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Regular Article

Comparison of thermally actuated retro-diels-alder release groups for nanoparticle based nucleic acid delivery

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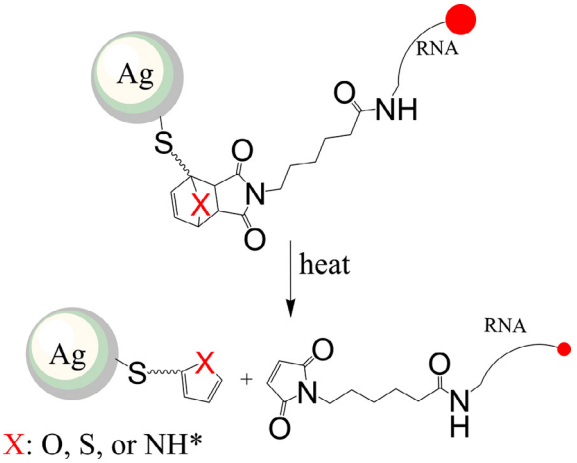
GRAPHICAL ABSTRACT



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ABSTRACT

The present study explores alternate pericyclic chemistries for tethering amine-terminal biomolecules onto silver nanoparticles. Employing the versatile tool of the retro-Diels-Alder (rDA) reaction, three thermally-labile cycloadducts are constructed that cleave at variable temperature ranges. While the reaction between furan and maleimide has widely been reported, the current study also evaluates the reverse reaction kinetics between thiophene-maleimide, and pyrrole-maleimide cycloadducts. Density Functional Theorem (DFT) calculations used to model and plan the experiments, predict energy barriers for the thiophene-maleimide reverse reaction to be greatest, and the pyrrole-maleimide barriers the lowest. Based on the computational analyses, it is projected that the cycloreversion rate would occur slowest with the thiophene, followed by furan, and finally pyrrole would yield the promptest release. These thermally-responsive linkers, characterized by Electrospray Ionization Mass Spectrometry, ^{1}H and ^{13}C NMR, are thiol-linked to silver nanoparticles and conjugate single stranded siRNA mimics with 5′

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Pyrrole
Thiophene
Furan

fluorescein tag. Second harmonic generation spectroscopy (SHG) and fluorescence spectroscopy are used to measure release and rate of release. The SHG decay constants and fluorescence release profiles obtained for the three rDA reactions confirm the trends obtained from the DFT computations.

1. Introduction

Delivery of short non-coding nucleic acid molecules as potential therapeutics has widely been investigated [1–7]. These molecules play a crucial role in regulating gene expression in numerous processes from embryonic to immune system development, and have been sought after as potential therapeutic agents relevant to human physiology and pathology. Clinical adoption, however, has been slow as a result of unidentified off-target effects as well as non-specific and inefficient delivery, requiring a high dosage for effective treatment. With the capability to respond to internal or external stimuli, nanoparticle delivery systems hold great promise for efficient spatiotemporal gene control of therapeutic delivery. Targeted delivery and release of therapeutics can be initiated via physical, chemical, or mechanical cues. Nanoparticle systems designed for nucleic acid caging and controlled release include polymer-based media [8], surface-linked or encapsulated nanoparticles [7,9], liposomal and viral vectors [10,11]. These systems can be tailored to employ various cues for nucleic acid delivery at targeted sites, including pH change [12], temperature [13] or electromagnetic-mediated stimuli [14]. Most of these methods rely on the linker or substrate chemical nature for stimuli response and drug release, typically through bond breaking and rearrangement.

For efficient temporal manipulation of small molecules and siRNA, click chemistry, most commonly reported between an azide and alkyne, can provide bioconjugation abilities of biomolecules with specific systems additionally providing a switch-like trigger for molecular release. These reactions are wide in scope, but pertain to reactions proceeding under mild conditions to give high yields and innocuous by-products [15,16]. Diels-Alder reactions meet this criteria, and involve formation of a cycloadduct product derived from a dienophile and diene in which more stable σ-bonds are formed from [4 + 2] π-bonds, based on overlapping electron levels between higher and lower occupied and unoccupied molecular orbitals [17,18,19]. At higher temperatures the cyloaddition reactions undergo reverse reaction pathways, referred to as the retro-Diels-Alder (rDA) reaction, to reproduce their diene and dienophile counterparts [20,21,22]. In Bakhtiari et al. [23], it was demonstrated that the cycloadduct between a furan and maleimide could be synthesized at room temperature over seven days, with the rDA reaction observed to occur at temperatures 60 °C and above. Using gold nanoparticles modified with fluorescein-tagged furan-maleimide linkers and irradiated at their plasmonic wavelength of 532 nm, an increase in solution fluorescence was observed due to localized plasmon–phonon heat generation, triggering the rDA reaction and releasing the markers. Other groups have utilized chiral auxiliary synthesis methods for enantiomer-selectivity of pyrrole-pyrimidine ring structures and microwave-inducible rDA reaction [24–26]. The rDA chemistry is a versatile tool for the delivery of short biological molecules utilizing simple chemical modifications, and provides a stimuli-responsive switch to trigger cargo release based on localized heat induction. By designing multiple linkers with alternate thermal responses, temporal delivery of more than one drug for gene therapy can be achieved.

The Diels-Alder forward reaction can be facilitated via an electron-enriched diene with an electron-poor dienophile, substituted with electron-donating and electron-withdrawing groups, respectively [27]. In the current study, we aim to build on the chemistry investigated previously [23], in which the bond breaking of 7-oxa-bicyclo-[2.2.1]hept-5-ene-2,3-dicarboxylic imide was studied, and explore the bicyclic reactions of alternate dienes. With distinct temperature ranges achieved in which to initiate the rDA reaction, multiplex delivery applications can be realized. In addition to the described furan-maleimide composition, substitution of the furan with both a pyrrole and thiophene-based diene was investigated, utilizing chemical modifications of the cycloadduct with a thiol terminal for nanoparticle conjugation, and a carboxyl terminal group for crosslinking with 3′ amine-modified nucleic acids (Fig. 1). Density functional theory was used to model the reaction barriers at various temperatures for the three different diene reactions with maleimide to aid in the rational design of the system and experimental conditions. To measure the rDA rates for each of the model compounds, second harmonic generation spectroscopy was utilized for nanoparticle surface measurements; the first time the technique is used, to our knowledge, to analyze the reversion of the DA linker.

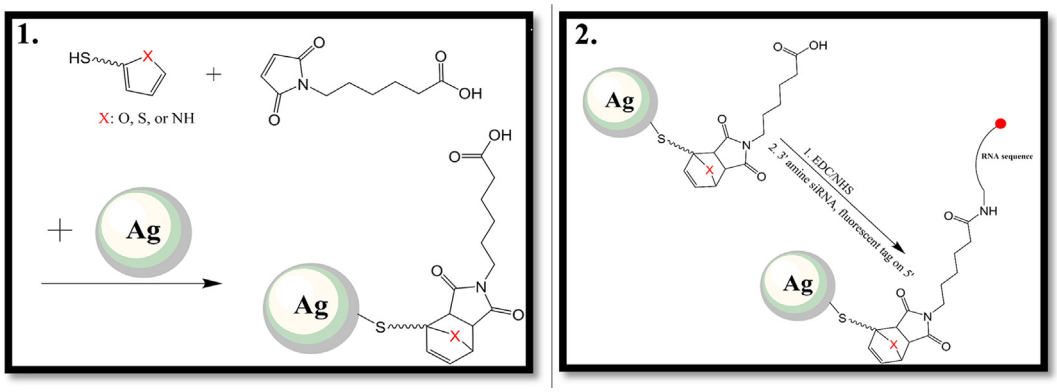

Fig. 1. Overall schematic illustrating pericyclic reaction between dienes with 6-maleimidohexanoic acid and conjugation onto nanoparticle via generic thiol linkage (1). EDC coupling chemistry was utilized to link amine-terminated siRNA to nanostructure (2).