Electronic Supplementary Material (ESI) for Faraday Discussions

Supplementary Data

Table S1 The amino acid sequences and DNA sequences of the 18Awt and 18A variants

Variants	Amino acid sequence	DNA sequence
18Awt	EWLKAFYEKVLEKLKELF	GAGTGGCTGAAAGCGTTCTACGAAAAGGTCCTGGAGAAACTGAAAGAACTGTTC
18A rev	KWLEAFYKEVLKELEKLF	AAATGGCTGGAAGCGTTCTACAAAGAAGTTCTGAAAGAACTGGAAAAACTGTTC
18Av1	KWLEAFYEKVLEKLKELF	AAATGGCTGGAAGCGTTCTACGAAAAGGTCCTGGAGAAACTGAAAGAACTGTTC
18Av2	EWLEAFYKKVLEKLKELF	GAGTGGCTGGAAGCGTTCTACAAAAAGGTCCTGGAGAAACTGAAAGAACTGTTC
18Av3	EWLKAFYEEVLKKLKELF	GAGTGGCTGAAAGCGTTCTACGAAGAAGTCCTGAAAAAACTGAAAGAACTGTTC
18Av4	EWLKAFYEKVLEELKKLF	GAGTGGCTGAAAGCGTTCTACGAAAAGGTCCTGGAGGAACTGAAAAAACTGTTC
18Av5	EWLKAFYEKVLKKLEELF	GAGTGGCTGAAAGCGTTCTACGAAAAGGTCCTGAAAAAACTGGAAGAACTGTTC
18Av6	KWLEAFYEEVLKKLKELF	AAATGGCTGGAAGCGTTCTACGAAGAAGTCCTGAAAAAACTGAAAGAACTGTTC
18Av7	KWLEAFYEKVLEELKKLF	AAATGGCTGGAAGCGTTCTACGAAAAGGTCCTGGAGGAACTGAAAAAACTGTTC
18Av8	EWLEAFYKEVLKKLKELF	GAGTGGCTGGAAGCGTTCTACAAAGAAGTCCTGAAAAAACTGAAAGAACTGTTC
18Av9	EWLEAFYKKVLEELKKLF	GAGTGGCTGGAAGCGTTCTACAAAAAGGTCCTGGAGGAACTGAAAAAACTGTTC
18Av10	EWLKAFYEEVLKKLEELF	GAGTGGCTGAAAGCGTTCTACGAAGAAGTCCTGAAAAAACTGGAAGAACTGTTC
18Av11	EWLKAFYEKVLKELEKLF	GAGTGGCTGAAAGCGTTCTACGAAAAGGTCCTGAAAGAACTGGAAAAACTGTTC
18Av12	KWLEAFYKKVLEELKKLF	AAATGGCTGGAAGCGTTCTACAAAAAGGTCCTGGAGGAACTGAAAAAACTGTTC
18Av13	KWLEAFYEKVLKELEKLF	AAATGGCTGGAAGCGTTCTACGAAAAGGTCCTGAAAGAACTGGAAAAACTGTTC
18Av14	EWLEAFYKEVLKELKKLF	GAGTGGCTGGAAGCGTTCTACAAAGAAGTCCTGAAAGAACTGAAAAAACTGTTC
18Av15	EWLEAFYKEVLKKLEELF	GAGTGGCTGGAAGCGTTCTACAAAGAAGTCCTGAAAAAACTGGAAGAACTGTTC
18Av16	EWLEAFYKKVLKELEKLF	GAGTGGCTGGAAGCGTTCTACAAAAAGGTCCTGAAAGAACTGGAAAAACTGTTC
18Av17	EWLKAFYEEVLKELEKLF	GAGTGGCTGAAAGCGTTCTACGAAGAAGTCCTGAAAGAACTGGAAAAACTGTTC
18Av18	KWLEAFYKEVLKKLEELF	AAATGGCTGGAAGCGTTCTACAAAGAAGTCCTGAAAAAACTGGAAGAACTGTTC

Table S2 DNA oligos used in this work

Primer name	Nucleotide sequence[a]	Description
18Arev-For	5'-ACGACGA***CATATG***GCTGAACACAATCCAGT-3'	Used for amplification of linker-18Arev
18Arev-Low	5'-TCGTT***CTCGAG***TCAGAACAGTTTTTCCAGTTCTTTCAGAACTTCTTTGTAGAACGCTTCCAGCCA TTTCGGCGTCGGGGTTGGGGTG-3'	
Linker-For	5'-ATGAA***AAGCTT***CCGACCCCACCGACCAC-3'	Used for amplification of linker-18A variants
Linker-oligos	5'-GCGTCGGGGTTGGGGTGGTTGGTGGCGTTGGCGTGGTCGGTGGGGTCG-3'	Used for assembly of linker-18A variants
18Av1	5'-CCCCAACCCCGACGCCGAAATGGCTGGAAGCGTTCTACGAAAAGGTCC-3'	Used for assembly of linker-18Av1
	5'-***CTCGAG***TCAGAACAGTTCTTTCAGTTTCTCCAGGACCTTTTCGTAGAACGCTTC-3'	
18Av2	5'-CCCCAACCCCGACGCCGGAGTGGCTGGAAGCGTTCTACAAAAAGGTCC-3'	Used for assembly of linker-18Av2
	5'-***CTCGAG***TCAGAACAGTTCTTTCAGTTTCTCCAGGACCTTTTTGTAGAACGCTTC-3'	
18Av3	5'-CCCCAACCCCGACGCCGGAGTGGCTGAAAGCGTTCTACGAAGAAGTCC-3'	Used for assembly of linker-18Av3
	5'-***CTCGAG***TCAGAACAGTTCTTTCAGTTTTTTCAGGACTTCTTCGTAGAACGCTTT-3'	
18Av4	5'-CCCCAACCCCGACGCCGGAGTGGCTGAAAGCGTTCTACGAAAAGGTCC-3'	Used for assembly of linker-18Av4
	5'-***CTCGAG***TCAGAACAGTTTTTTCAGTTCCTCCAGGACCTTTTCGTAGAACGCTTT-3'	
18Av5	5'-CCCCAACCCCGACGCCGGAGTGGCTGAAAGCGTTCTACGAAAAGGTCC-3'	Used for assembly of linker-18Av5
	5'-***CTCGAG***TCAGAACAGTTCTTCCAGTTTTTTCAGGACCTTTTCGTAGAACGCTTT-3'	
18Av6	5'-CCCCAACCCCGACGCCGGAGTGGCTGGAAGCGTTCTACAAAGAAGTCC-3'	Used for assembly of linker-18Av6
	5'-***CTCGAG***TCAGAACAGTTCTTTCAGTTTTTTCAGGACTTCTTTGTAGAACGCTTC-3'	
18Av7	5'-CCCCAACCCCGACGCCGGAGTGGCTGGAAGCGTTCTACAAAAAGGTCC-3'	Used for assembly of linker-18Av7
	5'-***CTCGAG***TCAGAACAGTTTTTTCAGTTCCTCCAGGACCTTTTTGTAGAACGCTTC-3'	
18Av8	5'-CCCCAACCCCGACGCCGGAGTGGCTGAAAGCGTTCTACGAAAAGGTCC-3'	Used for assembly of linker-18Av8
	5'-***CTCGAG***TCAGAACAGTTTTTCCAGTTCTTTCAGGACCTTTTCGTAGAACGCTTT-3'	
18Av9	5'-CCCCAACCCCGACGCCGGAGTGGCTGAAAGCGTTCTACGAAGAAGTCC-3'	Used for assembly of linker-18Av9
	5'-***CTCGAG***TCAGAACAGTTCTTCCAGTTTTTTCAGGACTTCTTCGTAGAACGCTTT-3'	

18Av10	5’-CCCCAACCCCGACGCCGAAATGGCTGGAAGCGTTCTACGAAAAGGTCC-3’	Used for assembly of linker-18Av10
	5’-***CTCGAG***TCAGAACAGTTTTTTCAGTTCCTCCAGGACCTTTTCGTAGAACGCTTC-3’	
18Av11	5’-CCCCAACCCCGACGCCGAAATGGCTGGAAGCGTTCTACGAAGAAGTCC-3’	Used for assembly of linker-18Av11
	5’-***CTCGAG***TCAGAACAGTTCTTTCAGTTTTTTCAGGACTTCTTCGTAGAACGCTTC-3’	
18Av12	5’-CCCCAACCCCGACGCCGAAATGGCTGGAAGCGTTCTACAAAAAGGTCC-3’	Used for assembly of linker-18Av12
	5’-***CTCGAG***TCAGAACAGTTTTTTCAGTTCCTCCAGGACCTTTTTGTAGAACGCTTC-3’	
18Av13	5’-CCCCAACCCCGACGCCGAAATGGCTGGAAGCGTTCTACGAAAAGGTCC-3’	Used for assembly of linker-18Av13
	5’-***CTCGAG***TCAGAACAGTTTTTCCAGTTCTTTCAGGACCTTTTCGTAGAACGCTTC-3’	
18Av14	5’-CCCCAACCCCGACGCCGGAGTGGCTGGAAGCGTTCTACAAAGAAGTCC-3’	Used for assembly of linker-18Av14
	5’-***CTCGAG***TCAGAACAGTTTTTTCAGTTCTTTCAGGACTTCTTTGTAGAACGCTTC-3’	
18Av15	5’-CCCCAACCCCGACGCCGGAGTGGCTGGAAGCGTTCTACAAAGAAGTCC-3’	Used for assembly of linker-18Av15
	5’-***CTCGAG***TCAGAACAGTTCTTCCAGTTTTTTCAGGACTTCTTTGTAGAACGCTTC-3’	
18Av16	5’-CCCCAACCCCGACGCCGGAGTGGCTGGAAGCGTTCTACAAAAAGGTCC-3’	Used for assembly of linker-18Av16
	5’-***CTCGAG***TCAGAACAGTTTTTCCAGTTCTTTCAGGACCTTTTTGTAGAACGCTTC-3’	
18Av17	5’-CCCCAACCCCGACGCCGGAGTGGCTGAAAGCGTTCTACGAAGAAGTCC-3’	Used for assembly of linker-18Av17
	5’-***CTCGAG***TCAGAACAGTTTTTCCAGTTCTTTCAGGACTTCTTCGTAGAACGCTTT-3’	
18Av18	5’-CCCCAACCCCGACGCCGAAATGGCTGGAAGCGTTCTACAAAGAAGTCC-3’	Used for assembly of linker-18Av18
	5’-***CTCGAG***TCAGAACAGTTCTTCCAGTTTTTTCAGGACTTCTTTGTAGAACGCTTC-3’	

[a]The underlined nucleotides indicate restriction sites.

Table S3 Protein quantification of GFP-18A fusions

	GFP-18A fusion[a]	Fusion protein (mg L^{-1} culture)	Intracellular localization of the aggregate[b]
1	GFP-18Av8	344.2	CM
2	GFP-18Av9	335.2	C
3	GFP native	330.7	-
4	GFP-18Av11	324.9	C
5	GFP-18Av16	309.2	C
6	GFP-18Av17	299.0	C
7	GFP-18Arev	291.1	C
8	GFP-18Av10	277.0	C
9	GFP-18Av2	259.7	M
10	GFP-18Av3	258.6	CM
11	GFP-18Av18	246.6	CM
12	GFP-18Av14	236.5	C
13	GFP-18Av6	233.8	M
14	GFP-18Av4	229.7	M
15	GFP-18Av13	222.9	M
16	GFP-18Av5	208.7	M
17	GFP-18Av15	195.4	C
18	GFP-18Awt	142.6	M
19	GFP-18Av7	136.2	M
20	GFP-18Av12	98.7	M
21	GFP-18Av1	69.9	M

[a]GFP-18A fusions were ranked by the level of expression from highest to lowest.

[b]C: aggregate located in the cytoplasm; M: aggregate located around the cell membrane; CM: aggregate located both in the cytoplasm and around the cell membrane.

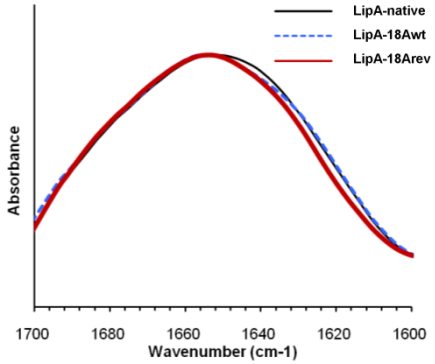

Fig. S1 FTIR spectra of wild type LipA protein (black), LipA-18Awt (blue dash line) and LipA-18Arev (red thick line). The amide region I between 1600 cm-1 and 1700 cm-1 are shown. The spectra are smoothed and scaled independently to be full scale on the absorbance axis.[53]

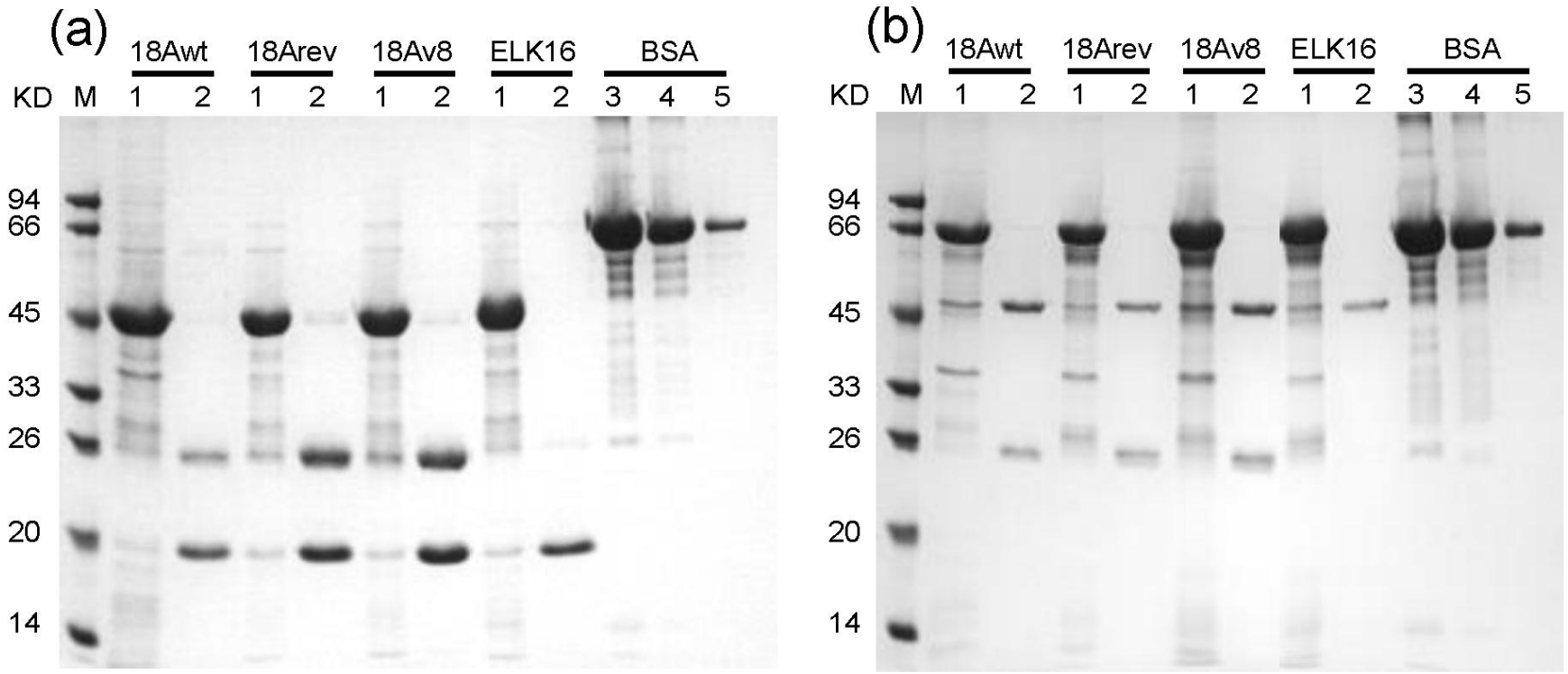

Fig. S2 Expression and cleavage of fusion proteins (target protein-intein-18Awt/18Arev/18Av8/ELK16). (a) LipA-I-18Awt/18Arev/18Av8/ELK16. (b) AMA-I-18Awt/18Arev/18Av8/ELK16. For both (a) and (b): lane 1, insoluble fraction of cell lysate; lane 2, soluble fraction of cleaved fusion protein; lane 3, 4 and 5, bovine serum albumin (BSA) standards, at 6, 3 and 0.75 μg per lane, respectively.