

**Supplemental Data for:**  
**Identification of Two Structural Elements Important for Ribosome-Dependent**  
**GTPase Activity of Elongation Factor 4 (EF4/LepA).**

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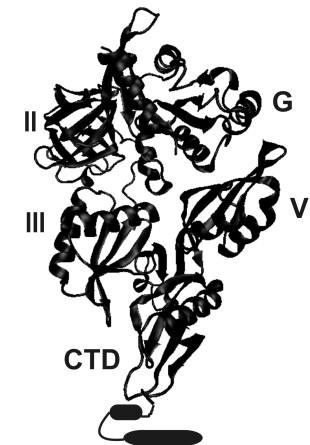
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## Supplemental Figures

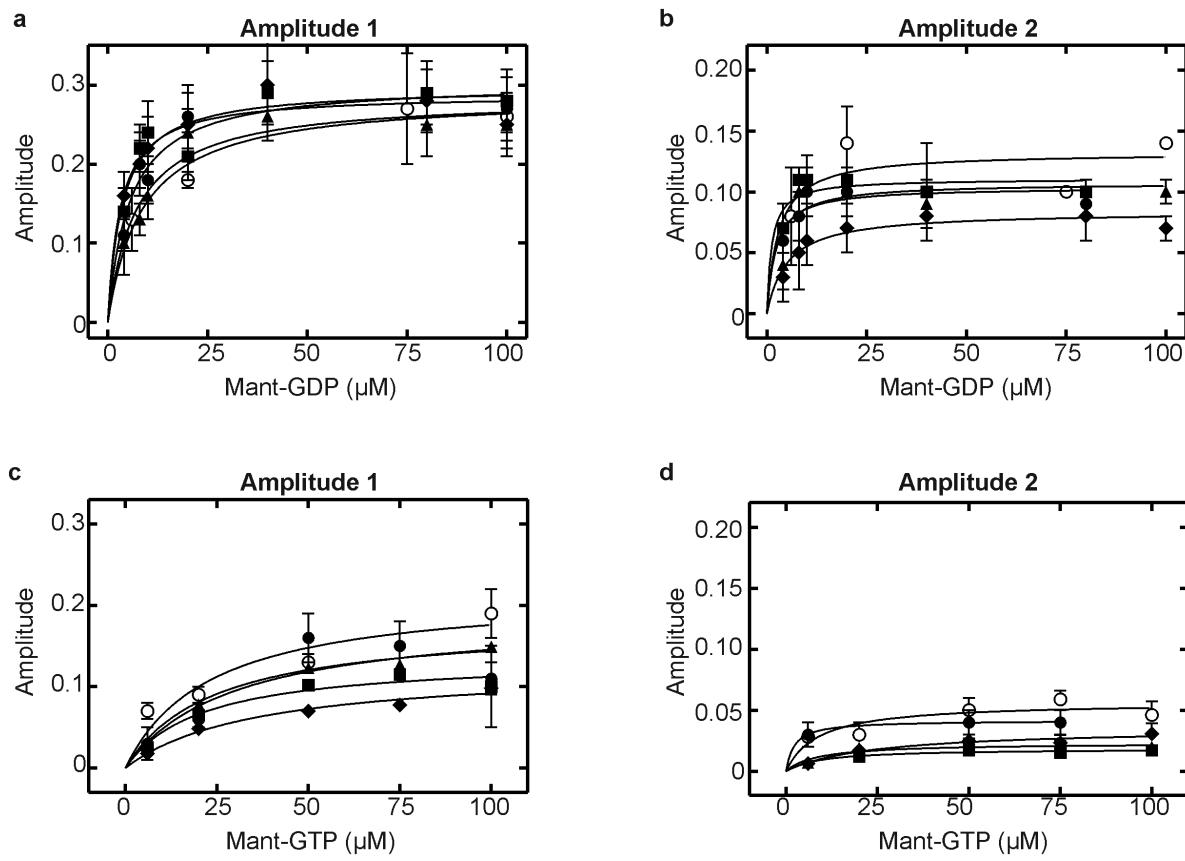
EF-G



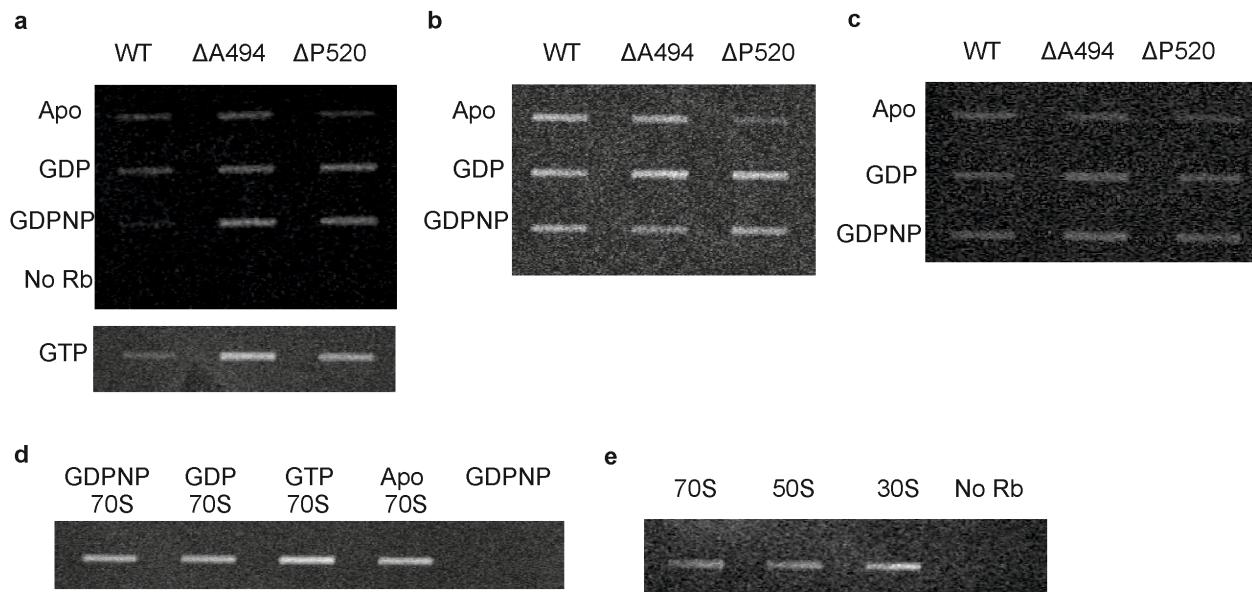
EF4



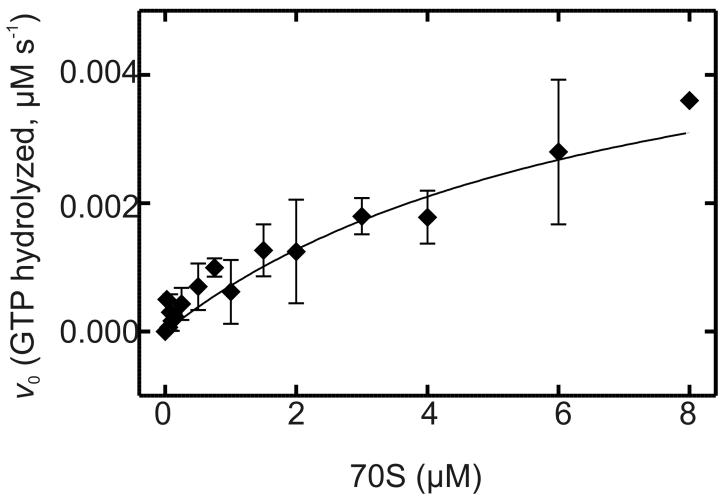
**Figure S1: Structural comparison of EF4 and EF-G.** The domain arrangement is represented on the left, the corresponding residues within the domain are indicated above the respective domain. Cartoon representations of EF-G from *Thermus thermophilus* (PDB ID 1FNM <sup>1</sup>) and EF4 from *Escherichia coli* (PDB ID 3CB4 <sup>2</sup>) are shown on the right. Domains are labelled as domain I (G-domain and G'-domain), domain II, domain III, domain IV, domain V and the CTD. The previously unresolved and likely flexible portion of the CTD (residues 556 to 599) is represented as a helix turn helix cartoon based on the recent X-ray crystallographic structure<sup>3</sup>.



**Figure S2: Amplitude of fluorescence change upon binding of mant-nucleotides to EF4.** EF4 wild type (●), EF4  $\Delta$ A494 (■), EF4  $\Delta$ P520 (▲), EF4  $\Delta$ G555 (◆) and EF4 H81A (○). Concentration dependence of amplitude 1 (a) and amplitude 2 (b) obtained from fluorescence time courses for mant-GTP and amplitude 1 (c) and amplitude 2 (d) obtained for mant-GDP association.

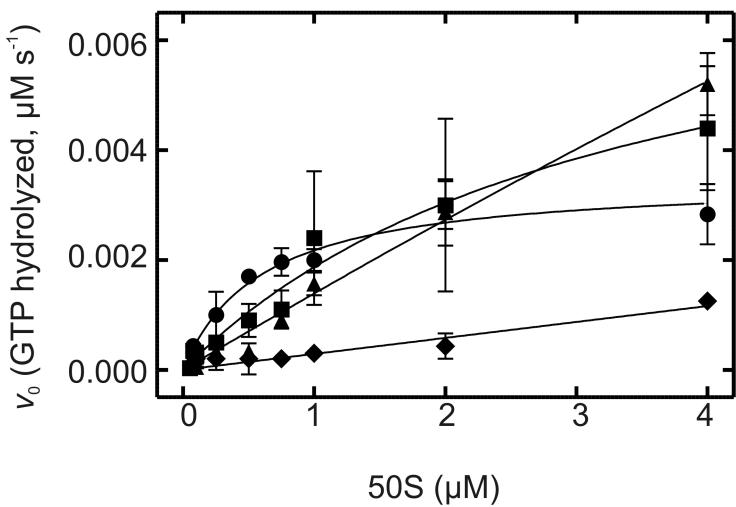


**Figure S3: Immunodetection of EF4 bound to the ribosome following ultracentrifugation.** (a) EF4 proteins (2  $\mu$ M) (indicated on top) bound to the 70S (0.1  $\mu$ M) ribosome in the presence of various guanine nucleotides (GTP, GDPNP, GDP (0.1 mM)) or *apo* indicated on the left. Pellet following ultracentrifugation of EF4 proteins bound to GDPNP in the absence of ribosome is shown (No Rb). (b) EF4 proteins (2  $\mu$ M) bound to the 50S (0.1  $\mu$ M) subunit in the presence of various guanine nucleotides (GDPNP, GDP (0.1 mM)) or *apo*. (c) EF4 proteins (2  $\mu$ M) bound to the 30S (0.1  $\mu$ M) subunit in the presence of various guanine nucleotides (GDPNP, GDP (0.1 mM)) or *apo*. (d) EF4 H81A (2  $\mu$ M) bound to the 70S (0.1  $\mu$ M) in the presence of various guanine nucleotides (GDPNP, GDP, GTP (0.1 mM)) or *apo* indicated on top. (e) EF4 ΔG555 protein (10  $\mu$ M) bound to the 70S, 50S and 30S (0.1  $\mu$ M) in the presence of GDPNP (0.1 mM).



**Figure S4: Michaelis-Menten titration of 70S ribosome stimulated GTPase activity of EF4  $\Delta$ G555.**

Initial rates of GTP hydrolysis are plotted as a function of increasing 70S ribosome concentration in the presence of (0.01  $\mu\text{M}$ ) EF4  $\Delta$ G555.



**Figure S5: Michaelis-Menten titration of 50S ribosome subunit stimulated GTPase activity of EF4.**

Initial rates of GTP hydrolysis are plotted as a function of increasing 50S ribosome concentration in the presence of (0.01  $\mu\text{M}$ ) EF4 wild type (●), EF4  $\Delta$ A494 (■), EF4  $\Delta$ P520 (▲), EF4  $\Delta$ G555 (◆).

## Supplemental Tables

**Table S1: Summary of dissociation rate constants determined for mant-GDP and mant-GTP from EF4.**

	EF4	ΔA494	ΔP520	ΔG555	H81A
$k_{off1(GDP)}$ y-int ( $s^{-1}$ )	11 ± 2	8 ± 3	13 ± 1	9 ± 2	8 ± 2
$k_{off1(GTP)}$ y-int ( $s^{-1}$ )	32 ± 3	39 ± 3	51 ± 3	76 ± 6	46 ± 6
$k_{off2(GDP)}$ ( $s^{-1}$ )	8 ± 2	4 ± 2	10 ± 2	3 ± 2	4 ± 2
$k_{off2(GTP)}$ ( $s^{-1}$ )	2.4 ± 1.5	1.0 ± 0.5	1.4 ± 0.6	7.2 ± 3.8	0.5 ± 0.4

**Table S2: 50S ribosome dependent GTPase activity of EF4 and three truncation variants.**

Protein	$K_M$ ( $\mu M$ )	$v_{max}$ ( $\mu M s^{-1}$ )	$k_{cat}$ ( $s^{-1}$ )	$k_{cat}/K_M$ ( $s^{-1} \mu M^{-1}$ )
EF4	0.6 ± 0.1	0.0035 ± 0.0003	0.4 ± 0.1	0.7 ± 0.2
ΔA494	3 ± 2	0.008 ± 0.003	0.8 ± 0.3	0.3 ± 0.2
ΔP520	>1	>0.006	>0.6	NA
ΔG555	>1	>0.001	>0.1	NA

## Sequence Alignment

Sequences (SwissProt) and Organisms used in the EF4/LepA multiple sequence alignment

Accession Number	Organism	Seq. identity to <i>E. coli</i> (%)
P60785	<i>Escherichia coli</i>	
Q9X1V8	<i>Thermotoga maritima</i>	50
Q72KV2	<i>Thermus thermophilus</i>	55
A0RIT7	<i>Bacillus thuringiensis</i>	56
C3P8M5	<i>Bacillus anthracis</i>	55
P37949	<i>Bacillus subtilis</i>	56
Q65H50	<i>Bacillus licheniformis</i>	56
A6QHC7	<i>Staphylococcus aureus</i>	54
C1CKU6	<i>Streptococcus pneumoniae</i>	54
Q5M4M2	<i>Streptococcus thermophilus</i>	56
Q831Z0	<i>Enterococcus faecalis</i>	56
Q03QU8	<i>Lactobacillus brevis</i>	53
Q03FQ4	<i>Pediococcus pentosaceus</i>	53
Q14NN1	<i>Spiroplasma citri</i>	53
A7FXL9	<i>Clostridium botulinum</i>	55
A6L744	<i>Bacteroides vulgatus</i>	53
A6LC18	<i>Parabacteroides distasonis</i>	52
Q2S5I1	<i>Salinibacter ruber</i>	53
A5U598	<i>Mycobacterium tuberculosis</i>	52
I7G5I5	<i>Mycobacterium smegmatis</i>	48
Q82BZ3	<i>Streptomyces avermitilis</i>	48
Q9Z8I4	<i>Chlamydia pneumoniae</i>	50
Q823H7	<i>Chlamydophila caviae</i>	49
Q9PKX6	<i>Chlamydia muridarum</i>	49
P60788	<i>Shigella flexneri</i>	100
P0A1W5	<i>Salmonella typhi</i>	96
A4TKY0	<i>Yersinia pestis</i>	92
Q6LMS0	<i>Photobacterium profundum</i>	83
Q3IDL4	<i>Pseudoalteromonas haloplanktis</i>	75
Q0VP16	<i>Alcanivorax borkumensis</i>	74
A9KF98	<i>Coxiella burnetii</i>	71
Q87C09	<i>Xylella fastidiosa</i>	67
A9III9	<i>Bordetella petrii</i>	66
B9J716	<i>Agrobacterium radiobacter</i>	57
B9JYH0	<i>Agrobacterium vitis</i>	55
Q8UIQ2	<i>Agrobacterium tumefaciens</i>	57
Q6G1F5	<i>Bartonella quintana</i>	56
Q0C5X0	<i>Hyphomonas neptunium</i>	56
Q5NLP5	<i>Zymomonas mobilis</i>	59
Q5FHQ1	<i>Ehrlichia ruminantium</i>	51
A8F140	<i>Rickettsia massiliae</i>	56
C4XND4	<i>Desulfovibrio magneticus</i>	60

**Multiple Sequence Alignment of the C-terminal domain of EF4/LepA.** Black indicates 100%, dark grey 80 - 100%, light grey 60 - 80% and white < 60% conservation. The start of the *E. coli* CTD is indicated (red arrow).

	520	*	540	*	560	*	580	*	600	*	620	*	640	*	660	*
P607851	: LTYEIPMAEVVLDFFDRLKTSRGYASLDYNFKRQASDMVRDVLDLINGERVDALALITHRDNSQRGRPIVEKMRDLIPROQFDIAQAAIGTHIIARSTVKOLRKNVIAKCYGGDISRKKEKLLQKQEGRKRMKQIGNVELPQAFIALIHVGKDNK-----	: 599														
Q9X1V8	: IRFADPLAEEIYYDFEDRMKAVSRGYASMDIEFKEIRRSDLVLKVTLILVNKEPVDAISFIVHRSKAYQMARKKIVEKILDLIPRFHQSCIFIQAKAGGRIIARADIKALRKDVLAKCYGGDVTRKKLLEKQKEGKKKLRIGRTVITPQAFIALIICKIGGEDEK-----	: 621														
Q72K2V	: ILYMPEPFAEELYYDFDHLRCKVSRGYASMDIEQIGYRPGDVLKVNLVHVGEVDALTFIAHEKAYTMRARAVDCKAIEVIRPCLFEPVIAQGKIIARAVVKALERKDVLAKCYGGDVTRKKLLEKQKEGKKRMLRAIGKWEVVPQAFALAVISAGRDPKG-----	: 610														
A0R1T7	: ILYEIPLSEIVYDFDQLKSNTKGYASDYEELIGKPSKLVKMDILLNSEQVDAISFIVHRSHDAYDRGKVIIVEKELIELIPRQFEEVPIQAIQGKVVARSITAKAMRKNVIAKCYGGDISRKKEKLLDQKEGKKRMKSVGSVSVQAFAMAVIKMDNN-----	: 607														
C3P8M5	: ILYEIPLSEIVYDFDQLKSNTKGYASDYEELIGKPSKLVKMDILLNSEQVDAISFIVHRSHDAYDRGKVIIVEKELIELIPRQFEEVPIQAIQGKVVARSITAKAMRKNVIAKCYGGDISRKKEKLLDQKEGKKRMKSVGSVSVQAFAMAVIKMDNN-----	: 607														
P37949	: IIYKQPLAETVYEFIDQLKSNTKGYASDYEELIGKPSKLVKMDILLNGEKIDALISFIVHRSHDAYERGKVIIVEKELIELIPRQFEEVVFVQAAGQKIVARSITAKAMRKNVIAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMAVIKMDDSGPKS-----	: 612														
Q65H50	: IVYMEPLAEVVYEFIDQLKSNTKGYASDYEELIGKPSKLVKMDILLNGEKIDALISFIVHRSHDAYERGKVIIVEKELIELIPRQFEEVVFVQAAGQKIVARSITAKAMRKNVIAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMAVIKMDDSGPKS-----	: 612														
A6QHC7	: IVYMEPLAEVVYEFIDQLKSNTKGYASDYEELIGKPSKLVKMDILLNGEKIDALISFIVHRSHDAYERGKVIIVEKELIELIPRQFEEVVFVQAAGQKIVARSITAKAMRKNVIAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMAVIKMDDSGPKS-----	: 607														
C1CKU6	: VIYQIPLAEVFDFFDQLKSNTKGYASDYEELIGKPSKLVKMDILLNGDKVDAISFIVHRSHDAYERGKVIIVEKELIELIPRQFEEVVFVQAAGQKIVARTNPKSGKNVIAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMAVIKMDDSGPKS-----	: 607														
Q5M4M2	: VIYQIPLAEVFDFFDQLKSNTKGYASDYEELIGKPSKLVKMDILLNGDKVDAISFIVHRSHDAYERGKVIIVEKELIELIPRQFEEVVFVQAAGQKIVARTNPKSGKNVIAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMAVIKMDDSGPKS-----	: 610														
Q831Z0	: IVYMEPLAEVYDFDFFDQLKSNTKGYASDYEELIGKPSKLVKMDILLNGDKVDAISFIVHRSHDAYERGKVIIVEKELIELIPRQFEEVVFVQAAGQKIVARSITAKAMRKNVIAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMAVIKMDDSGPKS-----	: 611														
Q03Q8U	: IIYNNPLSEIIFDFDQLKSNTKGYASDYEEMNGQAGDVLKIDILLNGDKVDAISFIVHRSHDAYERGKVIIVEKELIELIPRQFEEVVFVQAAGQKIVARSITAKAMRKNVIAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMAVIKMDDQNAK-----	: 610														
Q03FQ4	: VKYQIPPLSEIIFDFDQLKSNTKGYASDYEELGEYKVSDSLVKIDILLNGERVDALSFISHRDEAQCCRGNBETASLKEIIPFRNEEVEVQAAGQKIIARINIRAYRKDVTSKIHGDPRRAKLIDQKRGKRMKSVGVWSVVPQAFAMTVIKTDTEGGKG-----	: 613														
Q14NN1	: ILYMEPLINEVVFVDFDRLKTSISKGYASDYEFIGRPNPKVLMKDILLNNEIIDALSIIVHRSHDYERGKVIIVEKELIELIPRQFEEVQAAGQKIIARINIRAYRKDVTSKIHGDPRRAKLIDQKRGKRMKSVGVWSVVPQAFAMAVIKLDD-----	: 600														
I7FXL19	: ILYMEPLINEIYYDFDALKSNTKGYASDYEELIGKPSKLVKMDILLNNEADVDALSMIVPERAYAKGRNNAQKLEIIPRQFEEVPIQAAIGCAKIIARETAKAMRKDVLAKCYGGDISRKKEKLLDQKEGKKRMKQVGWSVVPQAFAMVLTKEE-----	: 602														
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A9III9	: ILYEIPMAEVVLDFFDQLKSNTKGVGSFDTKGSNTKLEIILNEFVDIFDSCIVLHRDKAESGRSICEKUVDVIIQOLEKIPQAAIGCAIARETAKVLPKLNKQIGNVELPQAFIALIHVGK-----	: 597														
B9J716	: ILYDIPLINEVVFDFDRLKTSRGYASDYNLTDRDSVLVKMSLVNAEFVDALSMVHSAAEKRGRMCEKLDIIECHMECIEIQQAAIGGKIIARETAKVLPKLNKQIGNVELPQAFIALIHVGK-----	: 610														
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Q6G1F5	: VTYNIPLINEVVFDFDRLKTSRGYASDYQMTDISEGDLVKMSLVNGESIDALSMVHTEAEKRGRMCEKLDIIECHMECIEIQQAAIGKIIARETAKVLPKLNKQIGNVELPQAFIALIHVGK-----	: 600														
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Q5FHQ1	: ILYKRPPLSSEVVVFDFDRLKTSRGYASDLOWEMDGMDSEPAKLTILNNEFVDALACIVHRSKVEQRGRBECIPLRDLIPRQFEEVPIQAAIGGKIIARETAKVLPKLNKQIGNVELPQAFIALIHVGDD-----	: 598														
A8F140	: IIYKUPPLAETVYDFDRLKTSRGYASDYNFKRQASDMVRDVLDILINGERVDALALITHRDNSQRGRPIVEKMRDLIPRQFEEVPIQAAIGMSGIIARETAKAMRKDVTAKCYGGDISRKKEKLLDQKEGKKRMQIGNVELPQAFIALIHVGAD-----	: 604														
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## References

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