SUPPLEMENT

Identification of permissive sites

The identification of permissive sites in *aadA* and *nat1* was performed in similar manner as for APH described in Material and Methods.

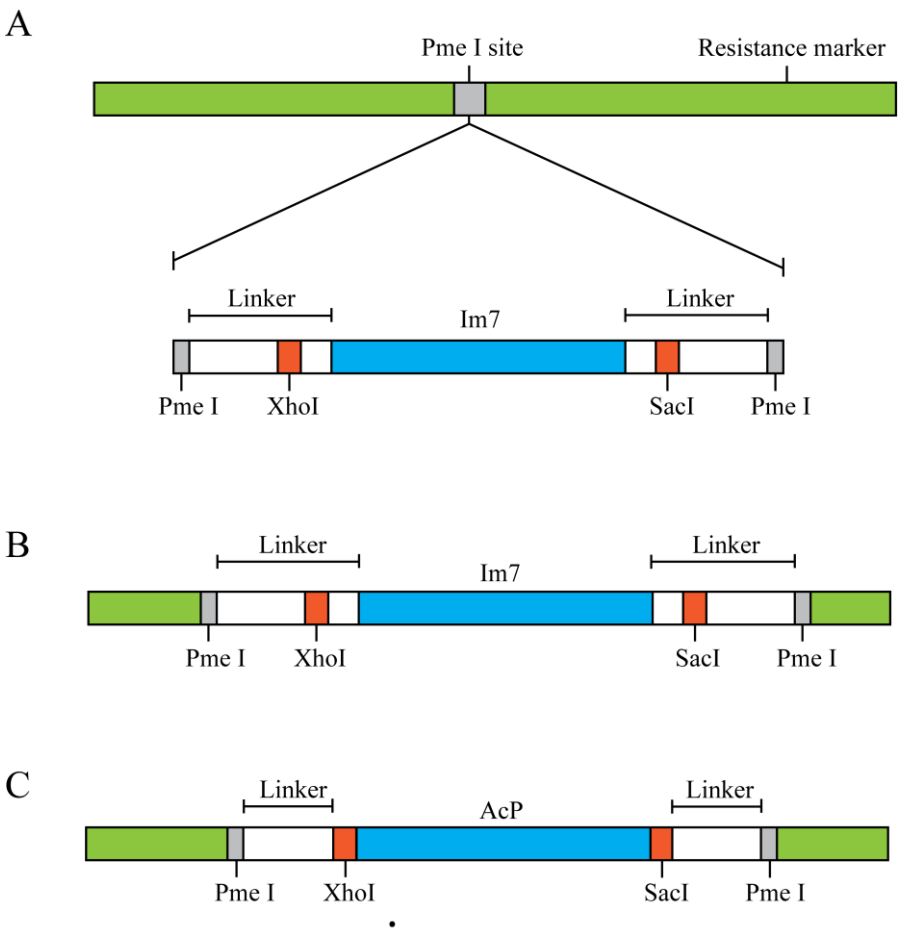
Successful transposon insertions in aadA could be detected loss of spectinomycin resistance. We identified 5000 clones that had lost their spectinomycin resistance, indicating transposon integration into aadA or its promoter region. To confirm the variability of our library, we sequenced a subset of clones and found that transposon insertions appeared to be distributed randomly throughout the ANT coding sequence (Fig. 6A). If these insertions are assumed to be completely random, we expect near complete coverage of the possible insertion sites within *aadA*, allowing us to screen through many possible sites for those permissive for insertion.

The plasmids of spectinomycin sensitive clones were pooled and the transposon was removed from *aadA* by digesting with restriction endonuclease Pme I. Again, after religation of the plasmids, a pentapeptide insertion scar that includes a Pme I restriction site remains at the former transposon integration site.

Transformation of the library and selection for regained resistance to 100 μg/ml spectinomycin only allows for the growth of colonies whose pentapetide insertion is in an ANT site that is at least partially permissive. Clones with insertions that significantly disturb ANT folding or function will not grow on 100 μg/ml spectinomycin. The wild-type pAMS1 plasmid confers resistance to more than 10,000 μg/ml spectinomycin. Sequencing of 40 resistant clones revealed three distinct hotspots in the protein that are permissive for pentapeptide insertion (Supplementary Fig. S4B)

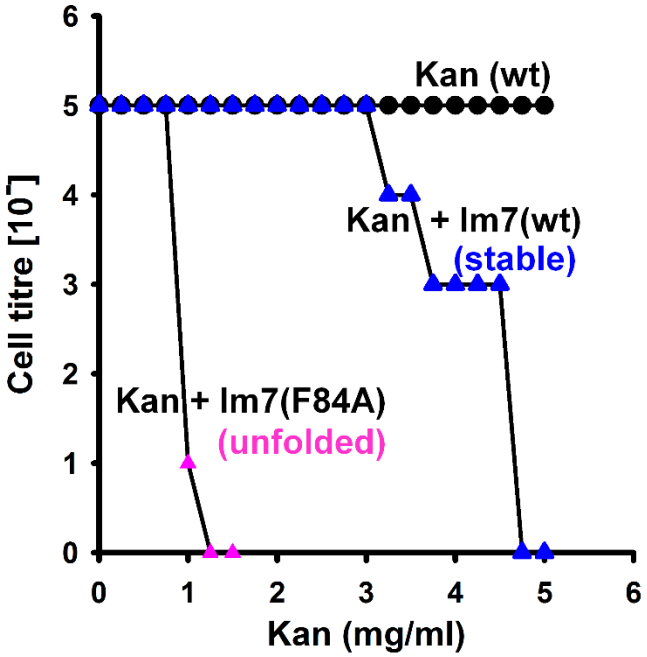
To clone Im7 into the pentapetide insertion library, plasmids from the library were pooled, purified, and digested with Pme I (which cuts inside the pentapetide insertion), then ligated with wild-type Im7 flanked by 17 amino acid GS linkers. The resulting plasmids were transformed into *E. coli* and selected on plates containing 100 μg/ml spectinomycin, only allowing growth of clones that had Im7 insertions in a permissive site in ANT.

Nat1 linker scanning was done with the Mutation Generation System (MGS) Kit (Thermoscientific), which inserts Not I-containing linkers instead of Pme I-containing linkers.



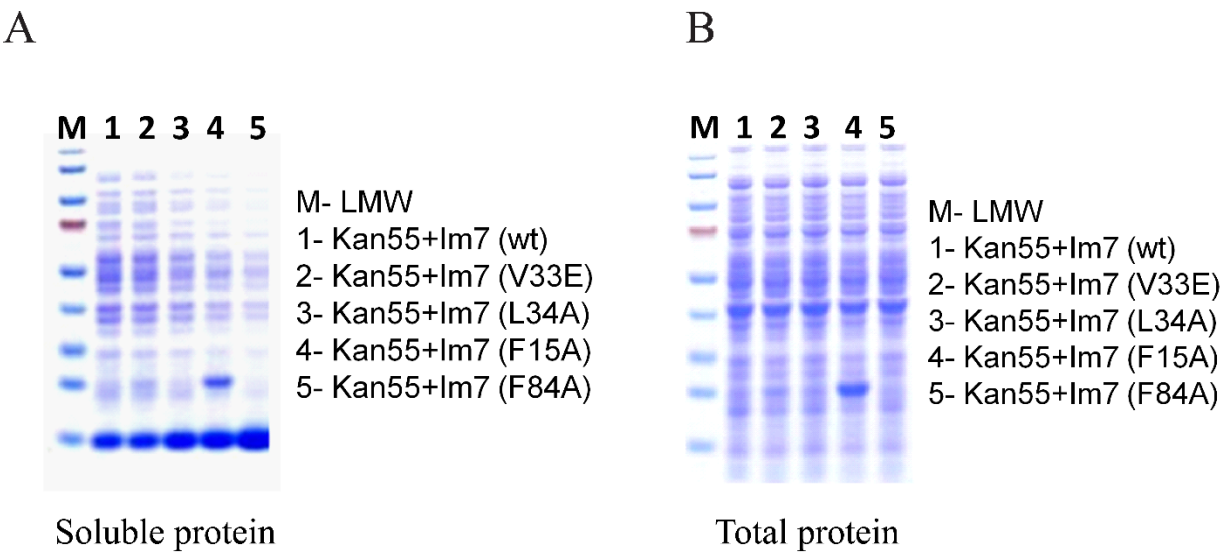
Supplementary Fig. S1:

A The pentapetide library of the resistance markers ANT and Kan^R (green) contains a Pme I site (grey) that is left behind after transposon excision. The Im7 construct we inserted into the pentapeptide library has Pme I sites (grey) on both sides of the protein and flexible GS-linkers (white). **B** The ANT biosensor after the insertion of the Im7 construct in the pentapeptide library (pAMS2 has the insertion at amino acid 155 of ANT). The Im7 construct is set up in such a way that the guest protein can be easily switched. The GS linkers contain Xho I and Sac I restriction sites (red), allowing directional cloning of new guest proteins. By digest with the two enzymes the majority of the linker is left behind in the resistance marker and allows simple substitution of new guest proteins. **C** ANT biosensor after Im7 was exchanged with AcP (pAMS34).



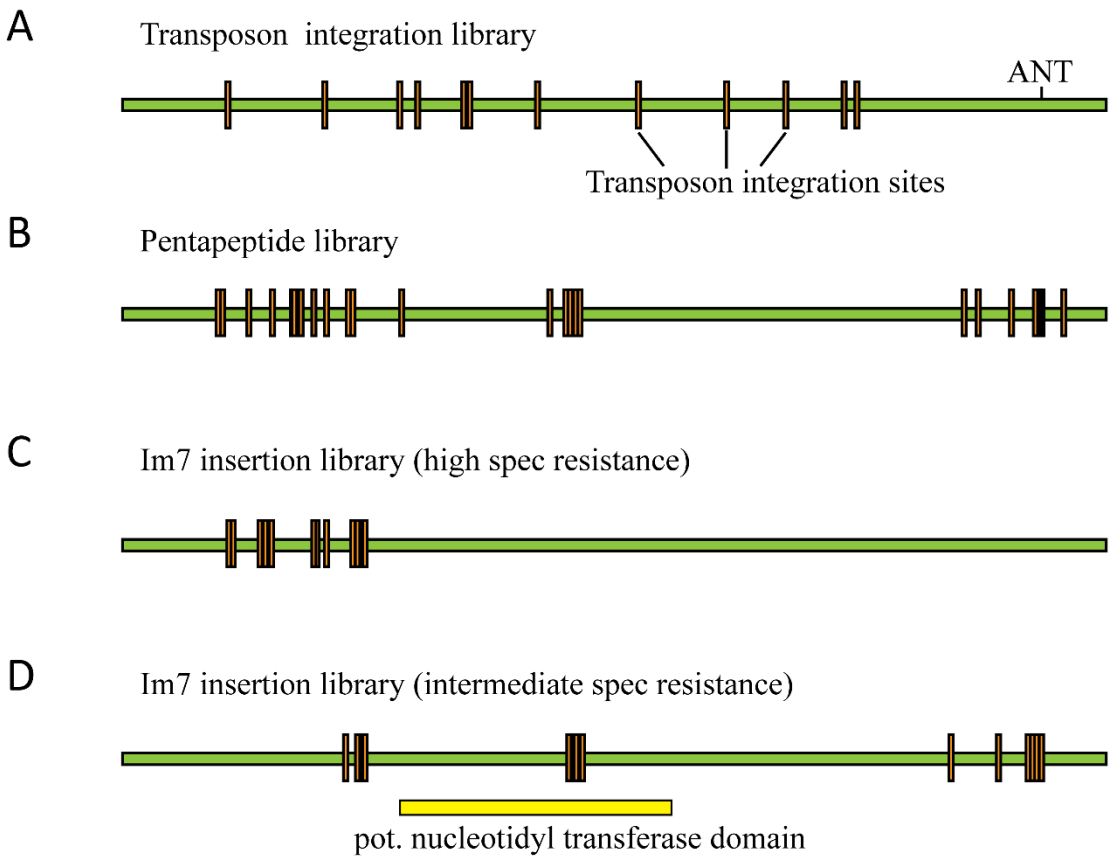
Supplementary Fig. S2

Comparison of antibiotic resistance in the kanamycin based biosensor. Cells expressing The unfolded Im7 variant F84A have a much lower antibiotic resistance than cells expressing the biosensor with Im7 wt. Maximal cell dilution of the cultures expressing Im7 (WT) and (F84A)-sandwich fusion at position 55 that allowed growth on different concentration of kanamycin shown with blue triangle and purple triangle, respectively. Bl21 (DE3)-RIPL strain expressing wt Im7 (stable) (AM235) and its unfolded variant F84A (AM236) fused at position 55 of the APH protein . Cultures were grown to mid-log phase and normalized to A_{600}=1. Cultures were 10 fold serially diluted from 10^0 to 10^5 were spotted on various concentration of kanamycin containing LB plates. Incubation was made 24 hrs at 37^{o}C. At each dilution, growth or no growth was counted on each kanamycin plates to calculate MIC values.



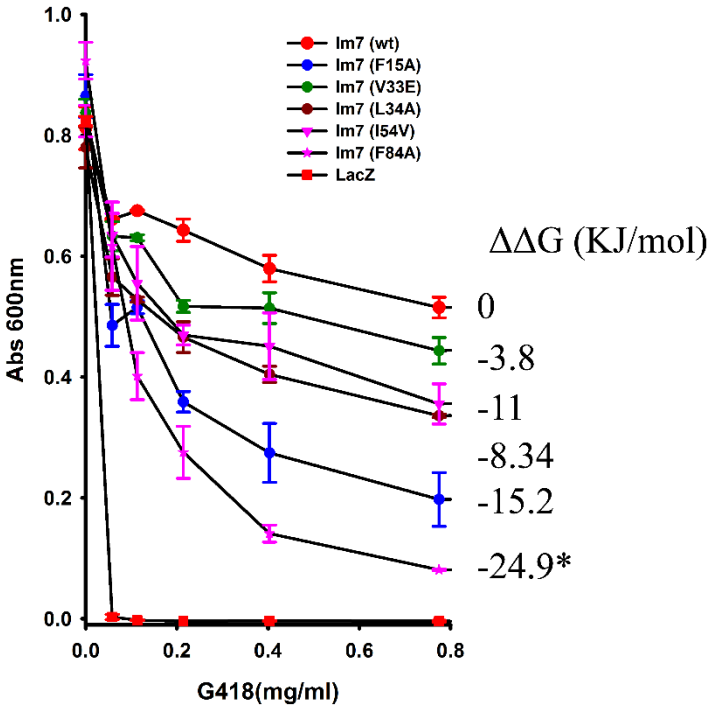
Supplement Fig. S3:

Loading control of soluble and total cell extract. (A) Soluble protein from cultures expressing Kan::Im7 variants was extracted by enzymatic treatment and glass bead grilling. Equal amount of soluble protein was analyzed on SDS-PAGE by Coomassie Blue staining (B) Whole cell expressing Kan::Im7 variants were boiled with SDS-loading dye and 15 ul was analyzed on SDS-PAGE by Coomassie Blue staining. For both A and B lane 1, AM235; lane 2, AM240; lane 3, AM 258; lane 4, AM247 and lane 5, AM236



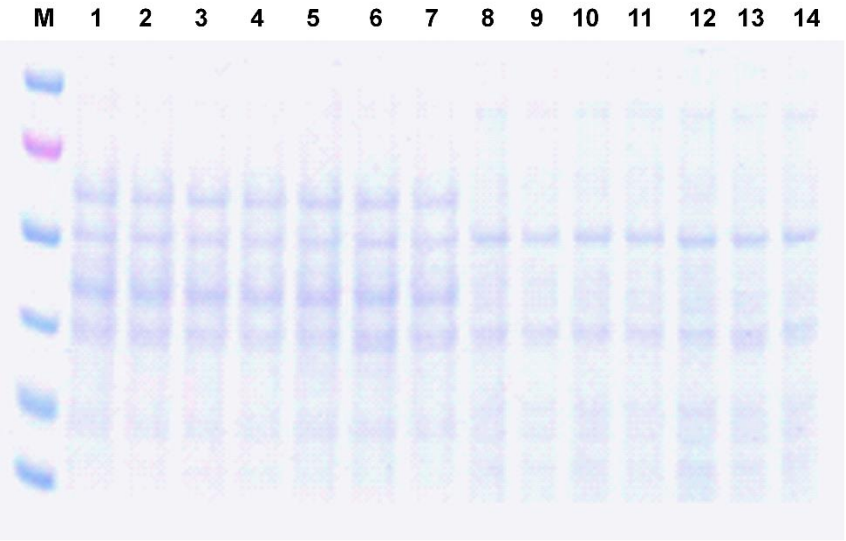
Supplementary Fig. S4

Transposon insertion sites during the identification of permissive sites in ANT. (A) Transposon integration sites (orange) seem to be randomly distributed throughout the ANT coding sequence (green). (B) Permissive sites in the pentapeptide library (orange) are found in three hotspots in ANT. (C) Im7 insertion sites (orange) and high spectinomycin resistance have insertions exclusively in the N terminus of ANT. (D) Im7 insertions with intermediate spectinomycin resistance have insertions in three hotspots of ANT. While insertions in the middle and the C terminus were all in-frame, all insertions in the N terminus led to frame shifts in ANT. It is possible that these clones use a downstream start codon to express a truncated version of ANT that confers lower levels of spectinomycin resistance.



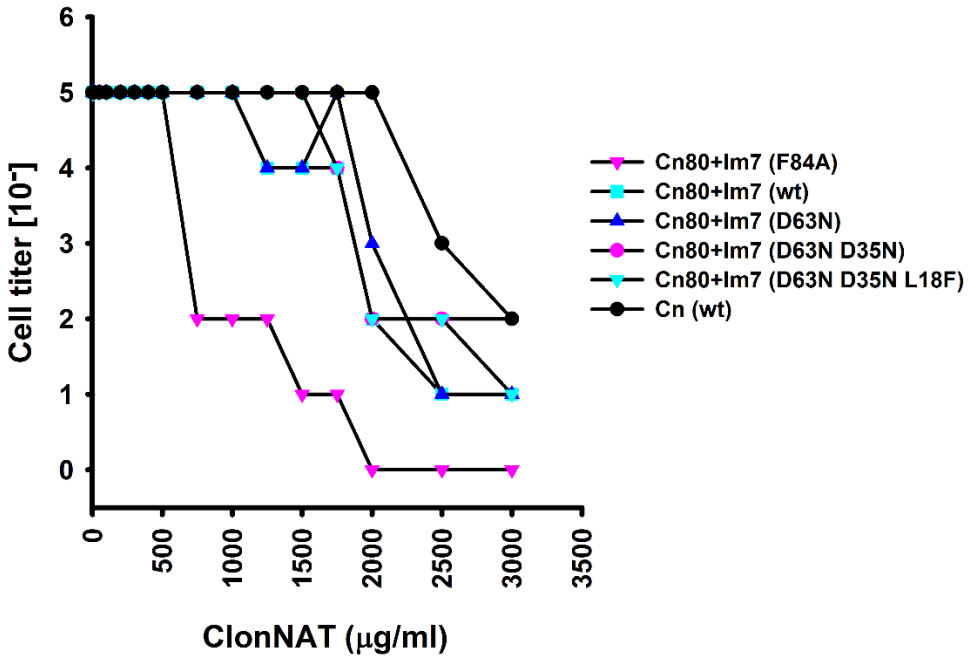
Supplementary Fig. S5:

Growth inhibition in S. cerevisiae expressing tripartite fusion under G418 selection pressure. From top to bottom in the graph, Im7 variants were wt, V33E, I54V, L34A, F15A and F84A, corresponding to strains ApYC 29, 34, 39,35, 30 and 42.Negative control expressing lacZ (ApYC43) showed with hexagon



Supplementary Fig. S6:

Loading control of soluble and total cell extract of S. cerevisiae. Lanes 1-7, soluble protein from S. cerevisiae expressing Kan::Im7 variants were isolated by treatment with yeast lysis solution; lanes 8-14, S. cerevisiae pellets boiled with SDS loading dye. From each sample, equal volume was loaded on SDS-PAGE and stained with Coomassie Blue. List all strains by number(Stains analysed in lanes 1-7 and 8-14 were AþYC29,34,35,30,42,44 and 46)



Supplementary Fig. S7:

Antibiotic resistance of Cn::Im7 variants. The antibiotic resistance of wild type Cn (AβYC53) showed with circle. Im7 wt fused at Cn80 (AβYC48) showed with cyan square, Im7 F15A (AβYC47) with inverted blue triangle, Im7 D63N (AβYC50) with blue triangle, Im7 D63N D35N (AβYC51) with pink circle and Im7 D63N D35N L18F (AβYC52) with inverted cyan triangle.

Supplement Table S1: Primers

P1	CAGTTTAAGGTTTAATCCTATAAAAGAG
P2	CTCTTTTATAGGATTAAACCTTAAACTG
P9	TTCAATTTCCTTAAGAAGTTGAACAGCCTCAGCCTCTGTGTAATCACTAATA
P10	TTCGAGTAACACATCTAACTCATCATCAGTTGCAGCAAC
P11	GCTGCAACTGATGATGTGTTAAATGTGTTACTCGAACACTTT
P12	GTTGCTGCAACTGATGATGTGGCAGATGTGTTACTCGAAC
P13	GTTCGAGTAACACATCTGCCACATCATCAGTTGCAGCAAC
P14	CTGAGCATCCAGATGGAACGGATGCGATTTATTATCCTAGTGATAATAG
P15	CTATTATCACTAGGATAATAAATCGCATCCGTTCCATCTGGATGCTCAG
P16	GCATCCAGATGGAACGGATCTGGTTTATTATCCTAGTGATAATAGA
P17	TCTATTATCACTAGGATAATAAACCAGATCCGTTCCATCTGGATGC
P18	GTCAAGACCGACCTGTCCGGATCCGCCCTGAATGAACTGC
P19	GCAGTTCATTCAGGGCGGATCCGGACAGGTCGGTCTTGAC
P20	GGATCCCAACAGCAGCAACAGCAACAACAG
P21	GGATCCCTGCTGTTGTTGCTGTTGCTGCTG
P22	GTCATGGTTGAACAAGATGGATTGCACGCAGGTTCTC
P23	TCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCGATG
P24	ATTGTTAATATACCTCTATACTTTAACGTC
P25	AGAATCGAGACCGAGGAGAGGGTTAGGGAT
P26	CCTAGTGATAATAGAGACAATAGCCCCGAAGGGA
P27	TCCCTTCGGGGCTATTGTCTCTATTATCACTAGG
P28	GTTGCTGCAACTGATGATGTGGCAGATGTGTTACTCGAACACTT
P29	AAGTGTTCGAGTAACACATCTGCCACATCATCAGTTGCAGCAAC
P30	CGGTTCCGGGAGCAGGGAAGCGAAAAATAGTATTAGTGATT
P31	AATCACTAATACTATTTTTCGCTTCCCTGCTCCCGGAACCG
P32	CATCCAGATGGAACGGATCTGGTCTATTATCCTAGTGATAATA
P33	TATTATCACTAGGATAATAGACCAGATCCGTTCCATCTGGATG
P34	GAGCATCCAGATGGAACGGATGCGATCTATTATCCTAGTGATAAT
P35	ATTATCACTAGGATAATAGATCGCATCCGTTCCATCTGGATGCTC
P36	GCTGCAACTGATGATGTGTTAAATGTGTTACTCGAACACTTT
P37	AAAGTGTTCGAGTAACACATTTAACACATCATCAGTTGCAGC
P38	ATTACACAGAGGCTGAGTTTGTTCAATTTCTTAAGGAAATTGAAAAAG
P39	CTTTTTCAATTTCCTTAAGAAATTGAACAAACTCAGCCTCTGTGTAAT
P40	TAAGGAAATTGAAAAAGAGAATGCTGCTGCAACTGATGATGTGTTAG
P41	CTAACACATCATCAGTTGCAGCAGCATTCTCTTTTTCAATTTCCTTA
P42	TGATTACACAGAGGCTGAGTTTGTTCAAGCTCTTAAGGAAATTGAAAAAGAG
P43	CTCTTTTTCAATTTCCTTAAGAGCTTGAACAAACTCAGCCTCTGTGTAATCA
P44	CTTAAGGAAATTGAAAAAGAGAAGGTTGCTGCAAATGATGATGTGTTAGATGTGT
P45	ACACATCTAACACATCATCATTTGCAGCAACCTTCTCTTTTTCAATTTCCTTAAG
P46	CTTAAGGAAATTGAAAAAGAGAAGGTTGCTGCAACTGATGATGTG
P47	CACATCATCAGTTGCAGCAACCTTCTCTTTTTCAATTTCCTTAAG

P48	GATCTGATCTATTATCCTAGGGATAATAGAGACGATAGCCC
P49	GGGCTATCGTCTCTATTATCCCTAGGATAATAGATCAGATC
P50	TATTAGTGATTACACAGAGGCTGAGGCTGTTCAACTTCTTAAGGAAATTGAA
P51	TTCAATTTCCTTAAGAAGTTGAACAGCCTCAGCCTCTGTGTAATCACTAATA
P52	GATAGCCCCGAAGGGATTGCCAAGGAAATTAAAGAATGG
P53	CCATTCTTTAATTTCCTTGGCAATCCCTTCGGGGCTATC
P54	GTCGCGTTCAAGGCGTCAGTTTTCGCATGTATACC
P55	GGTATACATGCGAAAACTGACGCCTTGAACGCGAC
P56	GCACAGTCCCTGAAATCGGTTGATTTTGAAGTCTTTGGT
P57	ACCAAAGACTTCAAAATCAACCGATTTCAGGGACTGTGC
P58	GGTCGCGTTCAAGGCGCCTGTTTTCGCATGTAT
P59	ATACATGCGAAAACAGGCGCCTTGAACGCGACC
P60	GCCCGAAGATAAAGTTAATTCCGCGAAATCTTGGTTGAGCAAAGTG
P61	CACTTTGCTCAACCAAGATTTCGCGGAATTAACTTTATCTTCGGGC
P62	AATTCCATGAAATCTTGGGTGAGCAAAGTGGGTTCGC
P63	GCGAACCCACTTTGCTCACCCAAGATTTCATGGAATT
P64	GATCGCACGAACTTTAGTAACGATAAGACCATTTCAAAATTGGAATA
P65	TATTCCAATTTTGAAATGGTCTTATCGTTACTAAAGTTCGTGCGATC
P134	GAATTCACATGGAGGCCCAGAATACCC
P135	CAGTATAGCGACCAGCATTCACGAATTC

Supplement Table S2: Thermodynamic stabilities of Im7 variants used in the biosensors

Im7 variant	Stability $\Delta\Delta G°_{UN}$ (KJ/mol)	ln (MIC $_{mut}$/MIC $_{WT}$) ANT system	SEM	ln (MIC $_{mut}$/MIC $_{WT}$) kan system	SEM
WT	0.00	0.00	0.00	0.00	0.00
V33E	-3.8			-0.04	0.03
L34A	-8.3	-0.60	0.05	-0.14	0.11
I54V	-6.4	-0.80	0.02	-0.48	0.09
L53A	-13.6			-0.73	0.05
F15A	-15.2	-2.02	0.02	-0.74	0.13
F84A	-24.9 *			-1.21	0.08
N26K S58R	2.05	0.35	0.11		
V69A	-2.30	-0.37	0.05		
N26K T30N S58R	5.89	0.45	0.02		
L3A	-4.2	-0.13	0.04		
I54A	-13.50	-1.57	0.01		
D35N D63N	5.97	0.28	0.05		
V27A D63N	3.93	0.30	0.04		
L18F D35N D63N	7.80	0.18	0.09		
L18A	-10.20	-1.94	0.02		

Supplement Table S3: Antibiotic resistance of Im7 variants inserted in different permissive sites of ANT

	Permissive site 153		Permissive site 157		Permissive site 289	
	ln (MIC $_{mut}$/MIC $_{WT}$)	SEM	ln (MIC $_{mut}$/MIC $_{WT}$)	SEM	ln (MIC $_{mut}$/MIC $_{WT}$)	SEM
WT	0.00	0.00	0.00	0.00	0.00	0.00
V69A	-0.06	0.09	-0.27	0.06	-0.31	0.12
F15A	-1.97	0.02	-2.24	0.05	-3.50	0.01
L34A	-0.63	0.02	-0.62	0.08	-0.78	0.05
N26K S58R	0.29	0.06	0.36	0.12	0.28	0.20
N26K T30N S58R	0.25	0.20	0.49	0.16	0.57	0.27

* Im7 F84A is so destabilized that it mainly populates the unfolded state *in vitro*. This precludes the accurate determination of its ΔG°UN values (27) For the purposes of this paper it is assumed to be fully unfolded and thus have a ΔG°UN value = 0 kJ/mol or 24.9 kJ/mol greater than wild type Im7 which has a ΔG°UN value = -24.9kJ/mol list all strains by number

Supplement Table S4: Thermodynamic stabilities and MICs of AcP variants tested in the ANT system

AcP variant	Stability $\Delta\Delta G°_{UN}$ (KJ/mol)	ln (MIC_{mut}/MIC_{WT})	SEM
C21S	0.00	0.00	0.00
Y11F C21S	1.80	0.20	0.07
C21S L65V	-22.40	-3.57	0.01
C21S E83D	-6.30	-0.43	0.08
C21S V20A	-1.20	-0.14	0.05
C21S M61A	-16.60	-1.84	0.04

Supplement Table S5 strains and plasmids used in this work

Strain or plasmid	Genotype	Relevant description or derivation
NEB10β	Δ*(ara-leu) 7697 araD139 fhuA* Δ*lacX74 galK16 galE15 e14-* ϕ*80*d*lacZ*Δ*M15 recA1 relA1 endA1 nupG rpsL* (Str^R) *rph spoT1* Δ*(mrr-hsdRMS-mcrBC)*	
AMS1	NEB10β pAMS1	
AMS2	NEB10β pAMS2	
AMS3	NEB10β pAMS3	
AMS4	NEB10β pAMS4	
AMS5	NEB10β pAMS5	
AMS6	NEB10β pAMS6	
AMS7	NEB10β pAMS7	
AMS8	NEB10β pAMS8	
AMS9	NEB10β pAMS9	
AMS10	NEB10β pAMS10	
AMS11	NEB10β pAMS11	
AMS12	NEB10β pAMS12	
AMS13	NEB10β pAMS13	
AMS14	NEB10β pAMS14	
AMS15	NEB10β pAMS15	
AMS16	NEB10β pAMS16	
AMS17	NEB10β pAMS17	
AMS18	NEB10β pAMS18	
AMS19	NEB10β pAMS19	
AMS20	NEB10β pAMS20	
AMS21	NEB10β pAMS21	
AMS22	NEB10β pAMS22	
AMS23	NEB10β pAMS23	
AMS24	NEB10β pAMS24	
AMS25	NEB10β pAMS25	
AMS26	NEB10β pAMS26	
AMS27	NEB10β pAMS27	
AMS28	NEB10β pAMS28	
AMS29	NEB10β pAMS29	
AMS30	NEB10β pAMS30	
AMS31	NEB10β pAMS31	
AMS32	NEB10β pAMS32	
AMS33	NEB10β pAMS33	
AMS34	NEB10β pAMS34	
AMS35	NEB10β pAMS35	
AMS36	NEB10β pAMS36	
AMS37	NEB10β pAMS37	
AMS38	NEB10β pAMS38	
AMS39	NEB10β pAMS39	

Strain or plasmid	Genotype	Relevant description or derivation
AMS40	NEB10β pAMS40	
BL21-codon plus (DE3)-RIPL	E. coli B F $^{-}$ ompT hsdS (r_B^{-} m_B^{-}) dcm + Tet^{r} gal λ (DE3) endA Hte [argU proL Cam^{r}] [argU ileY leuW Strep/Spec^{r}]	New England Biolabs
INVSc1	*MATa his3D1 leu2 trp1-289 ura3-52 MA*T *his3D1 leu2 trp1-289 ura3-52*	Dr. Anuj Kumar (University of Michigan)
AM101	NEB10b pAM103	
AM144	NEB10b pAM15	
AM152	NEB10b p4*339*	
AM200	NEB10b pAM79	
AM201	NEB10b pAM80	
AM235	BL21-codon plus (DE3)-RIPL pAM107	
AM236	BL21-codon plus (DE3)-RIPL pAM108	
AM240	BL21-codon plus (DE3)-RIPL pAM112	
AM242	BL21-codon plus (DE3)-RIPL pAM114	
AM243	BL21-codon plus (DE3)-RIPL pAM115	
AM246	BL21-codon plus (DE3)-RIPL pAM118	
AM247	BL21-codon plus (DE3)-RIPL pAM119	
AM248	NEB10b pAM120	
AM249	BL21-codon plus (DE3)-RIPL pAM121	
AM258	BL21-codon plus (DE3)-RIPL pAM130	
AM282	NEB10b pAM154	
AM283	NEB10b pAM155	
AM284	NEB10b pAM156	
AM285	NEB10b pAM157	
AM286	NEB10b pAM158	
AM288	NEB10b pAM160	
AM290	NEB10b pAM162	
AM291	NEB10b pAM163	
AM308	NEB10b pAM180	
AM323	NEB10b p416-103Q	
aYC-28	INVSc1 pAM154	
aYC-29	INVSc1 pAM154::ceiE7	
aYC-30	INVSc1 pAM154::ceiE7F15A	
aYC-34	INVSc1 pAM154::ceiE7V33E	
aYC-35	INVSc1 pAM154::ceiE7L34A	
aYC-39	INVSc1 pAM154::ceiE7I54V	
aYC-42	INVSc1 pAM154::ceiE7F84A	
aYC-43	INVSc1 pYES2.1/V5-His-TOPO::LacZ	
aYC-44	INVSc1 pAM154::Q20	
aYC-46	INVSc1 pAM154::Q87	
aYC-47	INVSc1 pYES2.1/V5-His-TOPO::nat1aa80::ceiE7 F15A	Im7 F15A inserted after amino acid 80 of nat1

Strain or plasmid	Genotype	Relevant description or derivation
aYC-48	INVSc1 pYES2.1/V5-His-TOPO::nat1aa80::ceiE7	Im7 wt inserted after amino acid 80 of nat1
aYC-50	INVSc1 pYES2.1/V5-His-TOPO::nat1aa80::ceiE7D63N	Im7 D63N inserted after amino acid 80 of nat1
aYC-51	INVSc1 pYES2.1/V5-His-TOPO::nat1aa80::ceiE7D63N,D35N	Im7 D63N, D35N inserted after amino acid 80 of nat1
aYC-52	INVSc1 pYES2.1/V5-His-TOPO::nat1aa80::ceiE7D63N,D35N,L18F	Im7 D63N, D35N, L18F inserted after amino acid 80 of nat1
aYC-53	INVSc1 pYES2.1/V5-His-TOPO::nat1	nat1 cloned on pYES2.1 vector
pAMS1	pBR322::aadA	aadA (encoding StrepR) from pBAD43 cloned into pBR322 using Hind III
pAMS2	pAMS1 aadAaa155::ceiE7	ceiE7 (gene encoding Im7) inserted after amino acid 155 of aadA
pAMS3	pAMS1 aadAaa155::linker	Linker inserted after amino acid 155 of aadA (encoding StrepR)
pAMS4	pAMS 2 aadAaa155::ceiE7L34A	Im7 L34A inserted after amino acid 155 of aadA
pAMS5	pAMS2 ceiE7I54V	Im7 I54V inserted after amino acid 155 of aadA
pAMS6	pAMS2 ceiE7F15A	Im7 F15A inserted after amino acid 155 of aadA
pAMS7	pAMS2 ceiE7N26K S58R	Im7 N26K S58R inserted after amino acid 155 of aadA
pAMS8	pAMS2 ceiE7V69A	Im7 V69A inserted after amino acid 155 of aadA
pAMS9	pAMS2 N26KT30NS58R	Im7 N26K T30N S58R inserted after amino acid 155 of aadA
pAMS10	pAMS2 ceiE7L3A	Im7 L3A inserted after amino acid 155 of aadA
pAMS11	pAMS2 ceiE7I54A	Im7 I54A inserted after amino acid 155 of aadA
pAMS12	pAMS2 ceiE7D35ND63N	Im7 D35N D63N inserted after amino acid 155 of aadA
pAMS13	pAMS2 ceiE7V27AD63N	Im7 V27A D63N inserted after amino acid 155 of aadA
pAMS14	pAMS2 ceiE7L18FD35ND63N	Im7 L18F D35N D63N inserted after amino acid 155 of aadA
pAMS15	pAMS2 ceiEL18A	Im7 L18A inserted after amino acid 155 of aadA
pAMS16	pBR322::aadAaa153::ceiE7	ceiE7 (gene encoding Im7) inserted after amino acid 153 of aadA
pAMS17	pAMS16 ceiE7V69A	Im7 V69A inserted after amino acid 153 of aadA
pAMS18	pAMS16ceiE7F15A	Im7 F15A inserted after amino acid 153 of aadA
pAMS19	pAMS16ceiE7L34A	Im7 L34A inserted after amino acid 153 of aadA
pAMS20	pAMS16 ceiE7N26KS58R	Im7 N26K S58R inserted after amino acid 153 of aadA
pAMS21	pAMS16 ceiE7N26KT30NS58R	Im7 N26K T30N S58R inserted after amino acid 153 of aadA
pAMS22	pBR322::aadAaa157::ceiE7	ceiE7 (gene encoding Im7) inserted after amino acid 157 of aadA
pAMS23	pAMS22 ceiE7V69A	Im7 V69A inserted after amino acid 157 of aadA
pAMS24	pAMS22 ceiE7F15A	Im7 F15A inserted after amino acid 157 of aadA

Strain or plasmid	Genotype	Relevant description or derivation
pAMS25	pAMS22 ceiE7L34A	Im7 L34A inserted after amino acid 157 of aadA
pAMS26	pAMS22 ceiE7N26KS58R	Im7 N26K S58R Im7 inserted after amino acid 157 of aadA
pAMS27	pAMS22ceiE7N26KT30NS58R	Im7 N26K T30N S58R Im7 inserted after amino acid 157 of aadA
pAMS28	pBR322::aadAaa289::ceiE7	ceiE7 (gene encoding Im7) inserted after amino acid 289 of aadA
pAMS29	pAMS28 ceiE7V69A	Im7 V69A inserted after amino acid 289 of aadA
pAMS30	pAMS28 ceiE7F15A	Im7 F15A inserted after amino acid 289 of aadA
pAMS31	pAMS28 ceiE7L34A	Im7 L34A inserted after amino acid 289 of aadA
pAMS32	pAMS28 ceiE7N26KS58R	Im7 N26K S58R inserted after amino acid 289 of aadA
pAMS33	pAMS28 ceiE7N26KT30NS58R	Im7 N26K T30N S58R inserted after amino acid 289 of aadA
pAMS34	pAMS1 aadAaa155::acpy2	Acyp2 encoding AcP inserted after amino acid 155 of aadA
pAMS35	pAMS34 acpy2C21S	AcP C21S AcP inserted after amino acid 155 of aadA
pAMS36	pAMS34 acpy2Y11FC21S	AcP Y11F C21S AcP inserted after amino acid 155 of aadA
pAMS37	pAMS34 acpy2C21SL65V	AcP C21S L65V AcP inserted after amino acid 155 of aadA
pAMS38	pAMS34 acpy2C21SE83D	AcP C21S E83D AcP inserted after amino acid 155 of aadA
pAMS39	pAMS34 acpy2V20AC21S	AcP C21S V20A AcP inserted after amino acid 155 of aadA
pAMS40	pAMS34 acpy2C21SM61A	AcP C21S M61A AcP inserted after amino acid 155 of aadA
pAM 15	pCR-Blunt II-TOPO- ccdB5	Point mutaion in *ccdB* gene at amino acid 5 to intoduce stop codon and NsiI digested and religated vector
pAM 18	pCR-Blunt II-TOPO- subclone	Subclone on pCR-Blunt II-TOPO- vector
pAM 79	pAM15aphA-2aa55::Q20	polyQ(20) inserted after amino acid 55 of aphA-2
pAM 80	pAM15aphA-2aa55::Q45	polyQ(45) inserted after amino acid 55 of aphA-2
pAM 81	pAM15aphA-2aa55::Q81	polyQ(87) inserted after amino acid 55 of aphA-2
pAM 103	pBR322::nat1	nat1 (encoding ClonNAT resistance) from plasmid p4339 (or pAM31) cloned into pBR322 using EcoRI
pAM 107	pAM15aphA-2aa55::ceiE7	ceiE7 (gene encoding Im7) inserted after amino acid 55 of aphA-2
pAM 108	pAM15aphA-2aa55::ceiE7 F84A	Im7 F84A inserted after amino acid 55 of aphA-2
pAM 112	pAM15aphA-2aa55::ceiE7 V33E	Im7 V33E inserted after amino acid 55 of aphA-2
pAM 114	pAM15aphA-2aa55::ceiE7 L53A	Im7 L53A inserted after amino acid 55 of aphA-2
pAM 115	pAM15aphA-2aa55::ceiE7 I54V	Im7 I54V inserted after amino acid 55 of aphA-2
pAM 118	pAM15aphA-2aa42::ceiE7 F84A	Im7 F84A inserted after amino acid 42 of aphA-2
pAM 119	pAM15aphA-2aa55::ceiE7 F15A	Im7 F15A inserted after amino acid 55 of aphA-2

Strain or plasmid	Genotype	Relevant description or derivation
pAM 120	pBR322 bla196::ceiE7 F84A	Im7 F84A inserted after amino acid 196 of bla in pBR322
pAM 121	pAM15aphA-2aa21::ceiE7 F84A	Im7 F84A inserted after amino acid 21 of aphA-2
pAM 130	pAM15aphA-2aa55::ceiE7 L34A	Im7 L34A inserted after amino acid 55 of aphA-2
pAM 154	pYES2.1/V5-His-TOPO::aphA-2	aphA-2 (encoding Kan resistance) cloned in pYES2.1/V5-His-TOPO
pAM 155	pYES2.1/V5-His-TOPO::aphA-2aa55::ceiE7	Im7 inserted after amino acid 55 of aphA-2 in pYES2.1/V5-His-TOPO
pAM 156	pYES2.1/V5-His-TOPO::aphA-2aa55::ceiE7 F84A	Im7 F84A inserted after amino acid 55 of aphA-2 in pYES2.1/V5-His-TOPO
pAM 157	pYES2.1/V5-His-TOPO::aphA-2aa55::ceiE7 F15A	Im7 F15A inserted after amino acid 55 of aphA-2 in pYES2.1/V5-His-TOPO
pAM 158	pYES2.1/V5-His-TOPO::aphA-2aa55::Q20	polyQ(20) inserted after amino acid 55 of aphA-2 in pYES2.1/V5-His-TOPO
pAM 160	pYES2.1/V5-His-TOPO::aphA-2aa55::Q87	polyQ(87) inserted after amino acid 55 of aphA-2 in pYES2.1/V5-His-TOPO
pAM 162	pAM15aphA-2aa55::ceiE7 V33E	Im7 V33E inserted after amino acid 55 of aphA-2 pYES2.1/V5-His-TOPO
pAM 163	pYES2.1/V5-His-TOPO::lacZ	lacZ gene coding for beta-galactosidase cloned in
pAM 180	pAM15aphA-2 (SDM41-43)aa55::Q87	polyQ(87) inserted in silent mutated aphA-2 variant after amino acid 55
p416-103Q		(14)
pGPS4	New England Biolab	
p4339	pCR-TOPO::natR-MX4	Dr. Charles Boone lab (U Toronto),

The source of all strains and plasmids is this study with the exception of NEB10β and BL21 which were obtained from New England Biolabs.