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Resistive-Pulse Measurements with Nanopipettes: Detection of Vascular Endothelial Growth Factor C (VEGF-C) Using Antibody-Decorated Nanoparticles

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Abstract

Quartz nanopipettes have recently been employed for resistive-pulse sensing of Au nanoparticles (AuNP) and nanoparticles with bound antibodies. The analytical signal in such experiments is the change in ionic current caused by the nanoparticle translocation through the pipette orifice. This paper describes resistive-pulse detection of cancer biomarker (Vascular Endothelial Growth Factor-C, VEGF-C) through the use of antibody-modified AuNPs and nanopipettes. The main challenge was to differentiate between AuNPs with attached antibodies for VEGF-C and antigen-conjugated particles. The zeta-potentials of these types of particles are not very different, and, therefore, carefully chosen pipettes with well-characterized geometry were necessary for selective detection of VEGF-C.

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The capacity of resistive-pulse sensors to detect particles or biomolecules that can enter a microscopic pore and partially block the flowing ion current has been widely employed in sensing applications^{1–3} from single-molecule detection⁴ to particle sizing^{5,6} to DNA sequencing.⁷ Although most reported resistive-pulse experiments were performed with biological or solid-state nanopores,² a few studies employing nanopipettes as the detecting platform have recently been reported.^{6,8–11} Nanopipettes are easy to pull from borosilicate or quartz capillaries, and their small physical size (the outer diameter of the tip can be as small as ~10 nm^{12,13}) and needle-like geometry make them suitable as probes for scanning probe microscopies,^{14–21} cell penetration, delivery, and in situ electrical measurements.^{22–25}

We have previously utilized nanopipettes for resistive-pulse sensing of gold nanoparticles (AuNPs), AuNPs coated with an allergen epitope peptide layer, and AuNP-peptide particles with bound anti-peanut antibodies. The selective detection of antibody-conjugated NPs was based on the difference in sizes and zeta-potentials of those particles.¹⁰ A conceptually similar strategy is employed here to develop a resistive-pulse sensor for a cancer biomarker—Vascular Endothelial Growth Factor C (VEGF-C). VEGF-C stimulates lymphangiogenesis,^{26–29} and overexpression of VEGF-C has been observed in various cancers and linked to lymph node metastasis.^{30,31} Serum concentrations of VEGF-C are typically in the nanogram per milliliter range.^{31–33}

For VEGF-C detection, monoclonal primary antihuman VEGF-C antibodies were immobilized onto carboxylate-functionalized gold nanoparticles (AuNPs).³⁴ After VEGF-C capture, AuNP–antibody and AuNP–antibody–VEGF-C nanoparticles coexist in a dispersion. In resistive-pulse experiments discussed below, both AuNP–antibody and AuNP–antibody–VEGF-C particles produced current blockages in nanopipettes with a wide range of radii. Careful selection of the pipettes with well-characterized geometry was essential for selective detection of VEGF-C because of relatively small differences in the pulses produced by the two kinds of particles.

EXPERIMENTAL SECTION

Chemicals and Materials

The following chemicals were used as received: 1,2-dichloroethane (DCE) and NaCl from Sigma-Aldrich; monosodium phosphate and potassium tetrakis-(4-chlorophenyl) borate (KTPBCl) from Alfa Aesar; disodium phosphate from J.T. Baker Chemical; tetrahexylammonium chloride (THACl) from Fluka. Tetrahexylammonium tetrakis-(4-chlorophenyl) borate (THATPBCl) was prepared by metathesis of KTPBCl with THACl and recrystallized from acetone. Aqueous solutions were prepared from deionized water (Milli-Q, Millipore Co.). A 10 mM sodium phosphate buffer (PB) solution at pH 7.3 was prepared and used for surface modification of gold colloids.

Sodium azide, TWEEN 20, sodium phosphate dibasic, and sodium phosphate monobasic (Sigma-Aldrich) were used for the synthesis of conjugated AuNPs. Citrate-stabilized gold nanoparticles (10 nm nominal diameter) were acquired from Ted Pella, Inc. Monoclonal mouse IgG_{2B} antibody for human VEGF-C (clone 193208) and recombinant human VEGF-C were received from R&D Systems. The particles were prepared with sterile 10 mM

phosphate buffer, pH 7.3. Then, 0.05% Tween-20 was added to phosphate buffer (PB-T) for washing and reconstituting the conjugated particles.

Preparation of Bioconjugated Particles

Gold nano-particle-mono-clonal antibody conjugates (AuNP-mAb) were prepared using a simple adsorption method as previously reported.³⁵ Briefly, 800 μL of AuNP stock solution ($\sim 8 \text{ nM}$) was taken and washed twice with 1 mL of PB-T and reconstituted in 1 mL of PB-T. Then, 100 μL of 100 $\mu\text{g/mL}$ of antibody (mAb) solution was added, and the AuNP-mAb mixture was incubated for 1 h on a rotator. Following incubation, 25 μL of 10% BSA solution was added to the AuNP-mAb mixture to block nonspecific binding sites on the AuNP-mAb bioconjugate. The mixture was incubated another 15 min on the rotator. Then, the mixture was centrifuged three times, and the AuNP-mAb bioconjugate was reconstituted in 1000 μL of PB-T containing 0.01% sodium azide.

The AuNP-mAb bioconjugate was mixed with 400 μL of 4 $\mu\text{g/mL}$ of VEGF-C antigen for 2 h to prepare VEGF-C antigen-conjugated particles (AuNP-mAb-VEGF-C). The mixture was centrifuged, and AuNP-mAb-VEGF-C bioconjugate was resuspended in PB-T containing 0.01% sodium azide to prevent any bacterial contamination. All colloidal sols were stored at 4 $^{\circ}\text{C}$ until use in further analyses. The bioconjugates in solution were stable for at least one month with no appreciable changes in solution color, zeta-potential, and measured DLS diameter. However, after about four months, the NPs seemed to aggregate, and the solution color changed from reddish to bluish.

All stock solutions were five times diluted with phosphate buffer, and the resulting concentration of AuNP was 0.95 nM. The ratio between AuNPs and the attached antibodies was about 1:6, and that between antibody and antigen was about 1:1.

Characterization of Particles

Nanoparticles and nano-particle bioconjugates were characterized using transmission electron microscopy (TEM), dynamic light scattering (DLS), atomic force microscopy (AFM), and zeta potential measurements. TEM was carried out using a Tecnai 12 TEM (FEI, Hillsboro, OR) with samples supported on 400 mesh copper grids coated with Formvar/carbon film (Ted Pella, Inc.). Briefly, 3 μL of sample was placed on the grid for 1 min. Grids were washed with water, and 1% uranyl acetate solution was added for 30 s for negative staining.¹⁰ Excess staining solution was soaked off grids using Whatman (grade 1) filter paper (GE Health Care Life Sciences, Piscataway, NJ), and grids were washed twice with distilled water and subsequently dried in the air before TEM analysis.

Stabilities of gold AuNPs and nanoparticle bioconjugates were examined using zeta potential analysis. Zeta (ζ) potential measurements were performed using ZetaPlus ζ -potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY). The reported zeta potential values were obtained by averaging three readings.

DLS experiments were conducted to find the size distributions of nanoparticles and bioconjugate particles in solution. Briefly, 1 mL of each sample was taken and filtered twice

with a 0.2 μm filter in a glass vial, and the experiment was performed using ALV/LSE-5004 (Langen, Germany).

Nanopipette Preparation and Characterization

Quartz capillaries with filament (o.d./i.d. ratio of 1.0/0.50; Sutter Instrument Co., Novato, CA) were cleaned in piranha solution, rinsed with copious amount of deionized water, and kept in the furnace overnight. The nanopipettes with a tip radii from 20 to 200 nm were pulled from quartz capillaries by a laser pipette puller (P-2000, Sutter Instruments). Representative pulling parameters for pulling ~ 150 nm-diameter quartz pipettes are HEAT = 760, FILAMENT = 3, VELOCITY = 16, DELAY = 128, PULL = 140.

The pair of pipettes pulled from the same capillary is expected to have the same radius (a) and similar taper angle (θ). The inner wall of one pipette from the pair was silanized in N,N-dimethyltrimethylsilylamine vapor and used for determining the a value. The pipettes were fixed in a mini-vacuum desiccator (Scienceware, Sigma-Aldrich), which was connected to a thermocouple gauge controller (KJLG 205 Series, Kurt J, Lesker Co.) and a pump (RV8, Edwards Co.). The desiccator was first evacuated by the pump, and then the silane vapor was delivered from the flask to the desiccator, where the pipettes were exposed to it for about 20 min. The system was evacuated again to remove the silane vapor before taking out the pipettes.

The pipettes were characterized by steady-state ion transfer (IT) voltammetry.¹⁰ A pulled pipette was backfilled with 1,2-dichloroethane (DCE) solution containing 10 mM THATPBCl using a 10 μL syringe and immersed in aqueous solution containing 0.1 M KCl and 2 mM TEACl. A 0.25 mm silver wire was inserted into each pipette from the back. The two-electrode setup was employed with another 0.25 mm Ag wire coated with AgCl serving as an aqueous reference electrode. IT voltammetry was performed in the following electrochemical cell:

Voltammograms were obtained with a BAS 100B/W electro-chemical workstation (Bioanalytical Systems, West Lafayette, IN).

TEM and SEM imaging

A JEOL JEM-2100 transmission electron microscope was used to characterize the geometry of the nanopipettes. The ~ 3 mm portion of the pipette adjacent to its tip was attached to the grid (PELCO Hole Grids, Copper) in such a way that its tip was exposed to the beam in the grid center hole, and the rest of the pipette was cut off. A relatively low electron beam voltage of 100 kV was used to reduce the charge/heat accumulating effects on the quartz surface. A Zeiss Supra 50VP scanning electron microscope was used to characterize the surface morphology of the fabricated nanopipettes. The 1.5–3 kV gun voltage was used with a typical working distance of 4 mm.

Resistive-pulse Experiments

The pipette filled with 15 mM NaCl and 10 mM PB was dipped into the same aqueous solution containing nanoparticles of interest. Each pipette was rinsed with deionized water before moving it between three different samples, including bare AuNPs, AuNP-mAb, and AuNP-mAb-VEGF-C. A Multiclamp 700B amplifier (Molecular Devices Corporation, CA) was used in the voltage-clamp mode to apply voltage between the Ag/AgCl reference electrode inside the nanopipette and the external Ag/AgCl reference facing the pipette orifice and to measure the resulting current. The signal was digitized using a Digidata 1440A analog-to-digital converter (Molecular Devices) at a sampling frequency of 100 kHz. A 10 kHz low pass filter was used. The recordings with a higher filter frequency, e.g., 20 kHz, as well as with no filtering were obtained in control experiments to verify that shorter current pulses have not been missed or filtered out.³⁶ The data were recorded and analyzed using pClamp 10 (Molecular Devices). The current-voltage (*i*-*V*) curves were also obtained using the Multiclamp 700B amplifier.

RESULTS AND DISCUSSION

Particle Characterization

From TEM images (Figure 1), the average diameter for the dried commercial AuNPs was 9.7 (± 0.7) nm based on around 20 measurements of individual, isolated particles. This is in good agreement with the nominal 10 nm diameter reported by the manufacturer. As expected, bioconjugated particles exhibited larger diameters than bare AuNPs. The AuNP-mAb bioconjugate was 13.3 (± 0.8) nm, and the AuNP-mAb-VEGF-C bioconjugate was 14.5 (± 0.5) nm.

The size distributions of nanoparticles and bioconjugate particles in solution found from DLS experiments are shown in Figure 2. The average hydrodynamic radius was 9.1 nm for commercial AuNPs, 35.4 nm for AuNP-mAb, and 45.6 nm for the AuNP-mAb-VEGF-C bioconjugate. Overall, hydrodynamic radii were thus 2 to 6 times larger than radii measured using TEM on dry particles. While DLS measures the diameter of the particles including protein coating, TEM images yield the particle core size³⁷ because soft material coating collapses.³⁸ As discussed previously,¹⁰ the DLS size was larger than the size of 10-nm-diameter AuNPs obtained from TEM.

In our previous resistive-pulse experiments, the difference in ζ -potentials was essential for the selective detection of antibody-conjugated particles.¹⁰ The zeta potentials of AuNPs and bioconjugate particles are summarized in Table 1. While the ζ -potential of the citrate-stabilized AuNP in PB was found to be highly negative (Table 1), the ζ -potential values measured for these NPs as well as for bioconjugate particles in the presence of 15 mM NaCl (i.e., in solution used in our resistive-pulse experiments) were much smaller and similar for all particles.

Characterization of Nanopipettes

Geometry parameters of pipettes (i.e., *a* and θ) were evaluated by electrochemical methods. Figure 3A shows four voltammograms of IT at the DCE/water interface obtained with four

different pipettes. For a silanized pipette with $RG = 1.5$ ($RG = r_g/a$, where r_g is the external glass radius at the tip), the diffusion limiting current follows eq 1.³⁹

$$i_d = 4 \times 1.16 z_i F D c a \quad (1)$$

where F is the Faraday constant and c , D , and z are the concentration, diffusion coefficient, and charge of the transferred ion, respectively. With $D = 1 \times 10^{-5} \text{ cm}^2/\text{s}$ for TEA^+ ,^{40,41} a can be calculated to be 23 nm (black), 45 nm (orange), 138 nm (blue), and 170 nm (green; Figure 3A).

The pipette angle, θ , was determined from the pipette resistance using eqs 2–4:^{10,22a}

$$R = R_{\text{int}} + R_{\text{ext}} \quad (2)$$

$$R_{\text{ext}} = 1 / (4 \kappa a) \quad (3)$$

$$R_{\text{int}} = 1 / \kappa \pi a \tan \theta \quad (4)$$

where R is the total resistance, R_{int} and R_{ext} are the resistances of the inner and outer solutions, respectively, and κ is the conductivity of the solution. R was extracted and can be evaluated from the slope of the current–voltage ($i - V$) curve recorded in aqueous solution containing 15 mM NaCl and 10 mM PB (Figure 3B). R_{ext} was calculated for the given radius a , and θ was then evaluated from the internal pipette resistance ($R_{\text{int}} = R - R_{\text{ext}}$) using eq 4. The θ values for the four pipettes in Figure 3 are 13.9° (black), 8° (orange), 5.1° (blue), and 8.7° (green), respectively. Pipettes with larger angles are preferable because they can produce larger signal-to-noise ratio in resistive-pulses measurements.¹⁰

For several pipettes, the radius value was validated by SEM or TEM images, as shown in Figure 4. The geometric parameters of the pipettes used in this study can be found in Table 2.

Selective Detection of NPs

The pipettes with radii ranging from 23 to 192 nm were used with three different types of NPs, and the importance of the orifice size for selective detection of different particles can be seen from Table 2. The bare AuNPs could only be detected with the 23-nm-radius pipette #1 (or smaller;¹⁰ not shown in Table 2). Neither AuNP–mAb nor AuNP–mAb–VEGF-C particles produced current blockages in recordings obtained with this pipette (Table 2). This observation is consistent with the mean radius values for both particles (36 and 45 nm, respectively, from DLS) being larger than 23 nm. Pipettes #2 to 4 with the radii ranging from 45 to 50 nm exhibited current pulses with AuNP–mAb, while AuNP–mAb–VEGF-C particles apparently were too large to pass through these pipettes' apertures.

The statistical analysis of the distribution of resistive pulses obtained with AuNP–mAb particles using pipette #4 is shown in Figure 5. The mean half-width duration was slightly less than 0.1 ms. The normalized current change values ($\Delta i/i$) range from 0.3% to 5.4%. This very broad range reflects significant polydispersity of AuNP–mAb particles. The mean

radius value of 15 nm, calculated using the previously reported treatment,¹⁰ was significantly smaller than 36 nm found from DLS measurement. The discrepancy is likely caused by two factors: DLS tends to overestimate the average particle size, while the resistive-pulse data may not fully account for larger particles, which are less likely to penetrate the comparably sized pipette orifice.

Both AuNP-mAb and AuNP-mAb-VEGF-C particles could be detected with pipettes #5 to #8, whose radii were between 60 and 90 nm. Both types of particles are polydisperse, and the distributions of their radii significantly overlap (Figure 2). The ζ -potentials of these NPs are also similar (Table 1). As expected from their somewhat larger mean radius, AuNP-mAb-VEGF-C produced slightly longer pulses (Figure 6A) with slightly larger mean magnitude (Figure 6B). However, these differences are too small to enable differentiation between these particles in a mixture, using an intermediate size pipette (e.g., 60–90 nm).

Antigen-conjugated NPs were selectively detected using larger pipettes (#9 to #15 in Table 2; 120 to 192 nm radius). The AuNP-mAb particles did not produce measurable current blockages with those large pipettes. (A small number of pulses, 1 pulse per recording, observed with pipettes #10 and #12, are probably due to the AuNP-mAb dimers.) Three successive recordings obtained with pipette #15 are shown in Figure 7. They were obtained by first immersing the pipette in a solution of AuNP-mAb-VEGF-C (Figure 7A), then transferring it to the solution of AuNP-mAb (Figure 7B), and then back to the solution of antigen-conjugated NPs (Figure 7C). Pulses produced by antigen-conjugated particles in Figure 7A disappeared in the AuNP-mAb solution (Figure 7B). The disappearance of the pulses was due to the lack of sufficiently large NPs rather than to clogging of the orifice or any other change in the pipette. This was confirmed by the reappearance of the signal after the pipette was returned to the AuNP-mAb-VEGF-C antigen solution (Figure 7C). Thus, pipette #15 selectively detected VEGF-C-conjugated particles. The statistical analysis of the recordings is shown in Figure 7D and E.

In the mixture of polydisperse NPs, different pipettes detect fractions of differently sized particles. This point is illustrated by Figure 8 that shows the dependence of the mean radius of the detected AuNP-mAb-VEGF-C particles on the radius of pipettes employed for resistive-pulse sensing (pipettes #5 to #15 in Table 2). The mean radius of the detected AuNP-mAb-VEGF-C particles in Figure 8 is proportional to the pipette radius. The smaller pipettes (#5 to #7) yielded the mean radius values of 16 to 20 nm, which are close to the lower edge in the particle size distribution measured by DLS (Figure 2C). The contribution of larger particles was diminished due to either their rejection by such a pipette or a low probability of penetrating the pipette orifice. In contrast, the mean radius of NPs measured with the largest pipettes (#13 to #15) was between 41.5 and 47.6 nm, i.e., close to the 45 nm mean value obtained by DLS. Such large pipettes could not sense the smallest antigen-conjugated particles, and therefore the 40+ nm values probably overestimate the average NP radius similarly to the DLS measurements.

The Effect of the Applied Voltage

The possibility of discriminating between different NPs by varying the applied voltage was also tested. Figure 9 shows the distribution of resistive pulses obtained with AuNP-mAb

(black) and AuNP–mAb–VEGF-C (red) particles at two potentials: –300 mV and –600 mV. At each potential, the difference between the distributions obtained for two types of NPs was not sufficiently large to differentiate between them. The measured pulse frequencies, i.e., 6 pulses/trace for AuNP–mAb and 4.8 pulses/trace for AuNP–mAb–VEGF-C at –600 mV and 2.5 pulses/trace for AuNP–mAb and 1.75 pulses/trace for AuNP–mAb–VEGF-C at –300 mV, were also similar. These results, which are consistent with similar zeta potentials of AuNP–mAb and AuNP–mAb–VEGF-C, suggest that the way to selectively detect these particles is by using sufficiently small or sufficiently large pipettes.

CONCLUSIONS

VEGF-C is a protein that promotes lymphangiogenesis, and overexpression of VEGF-C has been linked to lymph node metastasis of cancer. Resistive-pulse sensing of VEGF-C was accomplished using antibody-modified AuNPs. Differentiating between AuNP–mAb and AuNP–mAb–VEGF-C bioconjugates in these experiments is difficult because of similar sizes and zeta potentials of these particles. Nevertheless, by properly selecting the size of the nanopipette, it is possible to selectively detect either AuNP–mAb when $a < \sim 60$ nm and the orifice is too small for the translocation of antigen-conjugated NPs or AuNP–antibody–VEGF-C when $a > \sim 140$ nm and the small pulses produced by AuNP–mAb are obscured by the noise.

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Figure 1. TEM micrographs of different nanoparticles: (a) commercial AuNPs, (b) AuNP-mAb stained with 1% uranyl acetate, (c) AuNP-mAb-VEGF-C antigen stained with 1% uranyl acetate.

Figure 2.
Size distributions of different particles from DLS: (a) commercial AuNP, (b) AuNP-mAb, and (c) AuNP-mAb-VEGF-C.

Figure 3. Electrochemical characterization of pipette geometry. (A) Steady-state voltammograms and (B) $i-V$ curves obtained with silanized pipettes. a and $\theta = 23$ nm and 13.9° (black), 45 nm and 8° (orange), 138 nm and 5.1° (blue), and 170 nm and 8.7° (green).

Figure 4. SEM (A, B) and TEM (C, D) images of nanopipettes. Pipette orifice radius, a (nm) = 50 (A), 143 (B), 155 (C), and 192 (D).

Figure 5.

Statistical analysis of current pulses produced by translocation of AuNP-mAb through the 50 nm-radius pipette (#4 in Table 2). (A) Scatter plot of the normalized maximum current change ($\Delta i/i$) versus peak half-width ($\tau_{1/2}$). (B) Fraction of the total number of pulses at different $\Delta i/i$ (from 0 to 2%). Inset shows the fraction of pulses with larger current change (3% to 6%).

Figure 6. Statistical analysis of current pulses produced by translocation of AuNP–mAb (black) and AuNP–mAb–VEGF-C (gray) particles through a 60 nm-radius pipette (#5 in Table 2). (A) Scatter plot of the normalized maximum current change ($\Delta i/i$) versus peak half-width ($\tau_{1/2}$). (B) Fraction of the total number of pulses for different $\Delta i/i$. The inset shows the fractions of pulses with larger current change (3% to 18%).

Figure 7. Selective detection of AuNP-mAb-VEGF-C with a 193 nm-radius pipette (#15 from Table 2). Resistive pulse recordings obtained in the AuNP-mAb-VEGF-C solution before (A) and after (C) transferring the pipette to the AuNP-mAb solution (B). Distributions of the pulses: (D) current change ($\Delta i/i$) vs half width duration ($\tau_{1/2}$, ms) and (E) fractions of total pulses for different $\Delta i/i$.

Figure 8. Dependence of the mean radius of the AuNP-mAb-VEGF-C particles determined with a given nanopipette on the radius of the pipette orifice. The dashed straight line is shown as a guide.

Figure 9.

Statistical analysis of current pulses produced by translocation of AuNP-mAb (black) and AuNP-mAb-VEGF-C (red) through a 75-nm-radius pipette with the applied voltage of -300 mV (A) and -600 mV (B). The inset in B shows the enlarged portion of the distribution representing the majority of pulses.

Table 1**ζ-Potentials, Hydrodynamic Radii, and TEM Diameters of AuNPs and Bioconjugate Particles**

particle	ζ-potential (mV)	hydrodynamic radius (nm)	TEM diameter (nm)
AuNP ^a	-45 (±2.2)	9.1	9.7 (±0.7)
AuNP-mAb ^b	-18 (±1.3)	35.4	13.3 (±0.8)
AuNP-mAb-VEGF-C ^c	-15.1 (±0.9)	45.6	14.5 (±0.5)
AuNP ^d	-17 (±1.5)	9.1	9.7 (±0.7)
AuNP-mAb ^e	-15.9 (±0.8)	35.4	13.3 (±0.8)
AuNP-mAb-VEGF-C ^f	-14.0 (±0.5)	45.6	14.5 (±0.5)

^a 10 mM phosphate buffer with particle concentration of 7.2 nM.^b 10 mM phosphate buffer with particle concentration of 6.3 nM.^c 10 mM phosphate buffer with particle concentration of 5.4 nM.^d 10 mM phosphate buffer with 15 mM NaCl with particle concentration of 1.2 nM.^e 10 mM phosphate buffer with 15 mM NaCl with particle concentration of 1.05 nM.^f 10 mM phosphate buffer with 15 mM NaCl with particle concentration 0.9 nM.

Table 2

Resistive-Pulse Detection of AuNPs and Bioconjugated NPs with Nanopipettes of Different Geometry

no.	pipette geometry			# spikes/# traces/# spikes per trace		
	<i>a</i> (nm)	θ (deg)	method	AuNP	AuNP-mAb	AuNP-mAb-VEGF-C
1	23	13.9	voltammetry	30/10/3	0	0
2	45	8	voltammetry	0	43/27/1.6	0
3	48	16.4	voltammetry	0	45/13/4.1	0
4	50	8.6	SEM	0	122/11/11.1	0
5	60	8.9	voltammetry	0	165/13/12.7	56/14/4
6	60	11	voltammetry	0	62/24/2.6	101/19/5.3
7	75	13.6	voltammetry	0	120/20/6	92/19, 4.8
8	90	9.5	voltammetry	0	77/15/5.1	97/16/6.1
9	120	12.7	voltammetry	0	0	81/30/2.7
10	138	5.1	voltammetry	0	16/30/0.5	297/2.5/11.9
11	140	13.5	voltammetry	0	0	43/17/2.5
12	143	7.4	SEM	0	7/7/1	121/21/5.8
13	155	6.5	TEM	0	0	95/19/5.0
14	170	8.7	voltammetry	0	0	121/21/5.8
15	192	6.5	voltammetry, TEM	0	0	349/42/8.3