Electronic Supplementary Information

Mechanical unfolding of fluorescent protein enables self-reporting of damage in carbon-fibre-reinforced composites

Samuel Lörcher,[a] Thomas Winkler,[a] Katarzyna Makyła,[a,b] Claudiane Ouellet-Plamondon,[c] Ingo Burgert[c,d] and Nico Bruns*[a,e]

[a]Department of Chemistry, University of Basel, Klingelbergstrasse 80, 4056 Basel, Switzerland

[b]Department of General Chemistry, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Kraków, Poland

[c]Institute for Building Materials, ETH Zürich, Stefano-Franscini-Platz 3, 8093 Zürich, Switzerland

[d]EMPA – Swiss Federal Laboratories for Materials Science and Technology, Applied Wood Materials, Überlandstrasse 129, 8600 Dübendorf, Switzerland

[e]Adolphe Merkle Institute, University of Fribourg, Rte de l'Ancienne Papeterie, P.O. Box 209, 1723 Marly, Switzerland. E-mail: nico.bruns@unifr.ch; Fax: +41 26 300 9624; Tel: +41 4 26 300 9425

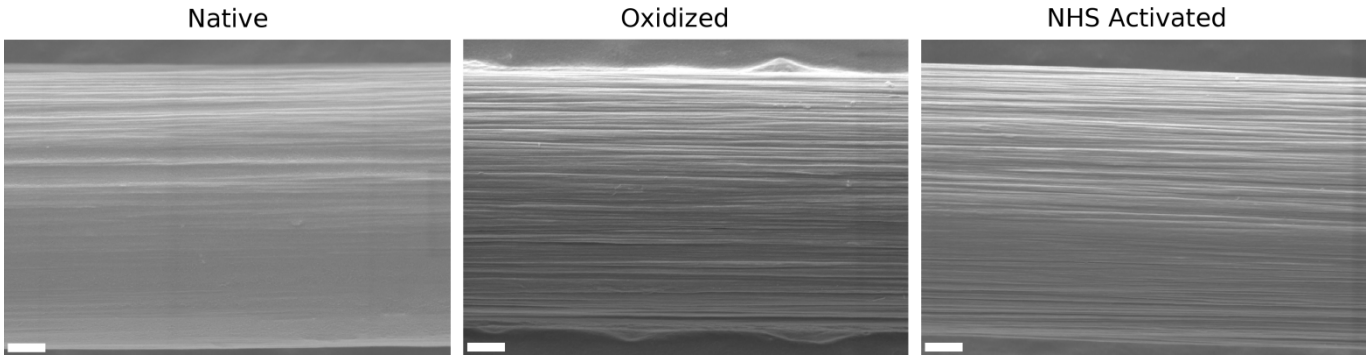

Figure S1: Scanning electron micrographs of a native carbon fibre, a carbon fibre after oxidation by nitric acid and a NHS-activated fibre. The observed increase in surface roughness is attributed to the removal of the fibre's sheath. The NHS activation of the carbon fibre surface is not causing any change in surface topology. Scale bars are 1 µm.

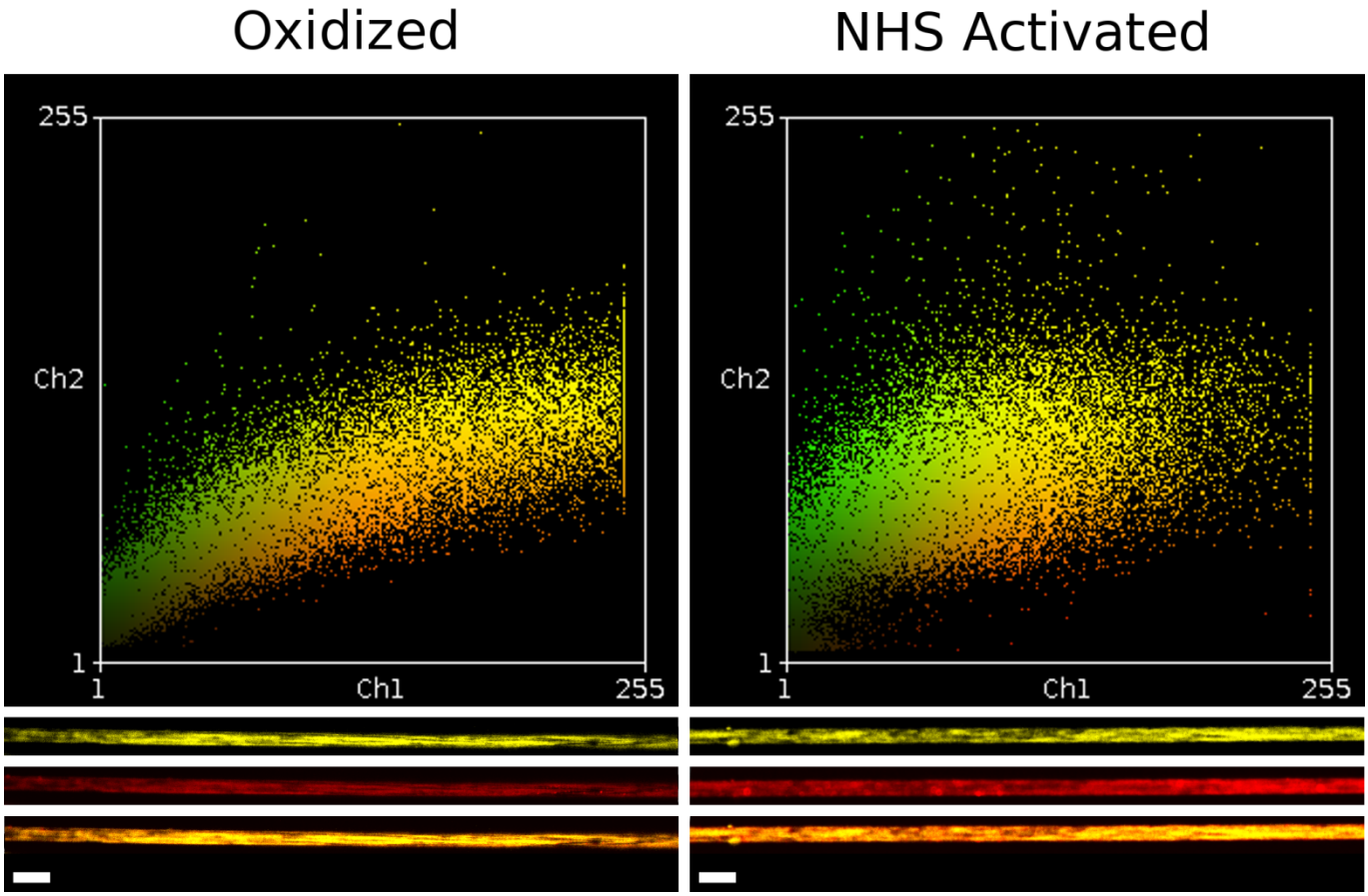

Figure S2: Colocalization analysis for anti-GFP stained eYFP adsorbed to oxidized carbon fibres and bound to NHS-activated carbon fibres, respectively. After image cropping to the size of a single fibre, the corresponding fluorescence information was transformed to a single channel 8-bit gray scale picture. Mander coefficients were calculated using the manders coefficient plugin in ImageJ with CH1 corresponding to eYFP and CH2 corresponding to Alexa 647 labelled anti-GFP fluorescence signal, revealing Manders coefficients of 0.999 and 1.000 on oxidized and NHS-activated fibres, respectively. Below the scatter plots the confocal microscopy images of the analyzed fibres are shown (yellow channel = eYFP; red channel = Alexa 647 labelled anti-GFP; overlay of both channels. Scale bars are 20 µm.).

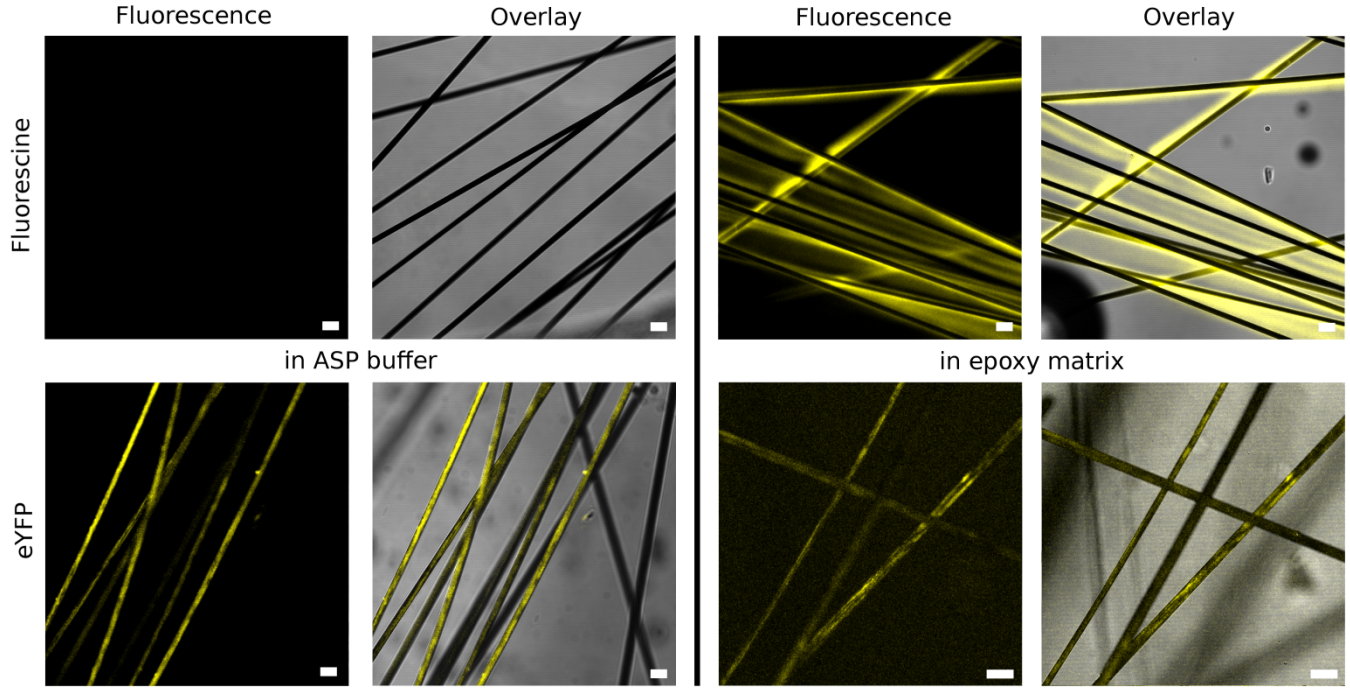

Figure S3: Comparison of fluorescence staining of carbon fibres with aminofluorescein and eYFP. Confocal laser scanning microscopy images of oxidized fibres with physisorbed aminofluorescein and physisorbed eYFP. Images of fibres submerged in ASP buffer and embedded in epoxy resin are shown. For aminofluorescein on carbon fibres no fluorescence signal is observed in buffer, while in epoxy resin a fluorescence halo surrounds the fibres. Fibres that were stained with eYFP fluoresced in buffer and in epoxy resin. In both environments the eYFP fluorescence is localized on the fibres. Scale bars are 20 µm.

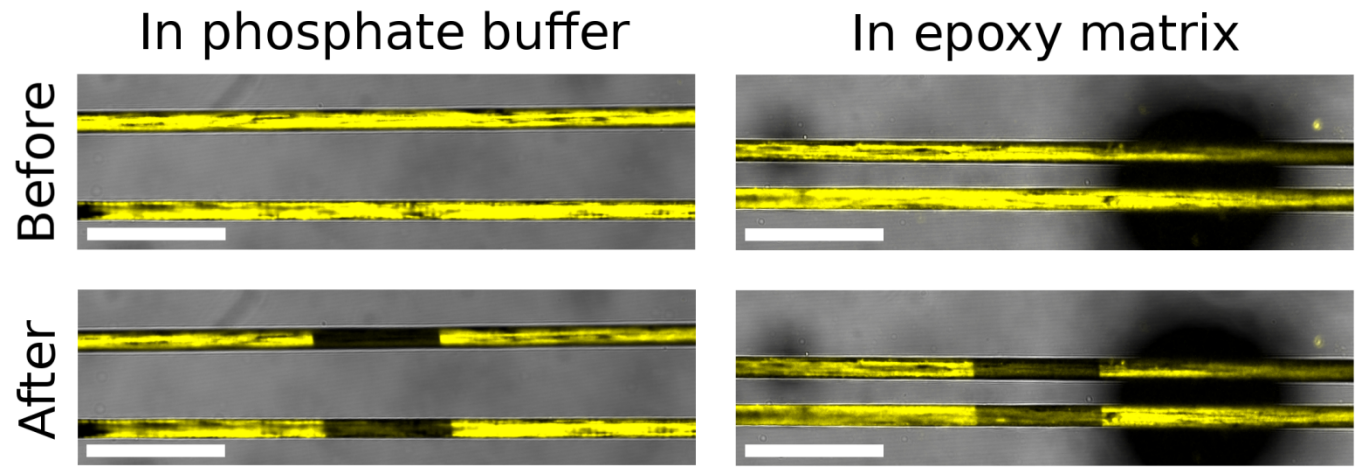

Figure S4: Fluorescence photobleaching experiments conducted on eYFP-modified fibres in phosphate buffer and on fibres embedded in the epoxy resin. The appearance of a dark, non-fluorescent part on the fibres after photobleaching proves the presence of fluorophores on the carbon fibres' surface. Scale bars are 20 µm.

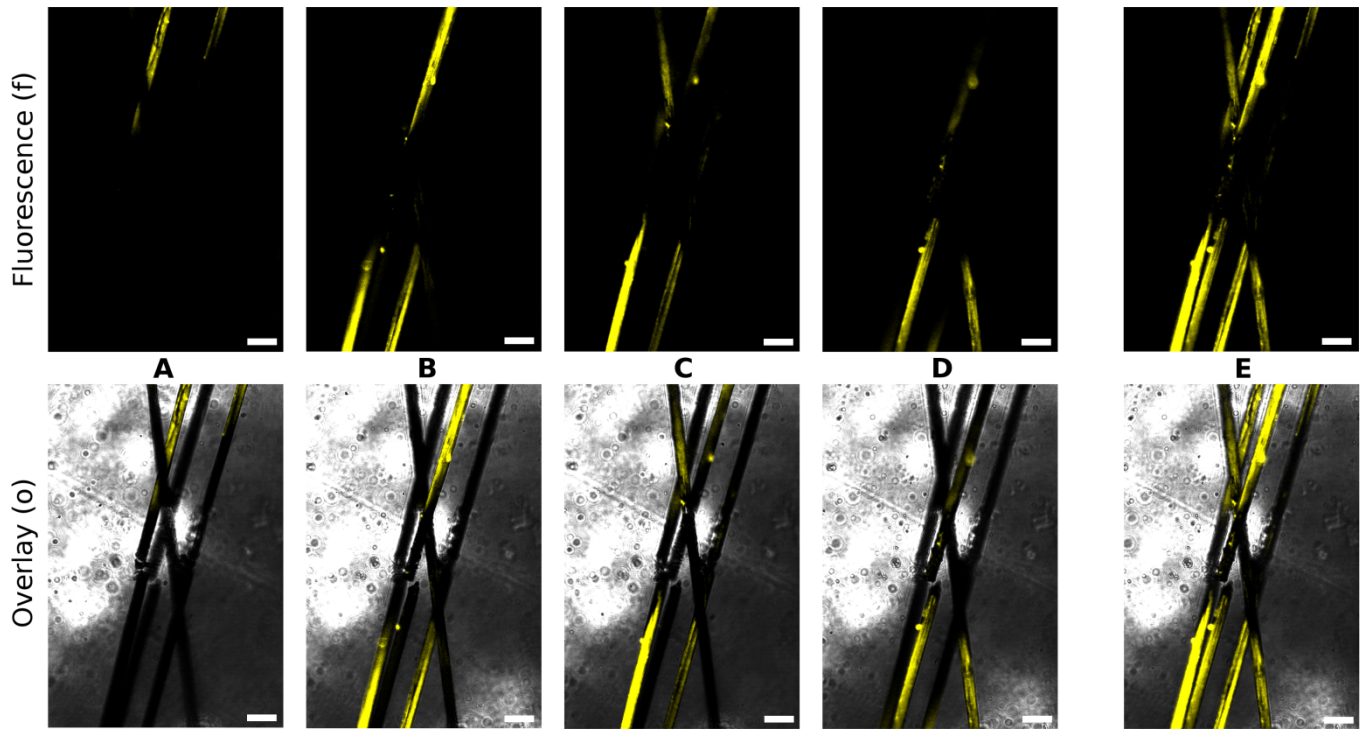

Figure S5: A-D) Confocal laser scanning microscopy images of a site of fibre fractures at several z-planes. The imaged area corresponds to the region that is indicated by a green arrow in Figure 4. E) Z-axis projection of this image stack. Fluorescence (f), and overlay (o) micrographs are shown. The images prove that the fluorescence loss seen in confocal micrographs is not due to out-of-focus signal, but due to a loss of eYFP fluorescence near fibre fractures. Scale bars are 20 µm.