

Plasmon enhanced silver quantum cluster fluorescence for biochemical applications

S. Bernard*, J. P. Kutter** and K. B. Mogensen*

* Technical University of Denmark (DTU), Dept. of Micro- and Nanotechnology
2800 Kgs. Lyngby, Denmark, klaus.mogensen@nanotech.dtu.dk

** University of Copenhagen (KU), Dept. of Pharmacy
2100 Copenhagen, Denmark, jorg.kutter@sund.ku.dk

ABSTRACT

Fluorescence microscopy of individual silver quantum clusters on the surface of silver nanoparticles reveals strong photoactivated emission under blue light excitation [1-4]. In this work, silver nanoparticles are produced by annealing silver thin films deposited on a glass substrate and silver quantum clusters are subsequently synthesized at the surface of the nanoparticles by photoactivation in presence of Ag^+ cations in solution. The photogeneration of these silver quantum clusters leads to a great increase in the fluorescent signal.

This photoactivated surface can then be used for sensing purposes. It was found, that in presence of a strong nucleophile (such as CN^-), silver quantum clusters are dissolved into non-fluorescing AgCN complexes, resulting in a fast and observable decrease of the fluorescent signal.

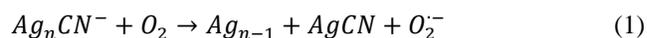
Keywords: silver quantum clusters, silver nanoparticles, plasmon enhanced fluorescence, optical cyanide detection

1 INTRODUCTION

Quantum clusters (QCs) are intermediates between atoms and nanoparticles (NPs) and can have very interesting luminescent properties [1-4]. AgQCs consisting of around 2-10 atoms can be highly fluorescing in the visible wavelength range [1]. AgQCs have been synthesized using chemical reducing agents in solution and conjugated with, e.g., DNA by a number of groups [2]. This approach has made it possible to produce novel types of fluorescent biosensors that possess a higher photostability and brightness than most organic fluorophores. An alternative strategy is, however, to use plasmonics for synthesis of the QCs, which takes advantage of the 'hot electrons' resulting from excitation of the plasmon band.

Only little work has been reported on attempts to utilize nanoparticle supported AgQC fluorescence for sensing purposes, e.g., by adsorption of proteins/surfactants on the surface, or tests with different organic solvents, where a minor difference in luminescence has been observed [5]. The novelty of this work is that here, a scheme based on oxidative dissolution is employed, where the QCs are dissolved upon interaction with the analyte, resulting in a

much bigger change in the fluorescent signal. Silver is dissolved by molecular oxygen in the solution with a very slow rate constant (half-life of several days). However, upon interaction with nucleophilic reagents, the rate is dramatically increased. The nucleophile donates an electron pair at the metal surface through a coordinative bond. The increase in electron density at the surface results in an increase in the free electron density at the bulk of the particle, thereby increasing the Fermi level (and decreasing the reduction potential). Hence, it is easier for the electrons to 'escape' from the metal, thereby promoting charge transfer to molecular oxygen in solution [6]. A possible reaction scheme is shown here, which is identical to the well-known extraction of gold from metal ores by cyanide.



Ag_n , denotes AgQCs , consisting of n atoms. The superoxide radical, O_2^- , is formed as a result of the oxidation. It should be kept in mind that the reaction does not stop here [7]. The oxygen radical is further reduced until it finally becomes water. This may involve a total of four electrons that all are donated from silver; hence the result of the reaction is properly not removal of a single Ag atom from the clusters, but complete dissolution, when they bind to CN^- .

2 EXPERIMENTAL

2.1 Chip design and fabrication

Here, plasmon-enhanced silver quantum cluster fluorescence is employed for chemical sensing in a simple microfluidic device (Figure 1). The bottom substrate is covered by plasmonic silver nanoparticles (AgNPs), fabricated by evaporating a 10 nm silver layer on a glass substrate and followed by annealing at 400°C in forming gas for 10 min. AgNPs are both used for quantum cluster photoactivation and indirect fluorescence detection of cyanide.

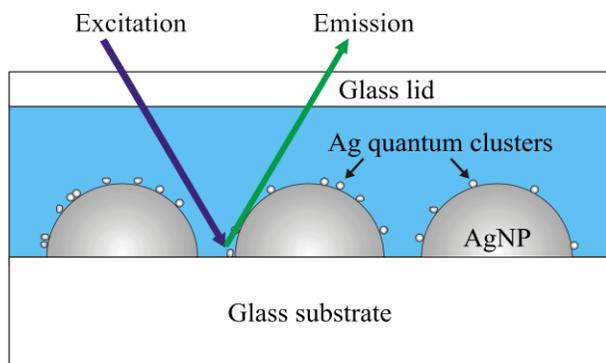


Figure 1: Cross sectional sketch of the microfluidic device, showing both AgNPs and Ag quantum clusters. The AgNPs are fabricated by high temperature annealing of vacuum deposited silver on a glass substrate, while a thin glass cover slip is sealed to the device using adhesive bonding.

A $\sim 100 \mu\text{m}$ thick glass cover slip is used as the lid in order to make $\times 100$ oil immersion microscopy of the individual QCs possible. Double sided adhesive tape is used to define the microfluidic channel and to seal the glass substrate with the cover slip. Laser ablation (CO_2 -laser) is used for fabricating an inlet hole in the lid and a glass reservoir is glued on top of the device for initial filling and exchange of the liquid. A steady fluid flow is established in the device due to evaporation of the liquid from the far side of the chip, thereby avoiding active pumping to replenish the reagents in the focal area.

2.2 Photoactivation

Initially, plasmon-enhanced photoreduction of Ag^+ cations in solution (1 mM AgNO_3 , $\text{pH}=6.0$) is employed for synthesis of the quantum clusters ($< 1\text{-}2 \text{ nm}$) at the surface of the larger AgNPs ($d=70\text{-}100 \text{ nm}$). This is done by excitation of the peak of the plasmon band using a laser diode ($\lambda=473 \text{ nm}$, 50 mW , Figure 2), which is well known to create a very high electric field density in the so-called 'hot spots' between the AgNPs. The resulting 'hot electrons' are utilized for reduction of Ag^+ cations in solution to fluorescing zero-valent Ag_n quantum clusters that reside on the plasmonic NPs.

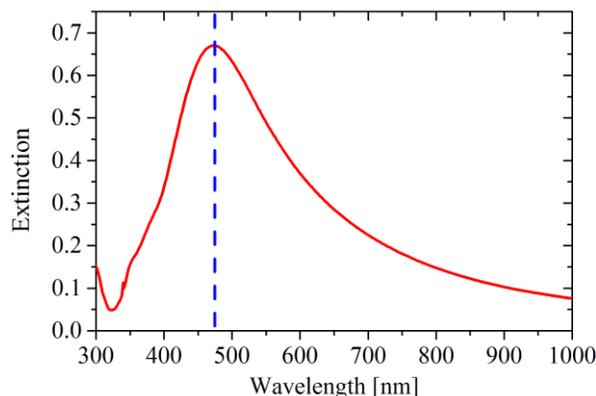


Figure 2: Plasmon band of a 10 nm Ag silver layer (nominal thickness) deposited onto a glass substrate after annealing. The particle size was around $70\text{-}100 \text{ nm}$ in in-plane diameter. This thickness was chosen in order to have a good overlap with the $\lambda=473 \text{ nm}$ excitation source (blue dashed line).

Oil-immersion microscopy is used through the glass lid of the chip ($\times 100$ objective), which makes imaging of individual silver clusters possible inside the channel. After several minutes of photoactivation, strong multicolored intermittent fluorescence can be observed under continuous blue excitation (Figure 3).

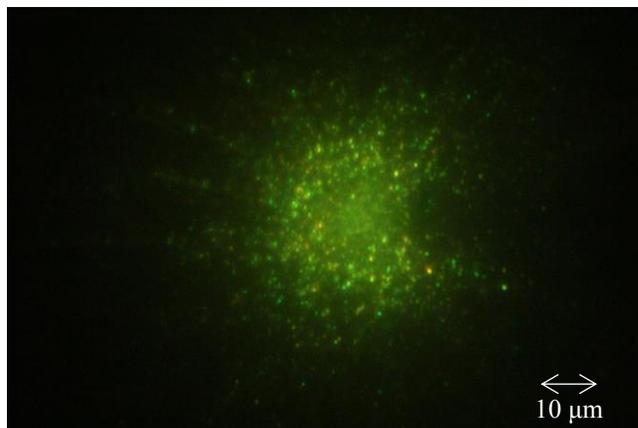


Figure 3: Multicolored fluorescence from a 10 nm Ag film in presence of 1 mM AgNO_3 and excited at $\lambda=473 \text{ nm}$.

The number of Ag atoms present in a given quantum cluster and its structure could be an explanation for the fluorescent color difference. The optical spectrum (Figure 4) indicates that at least three different species are present, where the NIR peak cannot be recorded with the camera (Figure 3).

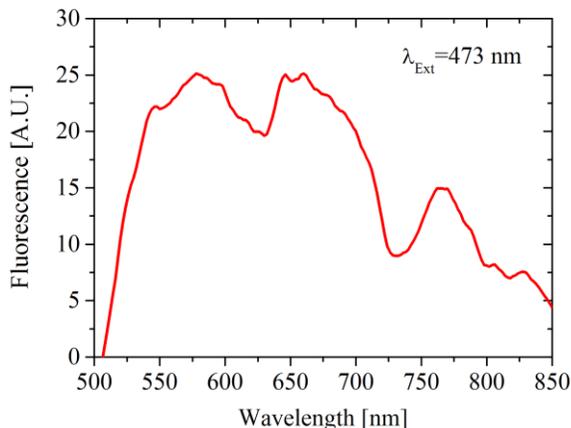


Figure 4: Optical spectrum of the silver quantum cluster emission, showing at least three bands: green, red and near infrared.

The HOMO-LUMO band gaps of Ag_n clusters in the $n=2-10$ range are known to vary significantly depending on the size and to show odd even behavior in terms of gap size depending on the specific electron configuration, with most cluster band gaps located in the NIR range.

Exceptions are, however, Ag_2 , Ag_6 and Ag_3^- that have been shown to have HOMO-LUMO gap energies in the $E=1.75-2.27$ eV range, while also possessing a high stability [8, 9]. Therefore these species could be candidates for the luminescent peak seen in the spectrum (Figure 4). It is, however, difficult to judge from these measurements, whether each peak is dominated by a single species or consists of several types.

In the future, synthesis of the clusters in the presence of ligands, such as sulfur containing thiol groups could be interesting, because the HOMO-LUMO gap changes significantly depending on the complexation with the ligand [8]. Hence, measurements of the change/shift in the optical spectrum may provide additional information about the type of clusters.

During photoactivation, the fluorescent signal emanating from AgQCs increases rapidly with time until it saturates at a level where the destruction rate of QCs equals the formation rate. The photogeneration is seen to obey Hill/Langmuir kinetics (Figure 5) with a fast time constant of 31 s.

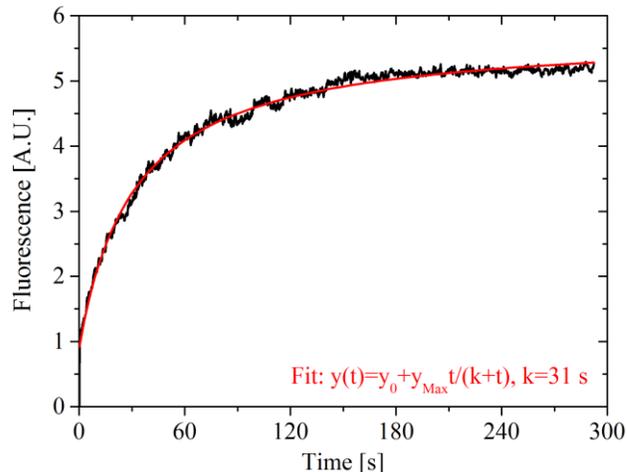


Figure 5: Plasmon-assisted photoactivation of quantum cluster fluorescence in a 1mM $AgNO_3$ solution (black curve) using laser diode excitation ($\lambda=473$ nm, $P=50$ mW). The photogeneration obeys Hill/Langmuir kinetics (red curve) with a time constant of 31 s.

The reducing power of the AgNPs was found to be highly dependent on the laser intensity, which will be studied in more detail in the future. Excitation of the plasmon band also results in highly localized heating and here the laser power was adjusted just below the boiling point of the aqueous $AgNO_3$ solution in order to avoid formation of gas bubbles in the focal spot.

2.3 Detection of cyanide

After photoactivation, the QC fluorescence was exploited for sensing purposes. Previously, plasmon enhanced quantum cluster fluorescence has only been slightly altered by adsorption of proteins/surfactants or by changing the solvents [5]. In this work, a more dramatic change in signal is exploited, by using an indirect fluorescence sensing scheme based on so-called nucleophile activated dissolution [7] of the AgQCs. Cyanide based dissolution of AuNPs and AgNPs has been used previously for colloidal solutions [10, 11], but not for plasmonic substrates, as in this work.

An example of a preliminary measurement of cyanide is shown in Figure 6. After 5 min photoactivation of a 10 nm Ag film in presence of 1mM $AgNO_3$ under $\lambda=473$ nm excitation, the medium was changed to a 10 μ M KCN solution at pH 10. A quick decrease of the fluorescence is observed.

Two time constants are needed to fit the exponential decay. Initially, there is a fast decay with a mean lifetime of only 15 s followed by a second slower decay.

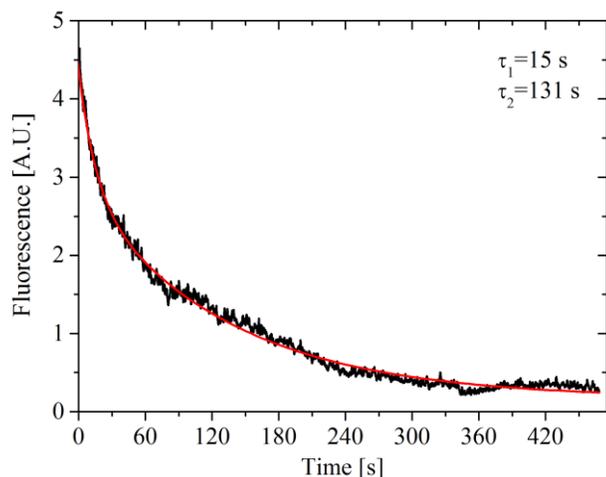


Figure 6: Decay of the fluorescence due to oxidative dissolution of the AgQCs, using 10 μ M cyanide (pH 10) as a nucleophile/ligand (black curve). Two time constants are necessary for the exponential fit (red curve), showing a fast initial decay (mean lifetime of only 15 s).

The necessity of using two time constants for the fit is an indication that the chemistry is more complex than given by equ. 1. This will be investigated in greater detail in the future, as well as measurements of different concentrations of cyanide, in order to establish the detection limits. A concentration of 10 μ M cyanide (Figure 6) is, however, already around the maximum health limit of cyanide in drinking water, recommended by WHO [12].

3 OUTLOOK AND CONCLUSIONS

The enhanced brightness due to coupling silver quantum clusters with plasmonic NPs on a surface is a distinct advantage compared to QCs in solution, because it enables easy visualization at a single cluster level using standard microscope setups. The long term goal is to control the chemistry on an individual cluster level, which could be provided by, e.g., using high speed cameras for the individual colors in order to track the luminescence of each cluster.

Another interesting aspect of QCs is that the sensitivity with respect to charge transfer reactions should be very high, because each cluster only consists of a few atoms/free electrons, so the relative effect of a single charge transfer reaction is high, potentially allowing detection on the single molecule level.

Overall, we strongly believe that quantum cluster fluorescence will receive increased interest within the scientific community, especially when coupled with plasmonic nanoparticles and possibly also integrated with microfluidics for improved control of the reaction kinetics.

REFERENCES

- [1] I. Diez and R. H. A. Ras, "Fluorescent silver nanoclusters" *Nanoscale*, 3, p. 1963, 2011.
- [2] Hangxun Xu and Kenneth S. Suslick, "Water-Soluble Fluorescent Silver Nanoclusters", *Adv. Mater.* 22, p. 1078, 2010.
- [3] L. A. Peyser, A. E. Vinson, A. P. Bartko and R. M. Dickson, "Photoactivated Fluorescence from Individual Silver Nanoclusters", *Science*, 291, p. 103, 2001.
- [4] L. A. Peyser, T.-H. Lee, and R. M. Dickson, "Mechanism of Ag_n Nanocluster Photoproduction from Silver Oxide Films", *J. Phys. Chem. B*, 106, no. 32, pp. 7725–7728, 2002.
- [5] A. Alqudami and S. Annapoorni, "Fluorescence From Metallic Silver and Iron Nanoparticles Prepared by Exploding Wire Technique", *Plasmonics*, vol. 2, no. 1, pp. 5–13, 2007.
- [6] A. Henglein, "Physicochemical properties of small metal particles in solution: 'microelectrode' reactions, chemisorption, composite metal particles, and the atom-to-metal transition", *J. Phys. Chem.*, vol. 97, no. 21, pp. 5457–5471, 1993.
- [7] C. M. Ho, C. K. Wong, S. K. W. Yau, C. N. Lok and C. M. Che, "Oxidative Dissolution of Silver Nanoparticles by Dioxide: A Kinetic and Mechanistic Study", *Chem. Asian J.* 6, p. 2506, 2011
- [8] A. F. Pedicini, A. C. Reber and S. N. Khanna, "The effect of sulfur covalent bonding on the electronic shells of silver clusters", *J. Chem. Phys.*, 139, p. 164317, 2013.
- [9] S. Fedrigo, W. Harbich and J. Buttet, "Optical response of Ag₂, Ag₃, Au₂, and Au₃ in argon matrices", *J. Chem. Phys.*, 99 (8), p. 5712, 1993
- [10] Y. Liu, K. Ai, X. Cheng, L. Huo and L. Lu, "Gold-Nanocluster-Based Fluorescent Sensors for Highly Sensitive and Selective Detection of Cyanide in Water", *Adv. Func. Mater.*, 20, p. 951, 2010.
- [11] X. Lou, Q. Zeng, Y. Zhang, Z. Wan, J. Qin and Z. Li, "Functionalized polyacetylenes with strong luminescence: "turn-on" fluorescent detection of cyanide based on the dissolution of gold nanoparticles and its application in real samples", *J. Mater. Chem.*, 22, p. 5581, 2012
- [12] "Cyanide in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality", WHO Press, World Health Organization, Switzerland, 2009