Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

Capacitance - Based Assay for Real - Time Monitoring of Endocytosis and Cell Viability

Rimi Lee, Jihun Kim, Sook Young Kim, Seon Mi Jang, Sun Mi Lee, In-Hong Choi, Seung Woo Park, Jeon-Soo Shin*, Kyung-Hwa Yoo*

Supplementary Note

According to the dielectric model, the dielectric constant at low frequencies in the linear regime of small electric fields is approximated as:

$$\varepsilon \approx \frac{9v\varepsilon_e}{16}\chi^2 a^2 \frac{(\mathrm{R}^+ - \mathrm{R}^-)^2 \mathrm{S}^2}{(\mathrm{R}+2)^2}$$

where

$$\chi = \sqrt{\frac{2\sigma_e}{(D^+ + D^-)\varepsilon_e}}$$

$$R^{\pm} = \frac{4}{\chi a}\left(exp\left(\mp\frac{e\xi}{2kT}\right) - 1\right)(1 + 3m^{\pm}) \pm \frac{6m^{\pm}e\xi}{\chi akT}$$

$$m^{\pm} = \frac{2\varepsilon_e}{3\eta D^{\pm}}\left(\frac{kT}{e}\right)^2$$

$$R = \frac{D^+R^+}{D^+ + D^-} + \frac{D^-R^-}{D^+ + D^-}$$

$$S = \frac{\frac{2(R+2)}{(R^+ + 2)(R^- + 2)}}{1 - P\frac{R+2}{(R^+ + 2)(R^- + 2)}}$$

$$P = \frac{D^+ + D^-}{2D^+D^-}\frac{48D^{\pm}m^{\pm}}{\chi a}\ln\left[\cosh\left(\frac{e\xi}{4kT}\right)\right]$$

Here, v is the volume fraction occupied by the cells; a is the cell radius; ε_e is the absolute permittivity, $\mathrm{D}^{\pm}$ are the diffusion coefficients of counterions and co-ions, ξ is the Zeta potential, and η is the viscosity. The electrolyte solution conductivity, σ_e, and absolute permittivity, ε_e, are considered to be independent of the frequency at low frequencies. Other constants include e = 1.602 × 10^{-19} C, k = 1.381 × 10^{-23} J/K, T = 310 K and ε_e= 78.5 ×

8.85×10^{-12} C^2/Nm^2 [1,2].

In order to calculate the dielectric constant, we must know the Zeta potential. Based on the Poisson-Boltzmann relation, the relative Zeta potential $|Z|=\xi_\beta/\xi_0$ in the presence of extracellular particles is given by

$$|Z| = \exp(m_{binding} \cdot \frac{\beta_{binding}}{2} - m_{int} \cdot \frac{\beta_{int}}{2}) \qquad \text{(Eq. S1)}$$

where $m_{binding}$ is the total mass of particles binding to the cell; m_{int} is the total mass of particles internalized within the cell, and $\beta_{binding}$ and β_{int} are the parameters that measure how the particles are bound to the cell and how the internalized particles affect the free energy of the ions at a distance from the surface, respectively[3,4]. In the Debye-Huckel approximation assuming the uniform distribution of nanoparticles in the cell interface, $\beta_{binding}$ and β_{int} are approximated to be constant. Then, from a Langmuir adsorption model, $m_{binding}$ and m_{int} are numerically estimated using the following differential equations.

$$\frac{dm_{int}(t)}{dt} = k_a C\big(m_0 - m_{binding}(t)\big) - k_d m_{binding}(t) - \frac{dm_{binding}(t)}{dt} \qquad \text{(Eq. S2)}$$

$$\frac{dm_{int}(t)}{dt} = k_i(\varphi_0 - \varphi_{int}(t))m_{binding}(t) \qquad \text{(Eq. S3)}$$

$$\frac{d\varphi(t)}{dt} = k_i(\varphi_0 - \varphi_{int}(t)) \qquad \text{(Eq. S4)}$$

where m_0 is the maximum mass of particles that can be attached to the cell membrane, k_a is the adsorption rate, k_d is the desorption rate, k_i is the internalization constant, φ_{int} is the fraction of the reactive surface being internalized, and φ_0 is the maximum fraction of reactive surfaces.

Reference

1. M. Tirado, C. Grosse, W. Schrader, and U. Kaatze, *J. Non-Cryst. Solids*, 2001, **305**, 373.

2. E. Prodan, C. Prodan, and J. H. Miller, *Biophys. J.*, 2008, **95**, 4174.

3. Y. Zhang, *et al.* Zeta , *Biomed. Microdevice*, 2008, **10**, 321.

4. Y. Zhang, *et al. Small*, 2009, **5**, 1990.

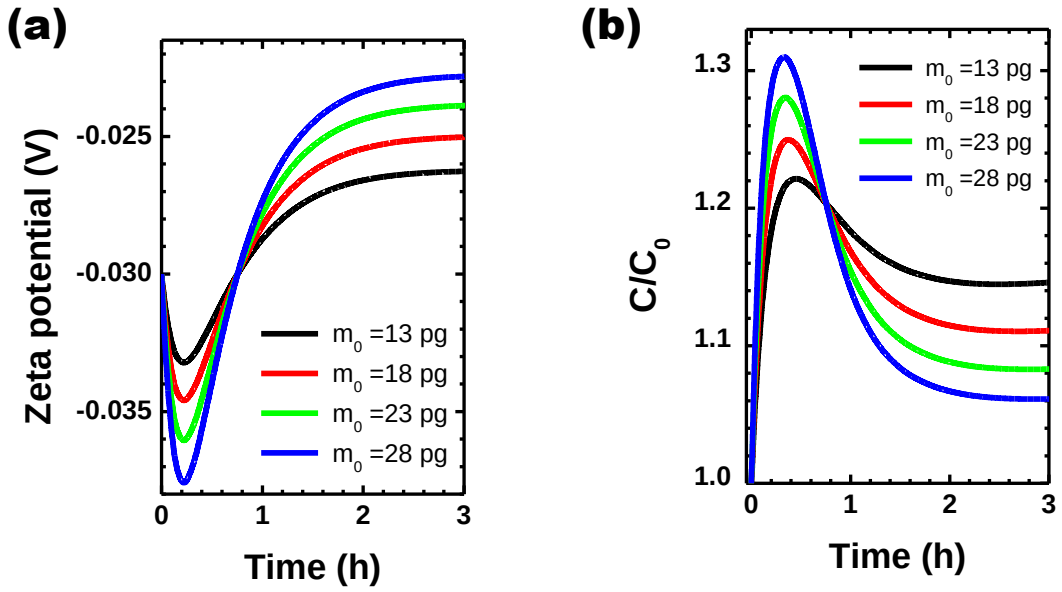

Fig. S1 Time dependence of Zeta potential (a) and capacitance (b) calculated for different values of m_0, assuming the parameters obtained by fitting the data from SK-BR-3 cells treated with Herceptin antibodies to Eq. 1.

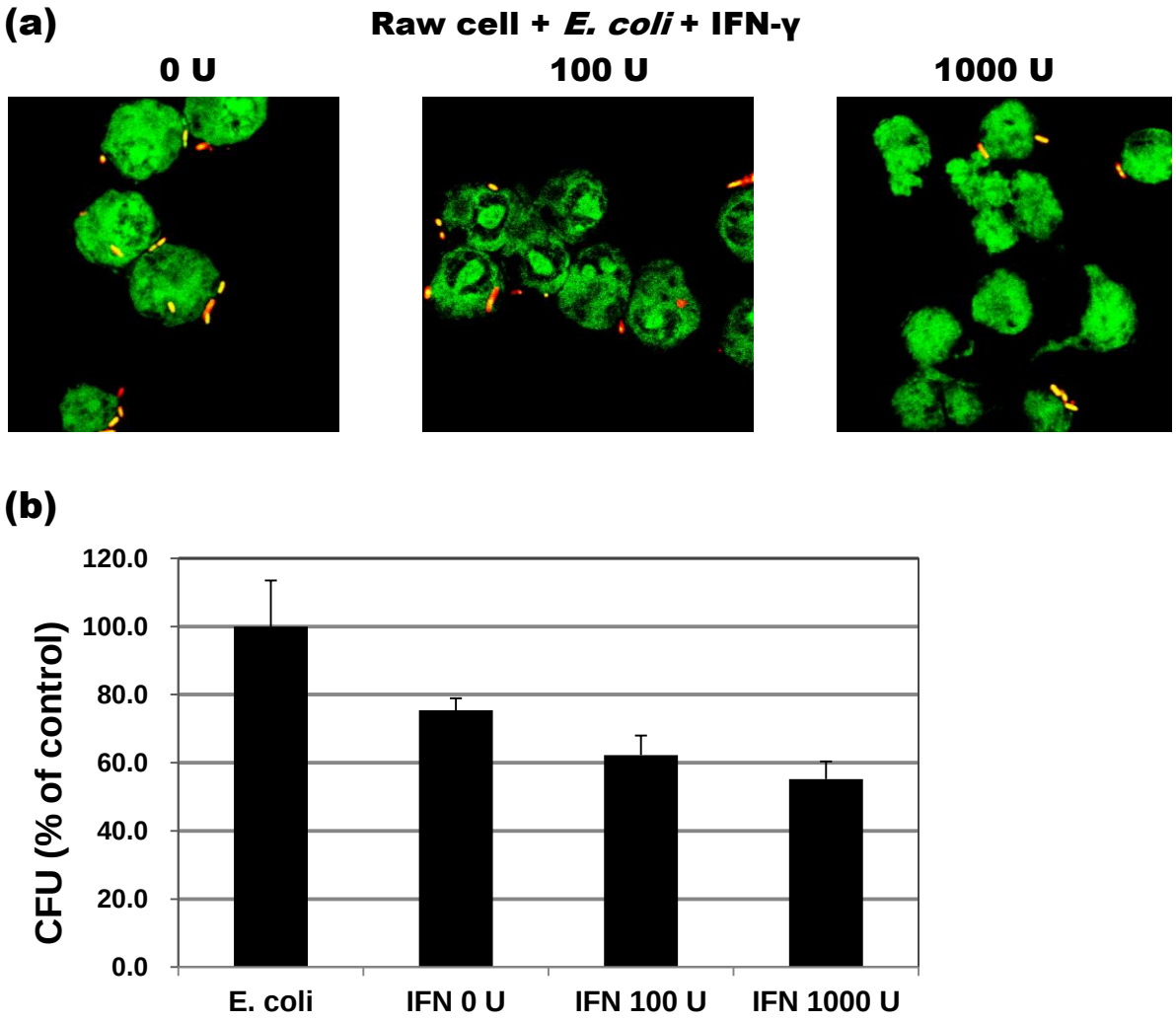

Fig. S2 RAW 264.7 cells were incubated with different concentrations of IFN-γ in the presence of 500 MOI of mCherry fluorescence protein-expressing ampicillin-resistant *E. coli* for 15 min, and then washed with PBS to observe cells by confocal microscopy after harvesting the culture supernatant. RAW 264.7 cells were stained with syto-9, green fluorescent nucleic acid stain, to observe the cells easily (a). The culture supernatants, which contained *E. coli* that was not endocytosed by RAW 264.7 cells, were harvested and cultured on the LB + ampicillin agar plate to measure the decrease of *E. coli* numbers (b).

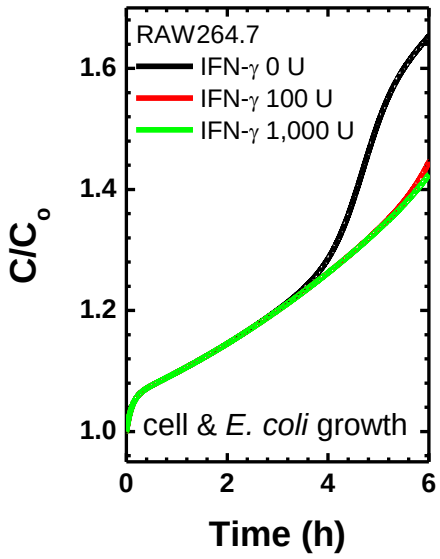

Fig. S3 Time dependence of C/C_0 for the growth of RAW 264.7 and *E. coli* assumed to fit the data in Fig. 3b to Eq. 1.

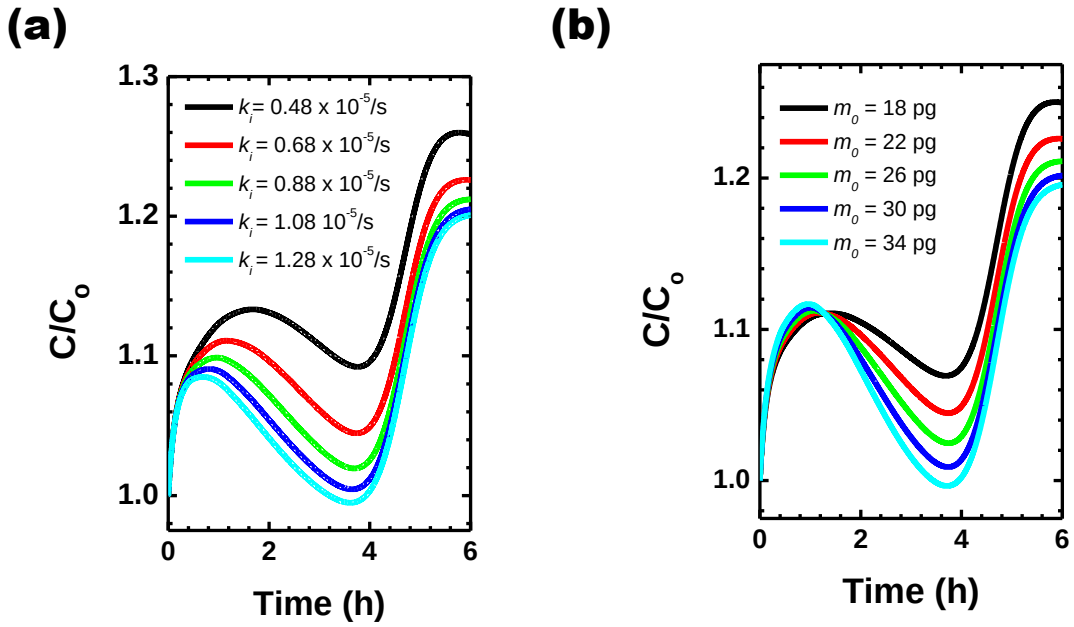

Fig. S4 Time dependence of C/C_0 calculated for different values of k_i (a) and m_0 (b), assuming the parameters obtained by fitting the data of RAW 264.7 cells treated with *E. coli* to Eq. 1.

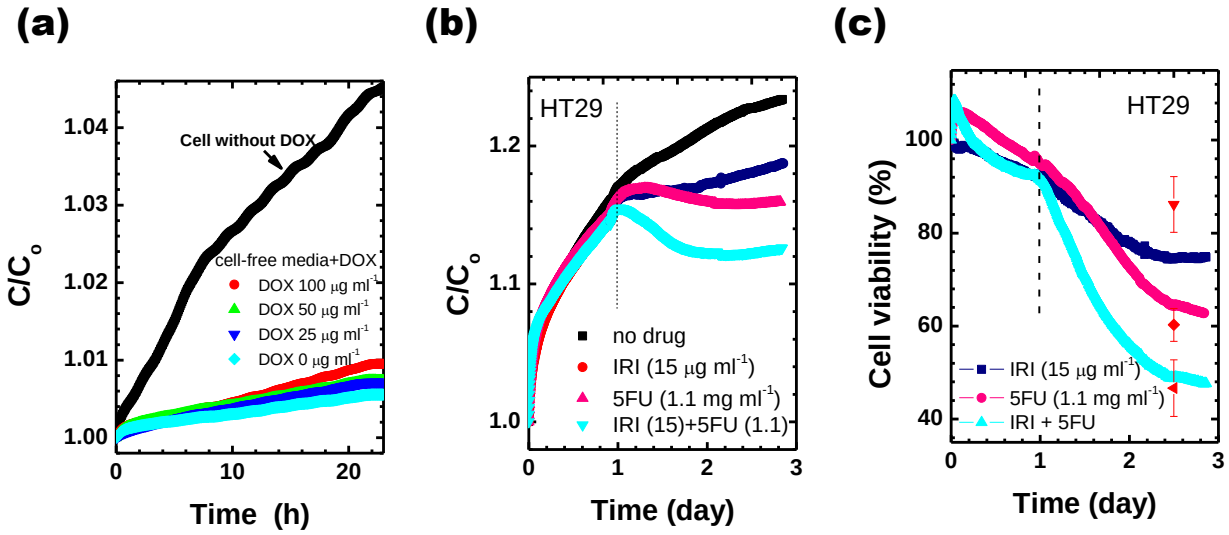

Fig. S5 (a) Time dependence of C/C_0 measured for cell-free media containing different concentrations of DOX and for SKMES-WT cells that were not treated with DOX. (b) Time dependence of C/C_0 measured for HT29 cells before and after adding irinotecan (15 μg ml^{-1}, IC 20), 5-fluorouracil (1.1 mg ml^{-1}, IC 50), and both irinotecan and 5-fluorouracil. The dashed line indicates the time that the drugs were added. (c) Time dependence of cell viability estimated by Eq. 2 for Panc-1 cells treated with cisplatin (6.25 μg ml^{-1}), gemcitabine (1.7 mg ml^{-1}), and both cisplatin and gemcitabine. The red symbols represent the cell viability measured using the MTT assay.

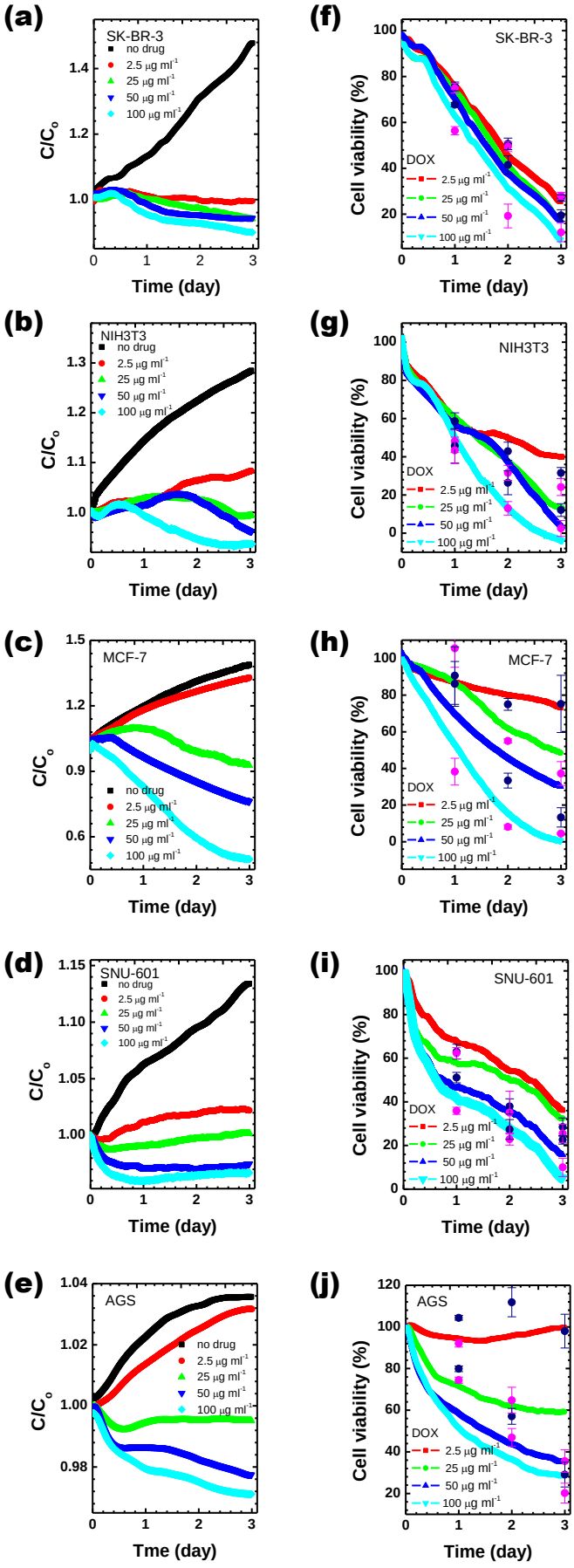

Fig. S6 Time dependence of C/C_0 measured for SK-BR-3 (a), NIH3T3 (b), MCF-7(c), SNU-601 (d), and AGS cells (e) treated with different concentrations of DOX (2.5, 10, 50, and 100 μg ml^{-1}). Time dependence of cell viability estimated by Eq. (2) for SK-BR-3 (f), NIH3T3 (g), MCF-7(h), SNU-601 (i), and AGS cells (j) treated with different concentrations of DOX (2.5, 10, 50, and 100 μg ml^{-1}). The symbols represent the cell viability measured using the MTT assay.

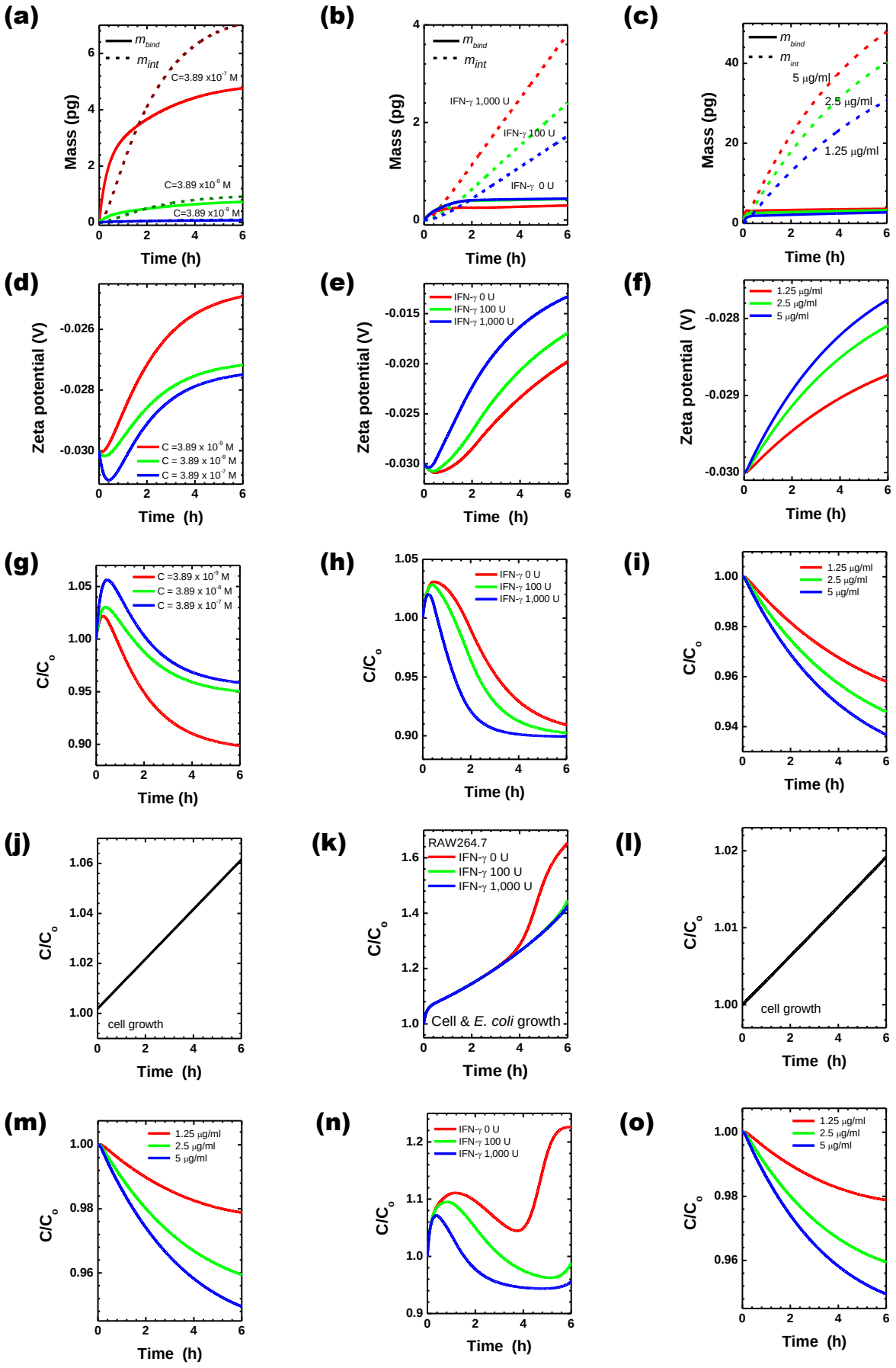
(a)
m_{bind}
m_{int}
C=3.89 x10^{-7} M
C=3.89 x10^{-8} M
C=3.89 x10^{-9} M
Mass (pg)
Time (h)
(b)
m_{bind}
m_{int}
IFN-γ 1,000 U
IFN-γ 100 U
IFN-γ 0 U
Mass (pg)
Time (h)
(c)
m_{bind}
m_{int}
5 μg/ml
2.5 μg/ml
1.25 μg/ml
Mass (pg)
Time (h)
(d)
Zeta potential (V)
C =3.89 x 10^{-9} M
C = 3.89 x 10^{-8} M
C = 3.89 x 10^{-7} M
Time (h)
(e)
IFN-γ 0 U
IFN-γ 100 U
IFN-γ 1,000 U
Zeta potential (V)
Time (h)
(f)
1.25 μg/ml
2.5 μg/ml
5 μg/ml
Zeta potential (V)
Time (h)
(g)
C =3.89 x 10^{-9} M
C = 3.89 x 10^{-8} M
C = 3.89 x 10^{-7} M
C/C$_o$
Time (h)
(h)
IFN-γ 0 U
IFN-γ 100 U
IFN-γ 1,000 U
C/C$_o$
Time (h)
(i)
1.25 μg/ml
2.5 μg/ml
5 μg/ml
C/C$_o$
Time (h)
(j)
C/C$_o$
cell growth
Time (h)
(k)
RAW264.7
IFN-γ 0 U
IFN-γ 100 U
IFN-γ 1,000 U
C/C$_o$
Cell & *E. coli* growth
Time (h)
(l)
C/C$_o$
cell growth
Time (h)
(m)
1.25 μg/ml
2.5 μg/ml
5 μg/ml
C/C$_o$
Time (h)
(n)
IFN-γ 0 U
IFN-γ 100 U
IFN-γ 1,000 U
C/C$_o$
Time (h)
(o)
1.25 μg/ml
2.5 μg/ml
5 μg/ml
C/C$_o$
Time (h)

Fig. S7 Simulated total mass of molecules binding to the cell surface (m_{bind}) and simulated total mass of molecules internalized into the cell (m_{int}) for receptor-mediated endocytosis (a), phagocytosis (b), and pinocytosis (c). Calculated Zeta potential using Eq. (S1) for receptor-mediated endocytosis (d), phagocytosis (e), and pinocytosis (f). Calculated capacitance using Eq. (1) without including the capacitance increase due to the growth of cells and *E. coli* for receptor-mediated endocytosis (g), phagocytosis (h), and pinocytosis (i). Assumed capacitance increases due to the growth of cells and *E. coli* for receptor-mediated endocytosis (j), phagocytosis (k), and pinocytosis (l). Calculated capacitance using Eq. (1) and including the capacitance increases due to the growth of cells and *E. coli* for receptor-mediated endocytosis (m), phagocytosis (n), and pinocytosis (o).