Electronic supplementary information for

Photochemical Ligation Meets Nanocellulose: A Versatile Platform for Self-Reporting Functional Materials

Daniel Hoenders,[a] Jiaqi Guo,[a] Anja S. Goldmann,[b] Christopher Barner-Kowollik,[b]* Andreas Walther[a]*

[a] Institute for Macromolecular Chemistry, Albert-Ludwigs-University Freiburg, Stefan-Meier-Straße 31, 79104 Freiburg, Germany

Freiburg Materials Research Center, Albert-Ludwigs-University Freiburg, Stefan-Meier-Straße 21, 79104 Freiburg, Germany

Freiburg Center for Interactive Materials and Bioinspired Technologies, Albert-Ludwigs-University Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany

Freiburg Institute for Advanced Studies (FRIAS), Albert-Ludwigs-University Freiburg, 79104 Freiburg, Albertstraße 19, 79104 Freiburg, Germany, andreas.walther@makro.uni-freiburg.de

[b] School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology (QUT), 2 George St, QLD 4000, Brisbane, Australia, christopher.barnerkowollik@qut.edu.au

Macromolecular Architectures, Institut für Technische Chemie und Polymerchemie, Karlsruhe Institute of Technology (KIT), Engesserstr. 18, 76128 Karlsruhe, Germany

Institut für Biologische Grenzflächen, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344, Eggenstein-Leopoldshafen, Germany, christopher.barner-kowollik@kit.edu

CONTENT:

1. Materials

Biotin-DEG-maleimide and Texas red-labelled Avidin were purchased from Thermo Fisher Scientific. 4-(dimethylamino)-pyridine (DMAP) and methoxypolyethylene glycol maleimide (M_n = 750 Da) were purchased from Sigma Aldrich. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimid hydrochloride (EDC-HCl), N-boc diamino ethane, triflouroacetic acid (TFA) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM) were purchased from ABCR. 8-Arm star-PEG-maleimide (sPEG-MI, $M_{n,star}$ = 10 kDa) was purchased from Creative PEGWorks. All chemical reagents and solvents were used without further purification unless otherwise stated.

2. Syntheses and Methods

Synthesis of N-(2-aminoethyl)-4-(2-(4-methoxyphenyl)-2H-tetrazol-5-yl)benzamide (tetrazole-NH_2)

The synthesis of tetrazole-NH_2 starts with the previously reported synthesis of 4-(2-(4-methoxyphenyl)-2H-tetrazol-5-yl)benzoic acid[1] (Tet-COOH) followed by aminolysis with the amine-linker. N-*boc* amino ethane (7.77 g, 48.5 mmol, 1.60 eq.) was dissolved in DCM (300 mL) and Tet-COOH (9.01 g, 30.4 mmol), EDC-HCl (22.32 g, 116.4 mmol, 3.83 eq.) and DMAP (14.25 g, 116.6 mmol, 3.84 eq.) were added subsequently. The reaction mixture was stirred at ambient conditions overnight. Afterwards, the mixture was concentrated *in vacuo* and dissolved in 400 mL ethyl acetate before washing with saturated $NaHCO_3$ solution (3 × 50 mL), HCl (0.1 M, 3 × 50 mL) and brine (3 × 50 mL). The organic phase was dried over $MgSO_4$ and finally concentrated *in vacuo* to obtain a slightly pink solid, protected tetrazole-NH-Boc (9.84 g, 96% yield). Subsequently, the tetrazole-NH-Boc was dissolved in DCM (100 mL) and cooled to 0 °C before TFA (10 mL) was added dropwise. After 1 h at 0 °C, the deprotection reaction was stirred for 3 h at ambient condition before it was concentrated *in vacuo*. The solid material was suspended in aqueous HCl (0.1M) and finally freeze-dried to remove residues of TFA. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 9.03 (t, J = 5.5 Hz, N*H*), 8.27 – 8.12 (m, 5H, NH_2, 4 × Ar *H*), 8.06 (d, J = 9.1 Hz, 2H, 2 × Ar *H*), 7.21 (d, J = 9.1 Hz, 2H, 2 × Ar *H*), 3.86 (s, 3H, O-CH_3), 3.58 (q, J = 5.9 Hz, 2H, amide-CH_2), 3.03 (q, J = 5.7 Hz, 2H, amine-CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, δ): 165.92 (C=O amide), 163.58 (tetrazole, C4), 160.42 (arom, C4), 135.81 (arom, C4), 129.46 (arom, C4), 128.98 (arom, C4), 128.43 (arom, 2 × C3), 126.33 (arom, 2 × C3), 121.65 (arom, 2 × C3), 115.07 (arom, 2 × C3), 55.69 (O-*C*H$_3$). 38.45 (amide-*C*H$_2$), 37.17 (amine-*C*H$_2$).

Decoration of TEMPO-oxidized cellulose nanofibrils (CNF) with tetrazole-NH_2 in dispersion and in bulk

The oxidation of bleached Kraft pulp by TEMPO/NaOCl/$NaClO_2$ system under neutral conditions was performed according to literature by PTS Hagenau (Dresden, Germany).[2] The resulting content of carboxyl groups is 0.94 mmol g^{-1} and the degree of polymerization (DP_v) is 650. A 1.3 wt% suspension of TEMPO-oxidized Kraft pulp was set to pH = 9 with NaOH and fibrillated by repeated shear cycles in a microfluidizer (Microfluidics corp. MRT CR5, 4 × 1000 bar). For decoration of CNF in dispersion, the gel-like CNF (1 wt%

in water) was diluted to 0.1 wt% in a solvent mixture of water and DMSO (800 mL, final w/w was 1/4) and vigorously stirred to obtain a clear homogeneous dispersion. Tetrazole-NH_2 (0.85 g, 2.26 mmol, 3 eq.) was dissolved in DMSO and added to the dispersion and next DMTMM (0.74 g, 2.26 mmol, 3 eq.) dissolved in water was added dropwise. The reaction mixture was stirred at ambient conditions for 24 h. Afterwards the dispersion was washed multiple times with DMSO and water by centrifugation to remove unreacted and adsorbed reactants. The final concentrated CNF-Tet was stored as aqueous dispersion in the fridge and under light protection until used. For the decoration of CNF bulk films, first, films were prepared by diluting the CNF dispersion (1 wt% in water) to 0.25 wt% (pH = 9) with water followed by film casting in a petri dish to obtain a self-standing film of ca. 20 μm thickness. A specimen with 1.5 cm diameter (3.9 mg, 3.69 μmol carboxyl groups) was punched out of a bigger film and was added to 10 mL of water/DMSO (1/4 w/w) mixture in a vial. Subsequently, tetrazole-NH_2 (4.2 mg, 11.05 μmol, 3 eq.) and DMTMM (3.6 mg, 11.05 μmol, 3 eq.) were added and the reaction was put in an overhead shaker for 24 h. For purification, the specimen was rinsed several times with DMSO and water and finally left for drying in air. Two control experiments were performed to prove covalent binding of the tetrazole-NH_2, to ensure absence of side reactions, and to verify the purification protocol. The first control contained CNF and tetrazole-NH_2, but without DMTMM, the second control contained CNF and DMTMM, but no tetrazole-NH_2.

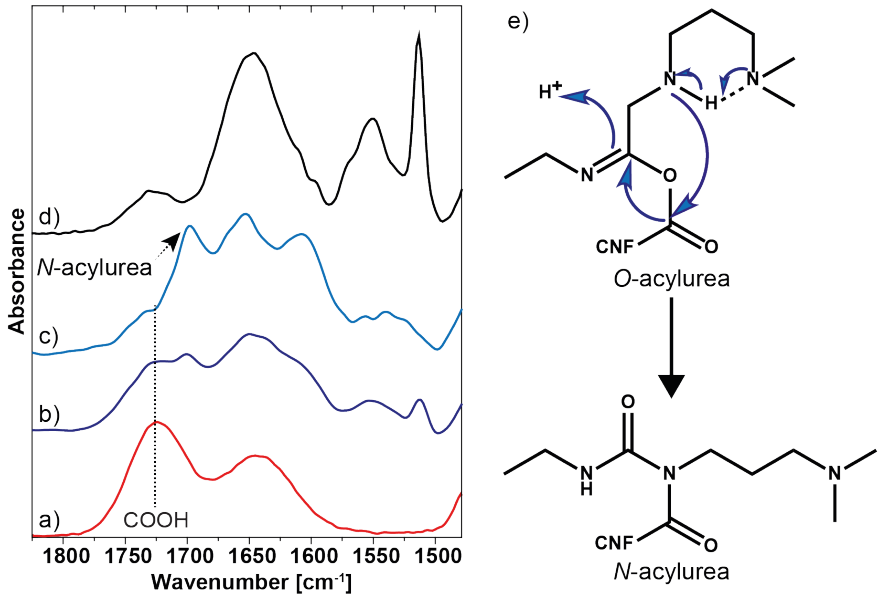

Figure S1: Influence of different coupling chemistries on product purity after exhaustive washing as seen by FTIR. (a) unmodified CNF, (b) modification with tetrazole-NH_2 using EDC/NHS coupling shows amide peaks and contamination with N-acylurea, (c) control experiment without addition of tetrazole-NH_2 demonstrates the side reaction with EDC to N-acylurea functionalized CNFs, (d) pure tetrazole-functionalized CNF as obtained by coupling using DMTMM. The reaction scheme (e) illustrates the undesirable conversion of O-acylurea to N-acylurea via an intramolecular acyl transfer (blue arrows).

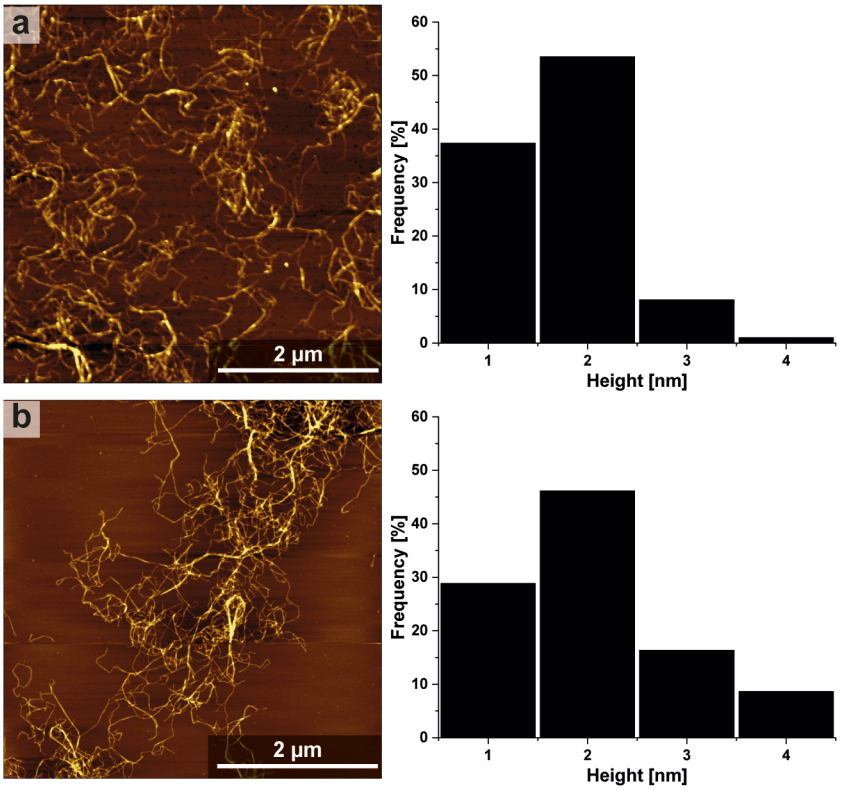

Figure S2: Comparison of AFM height images and corresponding size distribution of CNFs before and after functionalization with tetrazole-NH2. (a) unmodified CNF, (b) tetrazole-functionalized CNF as obtained by coupling using DMTMM (z-scale = 5 nm).

Photo-patterning of biorecognition units on bulk functionalized CNF-Tet films

Exemplary procedure: A solution of PEG-maleimide (M_n = 750 Da) in water (133.3 μM, 2.67 μmol, 20 μL) was added on the photo-active CNF-Tet film and left for 5 min to penetrate the film. Afterwards, a transparent foil with an inkjet printed pattern was put on top as a photomask and the film and the mask were squeezed between two glass slides and placed in the photoreactor for 30 min. After the reaction, the film was rinsed several times with DMSO and water and was dried afterwards. This procedure was repeated in the next step with Biotin-DEG-maleimide by adding again 20 μL (47.6 µM, 0.95 μmol) on the middle of the film followed by 5 min of incubation before the film was placed in the photoreactor without mask for another 30 min. After the reaction, the film was rinsed several times with DMSO and water and was dried. To verify the biorecognition functionality, 20 μL of a PBS buffered (pH = 7.2, 10 mM) solution of Avidin Texas red conjugate (1 mg mL^{-1}) was added to the film and after 5 min of incubation the film was washed several times with PBS buffered solution.

Photo-crosslinking of CNF-Tet in dispersion with sPEG-MI

First, CNF-Tet was diluted to a 0.4 wt% free flowing dispersion in water. CNF-Tet (1 mL, 1.21 μmol tetrazole) was mixed with 8-arm sPEG-MI ($M_{n,star}$ =10 kDa, 30 μL, 2.42 μmol maleimide, 2 eq. compared to tetrazole) for 1 h before it was placed in the photoreactor for 4 h. Solidification and stability in the gel inversion test

(Figure 3b) already works after 30 min, which corresponds to ca. 80% conversion considering the kinetic measurements by UV/Vis (Figure 3c).

Preparation of photo-crosslinkable nanocomposite films from CNF-Tet/sPEG-MI:

First, CNF-Tet was diluted to give a 0.25 wt% free flowing dispersion in water (pH = 9). Appropriate amounts of diluted sPEG-MI polymer were added to 60 mL of the CNF-Tet dispersion under rapid stirring to reach the target compositions. The polymer content in the nanocomposites was 0, 10, 20 and 50 wt%. After 24 h, the dispersions were filtered through a 50 μm pore size mesh sieve to remove dust and afterwards the mixtures were added to Petri dishes and dried to give films of ca. 20 µm thickness. The photo-crosslinking experiments were performed in the photoreactor, leaving the films for 3 h under UV irradiation.

3. Instrumentations

UV/Vis spectroscopy for kinetic studies: Dispersions: The absorption spectra (250 – 650 nm) of the CNF-Tet/sPEG-MI dispersions were recorded on a spectrophotometer (Scandrop, Analytik Jena) using chip cuvettes with 0.1 mm path length. Nanocomposite films: The absorption spectra (250 - 650 nm) of the CNF-Tet/sPEG-MI nanocomposite films were recorded on a spectrophotometer (Ocean Optics, QE Pro) equipped with a balanced deuterium halogen source (Ocean Optics, DH-2000-BAL) using a custom-made film holder.

Photo-reactions: The photo-conjugation experiments were performed in a custom-built photo-reactor equipped with a UV lamp (36 W, Arimed B6, λ_{max} = 305 nm, ca. 6 mW cm^{-2} at 5 cm distance to the lamp).[3]

Atomic force microscopy (AFM): AFM was performed on a MultiMode scanning probe microscope with a Nanoscope V controller (Digital Instruments) under ambient conditions operating in tapping mode. The samples were obtained from a diluted suspension in water (ca. 0.005 wt%) onto freshly cleaved mica.

Scanning electron microscopy (SEM): SEM was performed on a FEI Scios 2 DualBeam instrument after sputter-coating a thin Au/Pd layer.

Fourier Transform Infrared Spectroscopy (FTIR): All FTIR spectra were obtained using a Thermo Nicolet Nexus 470 spectrometer equipped with a smart split ATR single reflection Si crystal using a resolution of 4 cm^{-1}. Samples were studied as acidic form to avoid the superposition of the carboxylate peak with the water peak. For this, 1 drop of 1 M HCl was added to 1 mL of 0.8 wt% CNF-Tet and CNF sample and the dispersion was vigorously mixed and centrifuged and the precipitate was washed three times with 1 mL water, and measured.

Tensile tests: Tensile mechanical properties of the CNF-Tet/sPEG-MI nanocomposites were characterized using a Deben Minitester equipped with a 20 N load cell. The tensile test was performed at a controlled relative humidity of 50 % at 23 °C. Rectangular specimen strips of 12.5 mm in length, 2 mm in width, and a thickness in the range of 20-30 µm were tested at a strain rate of 0.1 mm min^{-1} and a gauge length of 10 mm. 7 specimens were tested for each sample. The Young's modulus was determined from the slope of the initial low strain region of the stress–strain curve.

4. References

1. C. Rodriguez-Emmenegger, C. M. Preuss, B. Yameen, O. Pop-Georgievski, M. Bachmann, J. O. Mueller, M. Bruns, A. S. Goldmann, M. Bastmeyer and C. Barner-Kowollik, *Adv. Mater.*, 2013, **25**, 6123-6127.
2. R. Tanaka, T. Saito and A. Isogai, *Int. J. Biol. Macromol.*, 2012, **51**, 228-234.
3. A. Hufendiek, A. Carlmark, M. A. R. Meier and C. Barner-Kowollik, *ACS Macro Lett.*, 2016, **5**, 139-143.