Structures of KEOPS bound to tRNA reveal regulatory roles of the kinase Bud32

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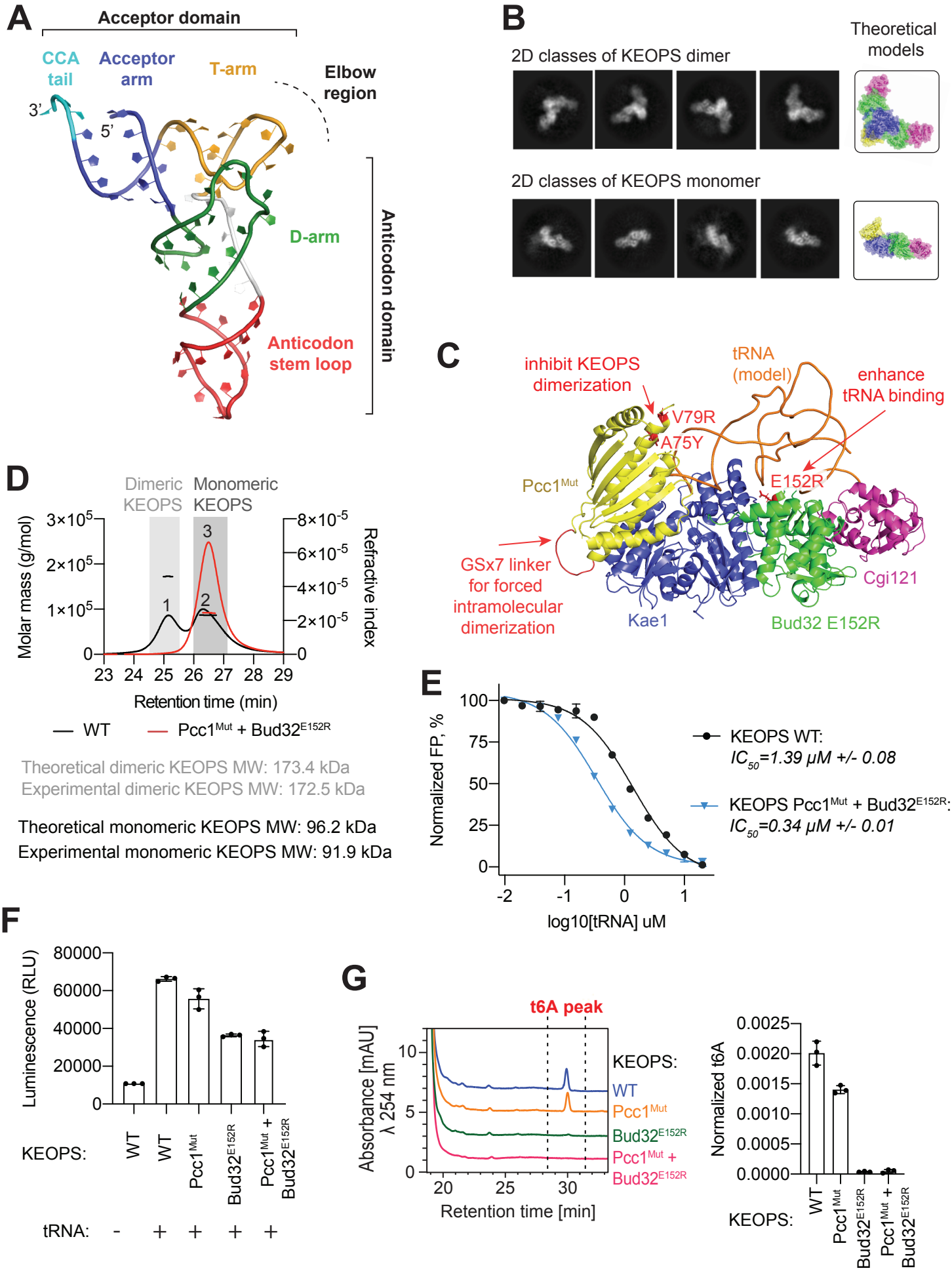
Supplementary Figure 1



Supplementary Figure 1. Cryo-EM sample preparation of KEOPS bound to a substrate tRNA

a Cartoon representation of the tRNA crystal structure of *M. jannaschii* $tRNA^{Lys}$ (PDB 7KJU) highlighting its conserved structural elements.

b (left) Representative 2D classes of cryo-EM images made with archaeal KEOPS reconstituted with WT Cgi121, Bud32, Kae1 and Pcc1 proteins in the presence of tRNA. 2D classes consistent with dimeric KEOPS and monomeric KEOPS are shown on the top and bottom panels respectively with no evidence of tRNA binding. (right) Surface representation of KEOPS dimer and monomer models are shown for comparison with 2D classes.

c Engineered mutations in KEOPS proteins to reduce monomer-dimer heterogeneity and to enhance tRNA binding in cryo-EM samples. The Bud32 E152R mutation, which enhances KEOPS tRNA binding affinity[40], orients towards tRNA in a composite enzyme-substrate model[40]. A seven GS dipeptide linker in $Pcc1^{Mut}$ joining two Pcc1 protomers is expected to promote an intramolecular dimer. The V79R and A75Y mutations on one of the Pcc1 protomers in $Pcc1^{Mut}$ disables one of two Kae1 binding surfaces thereby inhibiting KEOPS dimerization.

d SEC-MALS analysis of KEOPS WT (black) and KEOPS reconstituted with $Bud32^{E152R}$ and $Pcc1^{Mut}$ (red). KEOPS WT displays a monomeric-dimeric equilibrium while KEOPS with $Bud32^{E152R}$ and $Pcc1^{Mut}$ is exclusively monomeric.

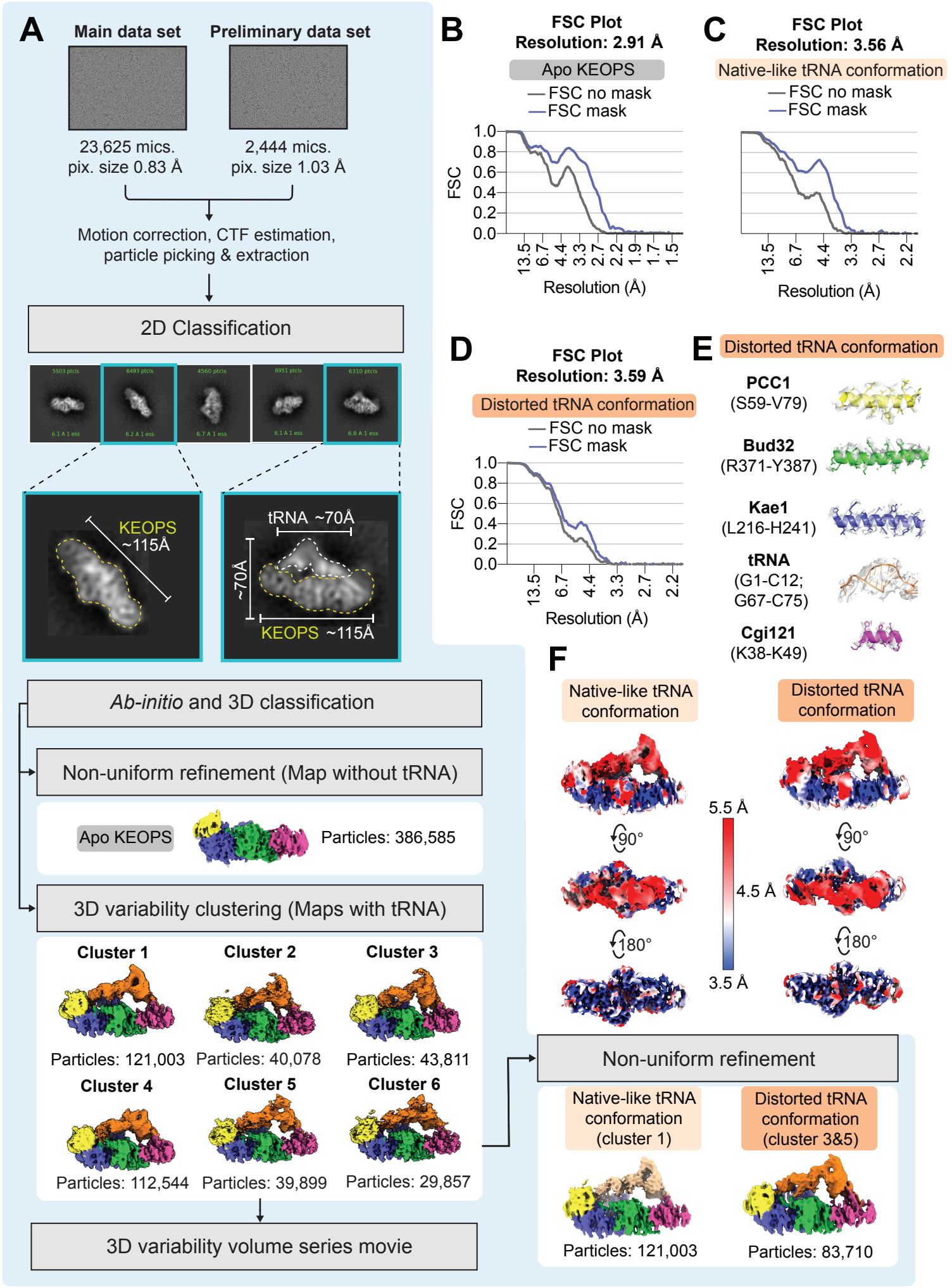
e Competitive displacement of an Alexa-647 labeled CCA-tail probe (647-CCA) from KEOPS WT or KEOPS reconstituted with the $Bud32^{E152R}$ and $Pcc1^{Mut}$ by titration of $tRNA^{Lys}$. Displacement of the 647-CCA probe was monitored by fluorescence polarization (FP). Respective IC_{50} values for the displacement are shown (n=3 technical replicates, bars indicate ±SD).

f ATPase activity analysis of the indicated KEOPS complexes in the presence and absence of tRNA, monitored using the ADP Glo assay. Displayed results represent the average luminescence for each reaction condition (n = technical replicates, ±SD).

g t^6A modification activity analysis of the indicated KEOPS complexes reconstituted with Bud32 WT and Pcc1 WT or the indicated mutants with $tRNA^{Lys}$ used as substrate. Representative HPLC profiles of nucleoside composition for each reaction are shown at

left. Quantification of average t^6A content normalized to the content of uridine is shown at right (n=3 technical replicates, ±SD).
Source data are provided as a Source Data file.

Supplementary Figure 2



Supplementary Figure 2. Cryo-EM workflow and map quality statistics of KEOPS with and without bound substrate tRNA

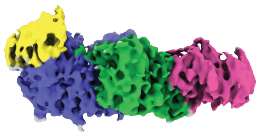
a Flow chart showing the cryo-EM multi-stage image processing workflow. Reconstruction of the data yielded density maps for apo KEOPS (2.91 Å), KEOPS bound to tRNA in a native-like conformation (3.56 Å) and KEOPS bound to tRNA in a distorted conformation (3.59 Å).

b-d Fourier Shell Correlation (FSC) curves of apo KEOPS, KEOPS bound to tRNA in its native-like conformation and KEOPS bound to tRNA in its distorted conformation (resolution at 2.91 Å, 3.56 Å, 3.59 Å, respectively).

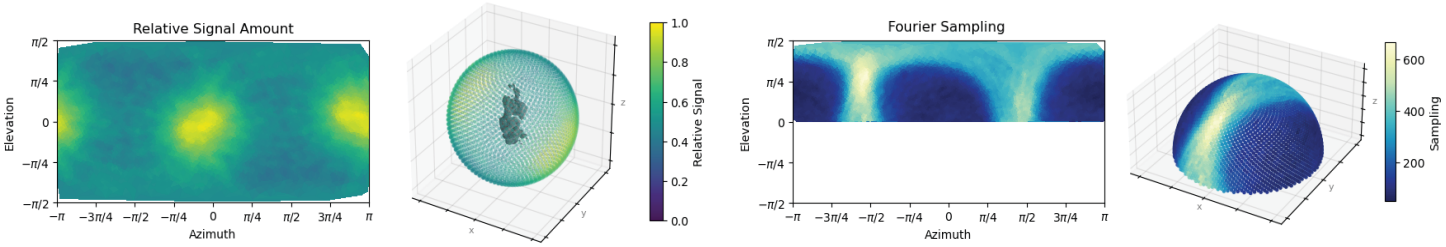
e Representative secondary structure regions illustrating the quality of the cryo-EM map for KEOPS bound to tRNA in a distorted conformation. Representative secondary structure regions of KEOPS bound to tRNA in a native-like conformation and apo KEOPS are not shown for simplicity but have a similar quality.

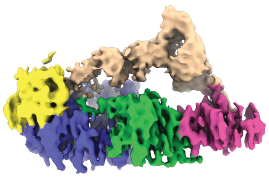
f Local resolution maps of KEOPS with the native-like (left) and distorted (right) tRNA conformations. Color gradient indicates the resolution per residue ranging from ~3.5 Å (blue) to 5.5 Å (red).

Supplementary Figure 3

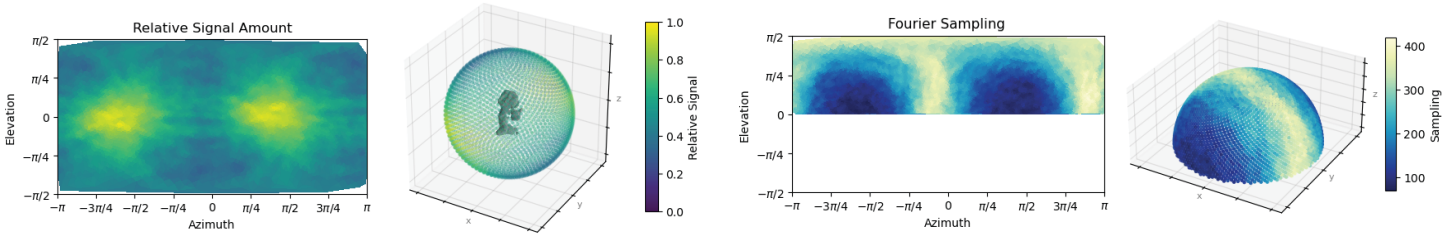


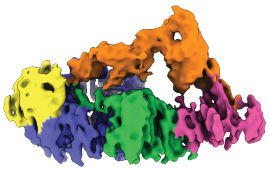
Apo KEOPS



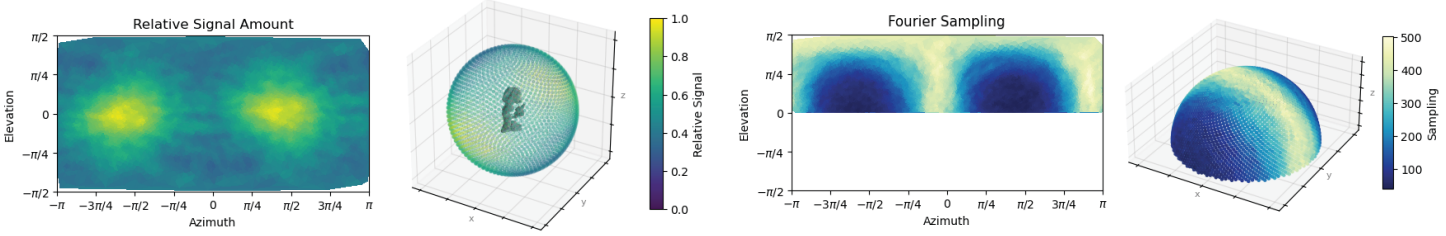


Native-like tRNA conformation (cluster 1)





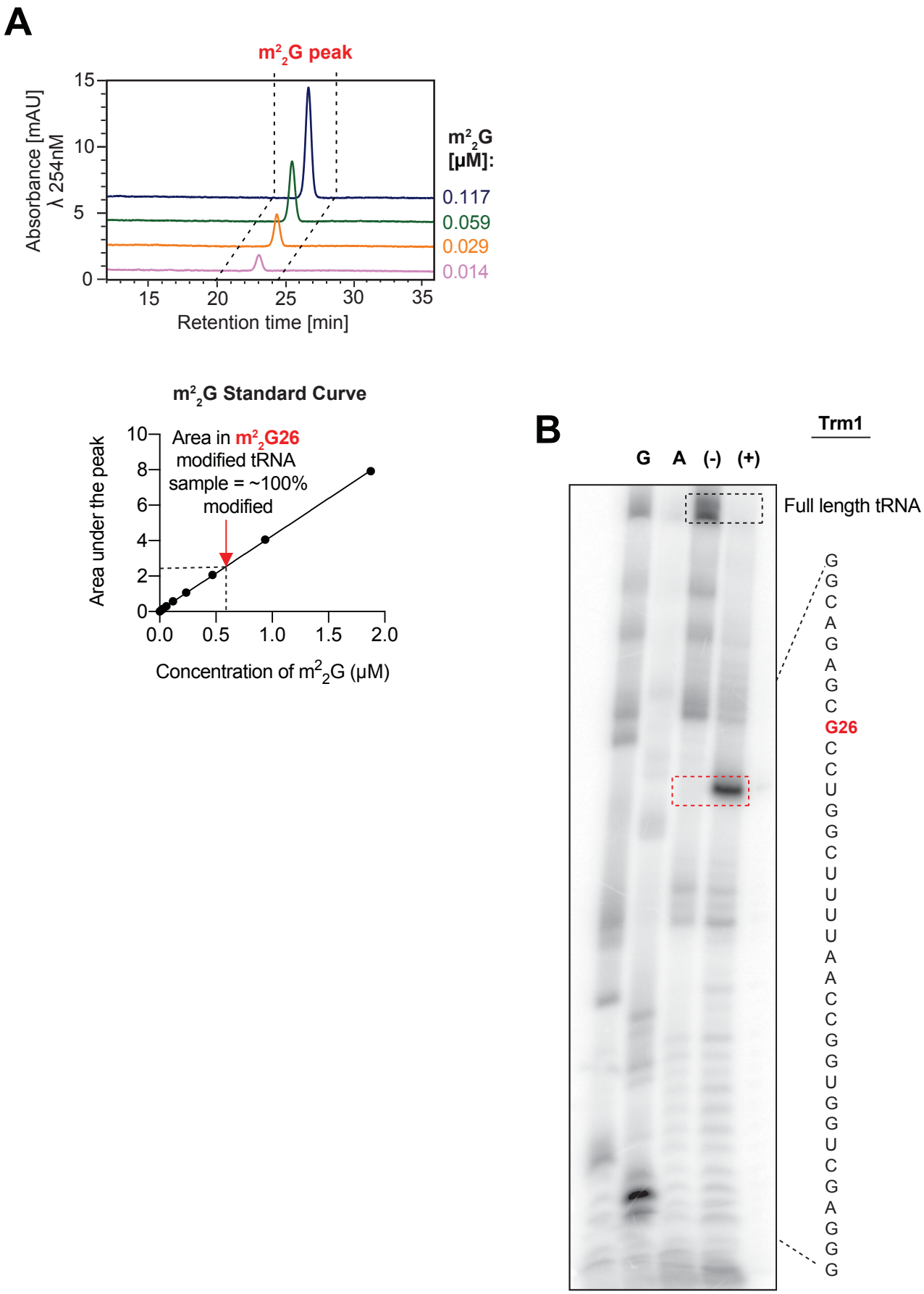
Distorted tRNA conformation (cluster 3&5)



Supplementary Figure 3. Map anisotropy analysis

Particle angular distribution Fourier Shell Correlation (FSC) profiles of apo KEOPS, KEOPS bound to tRNA in its native-like conformation and KEOPS bound to tRNA in its distorted conformation (resolution at 2.91 Å, 3.56 Å, 3.59 Å, respectively) calculated in cryoSPARC.

Supplementary Figure 4



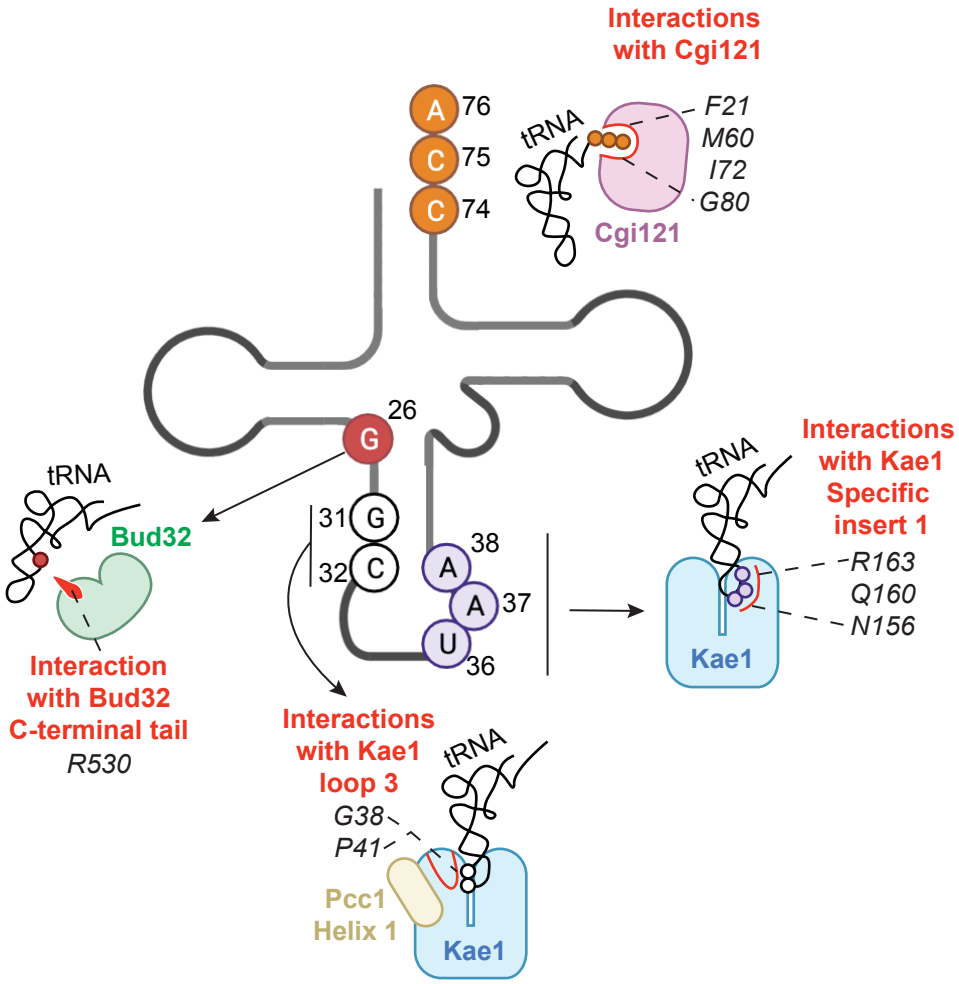
Supplementary Figure 4. *In vitro* m^2_2G-modification of $tRNA^{Lys}$ by Trm1.

a HPLC quantification of m^2_2G content of tRNA samples shown in **Fig. 4e**. (Top) Representative HPLC chromatograms of an m^2_2-guanine (m^2_2G) standard molecule at the indicated concentrations. (Bottom) The area beneath each peak was measured and plotted to obtain an m^2_2G standard curve. Red arrow indicates the quantified value for the samples shown in **Fig. 4e**, which corresponds to ~100 % m^2_2G-modification of $tRNA^{Lys}$.

b Primer extension analysis of m^2_2G-modified and non-modified $tRNA^{Lys}$. Red box indicates quantitative blockage of reverse transcriptase arising from complete m^2_2G modification at G26. G and A refer to sequencing lanes where ddCTP and ddTTP were added respectively. These lanes were used to assign positions to the bands in the reverse transcriptase reaction. Each band in the G lane for example corresponds to a G in the tRNA sequence (as read out by a ddCTP "stop"). By comparing the sequencing lanes to our experimental lanes, we can assign the strong primer extension stop in the +Trm1 lane to G26 as it occurs one base before the band in the G sequencing lane.

Source data are provided as a Source Data file.

Supplementary Figure 5



Supplementary Figure 5. Analysis of contacts between KEOPS and its substrate tRNA

Schematic representation depicting the interactions between KEOPS and the tRNA in either its native-like or distorted conformations.

(top) In both tRNA conformations, the CCA tail is engaged by Cgi121.

(left) In the distorted conformation, G26 interacts with the C-terminal tail of Bud32.

(middle) In the distorted conformation, the anticodon region is engaged by residues in a structured loop 3 of Kae1 and Helix 1 of Pcc1.

(right) In the native-like conformation, the anticodon region is engaged by residues from Kae1-specific-insert 1. A disordered loop 3 in Kae1 is denoted by dashed line.

For simplicity, only the proteins directly interacting with the tRNA in each conformation

Supplementary Table 1. Q-score analysis of side chains of interest resolvability in the cryo-EM maps. Q-score of 1 indicates ideal resolvability.

		Native-like	Distorted
Bud32	Asp451	0.43	0.178
	Arg530	0.32	0.378
Kae1	Gly38	Not modeled	0.158
	Pro41	0.25	0.235
	Asn156	0.40	0.393
	Gln160	0.24	0.332
	Arg163	0.38	0.343
	Arg237	0.46	0.227
Pcc1	Arg63	0.16	0.147
tRNA	C10	0.33	-0.03
	U11	0.24	0.23
	G24	0.30	-0.089
	C25	0.18	-0.051
	G26	0.16	0.299
	G31	0.10	0.07
	C32	0.05	0.107
	U36	0.21	Not modeled
	A37	0.29	Not modeled
	A38	0.27	Not modeled
	U44	0.19	0.249
	G45	0.15	-0.014

Supplementary Table 2. Primers for site directed mutagenesis used in this study.

Primer name	Target	Sequence
E152R forward	Bud32	GAAAGATTTCAAATCTTGATAGAGATAAGGCAGTTG
E152R reverse		CAACTGCCTTATCTCTATCAAGATTTGAAATCTTTC
D451R forward		CGATGTAATTCATAATCGCTTAACTACATCCAAC
D451R reverse		GTTGGATGTAGTTAAGCGATTATGAATTACATCG
R530D forward		GGATGTTGAAAGAGACGCAAGATATGTAGAGTAATAACTC
R530D reverse		GAGTTATTACTCTACATATCTTGCGTCTCTTTCAACATCC
P41A forward	Kae1	GGGTATTAATGCTAGAGAGGCTGCTGACC
P41A reverse		GGTCAGCAGCCTCTCTAGCATTAATACCC
N156A forward		GCTGTTGGTGCATGCTTAGACCAG
N156A reverse		CTGGTCTAAGCATGCACCAACAGC
Q160D forward		GTAACTGCTTAGACGACTTTGCAAGATATGTGAATTTGC
Q160D reverse		GCAAATTCACATATCTTGCAAAGTCGTCTAAGCAGTTAC
R163E forward		CTTAGACCAGTTTGCAGACTATGTGAATTTGCCACATCC
R163E reverse		GGATGTGGCAAATTCACATAGTCTGCAAACTGGTCTAAG
C25A forward	tRNA Lys	GCTCAGTCTGGCAGAGAGCCTGGC
C25A reverse		GCCAGGCTCTCTGCCAGACTGAGC
C10U+U11C forward		GGGCCCGTAGTCCAGTCTGGCAGAGC
C10U+U11C reverse		GCTCTGCCAGACTGGACTACGGGCCC
G45T forward		GGCTTTTAACCGGTTGTCGAGGGTTCAAATC
G45T reverse		GATTTGAACCCTCGACAACCGGTTAAAAGCC
G24A forward		CTGGCAGAACGCCTGGCTTTTAAC
G24A reverse		GTTAAAAGCCAGGCGTTCTGCCAG
G26T forward		CTGGCAGAGCTCCTGGCTTTTAAC
G26T reverse		GTTAAAAGCCAGGAGCTCTGCCAG
G26C forward		CTGGCAGAGCCCCTGGCTTTTAAC
G26C reverse		GTTAAAAGCCAGGGGCTCTGCCAG
G26A forward		CTGGCAGAGCACCTGGCTTTTAAC
G26A reverse		GTTAAAAGCCAGGTGCTCTGCCAG
34-CTG-37 to 34-UAA-37 forward	tRNA Ala	GAGCGCCGCATTGGTAATGCGGAG
34-CTG-37 to 34-UAA-37 reverse		CTCCGCATTACCAATGCGGCGCTC
34-CAA-37 to 34-UAA-37 forward	tRNA Val	CTATGATGCCGCCCTTAAACGGCGGTGGTCG
34-CAA-37 to 34-UAA-37 reverse		CGACCACCGCCGTTTAAGGGCGGCATCATAG

Supplementary Table 3. Sequences of DNA constructs used for in vitro transcription of tRNAs in this study. All tRNA constructs have a T7 promotor sequence at the 5' and a Ribozyme sequence at the 3'.

RNA name	Sequence
T7 promotor	TAATACGACTCACTATA
Ribozyme	GGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCA ACATTCCGAGGGGACCGTCCCCTCGGTAATGGCGAATGGGACC CAGGCTTAGTATAGCGAGGTTTAGCTACACTCGTGCTGAGCC
tRNA Lys	GGGCCCGTAAGCTCAGTCTGGCAGAGCGCCTGGCTTTTAACCG GTGGTCGAGGGTTCAAATCCCTTCGGGCCCGCCA
tRNA Val	GGGCTCGTGGTCTAGATGGCtATGATGCCGCCCTGACACGGCGG TGGtCGGGAGTTCGAATCTCCCCGAGCCCACCA
tRNA Ala	GGGCTGGTAGCTCAGACTGGGAGAGCGCCGCATTGGCTGTGCG GAGGCCGCGGGTTCAAATCCCGCCCAGTCCACCA
tRNA Arg	GCCCGGGTCGCCTAGCCAGGATAGGGCGCTGGCCTGCGGAGC CAGTTTTTTCAGGGGTTCAAATCCCCTCCCGGGCG
tRNA Asp	GCCCTGGTGGTGTAGCCCGGCCTATCATACGGGACTGTCACTCC CGTGACTCGGGTTCAAATCCCGGCCAGGGCGCCA
tRNA Arg Eng1	GCCCGGGTCCTCTAGCCAGGATAGGGCGCTGGCCTGCTAAGCC AGTGTTTTCAGGGGTTCAAATCCCCTCCCGGGCGCCA
tRNA Arg Eng 2	GCCCGGGTGCTCTAGCCAGGATAGAGCGCTGGCCTGCTAAGCC AGTGTTTTCAGGGGTTCAAATCCCCTCCCGGGCGCCA
tRNA Arg Eng3	GCCCGGGTGCTCAAGCCAGGACAGAGCGCCTGGCTTTTAACCA GGTGGTCTCAGGGGTTCAAATCCCCTCCCGGGCGCCA
tRNA Asp Eng1	GCCCTGGTGCTGTAGCCCGGCCTATCGCGCGGGACTGTTAATC CCGTGACTCGGGTTCAAATCCCGGCCAGGGCGCCA
tRNA Asp Eng 2	GCCCTGGTGCTCTAGCCCGGCCTATAGCGCGGGACTGTTAATCC CGTGACTCGGGTTCAAATCCCGGCCAGGGCGCCA
tRNA Asp Eng3	GCCCTGGTGCTCAAGCCCGGCCCAGAGCGCCTGGCTTTTAACC AGGTGGTCCGGGTTCAAATCCCGGCCAGGGCGCCA
tRNA Val Eng1	GGGCTCGTGCTCTAGATGGCTATGGCGCCGCCCTGATAAGGCG GTGGTCGGGAGTTCGAATCTCCCCGAGCCCACCA
tRNA Val Eng 2	GGGCTCGTGCTCTAGATGGCTATAGCGCCGCCCTGATAAGGCGG TGGTCGGGAGTTCGAATCTCCCCGAGCCCACCA
tRNA Val Eng3	GGGCTCGTGCTCAAGATGGCCAGAGCGCCTGGCTTTTAACCAG GTGGTCGGGAGTTCGAATCTCCCCGAGCCCACCA