

SUPPLEMENTARY INFORMATION FOR

A tripartite carbohydrate-binding module to functionalize cellulose nanocrystal

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This Supplementary Information contains 5 sections:

1. Cloning of the DNA sequences (Table S1-S2)
2. Dynamic of RAC sedimentation by magnetic beads grafted with fluorescent mSA-CBM3-AzF (Figures S2-S4). See Electronic Supplementary Information for Movie S1 and S2
3. Molecular modelling assesses the flexibility of mSA-CBM3 (Table S3 and Figure S7-S8)

4. Expression and purification of the chimeric CBM3 based proteins (Figures S8-S11, Table S5)

5. RAC is recovered using fluorescent mSA-CBM3-AzF grafted on magnetic beads (Figure S9-S10)

1. Cloning of the DNA sequences encoding the hybrid proteins

At position 153 of *CBM3a* (C to T exchange), HindIII restriction site at position 241 was removed (CTC → CTG). Undesired restriction sites present in the gene coding for mRFP1 were also removed by punctual mutation: HindIII at position 209 (GCT → GCG), NcoI at position 419 (ACC → ACG) and HindIII at position 431 (GCT → GCG).

The sequence coding for the monomeric hybrid streptavidin mSA2 from *Streptomyces avidinii* (Part:BBa_K1896000) was fused to the *N*-terminal of CBM3a with an additional sequence coding for a TEV protease site (GAA AAC CTG TAT TTT CAG GGC). The corresponding construction mSA2-Tev-linker1-CBM3a-linker2-His₆ (pET28-mSA2-CBM3 for short) is detailed in Table S2. In order to generate mSA2-Tev-linker1-CBM3a-linker2-AzF-His₆ (pET28-mSA2-CBM3a-AzF for short), codon TTT (Phe₃₆₄) was replaced by a stop amber codon TAG at position 1090 of *mSA2-Tev-linker1-CBM3a-linker2-His₆* (Part:BBa_K2668010, Table S2). Apart from point modifications of the DNA sequences (see Table S2 for details), all the genes correspond to the wild type sequences, except for CBM3, whose DNA sequence has been optimized to more closely reflect the codon usage of the recipient host organism (<http://parts.igem.org> as Part:BBa_K1321014).

Table S1: Primers used in construction of fusion proteins

pET28-mRFP1	For	5'- AGGAGATATACCATGGCTTCCTCCGAAGACGTTATCAAAG-3'
	Rev	5'- GTGCGGCCGCAAGCTTAGCACCGGTGGAGTGACG-3'
CBM3-RFP1	For	5'-GGACGATCCGATGGCTTCCTCCGAAGACG-3'
	Rev	5'-AGGAAGCCATCGGATCGTCCAATGATGGAGGAATC-3'
pET28-CBM3-mRFP1	For	5'- TAAGAAGGAGATATACCATGAATGCTACGCCAACTAAGG GTGC-3'
	Rev	5'- CTCGAGTGCGGCCGCAAGCTTAGCACCGGTGGAGTGACG-3'
CBM3-Met ₁₂₇ →Gly ₁₂₇	For	5'- CGTAAAGGGCAGCTCAAGCACAAATAACGC-3'
	Rev	5'- GAGCTGCCCTTTACGAATGTTCTTTTACATTT-3'
pET28-mSA-CBM3	For	5'- TAAGAAGGAGATATACCATGGCGGAAGCGGGTATCACC-3'
	Rev	5'- CTCGAGTGCGGCCGCAAGCTTCGGATCGTCTATGATGGAGG-3'
pET28-mSA-CBM3-AzF	For	5'- CCATCATAGGACGATCCGAAGCTTGCG-3'
	Rev	5'- ATCGTCCTATGATGGAGGAATCGTGGTAGCC-3'

Table S2: Details of the DNA sequences and corresponding primary sequences of the chimeric proteins used in this study. Linker1 sequence is in green, linker2 sequence is in blue, CBM3 sequence is in black, mRFP1 sequence is in purple, mSA sequence is in purple and TEV protease site sequence is underlined in black. Ambre codon in linker2 DNA sequence is in bold and corresponding emplacement in primary sequence is symbolized by *.

Construct	DNA sequence	Protein
linker1-CtCBM3a-linker2-RFP1-His ₆ Part:BBa_K2668020	atgaatgctacgccaactaag ggtgcaaccccgaccaacaca gcaacgcctacaaaaagcgct acagcaacacctacaagaccg tcagttcctacaaacacaccg actaacacaccggcaaataca cctgtttcaggcaacttgaag gtcgaatttttataactcaa ccgagtgatacaactaacagt attaatccgcagttttaagta acaatacaggatcaagtgca attgatcctttcaaagctgaca ttgagatactattacaccggt gatggccagaaggaccagact ttctggtgtgaccatgcagct atcataggtagcaacggctca tacaacggcatcacatcaa gtaaaaggaacattcgtaaag ggcagctcaagcacaataac gcagacacatacctcgaata	MNATPTKGATPTNTAT PTKSATATPTRPSVPT NTPTNTPANTPVSGNL KVEFYNSNPSTTNSI NPQFKVTNTGSSAIDL SKLTLRYYYTVDGQKD QTFWCDHAAIIGSNGS YNGITSNVKGTfVKGS SSTNNADTYLEISFTG GTLEPGAHVQIQGRFA KNDWSNYTQSNDSFK SASQFVEWDQVTAYLN GVLVWGKEPGGSVVPS TQPVTTTPPATTKPPAT TKPPATTIIPSLDDPM ASSEDVIKEFMRFKVR MEGSVNGHEFEIEGEG EGRPYEGTQTAKLKVT KGGPLPFAWDILSPQF QYGSKAYVKHPADIPD

	agtttcacaggtggcactttg gaacctggtgctcatgtacag atacagggtaggtttgcgaaa aatgactggagtaattataca cagtcaaattgattactcattt aagtcagcatcacagttcgta gaatgggatcaggttacagca tatttgaatggagtacttgta tggggtaaagaaccaggagga tcagtagttccgtcaacacag ccggtacaacccccaccggca acaaccaagccgcccagcaaca accaaaccaccggctaccacg attcctccatcagacgatccg atggcttcctccgaagacggt atcaaagagttcatgcgtttc aaagttcgtatggaaggttcc gttaacggtcacgagttcgaa atcgaaggtgaaggtgaaggt cgtccgtacgaaggtaccag accgctaaactgaaagttacc aaaggtggtccgctgccgttc gcttgggacatcctgtccccg cagttccagtacggttccaaa gcgtacgttaaacacccggct gacatcccggactacctgaaa ctgtccttcccgggaaggtttc aatgggaacgtgttatgaac ttcgaagacggtggtgttggt accgttaccaggactcctcc ctgcaagacggtgagttcatc tacaaagttaaactgcgtggt accaacttcccgtccgacggt ccggttatgcagaaaaaacG atgggttgggaagcgtccacc gaacgtatgtaccggaagac ggtgctctgaaaggtgaaatc aaaatgcgtctgaaactgaaa gacggtggtcactacgacgct gaagttaaaaccacctacatg gctaaaaaacgggttcagctg ccgggtgcttacaaaaccgac atcaaactggacatcacctcc cacaacgaagactacaccatc gttgaaacagtacgaacgtgct gaaggctcgtcactccaccggt gctaagcttgcggccgcactc gagcaccaccaccaccaccac	YKLSFPEGFKWERVM NFEDGGVVTVTDSSL QDGEFIYKVKLRGTNF PSDGPVMQKKTMGWEA STERMPEDGALKGEI KMRLKLKDGGHYDAEV KTTYMAKKPVQLPGAY KTDIKLDITSHNEDYT IVEQYERAEGRHSTGA KLAAALEHHHHHHH
mSA2-Tev-linker1-CtCBM3a-linker2-His ₆	atggcggaagcgggtatcacc ggcacgtggtacaaccagcat ggttctaccttcaccggttacc	MAEAGITGTWYNQHGS TFTVTAGADGNLTGQY ENRAQGTGCQNSPYTL

	gcggggtgcggacggtaacctg accggccagtagcggaaaccgt gcgcagggcactgggtgccag aactctccgtacaccctgacc ggccggttacaacgggtaccaa ctggaatggcgtggtgaaatg aacaactctaccgaaaactgc cactctcgtaccgaatggcgt ggtcagtagcagggtgggtgcg gaagcgcgtatcaacaccag tggaacctgacctacgaaggt ggttctgggtccggcgaccgaa cagggtcaggacaccttcacc aaagttaaaccgtctgcggcg <u>tccaccggtg</u>aaaacctgtat tttcagggcaatgctacgcc actaagggtgcaaccccgacc aacacagcaacgcctacaaaa agcgctacagcaacacctaca agaccgtcagttcctacaaac acaccgactaacacaccggca aatacacctgtttcaggcaac ttgaagggtcgaattttataac tcaaattccgagtgatacaact aacagtattaatccgcagttt aaagtaacaaatacaggatca agtgcgaattgatctttcaaag ctgacattgagatactattac accgttgatggccagaaggac cagactttctgggtgtgaccat gcagctatcataggtagcaac ggctcatacaacggcatcaca tcaaattgtaaaagggaacattc gtaaagggtcagctcaagcaca aataacgcagacacatacctc gaaataagttttcacagggtggc actttggaacctgggtgctcat gtacagatacagggtaggttt gcgaaaaatgactggagtaat tatacacagtcaaattgattac tcattttaagtcagcatcacag ttcgtagaatgggatcagggt acagcatatttgaaatggagta cttgtagtgggtgaaagaacca ggaggatcagtagttccgtca acacagccggtaacaacccca ccggcaacaaccaagccgcca gcaacaaccaaacaccgggt accacgattcctccatcattt gacgatccgaagcttgcgcc	TGRYNGTKLEWRVEWN NSTENCHSRTEWRGQY QGGAEARINTQWNLT EGGSGPATEQGQDTFT KVKPSAASTGENLYFQ <u>GNATPTKGATPTNTAT</u> PTKSATATPTRPSVPT NTPNTNPANTPVSGNL KVEFYNSNPSTTNSI NPQFKVTNTGSSAIDL SKLTLRYYYTVDGQKD QTFWCDHAAIIGSNGS YNGITSNVKGTfVKGS SSTNNADTYLEISFTG GTLEPGAHVQIQGRFA KNDWSNYTQSNdYSFK SASQFVEWDQVTAYLN GVLVWGKEPGGSVVPS TQPVTTPPATTKPPAT TKPPATTIPPSFDDPK LAAALEHHHHHHH
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	gcactcgagcaccaccaccac caccac	
mSA2-Tev-linker1-CtCBM3a- linker2-AzF-His ₆ Part:BBa_K2668010	atggcggaagcgggtatcacc ggcacgtggtacaaccagcat ggttctaccttcaccgttacc gcgggtgcggacggtaacctg accggccagtagcaaaaccgt gcgagggcactgggtgccag aactctccgtacaccctgacc ggccgttacaacgggtaccaa ctggaatggcgtggtgaatgg aacaactctaccgaaaactgc cactctcgtaccgaatggcgt ggtcagtagcagggtggtgcg gaagcgcgtatcaacaccag tggaacctgacctacgaaggt ggttctggtccggcgaccgaa cagggtcaggacaccttcacc aaagttaaaccgtctgcggcg tccaccggtgaaaacctgtat tttcagggcaatgctacgcc actaagggtgcaaccccgacc aacacagcaacgcctacaaaa agcgtacagcaaacactaca agaccgtcagttcctacaaac acaccgactaacacaccggca aatacacctgtttcaggcaac ttgaaggtcgaattttataac tcaaattccgagtatacaact aacagtattaatccgcagttt aaagtaacaaatacaggatca agtgaattgatctttcaaag ctgacattgagatactattac accgttgatggccagaaggac cagactttctggtgtgaccat gcagctatcataggttagcaac ggctcatacaacggcatcaca tcaaattgtaaagggaacattc gtaaagggcagctcaagcaca aataacgcagacacatacctc gaaataagtttcacaggtggc actttggaacctggtgctcat gtacagatacagggttaggttt gcgaaaaatgactggagtaat tatacacagtcaaattgattac tcatttaagtcagcatcacag ttcgtagaatgggatcaggtt acagcatattttgaatggagta cttgtagtggggtaaagaacca ggaggaacagtagttccgtca acacagccggtgaacaacccca	MAEAGITGTWYNQHGS TFTVTAGADGNLTGQY ENRAQGTGCQNSPYTL TGRYNGTKLEWRVEWN NSTENCHSRTEWRGQY QGGAEARINTQWNLT EGSGPATEQGQDTFT KVKPSAASTGENLYFQ GNATPTKGATPTNTAT PTKSATATPTRPSVPT NTPNTNPANTPVSGNL KVEFYNSNPSTTNSI NPQFKVTNTGSSAIDL SKLTLRYYYTVDGQKD QTFWCDHAAIIGSNGS YNGITSNVKGTfVKGS SSTNNADTYLEISFTG GTLEPGAHVQIQGRFA KNDWSNYTQSNDSYFK SASQFVEWDQVTAYLN GVLVWGKEPGGSVVPS TQPVTTTPPATTKPPAT TKPPATTIPPS*DDPK LAAALEHHHHHH

	ccggcaacaaccaagccgcca gcaacaaccaaaccaccggct accacgattcctccatca TAG gacgatccgaagcttgcggcc gcactcgagcaccaccaccac caccac	
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2. Dynamic of RAC sedimentation by magnetic beads grafted with fluorescent mSA-CBM3-AzF.

The imaging system is based on a acA1920-150 μm CMOS camera (Basler, Deutschland), with a sensor size of 1920×1200 pixels, a binarization of 12 bits (4096 levels of grey), allowing to acquire and grab 25 frames per second (fps) at full-frame. Associated with a 25 mm focal length lens, the system is designed to observe in transmission a field of 41.7 mm width $\times$ 14.6 mm height in a ROI (Region Of Interest) of 1000×350 pixels with a spatial sampling of 41.66 $\mu\text{m}/\text{pixel}$.

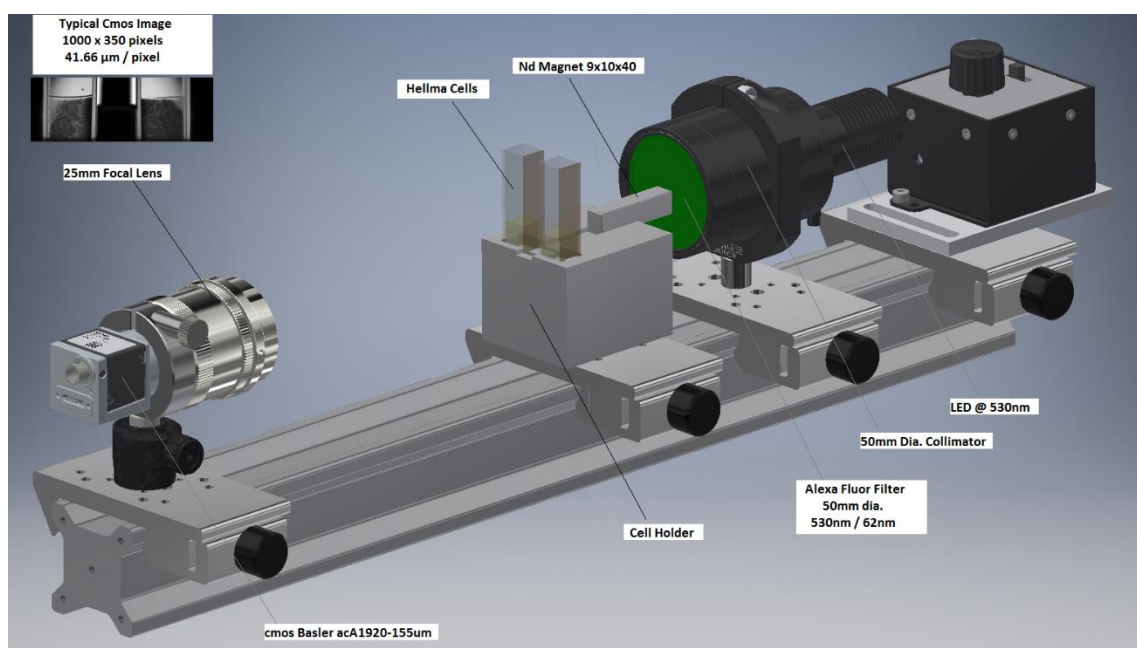


Figure S1: Experimental set-up in transmission

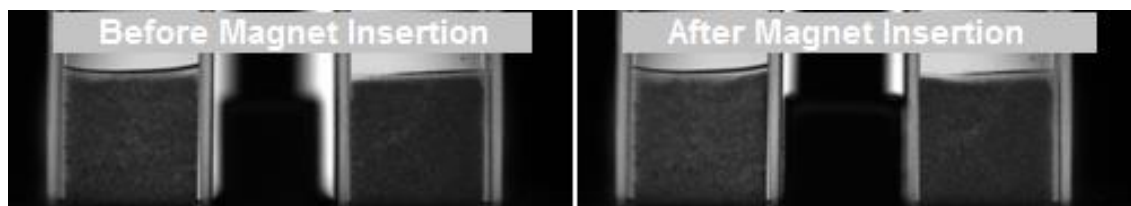


Figure S2: Two 1000×350 pixels acquired images. Left: before magnet insertion; Right: after magnet insertion.

Typically, samples to be compared are poured in individual sample cells, far from the magnet. Image acquisition is then started (two frequencies of 4 frames per second (fps) and 25 fps were used) and after about 10 s, beads recovery is initiated by insertion of the magnet. Acquisition is maintained for further 90 s (*i.e.* 360 images at 4 fps or 2250 images at 25 fps, Electronic Supplementary Information Movie S1). Treatment of the data is illustrated in Fig. S3. After background correction, two areas are isolated for each set of images (Fig. S3, in blue or in red, respectively). These areas are 220 pixels wide, pixel 0 being opposite to the position of the magnet, and 100 pixels high (delimited by the two white horizontal lines, Fig. S3A). The normalized intensity of each area is plotted, taking into account the intensity of the solution in each cuvette before and after complete sedimentation of the beads (Fig. S3B). The position of the sedimentation front in each sample corresponds to the value of the abscissa for 50% of the threshold level.

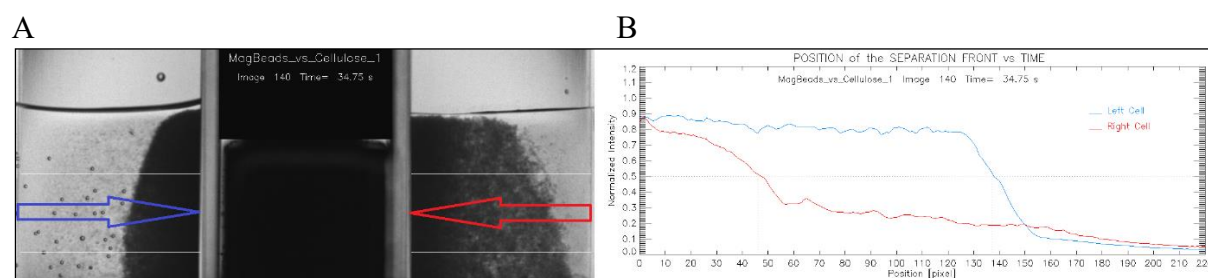


Figure S3: Example of the image data treatment (image 140 corresponding to 37.75 s). **A:** Position and direction of the selected area are represented by blue and red arrows. **B:** Screenshot

of the corresponding area plots intensity vs. pixel position. The 1 mL spectrophotometer semi-micro cuvettes were filled with 600 μ L of RAC (2%) in the presence of 80 μ L of magnetic beads grafted with TMR biocytin/mSA-CBM3-AzF (left cell, in blue) or bar beads (right cell, in red). Example of the evolution of the recovery front is provided here (Electronic Supplementary Information Movie S2)

The sedimentation front position of the different samples was plotted as a function of time (Fig. S4).

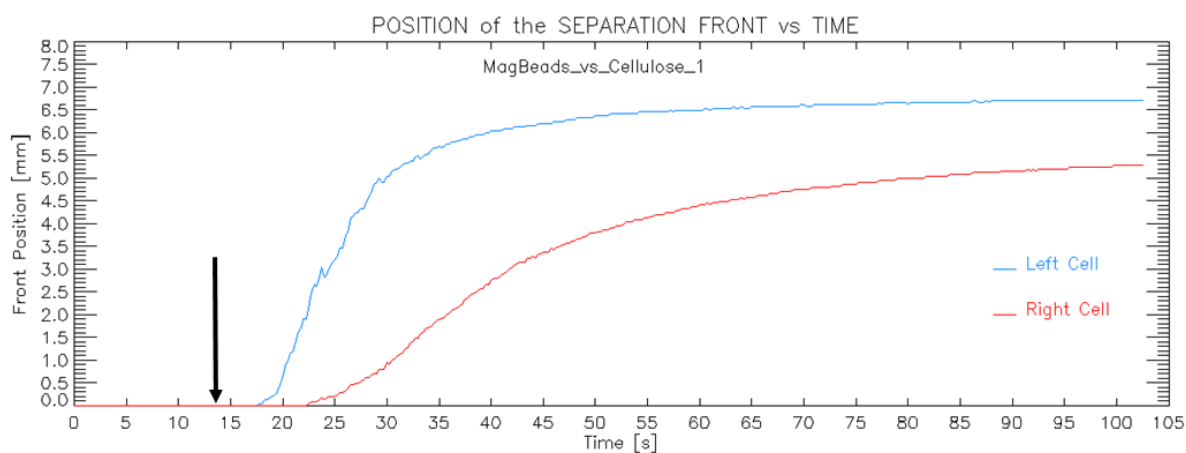


Figure S4: Evolution of the sedimentation front over the time, exemplified from run 1, at 4 fps. Black arrow corresponds to the insertion of the magnet. The cuvettes were filled with 600 μ L of RAC (2%) in the presence of 80 μ L of magnetic beads grafted with TMR biocytin/mSA-CBM3-AzF (left cell, in blue) or non-grafted beads (right cell, in red).

Curves were modeled as a classical first order step response time with the Equation 1:

$$Pos(t) = Pos(\infty) \left(1 - \exp\left(-\frac{(t-t_0)}{\tau}\right) \right) \quad \text{Eq. 1}$$

where t_0 is the starting time when the sedimentation front starts to move, $Pos(\infty)$ is the maximum position at "infinite" time and τ is time constant. The time constant that best fits the data is further called τ_{fit} .

From this first order model, the theoretical rising time T_r was obtained by the Equation 2:

$$T_r = T_{90\%} - T_{10\%} \quad \text{Eq. 2}$$

that can be linked to the time constant τ with regards to the Equation 3:

$$\tau = T_r / 2.2 \quad \text{Eq. 3}$$

3. Molecular modelling assesses the flexibility of mSA-CBM3

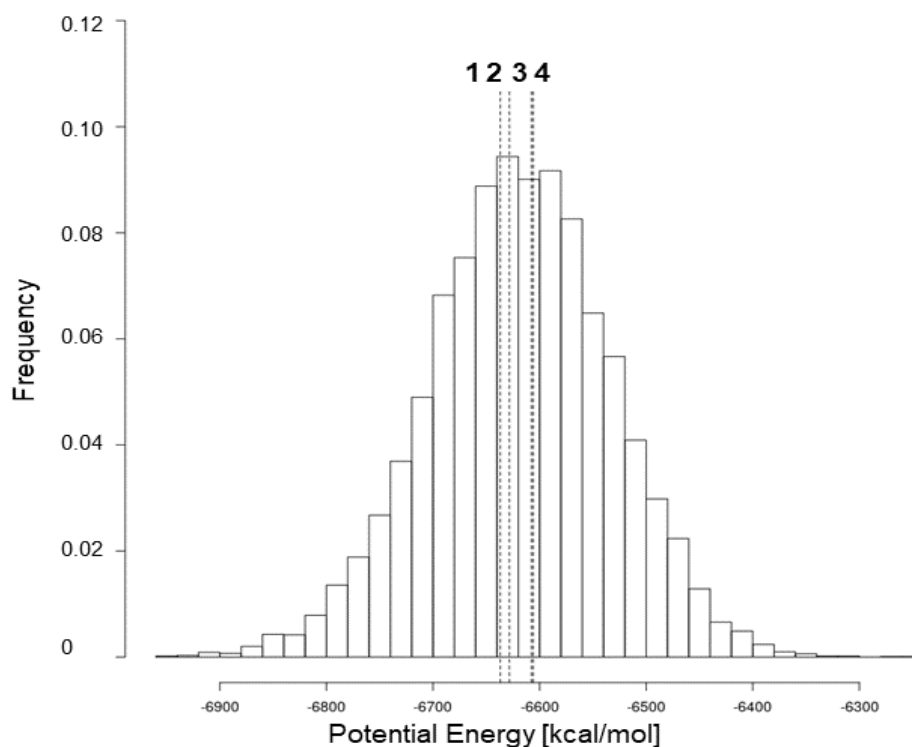


Figure S5: Potential energy distribution of the ensemble of conformations generated using the IDP conformational ensemble model generation method. Conformations (1, 2, 3, 4) selected for MD studies are indicated by vertical dashed lines.

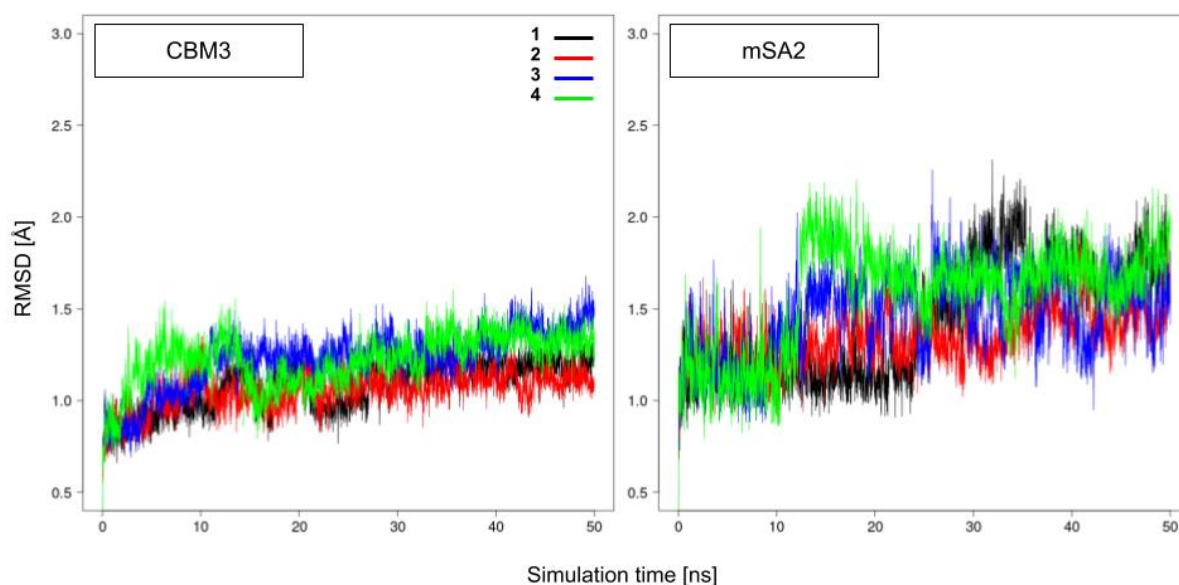
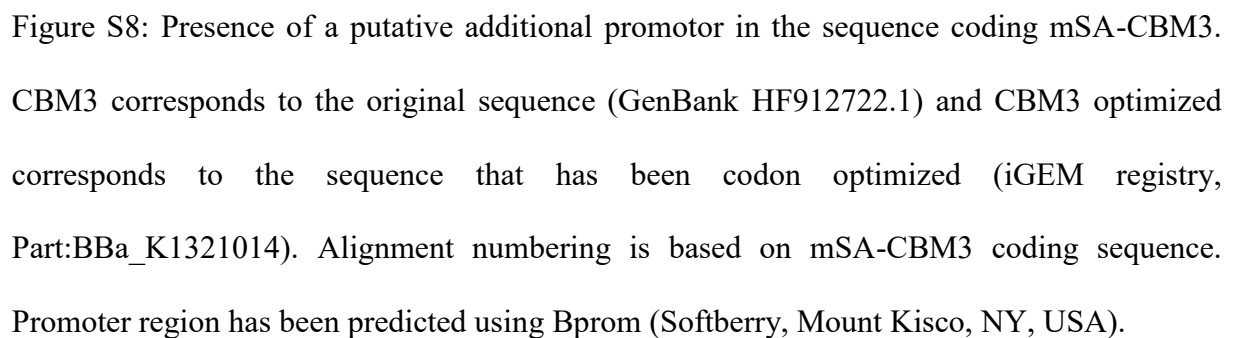
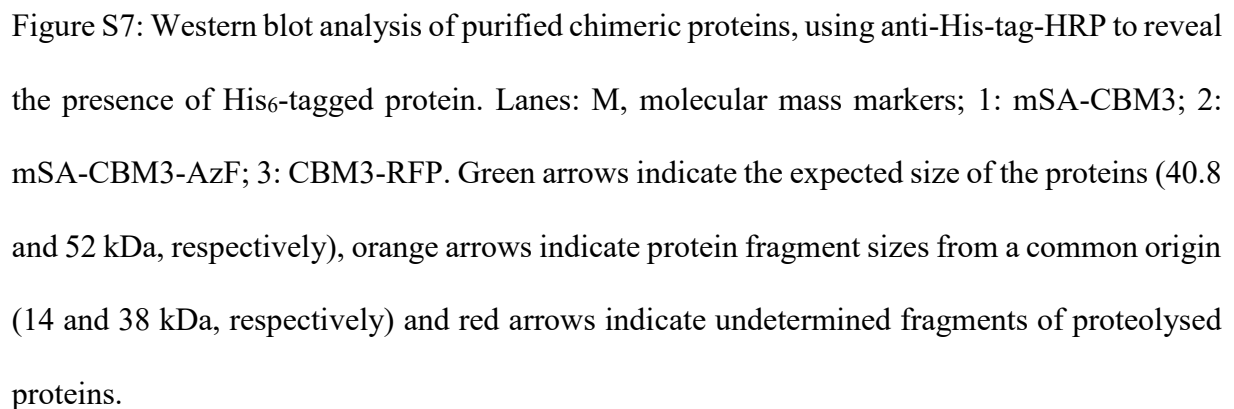


Figure S6: Backbone RMSD of globular domains (CBM3 on the left and mSA2 on the right) along 50 ns-MD simulations carried out using four starting conformational states of the protein platform extracted from the conformational ensemble model generated.

4. Expression and purification of the chimeric CBM3 based proteins

Table S3: Molar extinction coefficient and molecular weight of the proteins studied in this work. Theoretical molar extinction coefficients and molecular weight were calculated using ProtParam online software (<https://web.expasy.org/protparam/>)

Protein	Molar extinction coefficient ($\text{M}^{-1} \cdot \text{cm}^{-1}$)	Molecular weight (g/mol)
mRFP1	32,890	26,943
CBM3-RFP	68,300	52,164
mSA-CBM3	73,340	40,885
mSA-CBM3-AzF	73,340	40,885



5. RAC is recovered using fluorescent mSA-CBM3-AzF grafted on magnetic beads

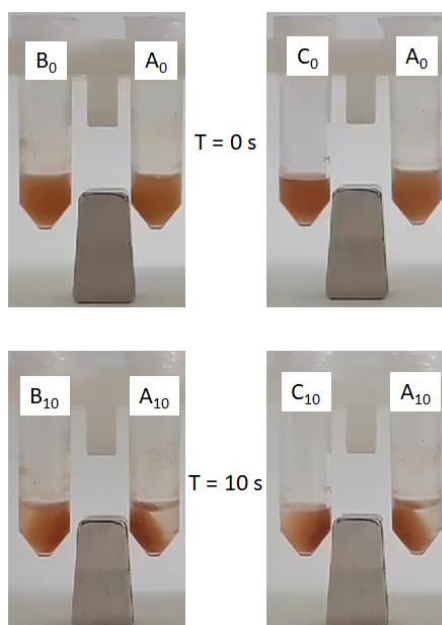


Figure S9: Recovery of magnetic beads previously incubated 1 h with 2% of RAC with a magnet after 10 s in presence of: **A**, magnetic bead grafted with mSA-CBM3-MB; **B**, magnetic beads in presence of mSA-CBM3; **C**, magnetic beads only. Brownish colour is due to the ferric cores of the magnetic beads. RAC in solution present a white cloudy aspect.

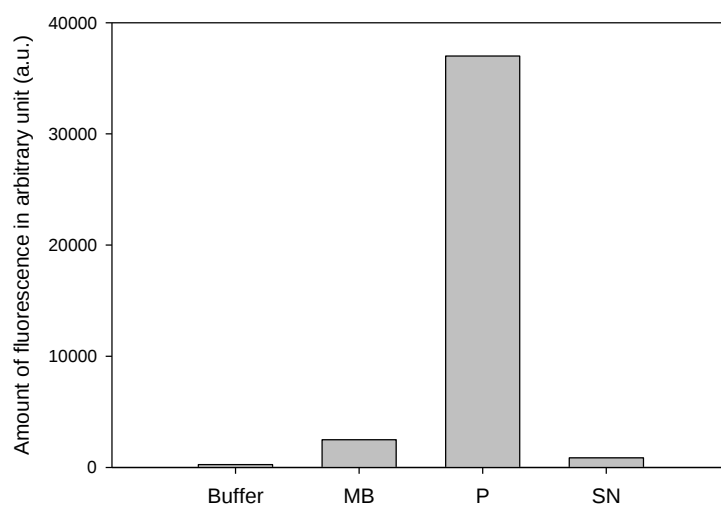


Figure S10: Amount of fluorescence measured after recovering of the beads with a magnet. TMR-mSA-CBM3-MB was incubated with 2% RAC for 1 h and the amount of fluorescence in the pellet (P) and the supernatant (SN) was measured after complete beads sedimentation. Fluorescence of the buffer and the magnetic beads (MB) was also measured.