**Recombinant peptide production softens *Escherichia coli* cells and increases their size during C-limited fed-batch cultivation**

**Supplementary materials**

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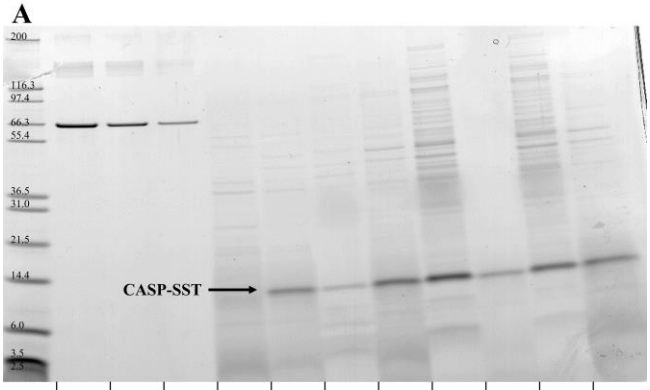
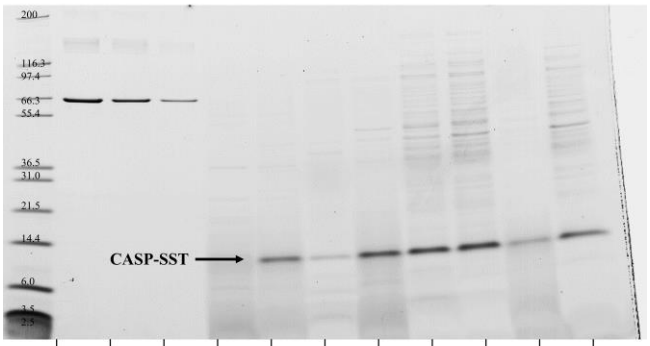
**A**



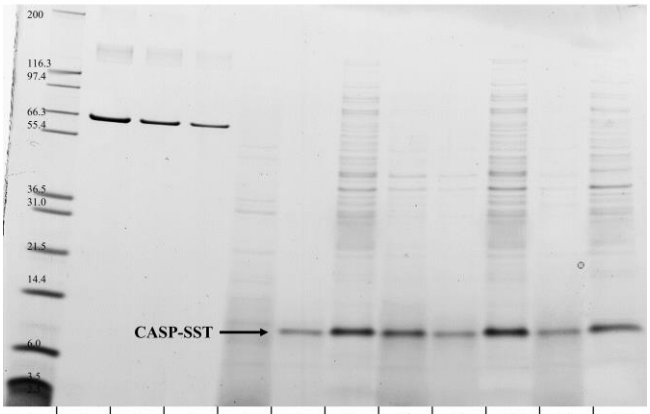

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | BSA 75 | BSA 50 | BSA 25 | IC 3h | IC 11h | EC 11h | IC 19h | EC 19h | IC 23h | EC 23h | IC 27h |
| Dilution | n.d. | n.d. | n.d. | n.d. | 1:2 | 1:5 | 1:2 | 1:12 | 1:4 | 1:12 | 1:2 |

**B**




| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | BSA 75 | BSA 50 | BSA 25 | IC 3h | IC 11h | EC 11h | IC 19h | EC 19h | EC 27h | IC 31h | EC 31h |
| Dilution | n.d. | n.d. | n.d. | n.d. | 1:2 | 1:5 | 1:2 | 1:12 | 1:12 | 1:2 | 1:15 |

**C**




| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | BSA 75 | BSA 50 | BSA 25 | IC 3h | IC 23h | EC 23h | IC 27h | IC 27h | EC 27h | IC 31h | EC 31h |
| Dilution | n.d. | n.d. | n.d. | n.d. | 1:4 | 1:12 | 1:2 | 1:4 | 1:12 | 1:2 | 1:15 |

**Figure S1.** SDS-PAGE gels (panels **A–C**) for quantification of intracellular (IC) and extracellular (EC) peptide content. All samples were analysed at least twice. 3 h samples represent uninduced reference samples. CASP-SST bands (black arrow) were quantified via ImageQuant™ using BSA standards (lanes

2-4, 75-25 µg mL$^{-1}$). Samples were diluted to fit the linear range of the standard, and non-diluted samples are indicated as n.d.

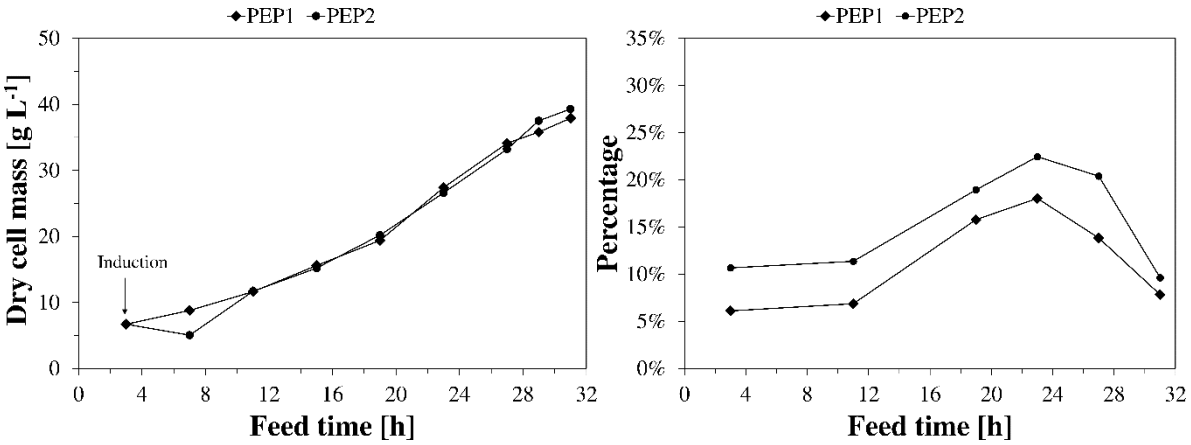



**Figure S2:** Growth curves (**A**) and cell lysis curves (**B**) of duplicate cultivations with PEP. Minor deviations in DCM (e.g. after 7 h) likely arise from errors during sample preparation. Cell lysis variation might arise from differences in timepoint of sample preparation and DNA measurement (DNA instability).

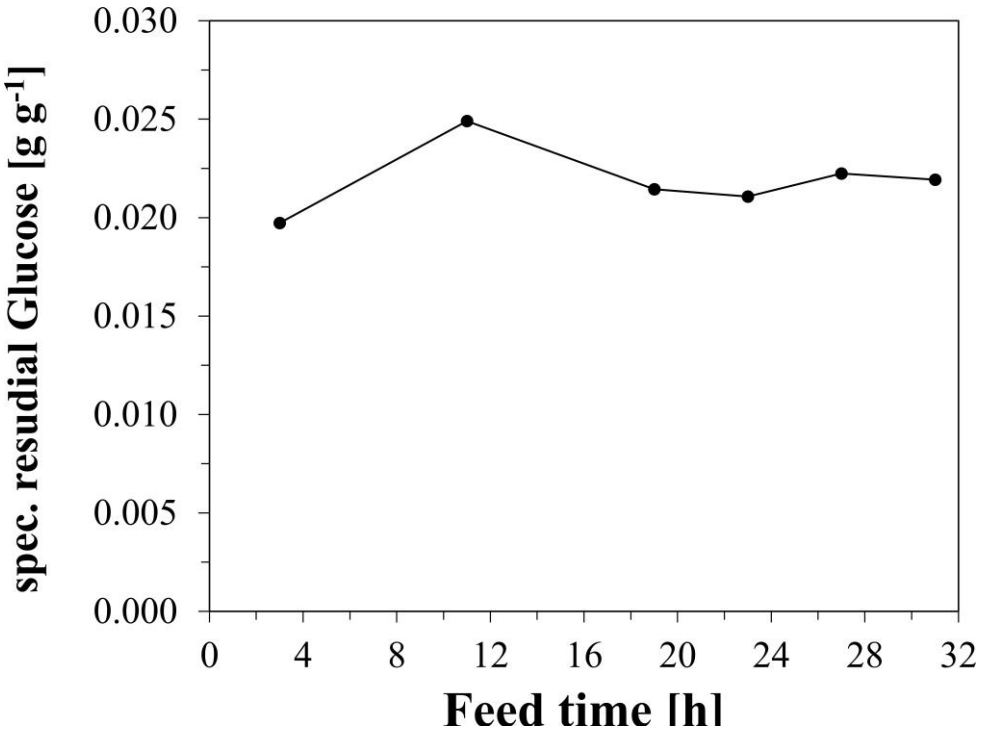



**Figure S3.** Specific residual glucose concentration in the cell-free cultivation medium of PEP. Glucose concentrations were analysed using HPLC as previously described by Marisch et al., 2013 (https://doi.org/10.1186/1475-2859-12-58).

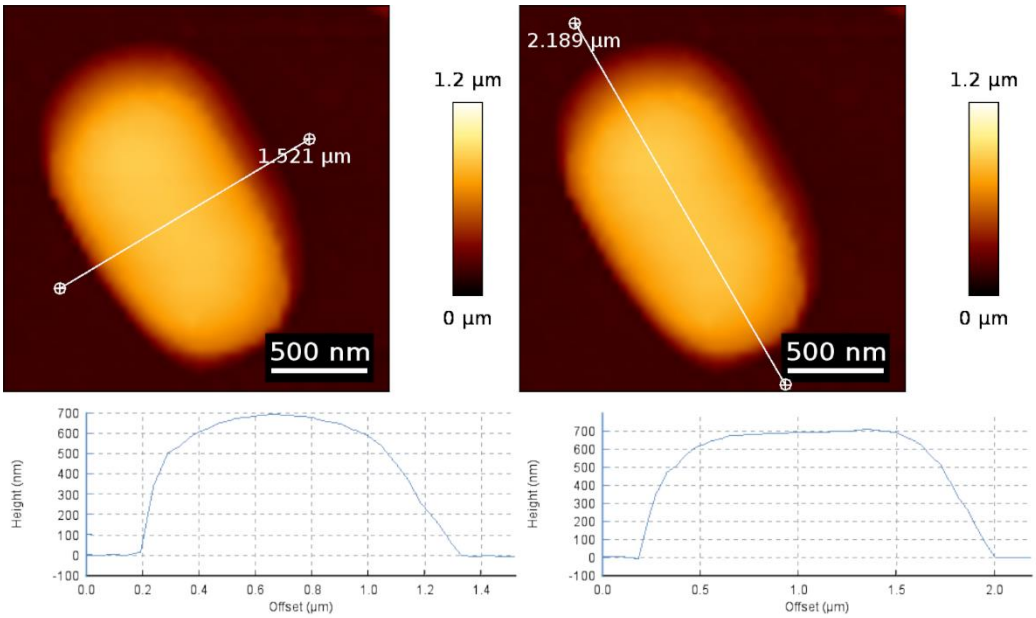



**Figure S4.** Analysis of cell width (**left**) and cell length (**right**) from AFM images.

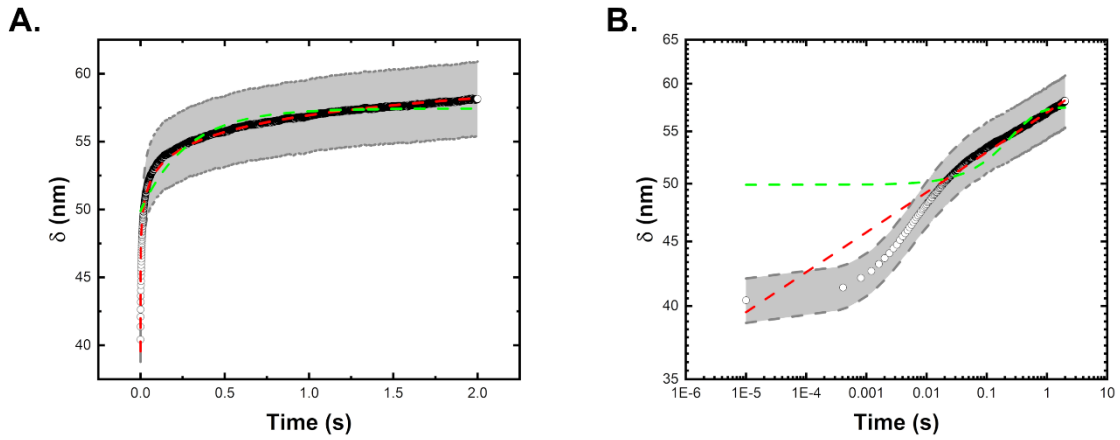



**Figure S5.** Performance of fitting procedures for creep curves (red power law, green standard linear solid) in normal representation (**A**) and logarithmic representation (**B**).

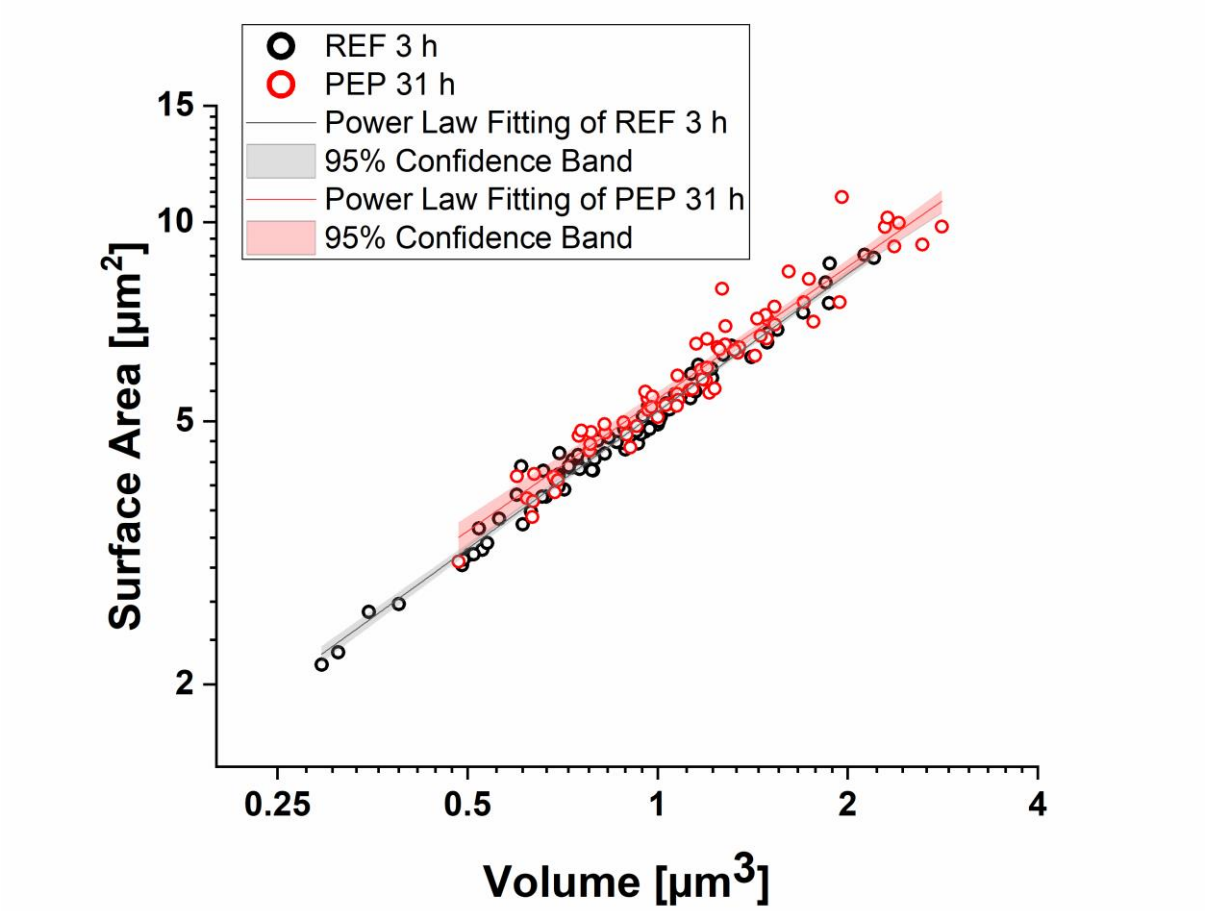



**Figure S6.** Surface area to volume scaling is conserved for the different samples. Here shown is REF 3h and PEP 31 h.

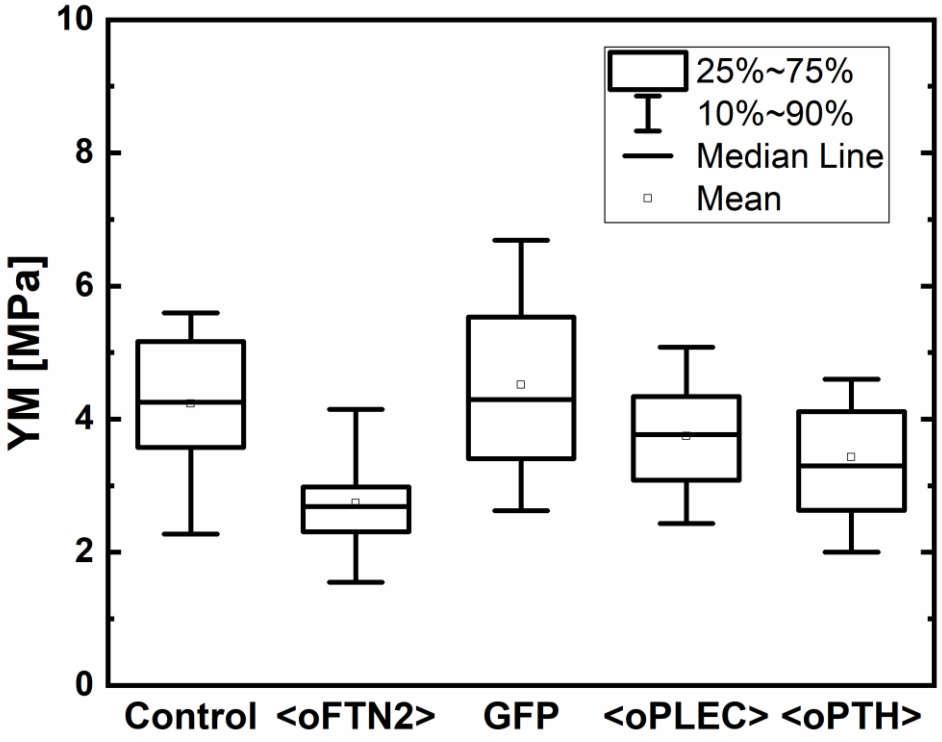



**Figure S7.** Apparent Young's Modulus of BL21(DE3) cells expressing recombinant Fab fragments (FTN2), green fluorescent protein (GFP), antimicrobial peptide plectasin (PLEC), and parathyroid hormone (PTH). Measurements of BL21(DE3) cells without any protein/peptide production are indicated as control. Brackets (< >) indicate genomic integration of the recombinant gene into the host. Translocation into the periplasmic space via the OmpA signal sequence of the respective protein/peptide is indicated as "o". Respective cultivations were carried out as described in the material and method section.