

Title	Cellulose-based scaffolds for fluorescence lifetime imaging-assisted tissue engineering
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Publication date	2018-09-25
Original Citation	O'Donnell, N., Okkelman, I. A., Timashev, P., Gromovykh, T. I., Papkovsky, D. B. and Dmitriev, R. I. (2018) 'Cellulose-based scaffolds for fluorescence lifetime imaging-assisted tissue engineering', Acta Biomaterialia, 80, pp. 85-96. doi:10.1016/j.actbio.2018.09.034
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.actbio.2018.09.034
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## Cellulose-based scaffolds for fluorescence lifetime imaging-assisted tissue engineering

Neil O'Donnell<sup>1\*</sup>, Irina A. Okkelman<sup>1\*</sup>, Peter Timashev<sup>2,3</sup>, Tatyana I. Gromovykh<sup>4</sup>, Dmitri B. Papkovsky<sup>1</sup>, Ruslan I. Dmitriev<sup>1,2</sup>

<sup>1</sup> School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland.

<sup>2</sup> Institute for Regenerative Medicine, I.M. Sechenov First Moscow State University, Moscow, Russian Federation

<sup>3</sup> Institute of Photonic Technologies, Research Center 'Crystallography and Photonics, Russian Academy of Sciences, Moscow, Russian Federation

<sup>4</sup> Department of Biotechnology, I.M. Sechenov First Moscow State University, Moscow, Russian Federation

### Supplementary info

#### Part 1. Nucleotide sequences produced during cloning:

```
atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATACCAACCTCGGCGACCCCGTCTCGTCTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCCGCTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
AACGGCAGCATCCCGACCGGCGGCACGGCGTCTGTTCCGGTTCAACGGCTCGTGGGCCGGGTCCAACCCGACG
CCGGCGTC
GTTCTCGTCAACGGCACCCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtacccccAGCTT
```

**CBDA(FTN)** in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

```
atgagaggatcgcatcaccatcaccatcacggatccGCTCCCGGCTGCCGCGTCGACTACGCCGTCACCAACCAGTGGCC
CGGCGGCTTCGGCGCCAACGTCACGATACCAACCTCGGCGACCCCGTCTCGTCTGGAAGCTCGACTGGAC
CTACACCG
CAGGCCAGCGcATCCAGCAGCTGTGGAACGGCACCCGCTCGACCAACGGCGGCCAGGTCTCCGTCACCAGCC
TGCCCTGG
AACGGCAGCATCCCGACCGGCGGCACGGCGTCTGTTCCGGTTCAACGGCTCGTGGGCCGGGTCCAACCCGACG
CCGGCGTC
GTTCTCGTCAACGGCACCCACCTGCACGGGCACCGTGCCGACGACCAGCCCCACGggtaccGGTACAGGTGGTT
CTGGTG
GTGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCCATCTGGTTCGAGCTGGACGGCGACGTAAACG
GCCACAAG
TTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACC
GGCAAGCT
GCCCCGTGCCCTGGCCACCCTCGTGACCACCCTGACCTGGGGCGTGACGTGCTTACGCCGCTACCCCGACCA
CATGAAGC
AGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGG
CAACTAC
AAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTC
AAGGAGGA
CGGCAACATCCTGGGGCACAAGCTGGAGTACAACCTACATCAGCCACAACGTCTATATACCCGCCGACAAGCAG
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GAACACC
CCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGAC
CCCAACGA
GAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC
AAGTCCG
GACTCAGATCTCGAGCTCAgGCTTCGAATTCTGCAGTCGACGGcACCGCGGGCccAggaTcTACCGGATCTAGAggt
acc
cccAGCTT
```

**CBD(G)-ECFP** in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWYTAGQRIQLWNGTASTNNGQVS  
VTSLPW  
NGSIPTGGTASFGFNWSWAGSNPTPASFSLNGTTCTGTVPPTSPTGTGTGGSGG VSKGEELFTGVVPILVELDGDVN  
GHK  
FSVSGEGEGDATYGLTLKFICTTGKLPVPWPTLVTTLTWGVQCFSTRYPDHMKQHDFFKSAMPEGYVQERTIFFKDD  
GNY  
KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNIYSHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQNT  
PIGDGPVLLPDNHYLSTQSALSQDPNEKRDHMLLEFVTAAGITLGMDELYKSGLSRAQASNSAVDGTAGPGSTGS  
RGT  
PS

Primary structure of **CBD(G)-ECFP protein**. 402 aa, 43,1 kDa, pI 6.6. Gly-rich linker is highlighted in yellow.

atgagaggatcgcaccatcaccatcaccggtaccGCTCCCGGCTGCCGCGTCTGACTACGCCGTCACCAACCAGTGGCC  
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TGCCCTGG  
AACGGCAGCATCCCGACCGGCGGCACGGCGTCTGTTCCGGTTCAACGGCTCGTGGGCCGGGTCCAACCCGACG  
CCGGCGTC  
GTTCTCGTCAACGGCACCCACTGCACGGGCACCGTCCGACGACCCAGCCCCACGgggtaccGGTACAGGTGGTT  
CTGGTG  
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CATGGTC  
GACTCATACGTCGTAAGTGAATAAGACAGGTCACGCAGTCAGAGCTATAGGTCCGGCTGAGCTCACTCGAGAA  
CGTCTA  
TATCATGGCCGACAAGCAGAAGAACGGCATCAAGGCGAAGTTCAGATCCGCCACAACATCGAGGACGGCGGC  
GTGCAGC  
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CACCCAG  
TCCAACTTTTCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGA  
TCACTCT  
CGGCATGGACGAGCTGTACAAGGGCGGcACCGGAGGGAGCATGGTGAGCAAGGGCGAGGAGCTGTTACCCGG  
GGTGGTGC  
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TGACCTA  
CGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCGAA  
GGCTACA  
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CACCCTG  
GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACA  
ACACGCG  
TGACCAACTGACTGAAGAGCAGATCGCAGAATTTAAAGAGGCTTTCTCCCTATTTGACAAGGACGGGGATGGGA  
CAATAA  
CAACCAAGGAGCTGGGGACGGTGTGCGGTCTCTGGGGCAGAACCCACAGAAGCAGAGCTGCAGGACATGA  
TCAATGAA  
GTAGATGCCGACGGTAATGGCACAATCGACTTCCCTGAGTTCCTGACAATGATGGCAAGAAAAATGAAAGACAC  
AGACAG  
TGAAGAAGAAATTAGAGAAGCGTTCCTGTGTTTGATAAGGATGGCAATGGCTACATCAGTGCAGCAGAGCTTC  
GCCACG  
TGATGACAAACCTTGGAGAGAAGTTAACAGATGAAGAGGTTGATGAAATGATCAGGGAAGCAGACATCGATGGG  
GATGGT  
CAGGTAAACTACGAAGAGTTTGTACAAATGATGACAGCGAAGgggtacccccAGCTT

**CBD(G)-GCaMP2** in pQE-30 vector (indicated in grey). *Kpn* I site used for cloning fusions and linker peptide is indicated in yellow.

MRGSHHHHHHGSAPGCRVDYAVTNQWPGGFGANVTITNLGDPVSSWKLDWYTAGQRIQLWNGTASTNNGQVS  
VTSLPW  
NGSIPTGGTASFGFNWSWAGSNPTPASFSLNGTTCTGTVPPTSPTGTGTGGSGG MASMTGGQQMGRDLYDDDDK  
DLATMV  
DSSRRKWNKTGHAVRAIGRLSSENVYIMADKQKNGIKANFKIRHNIEDGGVQLAYHYQQNTPIGDGPVLLPDNHYLS  
TQ  
SKLSKDPNEKRDHMLLEFVTAAGITLGMDELYKGGTGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGD  
ATY  
GKLTALKFICTTGKLPVPWPTLVTTLTWGVQCFSTRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKTRAEVKFEGD  
TL  
VNRIELKGIDFKEDGNILGHKLEYNTRDQLTEEQIAEFKEAFSLFDKDGDTITTKELGTVMRSLGQNPTAEALQDMIN  
E  
VDADGNGTIDFPEFLTMMARKMKDTSSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDG  
DG

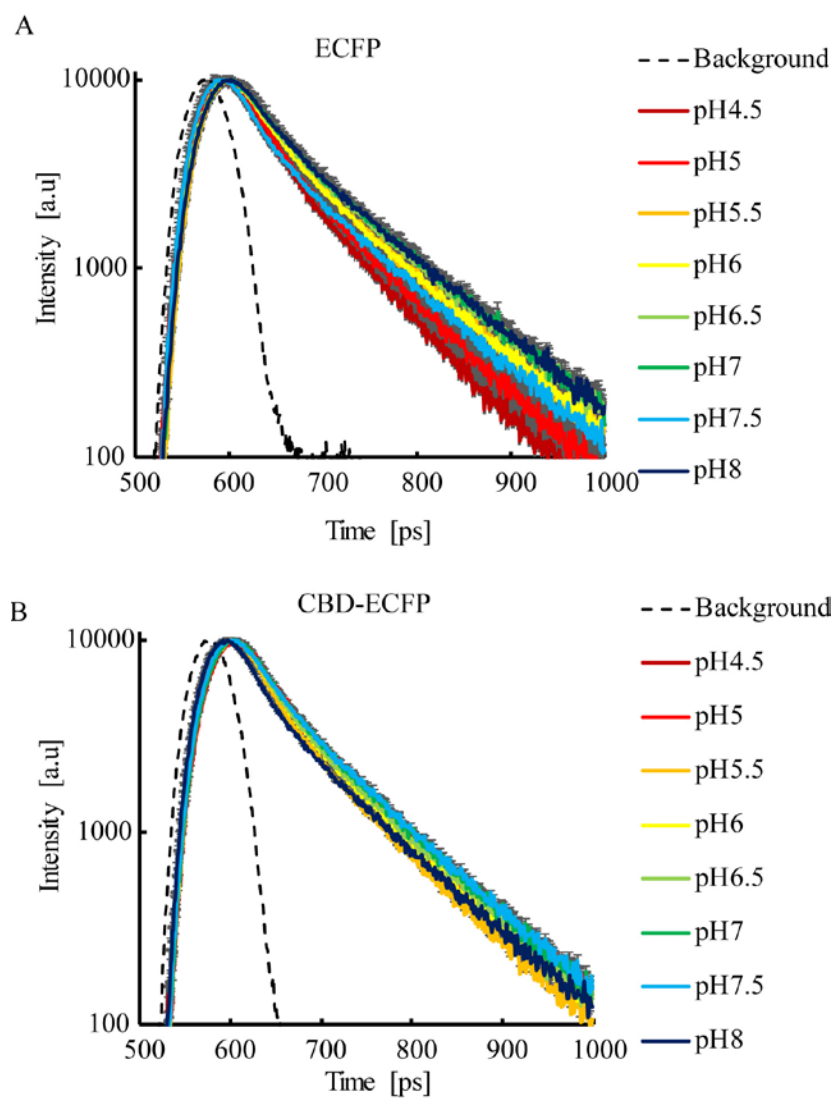
QVNYEEFVQMMTAKGTPS

Primary structure of **CBD(G)-GCaMP2** protein. 578 aa, 63,2 kDa, pI 4.9. Gly-rich linker is highlighted in yellow.

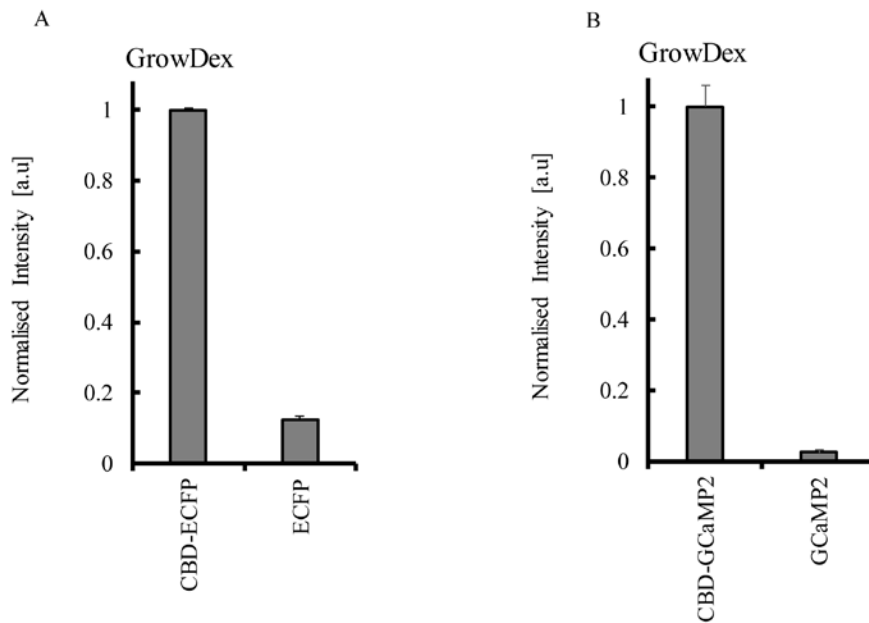
**Table S1.** Primer pairs used for cloning of ECFP and GCaMP2.

ECFP (no linker peptide)	Forward: 5'-GATCGGTACCGTGAGCAAGGGCGAGGA Reverse: 5'-GATCGGTACCTCTAGATCCGGTAGATCCT
GCaMP2 (no linker peptide)	Forward: 5'-GATCGGTACCATGGCTAGCATGACTGGTGGAC Reverse: 5'-GATCGGTACCCTTCGCTGTCATCATTGTA
ECFP (with linker peptide GTGGS GG)	Forward: 5'- GATCGGTACCGGTACAGGTGGTTCTGGTGGTGTGAGCAAGGGCGAGGA Reverse: 5'-GATCGGTACCTCTAGATCCGGTAGATCCT
GCaMP2 (with linker peptide GTGGS GG)	Forward: 5'- AGTCGGTACCGGTACAGGTGGTTCTGGTGGTATGGCTAGCATGACTGGT Reverse: 5'-GATCGGTACCCTTCGCTGTCATCATTGTA

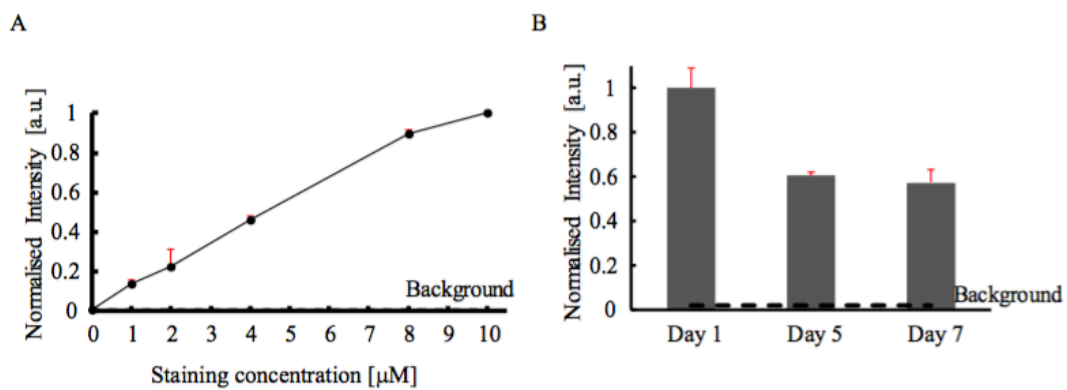
## Part 2. Supplementary figures



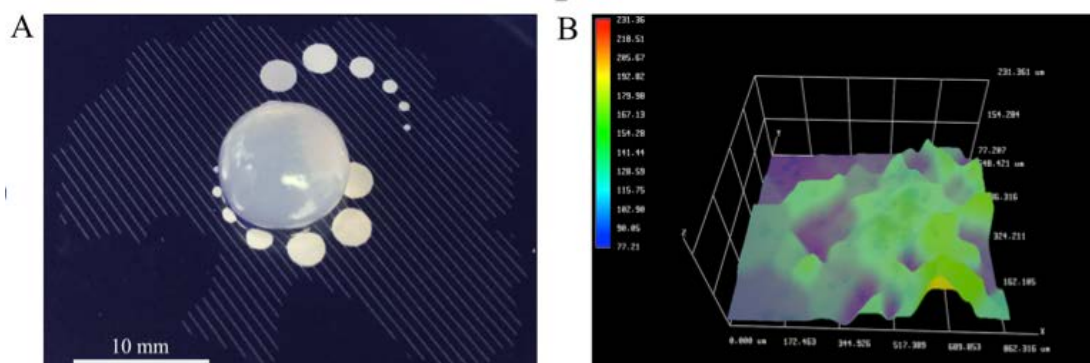
**Figure S1.** Fluorescence lifetime decays for ECFP and CBD-ECFP proteins measured in solution at different pH. A: Observed decay times for ECFP were 1.35 ns (pH 4.5) and 2 ns (pH 8). B: Observed decay times for CBD-ECFP were 1.89 ns (pH 4.5) and 2.18 ns (pH 8). Standard deviations are shown in gray. Prompt (background) fluorescence signals are also shown. N=3.



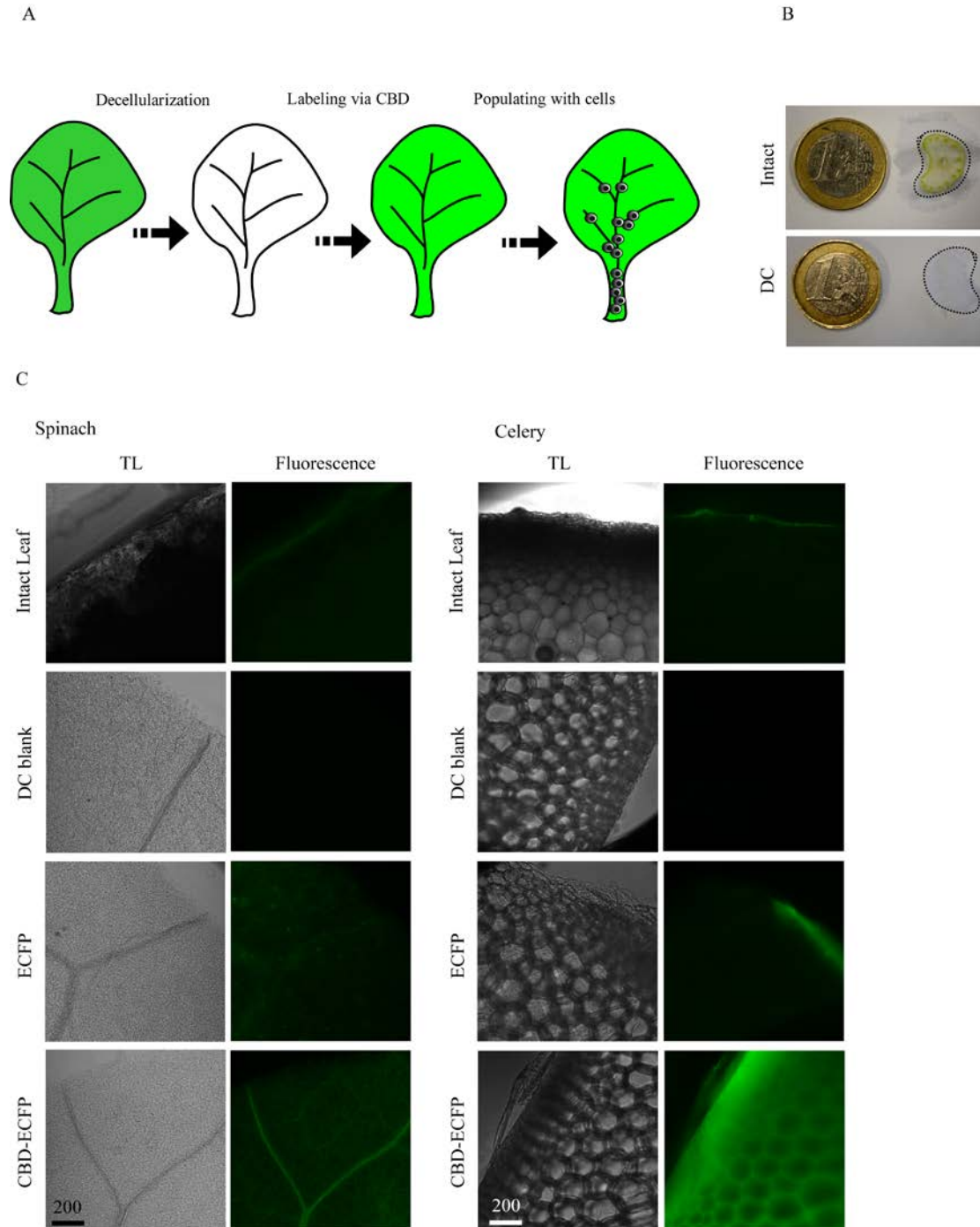
**Figure S2.** Comparison of specific / non-specific staining of GrowDex with CBD-ECFP (A) and CBD-GCaMP2 proteins (B) compared to untagged ECFP and GCaMP2 proteins. Matrices were incubated with 10  $\mu\text{M}$  of respective proteins for 15 min in PBS, washed and measured on fluorescence microscope (470 nm exc., 510-560 nm em.). N=3.



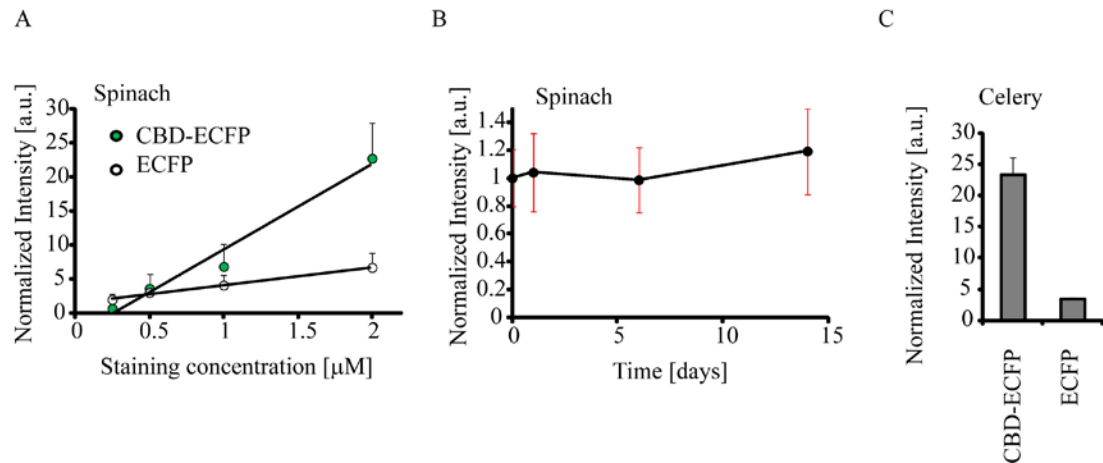
**Figure S3.** A: Concentration-dependent staining of GrowDex with CBD-GCaMP2 (incubation time 15 min, in PBS). B: Stability of stained (5  $\mu\text{M}$ , 15 min) GrowDex scaffold. Matrices were measured on a fluorescence microscope (470 nm exc., 510-560 nm em.), using the same acquisition settings for different experimental points. N=3.



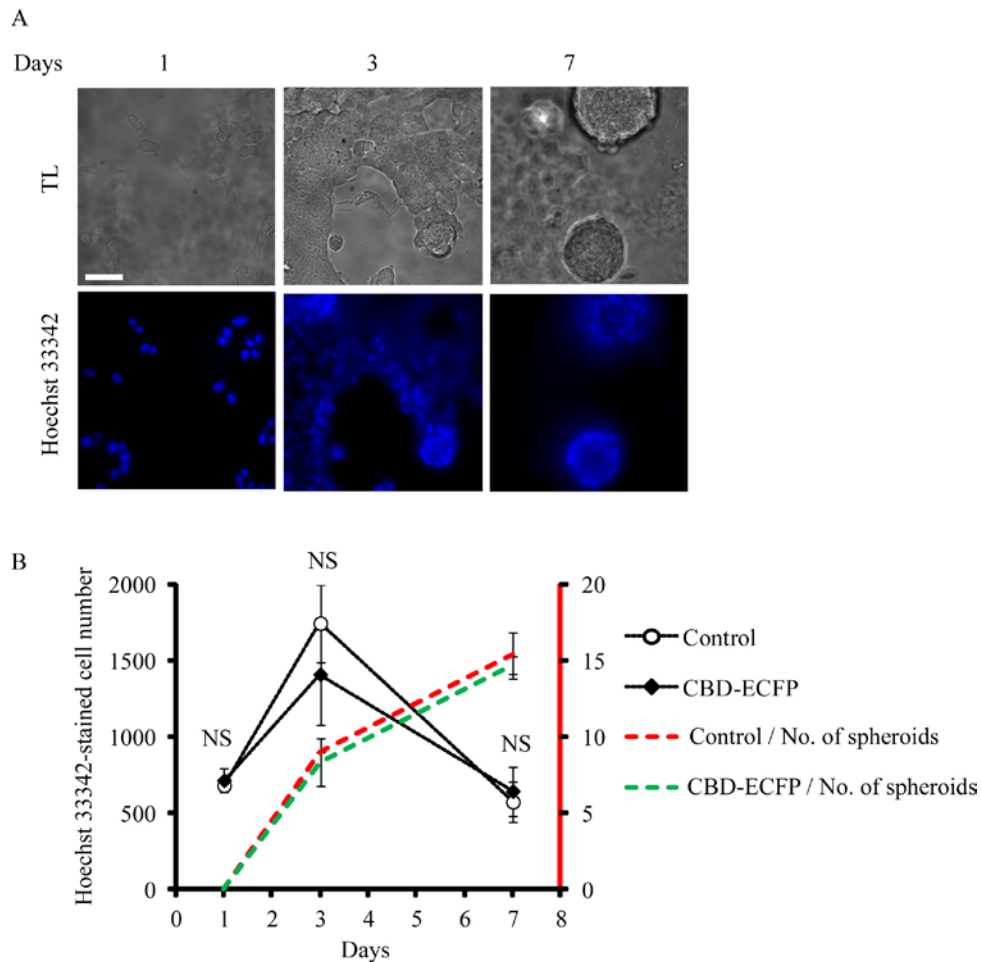
**Figure S4.** Characterization of bacterial cellulose. A: Photograph of cellulose sphere produced by *Gluconacetobacter hansenii* GH-1/2008 (VKPM B-10547). B: 3D microscopy and nanoindentation reveals homogenous surface of the bacterial cellulose. Young's modulus is  $1.93 \pm 0.76$  kPa.



**Figure S5.** Overview of plant decellularization (DC) procedure and studied examples. A: cartoon representation of the use of DC plant for cell-based application with CBD-tagged biosensors. B: Photographs of celery stem section before and after decellularization. 1 euro coin is shown for scale. C: Transmission light and widefield fluorescence (470 nm exc., 510-560 nm em.) microscopy images for spinach leaf and celery stem before (intact), after DC and following labeling either with ECFP (non-specific binding) or CBD-ECFP. The same acquisition settings were used for fluorescence images. Scale bar is in  $\mu\text{m}$ .



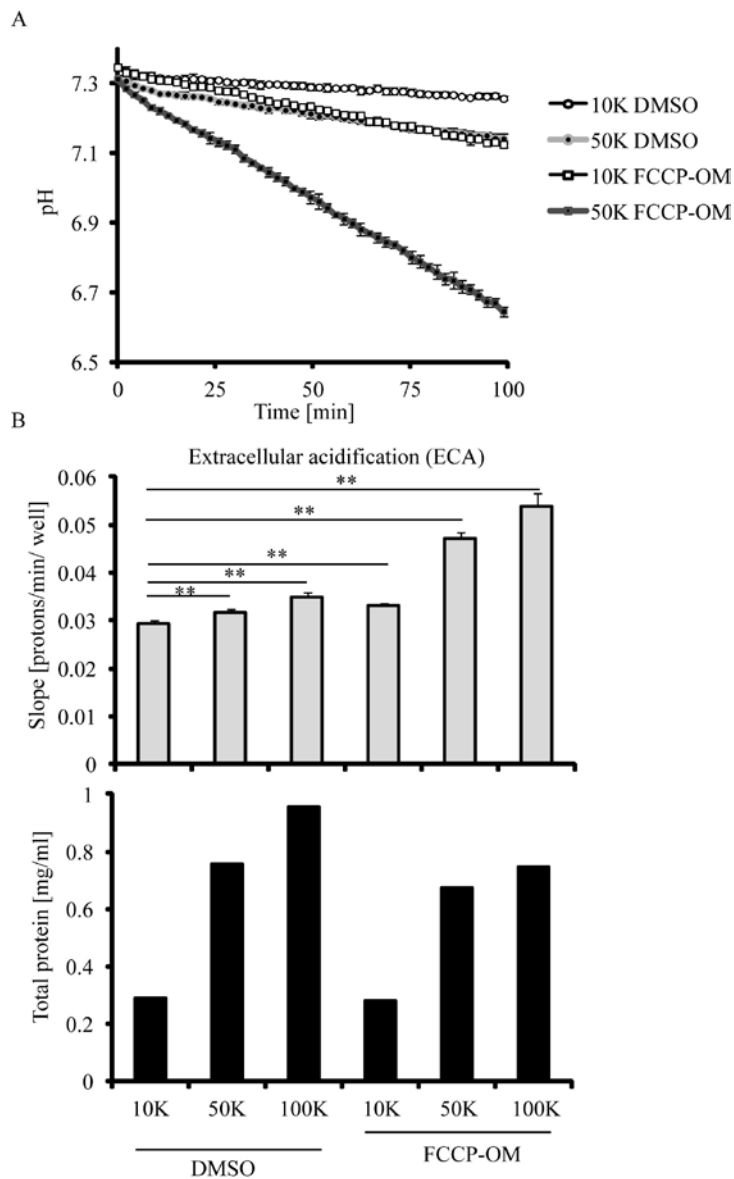
**Figure S6.** Staining and stability of decellularized (DC) celery and spinach. DC samples were stained with various concentrations of CBD-ECFP or ECFP and measured on a fluorescence microscope (470 nm exc., 510-560 nm em.). A: Concentration-dependence of staining of DC spinach (16 h incubation in PBS). B: Storage stability of stained DC spinach (5 μM, 16 h) over 2 weeks time. C: Staining of DC celery with CBD-ECFP (5 μM, 16 h). N=3.



**Figure S7.** Cell growth in unstained and CBD-ECFP-stained Growdex. HCT116 cells were seeded in stained (5 μM CBD-ECFP, 15 min) or unstained (control) Growdex matrices, grown for 0-7 days and quantified by using fluorescence microscopy with the help of Hoechst 33342 (1 μM, 30 min) staining. A: Fluorescence microscopy images showing cell growth (CBD-ECFP-stained sample). TL-transmission light. Scale bar is 50 μm. B: Calculated cell



numbers (per region of interest) for different time points of culture in Growdex. No significant differences (indicated as 'NS') between CBD-ECFP-stained and unstained samples were found for mean values ( $t$ -test,  $p=0.05$ ). Cells grown in Growdex cells also formed spheroids over time (shown as green and red dashed lines, red Y axis).  $N=3$ .



**Figure S8.** Dependence of extracellular acidification rates from cell number, measured on a microplate reader by ECA method. HCT116 cells seeded and grown at different densities (10,000, 50,000 and 100,000 designated as 10K, 50K and 100K, respectively) were measured by ECA method (unsealed system, glycolytic flux) in the presence of 1  $\mu$ M FCCP and 10  $\mu$ M Oligomycin (FCCP-OM, maximal acidification) and DMSO (mock). A: time-dependence of extracellular acidification. B: extracellular acidification rates (top) and average total protein concentrations (bottom).  $N=8$ . Asterisks indicate significant difference ( $t$ -test,  $p=0.001$ )