JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by ISTANBUL TEKNIK UNIV

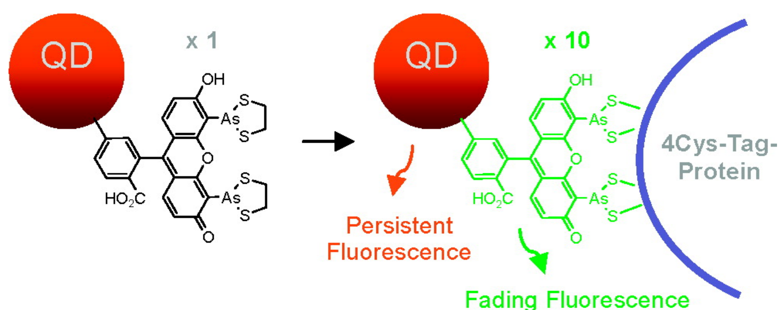
Communication

CrAsH#Quantum Dot Nanohybrids for Smart Targeting of Proteins

Emilie Genin, Olivier Carion, Benoit Mahler, Benoit Dubertret,
Nathalie Arhel, Pierre Charneau, Eric Doris, and Charles Mioskowski

J. Am. Chem. Soc., **2008**, 130 (27), 8596-8597 • DOI: 10.1021/ja802987q • Publication Date (Web): 13 June 2008

Downloaded from http://pubs.acs.org on February 8, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 06/13/2008

CrAsH–Quantum Dot Nanohybrids for Smart Targeting of Proteins

Emilie Genin,[‡] Olivier Carion,[§] Benoit Mahler,[§] Benoit Dubertret,[*,§] Nathalie Arhel,[||] Pierre Charneau,[||] Eric Doris,[*,‡] and Charles Mioskowski[†,‡]

CEA, iBiTecS, Service de Chimie Bioorganique et de Marquage, 91191 Gif sur Yvette, France, Laboratoire Photons Et Matière, UPR5 du CNRS, ESPCI, 10 rue Vauquelin, 75005 Paris, France, and Groupe de Virologie Moléculaire et Vectorologie, Institut Pasteur, 25–28 rue du Dr. Roux, 75724 Paris, France

Received April 23, 2008; E-mail: benoit.dubertret@espci.fr; eric.doris@cea.fr

The development of sensitive and specific probes that lack the intrinsic limitations of fluorogenic organic dyes is of considerable interest in many fields of research from molecular and cellular biology to medical imaging and diagnosis.[1] Semiconductor nanocrystals, also known as quantum dots (QDs), have received considerable attention due to their unique optical properties which include high quantum yield, large molar extinction coefficient, tunable fluorescence emission, and photostability.[2] These nanoparticles have already been conjugated to biomolecules such as peptides, antibodies, nucleic acids, or small ligands for applications as targeted fluorescent labels.[3] In addition, bisarsenical affinity probes FlAsH,[4] ReAsH,[5] and AsCy3[6] were recently introduced as innovative tools for assessing protein location or function.[7] These organic dyes are derivatized with two vicinal arsenic moieties which allow selective and complementary interactions with proteins that incorporate a tetracystein tag (Cys_2–$(X)_n$–Cys_2). Upon covalent interaction between the 4Cys tag of the protein and the bisarsenical probe, ligand exchange takes place on the two trivalent arsenic atoms. This induces a significant increase in the fluorescence of the probe. Targeted in vitro and in vivo imaging of recombinant 4Cys proteins is thus achievable using the smart FlAsH probe and derived systems. However, classical photobleaching of the organic dye is an inherent drawback that still remains to be addressed.

To overcome the above-mentioned limitation of FlAsH-based fluorophores, we report here the synthesis of new nanohybrids which combine the prominent features of both systems, being the persistent fluorescence of the QDs and the affinity of CrAsH (a FlAsH analogue) for Cys tags. The choice of CrAsH as a probe was governed by the fact that, contrary to FlAsH, it incorporates an additional carboxylic moiety in the 6-position that is amenable to conjugation. Our approach is based on the covalent anchoring of a controlled number of CrAsH molecules to red-emitting QD micelles and the evaluation of the resulting nanohybrid in the targeting of Cys-tagged proteins.

The synthesis of CrAsH started from 6-carboxyfluorescein which was prepared as a pure regioisomer according to the procedure of Burgess et al.[8] Mercuration of 6-carboxyfluorescein with mercuric oxide in trifluoroacetic acid followed by transmetalation with $AsCl_3$ and ligand exchange with ethanedithiol (EDT)[9] afforded CrAsH in 30% overall yield (Figure 1a). Spectral characterization of CrAsH in PBS indicated a maximum absorption at 508 nm and fluorescence emission at 520 nm. In parallel, core–shell CdSe/CdZnS nanocrystals were prepared as previously described.[10] These QDs, coated with hydrophobic ligands, were then encapsulated in poly(ethylene glycol) phospholipids to produce hydrosoluble and biocompatible QD micelles.[11] The phospholipids used for the encapsulation step consisted in a mixture of conventional phospholipid **1a** and variable amounts of NH_2 phospholipid **1b** (Figure 1b). The latter was to be used for the coupling of the QD micelles to CrAsH.

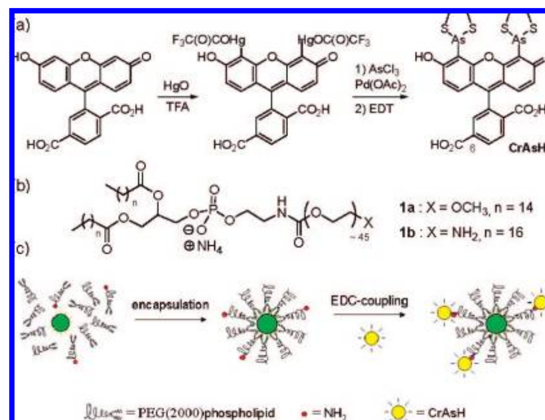


Figure 1. (a) Synthesis of CrAsH (FlAsH has the same chemical structure as CrAsH except for the carboxylic group in the 6-position). (b) Chemical structures of phospholipids **1a** and **1b**. (c) QD encapsulation and coupling to CrAsH.

Initial coupling experiments (Figure 1c) were conducted using QD micelles which incorporated 10 mol % of the NH_2 phospholipid **1b**. The 6-carboxyl group of CrAsH was first activated in THF with a carbodiimide (EDC) in excess, followed by addition of an aqueous solution of the micelle QD. Borate buffer (20 mM, pH 8) was then added, and the solution was further stirred for 4 h at room temperature. It is noteworthy that no QD aggregation was observed during the process.

The QD–CrAsH nanohybrids were then purified from EDC and CrAsH in excess by filtration through a NAP-5 column. The grafted QDs were then analyzed by UV/visible spectroscopy, which indicated a new band centered at ca. 500 nm (Figure 2a). The latter was attributed to absorption of CrAsH. The presence of CrAsH on the surface of QD micelles was confirmed by fluorescence measurements. As depicted in Figure 2b, the emission spectrum of the nanohybrid results from the linear superimposition of CrAsH and QD emission spectra. Coupling and purification thus did not alter the fluorescence properties of the QD nor that of CrAsH. To investigate whether nonspecific adsorption of CrAsH had taken place on the QD micelle, control experiments were run using the same coupling procedure but without EDC. The photophysical properties of the QD remained unchanged after Nap-5 purification, but no characteristic bands of CrAsH were detected either in the absorption or in the emission spectra. This blank experiment demonstrates that the signal observed in the case of the QD–CrAsH

[†] Deceased June 2, 2007.
[‡] Service de Chimie Bioorganique et de Marquage.
[§] Laboratoire Photons Et Matière.
[||] Institut Pasteur.

 10.1021/ja802987q CCC: $40.75 © 2008 American Chemical Society

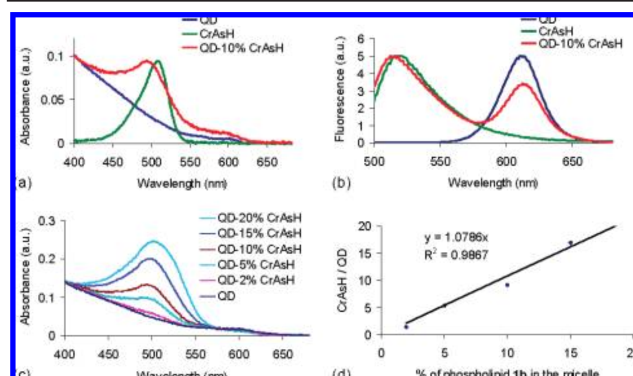


Figure 2. (a) Absorption spectra of CrAsH, QD, and QD−CrAsH in PBS. (b) Emission spectra of CrAsH, QD, and QD−CrAsH in PBS. (c) Absorption spectra of QD−CrAsH for increasing amounts of CrAsH coupled to the QD micelle. (d) Number of CrAsH per QD versus percentage of NH_2 phospholipids incorporated in the QD micelle. QDs were excited at 350 nm, whereas QD−CrAsH and CrAsH were excited at 485 nm.

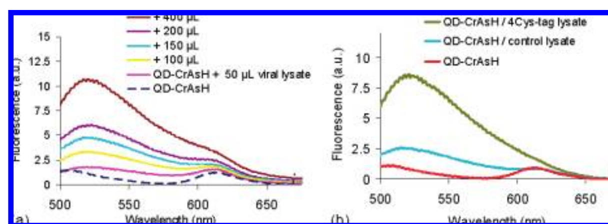


Figure 3. (a) Emission spectrum of QD-CrAsH (dotted line). Fluorescence spectra of QD−CrAsH micelles for increasing amount of viral lysate containing the 4Cys-tagged proteins (solid line). (b) Emission spectra of QD−CrAsH alone (red), incubated with the viral lysate with (brown) and without (blue) the 4Cys-tagged proteins. Spectra were recorded in PBS using QD loaded with 5% CrAsH. Excitation at 350 nm.

nanohybrid is not due to nonspecific adsorption of the dye but to the anticipated covalent linking of CrAsH to the micelle.

To investigate our methodology further, we repeated the same EDC-mediated coupling using variable amounts of the NH_2 phospholipid **1b** (2−20 mol % of **1b**/**1a**) in the encapsulation step of the QD. The number of CrAsH molecules attached to the micelle was then determined, in each case, by spectroscopy.[10] The intensity of the peak corresponding to CrAsH increases concurrently (Figure 2c) and linearly (Figure 2d) with the amount of **1b** initially added to the micelle. This relationship demonstrates that the coupling of CrAsH to the QD micelles was achieved in a controlled and quantitative manner.

The affinity of QD−CrAsH with regards to tetracystein-tagged proteins was then tested. Thus, increasing amounts of a viral lysate containing the target tagged protein (integrase protein) were added to a solution of QD−CrAsH micelles containing 5 mol % of CrAsH. After each addition, the solution was gently mixed for a few minutes before a fluorescence emission spectrum was recorded. As shown in Figure 3a, successive addition of the viral lysate induced a rise in the fluorescence of CrAsH. A ca. 10-fold increase was observed after addition of 400 μL of viral solution corresponding to approximately 10 ng of protein. Noteworthily, QD fluorescence remained unaffected throughout the sequential additions.

The selectivity of QD−CrAsH was also evaluated by comparing the above results with the fluorescence emission of the nanohybrid that was exposed to 400 μL of a similar viral lysate without the 4Cys-tagged protein (Figure 3b). In this case, only a 2.5-fold increase in fluorescence intensity was detected. Taken together, these data confirmed selective binding of QD−CrAsH to 4Cys-tagged proteins.

The resistance of the nanohybrid to photobleaching was assessed by continuous illumination of a QD−CrAsH micelle solution in interaction with the tagged protein. As anticipated, the fluorescence of CrAsH faded over several tens of seconds, while that of the QD remained mostly unaffected (see Supporting Information). Thus the persistent QD fluorescence emission could be used for detection/imaging long after photobleaching of the dye occurred. The initial fluorescence enhancement of CrAsH upon binding to the tagged protein combined with photostability of the QD makes our nanohybrid a superior system for smart targeted imaging.

In conclusion, we have reported the design and synthesis of a smart fluorescent nanohybrid probe for the targeting of Cys-tagged recombinant proteins. The nanohybrid was prepared by conjugation of CrAsH to hydrosoluble and biocompatible quantum dots. Introduction of variable amounts of amino poly(ethylene glycol) phospholipids in the micelle QD followed by EDC-mediated coupling permitted the covalent linkage of CrAsH to the QD. We have demonstrated that the QD fluorescence was resistant to photobleaching compared to CrAsH and that the QD-based nanohybrid binds efficiently and selectively to 4Cys-tagged proteins. This selective interaction could permit the imaging of tagged proteins in vivo with high sensitivity and over extended periods of time. Biomedical applications of these new multifunctional probes are currently under investigation.

Acknowledgment. This work is part of the “VISEN” project funded by the ANR. Dr. Alexander Yuen is gratefully acknowledged for helpful discussion.

Supporting Information Available: experimental procedures and spectral characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Alivisatos, P. *Nat. Biotechnol.* **2004**, *22*, 47–52. (b) Giepmans, B. N. G.; Adams, S. R.; Ellisman, M. H.; Tsien, R. Y. *Science* **2006**, *312*, 217–224.
(2) (a) Murray, C. B.; Norris, D. J.; Bawendi, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 8706–8715. (b) Hines, M. A.; Guyot-Sionnest, P. *J. Phys. Chem.* **1996**, *100*, 468–471.
(3) (a) Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. *Science* **2005**, *307*, 538–544. (b) Kim, J.; Park, H.-Y.; Kim, J.; Ryu, J.; Kwon, D. O.; Grailhe, R; Song, R. *Chem. Commun.* **2008**, 1910–1913.
(4) Griffin, B. A.; Adams, S. R.; Tsien, R. Y. *Science* **1998**, *281*, 269–272.
(5) Adams, S. R.; Campbell, R. E.; Gross, L. A.; Martin, B. R.; Walkup, G. K.; Yao, Y.; Llopis, J.; Tsien, R. Y. *J. Am. Chem. Soc.* **2002**, *124*, 6063–6076.
(6) Cao, H. S.; Xiong, Y. J.; Wang, T.; Chen, B. W.; Squier, T. C.; Mayer, M. U. *J. Am. Chem. Soc.* **2007**, *129*, 8672–8773.
(7) (a) Tour, O.; Meijer, R. M.; Zacharias, D. A.; Adams, S. R.; Tsien, R. Y. *Nat. Biotechnol.* **2003**, *21*, 1505–1508. (b) Langhorst, M. F.; Genisyuerek, S.; Stuermer, C. A. O. *Histochem. Cell. Biol.* **2006**, *125*, 743–747. (c) Yan, P.; Xiong, Y. J.; Chen, B. W.; Negash, S.; Squier, T. C.; Mayer, M. U. *Biochemistry* **2006**, *45*, 4736–4748. (d) Stenoien, D. L.; Knyushko, T. V.; Londono, M. P.; Opresko, L. K.; Mayer, M. U.; Brady, S. T.; Squier, T. C.; Bigelow, D. J. *Am. J. Physiol-Cell Ph.* **2007**, *292*, C2084–C2094.
(8) Ueno, Y.; Jiao, G. S.; Burgess, K. *Synthesis* **2004**, 2591–2593.
(9) Cao, H.; Chen, B.; Squier, T. C.; Mayer, M. U. *Chem. Commun* **2006**, 2601–2603.
(10) Carion, O.; Mahler, B.; Pons, T.; Dubertret, B. *Nat. Protocols* **2007**, *2*, 2383–2390.
(11) Dubertret, B.; Skourides, P.; Norris, D. J.; Noireaux, V.; Brivanlou, A. H.; Libchaber, A. *Science* **2002**, *298*, 1759–1762.

JA802987Q