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Single-cell immunosensors for protein detection

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Abstract

A single-cell detector is described that combines the natural signal amplification of whole-cell biosensors with the flexibility and specificity of immunological recognition. An immune cell that expresses receptors for the constant region of immunoglobulin G (IgG) is loaded with a Ca^{2+}-indicating dye and with antibodies directed against the protein of interest. Introduction of a multivalent protein antigen causes cross-linking of the receptors, which results in a detectable increase in the concentration of cytosolic Ca^{2+}. Some immune cell lines respond to stimulation with oscillations in their cytosolic Ca^{2+} levels that complicate their use as detectors. The human monocytic cell line U-937, when treated with the cytokine interferon-γ, produces a large, short-lived Ca^{2+} signal in response to cross-linking of its high-affinity IgG receptors. U-937 was therefore chosen for development as an immunity-based detector. Human and rabbit antibodies are found to effectively stimulate the cell, causing a prompt and transient response. The cell is able to respond to repeated stimulation, though the response diminishes during rapid stimulation. Ovalbumin can be detected in micromolar concentrations. Possible fundamental constraints on the size of a detectable analyte are discussed.

Keywords: Biosensor; Immunosensor; U-937; Single-cell; Detector cell; Whole-cell

1. Introduction

Living cells offer the possibility of being used as biosensors. They take advantage of the selective molecular recognition conferred by receptors in the cell membrane and can achieve high sensitivity through the natural amplification inherent in cellular signal transduction cascades. Detection platforms have been described in which the presence of the molecule of interest is reported by changes in cellular temperature resulting from metabolism (Johannessen et al., 2002), by changes in respiratory activity (Campanella et al., 1997), by acidification of the extracellular environment (McConnell et al., 1992), by the initiation of a Ca^{2+} spike (Luzzi et al., 2000; Shear et al., 1995), and by changes in membrane permeability to ions (Jardemark et al., 1997; Orwar et al., 1996). Arrays of single cells have been reported (Taylor and Walt, 2000; Zahn et al., 1999) and may hold promise for application in high-throughput screening.

One limitation of these whole-cell biosensors derives from the same attribute that confers their specificity, namely, that cell membrane receptors only bind a single molecule (or small number of closely related molecules). Therefore, if the cell being used as a detector lacks a receptor for the molecule of interest, that molecule will not be detected. One way to overcome this problem has been the injection into cells of mRNA to induce the expression of receptors for molecules of interest (Shear et al., 1995), but this technique is labor-intensive and only enables limited flexibility. A truly universal approach is reported here, in which the amplification ability of whole-cell biosensors is combined with the specificity and flexibility of immunological recognition. The resulting detector is referred to as a single-cell immunosensor.

The single-cell immunosensor is based on a monocytic cell, U-937 (Sundstrom and Nilsson, 1976), that expresses in its membrane receptors for the constant region of immunoglobulin G (IgG) (Allen and Seed, 1989). The role of these receptors *in vivo* is to communicate the presence of invaders to the cell. Monocytes

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and macrophages express high-affinity IgG receptors (FcγRI) as well as low-affinity IgG receptors (FcγRII and FcγRIII). Monomeric IgG can be immobilized in FcγRI and then cross-linked by binding to a multivalent antigen, whereas the low-affinity receptors bind IgG-containing immune complexes with high avidity. The cross-linking or clustering of these receptors activates a signaling cascade that involves an increase in cellular Ca^{2+} levels and culminates in the activation of cellular effector functions such as phagocytosis, oxide production, and the release of inflammatory mediators (Lin et al., 1994; Ravetch, 1994).

Because of the wide availability of fluorescent Ca^{2+}-indicating dyes such as fluo-4, it is possible to image the Ca^{2+} signal and therefore detect the exact moment of receptor cross-linking (Minta et al., 1989). As demonstrated in extensive studies by Allen and co-workers (Davis et al., 1993, 1994; Floto et al., 1995a,b), the particular shape of the Ca^{2+} signal depends on which receptor is involved. Whereas Ca^{2+} oscillations are seen in cells with high expression of FcγRII, a transient Ca^{2+} spike is seen in cells in which FcγRI has been up-regulated. A single spike is preferred as a response for a biosensor detector and therefore FcγRI levels are selectively up-regulated in the single-cell immunosensor.

The mammalian immune system is able to generate 10 million different antibody (or immunoglobulin) molecules, each of which recognizes with great specificity a particular epitope on an antigen. The specific and often high-affinity binding between antibodies and their antigens that forms the basis of the immune response also enables a huge number of analytical applications including clinical assays and purification procedures (de Frutos et al., 1996) and also forms the basis of the protein recognition ability of the single-cell immunosensor. By immobilizing in the cell membrane antibodies against the protein of interest, it is possible to create a specialized detector for that particular molecule. Fig. 1 illustrates the steps involved in the preparation and use of the single-cell immunosensor. Because FcγRI binds IgG molecules through the constant region (Fc), leaving the antigen binding fragments (Fab) available to interact with the protein they recognize, the cell response can be activated by the presence of any protein of interest, provided that antibodies against that protein are immobilized in the membrane.

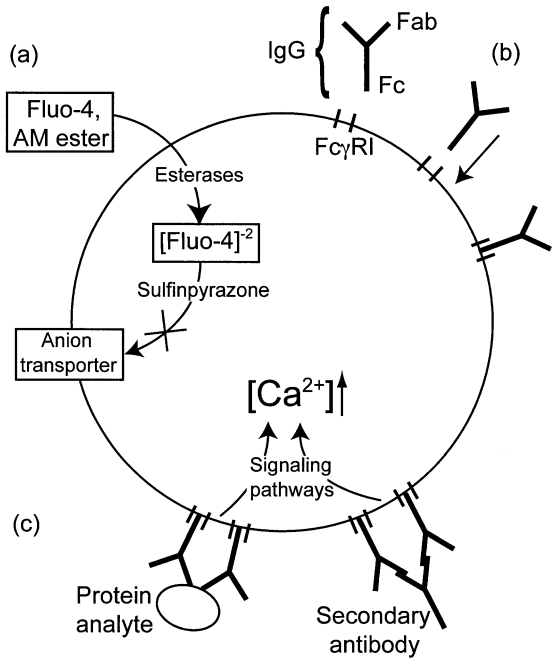

Fig. 1. Schematic showing the steps involved in the preparation and use of the U-937 immunosensor. (a) Cells are incubated with 3-μM fluo-4 acetoxymethyl ester (fluo-4, AM ester) which is able to permeate the membrane. Endogenous cellular esterases remove the ester groups, leaving the active, dianionic form of fluo-4 in the cells. Sulfinpyrazone (0.1 mM) is present at all times to inhibit the activity of cellular anionic transporters. (b) Cells are incubated with polyclonal IgG (human or rabbit; concentration varied between 3 and 15 μM.) The high-affinity Fcγ receptor (FcγRI) binds the constant fragment (Fc) of the antibodies, leaving the antigen-binding fragments (Fab) facing the bath solution. (c) Cross-linking protein (a secondary antibody or target of the primary antibody) was added.

2. Experimental

2.1. Cell culture

The human histiocytic lymphoma cell line U-937 (Sundstrom and Nilsson, 1976) and all solutions for cell culture were purchased from ATCC (Manassas, VA). Cells were maintained in a humidified atmosphere with 5% CO_2 in growth medium consisting of 90% modified RPMI 1640 medium (containing 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 10 mM HEPES, and 1 mM sodium pyruvate) and 10% fetal bovine serum. Cells were maintained at a density of 5×10^5–1×10^6 cell/ml and subcultured twice each week by diluting the cell solution 1:20 with fresh medium.

2.2. Materials

Interferon-γ (IFN-γ), sulfinpyrazone, bovine serum albumin (BSA), human IgG, goat anti-human IgG (Fab and Fc specific), rabbit IgG, goat anti-rabbit IgG, rabbit anti-ovalbumin, and ovalbumin (Grade V) were purchased from Sigma (St. Louis, MO), divided into aliquots, and stored as directed. The cell-permeant, acetoxymethyl ester of fluo-4 (fluo-4, AM), was purchased from Molecular Probes (Eugene, OR) and dissolved in anhydrous DMSO to make a 1 mg/ml stock solution that was stored at $-20\,^{\circ}C$, desiccated, and shielded from light.

2.3. Cell loading

Prior to using the U-937 cells as detectors, they were treated by overnight incubation with IFN-γ (150 ng/ml cell solution) to induce greater expression of the high-affinity Fcγ receptor. For each experiment, 1.5 ml cells were spun down at 200 g for 5 min, the supernatant was aspirated, and the pellet was resuspended in 1 ml Ringer's buffer (5 mM glucose, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM KCl, 160 mM NaCl, and 5 mM HEPES, pH 7.4) containing 0.1 mM sulfinpyrazone. Sulfinpyrazone was used to inhibit cellular anion transporters that would ordinarily shuttle out the active dianionic form of fluo-4 from the cell. Fluo-4, AM stock solution was added to make a 3 μg/ml (3 μM) final concentration in the resuspended cell solution. The cells were allowed to load fluo-4 at room temperature in the dark for 20 min, after which the cells were spun down, the supernatant was aspirated, and the cells were resuspended in clean Ringer's with sulfinpyrazone. The cells were allowed to equilibrate for 20 more min, then were spun down and resuspended again. Next, the cells were treated with primary antibody (either human IgG, rabbit IgG, or rabbit anti-ovalbumin in a concentration between 3 and 15 μM; conditions were optimized for each case) and 2 mg/ml BSA. The cell and antibody solution was kept on ice in the dark for 45 min to allow the antibodies to bind to the Fc receptors on the cell surface. Finally, cells were spun down, rinsed twice, and resuspended in 250 μl Ringer's with sulfinpyrazone to make the cell suspension. Fifty microliters of this cell suspension was placed onto a clean glass coverslip, and 450 μl Ringer's with sulfinpyrazone was added. The cells were then imaged and cross-linking protein (a secondary antibody or the target of the primary antibody) was added to induce a fluorescence signal.

2.4. Fluorescence microscopy

Cell imaging and epifluorescence microscopy were conducted on the stage of a Zeiss Axiovert 135 inverted microscope (Carl Zeiss, Thornwood, NY) equipped with narrow-band fluorescein filters (Chroma, Brattleboro, VT). Light from an arc lamp attached to the back of the microscope was passed through a filter that selected only 470–490 nm light that then bounced off a dichroic mirror. This excitation light was attenuated by a neutral density filter and directed through a 100× oil immersion objective onto the cell preparation. Any fluorescence from the cells was collected by the objective, passed through the dichroic mirror, and filtered by an emission filter that selected 510–530 nm light. A pinhole was placed in the path of the emitted light to minimize background fluorescence. In addition, it was important to use low-fluorescence immersion oil from Zeiss, because all-purpose immersion oil produced prohibitively large amounts of fluorescence. Emitted light was collected by a photomultiplier tube (PMT) (Hamamatsu USA, Bridgewater, NJ). Current from the PMT was converted to voltage by an auto-ranging picoammeter (Keithley, Cleveland, OH) that fed an analog-to-digital converter (National Instruments, Austin, TX) mounted in a PC. Data were collected using a virtual instrument created in LabVIEW (National Instruments) and analyzed using IGOR Pro (WaveMetrics, Lake Oswego, OR).

3. Results and discussion

3.1. Characterizing the single-cell response

U-937 cells were prepared to function as immunosensors by treatment with the Ca^{2+}-indicating dye fluo-4 (which accumulates in the cytosol) and polyclonal human IgG (which localizes in Fcγ receptors in the membrane). A single prepared cell was then chosen, positioned over the objective of a fluorescence microscope, and stimulated by the introduction of goat anti-human IgG. Fig. 2 shows three representative single-cell responses induced by receptor cross-linking. Once the loading conditions were optimized, addition of secondary antibody consistently produced single-cell responses. These responses, resulting from the transient increase of Ca^{2+} levels in the cytosol, were prompt (no detectable delay after the introduction of secondary antibody) and of short duration, usually returning to pre-stimulation levels within a few seconds. When 50 μl of goat anti-human IgG was used as the cross-linking agent (final concentration of 2.5 μM in the cell's environment), the mean area of the response peak was 0.7 ± 0.2 fluores-

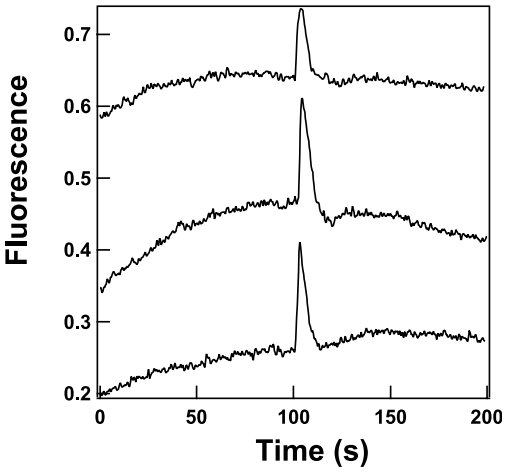

Fig. 2. Representative Ca^{2+} signals induced by receptor cross-linking in three different U-937 cells loaded with human IgG and fluo-4. Fifty microliters of goat anti-human IgG were added at 100 s. Fluo-4/Ca^{2+} fluorescence was excited at ~480 nm and detected at ~520 nm. Trace was smoothed once with a binomial algorithm.

cence units × s, and the mean width (full width at half the maximum signal; FWHM) was 5±2 s (replicate measurements, $n = 23$). When 25 μl of secondary antibody was used (1 μM final concentration in the cell's environment), the mean peak area was 0.5±0.2 fluorescence units × s and the FWHM was 4±2 s (replicate measurements, $n = 15$). In each case the reported error was determined as one S.D. of the mean. The duration of the Ca^{2+} spike reported here is consistent with that observed previously for populations of IFN-γ-treated cells (Davis et al., 1994).

3.2. Single-cell responses to multiple injections of secondary antibody

One desirable characteristic of a biosensor detector is the ability to respond repeatedly. Fig. 3 shows the response of a prepared single U-937 cell to multiple injections of goat anti-human IgG in 20 μl aliquots. When identical stimulation is provided at 40 s intervals, the cell fires a Ca^{2+} spike multiple times, but the response grows smaller with each stimulation. After three additions of secondary antibody, the signal can no longer be distinguished from the background noise (replicate measurements on different cells, $n = 15$). This decrease in cell biosensor response during repeated stimulation has been reported previously (Fishman et al., 1996) and was attributed to saturation of the binding sites on the cell surface and depletion of intracellular Ca^{2+} stores. Another process that will alter the future responsiveness of a stimulated cell is the endocytosis and recycling of cross-linked receptors. IgG-occupied FcγRI have been shown, in confocal fluorescence microscopy studies, to be significantly internalized within 5 min after cross-linking occurs (Harrison et al., 1994). The inconsistent detection of multiple identical analyte bands by a single-cell may present a significant problem that limits a cell as a detector for a separation. As we have previously demonstrated, an array of prepared detector cells can be interrogated, one at a time, by scanning the cells past the outlet of the separation column on the microscope stage (Fishman et al., 1996). For many applications, however, the ability to respond once to the presence of a single analyte is sufficient.

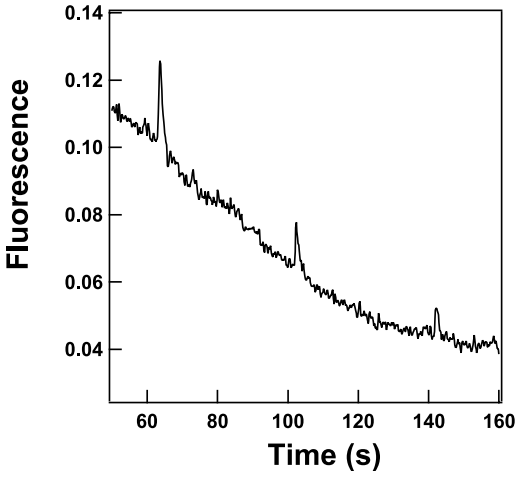

Fig. 3. Ca^{2+} response induced by receptor cross-linking in a single U-937 cell loaded with human IgG and fluo-4. Twenty microliters of goat anti-human IgG were added at 60, 100 and 140 s. Fluo-4/Ca^{2+} fluorescence was excited at ~480 nm and detected at ~520 nm. Trace was smoothed once with a binomial algorithm.

3.3. Single-cell responses using immobilized rabbit IgG

In the U-937 single-cell immunosensor, polyclonal antibodies immobilized in the Fcγ receptors confer the ability to recognize protein analytes. Because U-937 is a human cell line, studies on its physiology and Ca^{2+} signaling have used immobilized human IgG. Most commercially available IgGs are not prepared in humans, however, but are raised in other animals such as rabbit, mouse, or goat. In the original characterization of the high-affinity Fcγ receptor in U-937 (Allen and Seed, 1989), mouse IgG2a and rabbit IgG were shown to bind, but their ability to induce receptor cross-linking was not investigated. It was therefore of interest to characterize the Ca^{2+} signaling of U-937 cells loaded with a non-human IgG. U-937 cells were incubated with rabbit IgG, and 25 μl of goat anti-rabbit IgG was added, which resulted in a final concentration of secondary antibody in the cell environment of 5 μM. For replicate measurements ($n = 14$), the mean peak area was 0.8±0.4 fluorescence units × s and the FWHM was 4±2 s. The observation that the Ca^{2+} signaling induced by rabbit IgG cross-linking is indistinguishable from that induced by human IgG cross-linking is consistent with the idea that the mechanism of Ca^{2+} release depends on the proximity of the cytosolic region of the receptors and not on the nature of the extracellular cross-linking complex. The efficacy of rabbit IgG suggests that commercially available rabbit antibodies should be able to cross-link the receptors and therefore that to detect a protein it is only necessary to purchase or raise a polyclonal rabbit (or mouse) IgG against it.

3.4. Protein detection

We tested the idea that the binding of surface-immobilized IgG to a protein in the environment of a U-937 cell could induce a Ca^{2+} spike. For that purpose U-937 cells were loaded with polyclonal rabbit anti-ovalbumin. An aliquot of ovalbumin solution was then added such that the final ovalbumin concentration in the cell's environment was 150 μM, and the cell response was monitored. Fig. 4 shows two representative single-cell responses to ovalbumin addition. In replicate experiments on different single cells ($n = 9$), the addition of ovalbumin caused a Ca^{2+} spike with a mean area of

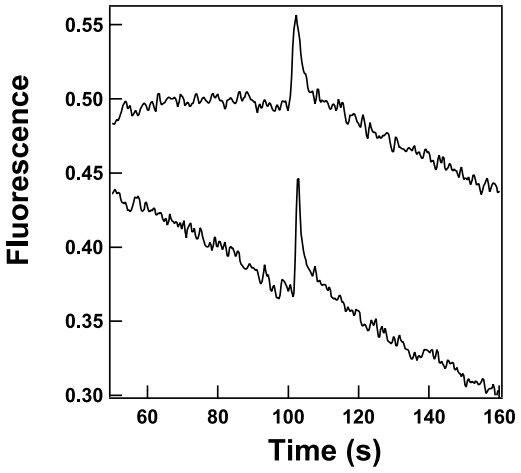

Fig. 4. Representative Ca^{2+} signals induced by receptor cross-linking in two different U-937 cells loaded with rabbit anti-ovalbumin and fluo-4. Fifty microliters of 84.5 mg/ml of ovalbumin were added at 100 s. Fluo-4/Ca^{2+} fluorescence was excited at ~480 nm and detected at ~520 nm. Trace was smoothed once with a binomial algorithm.

0.23 ± 0.08 fluorescence units × s and a FWHM of 2.5 ± 0.9 s. (Error determined as one S.D. of the mean.) This response is smaller in both magnitude and duration than the responses induced by secondary antibody cross-linking. It seems clear that U-937 cells can be used as immunosensors of proteins, but this observation raises interesting questions about the role of protein size in the cross-linking process.

In a current working model for FcγRI activation, the stoichiometry of IgG-FcγRI binding is 1:1 (Kato et al., 2000; Lin et al., 1994; Ravetch, 1994). Immobilized IgG is cross-linked by the binding of multivalent antigen. In the U-937 immunosensor, the protein analyte, though lacking a repeating antigenic motif, activates the cell by functioning as a multivalent antigen when immobilized polyclonal antibodies recognize their diverse cognate epitopes on the protein surface. In this model it is reasonable to expect a minimum size for the protein analyte that can be detected; if the protein is too small, it will be impossible for two antibodies to bind it, and thus the required cross-linking cannot occur. The molecular weight of ovalbumin is 45 kD; IgG is much heavier, with a molecular weight of 150 kD. As determined by crystallography (Stein et al., 1991), ovalbumin is an ellipsoid with dimensions 70 Å × 45 Å × 50 Å. The structure of IgG has been determined by X-ray crystallography (Marquart et al., 1980; Silverton et al., 1977), and by scanning tunneling microscopy (Leatherbarrow et al., 1991). These techniques give slightly different values for the exact molecular dimensions of IgG. Nevertheless, they agree that the molecule is Y-shaped with a longest dimension of approximately 120 Å. Our data suggest that proteins as small as 45 kD can cross-link the receptors and therefore be detected by the U-937 immunosensor, but that larger proteins can be detected at lower concentrations because they are more efficient cross-linking agents.

4. Conclusion

The strength of the single-cell immunosensor is how easily the detector may be customized to respond to the protein of interest. Within the constraints that the protein be large enough to induce cross-linking and that a polyclonal antibody against it be available, any protein can be detected. The immunosensor described here is a qualitative device. We have not investigated in detail the response versus concentration of a cell. We did note, however, that the difference in the areas and widths of the response when the smaller ovalbumin molecule is used compared to the larger IgG indicates that the size of the surface-bound immune complex can alter the magnitude and duration of the response.

Although the limits of detection of the immunosensor in the configuration described here are fairly high, it should be straightforward to improve the limit of detection by decreasing the volume surrounding the prepared detector cells and, therefore, the dilution of the injected sample. Recently, we have demonstrated the miniaturization of the single-cell immunosensor platform by performing the loading and cross-linking steps within a microfluidic device made from poly(dimethylsiloxane). In this microfluidic platform, reagent consumption is decreased by 10^5, with a corresponding improvement in limit of detection. A description of this microfluidic device appears in a report that has been accepted for publication in the journal *Analytical Chemistry*. The prompt, short-lived signal from the single-cell immunosensor suggests its potential use as a post-column detector in microscale separations, perhaps in the context of a microfluidic device. Moreover, the ease with which the single-cell immunosensor can be customized opens the possibility of the creation of single-cell immunosensor arrays, which might offer an effective way to screen for many analytes at once.

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References

Allen, J.M., Seed, B., 1989. Isolation and expression of functional high-affinity Fc receptor complementary DNAs. Science 243 (4889), 378–381.

Campanella, L., Favero, G., Mastrofini, D., Tomassetti, M., 1997. Further developments in toxicity cell biosensors. Sensor. Actuator. B 44 (1–3), 279–285.

Davis, W., Sage, S.O., Allen, J.M., 1993. Fc receptor mediated calcium signaling in differentiated and undifferentiated U937 cells. Biochem. Soc. Trans. 21 (4), 3365.

Davis, W., Sage, S.O., Allen, J.M., 1994. Cytosolic calcium elevation in response to Fc receptor cross-linking in undifferentiated and differentiated U937 cells. Cell Calcium 16 (1), 29–36.

de Frutos, M., Paliwal, S.K., Regnier, F.E., 1996. Analytical immunology. Methods Enzymol. 270 (1), 82–101.

Fishman, H.A., Orwar, O., Allbritton, N.L., Modi, B.P., Shear, J.B., Scheller, R.H., Zare, R.N., 1996. Cell-to-cell scanning in capillary electrophoresis. Anal. Chem. 68 (7), 1181–1186.

Floto, R.A., Mahaut-Smith, M., Allen, J.M., 1995a. IFN-γ and dibutyryl cAMP pre-treatment of U937 cells results in different Ca^{2+} signals triggered by antibody cross-linking of the human high affinity receptor for IgG (FcγRI). Biochem. Soc. Trans. 23 (1), S122.

Floto, R.A., Mahaut-Smith, M.P., Somasundaram, B., Allen, J.M., 1995b. IgG-induced Ca^{2+} oscillations in differentiated U937 cells; a study using laser scanning confocal microscopy and co-loaded fluo-3 and fura-red fluorescent probes. Cell Calcium 18 (5), 377–389.

Harrison, P.T., Davis, W., Norman, J.C., Hockaday, A.R., Allen, J.M., 1994. Binding of monomeric immunoglobulin G triggers FcγRI-mediated endocytosis. J. Biol. Chem. 269 (39), 24396–24402.

Jardemark, K., Orwar, O., Jacobson, I., Moscho, A., Zare, R.N., 1997. Patch clamp detection in capillary electrophoresis. Anal. Chem. 69 (17), 3427–3434.

Johannessen, E.A., Weaver, J.M.R., Bourova, L., Svoboda, P., Cobbold, P.H., Cooper, J.M., 2002. Micromachined nanocalorimetric sensor for ultra-low-volume cell-based assays. Anal. Chem. 74 (9), 2190–2197.

Kato, K., Fridman, W.H., Arata, Y., Sautes-Fridman, C., 2000. A conformational change in the Fc precludes the binding of two Fcγ receptor molecules to one IgG. Immunol. Today 21 (7), 310–312.

Leatherbarrow, R.J., Stedman, M., Wells, T.N.C., 1991. Structure of immunoglobulin G by scanning tunnelling microscopy. J. Mol. Biol. 221 (2), 361–365.

Lin, C.-T., Shen, Z., Boros, P., Unkeless, J.C., 1994. Fc receptor-mediated signal transduction. J. Clin. Immunol. 14 (1), 1–13.

Luzzi, V., Murtazina, D., Allbritton, N.L., 2000. Characterization of a biological detector cell for quantitation of inositol 1,4,5-trisphosphate. Anal. Biochem. 277 (2), 221–227.

Marquart, M., Deisenhofer, J., Huber, R., 1980. Crystallographic refinement and atomic models of the intact immunoglobulin molecule Kol and its antigen-binding fragment at 3.0 A and 1.9 A resolution. J. Mol. Biol. 141 (4), 369–391.

McConnell, H.M., Owicki, J.C., Parce, J.W., Miller, D.L., Baxter, G.T., Wada, H.G., Pitchford, S., 1992. The cytosensor microphysiometer: biological applications of silicon technology. Science 257 (5078), 1906–1912.

Minta, A., Kao, J.P.Y., Tsien, R.Y., 1989. Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores. J. Biol. Chem. 264 (14), 8171–8178.

Orwar, O., Jardemark, K., Jacobson, I., Moscho, A., Fishman, H.A., Scheller, R.H., Zare, R.N., 1996. Patch-clamp detection of neurotransmitters in capillary electrophoresis. Science 272 (5269), 1779–1782.

Ravetch, J.V., 1994. Fc receptors: rubor redux. Cell 78 (4), 553–560.

Shear, J.B., Fishman, H.A., Allbritton, N.L., Garigan, D., Zare, R.N., Scheller, R.H., 1995. Single cells as biosensors for chemical separations. Science 267 (5194), 74–77.

Silverton, E.W., Navia, M.A., Davies, D.R., 1977. Three-dimensional structure of an intact human immunoglobulin. Proc. Natl. Acad. Sci. USA 74 (11), 5140–5144.

Stein, P.E., Leslie, A.G.W., Finch, J.T., Carrell, R.W., 1991. Crystal structure of uncleaved ovalbumin at 1.95 A resolution. J. Mol. Biol. 221 (3), 941–959.

Sundstrom, C., Nilsson, K., 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer 17 (5), 565–577.

Taylor, L.C., Walt, D.R., 2000. Application of high-density optical microwell arrays in a live-cell biosensing system. Anal. Biochem. 278 (2), 132–142.

Zahn, M., Renken, J., Seeger, S., 1999. Fluorimetric multiparameter cell assay at the single cell level fabricated by optical tweezers. FEBS Lett. 443 (3), 337–340.