(SD2014-333) Highly Sensitive Detection Of Biomolecules Using Proximity Induced Bioorthogonal Reactions

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BACKGROUND

There is tremendous interest in the use of fluorogenic reactions for detecting and imaging nucleic acids, especially specific DNA and RNA sequences. Applications include time-resolved imaging of transcription, detection of disease-related single nucleotide polymorphisms, and tracking RNA fragments such as microRNAs. Despite advances in the use of molecular beacons, aptamers and antisense agents, the rapid detection and imaging of oligonucleotides in live cells and physiologically relevant media remains challenging. Current methods, although powerful, suffer from numerous drawbacks. For example, previous ligation reactions have been hampered by slow kinetics and autohydrolysis, often relying on nucleophilic/electrophilic reactions, which allow cellular or solvent nucleophiles to compete for reactivity.

Fluorogenic bioorthogonal ligations offer a promising route towards the fast and robust fluorescent detection of specific DNA or RNA sequences. Tetrazine bioorthogonal cycloadditions benefit from rapid tunable reaction rates and high stability against hydrolysis in buffer and serum. Furthermore, tetrazines act as both a fluorescent quencher and a reactive group, minimizing the complexity of fluorogenic ligation probe design.

TECHNOLOGY DESCRIPTION

Researchers from UC San Diego have developed a fluorogenic tetrazine-mediated transfer reaction using 7-azabenzonorbornadiene derivatives and have utilized this reaction to detect oligonucleotides with high sensitivity and sequence specificity. Critical to achieving signal amplification is template-driven turnover of antisense probes, which is enabled by spontaneous diazine release after the initial tetrazine ligation takes place. The use of a highly quenched alkenyl-fluorogenic tetrazine enables a >100-fold increase in fluorescence in response to the TMT reaction. By using RNA to template this transfer reaction with antisense probes, the researchers were able to detect mir-21 down to low-picomolar levels with the specificity to distinguish single-base mismatches in miRNA templates. The probes were capable of detecting endogenous mir-21 both in live cells and cell lysates. Oligonucleotide-templated fluorogenic TMT reactions will be useful for many applications that require detection of specific nucleic acids in either live cells or biological samples. These probes are likely to be applicable for profiling of endogenous miRNA levels in living cells, circulating exosomes, and tissues.

APPLICATIONS

TMT reactions may find wide application for amplified fluorescent detection of clinically relevant nucleic acid templates including:

Point of care diagnostics, detection of pathogens, clinical diagnostic tests, imaging biomarkers immunohistochemistry, molecular imaging/image guided surgery, telomere and telomerase assays

RELATED MATERIALS

- Haoxing W, BT Cisneros, CM Cole, and NK Devaraj. Bioorthogonal Tetrazine-Mediated Transfer Reactions Facilitate Reaction Turnover in the Nucleic Acid-Templated Detection of microRNA. J. Am. Chem. Soc., 2014, 136 (52), pp 17942–17945. - 12/12/2014

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OTHER INFORMATION

KEYWORDS

Aza Compounds, Fluorescent Dyes, Heterocyclic Compounds, 1-Ring, MicroRNAs; Biosensing Techniques; MCF-7 Cells; Nucleic Acid Conformation

CATEGORIZED AS

- Research Tools
  - Nucleic Acids/DNA/RNA

RELATED CASES

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