Supporting Information

Fabrication of modularly functionalizable microcapsules using protein-based technologies

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1. Protein Sequences

WT BslA	GPLGSMAESTSTKAHTESTMRTQSTASLFATITGASKTEWSFSD IELTYRPNTLLSLGVMEFTLPSGFTANTKDTLNGNALRTTQILNN GKTVRVPLALDLLGAGEFKLKLNNKTLPAAGTYTFRAENKSLSI GNKFYAEASIDVAKRSTPPTQ
C-terminally Spytagged BslA	GPLGSMAESTSTKAHTESTMRTQSTASLFATITGASKTEWSFSD IELTYRPNTLLSLGVMEFTLPSGFTANTKDTLNGNALRTTQILNN GKTVRVPLALDLLGAGEFKLKLNNKTLPAAGTYTFRAENKSLSI GNKFYAEASIDVAKRSTPPTQGGSGGSAHIVMVDAYKPTK
N-terminally SpyTagged BslA	GPAHIVMVDAYKPTKGGSGGSMAESTSTKAHTESTMRTQSTA SLFATITGASKTEWSFSDIELTYRPNTLLSLGVMEFTLPSGFTAN TKDTLNGNALRTTQILNNGKTVRVPLALDLLGAGEFKLKLNNKT LPAAGTYTFRAENKSLSIGNKFYAEASIDVAKRSTPPTQ
GST-BslA	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKER AEISMLEGAVLDIRYGVSRIAYSKDFLKVDFLSKLPEMLKMFEDR LCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCF KKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK SDLEVLFQGPLGSMAESTSTKAHTESTMRTQSTASLFATITGAS KTEWSFSDIELTYRPNTLLSLGVMEFTLPSGFTANTKDTLNGNA LRTTQILNNGKTVRVPLALDLLGAGEFKLKLNNKTLPAAGTYTFR AENKSLSIGNKFYAEASIDVAKRSTPPTQ
GST-BslA-SpyTag	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWR NKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKDFLKVDFLSKLPEMLKM FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFP KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPP KSDLEVLFQGPLGSMAESTSTKAHTESTMRTQSTASLFATITG ASKTEWSFSDIELTYRPNTLLSLGVMEFTLPSGFTANTKDTLN GNALRTTQILNNGKTVRVPLALDLLGAGEFKLKLNNKTLPAAG TYTFRAENKSLSIGNKFYAEASIDVAKRSTPPTQGGSGGSAHI VMVDAYKPTK
SpyCatcher-GFP	MSYHHHHHHDYDIPTTENLYFQGAMGSSKGEELFTGVVPILV ELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWP TLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFF KDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEY NYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQN TPIGDGPVLSPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAA GITHGMDELYKGGSGGSAMVDTLSRLSSEQGQSGDMTIEED SATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVK DFYLYPGKYTFCRNRSTRRYGGSTAIPYSMEQGQVTVMASN

2. Figures

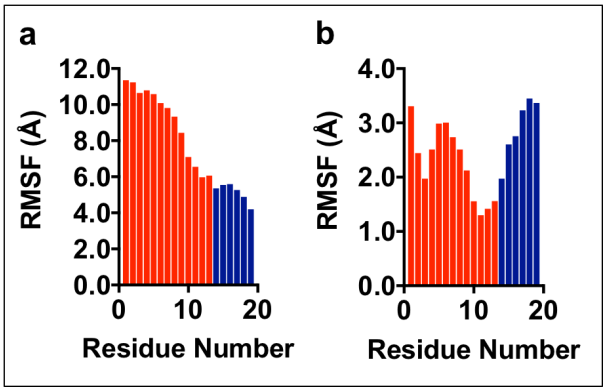

Figure S1. Root Mean Square Fluctuations for SpyTag (red) + linker (navy) residues in a) C-terminally SpyTagged BslA, and b) N-terminally SpyTagged BslA. The RMSFs for C-terminal SpyTag appear quite large because the RMSF was determined based on the alignment of backbone atoms of BslA residues only. However, these fluctuations capture the flexibility of the SpyTag with respect to the BslA when in the N- or C-terminal position. The fluctuations of the N-terminal SpyTag are much smaller than those for C-terminal SpyTag, and they are also closer to the values observed for the BslA residues, due to the hydrophobic interactions between N-terminal SpyTag and BslA.

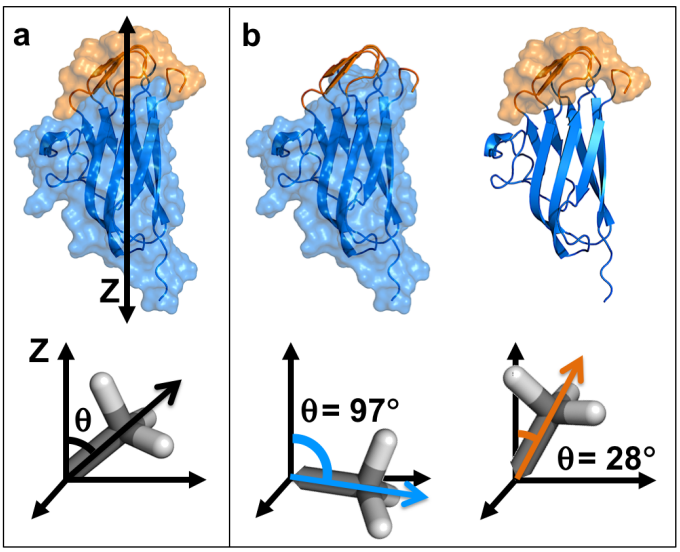

Figure S2. Orientation of methyl groups in hydrophobic and hydrophilic regions of BslA. (a) Top: Principle axis of WT BslA oriented along the Z-axis. Bottom: Schematic illustrating the total methyl group unit vector and angle with respect to the protein principle axis (θ) of WT BslA, which was determined by averaging the unit vector of each methyl group over each frame in the MD trajectory. (b) Top: Connolly surfaces of the hydrophilic (blue) and hydrophobic (orange) regions of BslA. Bottom: Orientation angle (θ) for the trajectory-averaged sum of individual methyl group unit vectors. (For the hydrophilic region, θ=97°, and for the hydrophobic region, θ=28°.)

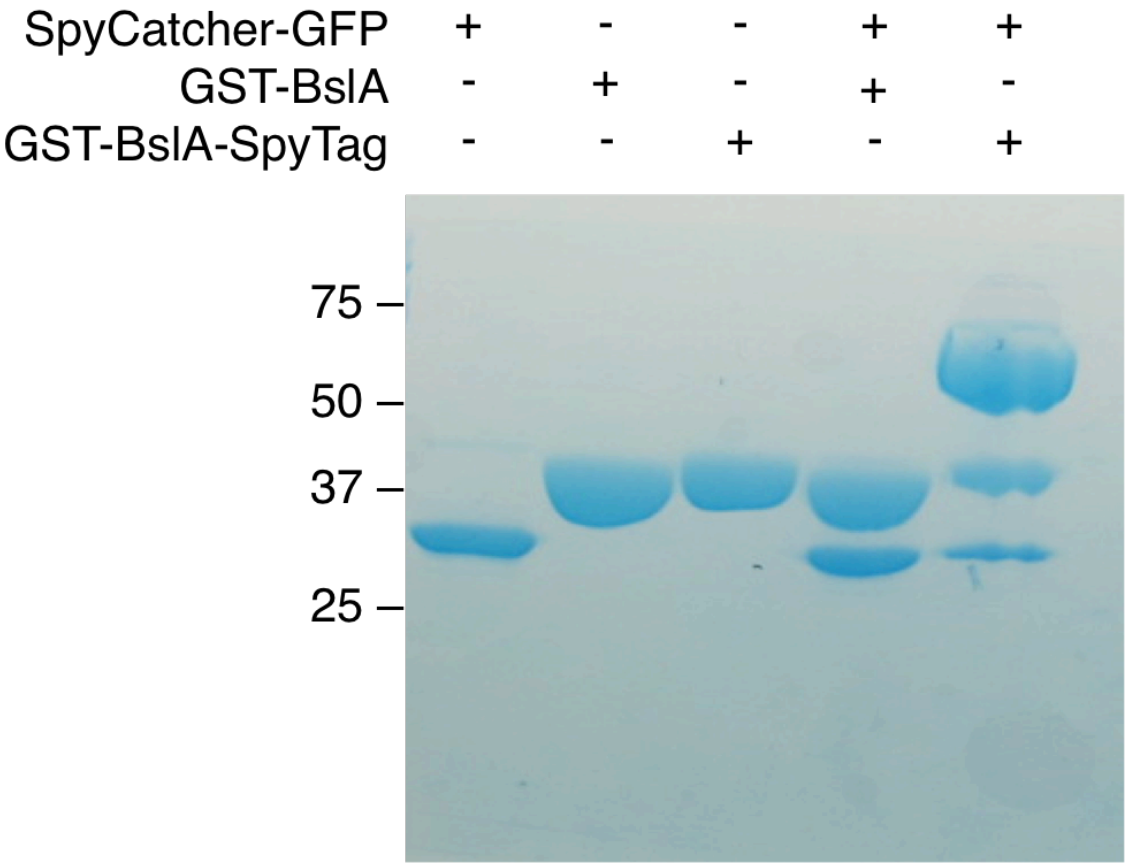

Figure S3. C-terminally SpyTagged BslA and SpyCatcher-GFP form a covalent linkage, detected by SDS PAGE analysis. **Lanes 1-3:** The protein components SpyCatcher-GFP, GST-BslA and GST-BslA-SpyTag. **Lane 4:** Unreacted control obtained by incubating SpyCatcher-GFP and GST-BslA together for one hour. The presence of two distinct bands and no new higher molecular weight band indicates that the two proteins do not react to form a covalent linkage. **Lane 5:** Reaction product obtained by incubating SpyCatcher-GFP and GST-BslA-SpyTag together for one hour. The appearance of a new, higher molecular weight band corresponds to the formation of the covalently linked SpyCatcher-GFP + GST-BslA-SpyTag protein complex. This conclusion is supported by the concomitant depletion of the bands corresponding to the unreacted proteins.

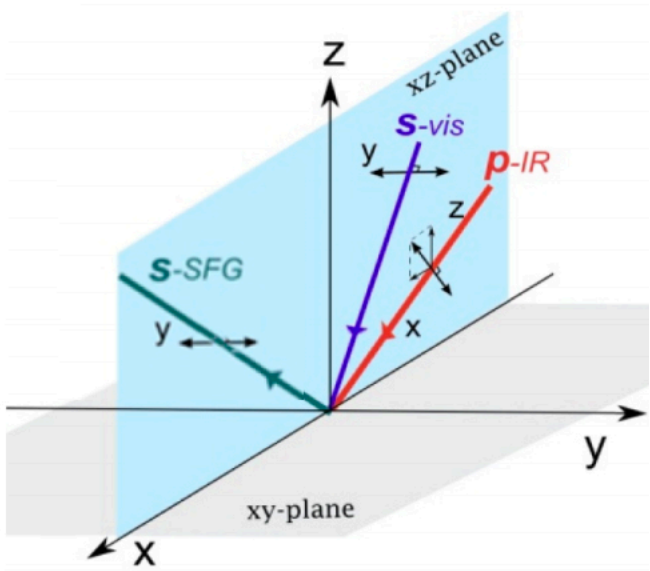

Figure S4. Schematic representation of ssp polarization settings for SFG experiments: the projection of the electric field of p-polarized and s-polarized light onto the laboratory coordinates.

3. Tables

Parameters	N-terminal SpyTagged BslA	C-terminal SpyTagged BslA	WT BslA	Assignment
χ_{NR} (a. u.)	0.0000±0.0086	0.0000±0.0068	0.0000±0.0047	Non resonance
ω (cm^{-1})	1675.0±1.45	1675.0±1.21	1675.9±0.2	β-turn
A (a. u.)	2.75±0.38	3.21±0.36	2.92±0.19	
Γ (a. u.)	17.71±2.22	18.58±1.80	15.16±0.98	
ω (cm^{-1})	1693.1±0.08	1692.3±0.67	1693.5±0.4	Antiparallel β-sheet B1 mode
A (a. u.)	0.23±0.15	0.28±0.14	0.29±0.08	
Γ (a. u.)	4.22±1.92	4.58±1.61	3.68±0.83	

Table S1a. Fitting parameters of SFG spectra of BslA proteins at the air-water interface in the amide I region.

Parameters	N-terminal SpyTagged BslA	C-terminal SpyTagged BslA	WT BslA	Assignment
χ_{NR} (a. u.)	0.0003±0.0040	0.0130±0.0058	0.0026±0.0018	Non resonance
ω (cm^{-1})	2858.0±0.6	2855.9±0.7	2857.2±0.2	CH_2 SS
A (a. u.)	0.37±0.08	1.03±0.29	0.32±0.03	
Γ (a. u.)	5.10±1.06	9.34±1.8	4.19±0.39	
ω (cm^{-1})	2883.8±0.4	2874.5±0.47	2875.7±0.2	CH_3 SS
A (a. u.)	1.67±0.11	1.86±0.23	2.13±0.05	
Γ (a. u.)	9.49±0.58	9.01±0.78	10.86±0.28	
ω (cm^{-1})	2913.7±1.4	2917.4±0.9	2915.1±0.6	CH_2 FR
A (a. u.)	0.54±0.17	1.27±0.17	0.82±0.09	
Γ (a. u.)	10.00±2.88	11.20±1.64	11.57±1.25	
ω (cm^{-1})	2946.1±0.6	2940.9±0.5	2942.7±0.3	CH_3 FR
A (a. u.)	3.79±0.20	0.88±0.14	1.78±0.09	
Γ (a. u.)	16.44±0.69	6.20±0.77	11.70±0.49	
ω (cm^{-1})	--	2970.9±1.1	2970.6±0.5	CH_3 AS
A (a. u.)	--	-0.69±0.19	-0.27±0.05	
Γ (a. u.)	--	7.80±1.93	5.86±1.07	

Table S1b. Fitting parameters of BslA SFG spectra at the air-water interface in the C-H stretch region.

System	RMSF of BslA alpha carbons (Å)		RMSF of BslA hydrophobic alpha carbons (Å)		RMSF of BslA hydrophilic alpha carbons (Å)	
	Average	Std dev	Average	Std dev	Average	Std dev
WT BslA	0.56	0.26	0.69	0.31	0.53	0.24
N-Terminally SpyTagged BslA	0.62	0.40	0.92	0.60	0.56	0.31
C-Terminally SpyTagged BslA	0.60	0.35	0.79	0.54	0.56	0.27

Table S2. Average root mean square fluctuations (Å) of the BslA alpha carbons over the MD trajectories.