Anal. Chem. **2007,** *79,* 2131−2136

Microwave-Accelerated Ultrafast Nanoparticle Aggregation Assays Using Gold Colloids

Kadir Aslan† and Chris D. Geddes*,†,‡

Institute of Fluorescence, Laboratory for Advanced Medical Plasmonics, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 West Lombard Street, Baltimore, Maryland 21201, and Center for Fluorescence Spectroscopy, Medical Biotechnology Center, University of Maryland School of Medicine, 725 West Lombard Street, Baltimore, Maryland 21201

In this paper, the proof of principle of microwave-accelerated aggregation assay technology, which shortens the solution-based aggregation assays' run time to seconds (>100-fold increase in kinetics) with microwave heating, was demonstrated using a model aggregation assay based on the well-known interactions of biotin and avidin. Biotinylated gold colloids were aggregated in solution with the addition of streptavidin, which takes 20 min at room temperature to reach >90% completion and only 10 s with microwave heating. The initial velocity (after 1-s microwave heating) of the biotinylated gold colloids reaches up to 10.5 m/s, which gives rise to greater sampling of the total volume but not a large increase in bulk temperature. The room-temperature, steady-state velocity of the colloids was <0.5 μm/s. In control experiments, where streptavidin preincubated with D-biotin in solution is added to biotinylated gold colloids and microwave heated, gold colloids did not aggregate, demonstrating that nonspecific interactions between biotinylated gold colloids and streptavidin were negligible.

The use of noble metal colloids in colorimetric assays for the detection of DNA hybridization[1,2] and immunoassays[3,4] has been proven to be revolutionary. Gold colloids are frequently used in a variety of aggregation assays due their exceptional optical and electronic properties.[5,6] Despite the promising progress in the development of aggregation-based assays using noble metal colloids, where many issues such as the reduction of nonspecific interactions and even reversibility of assays has been addressed,[7] no progress has been made to date pertaining to the assay run time. Current aggregation assays typically take between 10 and 30 min to complete.[7] In this regard, the development of a new technique that readily encompasses the current assays, but additionally reduces the assay run time, would certainly revolutionize the aggregation assays once again.

In this paper, the proof of principle of microwave-accelerated aggregation assay (MA-AA) technology, which shortens the solution-based aggregation assays' run time to seconds with low-power microwave heating, is demonstrated using a model aggregation assay based on the well-known interactions of biotin and avidin as depicted in Figure 1.[8]

Biotinylated gold colloids were aggregated in solution with the addition of streptavidin, which takes 20 min at room temperature (>90% completion) and only 10 s with microwave heating. In control experiments, where streptavidin preincubated with D-biotin in solution is added to biotinylated gold colloids, gold colloids did not aggregate, demonstrating that nonspecific interactions between biotinylated gold colloids and streptavidin were negligible. In this regard, the gold colloids are used as (1) a platform for carrying one of the binding partners (i.e., biotin), (2) an indicator of the aggregation process, and (3) a mediator for the localized delivery of the electromagnetic energy that significantly speeds up the aggregation process.

RESULTS AND DISCUSSION

As shown by Kogan et al.,[9] when exposed to weak microwave fields, gold colloids absorb and dissipate the electromagnetic energy at higher frequencies (>8 GHz) without any notable bulk heating. In this regard, to determine the effect of microwave heating (2.45 GHz) on the surface plasmon resonance of gold colloids, the absorption spectrum of gold colloids of varying concentrations was measured, Figure 2. Figure 2 shows that when exposed to 10-s microwave heating the absorption spectrum of gold colloids remains unchanged for all the concentrations used. This finding indicates that any change in the absorption spectrum of the colloids in the microwave-accelerated aggregation assays is due to other factors (i.e., binding events, etc). The effect of temperature on gold colloids was also studied by

* To whom correspondence should be addressed. E-mail: geddes@umbi.umd.edu.

† University of Maryland Biotechnology Institute.

‡ University of Maryland School of Medicine.

(1) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959−1964.

(2) Reynolds, R. A.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **2000**, *122*, 3795−3796.

(3) Sastry, M.; Lala, N.; Patil, V.; Chavan, S. P.; Chittiboyina, A. G. *Langmuir* **1998**, *14*, 4138−4142.

(4) Thanh, N. T. K.; Rosenzweig, Z. *Anal. Chem.* **2002**, *74*, 1624−1628.

(5) Cobbe, S.; Connolly, S.; Ryan, D.; Nagle, L.; Eritja, R.; Fitzmaurice, D. *J. Phys. Chem. B* **2003**, *107*, 470−477.

(6) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078−1081.

(7) Aslan, K.; Luhrs, C. C; Pérez-Luna, V. H. *J. Phys. Chem. B* **2004**, *108*, 15631−15639.

(8) Wilchek, M.; Bayer, E. A. Methods of Enzymology; Vol. 184, Academic Press, San Diego. 1990.

(9) Kogan, M. J.; Bastus, N. G.; Amigo, R.; Grillo-Bosch, D.; Araya, E.; Turiel, A.; Labarta, A.; Giralt, E.; Puntes, V. F. *Nano Lett.* **2006**, *6* (1), 110−115.

10.1021/ac0620967 CCC: $37.00
Published on Web 01/26/2007

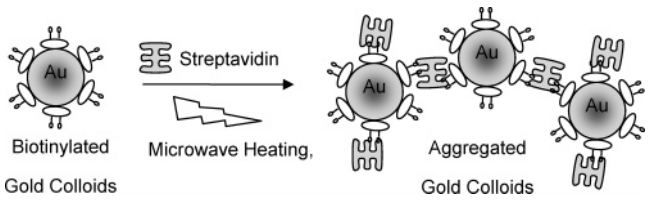

Figure 1. Model MA-AA using gold colloids. Biotinylated-BSA-coated 20-nm gold colloids cross-linked by streptavidin.

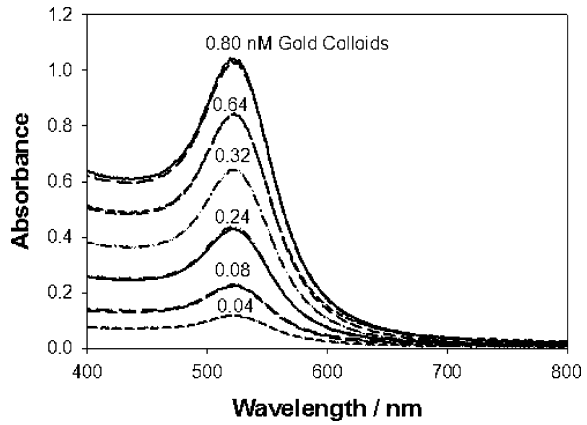

Figure 2. Absorption spectra of 20-nm gold colloids as a function of concentration before and after microwave (Mw) heating for 10 s. Note that the spectra before and after Mw heating are identical for all concentrations.

employing a conventional heating method. Similarly, the absorption spectrum of gold colloids remains unchanged after conventional heating (data not shown).

The size of the gold colloids used here is 20 nm in diameter, which corresponds to a surface of area of 1256 nm^2, although we do envisage that colloids of various sizes could be used with this platform technology. Thus, after the surface modification procedure, a single gold colloid can carry ~34 biotinylated-BSA molecules (considering 54.7% surface coverage, i.e., random sequential adsorption theory[10]). The aggregation of the gold colloids results in a broadening of the surface plasmon bands to longer wavelengths than those for the individual colloids, which is used as an indicator of the aggregation process.[7]

Figure 3 shows the change in absorbance of biotinylated-BSA 20-nm gold colloids (cross-linked by different additions of streptavidin, both without (20-min incubation) and after low-power microwave heating), as well as the change in absorbance at 650 nm for both the room-temperature-incubated (1 and 20 min) and microwave-heated (10 s) samples. The extent of the aggregation of biotinylated gold colloids was almost identical both at room temperature (20 min) and with microwave heating, while the extent of aggregation of biotinylated gold colloids was significantly less than either of these cases at room temperature when run for 1 min (Figure 3D). These results indicate that low-power microwave heating of the gold colloids results in the completion of the aggregation process in ~10 s, a 120-fold increase in kinetics compared to the room-temperature assay.

Control experiments, where the binding sites of streptavidin were blocked with free D-biotin (i.e., "blocked streptavidin") prior to the mixing with biotinylated gold colloids, showed that there was no aggregation of gold colloids due to the nonspecific interactions of biotinylated gold colloids and streptavidin induced by microwave heating and at room temperature (no microwave heating), Figure 4—left. In fact, previous studies have shown that microwave heating reduces the nonspecific interactions.[11−14] Figure 4 also shows that the absorption spectra of biotinylated-BSA 20-nm gold colloids in the presence of increasing concentrations of streptavidin after 1 min of incubation at room temperature did not change, indicating the incomplete aggregation process in 1 min at room temperature.

A recent report on the effects of microwaves (800 W, at 2.45 GHz for 10 s) on proteins showed that microwave photons interact with the tertiary structure of proteins but the chemical bonds are unaffected.[15] In addition, it was reported that microwaves (100 mW, 12 GHz for 8 h) accelerated the macromolecular aggregation of amyloid β-protein as well as dissolution of the protein aggregates in the presence of gold colloids with 99.99% of the microwave energy being absorbed by the gold colloids since water does not preferentially absorb the electromagnetic energy at these wavelengths, i.e., at 12 GHz.[9] In an another report by us,[11] fluorescence resonance energy transfer studies on proteins in proximity to silver nanoparticles revealed that proteins do not denature when exposed to microwaves (140 W, at 2.45 GHz for 30 s) despite the absorption of electromagnetic energy by water at 2.45 GHz that resulted in an increase in the temperature of the bulk medium by 8 °C. This was possible by the attenuation of the microwaves with the microwave-absorbing material present around the sample chamber.[11]

In the current study, the microwave power used for the aggregation assay was 140 W (at 2.45 GHz for 10 s), which resulted in an increase in the temperature of the bulk medium by 5 °C (Figure 8) primarily due to the direct absorption of energy by water at 2.45 GHz. The heat absorption and loss by the 0.8 nM gold colloids was calculated to be 10 560 J/mL (See Supporting Information). The dissipated heat from the gold colloids is much larger than the energy required for formation of biotin−avidin complex (88 J/mL, Supporting Information). While we cannot be certain about the extent of dissipated energy from the colloids that results in increase in kinetic energy of gold colloids and increase in bulk temperature (due to dissipation of the absorbed energy by the gold colloids as heat to the solution) at this time, we hypothesize that a significant part of the dissipated energy from the gold colloids is contributing to kinetic energy, which results in the ability of the biotinylated gold colloids to move about the solution volume at increased velocities. However, we note that, as previously hypothesized by Hamad-Schifferli et al.,[16] the increase in kinetic energy of gold colloids is a result of induced dipole torque on the colloids. The initial velocity (after 1-s microwave heating) of the biotinylated gold colloids reaches up to 10.5 m/s (Supporting Information), which gives rise to greater

(10) Feder, J.; Giaever, J. *J. Colloid Interface Sci.* **1980**, *78*, 144.
(11) Aslan, K.; Geddes, C. D. *Anal. Chem.* **2005**, *77*, 8057−8067.
(12) Aslan, K.; Geddes, C. D. *J. of Fluorescence* **2006**, *16*, 3−8.
(13) Aslan, K.; Malyn, S. N.; Geddes, C. D. *Biochem. Biophys. Res. Com.* **2006**, *348* (2), 612−627.
(14) Aslan, K.; Holley, P.; Geddes, C. D. *J. of Immunol. Methods* **2006**, *312*, 137−147.
(15) Bohr, H.; Bohr. *Phys. Rev. E* **2000**, *61*, 4310−4314.
(16) Hamad-Schifferli, K.; Schwartz, J. J.; Santos, A. T.; Zhang, S.; Jacobson, J. M. *Nature* **2002**, *415*, 152−154.

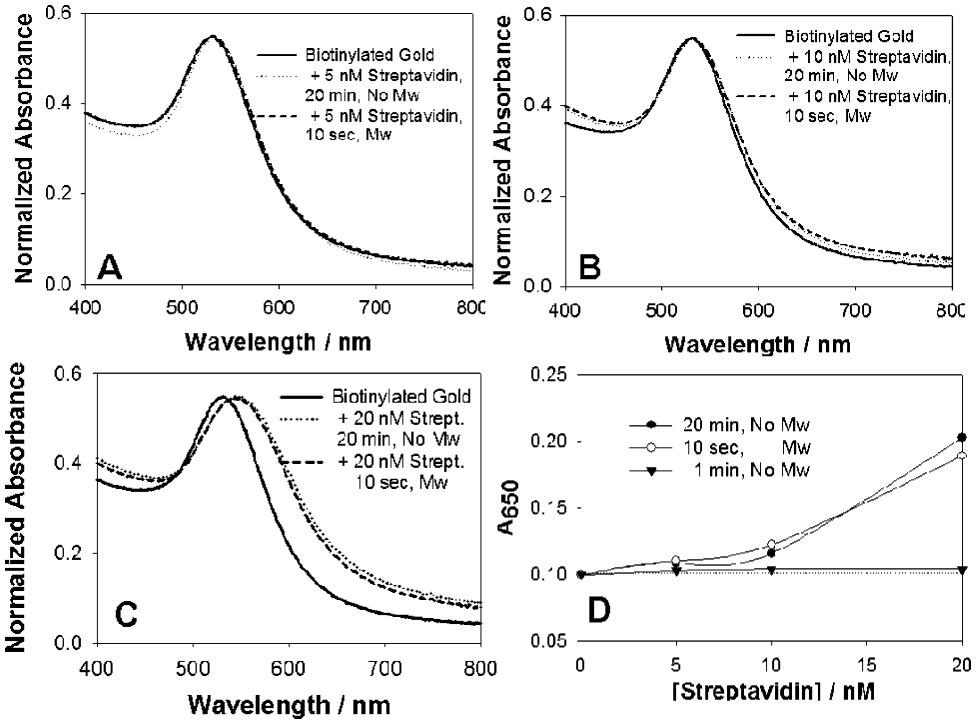

Figure 3. Change in absorbance of biotinylated-BSA 20-nm gold colloids cross-linked by different additions of streptavidin, both without (20 min incubation) and after low power microwave heating: (A) 5 , (B) 10, and (C) 20 nM streptavidin additions. Plot D shows the change in absorbance at 650 nm for both the room-temperature incubated and microwave-heated samples.

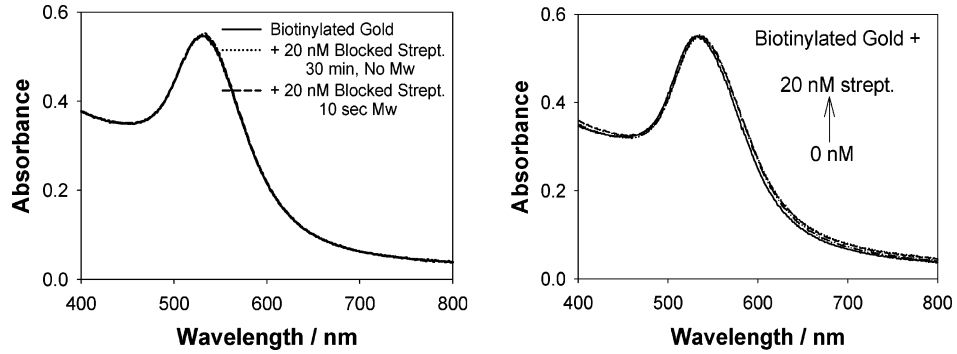

Figure 4. Control experiments: Absorption spectra of biotinylated-BSA 20-nm gold colloids (left) in the presence of blocked streptavidin, after both a 30-min incubation period and a 10-s microwave heating and (right) in the presence of increasing concentrations of streptavidin after 1 min of incubation at room temperature.

sampling of the total volume but not a large increase in bulk temperature (as is described in the next paragraph). In this regard, as the biotinylated gold colloids collide with free streptavidin molecules (and other biotinylated gold colloids) some of the energy is lost due to collisions and some for the formation of the biotin−streptavidin complex. As the biotinylated gold colloids−streptavidin aggregates increase in size more energy is used, and ultimately, the aggregation process stops after all dissipated energy is used.

In order to prove our hypothesis that the energy absorbed by gold colloids is dissipated local to the colloids, and this energy dissipation does not cause bulk heating, the temperature distribution around the gold colloids was calculated and plotted in Figure 5, and it was found to be in the order of a few microkelvin (Supporting Information). Figure 5 also shows the spatial distribution of the temperature rises inside and outside the gold colloids, which indicates the efficient dissipation of heat by the gold colloids without causing bulk heating. Thus, the primary source of increase in bulk temperature is due to the absorption of energy at 2.45 GHz directly by water. Thus, the only source of increase in bulk water temperature is the absorption of energy at 2.45 GHz, which we believe probably accounts, to some extent, for the faster aggregation kinetics. As shown before,[9] such a rapid acceleration in temperature did not denature the streptavidin molecules, and thus, the microwave-accelerated aggregation assays using gold colloids were made possible.

Microwave-Accelerated Aggregation Assay: A Platform Technology for Ultrafast Nanoparticle Assays. As a useful tool for other nanoparticle workers, and to show broad applicability of our approach, one can also calculate the expected temperature rise at the surface of the colloids ($r = R$) for various gold colloid sizes and various electromagnetic energy frequencies, Figure 6. As shown in Figure 6, the temperature rise increases with the increase in the sizes of the gold colloids, and the electromagnetic

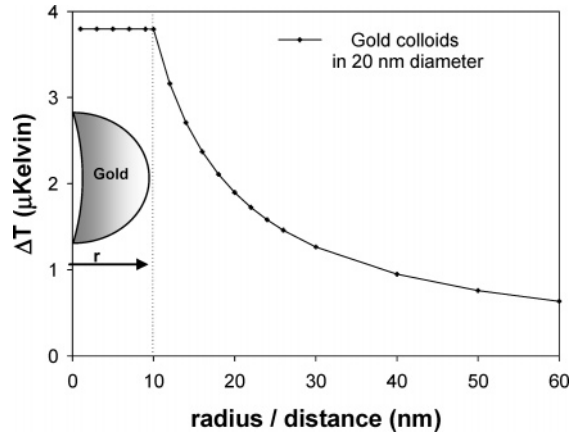

Figure 5. Spatial distribution of temperature rise (ΔT) inside (r = 0−10 nm) and outside (r = 10−60 nm) the surface of gold colloids at equilibrium. Note that while the temperature rise inside the gold colloids is constant, the temperature rise diminishes as the distance from the surface of gold colloids increases. Equations 8 and 9 (see Supporting Information) were used to calculate this figure.

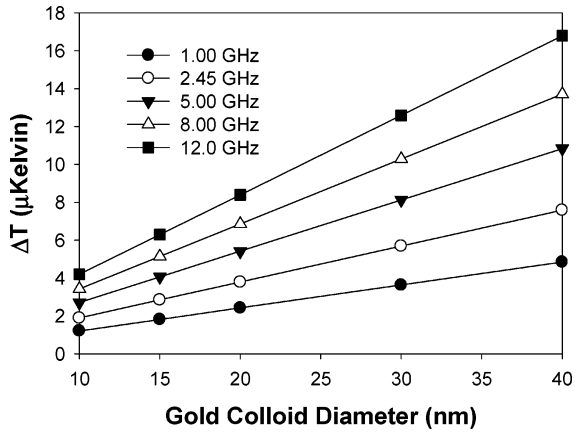

Figure 6. Calculated rise in temperature (ΔT) close to the surface ($r = R$) of various size of gold colloids using various frequencies of electromagnetic energy. Equation 9 (See Supporting Information) is used to plot this figure.

energy frequency. This shows that different sizes of gold colloids as well different microwave sources can be used in MA-AA assays without causing a large increase in the bulk temperature.

One can also calculate the initial velocity of the gold colloids right after (t = 1 s) the absorption of electromagnetic energy (after 1 s, from kinetic energy, KE = $P_{T,1s}$). As shown in Figure 7, the initial velocity of the gold colloids increases with the increase in the size of the gold colloids and the electromagnetic energy frequency. Different sizes of gold colloids (along with different electromagnetic energy) could be also used in MA-AA assays for even faster kinetics.

CONCLUSIONS

In this paper, we have demonstrated the microwave-accelerated aggregation assay technique, using a standard household microwave generator and gold colloids as localized energy "sinks", for ultrafast solution-based aggregation assays. We have found that the assay's run time is reduced from minutes to within a few seconds, a result of increased velocity (from on the order of μm/s[17] to 10 m/s) of the gold colloids. Our experimental observations

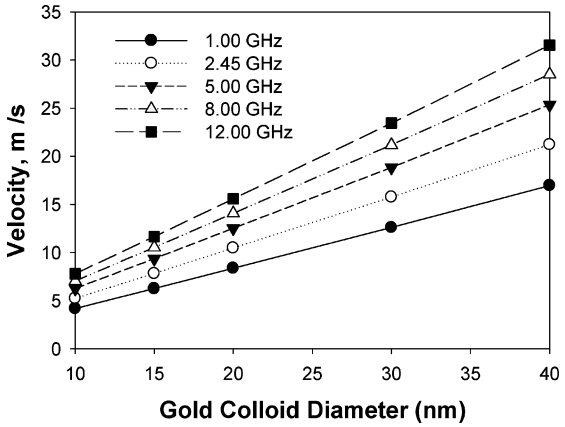

Figure 7. Calculated initial velocity of the biotinylated gold colloids after 1 s of microwave heating at different microwave frequencies.

and calculations show that the bulk heating of the solvent (water in our case) is negligible by the nanoparticles, the solvent directly heated by microwaves at 2.45 GHz. In addition, our calculations show that a 5 °C increase in bulk solution temperature, does not solely account for the fast reaction times, where the significant increase in nanoparticle velocity is thought to account for this. With an initial velocity of >10.5 m/s directly after heating, the particles are thought to sample a greater volume per unit time, accounting for the increased assay kinetics. Finally, we have also provided additional charts showing both the temperature increase and velocity, for a range of microwave frequencies and nanoparticle sizes. Work is currently underway to adapt MA-AA to solution-based DNA hybridization assays and will be reported in due course.

MATERIALS AND METHODS

Materials. Bovine biotinamidocaproyl-labeled albumin (biotinylated BSA), streptavidin, thymol blue, D-biotin, sodium phosphate monobasic, and phosphate-buffered saline (PBS) were obtained from Sigma-Aldrich. Gold colloids with 20- and 200-nm diameter size were purchased from Ted Pella. All chemicals were used as received.

Methods. (1) Preparation of Biotinylated BSA-Coated 20-nm Gold Colloids. The surface modification of 20-nm gold colloids was performed using an adapted version of the procedure found in the literature.[18] In this regard, 5 mL of the gold colloid solution was mixed with 0.05 mL of an aqueous solution of biotinylated BSA (1.44 mg/ml), and this mixture was incubated at room temperature for 2 h. The gold colloids/biotinylated BSA mixture was then centrifuged in an Eppendorf centrifuge tube equipped with a 100 000 MW cutoff filter for 10 min, using an Eppendorf microcentrifuge at 8000*g*, to separate the biotinylated BSA-coated gold colloids from the excess biotinylated BSA. The supernatant was carefully removed, and the pellet containing the biotinylated gold colloids was resuspended in 10 mM sodium phosphate buffer (pH 7). This was subsequently used in the aggregation assays.

(17) Ng, L. N.; Zervas, M. N.; Wilkinson, J. S.; Luff, B. J. *Appl. Phys. Lett.* **2000**, *76*, 1993−1995.
(18) Aslan, K.; Holley, P;, Davies, L;, Lakowicz, J. R.; Geddes, C. D. *J. Am. Chem. Soc.* **2005**, *127*, 12115−12121.

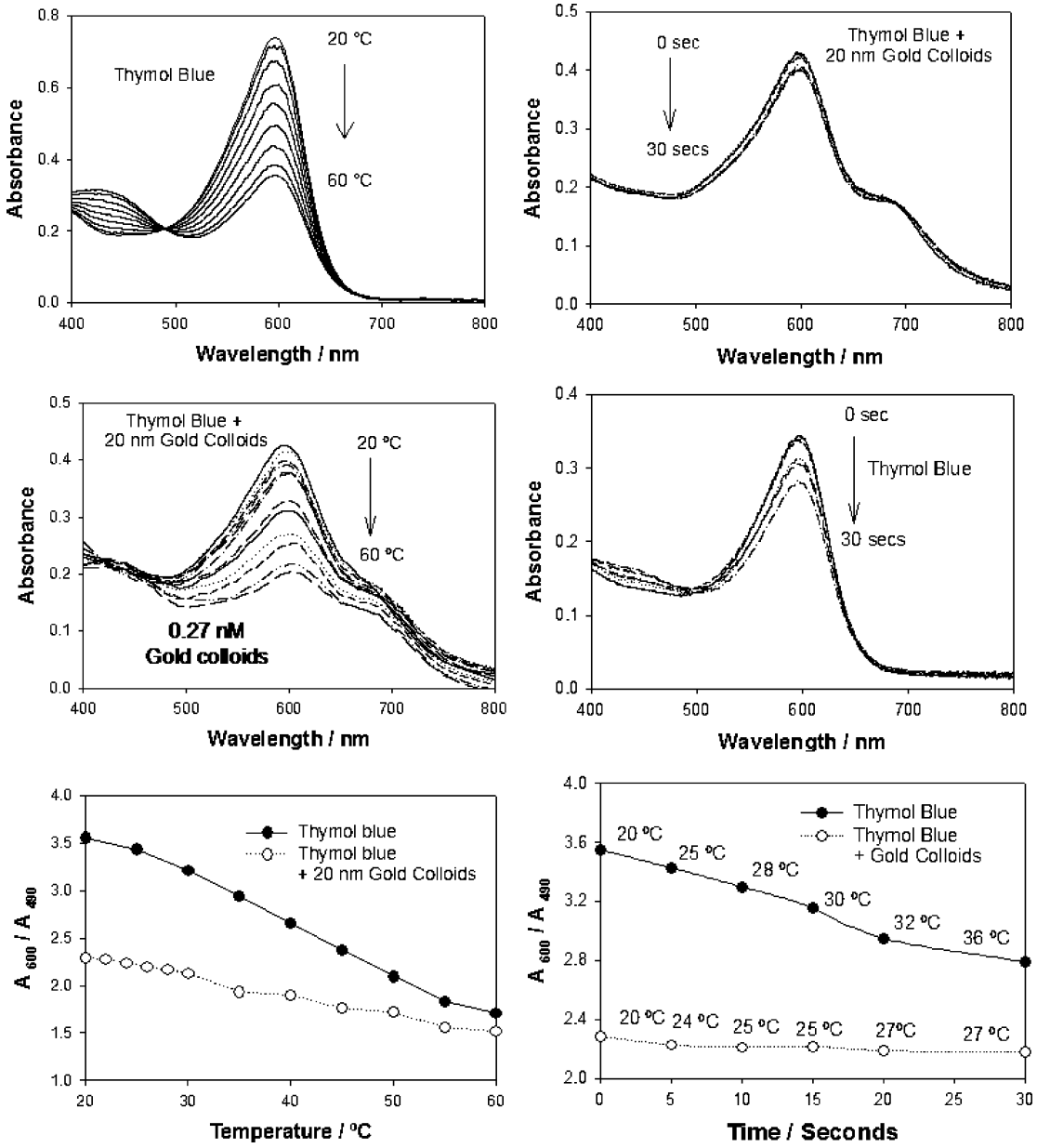

Figure 8. (Left) Absorption spectrum of thymol blue as a function of temperature, (left-top) the same solution containing 20-nm colloids (left-middle), and the respective A_{600}/A_{425} ratiometric plots vs temperature (left-bottom). (Right) Absorbance spectra of 450 μL of 20-nm gold colloids and thymol blue microwave heated in the black body for different times (right-top), 450 μL of thymol blue solely heated in the black body (right-middle), and the respective ratiometric plots (right-bottom).

(2) Aggregation Assays Using Biotinylated Gold Colloids and Streptavidin at Room Temperature and with Microwave Heating. The model aggregation assay was performed by mixing 0.8 nM biotinylated gold colloids (20 nm) with increasing concentrations of streptavidin in a quartz cuvette with microwave heating and at room temperature. It was previously found that the use of 0.8 nM gold colloids (20 nm) resulted in the largest change in absorption and thus higher sensitivity.[7] In this regard, a 1000 nM stock solution of streptavidin (prepared in PBS based on the specifications provided by the manufacturer, E1% at 282 nm = 31.0) was added to 0.5 mL of biotinylated gold colloid samples, and the final mixture was heated with microwaves (2.45 GHz, 140 W) for 10 s or incubated at room temperature for 30 min. In order to achieve the desired final streptavidin concentrations, predetermined volumes of streptavidin stock solution were used. The kinetics of aggregation (data not shown) and degree of aggregation were measured by recording the absorption spectrum of each sample (as with all other absorption measurements), using a Varian Cary 50 spectrophotometer.

Control experiments were performed similar to the procedure described above, the difference being the binding sites of streptavidin were blocked with free D-biotin (i.e., "blocked streptavidin") prior to the mixing with biotinylated gold colloids.

(3) Temperature Calibration in the Microwave Cavity. To achieve microwave power tunability and therefore assay temperature and completion time flexibility, a "black body" (black assembly tape) was employed around the commercially available microcuvette, which held ~500 μL of liquid. It was found that the presence of the black body had the desirable effect of substantially reducing the local cavity power, where without the black body

microcuvette, 500 μL of liquid quickly boiled and dried at 140 W. In essence, the use of the black bodied microcuvette afforded the tunability of the cavity power between the number 1 and 2 settings on the microwave device, alleviating the need for spending large monies on a tunable commercial instrument.

In order to calibrate the temperature change during low-power microwave heating, a simple pH thermoindicator system was used (0.5 mM thymol blue, in 50 mM tris acetate, pH 9.0). In this regard, 500 μL of a solution of thymol blue was placed in a quartz microcuvette that was covered with the black body except for two parallel sides to allow the passage of light for absorption measurements. The absorption spectra of the sample were recorded as the temperature was gradually increased from 20 to 85 °C, Figure 8. The color of the solution changed with temperature from deep magenta to pale yellow, due to the temperature dependence of the ionization constant of the Tris buffer. As the temperature was increased, the pH of the solution decreased and the distribution of the ionization states of the thymol blue dye changed, resulting in a color change as a function of temperature. The reversible color change is readily observed in the UV−visible spectrum via changes in the 425- and 600-nm spectral bands, Figure 8−left-top.

The calibration curve (A_{600}/A_{425} vs temperature), Figure 8−left-bottom, obtained from the above-mentioned calibration measurements, was used to determine the temperature of the sample during the microwave process: the absorption spectra of 500 μL of thymol blue in a quartz cuvette covered with the same black body was recorded both before and after microwave heating for up to 10 s, Figure 8−right-top, where the both the volume used and the black body were the same as actually used in the assays. The ratiometric response (A_{600}/A_{425}) obtained from these samples, Figure 8−right-bottom, was used to determine the temperature of the sample during microwave heating from the calibration curve. From the calibration plots, a 10-s, 140-W, 2.45-GHz microwave exposure, resulted in a temperature jump of ~5 °C (to ~25 °C) for 500 μL of sample. Hence, with this calibration curve, we are simply able to change the assay surface temperature.

ACKNOWLEDGMENT

The authors acknowledge UMBI for salary support. This work was also partially supported (salary contribution to C.D.G.) by the National Center for Research Resources, RR008119.

Note Added after ASAP Publication. This paper was published ASAP on January 26, 2007 with some minor text errors; the corrected version was published ASAP on February 5, 2007.

SUPPORTING INFORMATION AVAILABLE

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

Received for review November 8, 2006. Accepted December 22, 2006.

AC0620967

Supporting Information for the Manuscript:

"Microwave-Accelerated Ultra-Fast Nanoparticle Aggregation Assays using Gold Colloids" by Kadir Aslan and Chris D. Geddes*. E-mail: geddes@umbi.umd.edu

S.1. Preferential Heating of Gold Colloids with Electromagnetic Fields and Localized Dissipation of Heat by Gold Colloids.

As we mentioned in the main text, we hypothesize that the Microwave-Accelerated Aggregation Assays is possible due to two fundamental assumptions: 1) preferential heating of the gold colloids in the medium that they are in by the electromagnetic fields and (2) dissipation of the absorbed energy by local heat dissipation, which results in the ability of the biotinylated gold colloids to move about the solution at increased velocities. In order to prove our hypothesis that energy absorbed by gold colloids is dissipated local to the colloids, and this energy dissipation does not cause bulk heating, but indeed the bulk temperature increase by 5°C because of water absorption, several calculations were made: 1) Energy absorbed by water, 2) Energy absorbed / dissipated by gold colloids, 3) Energy required for the formation of avidin-biotin complex (from literature), and 4) the temperature distribution around the gold colloids.

K. Hamad-Schifferli *et al.* (Nature, **2002**, *415*, 152-154) and Kogan *et al* (Nano Letters, **2006**, *6(1),* 110-115) outlined the equations governing heat dissipation from gold colloids in water. Here, only the calculations related to this study are given and indeed used. The microwave power used in this study was 140 W at 2.45 GHz.

S.2. Energy Absorbed by Water

The absorption of electromagnetic energy by water depends on the energy input and the water absorption coefficient, and is given by:

$$P = P_0 \exp(-k_\upsilon * \rho * \Delta x) \textbf{ … Equation [1]}$$

where P is the absorbed energy, P_0 is the energy applied (140 W), k is water absorption coefficient (1 cm^{-1} at 2.45 GHz: from J.D. Jackson, Classical Electrodynamics, John Wiley &Sons, Inc., New York, 1975) and Δx is the cuvette diameter (1 cm).

Then,

$$P = (140\ W) \exp(-1*1*1) = 51\ W$$

However, since the cuvette had a "black body" around it, which absorbs some of the microwave energy, the real energy absorbed by water is calculated to be:

$$\Delta H = m * C_p * \Delta T \textbf{ … [2]}$$

where m is the mass (0.5 g, sample volume), Cp is molar heat capacity of water (4.18 J/ (g*C) and ΔT is temperature change (5 C) obtained from **Figure S3-Right-bottom**.

Then the energy absorbed by water is:

ΔH = (0.5 g) * [4.18 J/ (g*C)] * (5 C) = 10.45 J in 10 sec microwave heating or 1.05 W.

That is, in the system used here 1 W of energy is absorbed by water and resulted in the 5°C increase in the bulk temperature (assuming that the energy dissipated by gold colloids do not result in bulk heating, which is the case as explained in the next section)..

S.3. Energy Absorbed/Dissipated by Gold Colloids

K. Hamad-Schifferli *et al.* (Nature, **2002**, *415*, 152-154) described the governing equations for the energy dissipation by gold colloids. The mechanism of energy dissipation by gold colloids is due to inductive heating by Foucault currents. In this regard, firstly, the penetration depth (d_0) of the electromagnetic energy has to be calculated to determine the area of gold colloids that is interacting with it;

$$d_0 = \frac{1}{2\pi}\sqrt{\frac{\rho 10^7}{\mu_r \mu_0 f}} \quad \textbf{... [3]}$$

where μ_r is magnetic permeability (1), μ_0 is the permeability of free space (1), ρ is the material resistivity ($1/(4.1*10^7)$), and f is the frequency of the alternating magnetic field ($2.45*10^9$ Hz). For 20 nm gold colloids and 2.45 GHz electromagnetic energy $\underline{d_0 = 1586\ nm}$. That is, the penetration depth of the electromagnetic energy is two orders of magnitude larger than the diameter of the gold colloids, and thus the whole particle can dissipate the absorbed energy.

The power density (energy absorbed and dissipated) is calculated according to the following equation:

$$P = 4\pi H_e^2 \mu_0 \mu_r f F \frac{d_0}{d} \quad \textbf{... [4]}$$

where d is the gold colloid diameter, H_e is the electromagnetic field strength, and F is a transmission factor that has a sigmoidal dependence of d/d_0. Based on the assumption that H_e is 1 Gauss:

$$d/d_0 = 1586\ nm / 20\ nm = 79.3$$

$$f = 2.45 * 10^9\ Hz$$

$$\mu_r = \mu_0 = 1$$

$$H_e = 1\ Gauss$$

$$F = 0.27$$

$$\underline{P = 0.66*10^{11}\ W / m^3.}$$

Therefore, for 1 gold colloid:

$$P_{particle} = P * V_{particle} = 0.66*10^{11}\ W / m^3 * (4\pi\ (20*10^{-9})^3/3) \quad \textbf{... [5]}$$

$$\underline{P_{particle} = 220*10^{-14}\ J / s * particle}$$

Total energy loss from gold colloids (0.8 nM = $4.8*10^{14}$ particles/ ml = $N_{particles}$)

$$P_T = P * N_{particles} = [220*10^{-14}\ J / s*particle] * [4.8*10^{14}\ particles/ ml] \ldots \textbf{[6]}$$

$$P_T = 1056\ W / ml$$

The speed of gold colloids can be estimated from the energy absorbed. For 10 seconds microwave heating,

$$P_{T,\ 10\ sec} = 2200*10^{-14}\ J / particle$$

S.4. Use of Dissipated Energy by Gold Colloids

We believe that a significant part of the dissipated energy from the gold colloids is contributing to kinetic energy of gold colloids, which results in the ability of the biotinylated gold colloids to move about the solution at increased velocities. This gives rise to greater sampling of the total volume but not in increase in bulk temperature. In this regard, as the biotinylated gold colloids collide with free streptavidin molecules (and other biotinylated gold colloids) some of the energy is lost due to collisions and some for the formation of the biotin-streptavidin complex. As the biotinylated gold colloids-streptavidin aggregates increase in size more energy is used, and ultimately, the aggregation process stops after all dissipated energy is used.

The initial velocity of the gold colloids after the absorption of electromagnetic energy is calculated as below:

The mass of 1 gold colloid (density = 19.3 kg /L):

$$m = (19.3\ kg / 4.8*10^{14}\ particles).$$

Then the *initial* speed of the gold colloids right after (t=1 second) the absorption of electromagnetic energy will be calculated from kinetic energy (KE = $P_{T,\ 1\ sec}$ = $220*10^{-14}$ J / particle) term:

$$KE = \frac{mv^2}{2} \ldots \textbf{[7]}$$

$$(220*10^{-14}\ kg*m^2 / s^2*particle) = (19.3\ kg / 4.8*10^{14}\ particles) * v^2 / 2$$

$$v = 10.5\ m/s.$$

That is, the gold colloids can reach speeds up to of 10.5 m/s right after the absorption of electromagnetic energy for 1 second. However, since gold colloids carry biotin molecules and streptavidin molecules are present in the same solution, as biotinylated gold colloids start to move about the solution at high speed, they start to collide with streptavidin and other biotinylated gold colloids. This results in aggregation of the biotinylated gold colloids, the energy diminishing as the aggregations process continues until eventually all energy is lost and the aggregation stops.

One can calculate the extent of electromagnetic energy (10 sec) absorbed by the gold colloids (0.8 nM in 0.5 ml).

$$P_{T,\,10\,sec} = (1056\ J/s^{*}\ ml) * 10\ s$$

$$\underline{P_{T,\,10\,sec} = 10560\ J/ml}$$

Some of the energy dissipated gold colloids is used for the formation of avidin-biotin complexes and thus formation of aggregates. The energy required for the formation of 1 M avidin-biotin complex has been estimated before (T. Lazaridis *et al*. Proteins, 2002, 47, 194-208) as – 21 kcal / mol at 300K or 8800 J / mol.

$$P_{\text{av-biotin}} = (8800\ J/mol) * (1\ mol/L) * (1\ L/1000\ ml)$$

$$\underline{P_{\text{av-biotin}} = 88\ J/ml}$$

S.5. Localized Dissipation of Heat by Gold Colloids

In order to show our hypothesis that the energy absorbed by gold colloids is dissipated local to the colloids and that the energy dissipation does not cause bulk heating, the temperature distribution around the gold colloids is calculated using the following equations. These equations were derived for a simplified case of an energy absorbing sphere embedded in a homogeneous media of finite heat conductivity and are for *equilibrium temperature* (infinite exposure time) as a function of the distance *r* from the center of the colloids, as shown by R. Hergt *et al*, IEEE Trans. Magn. **1998**, *34*, 3745-3754 and also used by Kogan et al. Nano Letters, **2006**, *6(1),* 110-115.

The temperature distribution [ΔT (r)] inside the sphere of radius, R, when r < R:

$$\Delta T(r) = \frac{PR^2}{6\lambda_1}\left[1 - \frac{r^2}{R} + \frac{2\lambda_1}{\lambda_2}\right] \ldots \textbf{[8]}$$

The temperature distribution [ΔT (r)] outside the sphere of radius, R, when r > R:

$$\Delta T(r) = \frac{PR^2}{3\lambda_2}\,\frac{R}{r} \ \ldots \textbf{[9]}$$

P = Power density = $0.66*10^{11}$ W / m^3
R = Radius of the energy source = 10 nm
*λ_1 = gold thermal conductivity = 315 W / m*K*
*λ_2 = water thermal conductivity = 0.58 W / m*K*

Using equation 9 (r=R):

$$\underline{\Delta T\,(r) = 3.8 * 10^{-6}\ K}$$

That is, the temperature increase close to the surface of gold colloids is in the order of a few microKelvin.