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Structural and functional mapping of the archaeal multi-aminoacyl-tRNA synthetase complex

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Abstract

Methanothermobacter thermautotrophicus contains a multi-aminoacyl-tRNA synthetase complex (MSC) of LysRS, LeuRS and ProRS. Elongation factor (EF) 1A also associates to the MSC, with LeuRS possibly acting as a core protein. Analysis of the MSC revealed that LysRS and ProRS specifically interact with the idiosyncratic N- and C- termini of LeuRS, respectively. EF-1A instead interacts with the inserted CP1 proofreading domain, consistent with models for post-transfer editing by class I synthetases such as LeuRS. Together with previous genetic data, these findings show that LeuRS plays a central role in mediating interactions within the archaeal MSC by acting as a core scaffolding protein.

Keywords

aminoacyl-tRNA synthetase; translation; amino acid

1. Introduction

Protein translation based on a nucleic acid code relies on the correct pairing of amino acid and tRNA molecules and subsequent delivery to the ribosome. Aminoacyl-tRNA synthetases (aaRSs) play an essential role in protein synthesis by attaching the correct amino acid onto the cognate tRNA. Once synthesized, aa-tRNAs are bound by EF-1A as a ternary complex with GTP and ushered to the ribosome. AaRSs are commonly found in higher order complexes in eukaryotes and archaea. For example, a multi-aaRS complex (MSC) composed of nine aaRS activities (aminoacylating cognate lysine, leucine, proline, isoleucine, methionine, glutamine, glutamic acid, asparagine, and aspartic acid) is found in mammalian cells. Three auxiliary proteins (p38, p43, and p18) complete the complex, promoting the binding of tRNA and contributing to the stability of the complex. The association of aaRSs and auxiliary proteins in the macromolecular MSC requires a network of protein-protein interactions within the complex. Genetic and biophysical data suggest that most, if not all, aaRSs within the complex associate with p38 and/or p43, enhancing the stability of the complex. Several interactions

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Structured summary: MINT-6551032: *EF1A* (uniprotkb:O27132) *physically interacts* (MI:0218) with *LeuRS* (uniprotkb:O27552) by *surface plasmon resonance* (MI:0107)

within the MSC are known to be mediated via N- and C-terminal appended domains of the associated eukaryotic aaRSs, which are generally absent from their bacterial and archaeal counterparts [1]; for example, LeuRS associates with the mammalian MSC via N- and C-terminal extensions [2]. These appendages to the cores of aaRSs are non-catalytic and instead function to mediate protein-protein interactions or act as general RNA binding domains [3-5]. Conversely several aaRSs, such as LysRS and AspRS, associate with the MSC through their catalytic cores, since deletions of the appended domains have no effect on complex formation [6;7].

Methanothermobacter thermautotrophicus contains a smaller MSC composed of LysRS, LeuRS, and ProRS [8]. EF-1A also associates with LeuRS [9], which could act as a core protein to mediate stable complex formation of the archaeal MSC, since no homologues of the eukaryotic auxiliary proteins have been found in archaea. *M. thermautotrophicus* LeuRS possesses a C-terminal extension beyond the catalytic core; although shorter than its eukaryotic counterpart, this suggests that comparable regions of the structurally distinct C-terminus of archaeal LeuRS might also mediate interactions with other proteins within the MSC. This led us to more closely investigate the potential role of LeuRS in the archaeal MSC, which revealed an essential role for this aaRS as a core scaffolding protein.

2. Materials and methods

Protein and tRNA production and purification

His_6 fusion derivatives of N- and C-terminally truncated LeuRS, ΔCP1 LeuRS, LysRS, ProRS, EF-Tu and EF-1A were prepared as described, as were preparation and aminoacylation of tRNA and activation of elongation factors [9]. Both the full-length and truncation proteins were relatively labile during purification, presumably as a result of a significant level of misfolding, typically displaying 15-20% active fractions (Table 1).

Fluorescence anisotropy

Equilibrium dissociation constants were determined by measuring the fluorescence anisotropy of labeled proteins as a function of increasing concentrations of unlabeled proteins as previously described [9] with the following exceptions. Each of the LeuRS variants and EF-1A were labeled with Alexa Fluor (AF) 488 tetrafluorophenyl ester (Molecular Probes, Eugene, OR). LeuRS and EF-1A were chosen due to the significantly higher stability they displayed over LysRS and ProRS during labeling. LeuRS variants and EF-1A were labeled with AF-488 at a molar ratio of 1:15 enzyme:fluorophore as previously described [9]. Each fluorescently labeled LeuRS variant and EF-1A sample were visualized on a 10 % SDS-polyacrylamide gel, which confirmed that the final labeled product contained little or no free fluorophore. Prior to use in fluorescence anisotropy measurements, the activity of the labeled LeuRS variants and EF-1A were verified by aminoacylation and GDP-exchange assays, respectively, and protein concentrations determined by active site titration and dye binding (BioRad) (data not shown; [10]). Equilibrium dissociation constants were determined by measuring the fluorescence anisotropy of labeled EF-1A or LeuRS variants (50 nM each) as a function of increasing concentrations of unlabeled protein using a Fluorolog-3 spectrofluorimeter (Horiba Jobin Yvon) as previously described [9]. The following concentration ranges of unlabeled proteins were used: 0-2000 nM LysRS, and 0-2400 nM ProRS, full-length LeuRS, ΔCP1 LeuRS, LeuRS C-terminal deletion variants ΔC1 and ΔC2, and LeuRS N-terminal deletion variant ΔN1. All measurements were carried out at least three times. Titration curves were fitted assuming a 1:1 binding stoichiometry as previously described [11].

Surface plasmon resonance

Surface plasmon resonance (SPR) experiments were performed utilizing a Biacore T100 instrument (GE Healthcare). 2000 resonance units (RU) of EF-1A in 100 mM sodium acetate (pH 4.5) were immobilized to a carboxymethyl dextran chip according to the manufacturer's instructions. As a control, a flow cell was activated and blocked in the absence of protein, which was then used to subtract RU resulting from non-specific interactions and the bulk refractive index. Experiments were performed at 25 °C in HBS-EP+ buffer (0.01 M Na-Hepes (pH 7.4), 150 mM NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20), 10 mM $MgCl_2$, 50 mM KCl and 100 μM GDP at a flow rate of 10 μl/min. The chip was regenerated by flowing 10 mM glycine-HCl (pH 2.5) over it for 30 sec at a flow rate of 25 μl/min. Each cycle consisted of injection of 80 μl of analyte (full-length LeuRS or variant ΔC2) over the immobilized protein (EF-1A) and the control flow cell, 660 sec dissociation time in HBS-EP+ buffer, and regeneration prior to the next injection. RU values were determined following subtraction of the control from the protein of interest immobilized to the cell. R_{max} values for interactions between the analyte and immobilized protein were calculated according to the manufacturer's protocol. Dissociation constants were calculated using the average RU under steady-state conditions and data were fitted using the Binding Analysis Biacore T100 Evaluation Software.

ATP consumption editing assay

As a measure of *cis*-editing, LeuRS catalyzed ATP consumption was monitored in the presence of non-cognate amino acids. The reaction, carried out at 37 °C, contained 2 U/ml pyrophosphatase (Roche), 2 -10 mM leucine, methionine, valine, isoleucine, or norvaline, 5 μM $tRNA^{Leu}$, 2 mM [γ-^{32}P]ATP (5 cpm/pmol), 0.1 M Na-Hepes (pH 7.2), 30 mM KCl, 10 mM $MgCl_2$, and 1 μM ΔCP1 LeuRS or 1 μM LeuRS in the presence or absence of 5 μM EF-1A or EF-Tu. Aliquots were removed at various times and quenched in glacial acetic acid. Remaining [γ-^{32}P]ATP and [γ-^{32}P]Pi formed during the reaction were separated by TLC on a PEI cellulose plate (Sigma) prewashed with water. The TLC was subsequently developed in 0.7 M potassium phosphate (pH 3.5) and labeled products visualized and quantified on a Storm phosphorimager (Amersham Biosciences). Pi (mM) formed during the time-course reaction was estimated by multiplying the measured Pi/ATP ratio by the initial concentration of ATP (2 mM).

3. Results

Defining the roles of LeuRS N- and C-termini on complex formation

The LeuRS active site is composed of a Rossmann-fold domain containing the conserved sequence motifs HIGH and KMSKS near the N- and C-terminus (Fig. 1). To probe the role of LeuRS in the archaeal MSC five variants were created, truncated from the N-terminus to HIGH (ΔN1 and ΔN2) and from the C-terminus to KMSKS (ΔC1, ΔC2, and ΔC3; Fig. 1, Table 1). Variants adjacent to the HIGH and KMSKS motifs (ΔN2 and ΔC3) were inactive in aminoacylation and leucine activation (data not shown). LeuRS variants ΔN1, ΔC1 and ΔC2 were all able to bind and activate Leu, but only ΔN1 also retained aminoacylation activity. These three truncation variants were then utilized to investigate the domains of LeuRS responsible for mediating protein-protein interactions within the archaeal MSC. Full-length LeuRS bound LysRS, ProRS, and EF-1A with comparable affinities over a range of K_Ds between 80-170 nM (Table 2). Deletion of eight amino acid residues from the N-terminus of LeuRS (ΔN1 variant) had little effect on the binding of ProRS and EF-1A (K_Ds approximately 110 nM). Deletion of the LeuRS N-terminus increased affinity for LysRS three-fold, suggesting this region may modulate the LysRS:LeuRS interaction, in agreement with previous yeast two-hybrid analyses [8]. Attempts to further investigate LysRS:LeuRS binding in more detail were hindered by the lack of viable LeuRS variants further truncated from the N-terminus. LysRS and EF-1A each bound C-terminal truncated LeuRS variants (ΔC1 and ΔC2) with affinities

comparable to full-length LeuRS (Table 2). Truncation of the LeuRS C-terminus severely impaired ProRS binding (Table 2), indicating complex formation between these two aaRSs is at least partly mediated by the C-terminal extension of LeuRS, again consistent with previous yeast-two hybrid data [8].

Effects of the LeuRS CP1 editing domain on complex formation

In addition to N- and C-terminal truncations of LeuRS, the CP1 domain was deleted to investigate its role in complex formation. Located between the HIGH and KMSKS motifs, CP1 is an inserted editing domain which recognizes and hydrolyzes tRNAs misacylated with non-cognate amino acids. The ΔCP1 variant was active in leucine activation (Table1), but inactive in editing of non-cognate amino acids (see below) and in aminoacylation (data not shown). LysRS and ProRS bound the ΔCP1 LeuRS variant with affinities comparable to full-length LeuRS suggesting that the editing domain is not essential for complex formation (Table 2). Deletion of CP1 had a pronounced effect on EF-1A binding to LeuRS, suggesting that the editing domain is involved in mediating protein-protein interactions (Table 2). To further investigate the binding of EF-1A to LeuRS via the CP1 domain, SPR experiments were performed. Deletion of the LeuRS CP1 domain significantly impaired interactions with EF-1A (Fig. 2) again indicating that the CP1 editing domain specifically associates with EF-1A.

The effects of complex formation with EF-1A on the editing activities of LeuRS were monitored for the non-cognate substrates methionine, valine, isoleucine, and norvaline in the absence and presence of $tRNA^{Leu}$. Methionine, valine, and isoleucine were not editing substrates (data not shown), while wild-type LeuRS, but not the ΔCP1 variant, edited norvaline in a tRNA-dependent manner (Fig. 3A). Since EF-1A is known to adopt alternative conformations, the overall editing reaction was compared in the presence of EF-1A·GTP (required for aa-tRNA binding) and EF-1A·GDP. Regardless of the bound guanine nucleotide, LeuRS in complex with EF-1A displayed editing activities comparable to LeuRS alone (Fig. 3B), indicating that EF-1A does not effect the hydrolysis of non-cognate norvaline. Similarly, bacterial EF-Tu had no effect on LeuRS editing regardless of the GTP- or GDP-bound states (Fig. 3C), consistent with previous data that EF-Tu does not form specific interactions with *M. thermautotrophicus* LeuRS [9].

4. Discussion

LeuRS acts as the core protein of the archaeal MSC

The eukaryotic counterparts of LysRS, LeuRS, and ProRS associate into a higher order complex with six additional aaRS activities and three auxiliary proteins, homologues of which have not been observed in archaea. *M. thermautotrophicus* EF-1A associates with the archaeal MSC, through specific interactions with LeuRS, suggesting a central role of LeuRS as a platform for complex formation and assembly in archaea. Towards structural mapping of protein interactions within the MSC, variants of archaeal LeuRS were constructed including truncation mutants from the N- and C-terminus and deletion of the modular CP1 editing domain. Deletion of the N-terminus of LeuRS had a modest effect on LysRS binding, suggesting that the N-terminal region modulates the LysRS:LeuRS interaction, in agreement with previous yeast two-hybrid analyses. When examining the domains of LeuRS that mediate interactions with ProRS, it was discovered that truncation of the LeuRS C-terminus significantly decreased binding to ProRS, also consistent with yeast two-hybrid data.

Deletion of the LeuRS CP1 editing domain resulted in decreased binding by EF-1A compared to full-length or LeuRS truncation variants as assessed by both fluorescence and SPR experiments. Determination of binding kinetics was not possible under these experimental conditions, likely due to the instability of proteins over the lengthy association and dissociation

phases required for measurements of kinetic parameters in real time (data not shown). Overall, these data suggest that LysRS associates with the N-terminus of LeuRS, which in turn serves as a platform for the association of ProRS and EF-1A (Fig. 4). This potentially open structure is reminiscent of the extended V-shaped structure proposed for the far larger mammalian MSC [12], and would facilitate access of tRNA to the archaeal MSC consistent with the enhanced aminoacylation of all the aaRSs associated with the complex.

Functional interactions with EF-1A in the archaeal MSC

It was suggested that steric clashes with CP1 prevent class I aaRSs such as LeuRS from stably associating with EF-Tu ([13;14]). In the present study, however, we found that archaeal EF-1A makes specific contacts with the LeuRS CP1 editing domain. This may be explained by the fact that CP1 is a distinct globular domain flexibly linked to the catalytic body (Fig. 5). Consequently, the position of CP1 has been observed to depend on the orientation of the tRNA CCA-acceptor end as it enters the editing domain during post-transfer editing, rotating approximately 20° as observed in crystal structures of *P. horikoshii* and up to 35° in *T. thermophilus* LeuRS [15-17]. Given that the domain orientations of EF-1A and EF-Tu also differ (Fig. 5) reorientation of the CP1 domain may permit LeuRS association with EF-1A, resulting in enhanced synthesis of Leu-tRNA^{Leu}. No concomitant alteration in editing was detected as a result of the LeuRS:EF-1A interaction. This indicates that EF-1A has no effect on the rate-limiting step of editing, which is likely to be translocation of the misactivated or misacylated amino acid from the active site to the editing site [18]. These data indicate that EF-1A cannot directly abstract aa-tRNA^{Leu} from LeuRS, consistent with previous data which indicated that enhanced synthesis of Leu-tRNA^{Leu} could be specifically attributed to complex formation with EF-1A, since bacterial EF-Tu displayed no effect on aminoacylation despite its high affinity for Leu-tRNA^{Leu}. This supports the notion that EF-1A forms specific interactions with LeuRS which do not affect the overall editing reaction, but instead enhance the rate of Leu-tRNA^{Leu} synthesis, possibly by inducing conformational changes in the catalytic core of LeuRS.

Acknowledgements

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References

1. Mirande M. Aminoacyl-tRNA synthetase family from prokaryotes and eukaryotes: structural domains and their implications. Prog Nucleic Acid Res Mol Biol 1991;40:95–142. [PubMed: 2031086]
2. Ling C, Yao YN, Zheng YG, Wei H, Wang L, Wu XF, Wang ED. The C-terminal appended domain of human cytosolic leucyl-tRNA synthetase is indispensable in its interaction with arginyl-tRNA synthetase in the multi-tRNA synthetase complex. J Biol Chem 2005;280:34755–34763. [PubMed: 16055448]
3. Cahuzac B, Berthonneau E, Birlirakis N, Guittet E, Mirande M. A recurrent RNA-binding domain is appended to eukaryotic aminoacyl-tRNA synthetases. EMBO J 2000;19:445–452. [PubMed: 10654942]
4. Guigou L, Shalak V, Mirande M. The tRNA-interacting factor p43 associates with mammalian arginyl-tRNA synthetase but does not modify its tRNA aminoacylation properties. Biochemistry 2004;43:4592–4600. [PubMed: 15078106]
5. Robinson JC, Kerjan P, Mirande M. Macromolecular assemblage of aminoacyl-tRNA synthetases: quantitative analysis of protein-protein interactions and mechanism of complex assembly. J Mol Biol 2000;304:983–994. [PubMed: 11124041]
6. Agou F, Mirande M. Aspartyl-tRNA synthetase from rat: in vitro functional analysis of its assembly into the multisynthetase complex. Eur J Biochem 1997;243:259–267. [PubMed: 9030747]

7. Francin M, Kaminska M, Kerjan P, Mirande M. The N-terminal domain of mammalian lysyl-tRNA synthetase is a functional tRNA-binding domain. J Biol Chem 2002;277:1762–1769. [PubMed: 11706011]
8. Praetorius-Ibba M, Hausmann CD, Paras M, Rogers TE, Ibba M. Functional association between three archaeal aminoacyl-tRNA synthetases. J Biol Chem 2007;282:3680–3687. [PubMed: 17158871]
9. Hausmann CD, Praetorius-Ibba M, Ibba M. An aminoacyl-tRNA synthetase:elongation factor complex for substrate channeling in archaeal translation. Nucleic Acids Res 2007;35:6094–6102. [PubMed: 17766929]
10. Fersht AR, Ashford JS, Bruton CJ, Jakes R, Koch GL, Hartley BS. Active site titration and aminoacyl adenylate binding stoichiometry of aminoacyl-tRNA synthetases. Biochemistry 1975;14:1–4. [PubMed: 1109585]
11. An S, Musier-Forsyth K. Cys-tRNA^{Pro} editing by *Haemophilus influenzae* YbaK via a novel synthetase. YbaK.tRNA ternary complex. J Biol Chem 2005;280:34465–34472. [PubMed: 16087664]
12. Wolfe CL, Warrington JA, Treadwell L, Norcum MT. A three-dimensional working model of the multienzyme complex of aminoacyl-tRNA synthetases based on electron microscopic placements of tRNA and proteins. J Biol Chem 2005;280:38870–38878. [PubMed: 16169847]
13. Negrutskii BS, Shalak VF, Kerjan P, El'skaya AV, Mirande M. Functional interaction of mammalian valyl-tRNA synthetase with elongation factor EF-1alpha in the complex with EF-1H. J Biol Chem 1999;274:4545–4550. [PubMed: 9988688]
14. Zhang CM, Perona JJ, Ryu K, Francklyn C, Hou YM. Distinct kinetic mechanisms of the two classes of Aminoacyl-tRNA synthetases. J Mol Biol 2006;361:300–311. [PubMed: 16843487]
15. Tukalo M, Yaremchuk A, Fukunaga R, Yokoyama S, Cusack S. The crystal structure of leucyl-tRNA synthetase complexed with tRNALeu in the post-transfer-editing conformation. Nat Struct Mol Biol 2005;12:923–930. [PubMed: 16155583]
16. Fukunaga R, Yokoyama S. Aminoacylation complex structures of leucyl-tRNA synthetase and tRNA^{Leu} reveal two modes of discriminator-base recognition. Nat Struct Mol Biol 2005;12:915–922. [PubMed: 16155584]
17. Fukunaga R, Yokoyama S. The C-terminal domain of the archaeal leucyl-tRNA synthetase prevents misediting of isoleucyl-tRNA^{Ile}. Biochemistry 2007;46:4985–4996. [PubMed: 17407269]
18. Nomanbhoy TK, Hendrickson TL, Schimmel P. Transfer RNA-dependent translocation of misactivated amino acids to prevent errors in protein synthesis. Mol Cell 1999;4:519–528. [PubMed: 10549284]

Abbreviations

aaRS

aminoacyl-tRNA synthetase

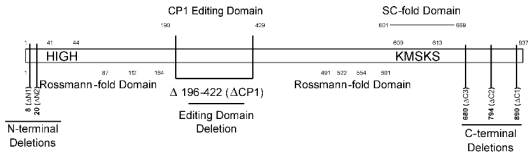

Figure 1. ***M. thermautotrophicus*** **LeuRS variants**

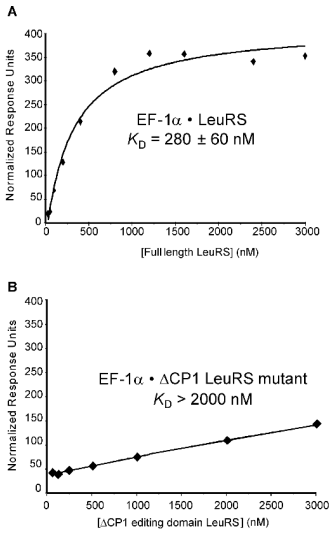

Figure 2. Surface plasmon resonance

EF-1A binding to (**A**) full-length LeuRS or (**B**) ΔCP1 variant.

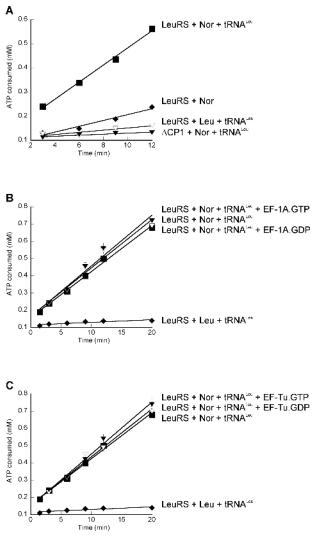

Figure 3. LeuRS editing

ATP consumption was monitored without elongation factor (**A**) or in the presence of EF-1A (**B**) or EF-Tu (**C**).

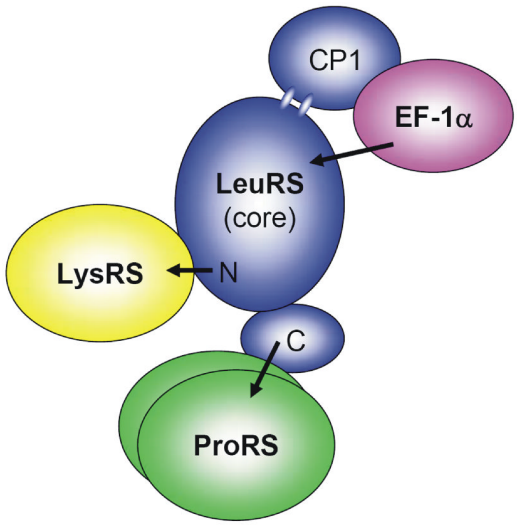

Figure 4. Schematic representation of the structural and functional association of the archaeal MSC

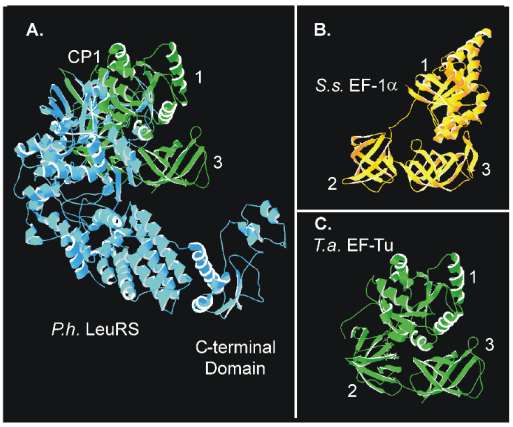

Figure 5. Structural modeling of a *P. horikoshii* LeuRS:*T. aquifex* EF-Tu complex

(**A**) Structural modeling of a LeuRS:EF-Tu complex was performed by aligning the tRNA backbones (not shown for effective protein visualization) from the structural models of *P. horikoshii* LeuRS:$tRNA^{Leu}$ and *T. aquifex* EF-Tu·GDPNP:$tRNA^{Phe}$. (**B**) Crystal structure of *Sulfolobus solfataricus* EF-1A·GDP and (**C**) *T. aquifex* EF-Tu.

Table 1

Activities of LeuRS variants.

LeuRS variant	Total protein concentration (BioRad assay)	Active LeuRS concentration (active site titration)	Active percentage of LeuRS variants
Full-length	110 μM	20 μM	18 %
ΔN1	260 μM	55 μM	21 %
ΔN2	200 μM	1 μM	< 1%
ΔC1	170 μM	20 μM	12 %
ΔC2	100 μM	17 μM	17 %
ΔC3	5 μM	< 0.01 μM	< 1 %
ΔCP1	190 μM	30 μM	16 %

Table 2

Binding affinities between components of the archaeal MSC determined by fluorescence anisotropy.

Unlabeled protein	Fluorescently labeled protein	K_D (nM)
LysRS	LeuRS	170±40
ProRS	LeuRS	80±10
LeuRS	EF-1A	90±10
LysRS	ΔN1 LeuRS	50±10
ProRS	ΔN1 LeuRS	110±20
ΔN1 LeuRS	EF-1A	110±30
LysRS	ΔCP1 LeuRS	40±10
ProRS	ΔCP1 LeuRS	110±10
ΔCP1 LeuRS	EF-1A	>2000
LysRS	ΔC2 LeuRS	160±30
ProRS	ΔC1 LeuRS	>2000
ProRS	ΔC2 LeuRS	>2000
ΔC1 LeuRS	EF-1A	150±30
ΔC2 LeuRS	EF-1A	110±20