Supplemental Material

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Supplemental Figures

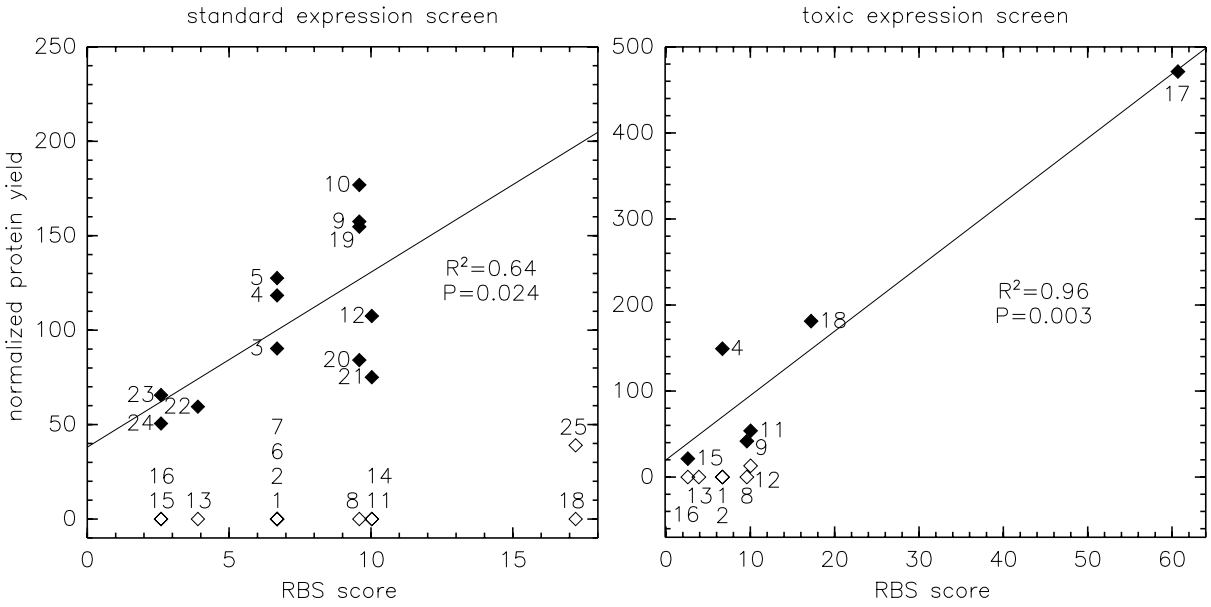

Figure S1: Control of protein expression by RBS obstruction. Total yields were normalized to cell density and are compared to the RBS calculator score reporting on the formation of mRNA secondary structure. Only filled data points above a low threshold were considered for the linear regression. The RBS score (here divided by 1000) considers only a sequence window surrounding the translation initiation site and is thus identical for constructs that share the same leading domain. Protein IDs are given next (above, below) to each data point (stacked if several constructs with zero expression share the same RBS score). Protein 17 showed no expression during the standard screen and is omitted from the left figure.

Supplemental Tables

Table S1: Yields of final assembly reactions.

ID	part A[a]		part B [b]	c.f.u.[c]	% correct[d]	failures[e] / comments
P01	T7>-FKBP∼ gLuc-frag 1	+	$\bowtie H_6 <$	200	100	
P02	T7>-FKBP∼ gLuc-frag 2	+	$\bowtie H_6 <$	200	25	
P03	T7>-FKBP∼ mCitrine	+	$\bowtie H_6 <$	200	75	
P04	T7>-FKBP∼ mCherry	+	$\bowtie H_6 <$	18	25	
P05	T7>-FKBP∼ mCerulean	+	$\bowtie H_6 <$	30	75	
P06	T7>-FKBP∼ bla-frag 1	+	$\bowtie H_6 <$	70	75	
P07	T7>-FKBP∼ bla-frag 2	+	$\bowtie H_6 <$	50	50	
P08	T7>-FRB∼ gLuc-frag 1	+	$\bowtie H_6 <$	70	50	
P09	T7>-FRB∼ gLuc-frag 2	+	$\bowtie H_6 <$	70	75	
P10	T7>-FRB∼ mCherry	+	$\bowtie H_6 <$	100	100	
P11	T7>-ZipE34∼ gLuc-frag 1	+	$\bowtie H_6 <$	200	50	
P12	T7>-ZipE34∼ mCerulean	+	$\bowtie H_6 <$	6	100	
P13	T7>-ZipR34∼ gLuc-frag 2	+	$\bowtie H_6 <$	2	100	
P14	T7>-ZipE34∼ bla-frag 1	+	$\bowtie H_6 <$	15	50	
P15	T7>-LOV2∼ gLuc-frag 1	+	$\bowtie H_6 <$	80	75	
P16	T7>-LOV2∼ gLuc-frag 2	+	$\bowtie H_6 <$	70	75	
P17	T7>-gLuc-frag 1∼ LOV2	+	$\bowtie H_6 <$	10	50	
P18	T7>-mCitrine∼ LOV2	+	$\bowtie H_6 <$	200(50)	100(75)	
P19	T7>-FRB∼ mCitrine	+	$\bowtie H_6 <$	6	50	
P20	T7>-FRB∼ mCerulean	+	$\bowtie H_6 <$	160	75	
P21	T7>-ZipE34∼ mCherry	+	$\bowtie H_6 <$	40(140)	50(25)	1
P22	T7>-ZipR34∼ mCitrine	+	$\bowtie H_6 <$	160	75	1
P23	T7>-LOV2∼ mCitrine	+	$\bowtie H_6 <$	11	100	2
P24	T7>-LOV2∼ mCerulean	+	$\bowtie H_6 <$	7	100	1
P25	T7>-mCerulean∼ LOV2	+	$\bowtie H_6 <$	190	100	
other constructs						
	T7>-FKBP	+	$\bowtie H_6 <$	150	75	ok but not used
	T7>-gLuc-frag1∼ LOV2	+	$\bowtie H_6 <$	50	0	2
	T7>-FRB∼ bla-frag1	+	$\bowtie H_6 <$	400	0	
	T7>-bla-frag2	+	$\bowtie H_6 <$	800	0	
	T7>-FRB∼ bla-frag2	+	$\bowtie H_6 <$	20	0	1
	T7>-ZipE34∼ bla-frag2	+	$\bowtie H_6 <$	1500	0	A and B sequenced

Success rate in the final round of assembly reactions. Assembly reactions were performed as described. Both part A and B were in one of three pSB1A*3F backbones and were assembled into pSB1AC3F or pSB1AK3F. 4 clones each were screened by colony PCR. Assemblies of additional target constructs which are not mentioned in this paper, are given as "other constructs".
[a] part A of assembly reaction, usually containing T7start, a domain, the 10aa linker (∼) followed by another domain; [b] part B of assembly reaction, always preSCsite-His6-T7stop ($\bowtie H_6 <$); (see table 3 for part nicknames). [c] colony forming units after transformation; [d] percent of positive clones (4 clones were screened per assembly); [e] number of previous failures to assemble A and B;

Table S2: Yields of intermediate assembly reactions involving pMA and pSB1AT3F vector backbones.

part A[a]	+	part B[b]	c.f.u.[c]	% correct[d]	comment
FKBP	+	∼GLuc-frag1	140	50	
FKBP	+	∼GLuc-frag2	40	75	
FKBP	+	∼Citrine	50	75	
FKBP	+	∼Cherry	60	50	
FKBP	+	∼Cerulean	n.d.	50	
FKBP	+	∼blac-frag1	n.d.	25	
FKBP	+	∼blac-frag2	n.d.	25	
FRB	+	∼GLuc-frag1	180	75	
FRB	+	∼GLuc-frag2	140	50	
FRB	+	∼Citrine	n.d.	0	later: T7start-FRB + ∼Citrine → 100% correct
FRB	+	∼Cherry	n.d.	25	
FRB	+	∼Cerulean	n.d.	50	
FRB	+	∼blac-frag1	51	50	
FRB	+	∼blac-frag2	n.d.	50	

Success rate in an intermediate round of assembly reactions where part A was provided in the pMA vector backbone and part B in the pSB1AT3F vector backbone. Reactions were performed as described. 4 clones each were screened by colony PCR.
[a] part A of assembly reaction (in pMA) [b] part B of assembly reaction, always 10aa linker (∼) plus a domain; (see table 3 for part nicknames). [c] colony forming units after transformation (n.d. = not determined); [d] percent of positive clones;

Table S3: Yields of initial assembly reactions involving short parts.

part A[a]	+	part B[b]	c.f.u.[c]	% correct[d]	failures[e]
StrepII (pMA)	+	T7stop (pMA)	n.d.	0	
GST (pMA)	+	T7stop (pMA)	n.d.	0	
preSC-Strep (pSB1AC3F)	+	T7stop (pMA)	2000	25	
preSC-GST (pSB1AC3F)	+	T7stop (pMA)	n.d.	50	
preSC (pMA)	+	His6 (pMA)	180	0	
preSC (pMA)	+	His6-T7stop (pSB1AT3F)	24	25	2

Success rate in initial rounds of assembly reactions where part A or B are short (except Gst, T7stop) or provided in pMA backbone. Reactions were performed as described. 4 clones each were screened by colony PCR.
[a] part A of assembly reaction, vector backbone given in brackets [b] part B of assembly reaction, vector backbone given in brackets; (see table 3 for part nicknames) [c] colony forming units after transformation (n.d. = not determined); [d] percent of positive clones; [e] number of previous failures to assemble A and B;

Sequences of final protein constructs

Note, sequences of basic parts and final constructs are deposited at the Registry of Standard Biological Parts (http://partsregistry.org) and are available under the accession numbers given in the main article. We here include the sequences of final protein constructs for reference. The sequences include the (modified, RFC 25) BioBrick prefix (uppercase), followed by the T7 start casette that also contains the leading ATG, the protein coding sequence with intervening assembly scars (uppercase) and the T7 terminator starting with two STOP codons. Each sequence ends with the RFC 25 BioBrick suffix (uppercase). Note that prefix and suffix sequences do not, technically, belong to the part.

Fasta headers of each sequence provide (1) the lab-internal part ID (on http://brickit.crg.es), (2) protein ID used in the article, (3) the MIT part ID (on http://partsregistry.org), (4) a short textual description of the part composition.

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