Supporting Information

Measuring binding kinetics of antibody-conjugated gold nanoparticles with intact cells

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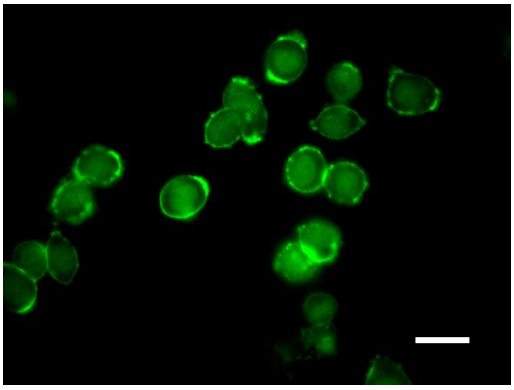

Figure S1. Immuno-fluoresence image of Her2 on SKBR3 cells. The fixed cells were incubated in 10 mg/mL Herceptin solution for 30 minutes, followed by another 30 minutes incubation with Alexa Fluor 488 goat anti-human IgG1 after 3 times rinses with PBS at room temperature, and then a set of optical filters (Ex420-480/Em515) to obtain immunofluorescence images after the well was rinsed twice by PBS buffer. Scale bar: 50 μm.

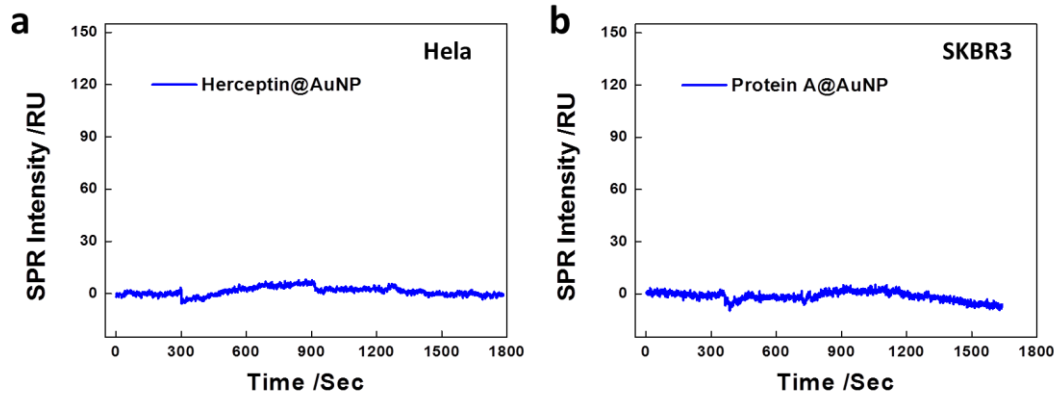

Figure S2. **Control experiments.** (a) The sensorgram of Herceptin@AuNP nano-conjugates binding to Her2 on Hela cells at 5.5 pM. These cells do not overexpress Her2 receptor, and the nano-conjugates show little binding signal. (b) The sensorgram of Protein A@AuNP nano-conjugates binding to Her2 on SKBR3 cells at 5.5 pM. These nano-conjugates are not coated with Herceptin, and the nano-conjugates show little binding signal, either.

Bivalent model. For a bivalent model, Herceptin@AuNP-cell interaction first occurred between one antibody (Herceptin) and one Her2 receptor on the cell membrane. Then an additional molecular binding occurred between another antibody molecule on the same nano-conjugate and an adjacent Her2 receptor to achieve a bi-valent attachment. Such an interaction can be expressed as:

$$A + 2L \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} LA + L \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} LLA \quad , \qquad \text{(S1)}$$

where A is the nano-conjugates, L is the receptor on the cell membrane. LA, k_{a1} and k_{d2} are the reaction product, the association and the dissociation rate constants for first binding, respectively. LLA, k_{a2} and k_{d2} are that for second binding.

The concentrations of all species in this reaction over time are listed as:

$$\frac{d[A]}{dt} = 0 \qquad \text{(S2)}$$

$$\frac{d[L]}{dt} = -(2k_{a1}[L][A] - k_{d1}[LA]) - (k_{a2}[LA][L] - 2k_{d2}[LLA]) \qquad \text{(S3)}$$

$$\frac{d[LA]}{dt} = (2k_{a1}[L][A] - k_{d1}[LA]) - (k_{a2}[LA][L] - 2k_{d2}[LLA]) \qquad \text{(S4)}$$

$$\frac{d[LLA]}{dt} = k_{a2}[LA][L] - 2k_{d2}[LLA] \qquad \text{(S5)}$$

$$RU = [LA] + [LLA] \qquad \text{(S6)}$$

Where [A] is the concentration of nano-conjugates and [L] is that for receptor protein on the surface; [AL] and [ALL] are the concentrations of reaction product for first and second binding, respectively. k_{a1}, k_{d2}, k_{a2} and k_{d2} are defined as above. RU is the plasmonic image response and t is elapsed time. A Matlab program is coded to solve the differential equations.

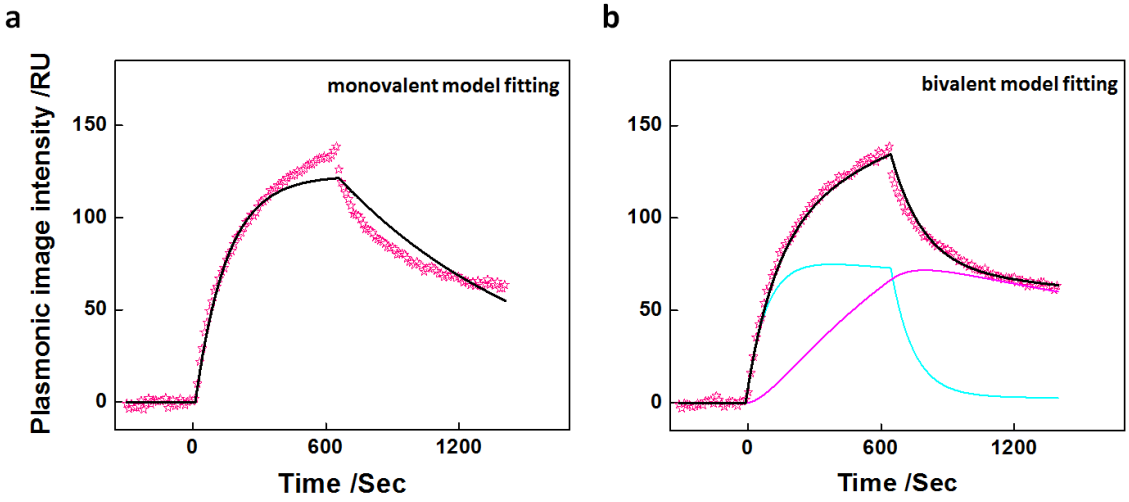

Figure S3: **Binding model test for the Herceptin@AuNP nano-conjugates**. Results of monovalent (a) and bivalent (b) model on fitting the sensorgram of Herceptin@AuNP nano-conjugates binding to Her2 on SKBR3 at the concentration of 27.5 pM. Pink curve is the experimental result and black curve is the fitting result with monovalent (left) or bivalent (right) model. The cyan curve represents the initial binding of Herceptin to Her2 on the cell membrane via one pair of Herception-Her2 interaction, and the magenta curve is the binding via a second pair of Herception-Her2 interaction. It is obvious that the monovalent model failed to fit this sensorgram but bivalent model worked perfectly.

Estimation of inter-molecular distance of Her2 receptor. In our previous work, we have calculated that 1 pg/mm^2 protein binding onto the cell membrane induced 0.5 RU plasmonic responses[3]. According to the molecular weight of Herceptin, 145.5 kDa, and the maximum plasmonic intensity for the association of Herceptin, 54 RU and 16 RU for SKBR3 and JIMT1 cells, respectively, we estimated the density of Herceptin binding onto the cells, which is the same as that of Her2 receptor based on the monovalent model. Thus, the inter-molecular distances of Her2 receptor on the cell membrane were obtained, as listed in Table S1.

Table S1: Estimation of inter-molecular distance of Herceptin on SKBR3 and JIMT1

	Maximum intensity (RU)	Receptor density (molecules/μm^2)	Receptor coverage area (nm^2/molecule)	inter-molecular distance (nm)
SKBR3	54	447	2237	47
JIMT1	16	132	7558	87

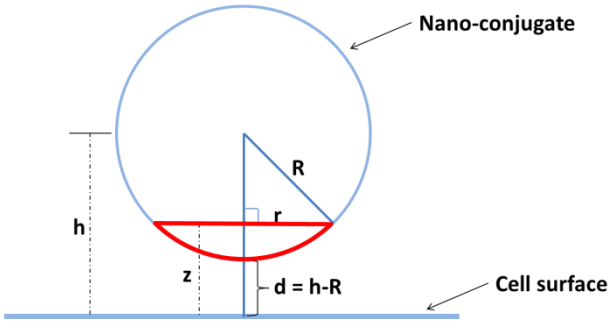

Figure S4. **Model for calculating the contact area of the nano-conjugates with intact cells proposed** by Mark E. Davis et.al.[1]. The cellular membrane with overexpression of Her2 was represented by a flat surface, and the nano-conjugate was simplified as a sphere of radius *R* whose center was above the surface with a height of *h* and bottom with a distance of *d* (*d* = *h*-*R*). The cross-section of the contact area on the sphere (red part) is a circle with radius of r and its vertical distance to the surface was recognised as *z*. The radius of the contact circle can be roughly estimated through $r \approx 2R\varepsilon^{1/2} = 2\sqrt{dR}$. (See the reference for the derivation details).

When we assumed that the distance of the nano-conjugates to cellular surface (*d*) is the height of Her2 receptor as 5.5nm [2], and that the radius of nano-conjugates is 37.5nm (Herceptin@AuNP conjugate consisted of a 25-nm gold nanoparticle core, a layer of protein A (10nm) and another layer of Herceptin (15nm)), the diameter of the contact circle (*l*) is 56nm ($l = 2r \approx 4\sqrt{dR}$). This value is comparable to the intermolecular distance of two Her2 receptors on the SKBR3 cells. Thus, we believed that the bivalent binding is dominant for the interaction of nano-conjugate with intact cells. Despite that multivalent binding might exist to some extent due to the inhomogeneous distribution of receptors on the cell surface, the calculation for binding affinity will be much more complicated if we introduce it. Based on above reasons, we finally used the bivalent binding model in this case.

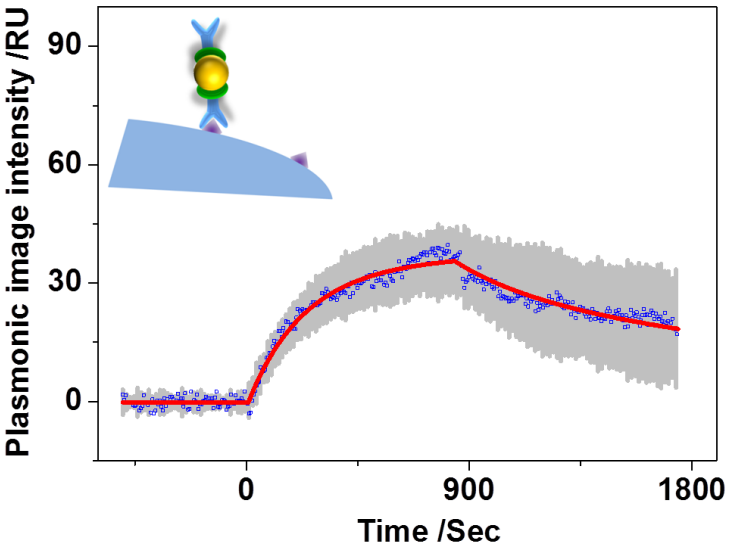

Figure S5: **Monovalent model validates for calculating the binding kinetics of nano-conjugate with 6nm AuNP** as illustrated with inset figure on top-left corner. At most two Herceptins conjugated to the gold nanoparticle based on the manufacturer's instruction. The blue dots and the red curve are the experimetal and the fitting results, respectively, and the grey background are individual sensorgrams of the nano-conjugates with multiple SKBR3 cells. The concentration of nano-conjugates is 2.09 nM. The association and dissociation rate sonstants, k_{a1} and k_{d1}, are $(1.3\pm0.4)\times10^{6}$ $M^{-1}s^{-1}$ and $(7.7\pm3.6)\times10^{-4}$ s^{-1}, respectively.

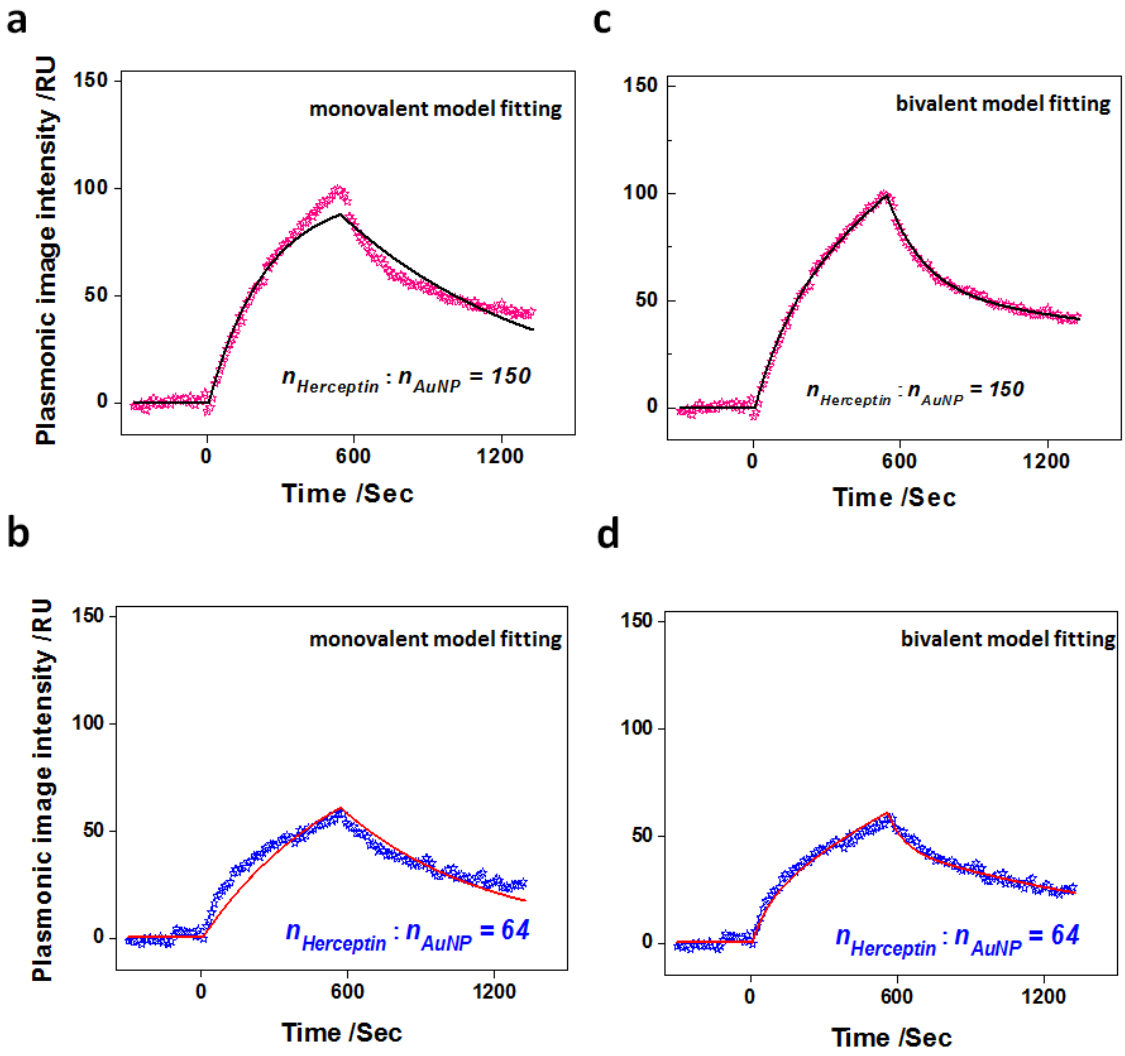

Figure S6: **Binding model test for the nano-conjugates at high molar ratio of Herceptin to AuNP**. Failure of monovalent model description of the binding kinetics at molar ratios of 150 (a) and 64 (b). Success of bivalent model description of the binding kinetics at molar ratios of 150 (c) and 64 (d).

Reference

[1] C.H.J. Choi, C.A. Alabi, P. Webster, M.E. Davis, *Proc. Natl. Acad. Sci. U. S. A.*, **2010**, *107*, 1235-1240.

[2] H.S. Cho, K. Mason, K.X. Ramyar, A.M. Stanley, S.B. Gabelli, D.W. Denney, D.J. Leahy, *Nature*, **2003**, *421*, 756-760.

[3] W. Wang, Y.Z. Yang, S.P. Wang, V.J. Nagaraj, Q. Liu, J. Wu, N.J. Tao, *Nat. Chem.*, **2012**, *4*, 846-853.