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# Release of Intracellular Proteins by Electroporation with Preserved Cell Viability

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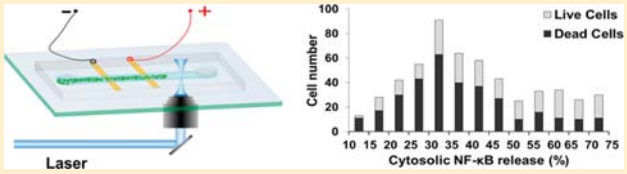
Supporting Information

**ABSTRACT:** Extraction of intracellular proteins from cells is often an important first step for conducting molecular biology and proteomics studies. Although ultrasensitive detection and analytical technology at the single molecule level is becoming routine, protein extraction techniques have not followed suit and still call for complete lysis that leads to cell death. In principle, with refined extraction techniques, intracellular proteins can potentially be extracted without killing the cell. In this Letter, we demonstrate that electroporation is capable of releasing intracellular proteins from adherent Chinese hamster ovary cells while preserving the cell viability. By tuning the duration and intensity of an electric pulse, we were able to control the average amount of protein release and the percentage of viable cells after the operation. Our results indicate that a substantial fraction of the cell population was able to release proteins under electroporation and survive the procedure. Interestingly, at the single cell level, the probability for cell death does not increase with more protein release. This work paves the way to extracting and analyzing intracellular proteins while keeping cells live.




Proteins constitute most of the dry mass of a cell and execute the vast majority of cell functions. Modern proteomics research has advanced to global scale analysis of protein expressions and functions.[1] The evaluation of abundance and modification/association state of cellular proteins enables the establishment of links between proteomics and physiological/pathological states. Such information has direct biomedical relevance to diagnosis/prognosis and drug discovery.

There have been a variety of methods developed over the time for extraction of cellular proteins for analysis, including chemical lysis,[2−4] optoporation,[5−7] and electroporation.[8−12] However, cell death is typically associated with the release of intracellular materials in these previous demonstrations. Although laser lysis[13] or tweezing[14] was used for subcellular disruption and manipulation due to its high spatial resolution and potentially offers preserved viability for cells, such technique requires high-precision manipulation and specialized laser setup. Cellular analysis with low invasiveness is desirable because such procedure potentially allows periodical sampling of the same cell(s) and continuous monitoring of cellular dynamics at the molecular level. The technological progress toward ultrasensitive molecular assays makes testing based on trace amount of molecules increasingly accessible. However, it remains a challenge to identify a method that can reproducibly extract a tiny amount of cellular proteins without killing the cells.

In this work, we explore using electroporation to release intracellular proteins while preserving the cell viability. Electroporation occurs when cells experience an external electrical field with its intensity beyond a threshold.[15,16] During electroporation, the electrical field opens nanoscale pores (or creates instability) in the cell membrane that permit uptake of outside macromolecules or release of intracellular molecules. In comparison to other methods, there are a couple of traits of electroporation that potentially permit minimally invasive probing of the intracellular contents: (1) Electroporation, when its parameters are tuned properly, can be a transient and reversible process. The electropores may eventually reseal themselves after removal of the field and allow cells to survive. (2) The main operational parameters of electroporation include field intensity and duration. They can be precisely and reproducibly controlled.

In our experiment, we applied electric pulses of defined intensity and duration to adherent Chinese hamster ovary (CHO) cells cultured in a microfluidic channel. The release of an intracellular protein (a transcription factor NF-κB tagged by

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green fluorescent protein) was quantified by laser-induced fluorescence (LIF) detection in the downstream under a slow sweeping flow. We found that a significant subset of the electroporated cells preserved their viability after protein release. Furthermore, when observed at the single cell level by imaging, a higher percentage of protein release does not coincide with higher probability for cell death. The results point to the feasibility of the use of electroporation for minimally invasive and real-time analysis of live cells.

Figure 1a shows the setup for the electroporation device. We used a simple microfluidic channel with two planar electrodes on the glass substrate surface. We cultured CHO cells that expressed NF-κB tagged by GFP in the channel with a monolayer of cells adherent to the glass substrate. NF-κB is a family of dimeric transcription factors (with the most abundant being p50/p65 heterodimer having a molecular weight of 115 kDa) whose subcellular localization can be varied between the cytosol and the nucleus.[12,17−19] Electric pulses with defined field intensity and duration were generated by a relay controlled by a LabVIEW program via a data acquisition card and a power supply that provided constant dc voltage. Figure 1b shows that there was drastic decrease in the cell fluorescence after the electroporation (one pulse of 800 V/cm and 100 ms) due to protein release. In order to quantify the amount of NF-κB released from cells, there was a constant flow in the channel established by a syringe pump. The GFP-tagged NF-κB released by an electric pulse was swept and detected in the downstream by laser-induced fluorescence (LIF), and its amount was quantified by integrating the area under the fluorescence intensity over the time curve (as shown in Figure 1c).

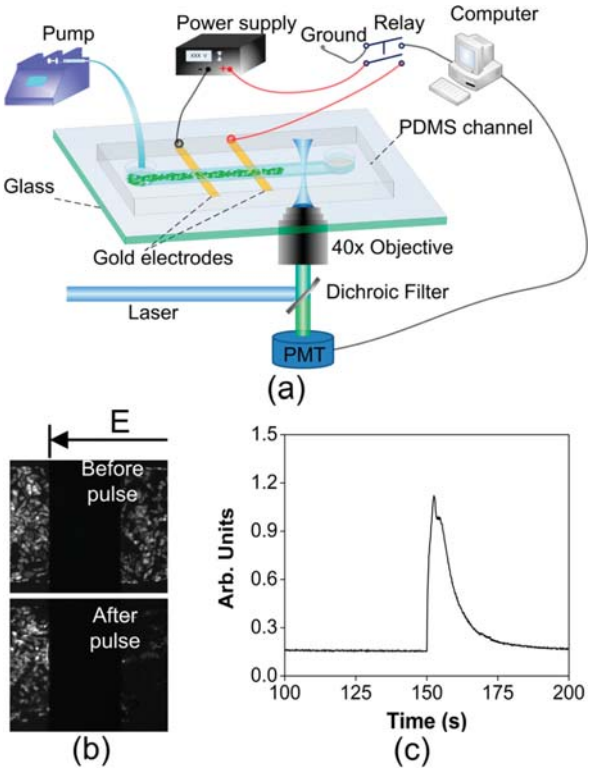


**Figure 1.** Experimental setup and detection of protein release. (a) Electroporation in a microfluidic channel. The channel was 7 mm long, 500 μm wide, and 36 μm deep. The electrodes were 300 μm wide, with the distance between the two electrodes being 3 mm. A constant flow (1 μL/min) was maintained in the channel to sweep the released protein to the downstream for LIF detection. The electric pulse was generated by a relay and a power supply that provided constant dc voltage. (b) The fluorescence images of the cells in the channel before and after a pulse of 800 V/cm and 100 ms. The dark area was one of the electrodes. (c) The LIF signal detected over time in the downstream after application of a pulse (600 V/cm and 50 ms).

We examined the percent release of NF-κB when it was in the cytosol and the cell viability after electroporation under various conditions (Figure 2). We first created a calibration curve that characterized the relationship between the number of cells lysed by chemical lysis (considered 100% release) and the peak area generated by the lysate (Supporting Information Figure S1). The percent release of NF-κB from a known number of cells could then be calculated by quantifying the peak area generated by electroporation-based release in reference to the calibration curve. NF-κB locates in the cytosol without activation.[19] NF-κB release here is expected to be representative of cytosolic proteins with similar molecular weights.[20] Figure 2a shows that the release of cytosolic NF-κB under a single pulse occurred more substantially under longer duration and higher field intensity. Electroporation has a threshold of 300−400 V/cm for CHO cells.[21] However, with a duration of 10 ms, a significant release of NF-κB was seen only when the field intensity was increased to 900 V/cm, presumably due to the molecule's relatively large size. The longer duration

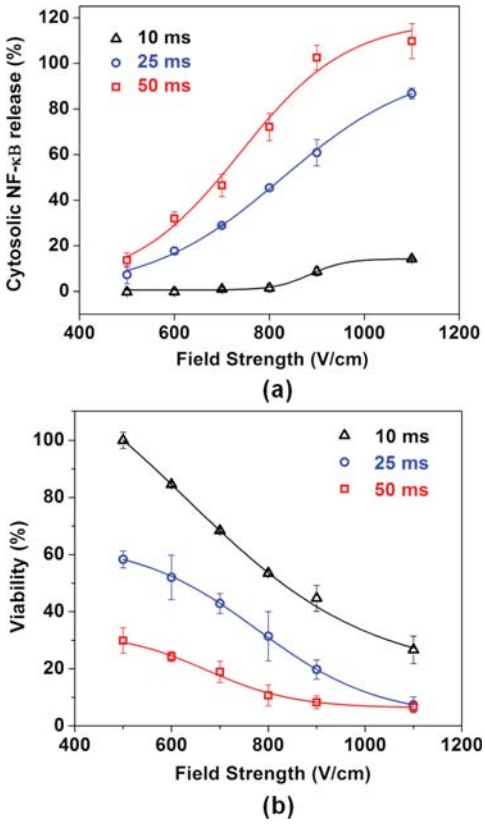


**Figure 2.** The percent release of cytosolic NF-κB (a) and the percent cell viability measured at 24 h after electroporation (b) after applying a single pulse with various field intensities and durations. The field intensity was calculated by the overall voltage divided by the distance between the two electrodes.


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substantially increased the amount released. At 1100 V/cm, the percent release was 10% at 10 ms duration, 82% at 25 ms, and 100% (shown as 110% due to error associated with the use of the calibration curve, detailed in Supporting Information) at 50 ms. Figure 2b shows that the cell viability decreased with higher field intensity and longer duration. The viability was 100% at 10 ms and 500 V/cm and decreased sharply to 58% at 25 ms and 29% at 50 ms with the same field intensity. The percent viability here was estimated on the basis of the entire cell population in between the two electrodes. It is worth noting that the cell death rate across the area was not uniform. Cells that were close to the electrodes were killed at a higher percentage than those at the center, presumably due to the stronger field in the immediate vicinity of the electrodes and pH variation introduced by water electrolysis. The results in Figure 2 are on extraction of NF-$\kappa$B when it is in the cytosol (not activated). As expected, when NF-$\kappa$B moves from the cytosol to the nucleus (i.e., after cells stimulated by IL-1$\beta$), the percent extraction became substantially lower under the same electric parameters (Supporting Information Figure S2).[12]

The above measurements were taken at the population level. We also examined the electroporation and the associated protein release at the single cell level. First, the images in Figure 3 confirm that a significant percentage of the cells were electroporated and then survived the procedure. As we can see from Figure 3a, electroporation occurred universally to all cells in the channel with a field intensity of 800 V/cm and duration of 25 ms, judged by the visible expansion in the nucleus.[22] By mapping the surviving cells against the original population (Figure 3b), we found that 61% of cells in this particular local area survived electroporation under 800 V/cm and 25 ms (it needs to be noted that the overall survival rate across the area between the two electrodes is around 31% as shown in Figure 2). Furthermore, the cell death in this case appears to be stochastic (i.e., there are no common visible characteristics among the surviving cells in terms of their sizes and morphology). Additional movies (Supporting Information) are available to show electroporation of cells under various conditions (one single pulse of 500 V/cm and 10 ms; 800 V/cm and 25 ms; 1100 V/cm and 50 ms).

In Figure 4, we examined the percent protein release from single cells when they were all exposed to a pulse of 800 V/cm and 25 ms (by comparing the fluorescence image of single cells before and after electroporation) and then compiled the histogram to link the protein release to the cell fate (live/dead). The cell population appears to roughly exhibit a normal distribution in terms of the percent release with the largest number of cells releasing a medium level of the protein (25–45%). However, there was no indication that a higher percent of protein release was associated with a higher probability for cell death at the single cell level. Counterintuitively, the cell death probability appears to be lower at the higher end of the protein release. This seems to suggest possible recovery mechanisms that are only triggered by larger amount of protein release and help maintain cell viability after the procedure. The details of such mechanisms will require further systematic studies.

Single cell analysis is important for understanding the stochastic cellular processes that often vary drastically from cell to cell[23−28] and observing temporal dynamics that lack synchronization among different cells.[29] Although protein dynamics can be observed using microscopic tools without breaching the membrane (e.g., when the protein is tagged by a fluorescent protein marker), protein release and subsequent labeling are typically necessary when primary cells are studied and the protein needs to be labeled by a means such as fluorescent antibody binding.[30] Our results suggest that

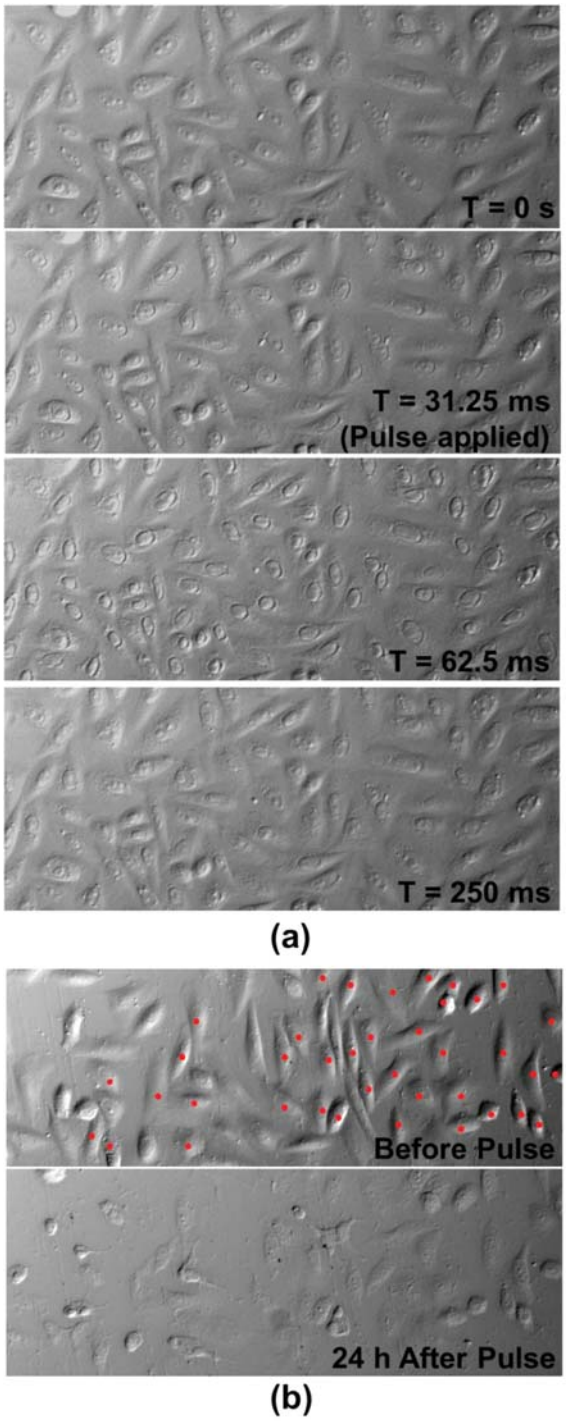


**Figure 3.** The DIC images of CHO cells before, during, and after electroporation when a pulse of 800 V/cm and 25 ms was applied. (a) The image series that showed the dynamic change in the cell morphology throughout the electroporation process. (b) The images of cells before and 24 h after the application of the pulse. The cells that were live 24 h after electroporation are marked by red dots in the upper image.


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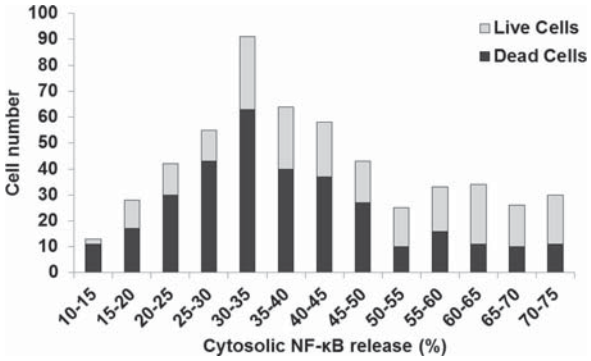


**Figure 4.** The histogram showing the percent protein release and the associated cell fate (live/dead) analyzed at the single cell level. The percent protein release was obtained by examining fluorescence images of single cells before and after electroporation. A pulse of 800 V/cm and 25 ms was applied. The cell viability was determined 24 h after electroporation.

releasing intracellular proteins while preserving cell viability is very feasible when electroporation is used. For example, starting with a population of cells, electroporation can be used to extract cellular proteins and analysis at the single cell level can be conducted. When operated with optimized parameters, a significant fraction of cells will survive the procedure and thus the same protein extraction procedure can be potentially carried out multiple times to observe temporal dynamics. Because the probability of cell death does not increase with more complete protein release, we can expect that, at least for some of the surviving cells, the protein extraction is substantial and allows meaningful single cell measurement. Depending on the cellular process under investigation, the impact of the electroporation as a perturbation may need to be studied and identified in order to correctly interpret the result. We envision that our approach may provide unique insights into cellular biology and proteomics at the single cell level.

## ■ ASSOCIATED CONTENT

### Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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