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Nucleic Acid Templated Uncaging of Fluorophores Using Ru-Catalyzed Photoreduction with Visible Light

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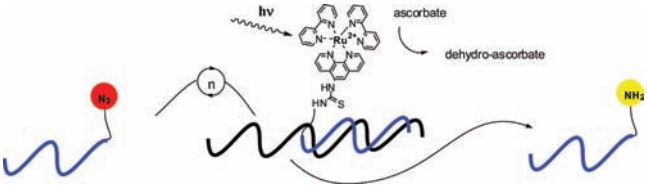
ABSTRACT



Hybridization-based reactions have attracted significant attention. The nucleic acid templated photocatalyzed azide reduction using catalytic amounts of a $[Ru(bpy)_2phen]^{2+}$ conjugate is reported. The reaction could be performed with as little as 2% of the Ru nucleic acid probe and was shown to productively unquench 7-azido-coumarin as well as uncage a small molecule.

Reactions that respond to a nucleic acid cue are attractive for nucleic acid sensing,[1] "smart therapeutics"[2] and more broadly in systems chemistry.[3] The Staudinger reaction between two nucleic acid probes derivatized with an azide and a phosphine respectively, which are brought to a high effective concentration through their hybridization onto a complementary template, has attracted particular attention by virtue of its orthogonality and biocompatibility.[4,5] Several studies have shown that the azide reduction could be coupled to a fluorescence readout[6−8] which has been implemented for nucleic acid sensing in bacteria[9] and eukaryotic cells.[10,11] An appealing feature of such a templated reaction is that signal amplification can be achieved through multiple turnover of the template.[12] However, one limitation of the Staudinger reaction is the sensitivity of phosphine to oxidation. This has generally been circumvented by using an excess of the phosphine probe relatively to the azide partner. This solution must however be balanced with the fact that an excess of phosphine probe does increase the untemplated bimolecular reaction. Recently, Liu and co-workers reported the discovery of a photocatalyzed reduction of azides using catalytic $[Ru(bpy)_3]^{2+}$ and stoichiometric ascorbate or NADPH (nicotinamide adenine dinucleotide phosphate)

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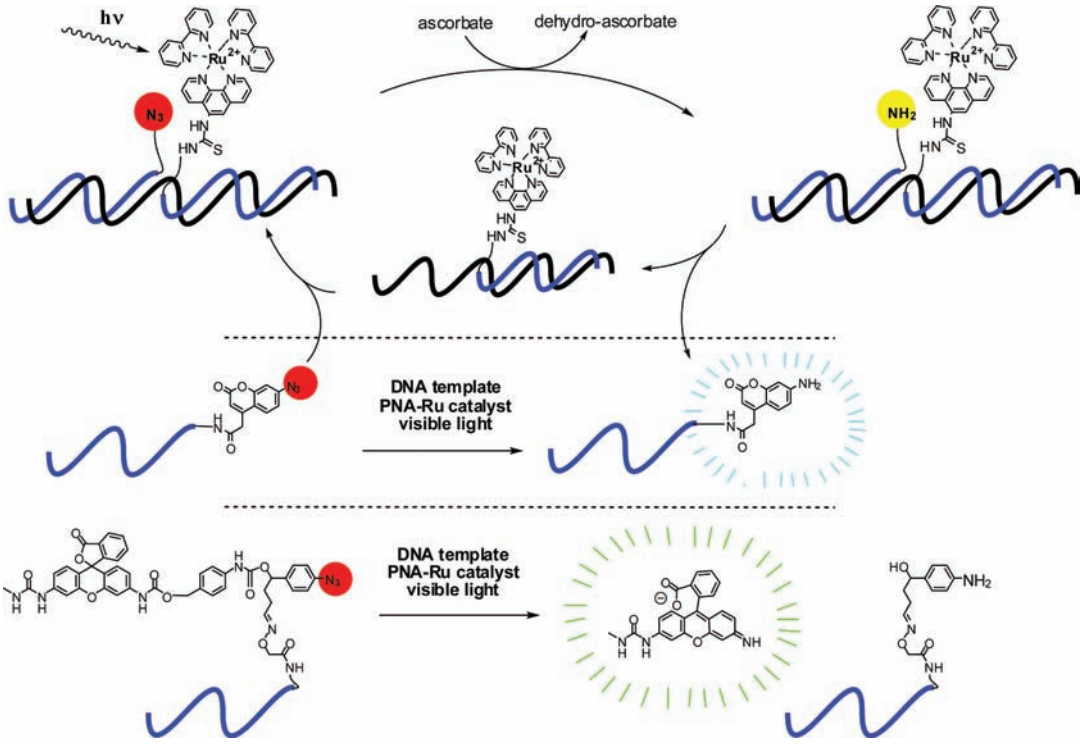

Figure 1. Schematic representation of light-triggered ruthenium promoted templated azide reduction.

as a reducing agent.[13] This new transformation was shown to be compatible with biomolecules raising the possibility that it could supersede the use of phosphines in templated Staudinger reactions. Inspired by the success of this reaction we asked if it could be used for nucleic acid sensing or nucleic acid triggered small molecule uncaging.

Based on our prior experience with peptide–nucleic acid[14] (PNA) probes in templated reactions and programmed self-assemblies,[15] we opted for this nucleic acid platform in the present study. To investigate the new azide reduction procedure in a nucleic acid templated reaction, we envisioned using a PNA derivatized with commercially available bis(2,2′-bipyridine)-(5-isothiocyanato-phenanthroline) ruthenium bis(hexafluorophosphate) as an analogue of $[Ru(bpy)_3]^{2+}$ and the second PNA bearing either of two different pro-fluorescent reporters: 7-azidocoumarin[7] or a pro-fluorescent rhodamine linked to the PNA through an immolative linker[8] (Figure 1). Preliminary experiments had shown that irradiation of an azido-profluorophore such as azido-coumarin or azido-rhodamine[11] at their respective absorption wavelengths in the presence of sodium ascorbate did lead to reduction. However, these molecules were stable if irradiated outside of their absorption window. Based on the fact that the ruthenium probe has an absorption maxima at 455 nm, using a 455 nm LED light source with no emission below 400 nm should preclude uncatalyzed photoreduction of azido-coumarin or the immolative linker but precluded the use of azidorhodamine. While the Ru-PNA probe has a broad emission band of 550–750 nm, it does not overlap with the emission spectra of either coumarin or rhodamine (emission maxima at 455 and 530 nm respectively). The PNA probes were prepared by a standard Fmoc-based strategy using GPNA[16] at every other position. The $Ru(bpy)_2$ phenanthroline isothiocyanate was coupled postcleavage from the resin to the side chain of a lysine residue incorporated at the C-terminus of the PNA. The azido-coumarin was coupled to the N-terminus of a PNA prior to cleavage while the immolative linker was conjugated in solution to a PNA bearing a hydroxylamine at the N-terminus (see SI for detailed procedures).

We first investigated the efficacy of the templated photoreduction of an azido-coumarin probe using a catalytic amount of the Ru-PNA probe. Preliminary experiments showed that a 15 mM concentration of sodium ascorbate was sufficient for the reaction to proceed. As shown in Figure 2, in the presence of 0.5 equiv of the Ru-PNA probe and DNA template, the reaction reached completion within 1 h whereas the reaction progressed marginally in the absence of DNA or sodium ascorbate. Control

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experiments in the absence of light did not yield any reaction. Ambient light within the laboratory was not sufficient to afford a detectable reaction within 1 h. Using the initial slope of the reaction after 10 min as an approximation of the first-order rate in comparison to the reaction in the absence of DNA (taking the slope for 210 min in order to average out the noise of the baseline), the templated reaction proceeded 56 times faster than the untemplated one. Lowering the template and Ru-PNA catalyst to a 25% loading resulted in a small reduction in reaction rate (50-fold rate acceleration). Gratifyingly, a 2% loading of the Ru-catalyst and template (10 nM) were sufficient to promote the reaction albeit at a reduced rate. At the 2 h time point, the reaction had proceeded to 30% suggesting a 15-fold turnover of the Ru-catalyst probe. Taken together, the data strongly support the hypothesis that the increase of fluorescence is a direct product of the Ru-mediated azide photoreduction enabled by the high effective concentration achieved in the supramolecular assembly and that the Ru-PNA probe can act catalytically.

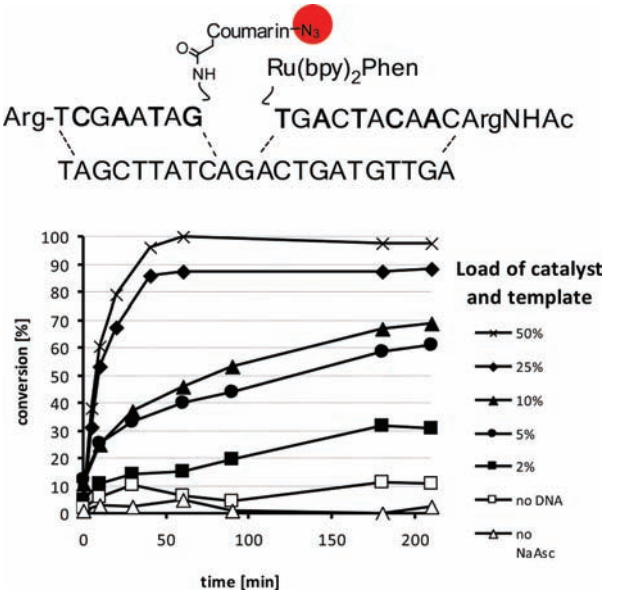

Figure 2. Kinetics of azidocoumarin reduction at different concentrations of Ru-PNA and DNA: 500 nM N_3-PNA, 15 mM ascorbate, LED 455 nm 1 W, 23 °C. Buffer: 500 mM Tris*HCl, pH 7.4, 0.05% tween (polyoxyethylene (20) sorbitan monolaurate). Bold letters in PNA sequences denote GPNA residues. Data points are the average of two experiments. Fluorescence measured at ex. 360 nm, em. 460 nm.

We next investigated the impact of mismatches in the template. As shown in Figure 3, a mismatch in the region of the azide-PNA probe or the Ru-PNA probe both led to a dramatic reduction in the rate of reaction. Taking the slope of the reaction after 15 min as an approximation of the first-order rate, the reaction was 18 times faster than the reaction with a template carrying a double mutation in the region complementary to the Ru-PNA probe whereas rate of reaction of the template carrying a mutation in the region complementary to the azide-PNA probe was comparable to the rate with a random DNA template. The slightly higher sensitivity to the mutation in the region complementary to the azide-PNA probe can be rationalized by the fact that this probe is two nucleobases shorter than the Ru-PNA probe and hence it will be more destabilized by a mismatch than the longer probe. While this discrimination may be further enhanced at higher temperature, this was not investigated at this stage.

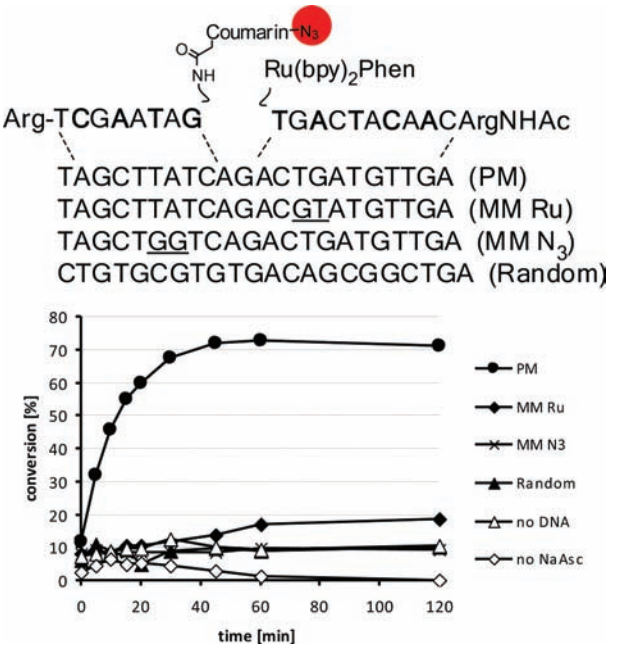

Figure 3. Kinetics of mismatch selectivity of azidocoumarin reduction: 500 nM N_3-PNA, 25% Ru-PNA and DNA (125 nM), 15 mM ascorbate, LED 455 nm, 1 W. Buffer: 500 mM Tris*HCl, pH 7.4, 0.05% tween (polyoxyethylene (20) sorbitan monolaurate). Bold letters in PNA sequences denote GPNA residues. Data points are the average of two experiments. Fluorescence measured at ex. 360 nm, em. 460 nm.

We then turned our attention to the ruthenium-catalyzed photoreduction of the azide to promote cleavage of the immolative linker. An asset of the immolative linker is that it is broadly applicable to uncage different fluorophores or bioactive small molecules. For the present study, we only focused on rhodamine as it provides a simple fluorescent readout of the reaction. Prior investigations into the kinetics of the self-immolation[8] had shown that the azide reduction was not the rate-limiting step and the half-life for self-immolation was under 11 min. As shown in Figures 4 and 5, the ruthenium-catalyzed photoreduction of the azide leading to rhodamine uncaging also proceeded efficiently. Comparing the rates of reactions after 45 min, the reaction in the presence of 20% of template and Ru-PNA probe was 25 times faster with a perfect match template than the one with a random template and 17 times faster than the one with the template carrying mismatches. Furthermore, the Ru-PNA probe did turn over providing

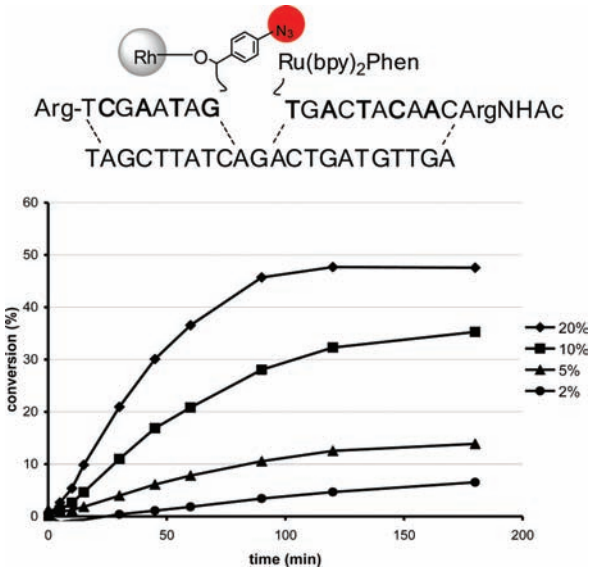

Figure 4. Kinetics of release of rhodamine in presence of different amounts of DNA and Ru-PNA: 250 nM N_3-PNA, 10 mM ascorbate, LED 455 nm, 1 W. Buffer: 500 mM Tris*HCl, pH 7.4, 0.05% tween (polyoxyethylene (20) sorbitan monolaurate). Bold letters in PNA sequences denote GPNA residues. Data points are the average of two experiments. Fluorescence measured at ex. 490 nm, em. 530 nm.

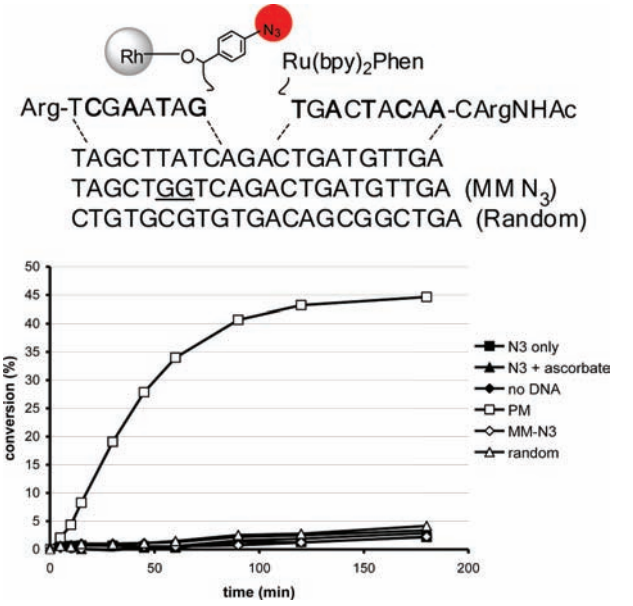

Figure 5. Kinetics of release of rhodamine in presence or absence of DNA, Ru-PNA, and ascorbate (250 nM N_3-PNA, 20% Ru-PNA and DNA, 10 mM ascorbate, LED 455 nm, 1 W). Buffer: 500 mM Tris*HCl, pH 7.4, 0.05% tween (polyoxyethylene (20) sorbitan monolaurate). Bold letters in PNA sequences denote GPNA residues. Data points are the average of two experiments. Fluorescence measured at ex. 490 nm, em. 530 nm.

yields in excess of its concentration (Figure 4) and providing a discernible reaction down to a 2% catalyst loading (5 nM).

In conclusion, we have demonstrated that the $[Ru(bpy)_3]^{2+}$ catalyzed photoreduction of azide discovered by Liu et al. is applicable to nucleic acid sensing. This strategy can be readily implemented with a commercially available 5-isothiocyanophenentroline analogue ($[Ru(bpy)_2(5\text{-NCSphen})]^{2+}$) which can be smoothly coupled to an amine in nucleic acid probes. In comparison to the templated Staudinger reaction, this catalyzed version can be carried out with just 2% of the Ru-derivatized probe rather than an excess of the phosphine probe. Furthermore, the fact that the reaction is enabled by the photoexcitation of the Ru-probe means that both components (azide and ruthenium probe) can be handled at high concentration in the absence of light without background reaction. This temporal control may be important in further implementation of this reaction in biological settings or other responsive systems.

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Supporting Information Available. Experimental procedures and characterization for the synthesis of the PNA probes. This material is available free of charge via the Internet at http://pubs.acs.org.