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Qualitative sensing of mechanical damage by a fluorogenic “click” reaction†

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A simple and unique damage-sensing tool mediated by a Cu(I)-catalyzed [3+2] cycloaddition reaction is reported, where a fluorogenic “click”-reaction highlights physical damage by a strong fluorescence increase accompanied by *in situ* monitoring of localized self-healing.

Visualizing mechanical stress¹ based on molecular probes is an important aspect in materials science, achievable *via e.g.* chemical reporters such as color and fluorescence changes in spiropyranes,² luminescence of dioxetanes³ or absorption and fluorescence changes of anthracenes.⁴ Only a few examples exist, where mechanical stress is directly transformed into a chemical reaction, requiring a link between the applied physical damage and the detectable (chemical) response, as *e.g.* in visualizing damage in coatings *via* rhodamine-based profluorophores,⁵ *via* stress-induced radical formation combined with optical reporting of hexaarylbiimidazole motifs⁶ or *via* mechanochromism followed by thiol-ene crosslinking reactions.⁷ However, often the initially present chromophore only highlights damage-induced capsule-rupture followed by the subsequent flow of the self-healing agents into the crack plane, while the self-healing reaction itself cannot be monitored *in situ*.⁸ We here report on a fluorogenic “click”-reaction as an *in situ* damage-sensing tool for the Cu(I)-catalyzed [3+2] azide-alkyne cycloaddition (CuAAC)-mediated crosslinking of multivalent polymer components,⁹ locally monitoring ongoing “click”-reactions suitable for self-healing applications *via* an increase of the fluorescence intensity after mechanical deformation.

Although fluorogenic “click”-reactions have been used extensively in the fields of (cell) biology, biochemistry and proteomics, less effort has been directed to the visualization of crosslinking reactions in self-healing materials,¹⁰ where the damage reporting is considered an as equally important element compared to damage restoration. Consequently, fluorogenic “click” reactions^{11–13} between non-fluorescent azide- or alkyne-functionalized anthracenes,¹⁴ naphthalimides^{15,16} and coumarins^{11,12,17–19} have gained increasing interest due to the absence of any disturbing fluorescence background, resulting in a “turn-on” fluorescence after successful triazole ring formation. Applications of this principle are therefore especially attractive for *in vivo* and *in vitro* probing of cellular components,^{11,15,18,20} the screening of reaction conditions for the optimization of “click”-bioconjugations²¹ and the fluorogenic conjugation of nanoparticles to polymers.¹⁹

We have designed a coumarin-functionalized linear polymer chain as a fluorogenic probe (PIB **1**, green), in turn exploring the fluorogenic “click”-reaction between the crosslinking components (azide- and alkyne-telechelic three-arm star polymers, red and blue) in the presence of Cu(I)-complexes in solution and in the solid state (see Fig. 1). Encapsulation of the components and the subsequent embedding in an epoxy-matrix ensured the stable placement within the final nanocomposite, probing the damage-induced “click” reaction locally by the fluorogenic activation only after capsule rupture by conversion of the initially non-fluorescent probe **1** to the fluorescent product **2**.

For the synthesis of the fluorogenic probe **1**, hydroxy-functionalized PIB was esterified with [(3-azido-2-oxo-2H-chromen-7-yl)oxy]acetyl chloride, prepared *in situ* by converting 3-azido-7-carboxymethoxycoumarin with oxalyl chloride. The successful endgroup-functionalization was proven on account of the detectable UV-trace within the gel permeation chromatogram at 330 nm, *via* ¹H-NMR-spectroscopy showing the resonances of the coumarin-scaffold between 6.81 ppm and 7.52 ppm, *via* IR-spectroscopy clearly indicating the azide vibration, and additionally *via* MALDI-ToF mass spectrometry displaying the sodium adduct of the fluorogenic probe **1** (see Fig. S1–S4, ESI†).

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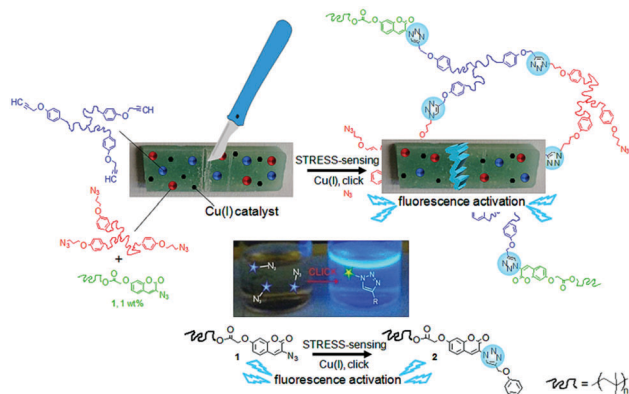


Fig. 1 Schematic representation of the fluorogenic “click”-reaction between the fluorogenic probe **1** and the crosslinking components (three-arm star PIB-azide and -alkyne) showing a fluorescence activation (yielding the fluorescent product **2**) integrated within a capsule-based self-healing nanocomposite suitable as a mechanical damage-sensing and -monitoring tool for CuAAC-mediated crosslinking.

To subsequently quantify the generated fluorescence intensities after the applied mechanical damage, the final fluorogenic “click” product **2** was generated by reacting the initial probe **1** with phenyl propargyl ether (see Fig. S5–S7 and S11, ESI[†]). Probing the fluorescence intensity of the fluorogenic product **2** in THF in the presence of different copper(I) catalysts such as CuI·P(OEt)₃, CuBr(PPh₃)₃ and CuF(PPh₃)₃ was required to quantify eventually occurring fluorescence quenching.²² While the fluorescence intensity of the fluorogenic product **2** in the presence of 0.3 equivalents (eq.) of CuF(PPh₃)₃ decreased only to 87% of its original value, a sharp exponential

decrease in the fluorescence intensity to approximately 7% in the presence of 0.3 eq. of CuI·P(OEt)₃ or CuBr(PPh₃)₃ was observed (Fig. S12, ESI[†]). Therefore, CuF(PPh₃)₃ and CuBr(PPh₃)₃ have been chosen for further investigations of the fluorogenic “click” reaction, mostly due to their high catalytic activity especially at room temperature revealing crosslinking within several hours.^{9,23}

For demonstrating sensing capabilities in solution (THF), a mixture of probe **1** and the crosslinking components together with a copper(I) catalyst was prepared and the fluorescence intensity was measured at defined time intervals (every 10 min). The fluorescence intensity increased within 290 min in the case of CuBr(PPh₃)₃ and within 40 min in the case of CuF(PPh₃)₃ (see Fig. 2(a) and (b)) followed by a plateau with a negligible decrease of the fluorescence intensity due to fluorescence quenching.²² Accordingly, the applicability of the fluorogenic “click” reaction as a sensing tool in solution was successfully demonstrated, while a stronger catalytic activity and thus a faster “click”-reaction was observed in the presence of CuF(PPh₃)₃.

Similar investigations were performed in the solid state, by blending the fluorogenic probe **1** and the crosslinking components together with the catalysts CuBr(PPh₃)₃ or CuF(PPh₃)₃. The finally obtained thin film was subsequently placed between two quartz glass plates in a specially designed solid state sample holder. The measured fluorescence intensity increased within 100 min in the case of CuBr(PPh₃)₃, therefore showing an even faster and stronger fluorescence increase in the polymer blend than in solution, attributed to predominantly auto-catalytic effects within the solid state.^{9,24} Interestingly, no increase of the fluorescence intensity was observed while applying CuF(PPh₃)₃ (see Fig. 2(c) and (d)), thus excluding this

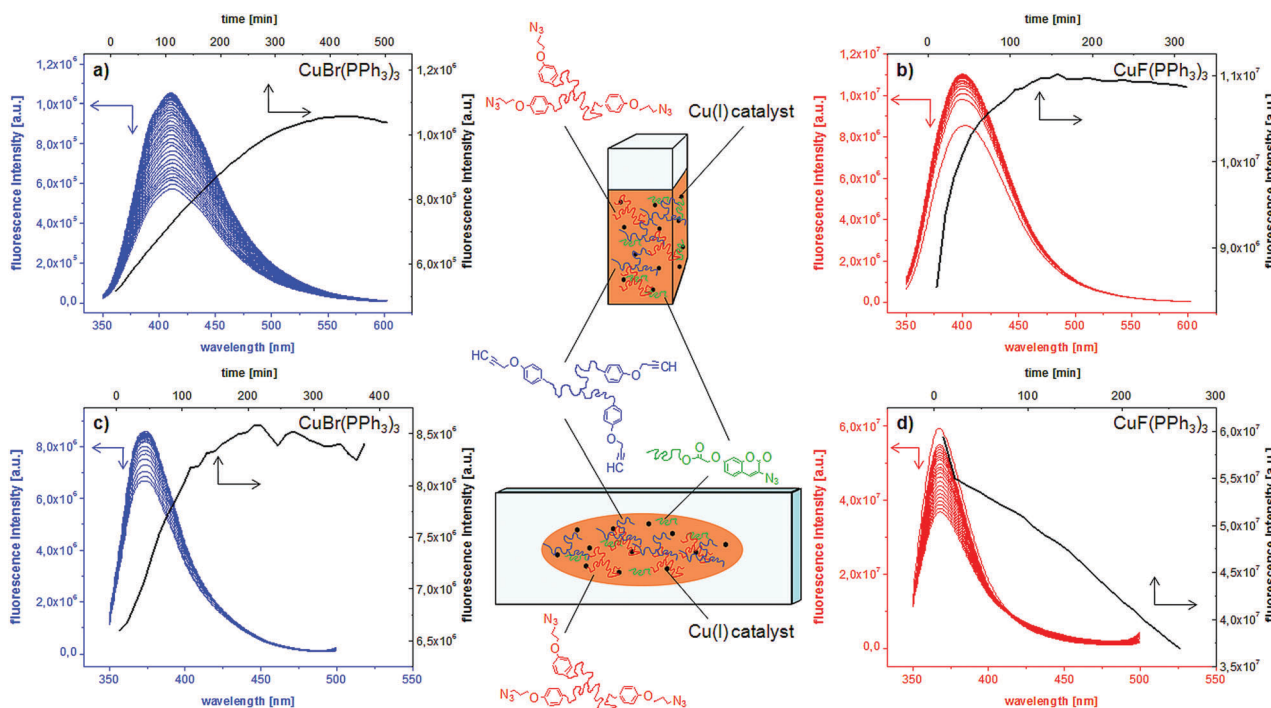


Fig. 2 Fluorogenic “click” reaction between fluorogenic probe **1** (1 wt%) and the crosslinking components at 20 °C in THF using (a) 0.3 eq. of CuBr(PPh₃)₃, (b) 0.3 eq. of CuF(PPh₃)₃, and in the solid state using (c) 0.3 eq. of CuBr(PPh₃)₃, (d) 0.3 eq. of CuF(PPh₃)₃.



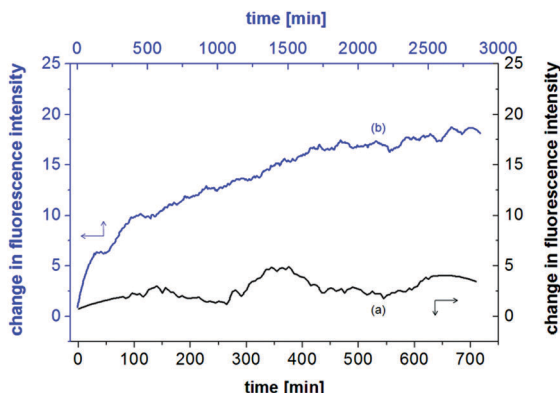


Fig. 3 Change in the fluorescence intensity during the fluorogenic “click” reaction within (a) an unscratched (lower curve, black) and (b) a scratched epoxy nanocomposite (upper curve, blue) containing PVF/PIB nanocapsules filled with the fluorogenic probe **1** (1 wt%) and the crosslinking components using 0.3 eq. of $\text{CuBr}(\text{PPh}_3)_3$ at 20 °C.

catalyst in the preparation of the subsequent epoxy nanocomposites due to its oxygen sensitivity.²⁵

Subsequent melt-rheology measurements proved the successful formation of a network in the presence of the fluorogenic probe **1** (1 wt%), which itself is not able to contribute to the crosslinking reaction. Furthermore, the obtained polymer resin showed fluorescence under a UV-lamp (366 nm), indicating the activation of the fluorescence probe during crosslinking (see Fig. S13, ESI†).

To finally demonstrate the applicability of the here presented fluorogenic “click” reaction as an *in situ* damage-sensing tool in the material itself, the system was integrated into an epoxy nanocomposite. Nanocapsules with an average hydrodynamic diameter of 204 nm (filled with the respective three-arm star PIB-azide or three-arm star PIB-alkyne), were generated *via* a miniemulsion solvent evaporation technique (for details see the ESI†)²⁶ and were homogeneously embedded within the epoxy matrix (see Fig. S16, ESI†). Thus, the crosslinking components were protected during the fabrication process of the epoxy nanocomposite, further separating them from the finely dispersed copper(i) catalyst ($\text{CuBr}(\text{PPh}_3)_3$), therefore avoiding premature crosslinking reactions. After cutting using a scalpel, in turn rupturing the capsules and triggering the fluorogenic “click”-reaction, the so generated fluorescence intensity was monitored *in situ* (see Fig. 3). While a control sample (undamaged) did not show any changes in the fluorescence intensity, a continuous increase in the fluorescence intensity was observed for the scratched sample, thus clearly indicating the progress of the fluorogenic “click”-crosslinking reaction within the epoxy nanocomposite.

Furthermore, the self-healing efficiency of an epoxy nanocomposite containing PVF nanocapsules separately filled with a low molecular weight trialkyne and triazide and $\text{CuBr}(\text{PPh}_3)_3$ as a catalyst (5 mol%) was investigated *via* dynamic mechanical analysis (DMA) measurements. Therefore, a 7 N bending force was applied to penetrate the crack and healing was investigated at room temperature by checking the tensile storage modulus (E') at low amplitude after defined time intervals (see Fig. S17, ESI†).

Thus, 48 hours after crack penetration nearly complete self-healing of the epoxy nanocomposite was observed.

In summary, we have presented the synthesis and characterization of a coumarin-functionalized fluorogenic polymer probe as a versatile *in situ* damage-sensing tool for the CuAAC-mediated crosslinking of multivalent, reactive polymers. Thus, the concept of the fluorogenic “click”-reaction for locally monitoring room temperature “click”-crosslinking was proven while avoiding the diffusion of interfering, free coumarins. During the course of the “click”-reaction a remarkable increase in the fluorescence intensity in solution, in the melt and directly within an epoxy nanocomposite was observed, strongly emphasizing its high application potential for monitoring localized self-healing reactions.

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