Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Antibacterial Activity of a Dual Peptide Targeting the *Escherichia coli* Sliding Clamp and the Ribosome

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Supplementary Information

SI.1a: HPLC and LCMS analyses of the different peptides synthesized

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SI.3: Typical ITC titration curves describing the interaction of the different peptides with ^{Ec}SC and ^{Ec}70S

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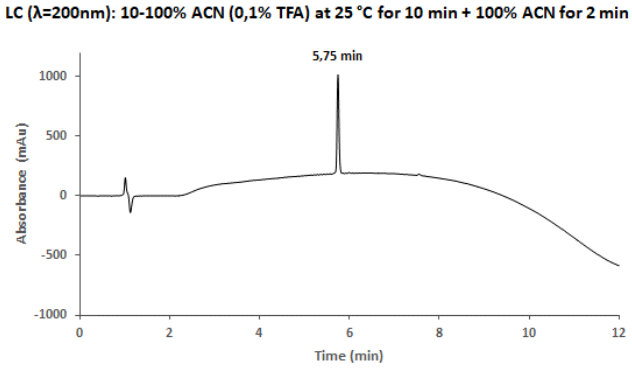
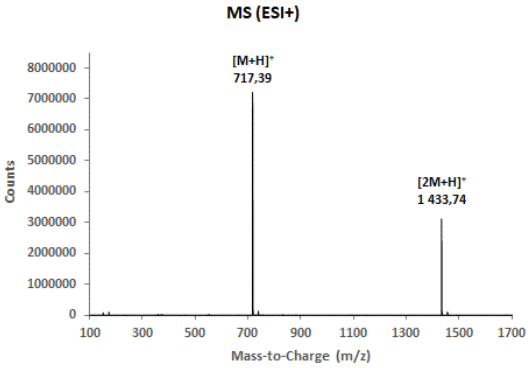
SI.6: Survival curves of *E. coli* infected *D. melanogaster* from three independent experiments

SI.1a: HPLC and LCMS analyses of the different peptides synthesized

P7: Ac-QXDLF-OH

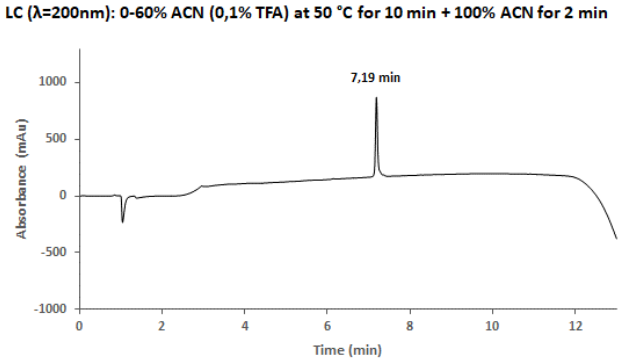
M_W = 716.82 g/mol

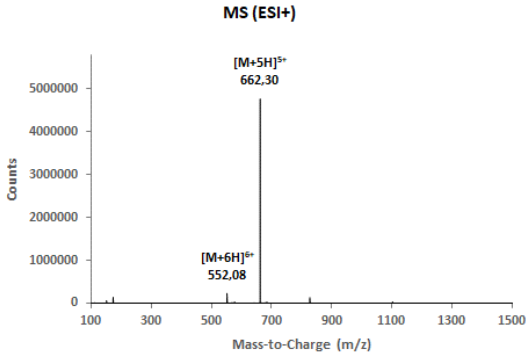




Py-P7: VDKGSYLPRPTPPRPIYNRNGPRQXDLF-OH

M_W = 3307.82 g/mol





Py-P7Scr: VDKGSYLPRPTPPRPIYNRNGPRXFQLD-OH

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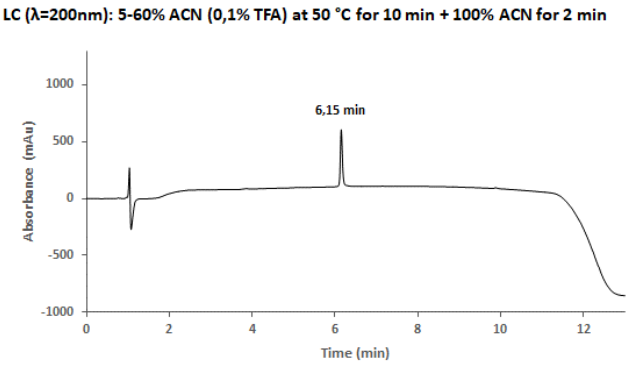
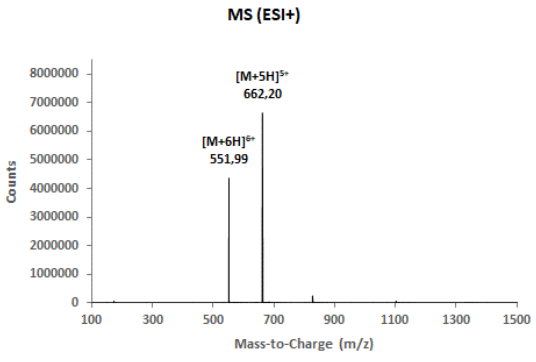
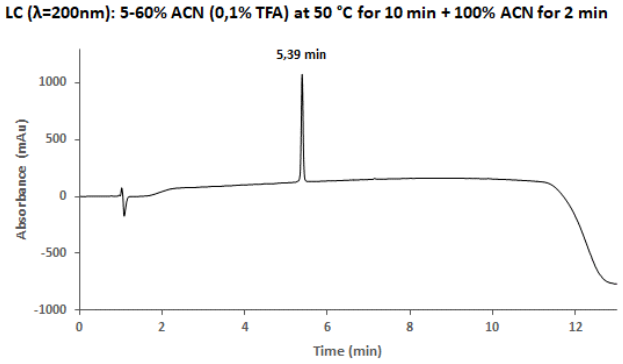
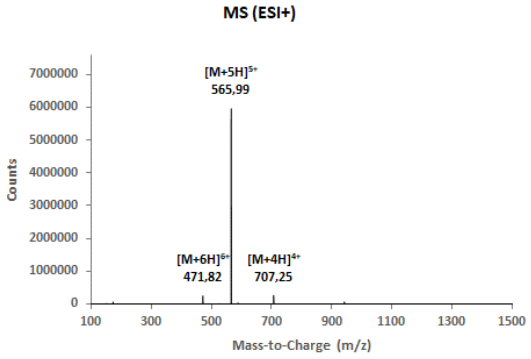




FITC-Py: FITC-KVDKGSYLPRPTPPRPIYNRN-NH_2

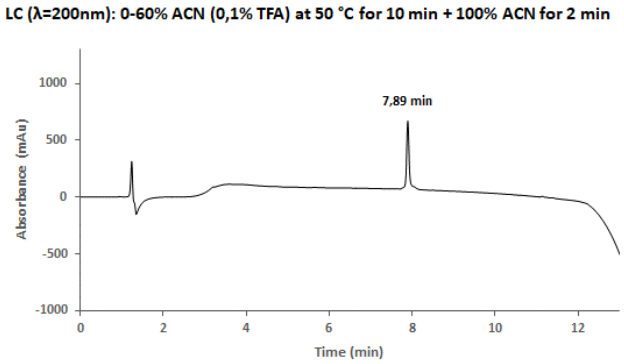
M_W = 2826.17 g/mol

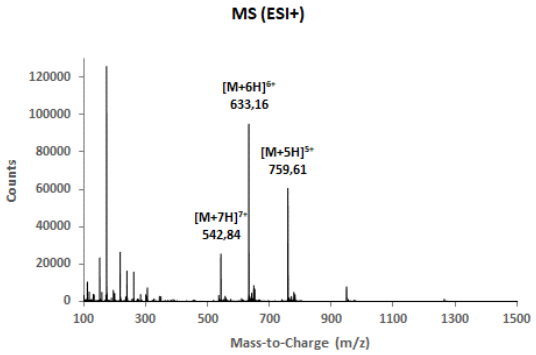




FITC-Py-P7: FITC-KVDKGSYLPRPTPPRPIYNRNGPRQXDLF-OH

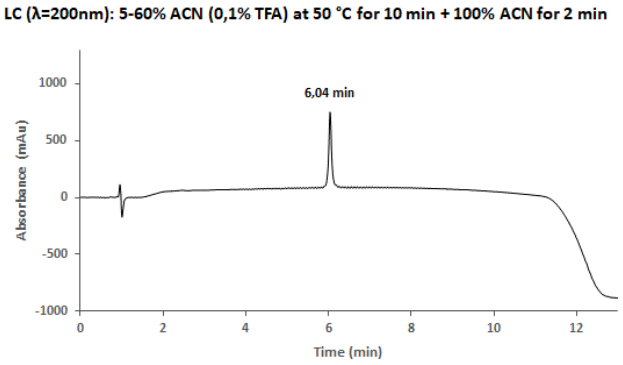
M_W = 3794.30 g/mol

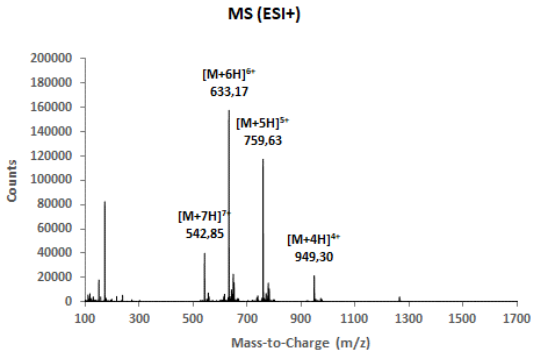




FITC-Py-P7Scr: FITC-KVDKGSYLPRPTPPRPIYNRNGPRXFQLD-OH

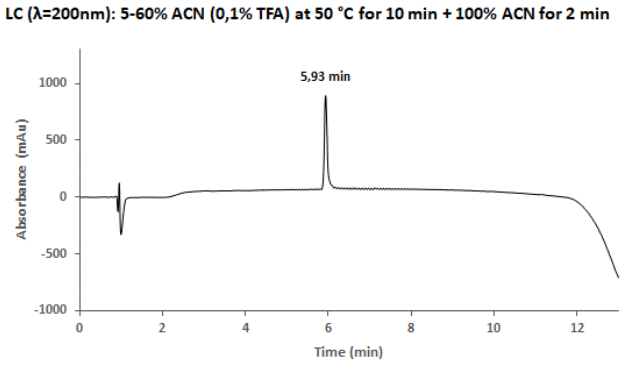
M_W = 3794.30 g/mol

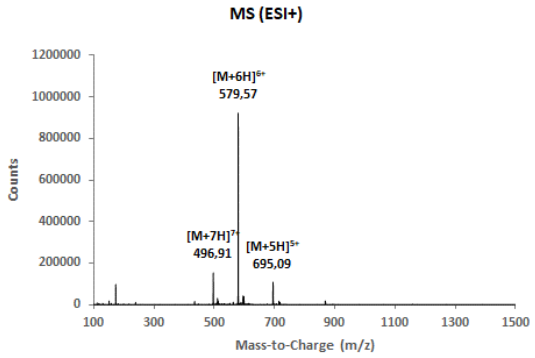




<u>Onc112-P7:</u> VDKPPYLPRPRPPRrIYNrNGPRQXDLF-OH

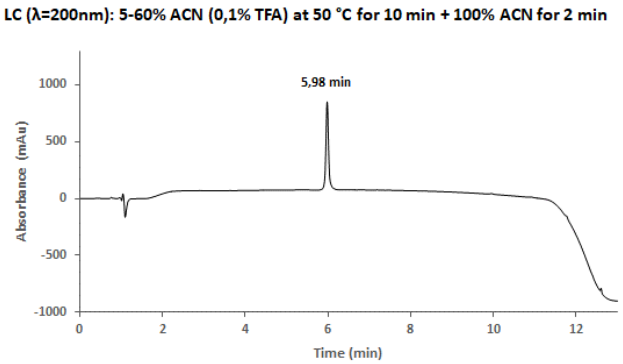
M_W = 3472.08 g/mol

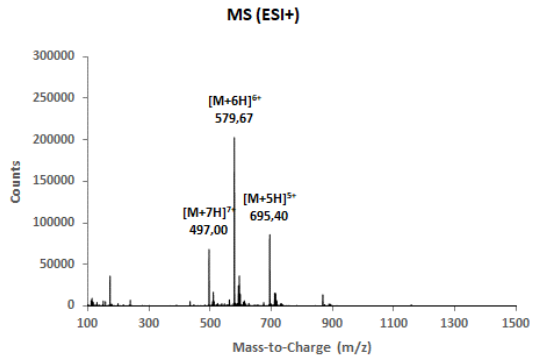




<u>Onc112-P7Scr:</u> VDKPPYLPRPRPPRrIYNrNGPRXFQLD-OH

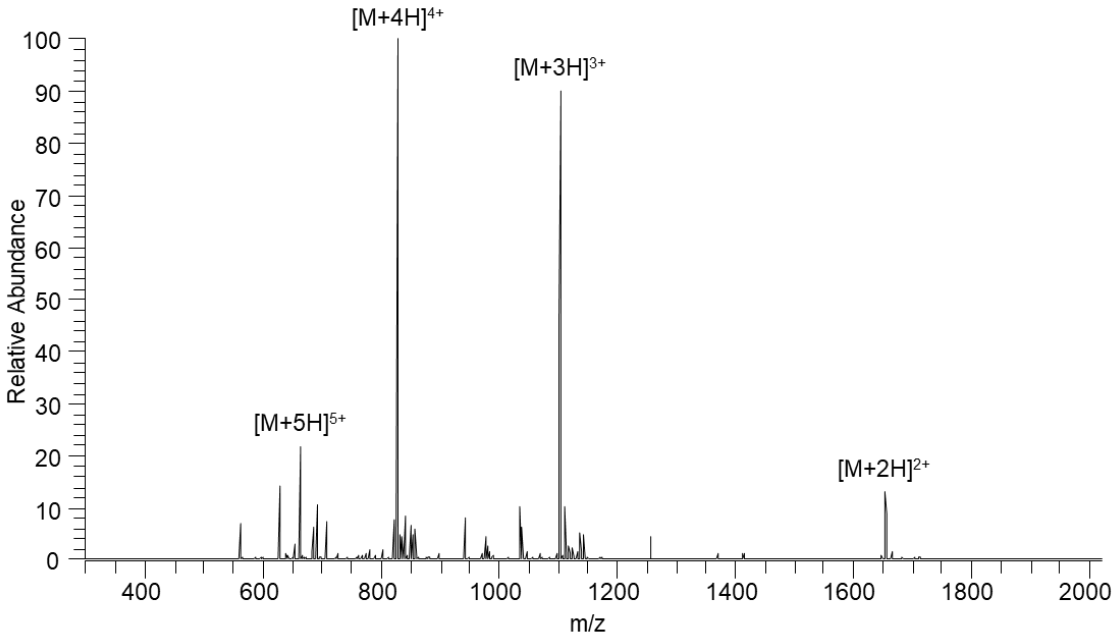
M_W = 3472.08 g/mol





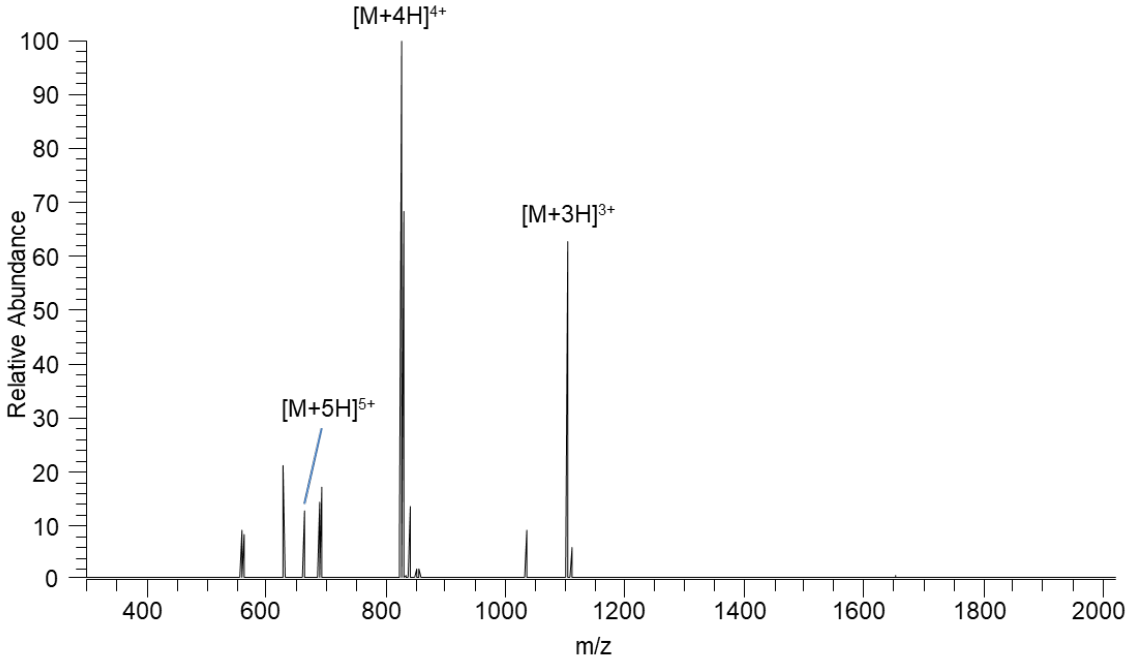
SI.1b: HRMS analyses of the peptides synthesized

Py-P7: VDKGSYLPRPTPPRPIYNRNGPRQXDLF-OH ($C_{151}H_{236}N_{44}O_{40}$)



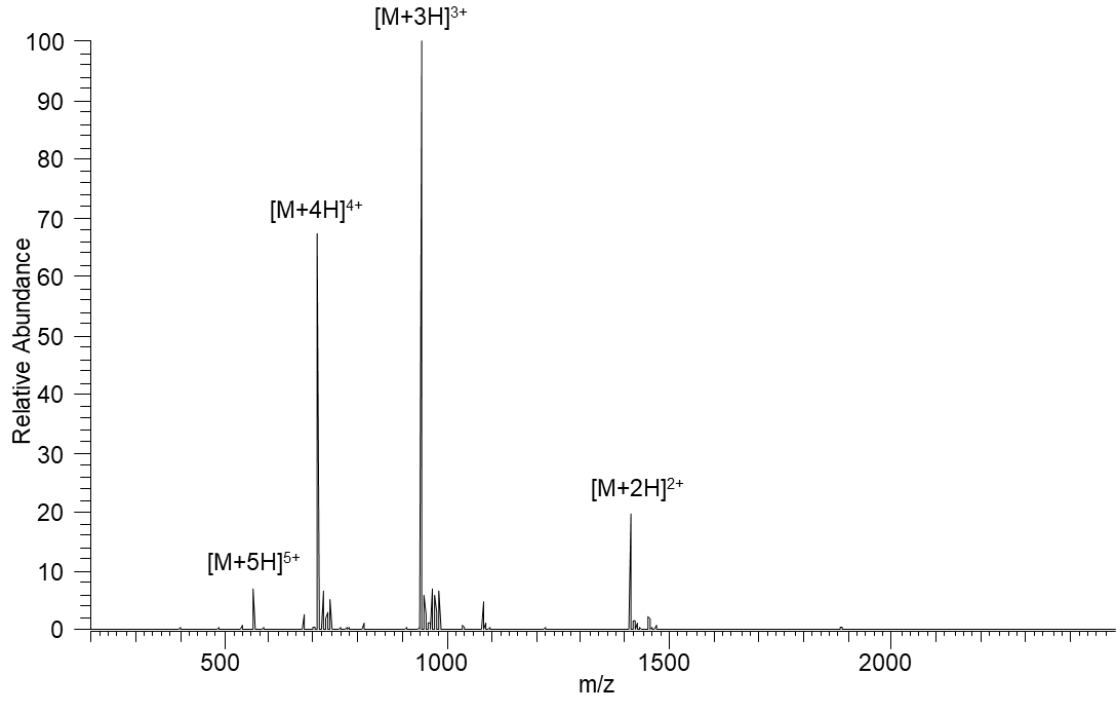
ESI+ HRMS (m/z): calcd 1653.89655 found 1653.89419 $[M + 2H]^{2+}$, calcd 1102.93346 found 1102.93591 $[M + 3H]^{3+}$, calcd 827.45191 found 827.45316 $[M + 4H]^{4+}$, and calcd 662.16299 found 662.16265 $[M + 5H]^{5+}$.

Py-P7Scr: VDKGSYLPRPTPPRPIYNRNGPRXFQLD-OH ($C_{151}H_{236}N_{44}O_{40}$)



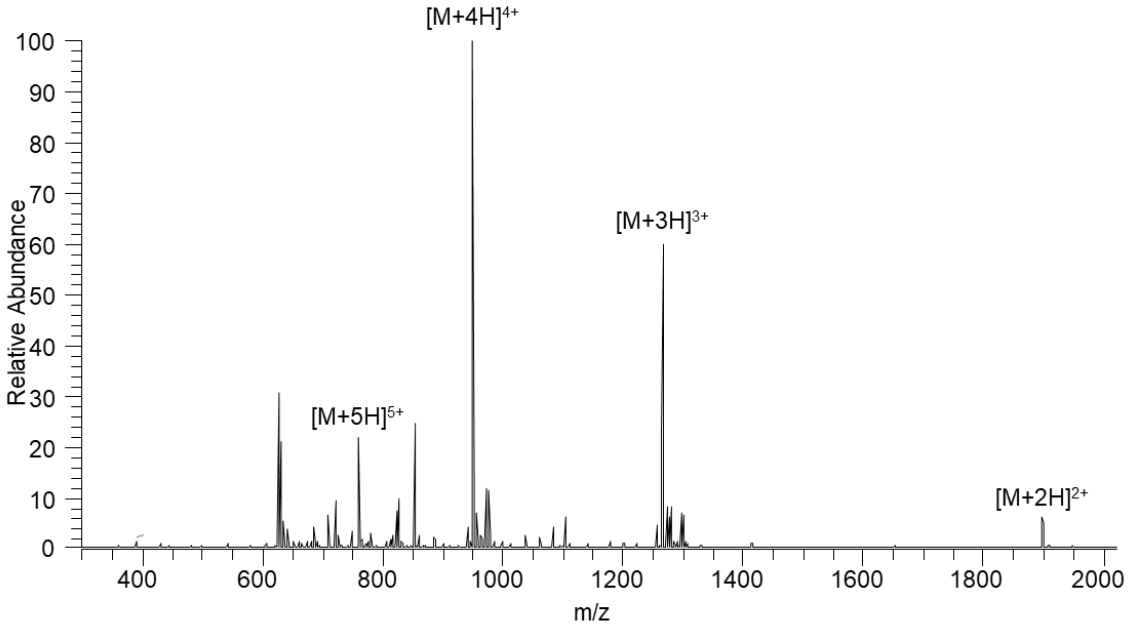
ESI+ HRMS (m/z): calcd 1102.93346 found 1102.94511 $[M + 3H]^{3+}$, calcd 827.45191 found 827.46011 $[M + 4H]^{4+}$, and calcd 662.16299 found 662.16796 $[M + 5H]^{5+}$.

FITC-Py: FITC-KVDKGSYLPRPTPPRPIYNRN-NH_2 ($C_{132}H_{189}N_{35}O_{35}$)



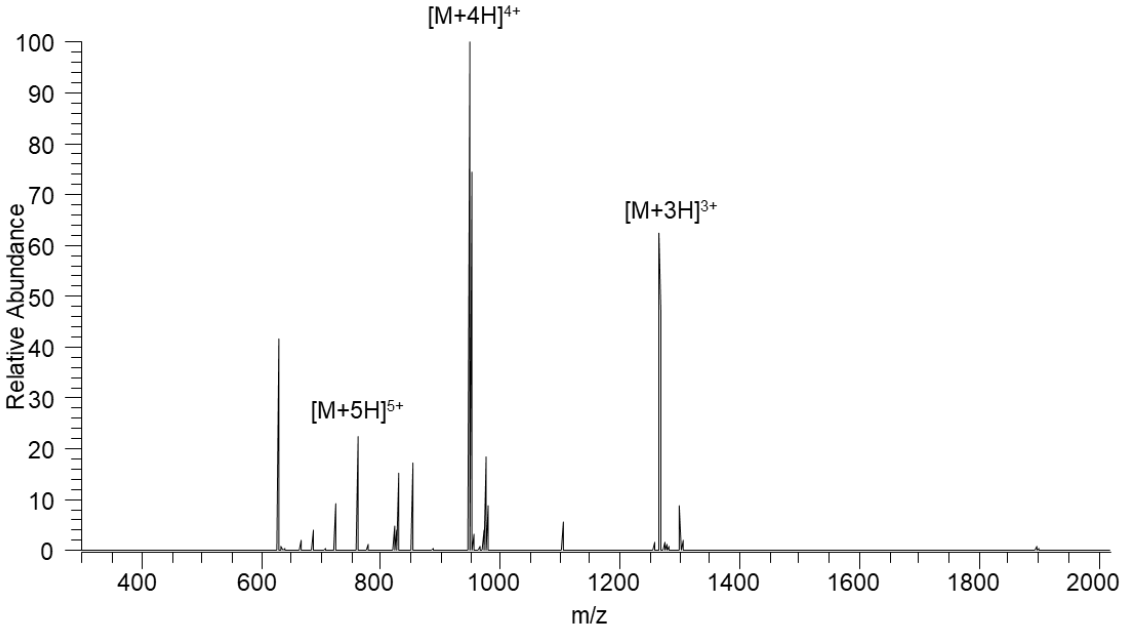
ESI+ HRMS (m/z): calcd 1413.21154 found 1413.20845 $[M + 2H]^{2+}$, calcd 942.47679 found 942.47697 $[M +3H]^{3+}$, calcd 707.10941 found 707.10943 $[M + 4H]^{4+}$, and calcd 565.88898 found 565.88725 $[M + 5H]^{5+}$.

FITC-Py-P7: FITC-KVDKGSYLPRPTPPRPIYNRNGPRQXDLF-OH ($C_{178}H_{258}N_{46}O_{47}$)



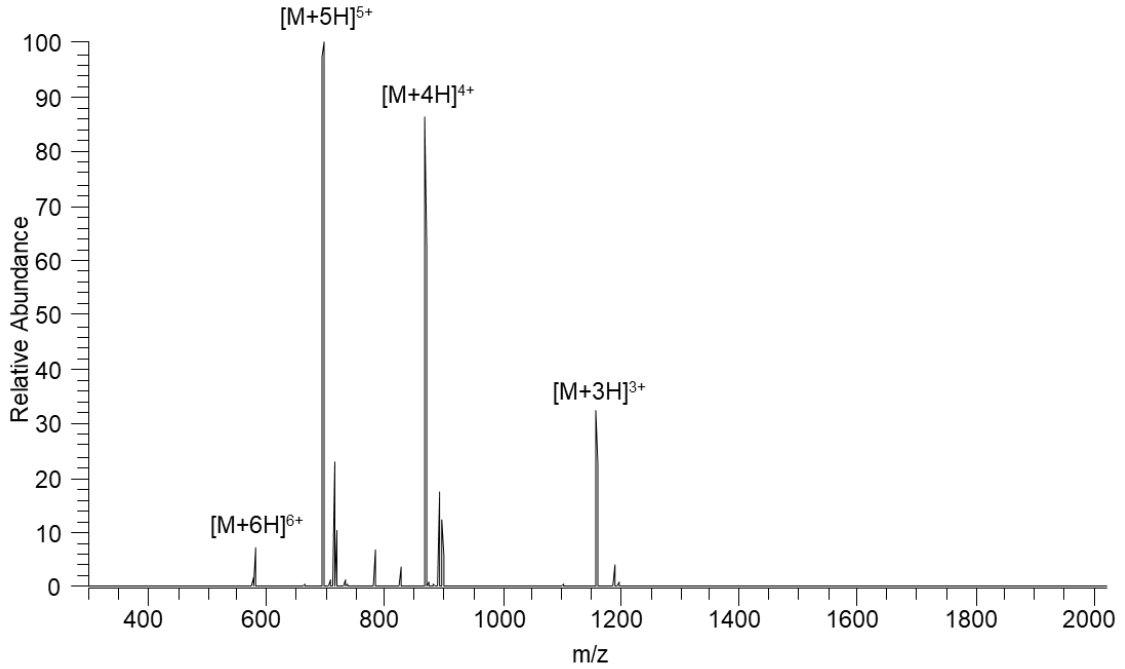
ESI+ HRMS (m/z): calcd 1896.96790 found 1896.97197 $[M + 2H]^{2+}$, calcd 1264.98103 found 1264.98645 $[M + 3H]^{3+}$, calcd 948.98759 found 948.99303 $[M + 4H]^{4+}$, and calcd 759.39153 found 759.39403 $[M + 5H]^{5+}$.

FITC-Py-P7Scr: FITC-KVDKGSYLPRPTPPRPIYNRNGPRXFQLD-OH ($C_{178}H_{258}N_{46}O_{47}$)



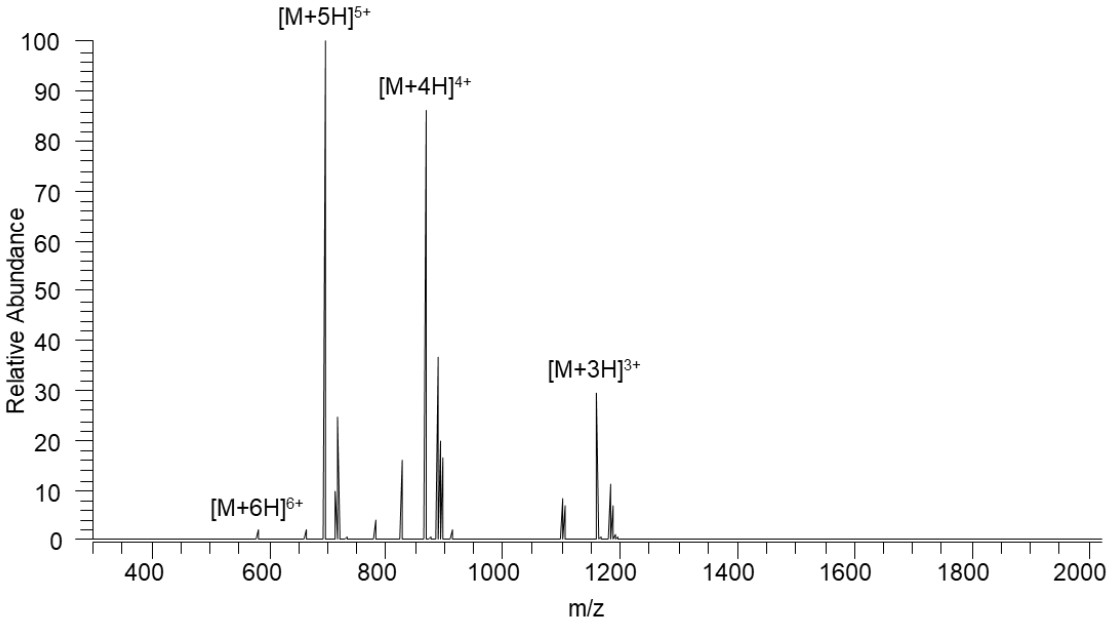
ESI+ HRMS (m/z): calcd 1264.98103 found 1264.99860 $[M + 3H]^{3+}$, calcd 948.98759 found 949.00171 $[M + 4H]^{4+}$, and calcd 759.39153 found 759.40126 $[M + 5H]^{5+}$.

Onc112-P7: VDKPPYLPRPRPPRrIYNrNGPRQXDLF-OH ($C_{159}H_{252}N_{50}O_{38}$)



ESI+ HRMS (m/z): calcd 1157.65140 found 1157.66745 $[M + 3H]^{3+}$, calcd 868.49037 found 868.50388 $[M + 4H]^{4+}$, calcd 694.99375 found 695.00511 $[M + 5H]^{5+}$ and calcd 579.32934 found 579.33641 $[M + 6H]^{6+}$.

Onc112-P7Scr: VDKPPYLPRPRPPRrIYNrNGPRXFQLD-OH ($C_{159}H_{252}N_{50}O_{38}$)



ESI+ HRMS (m/z): calcd 1157.65140 found 1157.66618 $[M + 3H]^{3+}$, calcd 868.49037 found 868.50235 $[M + 4H]^{4+}$, calcd 694.99375 found 695.00344 $[M + 5H]^{5+}$ and calcd 579.32934 found 579.33445 $[M + 6H]^{6+}$.

SI.2: Thermodynamic profiles describing the interaction of the different peptides used with ^{Ec}SC and ^{Ec}70S at 30°C

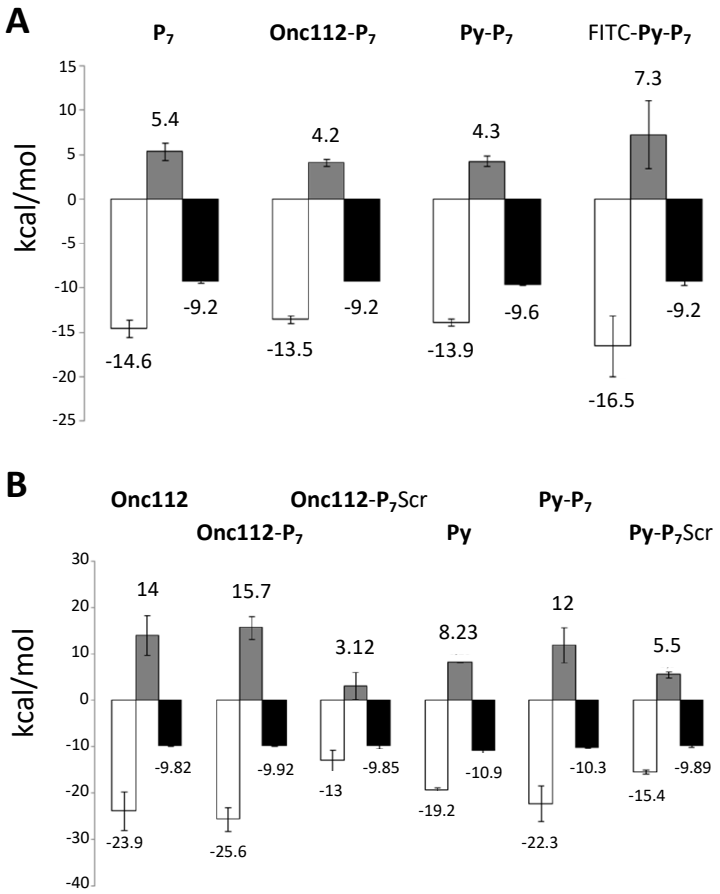


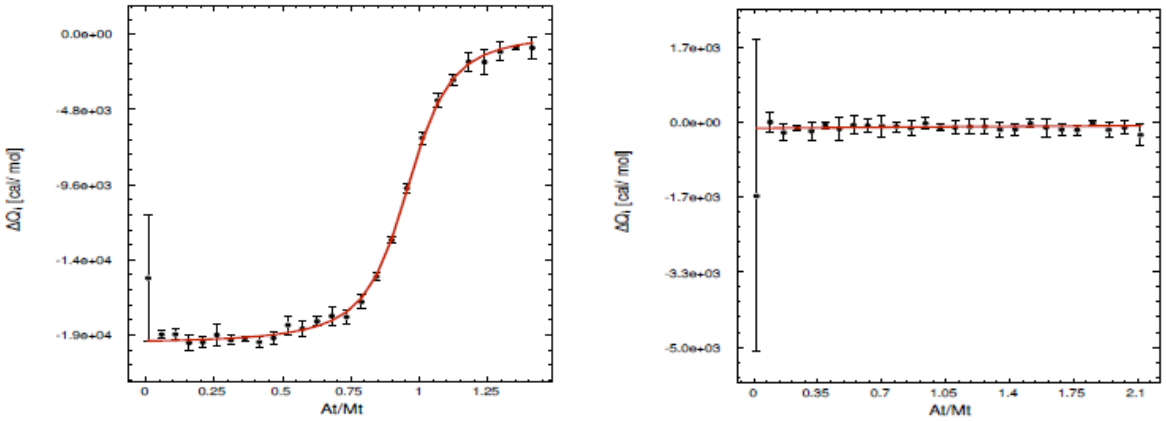
FIg. SI.2: (A): Interaction of **P$_7$**, **Onc112-P$_7$**, **Py-P$_7$** and FITC-**Py-P$_7$** with ^{Ec}SC at 30°C. (B): Interaction of **Onc112**, **Onc112-P$_7$**, **Onc112-P$_7$**Scr, **Py, Py-P$_7$** and **Py-P$_7$**Scr with ^{Ec}70S at 30°C. ΔH, -TΔS and ΔG (all in kcal/mol) are white, grey and black bars, respectively. Thermodynamic values are indicated for each bar. The interaction of **P$_7$** with ^{Ec}SC (FIg. SI.2 A) is a spontaneous enthalpy driven process as previously observed[1]. The unfavorable entropic factor can be partly related to the loss of freedom degrees due to peptide binding. The interaction of all peptides with ^{Ec}70S is also an enthalpy driven process (FIg. SI.2 B). It is characterized by an unfavorable entropic factor (-TΔS) that could be partly attributed to the loss of freedom degrees of the peptide upon binding into the 70S particle' PEC, and a Gibb's free energy that ranges around -10 kcal/mole, indicating the spontaneous nature of the interaction.

SI.3: Typical ITC titration curves describing the interaction of the different peptides with ^{Ec}SC and ^{Ec}70S

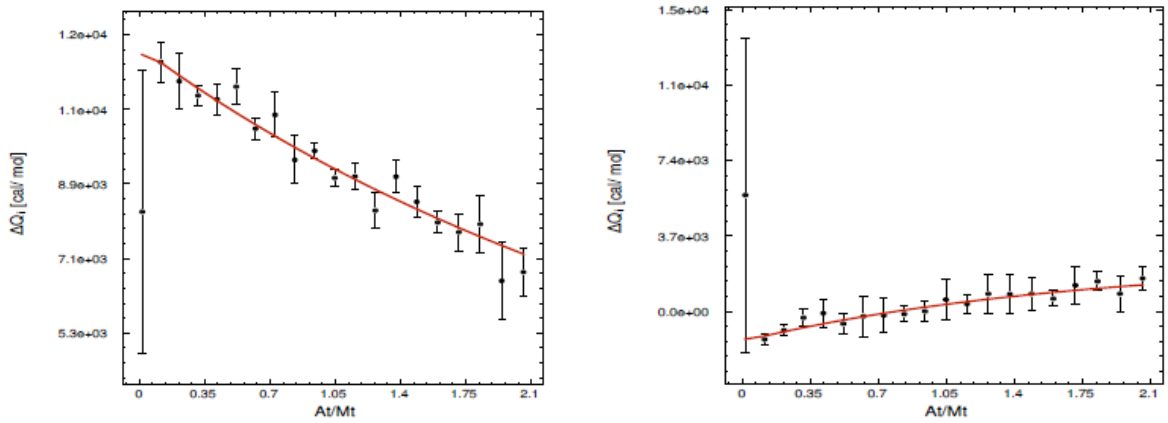
This section presents typical ITC titration curves of the interaction of ^{Ec}SC and ^{Ec}70S with the different peptides used in this study (Table 1 in the main text). All experiments were performed at 30°C. ITC raw data were treated by the AFFINImeter program online (https://www.affinimeter.com). Titration curves presented on the left column correspond to the ligand injection into the protein or ribosome solution. Titration curves on the right correspond to the ligand injection into buffer (ligand dilution control).

Interaction with ^{Ecwt}SC

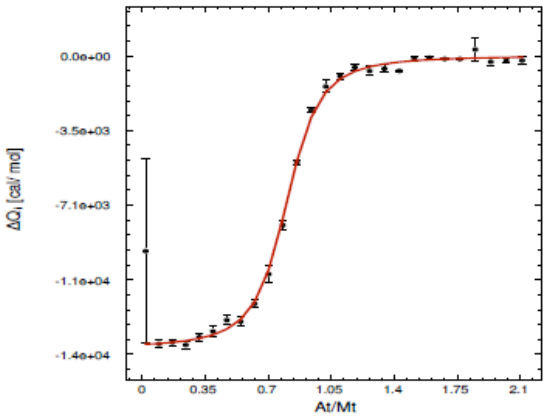
A. ^{Ec}SC/P$_7$

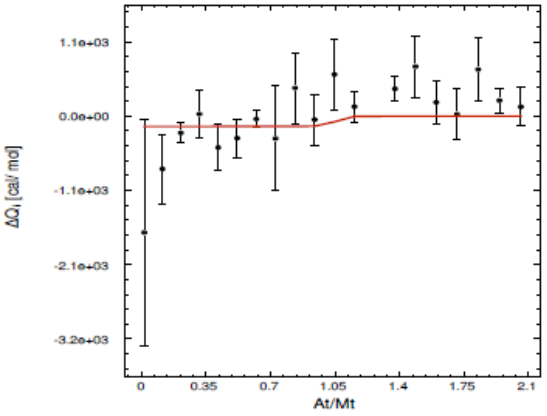


B. ^{Ec}SC/Onc112

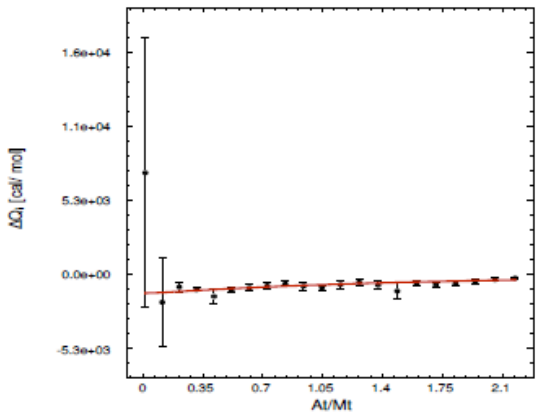


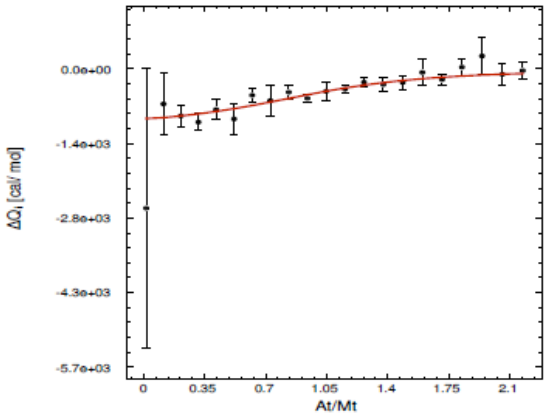
C. *Ec*SC/Onc112-P$_7$



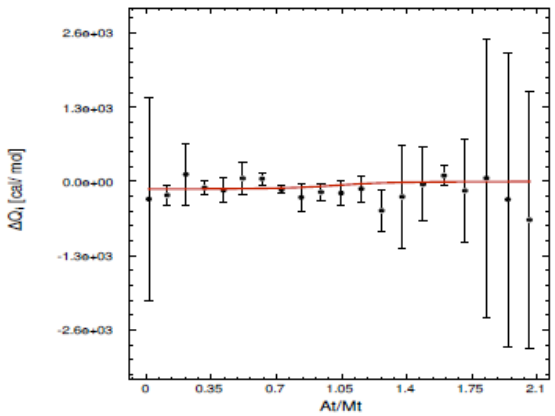


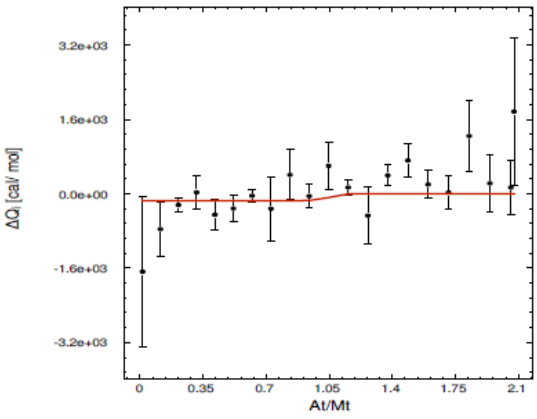
D. *Ec*SC/Onc112-P$_7$Scr



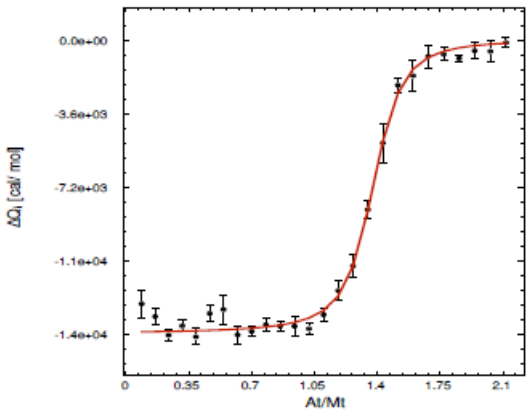


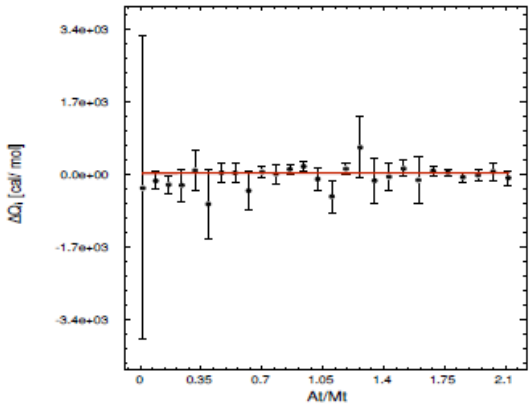
E. *Ec*SC/Py



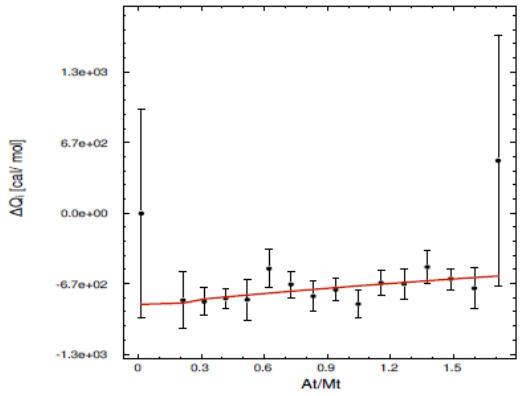


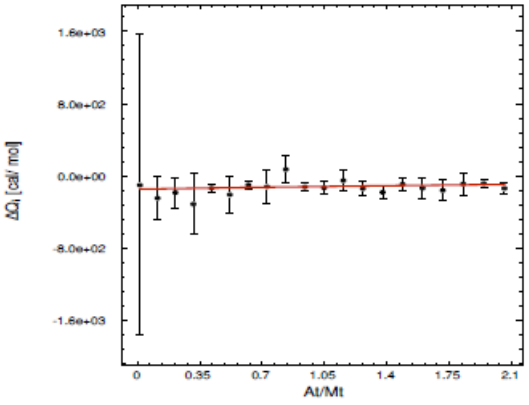
F. *Ec*SC/Py-P$_7$



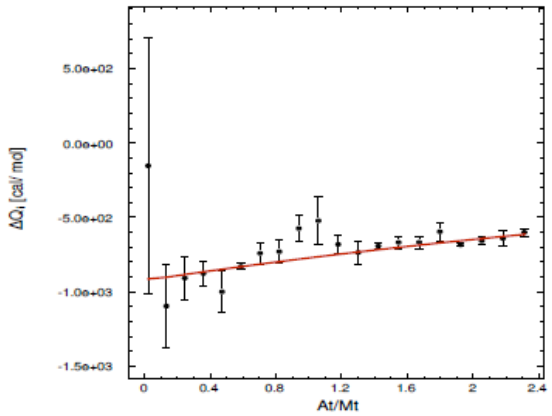


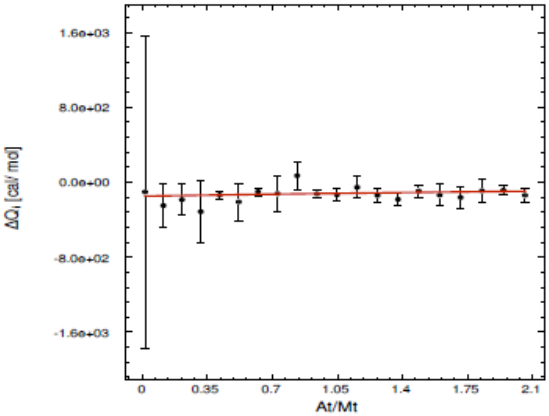
G. *Ec*SC/Py-P$_7$Scr



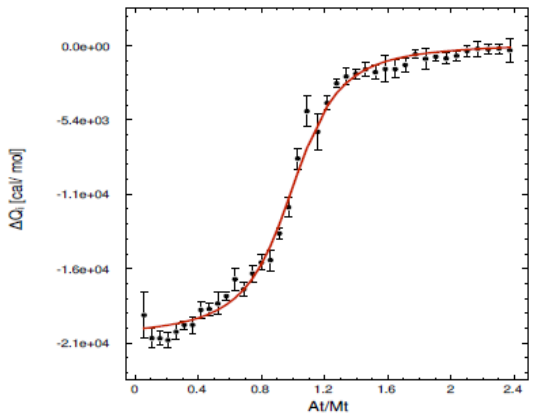


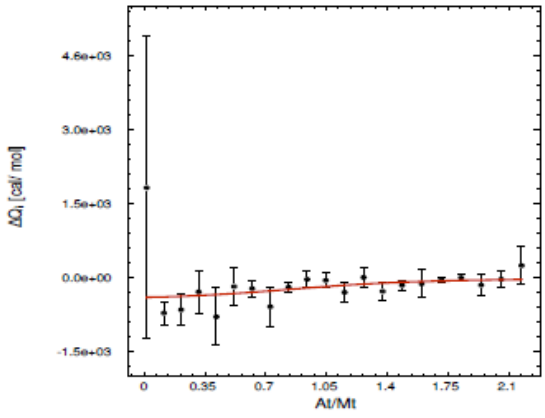
H. *Ec*SC/FITC-Py



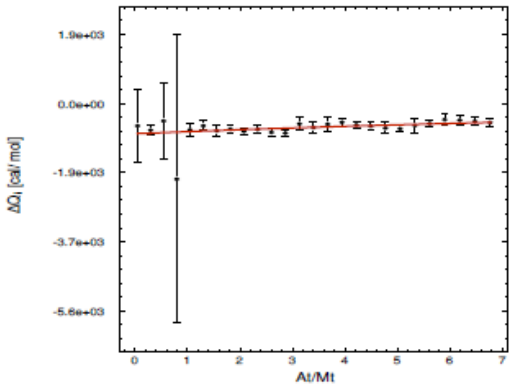


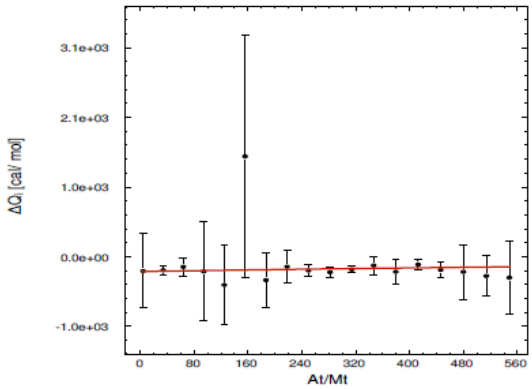
I. ^{Ec}SC/FITC-Py-P$_7$





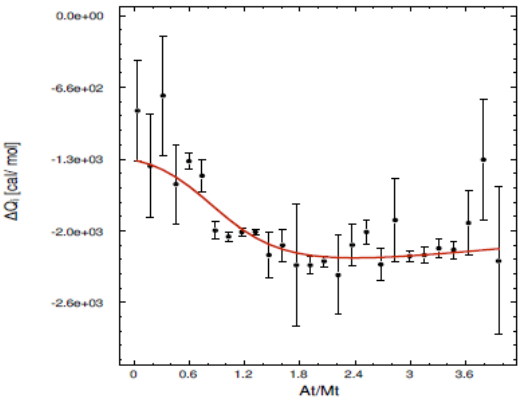
J. ^{Ec}SC/FITC-Py-P$_7$Scr

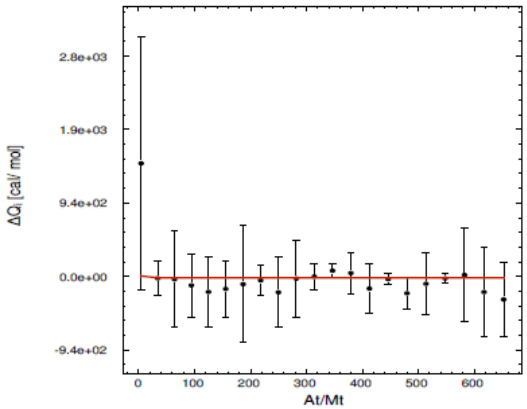




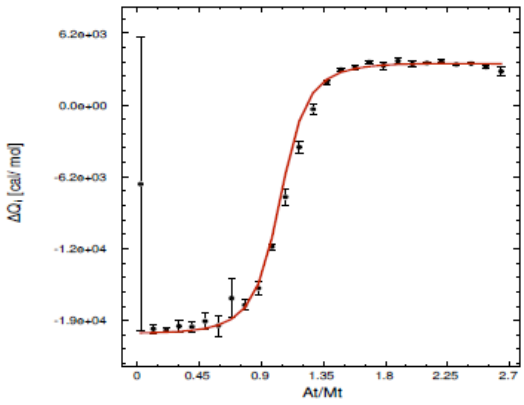
Interaction with ^{Ec}70S

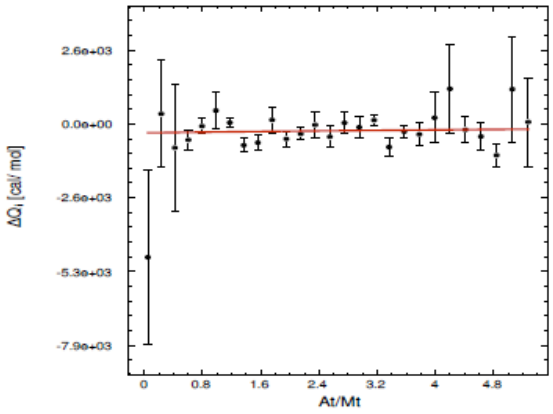
K. ^{Ec}70S /P$_7$



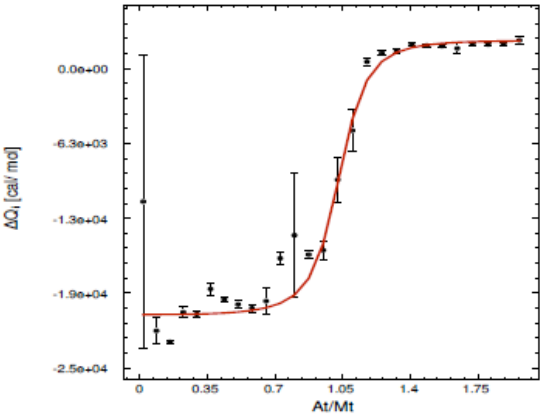


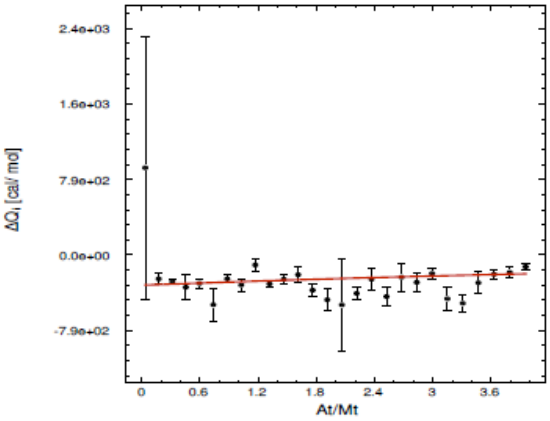
L. ^{Ec}70S /Onc112



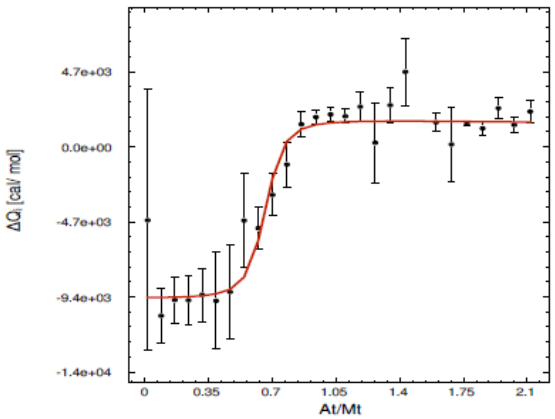


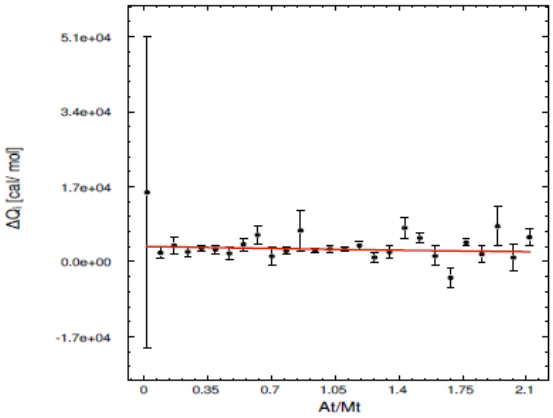
M. ^{Ec}70S /Onc112-P$_7$



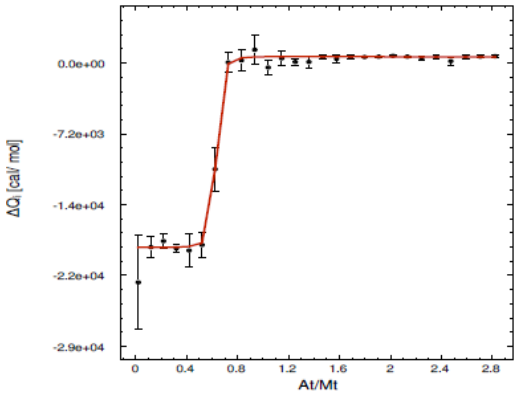


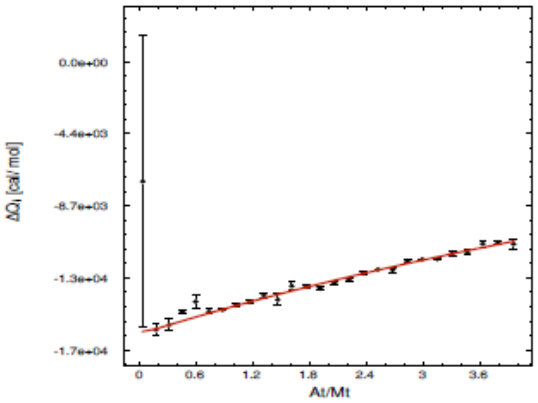
N. ^{Ec}70S /Onc112-P$_7$Scr



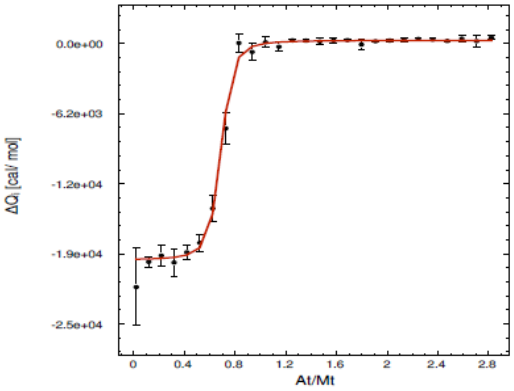


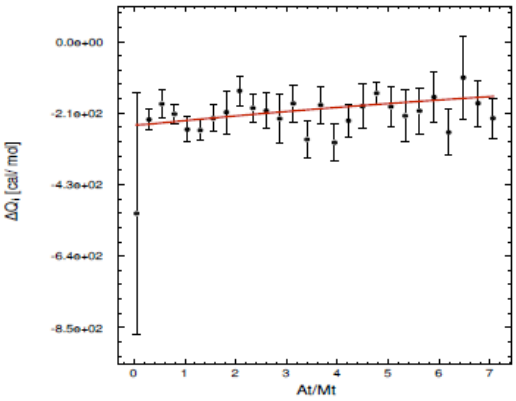
O. ^{Ec}70S /Py



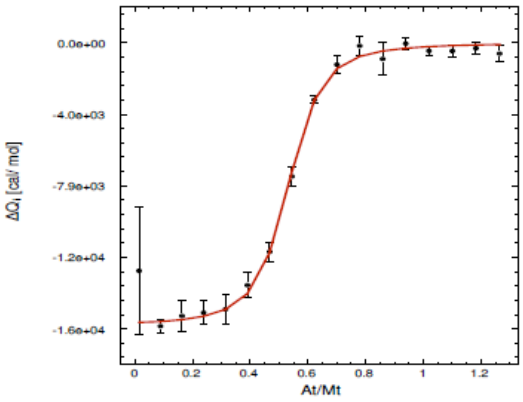


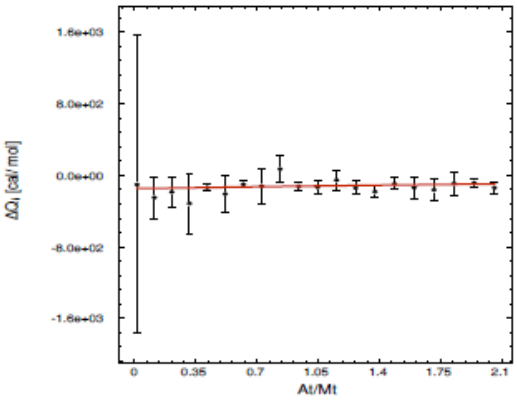
P. ^{Ec}70S/Py-P$_7$



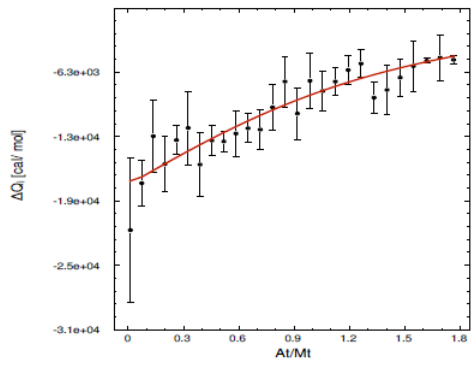


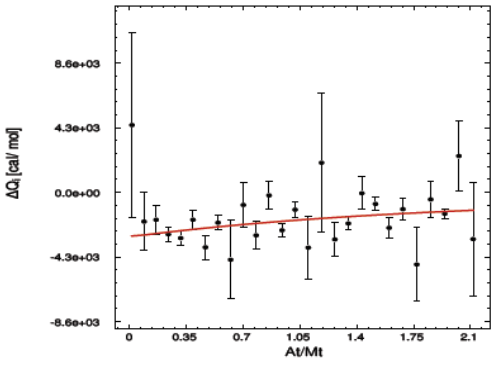
Q. ^{Ec}70S /Py-P$_7$Scr



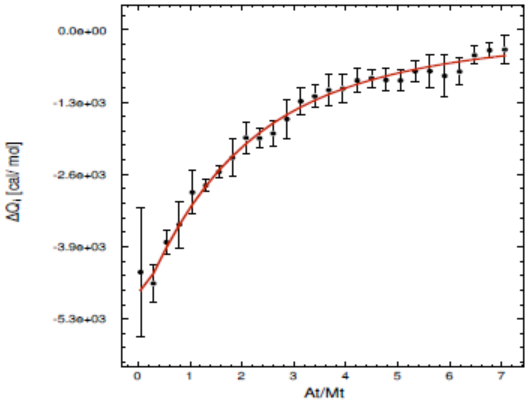


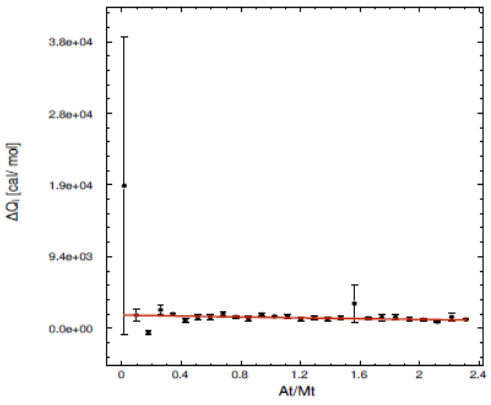
R. ^{Ec}70S /FITC-Py



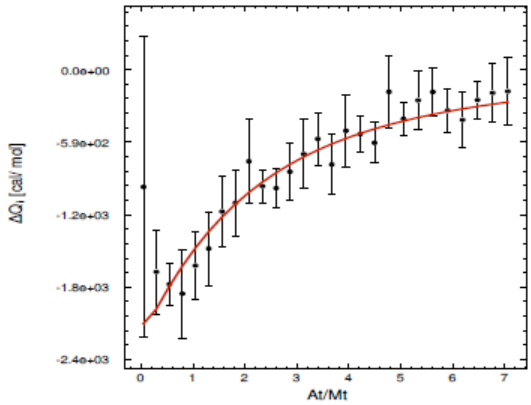


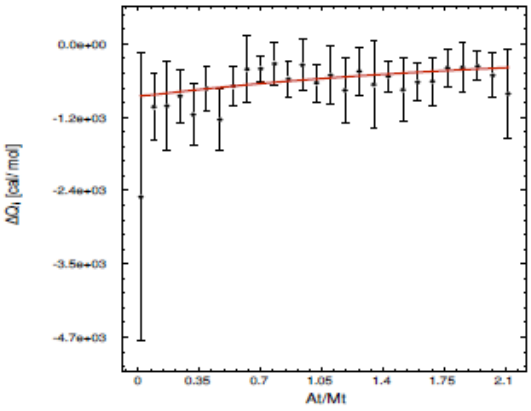
S. ^{Ec}70S /FITC-Py-P$_7$





T. ^{Ec}70S /FITC-Py-P$_7$Scr





SI.4: Interaction of the different peptides with ^{Ec}SC and ^{Ec}70S

The peptide interactions with their respective targets were monitored by ITC at 30°C. **P_7** interacts with ^{Ec}SC (Table SI.4 A, SI.3 A**)** as already observed in previous studies[1,2] but PrAMPs **Onc112** and **Py** do not (Table SI.4 A; SI.3 B and E). However, the fusion peptides, resulting from the C-terminal addition of **P_7** to each PrAMP, *i.e.* **Onc112**-**P_7** and **Py**-**P_7**, interact with ^{Ec}SC as efficiently as **P_7** alone, as indicated by the similar thermodynamic profiles (Table SI.4 A, FIg. SI.2 A and SI.3 C and F). In addition, these fusion peptides have a slightly increased affinity for ^{Ec}SC as compared to **P_7** (Table SI.4 A), which may result from additional interactions established by PrAMPs or linker residues, as previously observed for the linker R residue[3]. Control fusion peptides, **Onc112**-**P_7**Scr and **Py**-**P_7**Scr with a scrambled variant of **P_7**[1] (**P_7**Scr, XFQLD, X = cyclohexylalanyl, Cha) fail to interact with ^{Ec}SC (SI.3 D and G). The labeled fusion peptide FITC-**Py**-**P_7** similarly interacts with ^{Ec}SC when compared to the unlabeled peptides **P_7** and **Py**-**P_7** (Table 2 A, FIg. SI.2 A and SI.3 I). As expected from experiments with their unlabeled counterparts, the two other fluorescent peptides, FITC-**Py** and FITC-**Py**-**P_7**Scr do not interact with ^{Ec}SC (SI.3 H and J).

The interaction of each peptide with the ^{Ec}70S ribosomal particle was also characterized by ITC (Table SI.4 B, FIg. SI.2 B and SI.3 K-T). While **P_7** does not bind to ^{Ec}70S, PrAMPs interact with high affinity with their target (Table SI.4 B, SI.3 L and O), with K_D values in the order of 84 nM and 15 nM for **Onc112** and **Py**, respectively. The fusion peptides **Onc112**-**P_7** and **Py**-**P_7** also efficiently interact with ^{Ec}70S, similarly to their cognate monofunctional peptides (Table SI.4 B, FIg. SI.2 B and SI.3 M and P).

Although **Onc112** and **Py** present similar thermodynamic profiles, indicating an overall similar mode of interaction, their respective thermodynamic values are significantly different (Table SI.4 B). This suggests some differences between peptide interactions within the PEC and correlate with structural data where 16 **Py** residues, but only 12 for **Onc112**, are modeled in the *Thermus thermophilus* PEC (PDB ID: 5FUV)[4], indicating that **Py** might be more stable than **Onc112**. Unfortunately, the crystal structure does not identify additional contacts for **Py** that could account for the difference in thermodynamic parameters[4]. These differences are no longer observed with the corresponding bi-functional peptides **Onc112**-**P_7** and **Py**-**P_7**. Moreover, the **P_7** sequence does not alter the interaction of the PrAMPs with the ribosome (Table SI.4 B, FIg. SI.2 B, SI.3 M and P).

A

	P7	**Onc 112**	**Onc 112-P7**	**Onc 112-P7Scr**	**Py**	**Py-P7**	**Py-P7Scr**	**FITC-Py**	**FITC-Py-P7**	**FITC-Py-P7Scr**
ΔH (kcal/mole)	-14.6 (± 1)	Ni	-13.5 (± 0.4)	Ni	Ni	-13.9 (± 0.4)	Ni	Ni	-16.5 (± 3.4)	Ni
-TΔS (kcal/mole)	5.4 (± 1)	Ni	4.2 (± 0.4)	Ni	Ni	4.3 (± 0.5)	Ni	Ni	7.35 (± 3.8)	Ni
ΔG (kcal/mole)	-9.2 (± 0.2)	Ni	-9.28 (± 0.02)	Ni	Ni	-9.61 (± 0.1)	Ni	Ni	-9.2 (± 0.5)	Ni
K_D (nM)	194 (± 58)	Ni	200 (± 0.1)	Ni	Ni	120 (± 20)	Ni	Ni	300 (± 30)	Ni
n	10	3	2	5	2	2	2	2	4	2

B

	P7	**Onc 112**	**Onc 112-P7**	**Onc 112-P7Scr**	**Py**	**Py-P7**	**Py-P7Scr**	**FITC-Py**	**FITC-Py-P7**	**FITC-Py-P7Scr**
ΔH (kcal/mole)	Ni	-23.9 (± 4.1)	-25.6 (± 2.5)	-13 (± 2.2)	-19.2 (± 0.4)	-22.3 (± 3.8)	-15.4 (± 0.4)	-20.1 (± 13)	-13 (± 6.6)	-8.92 (± .2)
-TΔS (kcal/mole)	Ni	14.0 (± 4.3)	15.7 (± 2.4)	3.12 (± 2.9)	8.23 (±0.03)	12 (± 3.7)	5.5 (± 0.6)	13.3 (± 13.2)	6.7 (± 4.8)	2.94 (± 0.2)
ΔG (kcal/mole)	Ni	-9.82 (± 0.2)	-9.92 (± 0.1)	-9.85 (± 0.6)	-10.9 (± 0.5)	-10.3 (± 0.06)	-9.89 (± 0.2)	-6.91 (± 1.3)	-6.27 (± 0.2)	-5.98 (± 0.2)
K_D (nM)	Ni	84 (± 20)	70 (± 10)	100 (± 0.9)	15 (± 10)	37 (± 0.3)	76 (± 20)	$>10^4$	$>10^4$	$>10^4$
n	2	2	2	2	2	3	2	4	3	2

Table SI.4: A: peptides interaction with ^{Ec}SC. B: peptides interaction with ^{Ec}70S. All experiments were performed at 30°C (303.15 K). Data are means of *n* independent experiments. Ni: no specific interaction (see SI.3 for typical ITC titration curves). *n*: number of independent experiments performed.

Surprisingly enough, the enthalpic and entropic factors values drop dramatically for **Onc112**-**P_7**Scr and **Py**-**P_7**Scr peptides, indicating that, as compared to **P_7** fusion peptides, the **P_7** scrambled sequence affects the mode of interaction of the PrAMP with its ribosomal target. Nevertheless, these fusion peptides still interact with ^{Ec}70S (SI.3 N and Q), despite a slightly lower affinity (Table SI.4B). Finally, the N-terminal FITC moiety is highly deleterious for the interaction of **Py** and its derivatives with ^{Ec}70S (Table SI.4B, SI.3 R-T), indicating that this fluorescent probe strongly hinders the PrAMP entry into the PEC. The poor interaction of these labeled peptides with ^{Ec}70S precludes any serious analysis of the thermodynamic data.

SI.5: Whole gel images of the co-immunoprecipitation assays

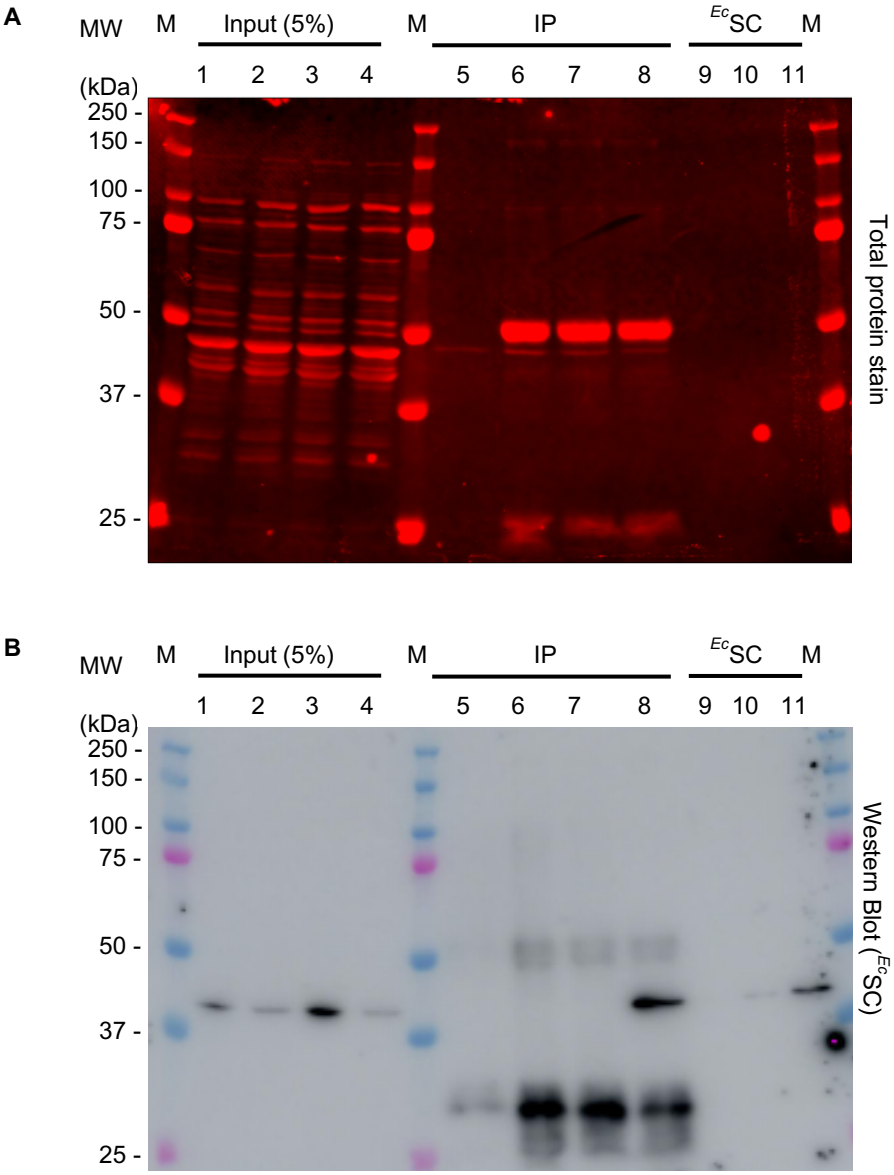


FIg. SI.5: Total protein stain (**A**) and ^{Ec}SC immuno-detection (**B**) of the immunoprecipitation assays. Lysates from *E. coli* cells exposed to either H_2O (lanes 1 and 5), FITC-**Py** (lanes 2 and 6), FITC-**Py**-$\mathbf{P_7}$Scr (lanes 3 and 7) or FITC-**Py**-$\mathbf{P_7}$ (lanes 4 and 8) were processed as described in the Experimental section in the main text. Lanes 9 to 11 were loaded with 5, 10 and 20 ng of purified ^{Ec}SC as control.

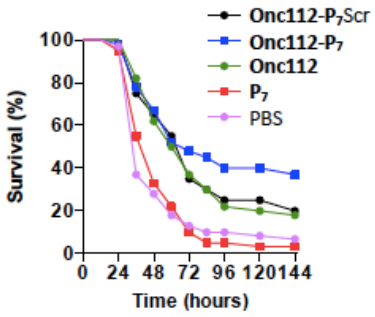
As shown in FIg. SI.5, there are no major differences between the protein profiles of the different inputs (total protein stain, lanes 1-4 FIg. SI.5A) although some variations in the ^{Ec}SC immunodetection assay signals are observed (lanes 1-4 FIg. SI.5B). No unspecific signals are detected in the inputs. Given

the profile of the ^{Ec}SC standard range (lanes 9-11 FIg. SI.5B) we can estimate the sensitivity of the anti-^{Ec}SC Ab246 antibody in the range of 10 ng of ^{Ec}SC under such conditions and an amount of about 15 to 30 ng of ^{Ec}SC per input sample. Majors bands of about 50 and 25 kDa, corresponding respectively to the molecular weigths of Ig heavy and light chains are detected in lanes 6-8 of both images. These signals are attributed to the presence of the goat anti-FITC antibody (ab 19224) in these samples (note that the control sample used to load lane 5 was not exposed to the goat anti-FITC antibody; see the Experimental section in the main text). A faint band, just under the 50 kDa MW marker of unidentified origin is also detected in all four samples treated with protein G sepharose by total protein staining but not by immunodetection (lanes 5-8, FIg. SI.5). Thus, the only anti-^{Ec}SC antibody-specific signal is observed in lane 8 (FIg. SI.5B) corresponding to the co-immunoprecipitation of the ^{Ec}SC bound to the FITC-labelled SC interacting peptide (FITC-Py-P7) by the anti-FITC antibody-protein G complex.

SI.6: Survival curves of *E. coli* infected *Drosophila melanogaster*

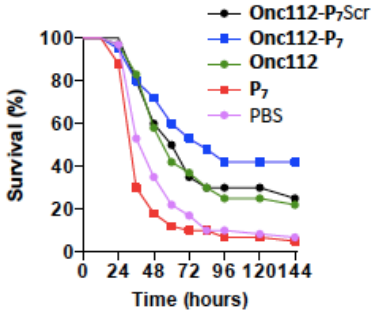
Three independent experiments have been performed (see the Experimental section in the main text). The resulting survival curves and the statistical analysis are presented below.

Experiment 1



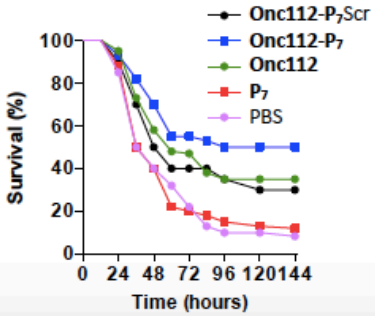
PBS *vs* **P$_7$**	ns	P = 0.8869
PBS *vs* **Onc112**	***	P = 0.0002
PBS *vs* **Onc112-P$_7$**	***	P = 8.7 10^{-7}
PBS *vs* **Onc112-P$_7$Scr**	***	P = 0.0001
Onc112-P$_7$ *vs* **Onc112**	ns	P = 0.0779
Onc112-P$_7$ *vs* **Onc112-P$_7$Scr**	ns	P = 0.1288
Onc112 *vs* **Onc112-P$_7$Scr**	ns	P = 0.8301

Experiment 2



PBS *vs* **P$_7$**	ns	P = 0.0590
PBS *vs* **Onc112**	**	P = 0.0012
PBS *vs* **Onc112-P$_7$**	***	P = 6.7 10^{-7}
PBS *vs* **Onc112-P$_7$Scr**	***	P = 0.0003
Onc112-P$_7$ *vs* **Onc112**	*	P = 0.0307
Onc112-P$_7$ *vs* **Onc112-P$_7$Scr**	ns	P = 0.0780
Onc112 *vs* **Onc112-P$_7$Scr**	ns	P = 0.6924

Experiment 3



PBS *vs* **P$_7$**	ns	P = 0.7828
PBS *vs* **Onc112**	***	P = 0.0003
PBS *vs* **Onc112-P$_7$**	***	P = 5.5 10^{-7}
PBS *vs* **Onc112-P$_7$Scr**	**	P = 0.0035
Onc112-P$_7$ *vs* **Onc112**	ns	P = 0.1102
Onc112-P$_7$ *vs* **Onc112-P$_7$Scr**	ns	P = 0.0225
Onc112 *vs* **Onc112-P$_7$Scr**	ns	P = 0.5043

FIg. SI.6: *In vivo* survival assay using *Drosophila melanogaster* infection model.

Seven-day-old female *kenny*^{C02831} flies were subjected to septic injury with a thin tungsten needle previously dipped in an *E. coli* suspension diluted in PBS and kept at 29 °C. 1 and 24 hours after injury, 18.4 nl of peptides (1 mM) diluted in PBS or PBS alone were injected into the flies body cavity. Each group was constituted of 60 flies and the experiment was conducted 3 times independently. For each experiment, results were analyzed by log rank analysis with the OASIS online application[5]. *P < 0.05, **P<0.01, ***P<0.001. ns: not significant.

References

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