containing a low concentration of radioligand was added to the dish, and the detector registered the intensity as a function of rotational position (Figure 2). After ~20 minutes, the concentration was increased by addition of a small amount of radioligand stock solution to the medium already present in the dish. The intensity was measured for another 20 minutes, followed by addition of stock solution. The procedure was repeated until a sufficiently high concentration had been reached. No washes of the cell dish were performed throughout the affinity measurement.

When the titration was completed, the peak heights were plotted versus the corresponding ligand concentrations, as shown in Figure 3. The affinity of the interaction was obtained by fitting an interaction model (monovalent binding) to the measured peak heights.

Results

The affinities of three interactions were measured using rotating RIA, as shown in Table 1. The interaction of epidermal growth factor (EGF) with its receptor has been characterized by numerous laboratories, and the affinities obtained using rotating RIA agrees with values reported in the literature. The affibody ZHER2 interacts with the receptor HER2. The interaction is strong, but the complex dissociates completely within 4-5 minutes. Thus, attempts to use standard protocol binding assays which include washes have failed to detect ZHER2 as a binder. Since rotating RIA is performed without washes, the affinity of the ZHER2-HER2 interaction could be accurately determined.

Conclusion

Rotating RIA is a simple and accurate method for affinity measurements. The required amount of consumables and reagents are reduced compared to manual protocols. Since rotating RIA technology eliminates all washes, valid affinities can be derived regardless of the dissociation rate of the interaction.

References

Bjorke H, Andersson K. *Measuring the affinity of a radioligand with its receptor using a rotating cell dish with in situ reference area.* Appl Radiat Isot. (in press)

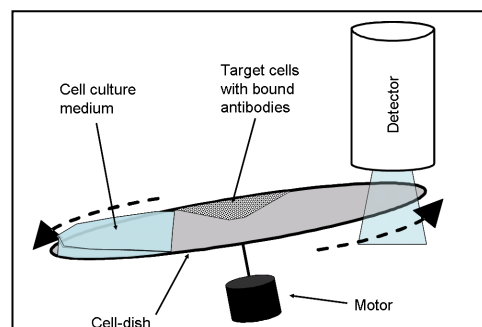


Figure 1. The principle of rotating RIA

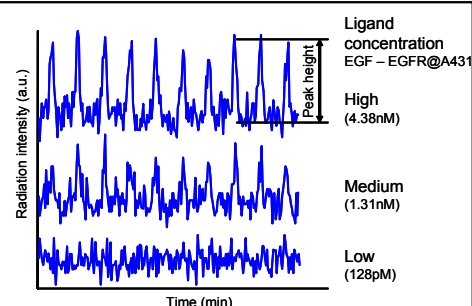


Figure 2. Radiation intensity versus time during ten revolutions of the cell dish for three different ligand concentrations.

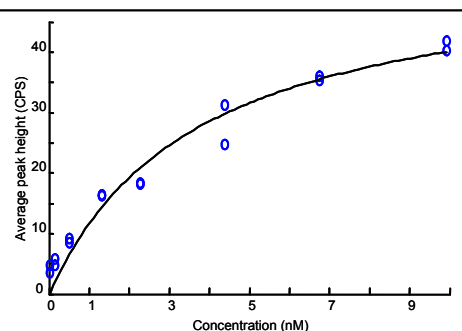


Figure 3. Peak height versus ligand concentration for EGF binding to the EGF receptor. The solid line represents the fitted interaction model.

Ligand	Receptor	Cell type	Affinity (nM)
EGF	EGFR	A431	3.2 ± 1.5
EGF	EGFR	U343	2.2 ± 0.5
ZHER2	HER2	SKOV-3	12 ± 1.9

Table 1. Affinities measured using rotating RIA. Each interaction was measured at least three times.