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# Metal-Enhanced Fluorescence-Based RNA Sensing

Kadir Aslan,[†] Jun Huang,[‡] Gerald M. Wilson,[‡] and Chris D. Geddes*,[†,§]

*Institute of Fluorescence, Laboratory for Advanced Medical Plasmonics, Medical Biotechnology Center, University of Maryland Biotechnology Institute, and Department of Biochemistry and Molecular Biology and Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Baltimore, Maryland 21201*

Received January 6, 2006; E-mail: geddes@umbi.umd.edu

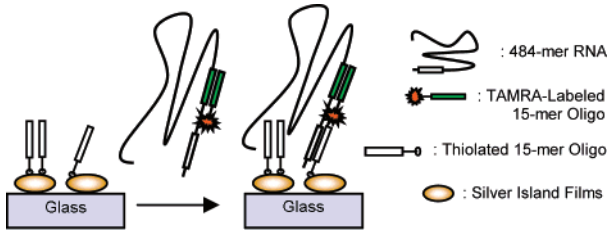
Quantitation of specific RNA molecules from biological samples is an essential tool for the study of regulated gene expression[1] and is routinely employed in studies of gene transcription,[2] RNA stability,[3] RNA transport, and a host of other biological processes.[4] In addition, RNA detection and quantitation also present an appealing strategy for rapidly identifying unknown biological agents (bacterial, viral, etc.).[5,6] Furthermore, they are of great utility for gene expression profiling in clinical settings, where the expression of a subset of genes within tissue (i.e., biopsy) or blood samples may be rapidly measured, revealing diagnostic information to direct patient-specific therapeutic strategies.[2,7]

All current techniques for quantifying specific RNAs exploit base pair complementarity between a target RNA and one or more nucleic acid probes, either in the form of extended DNA or RNA sequences (Northern blots,[1] RNase protection assays[8,9] [RPAs]) or short oligonucleotides (reverse transcription−PCR [RT−PCR],[10] RNA capture assays[11]). This principle allows for extremely precise target recognition, yet current methods of probe:target hybrid detection face a number of technological restrictions. In particular, the utility of RNA sensing in microbial detection and/or clinical gene expression profiling may be hindered by two principal constraints, namely, *sensitivity* and *rapidity*.[12]

RNA capture assays offer a simple and rapid approach to RNA quantitation. Target RNAs are selected based on complementarity to an oligonucleotide probe which is attached to a solid surface or matrix, then detected by annealing a radio- or chemically labeled probe at a distinct site on the target RNA.[11] At present, however, these assays are subject to the same sensitivity limitations as those described for Northern blots and RPAs, namely, that detection relies on the activity of radiolabels, the sensitivity of conjugated fluorophores, or the use of bright secondary chemiluminescent assays. These conditions make RNA capture assays currently useful only for abundant RNA species, thus limiting their general utility as a biosensor platform.[10]

Here we present the proof-of-principle of a new RNA sensing platform technology based on Metal-Enhanced Fluorescence (MEF), where the detected fluorescence emission is significantly amplified and we were able to trace the varying amount of RNA on the silver island films (SiFs) quantitatively as compared to the undetectable emission on the non-silvered glass surface at the femtomole level detection of RNA. In this regard, the detection of RNA is accomplished by annealing a target RNA, tagged with a fluorophore, to an oligonucleotide anchor probe in a single step on a solid surface, where the fluorescence signal is intrinsically enhanced by silver nanoparticles. The enhancement of fluorescence is, in part, due to the localized excitation of the fluorophores when in close proximity to the silver nanoparticles and results in improved photostability of the fluorophores.[13,14] When the metal (silver, aluminum, or gold) is a continuous 45 nm thick film, the spatially isotropic fluorescence emission can be converted into directional emission toward a detector, further improving the detectability.[16]

***Scheme 1.*** MEF-Based RNA Sensing Platform Technology




The RNA capture assay was constructed by covalently linking a thiolated oligo anchor probe (thiolated 15-mer oligo) onto SiFs via well-established self-assembled monolayer chemistry,[17] as shown in Scheme 1. The thiolated oligo anchor probe is designed by introducing thiol groups into the 15-base sequence from the 5′-end that specifically binds to silver nanoparticles with high affinity. A fluorescent probe (TAMRA-labeled 15-mer oligo) is annealed to a 484-mer RNA substrate containing the $\beta$-globin coding sequence (target RNA; see Figure S1, Supporting Information). The TAMRA-labeled oligo annealed to the RNA substrate was then hybridized with the thiolated oligo anchor probe on the surface of the SiFs. This procedure brings the fluorophore to a distance of approximately 4 nm from the surface of the SiFs where the fluorescence emission is expected to increase by MEF as described previously.[13,14]

The deposition of silver island films onto glass slides was performed as described previously.[18] In a typical SiF preparation, a solution of sodium hydroxide and ammonium hydroxide is added to a continuously stirred solution of silver nitrate at room temperature. Subsequently, the mixture is cooled in an ice bath, silane-prep glass slides (Sigma) are inserted, and a solution of D-glucose is added. As the temperature is increased, the color of the mixture turns yellow−brown and the SiF-deposited slides are removed from the mixture, washed with water, and sonicated for a few seconds at room temperature. SiF-deposited glass slides were stored in deionized water until they were used. Fluorescence emission spectra of TAMRA-labeled oligo with RNA substrate hybridized to the thiolated oligo anchor probe on SiFs are shown in Figure 1, left. The emission intensity peak of TAMRA-labeled oligo that was annealed to RNA substrates ranging from 25 to 500 fmol is clearly observed at 585 nm (Figure 1, left) and increased linearly as the amount of RNA substrate is increased (Figure 1, right). The fluorescence emission spectra of TAMRA shown in Figure 1, left (especially for the RNA substrates of 250 fmol or higher) appear slightly broader than the spectrum of TAMRA-labeled oligo anchor probe measured from a solution on plain glass (see Figure S2,

[†] Institute of Fluorescence, Laboratory for Advanced Medical Plasmonics.
[‡] Department of Biochemistry and Molecular Biology.
[§] Center for Fluorescence Spectroscopy.



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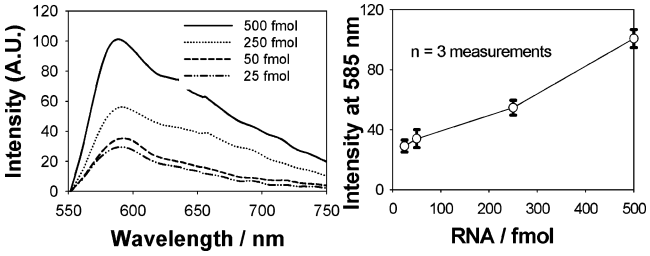


***Figure 1.*** (Left) Fluorescence emission spectra (intensity: arbitrary units) of TAMRA-linked oligo annealed to the RNA substrate that was hybridized with the thiolated oligo anchor probe on the surface of the SiFs. (Right) Fluorescence emission intensity measured at 585 nm versus the amount of RNA used in the RNA capture assay (signal-to-noise, S/N > 20) for three separate measurements (background fluorescence is 1.5 at 517 nm; see Figure S5).

Supporting Information) due to the background scattering from the SiF-coated glass slide.

The control experiments revealed that when the RNA sequence was changed (that is, control tRNA with random sequence is used in the RNA capture assay) the fluorescence emission from TAMRA-labeled oligo was not observed (see Figure S3, Supporting Information) since the control tRNA lacked the specific sequence that is required for the annealing of TAMRA-labeled RNA. In addition, when either of the other components of the RNA capture assay, thiolated oligo, or TAMRA-labeled oligo is omitted, almost no fluorescence emission was observed (see Figure S3, Supporting Information). Thus, the RNA capture assay is highly specific, and the contribution of the nonspecific interactions to the detected signal is minimal.

The lower detection limit (LDL) of the RNA capture assay described here was 25 fmol of RNA (S/N > 20) and made possible by the amplification of fluorescence emission intensity based on our previously described phenomenon of metal-enhanced fluorescence.[13,14] The amplification of fluorescence emission intensity is an inherent property of the silver nanoparticles deposited on the glass slides and thought to occur due to partial nonradiative energy transfer between the excited state of the fluorophore and the surface plasmons of the silver nanoparticles, as well as due to the spatially localized excitation of fluorophores created by the nanoparticles within close proximity.[19]

Although the LDL of the MEF-based RNA capture assay is 100−200-fold less sensitive than the current RNA capture assays,[11,22] the MEF-based RNA sensing method offers a considerably simpler, cheaper, and quicker alternative to RT−PCR since it does not require the amplification of the RNA target and can be performed relatively quickly. Given that the S/N > 3−4 for fluorescence-based assays is considered acceptable,[15] the actual lower detection limit of the MEF-based RNA capture assay is approximately 5 fmol.

The rapidity of the MEF-based RNA capture assays could be increased further with the help of low-power microwaves, as shown previously for the MEF-based protein and antibody assays that were completed within 20 s, that is, microwave-accelerated metal-enhanced fluorescence (MAMEF).[18,20] Similar to RT−PCR, the MEF-based RNA capture assays could potentially be multiplexed by simply using SiF-coated high-throughput screening (HTS) wells.[21] Ultimately, ultra-rapid MEF-based multiplexed RNA capture assays comparable to RT−PCR could be achieved by combining MAMEF technology with the use of SiF-coated HTS wells once the sensitivity of the MEF-based method is improved. In this regard, our laboratories recently reported MEF-based enhancements in excess of 3000-fold using fractal silver surfaces.[14] Accordingly, the timing is propitious for the application of metal-enhanced fluorescence to an RNA quantitation platform.

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**Supporting Information Available:** The experimental conditions for the preparation of silver island films, the preparation of the $\beta$-globin mRNA substrate, and the MEF-based RNA sensing assay are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Krumlauf, R. *Mol. Biotechnol.* **1994**, *2*, 227−242.
(2) Elkahloun, A. G.; Gaudet, J.; Robinson, G. S.; Sgroi, D. C. *Cancer Biol. Ther.* **2002**, *1*, 354−358.
(3) Wilson, G. M.; Deeley, R. G. *Plasmid* **1995**, *33*, 198−207.
(4) Kindler, S.; Wang, H.; Richter, D.; Tiedge, H. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 223−245.
(5) van Doorn, L. J.; Kleter, B.; Voermans, J.; Maertens, G.; Brouwer, H.; Heijtink, R.; Quint, W. *J. Med. Virol.* **1994**, *42*, 22−28.
(6) Call, D. R.; Borucki, M. K.; Loge, F. J. *J. Microbiol. Methods* **2003**, *53*, 235−243.
(7) Ramaswamy, S.; Golub, T. R. *J. Clin. Oncol.* **2003**, *20*, 1932−1941.
(8) Haines, D. S.; Gillespie, D. H. *Biotechniques* **1992**, *12*, 736−741.
(9) Rosenau, C.; Kaboord, B.; Qoronfleh. M. W. *Biotechniques* **2002**, *33*, 1354−1358.
(10) Bustin, S. A. *J. Mol. Endocrin.* **2002**, *29*, 23−39.
(11) Tsai, S. P.; Wong, A.; Mai, E.; Chan, P.; Mausisa, G.; Vasser, M.; Jhurani, P.; Jakobsen, M. H.; Wong, W. L. T.; Stephan, J.-P. *Nucleic Acids Res.* **2003**, *31*, e25.
(12) The *sensitivity* of RNA detection becomes limiting when only minute quantities of biological material are available. This is also reflected in poor signal-to-noise when the RNA of interest is expressed at very low levels relative to the bulk RNA population (e.g., expression of oncogene mRNAs as a function of total cell RNA mass, low levels of viral mRNAs in a blood sample from a patient with a latent infection). The *rapidity* is highly desirable in an RNA sensing system, particularly in microbial screening, since early identification of pathogens provides better opportunities for containment, decontamination, and treatment.
(13) Aslan, K.; Gryczynski, I.; Malicka, J.; Matveeva, E.; Lakowicz, J. R.; Geddes, C. D. *Curr. Opin. Biotechnol.* **2005**, *16*, 55−62.
(14) Parfenov, A.; Gryczynski, I.; Malicka, J.; Geddes, C. D.; Lakowicz, J. R. *J. Phys. Chem. B* **2003**, *107*, 8829−8833.
(15) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Kluwer: New York, 1999.
(16) Malicka, J.; Gryczynski, I.; Gryczynski, Z.; Lakowicz J. R. *J. Biomol. Screen.* **2004**, *9*, 208−215.
(17) Sastry, M.; Mayya, K. S.; Bandyopadhyay, K. *Colloids Surf. A* **1997**, *127*, 221−228.
(18) Aslan, K.; Geddes, C. D. *Anal. Chem.* **2005**, *77*, 8057−8067.
(19) Aslan, K.; Leonenko, Z.; Lakowicz, J. R.; Geddes, C. D. *J. Fluoresc.* **2005**, *15*, 643−654.
(20) Aslan, K.; Geddes, C. D. *J. Fluoresc.* **2006**, DOI:10.1007/s10895-005-0026-z.
(21) Aslan, K.; Holley, P.; Geddes, C. D. *J. Immun. Methods*, submitted.
(22) Xie, H.; Yu, Y. H.; Xie, F.; Lao, Y. Z.; Gao, Z. *Anal. Chem.* **2004**, *76*, 4023−4029.

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