Supplementary information

for

Expression of cell-penetrating peptides fused to protein cargo

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Table S1. Oligonucleotide sequences

Oligonucleotide name	Sequence (5′ to 3′)	CPP/cargo/ expression partner
SacI-ATG-GFP-F	GCGATGGAGCTCAGTAAAGGAGAAGAACTTTTC	GFP
GFP-NotI-R	AATAAAGCGGCCGCTTTGTATAGTTCATCCATGC	
SacI-MBP-F	ATTGGGAGCTCAAAATCGAAGAAGGTAAACTGG	MBP
NotI-MBP-R	ATTGGGCGGCCGCCAGTCTGCGCGTCTTT	
SacI-BCCP-F	GCGATGGAGCTCATGGAAGCGCCAGCAGCAG	BCCP
BCCP-NotI-R	AATAAAGCGGCCGCCTCGATGACGACCAGCGGCTC	
EcoRI-FactorXa-MPG-Top1	AATTCATTGAGGGACGCGGCGCACTTTTCTTAGGGTT CCTTGGAGCCGCCGGGAG	MPG
EcoRI-FactorXa-MPG-Bottom1	CGGCGGCTCCAAGGAACCCTAAGAAAAGTGCGCCGC GTCCCTCAATG	
SacI-G$_4$S-MPG-Top2	GATGGGTGCCTGGTCCCAGCCAAAGAAGAAACGTAA AGTAGGAGGCGGTGGAAGCGAGCT	
SacI-G$_4$S-MPG-Bottom2	CGCTTCCACCGCCTCCTACTTTACGTTTCTTCTTTGGCT GGGACCAGG	
SacI-G$_4$S-Hst-5-Bottom2	CGCTTCCACCGCCTCCGTACCCGCGATGACTGTGGTG CTTTTCGTG	Hst-5
SacI-G$_4$S-Hst-5-Top2	CAAGTTCCACGAAAAGCACCACAGTCATCGCGGGTAC GGAGGCGGTGGAAGCGAGCT	
EcoRI-FactorXa-Hst-5-Bottom1	GAACTTGCGCTTATACCCGTGATGGCGTTTTGCATGA GAATCGCGTCCCTCAATG	
EcoRI-FactorXa-Hst-5-Top1	AATTCATTGAGGGACGCGATTCTCATGCAAAACGCCA TCACGGGTATAAGCG	
FactorXa-SynB-Top-1	AATTCATTGAGGGACGCCGCGGGGGGCGGCTTAGCTA TTCAAGACGGC	SynB
FactorXa-SynB-Bottom-1	GTCTTGAATAGCTAAGCCGCCCCCCGCGGCGTCCCTC AATG	
FactorXa-SynB-G$_4$S-Top	GGTTCTCCACTTCCACGGGCCGTGGAGGCGGTGGAAG CGAGCT	
FactorXa-SynB-G$_4$S-bottom	CGCTTCCACCGCCTCCACGGCCCGTGGAAGTGGAGAA CCGCC	
BamHI-GST-F	GCGATGGGATCCATGTCCCCTATACTAGGTTATTG	GST
GST-EcoRI-R	AATAAAGAATTCACCAGAACCACTAGTTGAAC	

Table S2. SynB-MBP: Tukey's multiple comparisons test for constant temperature (see Figure 2)

Temperature	Time	Mean difference	95% confidence interval of difference	Significant?	Level of significance[a]
37 °C	6 h vs. 10 h	0.02294	-0.2997 to 0.3456	No	ns
	6 h vs. 24 h	0.6182	0.2956 to 0.9408	Yes	***
	10 h vs. 24 h	0.5952	0.2726 to 0.9179	Yes	***
30 °C	6 h vs. 10 h	0.4202	0.09761 to 0.7428	Yes	*
	6 h vs. 24 h	0.5556	0.2330 to 0.8783	Yes	***
	10 h vs. 24 h	0.1354	-0.1872 to 0.4580	No	ns
20 °C	6 h vs. 10 h	0.002444	-0.3202 to 0.3251	No	ns
	6 h vs. 24 h	-0.1666	-0.4892 to 0.1560	No	ns
	10 h vs. 24 h	-0.1691	-0.4917 to 0.1535	No	ns

[a] Significance levels: ns for $p > 0.05$, * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$, and **** for $p \leq 0.0001$

Table S3. SynB-MBP: Tukey's multiple comparisons test for constant induction time (see Figure 2)

Time	Temperature	Mean difference	95% confidence interval of difference	Significant?	Level of significance
6 h	37 °C vs. 30 °C	-0.3069	-0.6295 to 0.01573	No	ns
	37 °C vs. 20 °C	0.4472	0.1246 to 0.7698	Yes	**
	30 °C vs. 20 °C	0.7541	0.4315 to 1.077	Yes	****
10 h	37 °C vs. 30 °C	0.09039	-0.2322 to 0.4130	No	ns
	37 °C vs. 20 °C	0.4267	0.1041 to 0.7493	Yes	**
	30 °C vs. 20 °C	0.3363	0.01371 to 0.6589	Yes	*
24 h	37 °C vs. 30 °C	-0.3694	-0.6920 to -0.04682	Yes	*
	37 °C vs. 20 °C	-0.3376	-0.6602 to -0.01500	Yes	*
	30 °C vs. 20 °C	0.03182	-0.2908 to 0.3544	No	ns

Table S4. Hst5-MBP: Tukey's multiple comparisons test for constant temperature (see Figure 2)

Temperature	Time	Mean difference	95% confidence interval of difference	Significant?	Level of significance
37 °C	6 h vs. 10 h	0.8048	0.1365 to 1.473	Yes	*
	6 h vs. 24 h	1.126	0.4572 to 1.794	Yes	**
	10 h vs. 24 h	0.3207	-0.3475 to 0.9890	No	ns
30 °C	6 h vs. 10 h	-0.07835	-0.7466 to 0.5899	No	ns
	6 h vs. 24 h	0.1295	-0.5388 to 0.7977	No	ns
	10 h vs. 24 h	0.2078	-0.4604 to 0.8761	No	ns
20 °C	6 h vs. 10 h	-0.3876	-1.056 to 0.2806	No	ns
	6 h vs. 24 h	-0.3456	-1.014 to 0.3227	No	ns
	10 h vs. 24 h	0.04203	-0.6262 to 0.7103	No	ns

Table S5. Hst5-MBP: Tukey's multiple comparisons test for constant induction time (see Figure 2)

Time	Temperature	Mean difference	95% Confidence Interval of difference	Significant?	Level of significance
6 h	37 °C vs. 30 °C	0.7793	0.2324 to 1.326	Yes	**
	37 °C vs. 20 °C	1.351	0.8038 to 1.898	Yes	****
	30 °C vs. 20 °C	0.5714	0.02448 to 1.118	Yes	*
10 h	37 °C vs. 30 °C	-0.1038	-0.6507 to 0.4431	No	ns
	37 °C vs. 20 °C	0.1583	-0.3886 to 0.7052	No	ns
	30 °C vs. 20 °C	0.2621	-0.2848 to 0.8090	No	ns
24 h	37 °C vs. 30 °C	-0.2167	-0.7636 to 0.3302	No	ns
	37 °C vs. 20 °C	-0.1204	-0.6673 to 0.4265	No	ns
	30 °C vs. 20 °C	0.09629	-0.4506 to 0.6432	No	ns

Table S6. MPG-MBP: Tukey's multiple comparisons test for constant temperature (see Figure 2)

Temperature	Time	Mean difference	95% confidence interval of difference	Significant?	Level of significance
37 °C	6 h vs. 10 h	0.2672	-0.2934 to 0.8278	No	ns
	6 h vs. 24 h	0.4546	-0.1059 to 1.015	No	ns
	10 h vs. 24 h	0.1874	-0.3732 to 0.7480	No	ns
30 °C	6 h vs. 10 h	-0.3747	-0.9352 to 0.1859	No	ns
	6 h vs. 24 h	-0.1389	-0.6994 to 0.4217	No	ns
	10 h vs. 24 h	0.2358	-0.3248 to 0.7964	No	ns
20 °C	6 h vs. 10 h	-0.4550	-1.016 to 0.1056	No	ns
	6 h vs. 24 h	-0.4688	-1.029 to 0.09177	No	ns
	10 h vs. 24 h	-0.01383	-0.5744 to 0.5467	No	ns

Table S7. MPG-MBP: Tukey's multiple comparisons test for constant induction time (see Figure 2)

Time	Temperature	Mean difference	95% confidence interval of difference	Significant?	Level of significance
6 h	37 °C vs. 30 °C	0.3613	-0.08208 to 0.8046	No	ns
	37 °C vs. 20 °C	0.4847	0.04135 to 0.9280	No	ns
	30 °C vs. 20 °C	0.1234	-0.3199 to 0.5668	No	ns
10 h	37 °C vs. 30 °C	-0.2806	-0.7240 to 0.1627	No	ns
	37 °C vs. 20 °C	-0.2375	-0.6808 to 0.2058	No	ns
	30 °C vs. 20 °C	0.04311	-0.4002 to 0.4865	No	ns
24 h	37 °C vs. 30 °C	-0.2322	-0.6756 to 0.2111	No	ns
	37 °C vs. 20 °C	-0.4387	-0.8821 to 0.004601	No	ns
	30 °C vs. 20 °C	-0.2065	-0.6498 to 0.2368	No	ns

Table S8. All CPP-MBP constructs: Tukey's multiple comparisons test for constant temperature and induction time (see Figure 3)

Constructs	Mean difference	95% confidence interval of difference	Significant?	Level of significance
MBP vs. SynB-MBP	0.5826	-0.2880 to 1.453	No	ns
MBP vs. Hst5-MBP	-0.2598	-1.130 to 0.6108	No	ns
MBP vs. MPG-MBP	0.3084	-0.5621 to 1.179	No	ns
SynB-MBP vs. Hst5-MBP	-0.8424	-1.713 to 0.02820	No	ns
SynB-MBP vs. MPG-MBP	-0.2741	-1.145 to 0.5964	No	ns
Hst5-MBP vs. MPG-MBP	0.5682	-0.3023 to 1.439	No	ns

Table S9. SynB-GFP: Tukey's multiple comparisons test for constant temperature (see Figure 4)

Temperature	Time	Mean difference	95% confidence interval of difference	Significant?	Level of significance
37 °C	6 h vs. 10 h	-0.5043	-1.147 to 0.1390	No	ns
	6 h vs. 24 h	-0.05636	-0.6996 to 0.5869	No	ns
	10 h vs. 24 h	0.4479	-0.1953 to 1.091	No	ns
30 °C	6 h vs. 10 h	-0.2950	-0.9382 to 0.3482	No	ns
	6 h vs. 24 h	0.1632	-0.4800 to 0.8065	No	ns
	10 h vs. 24 h	0.4583	-0.1850 to 1.101	No	ns
20 °C	6 h vs. 10 h	-0.4327	-1.076 to 0.2106	No	ns
	6 h vs. 24 h	0.04216	-0.6011 to 0.6854	No	ns
	10 h vs. 24 h	0.4748	-0.1684 to 1.118	No	ns

Table S10. SynB-GFP: Tukey's multiple comparisons test for constant induction time (see Figure 4)

Time	Temperature	Mean difference	95% confidence interval of difference	Significant?	Level of significance
6 h	37 °C vs. 30 °C	-0.02688	-0.7052 to 0.6515	No	ns
	37 °C vs. 20 °C	0.2870	-0.3914 to 0.9653	No	ns
	30 °C vs. 20 °C	0.3139	-0.3645 to 0.9922	No	ns
10 h	37 °C vs. 30 °C	0.1823	-0.4960 to 0.8607	No	ns
	37 °C vs. 20 °C	0.3586	-0.3198 to 1.037	No	ns
	30 °C vs. 20 °C	0.1762	-0.5021 to 0.8546	No	ns
24 h	37 °C vs. 30 °C	0.1927	-0.4856 to 0.8711	No	ns
	37 °C vs. 20 °C	0.3855	-0.2928 to 1.064	No	ns
	30 °C vs. 20 °C	0.1928	-0.4856 to 0.8711	No	ns

Table S11. Hst5-GFP: Tukey's multiple comparisons test for constant temperature (see Figure 4)

Temperature	Time	Mean difference	95% confidence interval of difference	Significant?	Level of significance
37 °C	6 h vs. 10 h	-0.06067	-0.5441 to 0.4228	No	ns
	6 h vs. 24 h	0.1137	-0.3698 to 0.5971	No	ns
	10 h vs. 24 h	0.1743	-0.3091 to 0.6578	No	ns
30 °C	6 h vs. 10 h	-0.2430	-0.7265 to 0.2405	No	ns
	6 h vs. 24 h	-0.09200	-0.5755 to 0.3915	No	ns
	10 h vs. 24 h	0.1510	-0.3325 to 0.6345	No	ns
20 °C	6 h vs. 10 h	-0.1280	-0.6115 to 0.3555	No	ns
	6 h vs. 24 h	-0.4080	-0.8915 to 0.07547	No	ns
	10 h vs. 24 h	-0.2800	-0.7635 to 0.2035	No	ns

Table S12. Hst5-GFP: Tukey's multiple comparisons test for constant induction time (see Figure 4)

Time	Temperature	Mean difference	95% confidence interval of difference	Significant?	Level of significance
6 h	37 °C vs. 30 °C	-0.2447	-0.7281 to 0.2388	No	ns
	37 °C vs. 20 °C	0.2263	-0.2571 to 0.7098	No	ns
	30 °C vs. 20 °C	0.4710	-0.01247 to 0.9545	No	ns
10 h	37 °C vs. 30 °C	-0.4270	-0.9105 to 0.05647	No	ns
	37 °C vs. 20 °C	0.1590	-0.3245 to 0.6425	No	ns
	30 °C vs. 20 °C	0.5860	0.1025 to 1.069	Yes	*
24 h	37 °C vs. 30 °C	-0.4503	-0.9338 to 0.03314	No	ns
	37 °C vs. 20 °C	-0.2953	-0.7788 to 0.1881	No	ns
	30 °C vs. 20 °C	0.1550	-0.3285 to 0.6385	No	ns

Table S13. MPG-GFP: Tukey's multiple comparisons test for constant temperature (see Figure 4)

Temperature	Time	Mean difference	95% confidence interval of difference	Significant?	Level of significance
37 °C	6 h vs. 10 h	0.3703	-0.2678 to 1.008	No	ns
	6 h vs. 24 h	0.4350	-0.2031 to 1.073	No	ns
	10 h vs. 24 h	0.06472	-0.5734 to 0.7028	No	ns
30 °C	6 h vs. 10 h	0.7439	0.1058 to 1.382	Yes	*
	6 h vs. 24 h	0.8754	0.2373 to 1.513	Yes	**
	10 h vs. 24 h	0.1315	-0.5065 to 0.7696	No	ns
20 °C	6 h vs. 10 h	0.1496	-0.4884 to 0.7877	No	ns
	6 h vs. 24 h	-0.6738	-1.312 to -0.03572	Yes	*
	10 h vs. 24 h	-0.8234	-1.462 to -0.1854	Yes	*

Table S14. MPG-GFP: Tukey's multiple comparisons test for constant induction time (see Figure 4)

Time	Temperature	Mean difference	95% confidence interval of difference	Significant?	Level of significance
6 h	37 °C vs. 30 °C	-1.211	-1.789 to -0.6341	Yes	***
	37 °C vs. 20 °C	-0.8813	-1.459 to -0.3041	Yes	**
	30 °C vs. 20 °C	0.3301	-0.2472 to 0.9073	No	ns
10 h	37 °C vs. 30 °C	-0.8378	-1.415 to -0.2606	Yes	**
	37 °C vs. 20 °C	-1.102	-1.679 to -0.5248	Yes	***
	30 °C vs. 20 °C	-0.2642	-0.8414 to 0.3131	No	ns
24 h	37 °C vs. 30 °C	-0.7710	-1.348 to -0.1938	Yes	**
	37 °C vs. 20 °C	-1.990	-2.567 to -1.413	Yes	****
	30 °C vs. 20 °C	-1.219	-1.796 to -0.6419	Yes	***

Table S15. All CPP-GFP constructs: Tukey's multiple comparisons test for constant temperature and induction time (see Figure 5)

Constructs	Mean difference	95% confidence interval of difference	Significant?	Level of significance
GFP vs. Hst-5-GFP	0.2477	-0.7626 to 1.258	No	ns
GFP vs. MPG-GFP	1.183	0.1725 to 2.193	Yes	*
GFP vs. SynB-GFP	1.774	0.7634 to 2.784	Yes	**
Hst-5-GFP vs. MPG-GFP	0.9352	-0.07519 to 1.946	No	ns
Hst-5-GFP vs. SynB-GFP	1.526	0.5156 to 2.536	Yes	**
MPG-GFP vs. SynB-GFP	0.5908	-0.4195 to 1.601	No	ns

Table S16. All CPP-BCCP constructs: Tukey's multiple comparisons test for constant temperature (see Figure 6)

Time	Constructs	Mean difference	95% confidence interval of difference	Significant?	Level of significance
6 h	SynB-BCCP vs. MPG-BCCP	-0.02954	-0.6309 to 0.5718	No	ns
	SynB-BCCP vs. Hst5-BCCP	1.287	0.6852 to 1.888	Yes	****
	MPG-BCCP vs. Hst5-BCCP	1.316	0.7148 to 1.918	Yes	****
8 h	SynB-BCCP vs. MPG-BCCP	-0.5593	-1.161 to 0.04212	No	ns
	SynB-BCCP vs. Hst5-BCCP	1.363	0.7613 to 1.964	Yes	****
	MPG-BCCP vs. Hst5-BCCP	1.922	1.321 to 2.523	Yes	****
24 h	SynB-BCCP vs. MPG-BCCP	-0.6391	-1.240 to -0.03768	Yes	*
	SynB-BCCP vs. Hst5-BCCP	0.6930	0.09165 to 1.294	Yes	*
	MPG-BCCP vs. Hst5-BCCP	1.332	0.7307 to 1.933	Yes	****

Table S17. All CPP-BCCP constructs: Tukey's multiple comparisons test with comparisons at different induction times (see Figure 6)

Constructs	Time	Mean difference	95% confidence interval of difference	Significant?	Level of significance
SynB-BCCP	6 h vs. 8 h	-0.05898	-0.7586 to 0.6407	No	ns
	6 h vs. 24 h	0.7096	0.009950 to 1.409	Yes	*
	8 h vs. 24 h	0.7686	0.06893 to 1.468	Yes	*
MPG-BCCP	6 h vs. 8 h	-0.5887	-1.288 to 0.1110	No	ns
	6 h vs. 24 h	0.1001	-0.5996 to 0.7997	No	ns
	8 h vs. 24 h	0.6888	-0.01087 to 1.388	No	ns
Hst5-BCCP	6 h vs. 8 h	0.01706	-0.6826 to 0.7167	No	ns
	6 h vs. 24 h	0.1160	-0.5836 to 0.8157	No	ns
	8 h vs. 24 h	0.09898	-0.6007 to 0.7986	No	ns

Table S18. All CPP-cargo constructs: Tukey's multiple comparisons test for constant temperature and induction time (see Figure 7)

Constructs		Mean difference	95% confidence interval of difference	Significant?	Level of significance
SynB-MBP	vs. Hst-5-MBP	-0.2381	-0.8350 to 0.3589	No	ns
	vs. MPG-MBP	-0.3939	-0.9909 to 0.2030	No	ns
	vs. SynB-GFP	0.8750	0.2780 to 1.472	Yes	**
	vs. Hst-5-GFP	-0.04628	-0.6433 to 0.5507	No	ns
	vs. MPG-GFP	-0.08235	-0.6793 to 0.5146	No	ns
	vs. SynB-BCCP	-0.3570	-0.9540 to 0.2400	No	ns
	vs. Hst5-BCCP	0.9410	0.3440 to 1.538	Yes	***
	vs. MPG-BCCP	-0.6737	-1.271 to -0.07670	Yes	*
Hst-5-MBP	vs. MPG-MBP	-0.1559	-0.7529 to 0.4411	No	ns
	vs. SynB-GFP	1.113	0.5161 to 1.710	Yes	***
	vs. Hst-5-GFP	0.1918	-0.4052 to 0.7888	No	ns
	vs. MPG-GFP	0.1557	-0.4413 to 0.7527	No	ns
	vs. SynB-BCCP	-0.1189	-0.7159 to 0.4781	No	ns
	vs. Hst5-BCCP	1.179	0.5821 to 1.776	Yes	****
	vs. MPG-BCCP	-0.4356	-1.033 to 0.1614	No	ns
MPG-MBP	vs. SynB-GFP	1.269	0.6720 to 1.866	Yes	****
	vs. Hst-5-GFP	0.3477	-0.2493 to 0.9446	No	ns
	vs. MPG-GFP	0.3116	-0.2854 to 0.9086	No	ns
	vs. SynB-BCCP	0.03697	-0.5600 to 0.6339	No	ns
	vs. Hst5-BCCP	1.335	0.7380 to 1.932	Yes	****
	vs. MPG-BCCP	-0.2797	-0.8767 to 0.3173	No	ns
SynB-GFP	vs. Hst-5-GFP	-0.9213	-1.518 to -0.3243	Yes	**
	vs. MPG-GFP	-0.9573	-1.554 to -0.3604	Yes	***
	vs. SynB-BCCP	-1.232	-1.829 to -0.6350	Yes	****
	vs. Hst5-BCCP	0.06600	-0.5310 to 0.6630	No	ns
	vs. MPG-BCCP	-1.549	-2.146 to -0.9517	Yes	****
Hst-5-GFP	vs. MPG-GFP	-0.03607	-0.6330 to 0.5609	No	ns
	vs. SynB-BCCP	-0.3107	-0.9077 to 0.2863	No	ns
	vs. Hst5-BCCP	0.9873	0.3903 to 1.584	Yes	***
	vs. MPG-BCCP	-0.6274	-1.224 to -0.03042	Yes	*
MPG-GFP	vs. SynB-BCCP	-0.2746	-0.8716 to 0.3223	No	ns
	vs. Hst5-BCCP	1.023	0.4264 to 1.620	Yes	***
	vs. MPG-BCCP	-0.5913	-1.188 to 0.005652	No	ns
SynB-BCCP	vs. Hst5-BCCP	1.298	0.7010 to 1.895	Yes	****
	vs. MPG-BCCP	-0.3167	-0.9137 to 0.2803	No	ns
Hst5-BCCP	vs. MPG-BCCP	-1.615	-2.212 to -1.018	Yes	****