T. Pellegrino[1,2,3,#], R. A. Sperling[1,#], A. P. Alivisatos[2], W. J. Parak[1,2,*]

[1] Center for Nanoscience, Ludwig Maximilians Universität München, München, Germany
[2] Department of Chemistry, University of California and Lawrence Berkeley National Lab, Berkeley, California, USA
[3] National Nanotechnology Lab of INFM, Lecce, Italy
[#] Both authors contributed equally to this work

[*]to whom correspondence should be addressed
Dr. Wolfgang J. Parak
Wolfgang.Parak@physik.uni-muechen.de

Gel electrophoresis of Gold-DNA nano-conjugates

Abstract

Single stranded DNA of different lengths and different amounts was attached to colloidal phosphine stabilized Au nanoparticles. The resulting conjugates were investigated in detail by a gel electrophoresis study based on 1200 gels. We demonstrate how these experiments help to understand the binding of DNA to Au particles. In particular we compare specific attachment of DNA via gold-thiol bonds with nonspecific adsorption of DNA. The maximum number of DNA molecules that can be bound per particle was determined. We also compare several methods to used gel electrophoresis for investigating the effective diameter of DNA-Au conjugates, such as using a calibration curve of particles with known diameters and Ferguson plots.

Introduction

DNA functionalized gold nanoparticles are an interesting system with applications ranging from biological sensors to the construction of self-assembled materials. Experiments are based on attaching single stranded DNA molecules via thiol-gold bonds to the surface of Au nanoparticles and a subsequent self-assembly process of these conjugates based on base pairing of complementary DNA molecules {Fritzsche, 2003 #9366; Mirkin, 2000 #4683; Dujardin, 2002 #8339; Wang, 2005 #10519}. As example, by employing DNA-gold conjugates several groups have developed schemes to detect target DNA sequences {Elghanian, 1997 #3441} and to assemble nanoparticles into macroscopic materials {Mirkin, 1996 #3440; Alivisatos, 1996 #3342}. DNA-functionalized Au nanoparticles are the building blocks for the above mentioned

experiments. Therefore it is of great interest to investigate the properties of these conjugates in detail.

Due to the high affinity of thiol-groups to gold surfaces thiol-modified DNA molecules can be directly bound to the surface of citrate- or phosphine-stabilized Au nanoparticles {Loweth, 1999 #5407; Letsinger, 2000 #9532}. Although commonly a random number of DNA molecules is attached per Au nanoparticle {Fritzsche, 2003 #9366} also particles with an exactly defined number of one, two, or three attached DNA molecules per nanoparticles can be obtained {Zanchet, 2001 #5789; Sung, 2004 #9730; Jhaveri, 2004 #9681; Ackerson, 2005 #10555}. Certainly the following parameters have significant influence on the properties of DNA-Au conjugates: coverage of the Au surface with DNA, configuration of the attached DNA molecules, and hybridization efficiency of DNA attached to Au surfaces. These parameters are strongly connected. The degree of DNA coverage will influence the DNA conformation which in turn will affect the hybridization efficiency. Also nonspecific adsorption has to be considered.

A body of experiments investigating these parameters has been reported for DNA attached to flat Au surfaces using different techniques such as atomic force microscopy (AFM) {Mourougou-Candoni, 2003 #9210; Huang, 2001 #9336; Huang, 2000 #6641}, surface plasmon resonance (SPR) spectroscopy {Peterson, 2001 #9341; Peterson, 2002 #9212; Peterlinz, 1997 #6539}, radioisotopic techniques {Steel, 2000 #5064; Herne, 1997 #4422}, ellipsometry {Herne, 1997 #4422}, and by X-ray photoelectron spectroscopy (XPS) {Herne, 1997 #4422}. These experiments allow for a detailed picture of DNA bound to planar gold surfaces and the results have clarified the binding mechanism, the surface coverage, the hybridization efficiency, and the role of nonspecific adsorption, all in dependence of the length of the DNA.

Since the effect of curvature has to be taken into account {Leff, 1996 #9283} the results obtained for planar Au surface can be transferred to spherical Au nanoparticles only under certain restrictions. The surface coverage of Au nanoparticles with DNA has been investigated using the displacement of fluorescence labeled DNA molecules with mercaptoethanol {Demers, 2000 #6442} and by gel electrophoresis {Sandström, 2003 #9282}. Also the conformation of bound DNA {Parak, 2003 #8595; Park, 2004 #9830}, hybridization {HAMAD-SCHIFFERLI, 2002 #7564}, and the role of nonspecific adsorption {Storhoff, 2002 #8374; Sandström, 2003 #9282; Park, 2004 #9830} have been investigated for Au nanoparticles.

In this report we present a detailed study of electrophoretic mobility of DNA-Au conjugates. We will describe that this is convenient method to investigate specific and nonspecific attachment of DNA to the surface of Au nanoparticles. In particular we investigate the surface coverage dependent on the length of the DNA.

Materials and Methods

Sample preparation

Citrated coated gold nanoparticles of 5, 10 and 20 nm diameter were purchased from TED Pella (Redding, CA, USA). In order to improve their stability in buffer solution adsorbed citrate molecules were replaced by (bis(p-sulfonatophenil)phenylphosphine dehydrate, dipotassium salt) {Zanchet, 2001 #5789}. All experimental procedures are described in detail in the Supporting Information (SI). The concentration of the Au nanoparticles was determined by UV-VIS spectroscopy by using the molecular extinction coefficient of their absorption at the plasmon peak. Thiol- and Cy5- modified and unmodified single-stranded DNA were purchased from IDT (Coralville, IA USA; sequences see SI) or Metabion (München, Germany). The concentration of the DNA was determined by UV-VIS spectroscopy by using the molecular extinctions coefficient of their absorption at 260 nm. The thiol-modified and plain DNA was added to the phosphine coated Au nanoparticles at pH = 7.3, c(NaCl) = 50 mM, and samples were incubated for some hours up to several days {Zanchet, 2001 #5789; Parak, 2003 #8595} (SI).

Gel electrophoresis experiments

The resulting DNA-Au conjugates were loaded on 0.5% - 6% agarose gels (agarose: Gibco BRL, # 15510-027; 0.5 x TBE buffer, pH 9) and run for one hour at 100 V {Zanchet, 2001 #5789; Parak, 2003 #8595} (SI). As reference always unconjugated Au nanoparticles of the same diameter were run on the same gel. In addition gels with unconjugated Au nanoparticles of different diameter and free DNA of different length were run. The bands of the plain and DNA-conjugated Au nanoparticles were directly visual by the read color of the Au colloid and the free DNA was visualized by an attached fluorescence label (fluorescein, Cy3, or Cy5). The bands of the gels were photographed using a digital camera system (Eagle Eye II, Biorad, USA). The mobility of each sample was determined by measuring the position of each band referring to the start position where the samples had been loaded. This resulted in a comprehensive set of data which relates the mobility of DNA-Au conjugates to the diameter of the Au particles, the amount and the length of the attached DNA, to nonspecific or specific attachment via thiol-gold bonds, and the gel percentage.

Calculation of the efficient diameter of the DNA-Au conjugates

Since mobility is not an illustrative quantity we have converted the mobilities of DNA-Au conjugates in effective diameters {Parak, 2003 #8595}. The evaluation of the gels in which plain Au-nanoparticles of known diameter were run yielded a calibration curve in which the mobility is plotted versus the diameter {Parak, 2003 #8595}. By using this calibration curve the mobility of the DNA-Au conjugates could be directly converted into effective diameters {Parak, 2003 #8595}. Alternatively the mobility of DNA-Au conjugates at different agarose concentrations was used to obtain Ferguson plots {Ferguson, 1964 #10757} and a fits of the Ferguson plots yielded the retardation

coefficients {Park, 2004 #9830}. First this procedure was performed for plain Au-nanoparticles of known diameter and a calibration curve in which the retardation coefficients were plotted versus the particle diameter was obtained {Park, 2004 #9830}. By using this calibration curve the effective diameters of DNA-Au conjugates could be derived from the retardation coefficients derived from the Ferguson plots of the DNA-Au conjugates{Park, 2004 #9830}.

Determination of the maximum number of attached DNA molecules per particle

We have also quantified the maximum number of DNA strands that can be attached per gold nanoparticle for particles with 5 nm and 10 nm diameter and single stranded DNA with 8 and 43 bases. For this purposed single stranded DNA that had been modified with a thiol group on one and a Cy5 dye on the other end has been attached via formation of thiol-Au bonds to the surface of Au particles. DNA was added in different DNA to Au ratios and the conjugates were run on an agarose gel. The more DNA bound per Au nanoparticle the more the band of this conjugate was retarded on the gel {Parak, 2003 #8595}. At a certain amount of added DNA the retardation of the band of the conjugates did not further increase, which indicates that the Au surface is fully saturated with DNA {Parak, 2003 #8595}. The bands were extracted from the gel by cutting out the agarose piece that contained the band and putting it into 0.5x TBE buffer solution. After two days the DNA-Au conjugates had diffused out of the gel in the buffer. The cutting procedure ensures that all DNA is really attached to the Au particles, since free DNA migrates in a much faster band. UVVIS spectra were recorded of the extracted DNA-Au conjugates. For each of the conjugates the DNA concentration was determined by the Cy5 absorption and the Au concentration was determined by the absorption at the plasmon peak and from both concentrations the number of attached DNA molecules per particles was derived.

Results and Discussion

The attachment of DNA to particles increases the effective diameter and thus lowers the electrophoretic mobility

The attachment of DNA to Au nanoparticles can be clearly observed by gel electrophoresis {Loweth, 1999 #5407; Zanchet, 2001 #5789; Zanchet, 2002 #7246; Parak, 2003 #8595; Sandström, 2003 #9282; Sandström, 2004 #9868; Park, 2004 #9830; Aubin, 2005 #10068; Jhaveri, 2004 #9681}. The mobility of particles on the gel depends on two factors: size and charge. The bigger the size the slower and the higher the charge the faster particles will migrate. In the case of negatively charged Au particles (e.g. with citrate or phosphine molecules adsorbed to the particles) the attachment of negatively charged DNA molecules causes in first place an increase of size that can be seen as a retardation of the band of the gel {Parak, 2003 #8595}. If an increase in charge dominated, then the mobility of the Au particles should be increased. Although this effect has been observed for different systems {Parak, 2002 #7047} it has not been observed for

the DNA-Au conjugates used in this study. Upon attachment of DNA the mobility of the resulting conjugates was always decreased. Therefore, in agreement with previous reports, we assume throughout this manuscript that attachment of DNA to Au nanoparticles in first order increases the effective diameter of the conjugates which can be directly seen in the retardation of the band of the conjugates in gel electrophoresis experiments {Loweth, 1999 #5407; Zanchet, 2001 #5789; Zanchet, 2002 #7246; Parak, 2003 #8595; Sandström, 2003 #9282; Sandström, 2004 #9868; Park, 2004 #9830; Aubin, 2005 #10068}.

Generation of a calibration curve that related electrophoretic mobilities to effective diameters

One aim of this study was to obtain calibration curves in which measured electrophoretic mobilities m can be related to effective diameters d_{eff}. By running phosphine stabilized Au particles of known diameter on gels, by measuring their mobility, by fitting the data with an exponential function, and by using the inverse of the fit function we obtained a function in which the effective diameter of Au particles and DNA-Au conjugates can be directly calculated from their electrophoretic mobility:

$$d_{eff}(m) = -T_y * \ln((m/m_{10nm,y}) /A_y) + 6 \text{ nm}$$

The parameters for y = 0.5%, 1%, 2%, 3%, 4%, 5%, and 6% agarose gels are listed in Table 1. In order to enhance the accuracy by making relative instead of absolute measurements we always normalized the mobilities m to the mobilities $m_{10nm,y}$ of plain phosphine stabilized Au particles of 10 nm core diameter on the same gel. Therefore, although the primary data of all electrophoresis measurements are electrophoretic mobilities we are discussing the experimental results in terms of effective diameters. The diameters have been obtained with the above described formula from the mobility data. We believe that diameters are easier to rationalize than mobilities.

Since obviously the effective diameter of DNA-Au conjugates is a fixed physical property it should not depend on the form of measurement and analysis. We therefore compared the effective diameters derived from 1%, 2%, 3% gels via the respective mobility-diameter calibration curves and from Ferguson plots {Ferguson, 1964 #10757}. For the Ferguson plots the mobility data from all gel percentages are required. For a detailed description of the analysis we refer to the Supporting Information.

Evaluation of the accuracy of effective diameters obtained from electrophoretic mobilities via mobility-diameter calibration curves

The determined effective diameters for DNA-Au conjugates for Au particles saturated with DNA and for Au particles with only few DNA strands attached per particle are plotted in Figures 1 and 2 for DNA of different length. In all cases, regardless the length of the DNA, whether DNA was attached by specific thiol-gold linkage or by nonspecific adsorption, or whether only a few or a many as possible DNA molecules were bound per

Au nanoparticles, the effective diameters derived with the mobility-diameter calibration curves are different for different gel percentages. Sometimes the effective diameters derived from gels with higher percentage are higher than the ones obtained from gels with lower percentage, and vice versa (see for example Figure 2). The effective diameters derived from Ferguson plots were always smaller than the ones derived from the mobility-diameter curves. This clearly demonstrates a severe limitation of deriving effective diameters from electrophoretic mobilities. If always the effective diameters derived from the gels of higher percentage were smaller than the one derived from gels with lower percentage one could have argued that the soft DNA shell around the rigid Au cores would be squeezed or compressed more while migrating through the gel, which would lead to smaller effective diameters. However, since no correlation between the gel concentration and the derived effective diameters was observed we have to consider the difference between the effective diameters that have been obtained from gels of different concentrations as error bars. The bigger the Au particles become due to attachment of DNA, the bigger the error in deriving their effective diameter from electrophoretic mobilities becomes. To give an example: according to Figure 1 the effective diameters of 10 nm Au particles saturated with 100 bases DNA that is specifically linked via thiol-Au bonds are 66.3 nm, 69.5 nm, and 58.5 nm as determined from 1%, 2%, and 3% gels. We believe that from these data we can assume that the effective diameter of these conjugated is around 60 nm with an error bar of around 10 nm. In the Supporting Information the data sets obtained from 1200 gels are shown. From these data we conclude that deriving absolute effective diameters from electrophoretic mobilities via mobility-diameter calibration curves is possible only under certain restrictions. It is not enough to extract the data just from gels of one percentage. Only by using gels of different percentage an average value for the effective diameter and an estimate about the error can be obtained. Part of this limitation might be due to our principal assumption that in the case of phosphine stabilized Au particles conjugated with DNA the electrophoretic mobility is in first order only determined by the size of the conjugates. Charge effects may hamper obtaining more precise data for effective diameters. For other systems in which charge effects certainly will play a more important role {Parak, 2002 #7047} it might even be impossible to derive effective diameters from electrophoretic mobilities with the here reported mobility-diameter calibration curves.

Evaluation of the accuracy of effective diameters obtained from Ferguson plots

We have also evaluated the possibility to obtain effective diameters of DNA-Au conjugates via Ferguson plots. From Figures 1 and 2 it is evident that the effective diameters obtained from Ferguson plots are always significantly smaller than the ones obtained from mobility-diameter calibration curves. It has to be pointed out that both evaluation methods are based on the same set of experimentally obtained mobilities. In a classical Ferguson plot, for example for free DNA, the logarithm of the mobilities is linear to the gel percentage. However, in the case of Au and Au-DNA conjugates this linearity holds no longer true, in particular for gels of higher percentage (see the Supporting Information). We therefore had to restrict our analysis to gels from 1% to 3%, although in some cases also data for 4% to 6% had been available. Though theories for

nonlinear, convex Ferguson plots exit {Tietz, 1986 #9703; Tietz, 1992 #10712} we did not try to apply them here. Due to the significant deviation from the data obtained with the Ferguson plots to the data obtained with mobility-diameter calibration curves and due to the above mentioned limitations we conclude that the linear Ferguson analysis is less suited to obtain absolute effective diameters. However, as with the mobility-diameter curves relative increases in size due to binding of molecules can be observed with sufficient resolution.

Specific thiol-Au bond mediated attachment of DNA versus nonspecific DNA adsorption

Although the absolute numbers derived for effective diameters for DNA-Au conjugates are afflicted with significant error bars as described above these data nevertheless yield valuable information about the binding of DNA to Au particles. Any attachment of DNA leads to an increase in the effective diameter, dependent on the nature of attachment, the amount of bound DNA, and the length of each DNA molecule, see Figure 1. With very easy models we can assume that DNA attached to the surface of Au particles can adopt two basic types of conformation {Parak, 2003 #8595}. In the first case the confirmation of DNA is not effected by the presence of the Au particles and it will be existent in random coil conformation. In the second case DNA has to compete for the binding places at the gold surface and thus in order to bind as many DNA molecules per area as possible the DNA has to be fully stretched. In reality a combination of both models will describe the reality best. In Figure 1 the effective diameters for the different models (randomly coiled DNA, fully stretched DNA, and DNA that is stretched for the first 30 bases and randomly coiled for the rest of the bases) are plotted versus the DNA length for Au particles that are saturated with DNA. Clearly thiol-gold-bond specific attachment can be distinguished from nonspecific adsorption of DNA. Similar observations have been reported also before by Sandström et al. {Sandström, 2003 #9282; Sandström, 2004 #9868}. First, the increase in the effective diameter tells that also DNA without thiol-modification can be adsorbed to the surface of phosphine stabilized Au nanoparticles. Second, a comparison with the effective diameters of the theoretical models clearly proves that nonspecifically adsorbed DNA does not exist in a stretched configuration perpendicular to the Au surface. The data rather indicate that even when the particle surface is saturated with nonspecifically attached DNA only parts of the DNA molecules will be existent in random coil configuration, as the experimentally obtained effective diameters are smaller than the diameter of conjugates in which the adsorbed DNA is randomly coiled. From this one can conclude that due to nonspecific DNA-Au interaction the adsorbed DNA is at least partly wrapped around the surface of the Au particles. In case of Au surfaces saturated with thiol-modified DNA the effective diameters are significantly bigger compared to nonspecifically adsorbed DNA, see Figure 1. By comparison with basic models we conclude in agreement to our previous study that specifically bound DNA adopts a stretched configuration so that as many DNA molecules as possible can bind to the Au surface. Due to the spherical geometry DNA longer than around 30 nm only needs to be stretched due to this space limitation within around the first 30 bases, whereas the parts of the DNA molecules further away from the Au particle are not affected by space limitation and thus can be randomly coiled. These results again

shown the possibilities and limitations of the here described method. Though it is complicated to derive accurate absolute effective diameter of DNA-Au conjugates the binding of DNA molecules can be clearly seen by increases in the effective diameters and a comparison with theoretical models can give indications about the conformation of the attached DNA. These types of binding assays via gel electrophoresis are an attractive complementary method compared to other techniques such as light scattering {Cardenas, 2006 #10660}.

Does an organic fluorophore attached to the DNA change the DNA's binding to Au particles?

When organic fluorophores are attached to DNA-Au conjugates at the free end of the DNA that is pointing towards solution then energy transfer between the fluorophore and the Au nanoparticle can be observed {Dulkeith, 2005 #9845}. This effect can be for example employed for DNA sensors {Kim, 2006 #10631}. Since energy transfer depends on the distance between the organic fluorophore and the Au surface {Dulkeith, 2002 #8667; Dulkeith, 2005 #9845} certainly the configuration of the bound fluorophore-modified DNA is important for this process. In case of nonspecific adsorption of the fluorophore to the Au surface the distance between the fluorophore and the Au would be much smaller than for the case, in which the DNA is linked with its thiol-modified end, see Table 2. In this study we have shown, that the attachment of Cy5 to the free end of thiol-modified DNA does not change the effective diameter of Au particles saturated with DNA, see Table 2. These results demonstrate that the direct adsorption of Cy5 to the Au surface is much less probable than the formation of thiol-Au bonds and that therefore the dye points towards the solution.

Counting the maximum number of DNA molecules that can be bound per one Au particle

The number of bound DNA molecules per Au particles has already been determined with several methods {Demers, 2000 #6442; Sandström, 2003 #9282}. Compared to methods in which the number of DNA molecules is quantified by the fluorescence of attached fluorophores counting of DNA via absorption measurements (as reported in this study) is not affected by photobleaching and quenching effects. Extracting the DNA-Au conjugates from the gel also helps that no unbound excess DNA is present in the solution, as still might be possible in the case of purification with filter membranes. The results of this study are summarized in Table 2 and correspond well with the results obtained by other groups {Demers, 2000 #6442; Sandström, 2003 #9282}. In our measurements we could not any effect of the different curvature between 5 nm and 10 nm gold particles on the density of attached DNA molecules.

Attachment of an exactly known number of DNA molecules per Au particle

As already reported in earlier publication gel electrophoresis allows for a separation of DNA-Au conjugates with 0, 1, 2, ... DNA molecules attached per particle {Loweth, 1999 #5407; Zanchet, 2001 #5789}. In Figures 2 and 3 the effective diameters of such conjugates as determined from their electrophoretic mobilities are presented. The dependence of DNA length and Au core diameter on the effective diameter is as expected. The longer the DNA the more the effective diameter of DNA-Au conjugates upon attachment of another DNA molecule to one gold particle is increased (see Figure 2). The more long DNA strands are attached per individual gold particle, the fewer the effective diameter of the DNA-Au conjugated depends on the initial diameter of the Au core (see Figure 3). So far we are not aware of another separation technique (such as HPLC) that can resolve Au particles with an individual number of attached DNA molecules as it is possible with gel electrophoresis. The concept of separating conjugates of particles with a discrete number of attached molecules by gel electrophoresis could be also generalized and used besides for DNA-gold conjugates for other systems {Sperling, 2006 #10309}. Because of their defined composition we think that such conjugates are very interesting model systems and several applications have been already demonstrated {Sharma, 2006 #10613; Zheng, 2006 #10760}.

Conclusions

In this manuscript the analysis of DNA-Au conjugates by gel electrophoresis is analyzed. Whereas the principal effects are already known by our previous studies and reports by other groups the aim of this work was the detailed analysis about the possibilities and limitations of this technique. For this purpose an extensive study with 1200 gels was performed. The results of the complete data set can be seen in the Supporting Information. From these data we can conclude that the determination of absolute effective diameter from electrophoretic mobilities has clear limitations. In order to get an estimate about the accuracy the data of gels of different percentages have to be compared. The deviation between these data sets is an indicator for the error bars in the derived effective diameters. We believe that this strategy leads to more reliable values for effective diameters than Ferguson analysis. Though the extraction of absolute values for effective diameters from the mobility data has limited accuracy, the attachment of molecules to particles can on the other hand be detected with high sensitivity as increment in the effective diameters. In this way even the attachment of single molecules can be resolved. Besides such binding assays also indications about the conformation of the DNA molecules that are bound to the particles can be derived from the obtained effective diameters. In this way we believe that gel electrophoresis, and in particular deriving effective diameters from electrophoretic mobilities, is a very powerful method to investigate the attachment of DNA molecules to Au nanoparticles, although conclusive results only can be obtained with a detailed knowledge of the limitations of this technique.

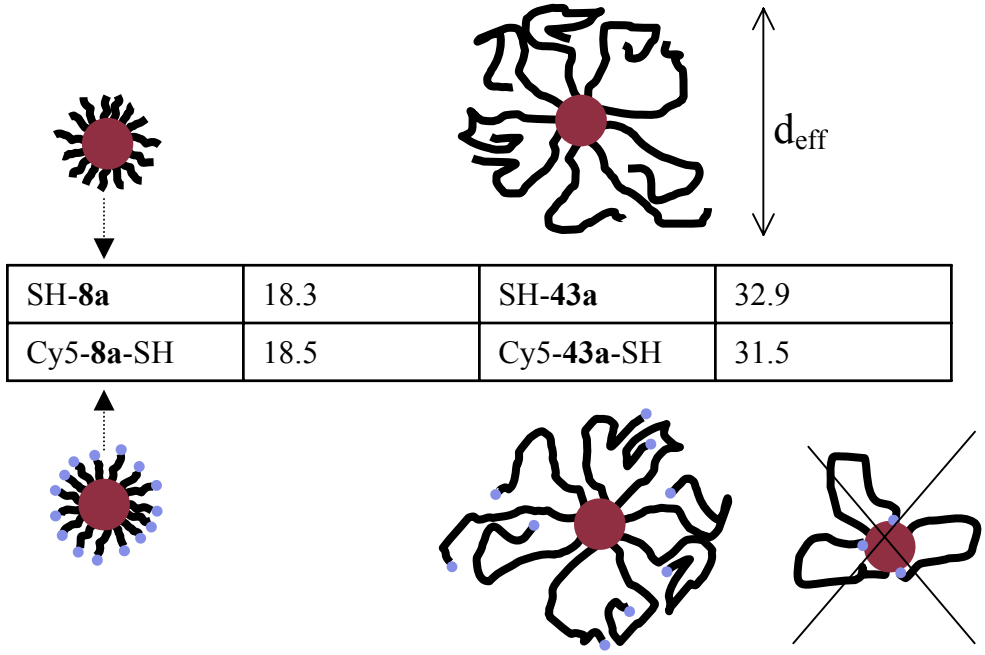
Acknowledgement

The authors are grateful to Dr. Eric Dulkeith for helpful comments. This work was supported by the German Research Foundation (DFG, Emmy Noether program), the European Union (STREP program NANO-SA) and the Center for Nanoscience (CeNS).

References

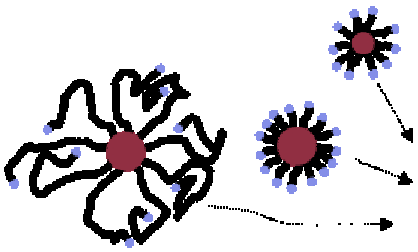
y	A_y	T_y
0.5%	1.017 ± 0.015	189 ± 19
1%	1.049 ± 0.012	85.0 ± 3.7
2%	1.120 ± 0.024	37.7 ± 1.9
3%	1.236 ± 0.025	18.8 ± 0.8
4%	1.476 ± 0.061	10.3 ± 0.9
5%	1.759 ± 0.079	7.16 ± 0.66
6%	2.073 ± 0.083	5.77 ± 0.49

Table 1: Experimentally obtained parameters for deriving effective diameters from electrophoretic mobilities for different gel percentages y (see also the Supporting Information).



SH-**8a**	18.3	SH-**43a**	32.9
Cy5-**8a**-SH	18.5	Cy5-**43a**-SH	31.5

Table 2: 10 nm diameter Au particles have been saturated with thiol-modified single stranded DNA of 8 (sequences SH-**8a** and Cy5-**8a**-SH) and 43 bases lengths (sequences SH-**43a** and Cy5-**43a**-SH) and have been run on 2% agarose gels. From the resulting mobilities effective diameters were derived via a mobility-diameter calibration curve (for 2% agarose gels). In the table the effective diameters of particles are given in nm. In the upper row the data for DNA modified at one end with a -SH group are shown. In the lower row the data for DNA modified at one end with a -SH and at the other end with a -Cy5 organic fluorophore are shown. The results are within the error bars identical for DNA with and without Cy5, which indicates that the Cy5 at the free end does not interfere with the binding process of the DNA to the Au particle surface.



d(Au) [nm]	number of bases per DNA	maximum number of DNA molecules per Au particle	maximum DNA density on the particle surface [nm^{-2}]
5	8	13	0.041
10	8	53	0.042
10	43	43	0.033

Table 3: Maximum number of thiol-modified single stranded DNA molecules that can be bound to the surface of phosphine-stabilized Au particles. Au particles of different core-diameter (d = 5 nm, 10 nm) and thiol modified single stranded DNA of different length (8 bases: sequence Cy5-**8a**-SH, 43 bases: sequence Cy5-**43a**-SH) have been used. The maximum possible number of DNA molecules per Au particle and the maximum surface density (in DNA per particle surface) are given.

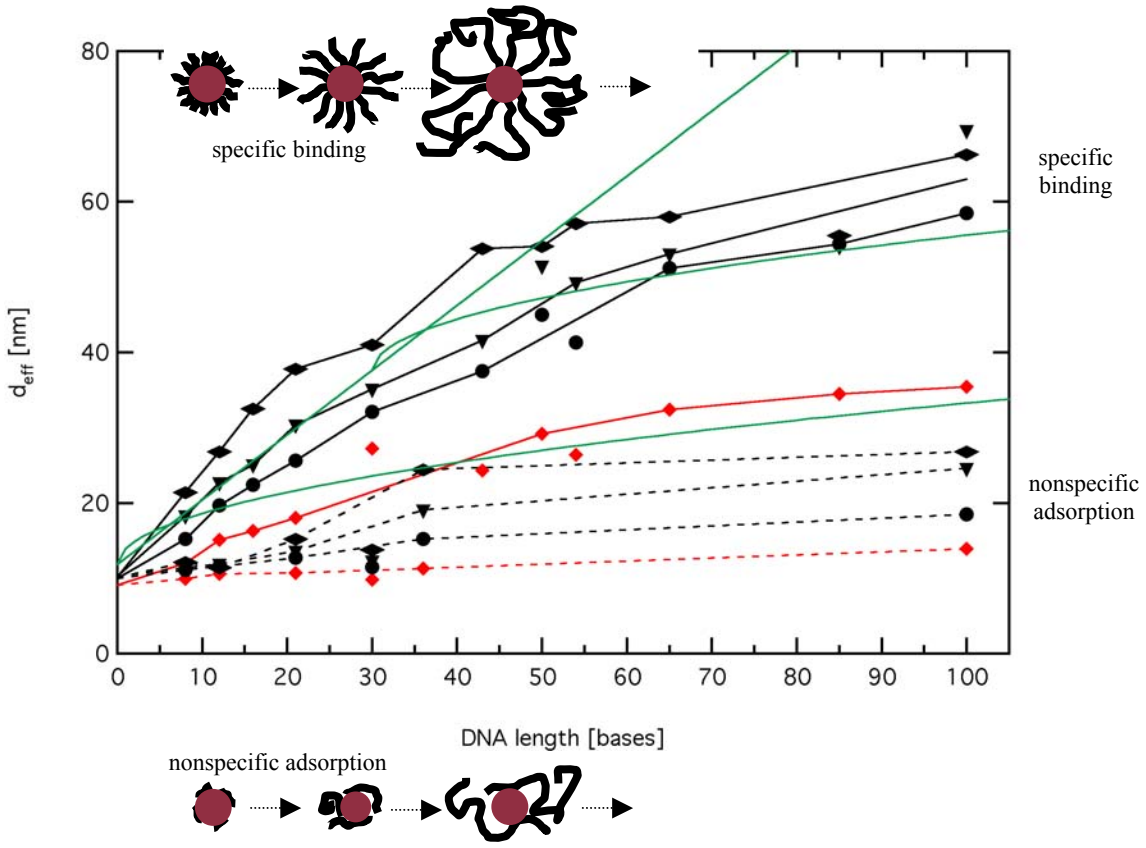


Figure 1: Effective diameter d_{eff} of Au-DNA conjugates for Au surfaces saturated with DNA. The surface of 10 nm phosphine stabilized Au nanoparticles was saturated with single stranded DNA of different lengths and the conjugates were run on 1%, 2%, and 3% gels. From the measured mobilities the effective diameters of the conjugates were determined as described in detail in the Supporting Information. The effective diameters obtained from 1%, 2%, and 3% gels are plotted in black with diamond, triangle, and circle symbols, the effective diameters obtained from Ferguson analysis are plotted in red. The effective diameters of conjugates in which the DNA was linked to the Au particles via specific thiol-gold bonds are connected with straight lines, the effective diameters of conjugates in which the DNA is nonspecifically adsorbed to the Au particles are connected with dotted lines. The green lines correspond to rudimentary theoretical models of the effective diameters of DNA molecules attached via thiol-gold to Au particles {Parak, 2003 #8595}. For fully stretched DNA (bottom curve) $d_{eff,linear}(N)$ = 10nm + 2•(0.92nm + N•0.43 nm), for randomly coiled DNA (top curve) $d_{eff,coil}(N)$ = 10nm + 2•(0.92nm + 2•$[3^{-1}$•N•0.43nm•2nm$]^{1/2}$), and for DNA partly stretched and partly coiled DNA (middle curve) $d_{eff,mixed}(N)$ = 10nm + 2•(0.92nm + 30•0.43nm + 2•$[3^{-1}$•(N-30)•0.43nm•2nm$]^{1/2}$) was used {Parak, 2003 #8595}. We assumed 0.92 nm for the length of the thiol-hydrocarbon (C_6) spacer at the reactive end of the DNA, 0.43 nm per base for the contour length and 2 nm for the persistence length {Tinland, 1997 #7932; Rivetti, 1996 #4406}. N corresponds to the number of bases.

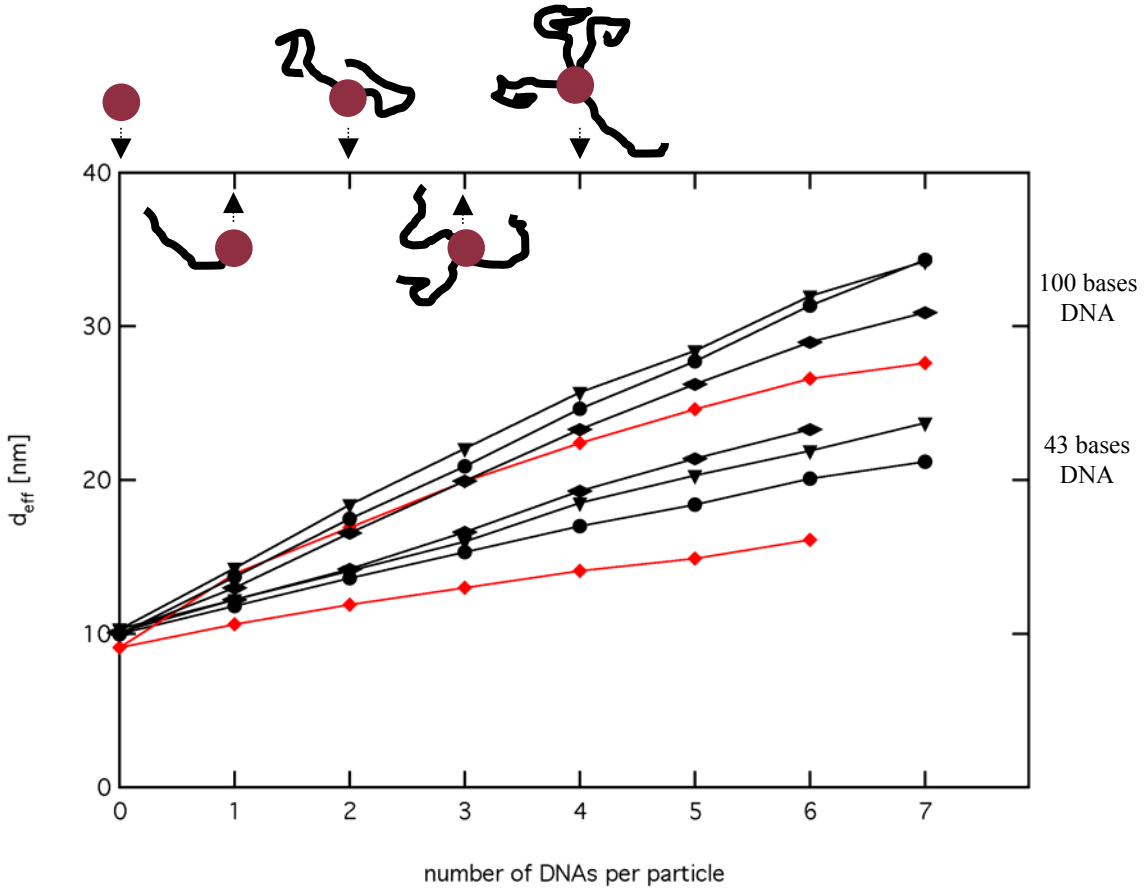


Figure 2: Effective diameter of DNA-Au conjugates with a discrete number of DNA molecules attached per Au nanoparticle. 10 nm Au particles were incubated with thiol-modified single stranded DNA of 43 bases (sequence SH-**43a**) and 100 bases length (sequence SH-**100a**) and run on 15, 2%, and 3% agarose gels. On the gels particles with exactly 0, 1, 3, 4, ... DNA molecules attached per Au particle could be identified as discrete bands. From the mobilities of the bands on the gels the effective diameters d_{eff} were derived by using a calibration curve that relates mobilities and diameters. The effective diameters corresponding to effective diameters derived from 1%, 2%, and 3% gels are plotted in black with diamond, triangle, and circle symbols, respectively. From the mobility data of the gels of different percentage effective diameters were also obtained by the Ferguson method and are plotted in red. The upper and lower sets of curves belong to the Au-DNA conjugates with 100 bases and 43 bases DNA, respectively.

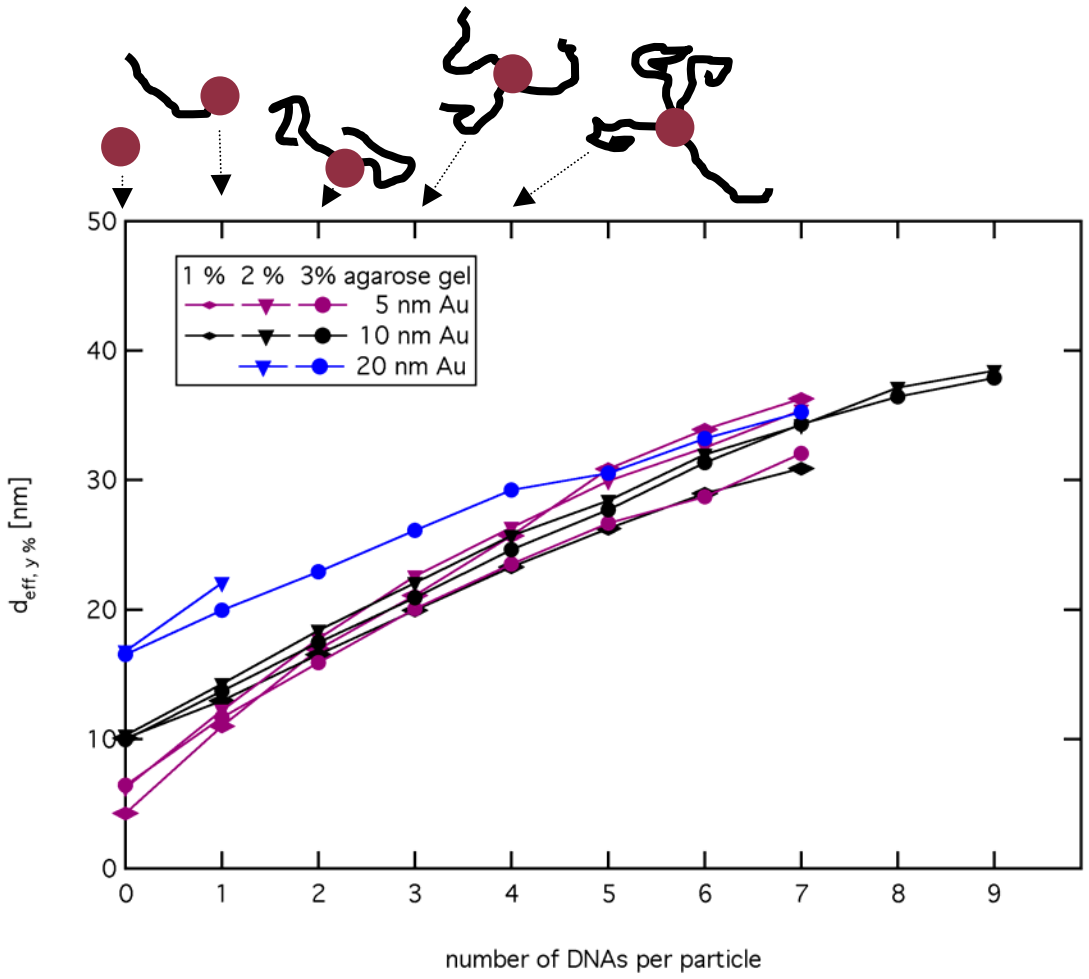


Figure 3: Effective diameters d_{eff} of Au-DNA conjugates with a discrete number of DNA molecules per particle for Au particles of different diameter. Single stranded DNA (100 bases, sequence SH-**100a**, see Supporting Information) had been specifically attached via thiol-gold bonds to the surface of 5 nm, 10 nm, and 20 nm Au particles. The conjugates were run on 1%, 2%, and 3% agarose gels and their effective diameters d_{eff} were derived from the measured electrophoretic mobilities. Here the effective diameters for Au particles with a discrete number of attached DNA molecules (100 bases) per particle are shown. Data for 5 nm, 10 nm, and 20 nm particles are plotted in violet, black, and blue, respectively. Data derived from 1%, 2%, and 3% gels are plotted with diamond, triangle, and circle symbols.