

Nanoscale Engineering of Molecular Beacons for Imaging of RNA in Living Systems

Stephen R. Jackson¹ and Ashwath Jayagopal^{2*}

¹Department of Chemistry, Vanderbilt University, USA

²Vanderbilt Eye Institute, Vanderbilt University Medical Center, USA

Increasing evidence suggests that RNA is responsible for a wide range of functions in living cells. [1,2] The important role RNA plays in dictating cell behavior has led to the development of numerous methods for detecting RNA in living systems. A promising tool for RNA detection is molecular beacon (MB) technology. MBs were initially developed as a probe for quantifying nucleic acids in real-time [3]. MBs combine the specificity of nucleic acid hybridization with the ability of nucleic acids to take on distinct conformations to create hairpin-shaped oligonucleotides with a fluorophore at one end and a proximity-dependent quencher at the other end. When target nucleic acids are not present, the hairpin structure keeps the fluorophore in close proximity to the quencher and the probes are in a “dark” state. When the probes bind to their targets, they undergo a conformational reorganization that separates the fluorophore from the quencher, resulting in a bright fluorescent signal that indicates the presence of the target. Since their inception, MBs have found wide application in molecular and cellular biology, pathogen detection, and molecular diagnostics [4-7].

Advances in MB design and modifications have enabled imaging of RNA in living cells [8,9]. However, challenges encountered in using traditional MBs for *in vivo* imaging include nonspecific background fluorescence, potential for nuclease degradation and reduced half-life, and limited approaches for non-invasive intracellular delivery mechanisms. To address these limitations, continued development of MBs has resulted in improvements in these aspects which we discuss below.

Improved MB Signal to Noise Ratio

Key issues in using MBs for imaging are maximization of quenching efficiency as well as fluorophore quantum efficiency. One strategy to improve the signal-to-background ratio is to increase the brightness of the signaling moiety. Replacement of organic fluorophores with brighter signaling materials, such as quantum dots, has been reported [10]. A second strategy is to minimize the fluorescence background using a more efficient quencher. While standard organic quenchers have demonstrated effectiveness in the quenching of some fluorophores, their quenching efficiencies vary significantly from one dye to another. For example, 4-((4-(dimethylamino)phenyl)azo)benzoic acid (DABCYL) efficiently quenches fluorescein (FAM) but is much less efficient for dyes emitting at longer wavelengths such as Cy5 and Texas Red [11]. Black Hole Quenchers (BHQ) have no native fluorescence and a broad effective range of absorption, but it is still important to match the BHQ with the appropriate dye. Pairing multiple assembled quenchers to one fluorophore has also been shown to provide better quenching efficiency [12]. The development of quenchers which work effectively within the near infrared range open up exciting possibilities for imaging of RNA with minimal cell or tissue autofluorescence.

Nuclease Degradation

MBs are vulnerable to intracellular enzymatic degradation, such as DNase/RNase cleavage and non-specific opening by single-

strand binding proteins [13] leading to false-positives. To improve the resistance of enzymatic activities, non-standard nucleic acids have been explored to design MBs, such as 2'-O-methyl-modified RNAs, peptide nucleic acids (PNAs), and locked nucleic acids (LNAs). 2'-O-methyl MBs show higher affinity, increased specificity, and faster hybridization kinetics [14-16]. However, evidence suggests that not all 2'-O-methyl MBs retain a stem-loop structure inside living cells, possibly due to nucleic acid binding proteins [17]. PNAs have neutral polyamide backbones, are not degraded by nucleases, and have faster hybridization kinetics than DNA probes [18]. However, PNAs have not been widely used mainly because they have poor water solubility causing aggregation in biological environments [19]. LNAs are nucleic acid analogues, in which the ribose ring is locked into a rigid C3'endo or Northern-type conformation by a simple 2'-O, 4'-C methylene bridge. LNAs as well as LNA-MBs have many attractive properties, such as high binding affinity, excellent base mismatch discrimination capability, and decreased susceptibility to nuclease digestion, making them important tools for synthesizing MBs [20].

Approaches for Delivery of MBs in Live Cells

A limitation of traditional MBs is the need to permeabilize the cell through microinjection or use of pore-forming reagents, such as streptolysin O, for effective delivery and target hybridization [8]. The use of cell penetrating peptides conjugated to MBs was recently reported to provide for translocation of MBs into cells without pore-forming reagents; however, Tat-linked MBs have been observed to enter the nucleus, causing nonspecific background signal due to opening of the construct within that compartment [21]. In a different approach to achieve delivery of MBs, Seferos et al. [22], developed a MB-based technique based on oligonucleotide-modified AuNPs that take advantage of the fact that AuNPs functionalized with oligonucleotides efficiently enter live cells without the aid of transfection/permeabilization agents. Their probe consists of a AuNP conjugated to many sequences of single stranded DNA (ssDNA), which are complementary to the RNA sequence of interest. Short “reporter” strands of fluorescently tagged ssDNA are hybridized to the probe DNA. In this state, the organic fluorophore is quenched as a result of its proximity to the AuNP surface. Upon hybridization of the probe strand with the target RNA complement, the reporter strand

*Corresponding author: Ashwath Jayagopal, Vanderbilt Eye Institute, Vanderbilt University Medical Center, 8010 Medical Center East, North Tower Nashville, TN 37232-8808, USA, E-mail: ash.jayagopal@vanderbilt.edu

Received September 22, 2012; Accepted September 22, 2012; Published September 24, 2012

Citation: Jackson SR, Jayagopal A (2012) Nanoscale Engineering of Molecular Beacons for Imaging of RNA in Living Systems. J Nanomed Nanotechnol 3:e119. doi:10.4172/2157-7439.1000e119

Copyright: © 2012 Jackson SR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

is displaced, spatially separating it from the AuNP and resulting in a fluorescent signal that can be correlated to the presence of the RNA sequence of interest. This design yields a signal that is separated from the hybridized RNA/nanoflare construct, consequently important spatial information is lost in live cell microscopy applications. DNA hairpin-gold nanoparticles (hAuNPs) provide spatiotemporal information about target oligonucleotides when used in live cells [23]. hAuNPs utilize the fluorescence quenching ability of a 15 nm AuNP, where DNA hairpins are attached to the particle via a gold-thiol bond through a 5' hexane thiol linker. A 3' fluorophore is quenched by the AuNP until the hairpin binds a complementary sequence, extending the fluorophore past the quenching distance of the AuNP. This results in a positive fluorescent signal, allowing high specificity and high sensitivity imaging of messenger RNA (mRNA) in live cells for elucidating spatial and temporal aspects of mRNA expression and trafficking.

Outlook

Since their introduction in 1996, much progress has been made in improving applications of MBs. The flexibility of their chemical modification and their cost-effectiveness has led to applications in biology, chemistry, biomedicine, and biotechnology. Continuing efforts to improve MB-based technology, such as the optimization of signal transduction, use of modified bases, and AuNP-based constructs will surely catalyze the development of new biochemical and biomedical applications.

References

1. Kleinman ME, Yamada K, Takeda A, Chandrasekaran V, Nozaki M, et al. (2008) Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. *Nature* 452: 591-597.
2. Zhou Q, Gallagher R, Ufret-Vincenty R, Li X, Olson EN, et al. (2011) Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23~27~24 clusters. *Proc Natl Acad Sci U S A* 108: 8287-8292.
3. Tyagi S, Kramer FR (1996) Molecular beacons: Probes that fluoresce upon hybridization. *Nat Biotechnol* 14: 303-308.
4. Sandhya S, Chen W, Mulchandani A (2008) Molecular beacons: a real-time polymerase chain reaction assay for detecting *Escherichia coli* from fresh produce and water. *Anal Chim Acta* 614: 208-212.
5. Santangelo PJ (2010) Molecular beacons and related probes for intracellular RNA imaging. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2: 11-19.
6. Santangelo PJ, Alonas E, Jung J, Lifland AW, Zurla C (2012) Probes for intracellular RNA imaging in live cells. *Methods Enzymol* 505: 383-399.
7. Yeh HY, Yates MV, Mulchandani A, Chen W (2008) Visualizing the dynamics of viral replication in living cells via Tat peptide delivery of nuclease-resistant molecular beacons. *Proc Natl Acad Sci U S A* 105: 17522-17525.
8. Santangelo P, Nitin N, Bao G (2006) Nanostructured probes for RNA detection in living cells. *Ann Biomed Eng* 34: 39-50.
9. Santangelo PJ, Nitin N, Bao G (2005) Direct visualization of mRNA colocalization with mitochondria in living cells using molecular beacons. *J Biomed Opt* 10: 44025.
10. Kim JH, Morikis D, Ozkan M (2004) Adaptation of inorganic quantum dots for stable molecular beacons. *Sensors and Actuators B-Chemical* 102: 315-319.
11. Adams NM, Jackson SR, Haselton FR, Wright DW (2012) Design, synthesis, and characterization of nucleic-acid-functionalized gold surfaces for biomarker detection. *Langmuir* 28: 1068-1082.
12. Yang CJ, Lin H, Tan W (2005) Molecular assembly of superquenchers in signaling molecular interactions. *J Am Chem Soc* 127: 12772-12773.
13. Fang XH, Li JJ, Tan WH (2000) Using molecular beacons to probe molecular interactions between lactate dehydrogenase and single-stranded DNA. *Anal Chem* 72: 3280-3285.
14. Bratu DP, Catrina IE, Marras SA (2011) Tiny molecular beacons for in vivo mRNA detection. *Methods Mol Biol* 714: 141-157.
15. Catrina IE, Marras SA, Bratu DP (2012) Tiny Molecular Beacons: LNA/2'-O-methyl RNA chimeric probes for imaging dynamic mRNA processes in living cells. *ACS Chem Biol*.
16. Perlette J, Tan W (2001) Real-time monitoring of intracellular mRNA hybridization inside single living cells. *Anal Chem* 73: 5544-5550.
17. Molenaar C, Marras SA, Slats JC, Truffert JC, Lemaitre M, et al. (2001) Linear 2' O-Methyl RNA probes for the visualization of RNA in living cells. *Nucleic Acids Res* 29: E89.
18. Xi CW, Balberg M, Boppart SA, Raskin L (2003) Use of DNA and peptide nucleic acid molecular beacons for detection and quantification of rRNA in solution and in whole cells. *Appl Environ Microbiol* 69: 5673-5678.
19. Braasch DA, Corey DR (2001) Synthesis, analysis, purification, and intracellular delivery of peptide nucleic acids. *Methods* 23: 97-107.
20. Vester B, Wengel J (2004) LNA (locked nucleic acid): high-affinity targeting of complementary RNA and DNA. *Biochemistry* 43: 13233-13241.
21. Chen AK, Behlke MA, Tsourkas A (2008) Efficient cytosolic delivery of molecular beacon conjugates and flow cytometric analysis of target RNA. *Nucleic Acids Res* 36: e69.
22. Seferos DS, Gijjohann DA, Hill HD, Prigodich AE, Mirkin CA (2007) Nanoflares: probes for transfection and mRNA detection in living cells. *J Am Chem Soc* 129: 15477-15479.
23. Jayagopal A, Halfpenny KC, Perez JW, Wright DW (2010) Hairpin DNA-functionalized gold colloids for the imaging of mRNA in live cells. *J Am Chem Soc* 132: 9789-9796.