Supporting information of

Elastic and pH responsive hybrid interfaces created with engineered resilin and nanocellulose

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Construction of resilin expression plasmids and T. reesei strains

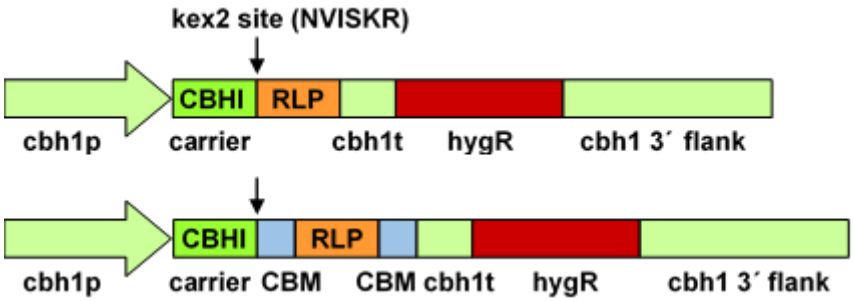

Figure S1. The design of the RLP and CBM-RLP-CBM expression constructs. The expression is driven by the cbh1 promoter (cbh1p) and terminated with the cbh1 terminator (cbh1t). The CBHII CBM is located before the RLP and the CBHI CBM after the RLP polypeptide. The constructs contain an N-terminal His(8)-tag and a C-terminal WSHPQFEK Strep tag. The proteins are expressed as cleavable fusion proteins with CBHI carrier. The fusion protein is cleaved after the NVISKR sequence by the KEX2 protease, residing in late golgi. The expression constructs contain a hygromycin selection marker (hygR) and is targeted to the cbh1 locus with the cbh1p sequence at the 5´end and cbh1 3´flank sequence at the 3´ end.

Table S1. PCR-primers used for screening of *T. reesei* transformants.

Name	Sequence	Use
T095	GCTGTTCCTACAGCTCTTTC	5’ end integration in *cbh1*
T096	AGCCGCACGGCAGC	5’ end integration in *cbh1*
T008	GGTTGACTTACTCCAGATCG	3’ end integration in *cbh1*
T047	CCTATGAGTCGTTTACCCAGA	3’ end integration in *cbh1*
T685	GCCTTTGGGTGTACATGTTTG	*cbh1* ORF
T908	TGGCCAGTCAGCTGGGAGCC	*cbh1* ORF

QCM-D experiments

As shown in Figure S1, the CBM-RLP-CBM has very clear pH responsive properties. The CNF reference surface did not response to different pHs and it was only a little swollen at pH 11. The frequency decreased clearly when the DCBM solution was introduced, indicated the adsorption of DCBM to cellulose surface, but it did not show any pH responsive properties.

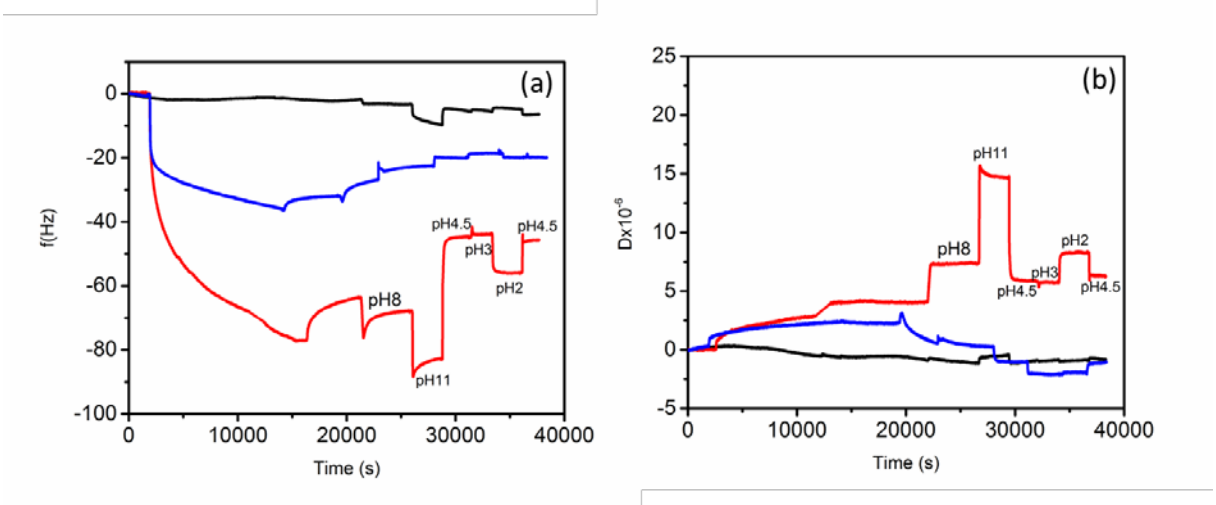

Figure S2. Responsiveness of immobilized CBM-RLP-CBM (red) and DCBM (blue) layer formed at pH 4.5 and exposed to various pH values. The changes of frequency and dissipation were shown in (a) and (b) respectively. The black curve is CNF reference.

The RLP protein without CBMs was used as reference in this work and the results were shown in Figure S2. There is certain amount of RLP adsorbed on the cellulose surface, but a large fraction was desorbed when it was washed with buffer. This confirmed that the adsorption of CBM-RLP-CBM to cellulose is mainly from the CBMs. The original thickness of RLP layer is around 18 nm, which is very close to the CBM-RLP-CBM layer, indicates their conformations are the same.

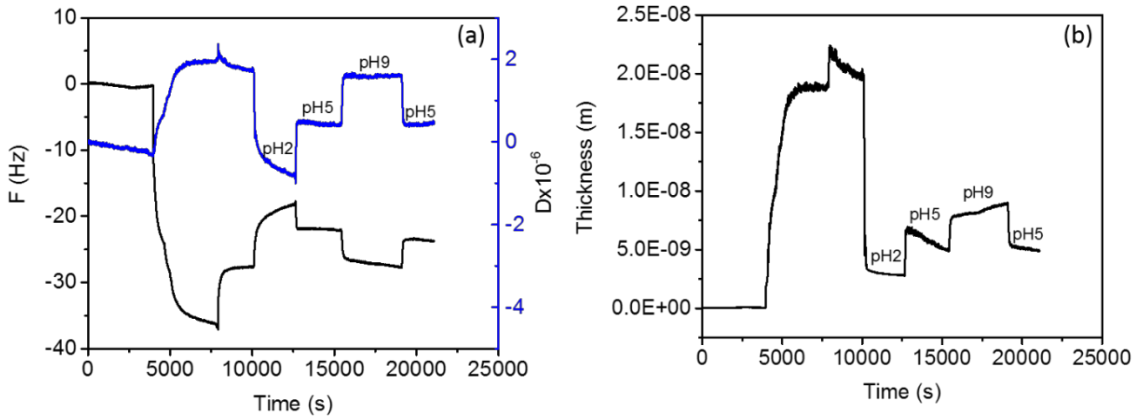

Figure S3. Response of RLP reference protein absorbed on cellulose surface to pH changes (a) and the estimated thickness based on Voigt model (b). The surface was first stabilized with buffer at pH 5 and the protein was then injected in similar buffer.

Size of the proteins

The sizes of the proteins were estimated by calculating the radius of gyration based on the protein sequence.

$$R_G = \sqrt{\frac{Nl}{6}}$$

Where *N* is the number of the amino acids and *l* is the persistence length of the protein chain taken as 0.36 nm.[1] Each module and linker were regarded as individual random coils and the total diameter of the protein was obtained as the sum of the individual modules. The results for the CBM-RLP-CBM and the dCBM proteins are summarized in the Tables S1 and S2. It is good to notice that this estimation is only giving a rough estimate of the size of the proteins, because the CBMs are not random coil conformers but have compact conformation, meaning that their actual size is smaller than estimated here.

Table S2. Estimated size of the CBM-RLP-CBM and the sizes of the modules.

CBM-RLP-CBM	CBM_{CBHII}	RLP	CBM_{CBHI}	strep-tag	Total
N	46.0	322.0	38.0	8.0	414.0
Extended length (nm)	16.6	115.9	13.7	2.9	149.0
$2 \times R_G$ (nm)	3.3	8.8	3.0	1.4	16.5

Table S3. Estimated size of the dCBM and the sizes of the modules.

dCBM	CBM_{CBHII}	linker	CBM_{CBHI}	Total
N	38.0	48.0	36.0	122.0
Extended length (nm)	13.7	17.3	13.0	43.9
$2 \times R_G$ (nm)	3.0	3.4	2.9	9.4

Tensile tests of the nanopapers

With addition of CBM-RLP-CBM, the Young's modulus and yield stress of CNF film increased significantly, but the maximum stain decreased. RLP without CBMs was used as reference. The yield stress of CNF/RLP was much lower than that of the CNF/CBM-RLP-CBM film, which indicates there is no cross linking of the fibrils.

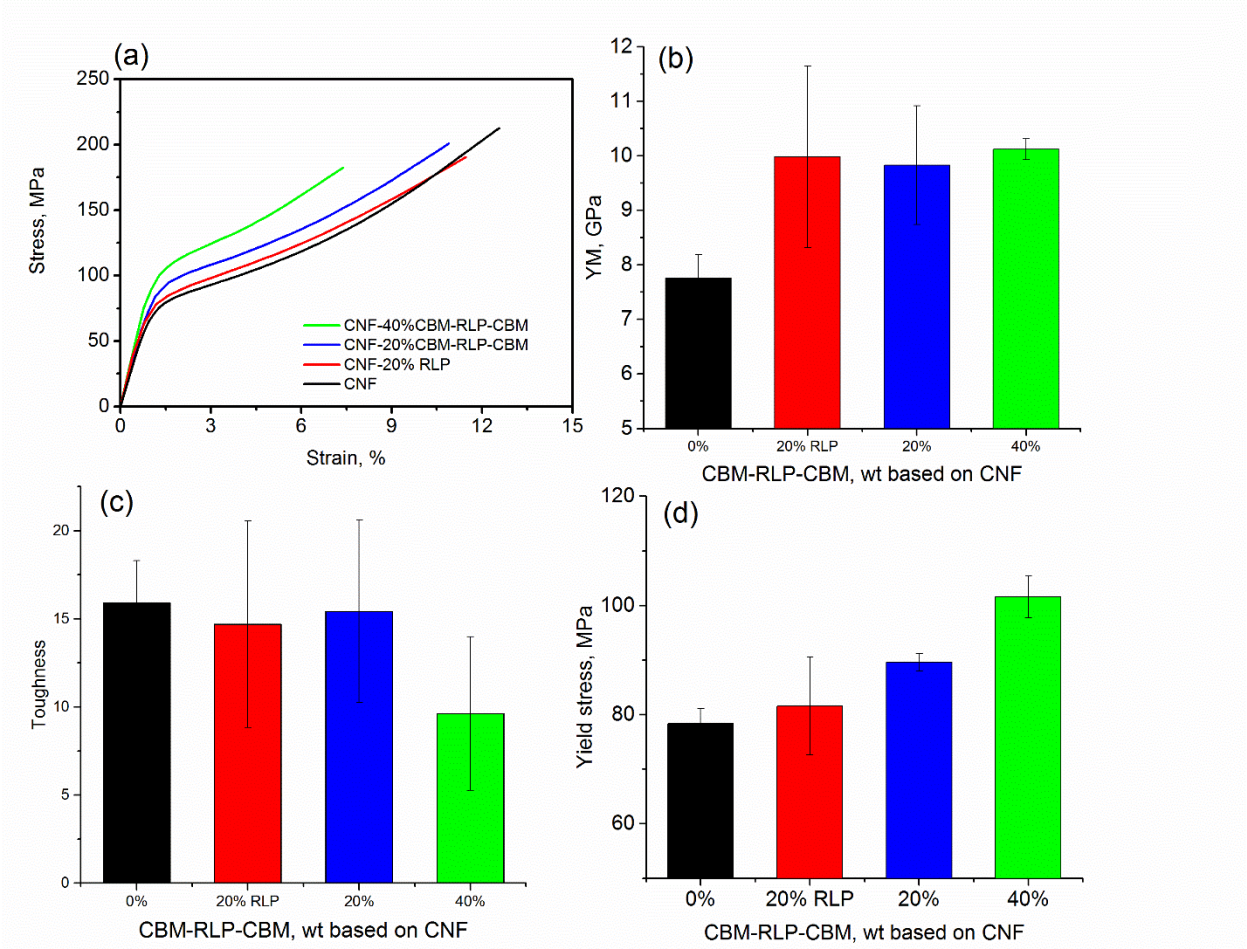

Figure S4. Tensile strain-stress curves of CNF and CBM-RLP-CBM/RLP nanocomposites at 50% RH (a). The Young's modulus, toughness and yield stress values are shown in (b), (c) and (d) respectively.

References

[1] Sbrana F, Lorusso , Canale C, Bochicchio B, Vassalli M, "Effect of chemical cross-linking on the mechanical properties of elastomeric peptides studied by single molecule force spectroscopy", *Journal of Biomechanics*, *44* (2011) 2118–2122.