Photochemistry Hot Paper

International Edition: DOI: 10.1002/anie.201606425
German Edition: DOI: 10.1002/ange.201606425

Near-Infrared Photoinduced Coupling Reactions Assisted by Upconversion Nanoparticles

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Abstract: *We introduce nitrile imine-mediated tetrazole–ene cycloadditions (NITEC) in the presence of upconversion nanoparticles (UCNPs) as a powerful covalent coupling tool. When a pyrene aryl tetrazole derivative ($\lambda_{abs,\,max} = 346$ nm) and UCNPs are irradiated with near-infrared light at 974 nm, rapid conversion of the tetrazole into a reactive nitrile imine occurs. In the presence of an electron-deficient double bond, quantitative conversion into a pyrazoline cycloadduct is observed under ambient conditions. The combination of NITEC and UCNP technology is used for small-molecule cycloadditions, polymer end-group modification, and the formation of block copolymers from functional macromolecular precursors, constituting the first example of a NIR-induced cycloaddition. To show the potential for in vivo applications, through-tissue experiments with a biologically relevant biotin species were carried out. Quantitative cycloadditions and retention of the biological activity of the biotin units are possible at 974 nm irradiation.*

Light-induced reactions are an important tool in the field of contemporary chemical synthesis as they afford spatial[1] and temporal reaction control.[2] A large number of functionalities can be found in literature which are labelled photo-sensitive, that is, having the capability of undergoing a light-stimulated elimination, isomerization, or internal electronic activation to participate in a chemical reaction.[3] The most ideal of these reactions also fulfil the criteria required for a click reaction, namely equimolarity, full reagent conversion, exclusive product formation, short reaction times, functional-group orthogonality, and mild reaction conditions.[4] This class of reactions has been termed as "photo-click" reactions.[2,5] While a number of these photo-click reactions have been reported, they almost exclusively require UV-light as a trigger. The use of such high-energy UV photons can be detrimental to a large proportion of organic, inorganic, and biological species which are sensitive to UV irradiation, leading to unwanted side-reactions. Thus, efficient photo-reactions triggered by substantially lower energy photons (e.g. wavelengths in the visible or infrared regime) are highly sought.[6] This wavelength regime is of critical importance for in vivo biological applications as tissue is more optically transparent in this region, allowing for overall deeper light penetration. One example of a photo-click moiety that has been applied in polymer chemistry,[7] for the modification of surfaces,[8] or in the field of biological-ligation[9] is the diaryl tetrazole. Triggered via a light stimulus, the tetrazole undergoes a rapid elimination of nitrogen to yield a reactive nitrile imine intermediate, which will undergo a subsequent equimolar cycloaddition with a dipolarophile in quantitative yields. Recently, some of us have reported that the inclusion of a pyrene chromophore at the N-aryl moiety of the tetrazole substantially red-shifted the wavelength at which the formation of the nitrile imine was triggered.[10] Block copolymers via polymer NITEC coupling were achieved through irradiation with an LED at 410–420 nm, constituting a shift of over 70 nm compared with the diaryl tetrazole system. To date this is the longest wavelength single-photon triggering of a NITEC reaction. Two photon excitation has also been successfully applied in the literature with naphthalene-based tetrazoles efficiently activated by a 700 nm femtosecond pulsed laser.[11] However, two-photon absorption requires high-intensity pulsed lasers (typical pulse intensity: $> 10^6$ W cm^{-2})[12] and is of low efficiency even when femtosecond lasers are employed. Two-photon absorption only occurs at the laser focus. As the femtosecond laser will defocus while passing through the tissue, the two-photon absorption strategy is impractical for deep tissue trials.

Recently, upconversion nanoparticles (UCNPs) were used as upconverters to efficiently assist various NIR-induced photoreactions, including photolysis, photoisomerization and photopolymerization.[6b,13] UCNPs consist of a crystalline host matrix (e.g., $NaYF_4$, LaF_3, Y_2O_3) doped with lanthanide sensitizer ions (e.g., Yb^{3+}) and activators (e.g., Er^{3+}, Tm^{3+}). Upon NIR irradiation, the sensitizer ion absorbs and transfers NIR energy to the activator ion, resulting in multiple luminescence emissions at various wavelengths, including

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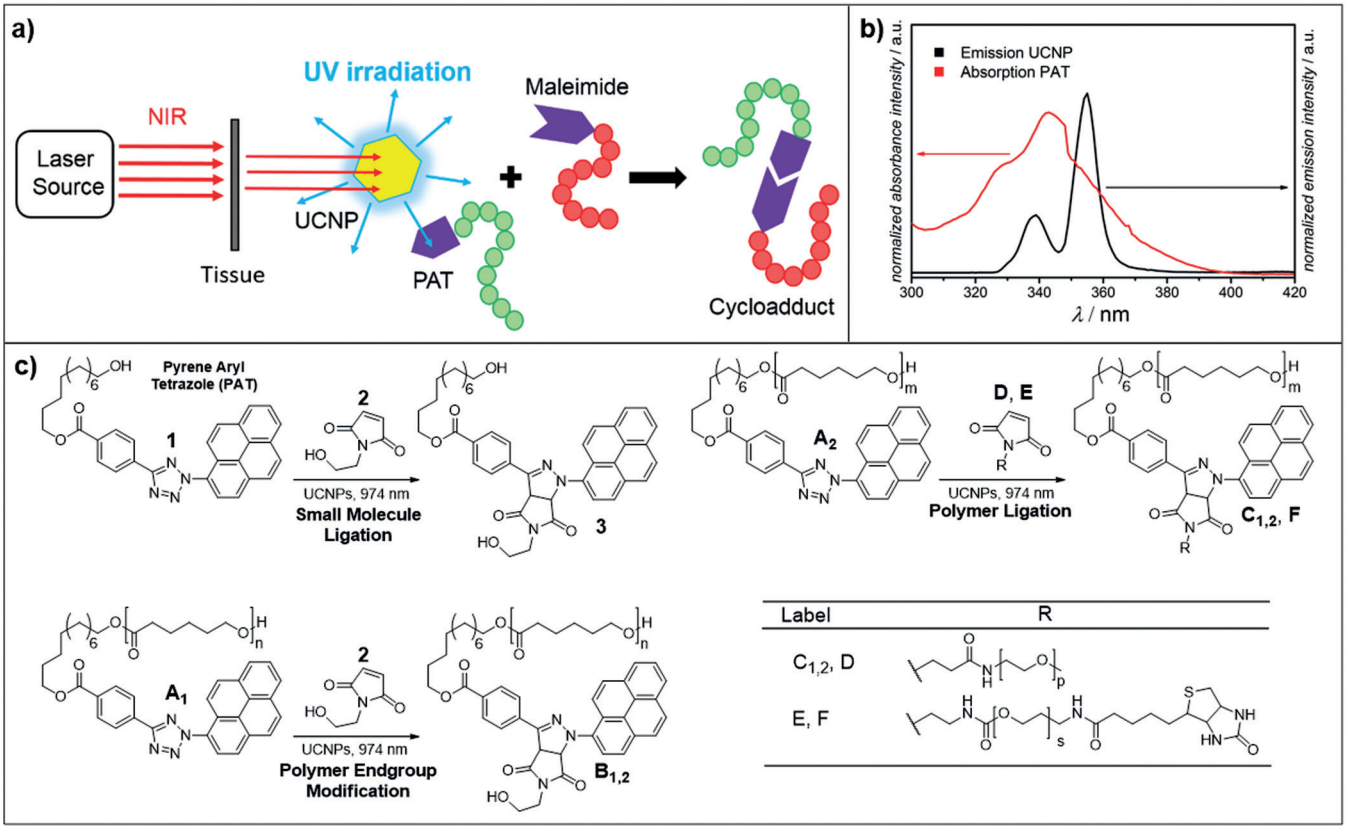
Supporting information for this article can be found under: http://dx.doi.org/10.1002/anie.201606425.

UV, blue, green, red, and NIR. The required light intensity to induce photoreactions using upconversion (hundred $mW\,cm^{-2}$ to some hundred $W\,cm^{-2}$) is several orders of magnitude lower than those using two-photon absorption.[6b]

Herein, we introduce a new type of reaction, that is, UCNP-assisted photoinduced coupling chemistry, triggered with a 974 nm light source (14 W) by combining upconversion and NITEC technology. To our knowledge, the technique is the only example of a photoinduced coupling reaction using a near-infrared source at such a long wavelength, providing an efficient and rapid linkage method. Especially in the field of biology, the method has significant potential due to the deep tissue penetration ability of the light source. Furthermore, the formed pyrazoline cycloadduct exhibits near-infrared fluorescence, allowing for potential theranostic applications by in vivo imaging/tracking of the ligated species. Herein, the concept of upconversion photoinduced coupling chemistry is applied for small-molecule ligation, polymer-end-group modification, and block-copolymer formation via functional polymer block linkage (refer to Scheme 1). Further—to demonstrate in vivo penetration—upconversion photoinduced coupling reactions induced by NIR light were carried out with a tissue spacer placed between the reaction vessel and irradiation source. In addition—to demonstrate the bioorthogonality of the upconversion photoinduced coupling chemistry—photoinduced coupling experiments in the presence of a biotin derivative were performed and the retained bio-activity confirmed.

The synthesis and applications of the UCNPs and the pyrene aryl tetrazole (PAT) have both been previously described.[6b, 10, 13, 14] For efficient NITEC reaction to occur, sufficient overlap between the emission of UCNPs and the absorbance of the PAT must be ensured, leading to nitrile imine formation. As shown in Scheme 1, when $NaYF_4$:TmYb@$NaYF_4$ upconversion nanoparticles (UCNPs) are employed, there is significant overlap in the region of 330–370 nm. Although the PAT species has been reported to undergo 410–420 nm light triggered cycloadditions, it can also be activated in UV wavelength regime. Thus, the observed joint activation region has the potential for near-infrared light triggering of NITEC systems.

Initially, model experiments were undertaken involving small molecules **1** (1.0 equiv.) and **2** (1.1 equiv.) to validate the concept of upconversion photoinduced coupling chemistry, as well as to quantify the efficiency of the reaction compared to established photoligations via NITEC.[10,15] Therefore, photoactive PAT **1** and hydroxy-functionalized maleimide **2** were dissolved in acetonitrile (MeCN) in the presence of UCNPs



Scheme 1. a) Schematic illustration of near-infrared photoinduced coupling reactions assisted by upconversion nanoparticles. Tissue was optionally placed between the beam and reaction vessel to demonstrate the penetration capability of the near-infrared light. b) Magnification of the 300–420 nm region of the emission spectra of UCNP (black) and absorption spectra of PAT (red), as well as transmission electron microscopy image of UCNPs. c) Synthetic path for near-infrared photoinduced coupling reactions to form small-molecule cycloadduct **3**, end-group modified PCL $\mathbf{B_1}$ (no tissue used)/$\mathbf{B_2}$ (tissue used), block copolymers $\mathbf{C_1}$ (no tissue used)/$\mathbf{C_2}$ (tissue used), and biotin functional block copolymer PCL-*b*-PEG **F**. Refer to Section S2,S5 in the Supporting Information for the reaction details.

and irradiated at 974 nm (refer to Scheme 1). The kinetics of the NITEC reaction were monitored using fluorescence spectroscopy via the emission of the generated pyrazole at 570 nm (refer to the Supporting Information, Section S3). The resulting product stream was investigated via HPLC without any further purification (refer to Figure 1). Full conversion of

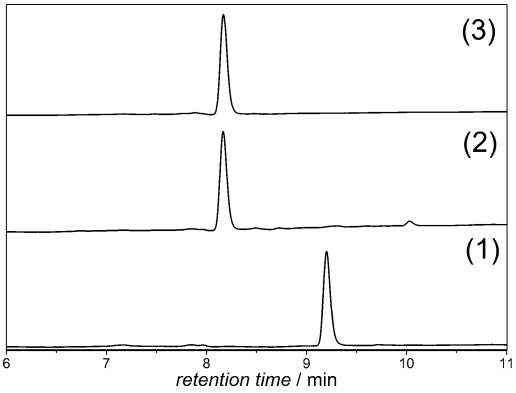


Figure 1. Normalized HPLC traces of PAT **1** (1), crude reaction mixture of PAT **1**, hydroxy-functionalized maleimide **2** and UCNPs, irradiated at 974 nm in MeCN for 30 min (2), and reference sample of cycloadduct **3** synthesized according to the literature in the absence of UCNPs (3) (refer to Scheme 1 for the reaction details); THF/MeCN/H_2O + 0.1% trifluoroacetic acid was used as eluent and a 220 nm UV detector was employed.

PAT **1** and the exclusive formation of the desired pyrazoline cycloadduct under NIR irradiation was observed. Only very minimal side reactions were detected in the crude NIR-irradiated reaction mixture. Trace (1) in Figure 1 shows the tetrazole signal before irradiation eluting at 9.2 min. A shift of the signal to 8.2 min was observed in trace (2), indicating full conversion of the tetrazole species and exclusive formation of the desired cycloadduct. Trace (3) shows the purified cycloadduct **3**, synthesized according to the literature,[10] and employed as a reference. The structure of the formed pyrazoline **3** was confirmed by ESI-MS (see Section S2). The corresponding control experiment in the absence of UCNPs did not yield any of the desired cycloadduct (see Figure S2).

After the successful upconversion photoinduced coupling chemistry for small molecules, the concept was extended as a tool for macromolecular end-group modification and block-copolymer formation. The NITEC reaction was carried out in the presence of poly(ε-caprolactone) (PCL) and polyethylene glycol (PEG) polymers since both species are relevant in the field of biology because of their non-toxicity and biocompatibility.[16]

In the first approach, a PAT from which a PCL chain had been polymerized, $\mathbf{A_1}$, was employed for end-group modification. The tetrazole-containing polymer (1.0 equiv.) was irradiated in the presence of maleimide **2** (1.5 equiv.) in MeCN at 974 nm for 40 min (refer to Scheme 1). Experiments both with direct irradiation, $\mathbf{B_1}$, and in the presence of a tissue-shielded light source, $\mathbf{B_2}$, were carried out. The resulting reaction mixtures were investigated via ESI-MS

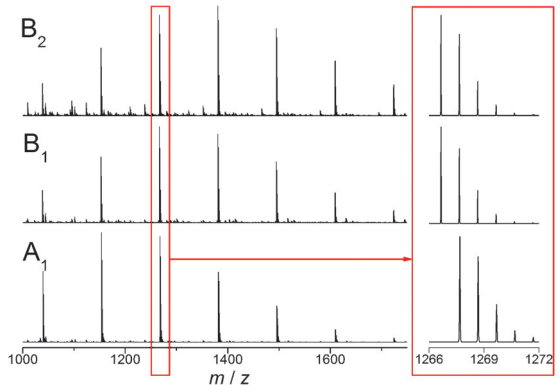


Figure 2. Magnified view in the region of *m*/*z* 1000–1750 of the high-resolution ESI-MS spectrum of PAT end-capped poly(ε-caprolactone) (PCL) $\mathbf{A_1}$ and the cycloadducts $\mathbf{B_1}$ (no tissue used) and $\mathbf{B_2}$ (tissue used). Inset: expanded view of the isotopic patterns for $\mathbf{A_1}$ and $\mathbf{B_{1,2}}$. Refer to Scheme 1 for the structures of the formed cycloadducts $\mathbf{B_{1,2}}$ and the reaction conditions; refer to Table S2 for the exact theoretical and experimental masses of the depicted polymer species $\mathbf{A_1}$ and $\mathbf{B_{1,2}}$. Note that only a signal shift of approximately 1 Da results.

(refer to Figure 2). Full conversion of the PAT end capped polymer and exclusive formation of the cycloadducts $\mathbf{B_{1,2}}$ under the employed conditions was observed. For the tissue shielded experiment $\mathbf{B_2}$, a prolonged reaction time (60 min) was necessary due to the strength of the irradiation stimulus being reduced by tissue absorbance. However, the presence of the tissue had no significant influence on the final level of conversion or the products formed by the coupling. The structures of the modified polymers, $\mathbf{B_{1,2}}$, were confirmed by ^{1}H NMR (see Figures S4 and S7). Interestingly, the fluorescence excitation of the pyrazoline containing PCL formed via NITEC can potentially be excited by infrared light, as long as the UCNP are in close proximity. Combination of the fluorescence properties of the cycloadduct revealing a strong tail into the near-infrared region and UCNP provides the opportunity of fluorophore excitation and fluorescence detection involving near-infrared light only.

Subsequently, upconversion assisted polymer–polymer coupling was attempted. PAT-functional PCL $\mathbf{A_2}$ (1.0 equiv.) was irradiated in the presence of a maleimide functional PEG (1.5 equiv.) and UCNPs at 974 nm for 60 min to form PCL-*b*-PEG block copolymer $\mathbf{C_1}$ (refer to Scheme 1). Again, both standard NIR irradiation (cycloadduct $\mathbf{C_1}$) and a tissue-shielded irradiation (cycloadduct $\mathbf{C_2}$) were attempted, to mimic differences between an in vitro and in vivo experiment. The corresponding reaction mixtures were analyzed by size exclusion chromatography (SEC; refer to Figure 3). Inspection of Figure 3 indicates that full conversion of the tetrazole-terminated PCL and exclusive formation of the block copolymer is observed. For both experiments ($\mathbf{C_1}$ and $\mathbf{C_2}$) a significant shift to lower retention times combined with a decrease of $Đ$ were observed. The structures of the cycloadducts formed were confirmed by ^{1}H NMR spectroscopy (see Figures S10–S12). Similar to the end-group modification experiments, prolonged irradiation times were necessary for the coupling reaction in the presence of the tissue shield to proceed to full conversion. However, the extended

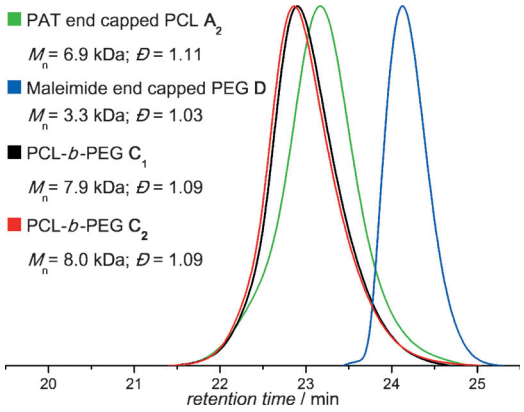


Figure 3. Normalized SEC traces of PAT functional PCL $\mathbf{A_2}$ (green), maleimide functional PEG (blue), and block copolymers $\mathbf{C_1}$ (no tissue used, black) and $\mathbf{C_2}$ (tissue used, red) formed by upconversion photoinduced coupling reactions. M_n and $Đ$ were determined by SEC in THF using poly(styrene) calibration standards.

reaction times did not result in the formation of additional side products, for example, due to the decomposition of the cycloadduct formed. To demonstrate the feasibility of upconversion photoinduced coupling chemistry for the preparation of block copolymers carrying a photosensitive, bioactive species, a polymer functionalized with a biotin terminus was used. PCL $\mathbf{A_2}$ was irradiated at 974 nm in the presence of UCNPs and a bifunctional biotin/maleimide PEG **E** for 60 min under ambient conditions (Scheme 1). Again, a tissue was placed between the irradiation source and the reaction vessel to demonstrate the penetration ability of the NIR light. The conversion of the PAT moiety ($\lambda_{em} = 431$ nm) and formation of the pyrazoline containing block copolymer **F** ($\lambda_{em} = 571$ nm) was detected by fluorescence spectroscopy from the crude reaction mixture after removing the UCNPs (Figure 4). Full conversion of the PAT moiety and formation of the desired cycloadduct was observed. The fluorescence band of the tetrazole species (Figure 4; black line) vanishes, while a new band associated with the cycloadduct appears (Figure 4; blue line). Importantly, the biotin retains its bioactivity after the upconversion photoinduced coupling reaction with 88 % of the block copolymer **F** found to undergo non-covalent bonding with avidin protein (see Section S5).

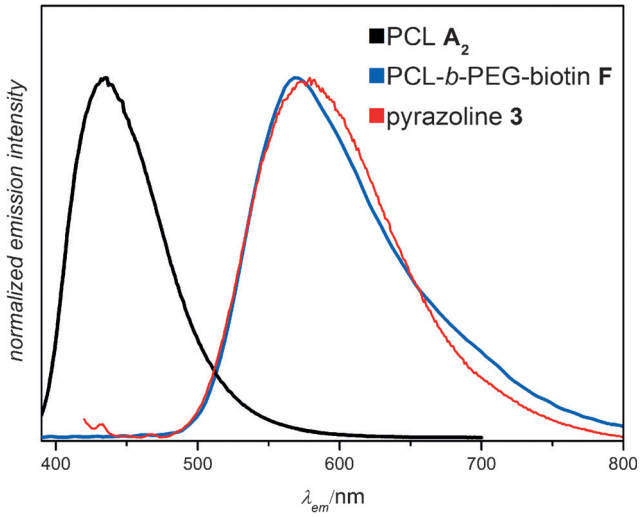


Figure 4. Normalized fluorescence spectra of PCL $\mathbf{A_1}$ (black), PCL-*b*-PEG-biotin **F** (blue),and pyrazoline **3** (red).

In summary, we introduce a mild, efficient, rapid, photoinduced coupling strategy at 974 nm. The methodology was shown to be suitable for small molecule ligation, polymer end-group modification, as well as polymer–polymer linkage. For all performed reactions, full conversion of the photo-active tetrazole species was observed, leading to the exclusive formation of the desired cycloadduct and no visible side-reactions. In addition, the formed cycloadducts display strong fluorescence reaching into the near-infrared region. This is the first example of a NITEC reaction and, more broadly, a photoinduced coupling reaction that can be triggered by NIR light, offering a new tool for bio-orthogonal labelling.

Acknowledgments

C.B.-K. and J.B. acknowledge the DAAD/ATN for funding, facilitating a research stay of P.L. at the Karlsruhe Institute of Technology (KIT). C.B.-K. acknowledges the Sonderforschungsbereich 1176 (project B4) and the GRK 2039 (project A1) funded by the German Research Council (DFG) for support. P.L. acknowledges the Queensland University of Technology (QUT) and the KIT for funding his PhD studies. C.B.-K. acknowledges continued support from the KIT in the context of the Helmholtz STN and BIFTM programs. Z.C.'s PhD studies were supported by a CSC scholarship. S.W. acknowledges the DFG (WU 787/2-1) and the Fonds der Chemischen Industrie (FCI, No. 661548) for financial support.

Keywords: near infrared · NITEC · photoinduced cycloaddition · polymer coupling · upconversion

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 12195–12199
Angew. Chem. **2016**, *128*, 12382–12386

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Received: July 2, 2016
Published online: August 25, 2016

