Supplementary Information

Enzyme immobilisation on wood-derived cellulose scaffolds via carbohydrate-binding module fusion constructs

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Further experimental details

CBM-fluorescent protein construct design, expression and purification

Construct design and cloning

Genes encoding the fusion proteins were codon optimised for expression in *E. coli* using PartsGenie\(^1\) and synthesised (Twist Bioscience). For protein expression the genes encoding the fusion protein were cloned into expression vector pBbA1c.\(^2\) The genes of interested were amplified by PCR using CloneAmp polymerase premix (ClonTech) and the primers detailed in Table S2. The template was removed by DpnI (New England Biolabs) digest and the DNA purified using a Qiaquick PCR purification kit (Qiagen). The PCR products were cloned into Ndel/Xhol (New England Biolabs) linearised pBbA1c using Infusion HD (Clontech). DNA was transformed into NEB5alpha cells (New England Biolabs).

Protein expression and purification

An overnight preculture was grown in LB medium supplemented with 34 µg ml\(^{-1}\) chloramphenicol at 37°C. 500 ml phosphate buffered terrific broth (Formedium) supplemented with 34 µg/ml chloramphenicol at 37°C was inoculated with 5 ml pre-culture and grown at 37°C. Once the culture had reached an OD\(_{600}\) of 0.5-1 cultures were induced with 1 mM IPTG and grown at 37°C overnight. In the case of the CBM2a-mEGFP construct protein solubility was increased by dropping the incubation temperature to 25°C post-induction. The following morning cells were harvested by centrifugation and stored at -20°C until required.

Cells were resuspended in buffer A (200 mM KCl, 50 mM Tris pH 7.5) supplemented with protease inhibitor cocktail and DNAase (Sigma) and lysed by cell disruption at 20 KPsi. Lysate was clarified by ultracentrifugation at 14,000 g for 1 hour and the supernatant applied to a Ni-NTA agarose column (Qiagen). The column was washed with 5 column volumes of
buffer A supplemented with 10 mM imidazole and then 40 mM imidazole. The protein was eluted with buffer A supplemented with 250 mM imidazole. If required, the purified protein was concentrated in a 10k MWCO Vivaspin20 centrifugal device before being desalted into buffer A using a desalting column in order to remove the imidazole.

Protein concentration was quantified using a Cary 60 UV-vis spectrophotometer (Agilent). Extinction coefficients are detailed in Table S9. Values for $A_{280}$ were calculated from the amino acid sequence using the Protparam tool on the ExPASy proteomics server (https://web.expasy.org/protparam/, accessed 4th March 2021) whilst extinction coefficients for the chromophores were taken from the fluorescent protein database (https://www.fpbase.org, accessed 4th March 2021).

**Cellulose scaffold synthesis before DOE-based optimisation**

A block of *Tilia Americana* basswood (Stockport Hobbycraft Store, UK) was sawn into 10 x 10 x 50 mm portions with the grain of the wood following the long (50 mm) axis (Figure S1). Each wood portion weighed approximately 0.9 – 1 g and was gently sanded by hand with abrasive paper to remove any residual wood flakes. Separately, 50 g NaOH and 25.2 g Na$_2$SO$_3$ were dissolved in DI water to a final volume of 500 ml (2.5 M NaOH, 0.4 M Na$_2$SO$_3$). 25 portions of wood were added to a 1 L round-bottomed flask before addition of the 500 ml NaOH/Na$_2$SO$_3$ solution. The mixture was then refluxed at 140 °C for 16 h with gentle magnetic stirring. The solution was then drained and the wood portions (now CSs) were washed with cold DI water 3 x times, before being refluxed with DI water (500 ml) at 125 °C for 1 hour a single time. The CSs were then refluxed in 500 ml H$_2$O$_2$ (5 %, Sigma UK) for 2 hours at 125 °C along with ca 5 drops of antifoam-204 (Sigma-Aldrich) (caution: significant foaming would still occur even with antifoaming agent). This H$_2$O$_2$ treatment was repeated two further times. The CSs were then washed in cold DI water 3 x times before being placed in 200 ml absolute ethanol overnight. DI water was then added to bring the final volume to 1 L (20 % v/v EtOH) where the CSs were then stored at room temperature (RT) until use.

Informative YouTube videos of similar procedures are available online.$^{3,4}$
**CBM3A-mCherry immobilisation before DOE-based optimisation**

Transparent PVC tubing (ID 8 mm, OD 12 mm) was cut to a length of approximately 80 mm. A CS would then be trimmed slightly with a razor blade into a cylindrical shape (initially cuboidal) and inserted into the tubing ensuring a tight fit and sitting in the centre of the tubing (Figure S2a). A 50 ml plastic Luer-Lock syringe was connected to one end of the PVC tubing using a 3-way Luer Lock valve (for loading/discharging solutions), male/female Luer Lock attachments and poly(vinylidene fluoride) (PVDF) tubing (Figure S2b). 30 ml of buffer solution (50 mM TRIS, 200 mM KCl, pH 7.5) was then flushed through the CS at a rate of 250 µL min⁻¹ to flush out residual EtOH. 5 ml of CBM3A-mCherry (2 mg ml⁻¹) was then flushed through the CS at a rate of 100 µL min⁻¹ (Aladin syringe pump, World Precision Instruments) and collected. A further 2 ml of buffer was flushed through and collected to wash out non-adsorbed CBM3-mCherry. The CBM3-mCherry concentration before (ca 2 mg ml⁻¹) and after passing though the CS (including 2 ml additional flush) was measured via UV-visible spectrophotometry (Abs [587], ε = 72000 M⁻¹ cm⁻¹, Mₚ = 48892.28 g mol⁻¹). The experiments were conducted at room temperature (RT, typically 19 ±3 °C).

**Details of the initial CS immobilisation scoping trials**

Methyl-red, a water-soluble pH sensitive dye, was employed for these trials due to availability and for its intense colouration allowing facile concentration determination by UV-Vis spectrophotometry. The pH sensitivity would also roughly indicate the pH of the CSs (red at pH 4.4 and below, yellow at pH 6.2 and above) and therefore if any residual NaOH remained from the Kraft process. The trial experiments revealed the adsorption of a saturated solution of Methyl-red (20 ml vol., flow rate 1 ml min⁻¹) to the CSs and a pH-induced colour change from yellow to red – indicating an acidic nature of the CSs (likely from carboxylic acid groups). The dye appeared to have stronger adsorption at the end of the CS where the dye entered than the end where the dye exited, forming a gradient (see image below).
After establishing a working procedure with Methyl-red, small quantities of the recombinant CBM-FP proteins produced and purified from the expression trials were flowed through the CSs, with concentration being measured before and after to determine immobilisation efficiency and total protein loading (see image below). The volume of each CBM-FP was made up to 5 ml, but differed in concentration due to variability in expression yield (Table S4). The results revealed successful immobilisation of each CBM-FP construct, with some having concentrations too low for detection after flushing through (implying close to 100 % immobilisation efficiency), and after an additional 1.5 ml of buffer to flush out any non-adsorbed protein. The variability in initial concentration made comparisons between CBM-FPs difficult, but CBM3A-mCherry was selected for use in the optimisation trials due to its relatively high expression yield in comparison to the other CBM-FP constructs, and its relatively high total loading (0.4 wt. %) and immobilisation efficiency (59.8 %) determined from this trial.
a) CBM-FP proteins purified from expression trials (prior to dilution to 5 ml): 1. mCitrine-CBM1, 2. CBM30-mOrange, 3. CBM3A-mCherry, 4. mNeptune-CBM28, 5. mECFP-CBM2A and 6. CBM2a-mEGFP. 

b) 96-well plate of the CBM-FP solutions before and after CS flowing through (FT), and after a subsequent 1.5 ml FT of buffer, under ordinary light (left) and a black light (right).

c) UV-vis spectrophotometry traces of the CBM-FPs before (left) and after (centre) FT, and after a further 1.5 ml FT of buffer (right). 

d) Visible light (above) and black light (below) images of the cut-up CS’s after FT and 1.5 ml buffer flush.
Details of the Bradford-chromophore protein concentration normalisation factor calculations

The calculated concentration of CBM2a-mEGFP differed significantly when measured using via the Bradford method or via its chromophore absorbance. Since the CBM2a-ωTA construct could only be measured via the Bradford method (since it has no chromophore), a normalisation factor was employed to allow direct comparison between CBM2a-ωTA and the other CBM-FP constructs (see section 3.6.1 for details).

The normalisation factor (NF) was calculated using the following equation:

\[
NF = \frac{[FP]_{Abs\ 488}}{[FP]_{Bradford}}
\]

Where \([FP]_{Abs\ 488}\) and \([FP]_{Bradford}\) are the calculated CBM2a-mEGFP concentrations via absorbance at 488 nm and via the Bradford method, respectively. The normalised concentration of CBM2a-ωTA construct ([ωTA]\text{norm}) was then calculated using the following equation:

\[
[ωTA]_{norm} = [ωTA]_{Bradford} \times NF
\]

Where \([ωTA]_{Bradford}\) is the concentration of the CBM2a-ωTA construct determined by the Bradford method. The value of the normalisation factor was calculated to be 2.93.
Figure S1. a) – e) Schematic depicting the initial CS synthesis protocol. f) Basswood portion (left) and processed CS (right) for comparison.

Cut into 10x10x50 mm portions
2.5 M NaOH
0.4 M Na₂SO₃
Reflux 16h
5% H₂O₂
Reflux 2h
5% H₂O₂
Reflux 2h
x3

Figure S2. a) A representative CS inserted into 80 mm length PVC tubing. b) Image of syringe-tubing connection via Luer-lock attachments.
Figure S3. Characterisation of CBM-Reporter Constructs: a) Schematic of the six CBM-reporter constructs. b) Purified proteins under (i) white light and (ii) UV (from left to right, mECFP-CBM2A, CBM2a-mEGFP, mCitrine-CBM1, CBM30-mOrange, CBM3A-mCherry and mNeptune-CBM28. c) UV-vis spectra of the purified proteins.

Figure S4. Characterisation of CBM2a-ωTA fusion construct. a) Schematic of the CBM-ωTA construct. b) UV-vis spectra of the purified protein. Inset, close up of the spectral features in 300 – 800 nm region resulting from the PLP cofactor.
Figure S5. Numbers 1 – 14 represent the CS synthesis conditions given in Table 2. a) Visible light images of CSs soaked in acetone and b) after drying. c) FEG-SEM images of the end of each CS showing loss of ordered structure (fibrillation). Scale bar = 100 µm d) Plot showing variation of CS porosity with increasing NaOH concentration during Kraft process.
Figure S6. Visible light images of CS’s produced under the synthesis conditions given in Table 2 after flowing-through CBM3-mCherry solution and cutting up.
Figure S7. a) Immobilisation yield and b) loading results for the flow conditions scoping trials showing minimum (Min) and maximum (Max) forcing and centre-point (CP) conditions.

Figure S8. Experiments 14, 15 and 16 of the flow conditions optimisation DOE: immobilisation yield (red dots) and loading (blue triangles) with increasing buffer concentration.
Figure S9. Reaction scheme depicting the conversion of (S)-α-MBA into acetophenone via the CS immobilised CBM2a-ωTA enzyme

Figure S10. Bradford assay calibration curve employing BSA as a standard

Figure S11. a) Individual calibration curves for acetophenone, pyruvate and α-MBA at 245 nm based on theoretical conversion extent. b) Summed calibration curves allowing monitoring of acetophenone production.
Figure S12. Visible light images of the CS’s after having cell lysate containing mCherry (above) and CBM3a-mCherry (below) flowed through.

Figure S13. Camera images of Basswood (left) and Oak (right) derived CSs. b) camera images of the above before (left) and after (right) flowing through CBM3a-mCherry.
Table S1. Summary of previously reported enzyme immobilisation techniques employing CBM-based affinity binding in comparison with results from this study. NR = Not reported. Numbers in parentheses denote values after a 10 ml buffer flush.

| Cellulose support | Protein/Enzyme | CBM | Loading (wt. %) | Immobilisation efficiency (%) | Ref |
|-------------------|----------------|-----|----------------|-------------------------------|-----|
| Microcrystalline cellulose (Avicel®) | Cis-epoxysuccinic acid hydrolase (CESH) from *Rhodococcus opacus* | CcCBM2a from *Clostridium cellulovorans* (EngD) | 1.97 ±0.12 | NR | 5 |
| | | CcCBM3 from *Clostridium cellulolyticum* (CipC) | 2.00 ±0.05 | | |
| | | GiCBM3 from *Clostridium thermocellum* (CipA) | 1.22 ±0.26 | | |
| | | CiCBM11 from *C. thermocellum* (CelH) | 1.26 ±0.07 | | |
| | | CiCBM30 from *C. thermocellum* (CelJ) | 0.79 ±0.06 | | |
| Microcrystalline cellulose (Avicel®) | Lipase from *Bacillus stearothermophilus* | ThCBM from *Trichoderma hazianum* | 0.112 ±0.04 | NR | 6 |
| Bacterial microcrystalline cellulose | Lipase B from *Candida antarctica* | NpCBM from *Neocallimastix patriciarum* (Cel6A) | NR | NR | 7 |
| Microcrystalline cellulose (unspecified source) | Glucoamylase from *Neurospora crassa* | NcCBM from *N. crassa* (Cbh-1) | NR | NR | 8 |
| Microcrystalline cellulose (Sigmacell® Type 20) | Atrazine chlorohydrolase | CiCBM from *C. thermocellum* | NR | NR | 9 |
| | | CcCBM from *C. cellulovorans* | NR | NR | |
| Microcrystalline cellulose (Sigmacell® Type 20) | Horseradish peroxidase from *Armoracia rusticana* | CcCBM from *C. cellulovorans* (CbpA) | 2.2 | NR | 10 |
| Microcrystalline cellulose (Avicel®) | Organophosphate hydrolase from *Flavobacterium* | CcCBM from *C. cellulovorans* (CbpA) | NR | NR | 11 |
| Microcrystalline cellulose (Avicel®) | Lipase B from *Candida antarctica* | NpCBM from *N. patriciarum* (Cel1A) | NR | NR | 12 |
| Bacterial cellulose membrane | β-galactosidase from *T. maritima* | PICBM2 from *P. furiosus* chitinase | 1.85 | 81 ±4 | 13 |
| Insoluble β-1,3-glucan (Curdlan) | Chitosanase from *Gynuella sunshinyii* | CBM56 (β-1,3-glucan-binding domain) from *Paenibacillus barengoltzii* | NR | 90.1 | 14 |
| Cellulose nanogel from microcrystalline cellulose | Lipase from *Geobacillus stearothermophilus* | CBM (unspecified) | NR | ~92 | 15 |
| Microcrystalline cellulose (Avicel® and Sigmacell®) | Lipase S-2 from *Cryptococcus sp.* | CBM from *T. reesei* (Cbh-1) | NR | ~85 – 90 | 16 |
| Microcrystalline cellulose powder, 25 µm (Aladdin Chemistry®) | β-glucosidase Bgl1A(A245/F297Y) | CBM3 from *Trametes sp.* AH28-2 | NR | NR | 17 |
| Wood-derived aligned CSs: conditions for high loading | CBM67 from Ruminiclostridium thermocellum (CCV01467.1) | CBM91 from Cellulosilyticum ruminicola (JCM 14822) | CBM92 from R. thermocellum, (AAA20892.1) |
|---|---|---|---|
| mEGFP | CcCBM2a from C. cellulovorans (EngD) | 4.59 (3.94) | 91.8 (78.7) |
| mCherry | CtCBM3A from C. thermocellum (CipA) | 5.24 (4.63) | 79.4 (70.1) |
| mOrange | CtCBM30 from C. thermocellum (CelU) | 3.22 (1.88) | 51.8 (30.2) |
| mCitrine | TrCBM1 from Trichoderma reesei (Cell17A) | NR | NR |
| mECFP | AcCBM2A from Acidothermus cellulolyticus (GH5) | NR | NR |
| mEGFP [control] | None | 3.43 (1.05) | 78.1 (24.0) |
| mCherry [control] | None | 2.02 (0.34) | 40.9 (6.9) |
| ωTA from B. megaterium | CcCBM2a from C. cellulovorans (EngD) | 3.99±0.66 | 62.1±8.9 |

**Wood-derived aligned CSs: conditions for high immobilisation efficiency**

| mEGFP | CcCBM2a from C. cellulovorans (EngD) | 2.88 (2.38) | 97.1 (80.2) |
| mNeptune | BsCBM28 from Bacillus sp. 1139 (Cel5a) | 2.11 (1.60) | 69.5 (52.9) |
| ωTA from B. megaterium | CcCBM2a from C. cellulovorans (EngD) | 2.34 (1.72) | 81.4 (60.3) |

**Wood-derived non-aligned cellulose scaffolds [control]: conditions for high loading**

| mCherry | CtCBM3A from C. thermocellum (CipA) | 5.91 (4.15) | 91.3 (64.1) | This work |
| Name               | Sequence                                      |
|--------------------|-----------------------------------------------|
| pBbA1cvCATHisFw    | AAGGAGATACATATGgcacaccaccacc                 |
| pBbA1cmNeptuneFw   | AAGGAGATACATATGgtaaatgacagaagaactg           |
| pBbA1cmECFPFw      | AAGGAGATACATATGgttagcaagggagggaaac          |
| pBbA1cmCitrineFw   | AAGGAGATACATATGgtatcgaaggtgaggaattg         |
| pBbA1cXhoIsuffixRv | GAGATCCTTTACTCGAGtttgtgatccgtg             |
| CcCBM2aEngDBMwTAF  | GGCAGTGGAGAGCCTGAGCTTGAGCCTGACCCTGGCAG      |
| HindIIISuffixBmwTAR2 | AGTCTACAAAAGCTTTTATGCCTGACCCTGCCACCC       |
| HindIIISuffixF     | AAGCTTTTGTAGACTCGGATCCA                    |
| HisCcCBM2aEngDR    | GAGCTCTCCACTGCGGCT                         |

Table S3. Summary of the design of the CBM-FP constructs

| Name                | N-term               | Linker                           | C-term               |
|---------------------|----------------------|----------------------------------|----------------------|
| CBM2a-mEGFP         | His-CcCBM2a(EngD)   | GSSAGSSAAGSGSG                  | mEGFP                |
| CBM30-mOrange       | His-CtCBM30(CelJ)   | GSSAGSSAAGSGSG                  | mOrange              |
| CBM3A-mCherry       | His-CtCBM3A(CipA)   | RGTTTRRPATTTGSSPGPTQS           | mCherry              |
| mCitrine-CBM1       | mCitrine             | RGTTTRRPATTTGSSPGPTQS           | TrCBM1(Cel17A)-His   |
| mECFP-CBM2A         | mECFP               | RGTTTRRPATTTGSSPGPTQS           | AcCBM2A(GH5)-His     |
| mNeptune-CBM28      | mNeptune             | RGTTTRRPATTTGSSPGPTQS           | BsCBM28(Cel5a)-His   |
Table S4. Summary of initial scoping trials probing CBM-FP immobilisation to CS’s

| Protein          | Expression yield (mg L⁻¹) | Initial concentration (µg ml⁻¹) | Amount immobilised (µg) | Immobilisation yield (%) | Loading (wt. %) |
|------------------|----------------------------|-------------------------------|-------------------------|---------------------------|-----------------|
| 1 mCitrine-CBM1   | 3.67                       | 733.8                        | 935.0                   | 31.9                      | 0.148           |
| 2 CBM2-mOrange    | 0.20                       | 40.20                        | 160.8                   | 100                       | 0.0267          |
| 3 CBM3-mCherry    | 5.1                        | 1020                         | 2443                    | 59.8                      | 0.400           |
| 4 mNeptune-CBM4   | 1.34                       | 268.4                        | 333.6                   | 31.1                      | 0.0543          |
| 5 CFP-CBM5        | 0.61                       | 121.0                        | 483.9                   | 100                       | 0.0765          |
| 6 CBM6-GFP        | 0.17                       | 33.60                        | 134.4                   | 100                       | 0.0226          |

Table S5. Summary of main factors involved in the CS synthesis DSD

| Factor                          | Categorisation | Inclusion in DSD? (Y/N) | Value and range | Units |
|---------------------------------|----------------|-------------------------|-----------------|-------|
| NaOH conc.                      | Numerical      | Y                       | 2.5 – 7.5       | M     |
| Na₂SO₃ conc.                    | Numerical      | Y                       | 0.4 – 1.2       | M     |
| Sulphite processing vol.        | Numerical      | Y                       | 100 - 250       | ml    |
| H₂O₂ conc.                      | Numerical      | Y                       | 4 - 12          | vol. %|
| H₂O₂ vol.                       | Numerical      | Y                       | 100 – 250       | ml    |
| Mass of wood portions           | Numerical      | N                       | 4.5 – 5         | g     |
| Flask size                      | Categorical    | N                       | 1000            | ml    |
| Sulphite processing duration    | Numerical      | N                       | 16 – 18         | h     |
| No. of water washes (RT)        | Categorical    | N                       | 3               | n     |
| No. of water washes (reflux)    | Categorical    | N                       | 3               | n     |
| No. of H₂O₂ treatments (reflux) | Categorical    | N                       | 1               | n     |
| Protein type                    | Categorical    | N                       | CBM3-mCherry    | n/a   |
| Immobilisation conditions       | mix            | N                       | Initial immobilisation procedure | n/a |
| CS storage conditions            | mix            | N                       | 20% EtOH        | n/a   |
Table S6. Summary of output variables and other characteristics of the CS’s produced from the CS synthesis DSD

| Experiment No. | Protein loading (wt. %) | Immobilisation yield (%) | Porosity (%) | $I_{\text{max}}/I_{110}$ | Cellulose polymorph | Shrinkage on drying |
|----------------|-------------------------|--------------------------|--------------|--------------------------|---------------------|---------------------|
| 1              | 0.71                    | 42.3                     | 70.9         | 2.88                     | II                  | High                |
| 2              | 0.52                    | 29.5                     | 78.0         | 5.43                     | Iβ                  | Low                 |
| 3              | 0.64                    | 39.7                     | 72.1         | 3.57                     | Iβ                  | High                |
| 4              | 0.69                    | 47.1                     | 78.1         | 3.85                     | Iβ                  | Low                 |
| 5              | 0.35                    | 28.8                     | 70.9         | 3.73                     | Iβ                  | Medium              |
| 6              | 0.54                    | 41.7                     | 69.1         | 3.75                     | II                  | High                |
| 7              | 0.60                    | 34.5                     | 70.4         | 5.79                     | Iβ                  | Low                 |
| 8              | 0.81                    | 52.4                     | 68.9         | 3.77                     | II                  | High                |
| 9              | 0.59                    | 42.6                     | 69.6         | 5.03                     | II                  | High                |
| 10             | 0.52                    | 39.2                     | 73.0         | 5.22                     | Iβ                  | Medium              |
| 11             | 0.25                    | 15.9                     | 77.3         | 4.13                     | Iβ                  | Low                 |
| 12             | 0.26                    | 18.4                     | 70.7         | 3.64                     | II                  | High                |
| 13             | 0.28                    | 17.5                     | 76.6         | 3.79                     | Iβ                  | Medium              |
| 14             | 0.18                    | 11.8                     | 74.5         | 3.43                     | Iβ                  | Low                 |
| 15             | 0.83                    | 49.4                     | -            | 3.55                     | Iβ                  | -                   |
| 16             | 1.01                    | 59.5                     | -            | -                        | -                   | -                   |
| 17             | 1.15                    | 67.1                     | -            | -                        | -                   | -                   |
| 18             | 1.09                    | 70.5                     | -            | -                        | -                   | -                   |
| PB             | -                       | -                        | 72.3         | -                        | -                   | -                   |
| 4 no H$_2$O$_2$ | -                       | -                        | 72.6         | 6.14                     | Iβ                  | Low                 |

Table S7. Summary of main factors involved in the flow conditions optimisation DSD

| Factor                        | Categorisation | Inclusion in DSD? (Y/N) | Value and range | Units     |
|-------------------------------|----------------|-------------------------|-----------------|-----------|
| Protein conc.                 | Numerical      | Y                       | 0.5 – 5         | mg ml$^{-1}$ |
| Protein vol.                  | Numerical      | Y                       | 2.5 – 7.5       | ml        |
| pH                            | Numerical      | Y                       | 7.1 – 9.1       | n/a       |
| Flow rate                     | Numerical      | Y                       | 50 – 250        | µl min$^{-1}$ |
| Salt conc.                    | Numerical      | Y                       | 10 – 300        | mM        |
| Buffer conc.                  | Numerical      | N                       | 50              | mM        |
| Salt type                     | Categorical    | N                       | KCl             | n/a       |
| Buffer type                   | Categorical    | N                       | TRIS            | n/a       |
| Protein type                  | Categorical    | N                       | CBM3-mCherry    | n/a       |
| Buffer type                   | Categorical    | N                       |                 |           |
| CS synthesis conditions       | Mix            | N                       | Initial CS synthesis conditions | n/a |
| Temperature                   | Nuisance       | N                       | 19 ±2           | °C        |
Table S8. Summary of the CS immobilisation data for the CBM2a-ωTA and CBM2a-mEGFP constructs

| Construct        | Quantification method | Initial protein concentration (mg ml⁻¹) | Initial loading (wt. %) | Loading after 10 ml flush (wt. %) | Initial immobilisation efficiency (%) | Immobilisation efficiency after 10 ml flush (%) |
|------------------|-----------------------|----------------------------------------|-------------------------|-----------------------------------|---------------------------------------|-----------------------------------------------|
| CBM2a-ωTA        | Bradford assay        | 2.25                                   | 0.80                    | 0.59                              | 81.4                                  | 60.3                                          |
| CBM2a-mEGFP      | Bradford assay        | 1.7                                    | 0.71                    | 0.53                              | 95.5                                  | 71.6                                          |
| CBM2a-ωTA        | Abs 488 nm            | 4.98                                   | 1.94                    | 1.30                              | 94.2                                  | 68.7                                          |
| CBM2a-ωTA        | [Normalised]          | 6.68                                   | 2.34                    | 1.72                              | 81.4                                  | 60.3                                          |

Table S9. UV-vis parameters used for protein quantification

| Construct        | Ext. Coeff. 280nm (cm⁻¹M⁻¹) | Chromophore | Wavelength (nm) | Ext. Coeff. Chromophore (cm⁻¹M⁻¹) |
|------------------|------------------------------|-------------|-----------------|-----------------------------------|
| mNeptune-CBM28   | 70820                        | mNeptune    | 600             | 60,000                            |
| CBM30-mOrange    | 79300                        | mOrange     | 548             | 71,000                            |
| mECFP-CBM2A      | 52370                        | mECFP       | 433             | 32,500                            |
| mCitrine-CBM1    | 29340                        | mCitrine    | 516             | 77,000                            |
| CBM3A-mCherry    | 69790                        | mCherry     | 587             | 72,000                            |
| CBM2a-mEGFP      | 48360                        | mEGFP       | 488             | 56,000                            |

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