

## **High complexity of Glutamine synthetase regulation in *Methanosarcina mazei*: Small protein 26 interacts and enhances glutamine synthetase activity**

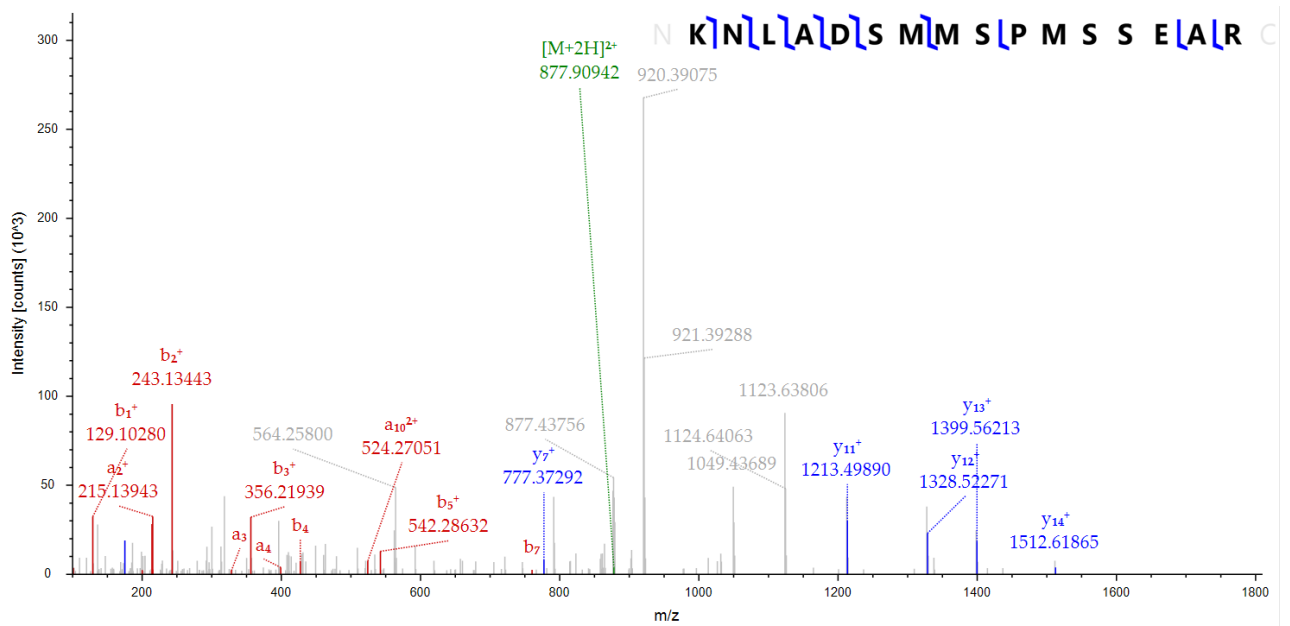
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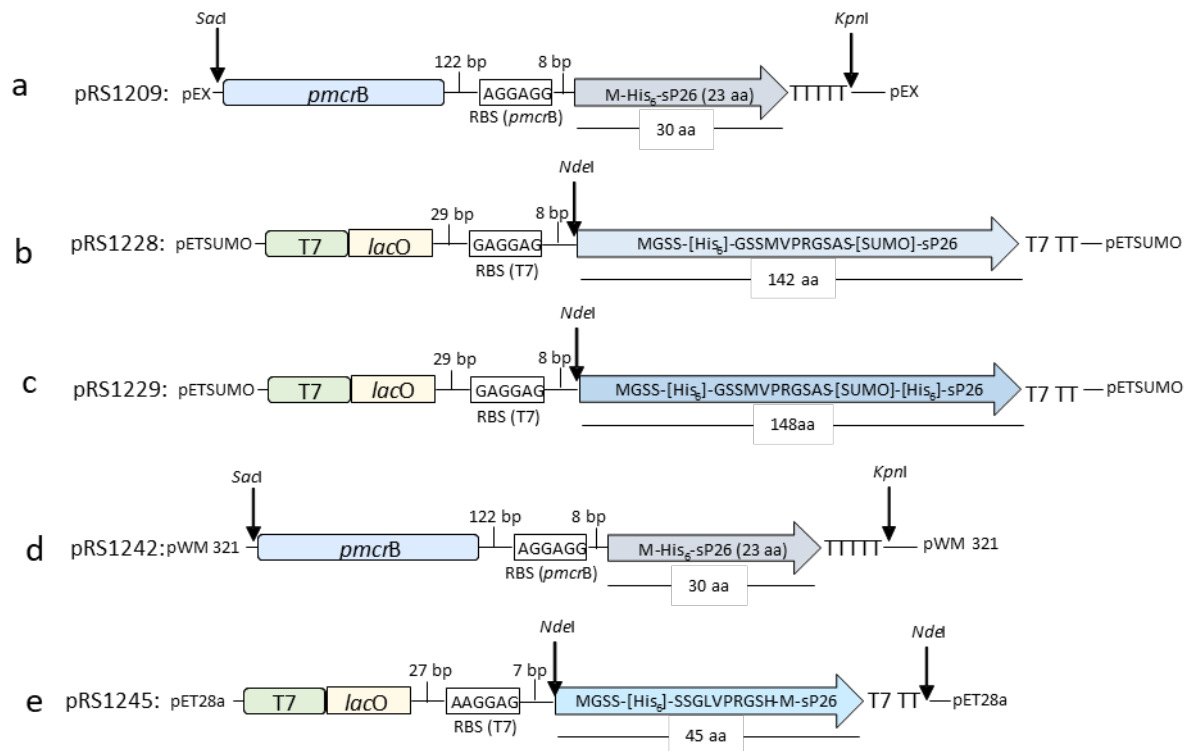
**Supplemental Material:**

**Supplemental figures 1+2**

**Supplemental Table 1 + 2**



**Suppl. Fig. 1. Annotated spectra for the proteotypic peptide KNLADSMMSSEAR** (MH<sup>+</sup>: 1754.78093 Da; +2 monoisotopic m/z: 877.89410 Da (-3.46 ppm) from sp26 identified by C-MS/MS after tryptic digestion of the *M. maezi* proteome [8]. The peptide was identified with high confidence at the peptide level (peptide level FDR <1%), the inferred identification of sp26 had a protein level FDR of <5%. Inserted peptide sequence showing the b and y ion fragmentation positions identified for the peptide.



**Suppl. Fig. 2: Schematic plasmid maps of expression vectors:** (a) pRS1209 containing His<sub>6</sub>-SORF26 in pEX (gene synthesis, Eurofins Scientific, Nantes). (b) pRS1228 containing SUMO-sP26 for heterologous expression in *E. coli*. (c) pRS1229 containing SUMO-His<sub>6</sub>-sP26 for heterologous expression in *E. coli* and pulldown experiments. (d) pRS1242 containing His<sub>6</sub>-sP26 for expression in *M. mazei*. (e) pRS1245 containing His<sub>6</sub>-sP26 for heterologous expression in *E. coli*. TTTT, transcriptional terminator for *M. mazei*; T7 TT, transcriptional terminator T7 RNA polymerase.

**suppl. Table 1:** GlnA<sub>1</sub> activity determined as described in Methods using purified proteins of independent purifications

Experimental conditions		App. spec. activity (U/mg)	activation (n-fold)
+ His <sub>6</sub> -sP26	0.472 μmol GlnA <sub>1</sub>	0.0175	1.00
	+ 2.2 μmol sP26	0.0460	2.64
	+ 4.4 μmol sP26	0.115	6.60
	+ 8.8 μmol sP26	0.172	9.82
	0.95 μmol GlnA <sub>1</sub>	0.035	1.00
	+ 2.2 μmol sP26	0.047	1.36
	+ 8.8 μmol sP26	0.055	1.60
	0.95 μmol GlnA <sub>1</sub>	0.04	1.00
	+ 2.2 μmol sP26	0.05	1.20
	+ 17.9 μmol sP26	0.08	2.10
	0.95 μmol GlnA <sub>1</sub>	0.008	1.00
	+ 1.6 μmol sP26	0.029	3.50
	0.95 μmol GlnA <sub>1</sub>	0.013	1.00
	+ 2.9 μmol μg sP26	0.0292	2.22
+ 5 mM 2-OG + His <sub>6</sub> -sP26	0.11 μmol GlnA <sub>1</sub> + 2-OG	4.80	1.00
	+ 2.2 μmol μg sp26	10.60	2.20
	0.95 μmol GlnA <sub>1</sub> + 2-OG	0.55	1.00
	+ 2.2 μmol μg sP26	0.70	1.28
	+ 17.9 μmol sP26	2.78	5.07
	0.95 μmol GlnA <sub>1</sub> + 2-OG	1.17	1.00
	+ 2.9 μmol sP26	2.47	2.12
+ His <sub>6</sub> -sP26 + His <sub>6</sub> -GlnK <sub>1</sub>	0.95 μmol GlnA <sub>1</sub>	0.008	1.00
	+ 3.3 μmol GlnK <sub>1</sub>	0.026	3.16
	+ 3.3 μmol GlnK <sub>1</sub>		
	+ 1.6 μmol sP26	0.042	5.10
	0.95 μmol GlnA <sub>1</sub>	0.013	1.00
	+ 0.65 μmol GlnK <sub>1</sub>	0.025	1.91
	+ 0.65 μmol GlnK <sub>1</sub> + 2.9 μmol sP26	0.043	3.29
+ 5 mM 2-OG + His <sub>6</sub> -GlnK <sub>1</sub> + His <sub>6</sub> -sP26	0.11 μmol GlnA <sub>1</sub> + 2-OG	4.82	1.00
	+ 0.54 μmol GlnK <sub>1</sub>	7.61	1.58
	+ 0.54 μmol GlnK <sub>1</sub>		
	+ 2.2 μmol sP26	11.76	2.44
	0.95 μmol GlnA <sub>1</sub> + 2-OG	1.17	1.00
	+ 0.65 μmol GlnK <sub>1</sub>	1.45	1.24
	+ 0.65 μmol GlnK <sub>1</sub> + 2.9 μmol sP26	2.54	2.17

suppl. Table 2. Strains and plasmids used

Strain or plasmid	Genotype or description	Source of reference
<b>Strains</b>		
<i>Methanosarcina mazei</i> strain GÖ1	wild type; DSM No. 3647	DSMZ, Braunschweig, Germany
<i>Escherichia coli</i> BL21 (DE3)	general expression strain	Invitrogen, Carlsbad, USA
<i>Escherichia coli</i> BL21-CodonPlus®-RIL	general expression strain containing the pRIL plasmid (ileW, leuY, proL)	Stratagene, La Jolla, USA
<b>Plasmids</b>		
pET28a	general cloning vector providing N-terminal His <sub>6</sub> -tag	Merck KGaA, Darmstadt, Germany
pET-SUMO	general cloning vector providing N-terminal SUMO-tag	Invitrogen, Carlsbad, USA
pCR®II –TOPO® vector	general cloning vector	Life Technologies, Darmstadt, Germany
pRS196	<i>MM0964 (glnA<sub>1</sub>)</i> under the control of T7 in pET28a	[32]
pRS203	<i>MM0732 (glnK<sub>1</sub>)</i> under the control of T7 in pET28a	[32]
pRS375	pRS196 with exchanged His <sub>6</sub> -Tag by Strep-tag	this work
pRS1209	His <sub>6</sub> -sORF26 under the control of <i>pmcrB</i> in pEX	Eurofins Scientific, Nantes, Luxemburg
pRS1228	SUMO-sORF26 under the control of T7 in pET-SUMO	this work
pRS1229	SUMO-His <sub>6</sub> -sORF26 under the control of T7 in pET-SUMO	this work
pRS1242	His <sub>6</sub> -sORF26 under the control of <i>pmcrB</i> in pWM321	this work
pRS1244	sORF26 in pCR®II-TOPO®	this work
pRS1245	His <sub>6</sub> -sORF26 under the control of T7 in pET28a	this work