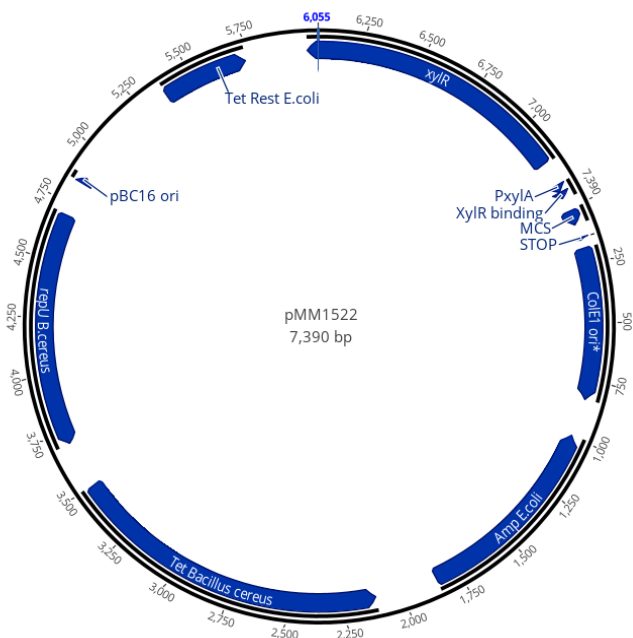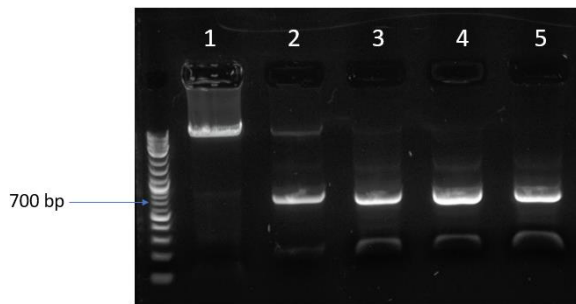# Supplemental data

A. pMM1522 Plasmid map and xylose-inducible adhesion constructs

Table A. Xylose- Inducible Adhesion Constructs.

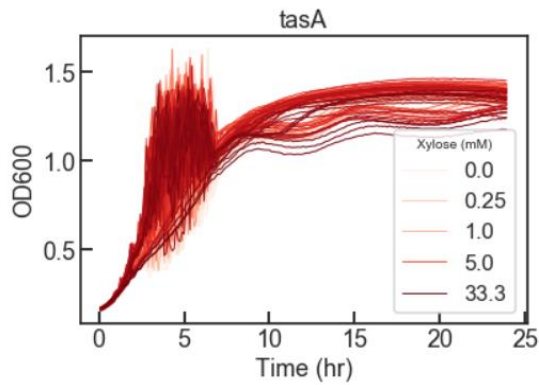| Construct name	Description	Transformed into *E. coli*	Transformed into *B. megaterium*
csgA	Major curli subunit	✔	✔
csgA + linker	*csgA* and linker sequence	✔	✔
csgB	Minor curli subunit	✔	✔
csgB + linker	*csgB* and linker sequence	✔	✔
csgAB	*csgA* followed by *csgB*	✔	✔
tasA	*B. subtilis* amyloid	✔	✘
tasA + linker	*tasA* and linker sequence	✔	✔

B. Gel image of parts for Gibson assembly

Figure B1. Gel image of parts for Gibson assembly. Picture of agarose gel bands for (1) the backbone piece, (2) csgA, (3) csgB, (4) csgA + linker, (5) csgB + linker.

C. Media optimization

Plate reader induction experiment and visual phenotype reveal B. megaterium forms clusters when grown in LB

In order to further investigate the relationship between amyloid protein expression and cell growth dynamics, we measured growth over time at different induction levels for each of our constructs. The growth curves for cells grown in LB showed a high degree of variation in OD600 between 2 and 7 hours (Fig. 9). We surmise that this variation may be due to cell clumping. We observed visual clumping for cells grown in 5 mL of LB, at mid-log phase (3 hours). These cultures returned to homogeneity at stationary phase. These observations mirror those recorded in the plate reader. We could not find anything in the literature that explains why this is happening.

Figure C1. OD600 measurements of tasA-containing cells grown in LB in different xylose concentrations. tasA-containing cells were grown in LB for 24 hours in xylose concentrations from 0 to 33.3 mM in triplicates. All concentrations and triplicates are plotted. Around 3-5 hours, the OD600 measurements are very noisy for all induction concentrations due to cell clumping in the logarithmic growth phase.

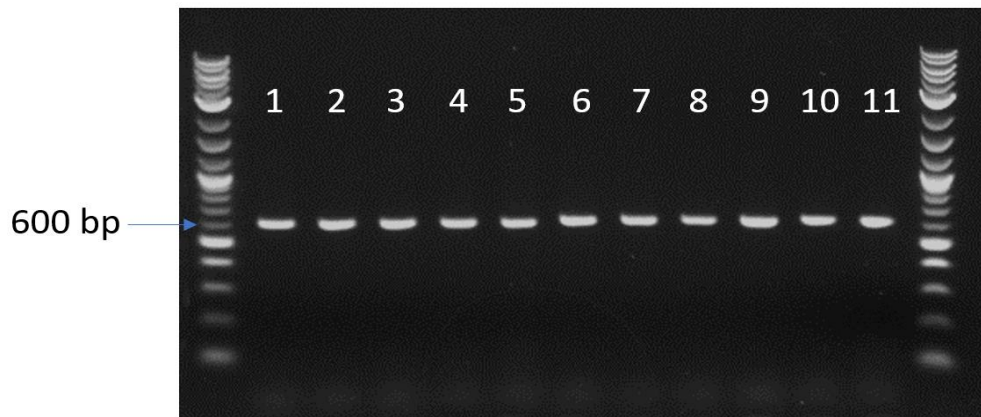## D. Follow-up experiments on plate reader induction experiment

Growing cells in the supernatant suggests xylose is being consumed after 7 hours of growth

After excluding the possibility that the circuit had incurred mutations, we reasoned the biphasic nature of the growth curves may be due to metabolism of the xylose, thereby repressing expression of the csg proteins. To test this, *csgA-* and *csgB*-containing cells were grown in beef extract containing xylose concentrations ranging from 0-33.3 mM. One row in the plate was filled with 300 µl beef extract (33.3 mM xylose) without any cells to make sure heating of the medium did not affected the xylose. The cells were grown at 37ºC for 22 hours and then centrifuged to separate the cells from the supernatant. The supernatant, including the media without added cells, was then transferred to a new plate and fresh cells, grown to mid-log, were added to the plate and grown for 24 hours. The fresh cells that grow in the supernatant from cells grown in 33.3 mM xylose do not show biphasic growth behavior, while cells grown in the 33.3 mM xylose medium have the same recovery at 7 hours as the cells in the first plate. This suggests that the xylose is completely consumed at 7 hours when the cells can start to grow normally again.
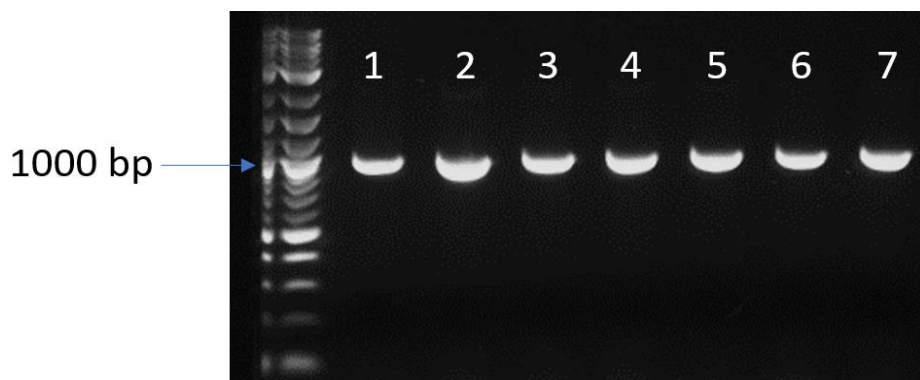
Sequencing results reveal the csgA-circuit is intact after 24 hours of growth

To determine if the biphasic growth curve shape (Fig. 4A and 4C) could be explained by a large deletion or insertions in the genetic circuit after 7-8 hours, the *csg* gene was PCR-amplified from construct containing cells. The PCR product was separated on an agarose gel. Bands of the expected molecular weight were present for all xylose concentrations (Fig. D1 and D2). The PCR product was then sequenced to determine if any point mutations had

occurred. The sequencing results showed that the construct is unchanged after the construct-containing cells have been grown for 24 hours.

Figure D1. csgA plate reader PCR. *(1) 0 mM xylose in LB, (2) 2 mM xylose in LB, (3) 6 mM xylose in LB, (4) 20 mM xylose in LB, (5) 25 mM xylose in LB, (6) 0 mM xylose in beef extract, (7) 2 mM xylose in beef extract, (8) 6 mM xylose in beef extract, (9) 20 mM xylose in beef extract, (10) 25 mM xylose in beef extract and (11) individual csgA-containing cell colony from agar plate. The expected length of csgA is 654 base pairs.*

Figure D2. tasA plate reader PCR. *(1) 0 mM xylose in beef extract, (2) 2 mM xylose in beef extract, (3) 6 mM xylose in beef extract, (4) 20 mM xylose in beef extract, (5) 25 mM xylose in beef extract, (6) 33.3 mM xylose in beef extract and (7) individual tasA-containing cell colony from an agar plate. The expected length of tasA is 1002 base pairs.*

E. Nickel bead column assay - data

Table E. Ratios (Retained cells/All cells) for induced and uninduced cells in the Nickel column assay and calculated p-value from permutation test.

Replicate	Uninduced	Induced
1	0.296	0.333
2	0.272	0.462
3	0.276	0.3425
Mean	0.282	0.379
p-value	0.01655	

F. DNA sequences

Name	Description	Sequence
UNS3F_seq	Forward primer for sequencing of constructs	CGCACTGGAAACATC
UNS3R_seq	Reverse primer for sequencing of constructs	GTTTGTTTCCGTCTACG
UNS3_FOR	Forward primer for adding UNS3 to 5'-end of construct	GCACTGAAGGTCCTCAATC GCACTGGAAACATCAAGGT CGTAGAGTATAAGGAGGTT ACT
UNS2_REV	Reverse primer for adding UNS2 to 3'-end of construct	GCTTGGATTCTGCGTTTGTT TCCGTCTACGAACTCCCAG CTTAATGGTGATGGTGATG GT
UNS4_FOR	Forward primer for adding UNS4 to 5'-end of construct	CTGACCTCCTGCCAGCAAT AGTAAGACAACACGCAAA GTCTAGAGTATAAGGAGGT TACT
UNS4_REV	Reverse primer for adding UNS4 to 3'-end of construct	GACTTTGCGTGTTGTCTTAC TATTGCTGGCAGGAGGTCA GTTAATGGTGATGGTGATG GT
UNS2_pMM1522_FOR	Forward primer for adding UNS2 to the linear plasmid backbone	GCTGGGAGTTCGTAGACGG AAACAAACGCAGAATCCA AGCACCTCGCTAACGGATT CACC
UNS3_pMM1522_REV	Reverse primer for adding	CGACCTTGATGTTTCCAGT

	UNS3 to the linear plasmid backbone	GCGATTGAGGACCTTCAGT GCATTGAGTTAGTTTGTTT ATC
SPlipA export tag	Export tag sequence	ATGGCCAATCAACCGACGA AATACCGCAAGTTTGTTGT AGGAGCGGCGAGCGCGGC CTTAGTGGCTTCAGCGGTC GCGCCTGTCGCGTTT
SPlipA linker sequence	Linker sequence	GAATCAGTACATAAT

G. Media

2x AB3 (1 L)
Autoclave for 15 minutes
- 3 g Beef Extract
- 3 g Yeast Extract
- 10 g peptone
- 1 g glucose
- 7 g NaCl
- 7.36 g K_2HPO_4
- 2.64 g KH_2PO_4

2x SMM
pH adjust to pH 6.5 and filter sterilize

- 1 M Sucrose
- 40 mM maleic acid
- 40 mM $MgCl_2$

PEG-P
Autoclave for 15 minutes:
- 40% PEG 6000 in 1x SMM

SMMP
2 x AB3 and 2 x SMM; mixed 1:1

Solution A
pH adjust to pH 7.3 and filter sterilize
- 602 mM
- 58 mM MOPS
- 30 mM NaOH

Solution B
Autoclave 15 minutes

- ■ 4 % (w/v) agar agar
- ■ 0.2 % (w/v) casamino acids
- ■ 10 % (w/v) yeast extract

8 x cR5-salts

Autoclave

- ■ 11 mM K_2SO_4
- ■ 394 mM $MgCl_2$ x 6 H_2O
- ■ 3 mM KH_2PO_4
- ■ 159 mM CaCl2

cR5 top agar (2.5 ml)

- ■ 1.25 ml Solution A
- ■ 713 µl Solution B
- ■ 288 µl cR5-salts
- ■ 125 µl 12 % (w/v) proline
- ■ 125 µl 20 % (w/v) glucose