## Structural characterization of LsrK as a quorum sensing target and a comparison between X-ray and homology models

Prasanthi Medarametla<sup>1</sup>, Thales Kronenberger<sup>1,2</sup>, Tuomo Laitinen<sup>1</sup>, Antti Poso<sup>1,2\*</sup>

- School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland email: <u>antti.poso@uef.fi</u>
- Department of Oncology and Pneumonology, Internal Medicine, University Hospital Tübingen, Otfried-Müller-Straße 10, DE 72076 Tübingen, Germany

sp P77432 LSRK_ECOLI	1 10	β1 β:	2 	β3 40	TT 50	<u>0000000</u> 60
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	MARLETLSESKYY Marlothies <mark>ghy</mark>	LMALDAGTGS <mark>T</mark> RA LMALDAGTGSVRA	VIFDLEGNQI VIFDL <mark>QG</mark> KQI	AVGQAEW <mark>RFLA</mark> AVGQAEW <mark>Q</mark> HLA	VPDVPGS ME VPDVPGSME	FDL <mark>NKNWQL</mark> FDL <mark>AKNWQL</mark>
sp P77432 LSRK_ECOLI	αl <u>202020202000</u> 70	η <u>1</u> β4 εο οο οο	TT2	η2 β6 22	η3 <u>00000000</u> 120	α2 100000000 130
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	ACECMRQALHNAG ACOCIRQALOKAA	IAPEY <mark>IAAVSACS</mark> IPATA <mark>IAAVSACS</mark>	MREGIVLYNN MREGIVIYDS	E <mark>GAPIWACANV</mark> N <mark>GEPIWACANV</mark>	DARAAREVS DARAAHEVS	ELKELHNNT ELKELYD <mark>NT</mark>
sp P77432 LSRK_ECOLI	α3 202020000 140	α4 000000000 150	α5 2 <u>2222</u> 160	β7 170 170	000 180	α7 η4 <u>0000000</u> 190
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	FENEVYRATGQTL FEEEVYRCSGQTL	ALSAIPRLLWLAH ALSAIPRLLWLAH	HRSDIYRQAS HRPDIYHRAS	TI <mark>TMISDWLA</mark> Y TVTMISDWMAF	MLSGELAVI MLSGELAVI	PSNAGTTGL PSNAGTTGL
sp P77432 LSRK_ECOLI	$\xrightarrow{\beta8}_{200} \xrightarrow{\beta9}_{2000} \xrightarrow{\alpha8}_{2000}$	8 α9 <u>000 0000</u> 10 <b>22</b> 0	TT230	α10 <u>0000000</u> 240	TT <u>β11</u> 250	α11 ►00000000 260
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	LDLT <b>TR</b> DWKPALL LDLVTRNWKRSLL	DMAGLRADILSPV QMAGLRS <mark>DILSPV</mark>	KETGTLLGVV KETGTLLGHI	SSQAAELCGLK SQK <mark>AAEQC</mark> DLQ	AGTPV <mark>V</mark> VGG AGTPV <mark>I</mark> VGG	GDVQLGCLG
sp P77432 LSRK_ECOLI	$\mathbf{TT} \xrightarrow{\beta 12}_{270}$	β13 280	290 <sup>TT</sup> β	14 300 TTβ	15 310 20	α12 1000000000 320
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	LGVVRPAQTAVLG LGVVRPAQTAVLG	GTFWQQVVNLAAP GTFWQQVVNL <mark>P</mark> AP	VTDPEMNVRV VTDP <mark>NMNVR</mark> I	NPHVIPGMVQA NPHVIPGMVQT	ESISFFTGI ESISFFTGI	TMRWFRDAF TMRWFRDAF
sp P77432 LSRK_ECOLI	α13 <u>000000000</u> <b>330 3</b>	α14 <u>000000000</u> 40 350	$\begin{array}{c} \eta 5 \\ 0 0 0 \\ 3 6 0 \end{array} \xrightarrow{\beta 16} 3 6 0 \end{array}$	η6 222 370	β17 380	ي عەن
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	CAEEKLIAERLGI CAEEKLIAERLGI	DTYTLLEEMASRV DAYSLLEDMASRV	PPG <mark>SWGVMPI PPGA<mark>Y</mark>GVMPI</mark>	FSDRMRFKTWY FSDWMRFKRWY	HAAPSFINI HAAPSFINI	SIDP <mark>DKCNK</mark> SIDP <mark>E</mark> KCNK
sp P77432 LSRK_ECOLI	α15 222222222222222222222222222222222222	410	420 420	η7 α 222 22222 430	16 222222 449	β19 450
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	ATLFRALEENAAI ATLFRALEENAAI	VSACNLQQIADFS VSACNLQQIA <mark>A</mark> FS	NIHPS <b>SLVFA</b> G <mark>V</mark> QAD <mark>SLVFA</mark>	GGGSKGKLWSQ GGGSKGKLWSQ	ILADVSGLF ILADVTGL	VNIPVVKEA VH <u>V</u> PVVKEA
sp P77432 LSRK_ECOLI	α17 2020202020200 <b>46</b> 0 <b>4</b>	α18 <u>00000000</u> 70 <b>48</b> 0	β20 490	α19 222222222 500	510	520
sp 277432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	TALGCAIAAGVGA TalgcaiaagvgV	GIHSSMAETGERL GVWPSLAETGEKL	VRWERTHTPD VRWDR <mark>E</mark> HKPN	PEKHELYQDSR PENFAVYQQAR	DIKWQAVYQI EKWQAVYQI	IQLGLVDHGL QRALVDGGL
sp P77432 LSRK_ECOLI	530					
sp P77432 LSRK_ECOLI sp Q8ZKQ6 LSRK_SALTY	TTSLWKAPGL TTSLWKAPG.					

Figure S1. Sequence alignment of E. coli LsrK kinase and S. typhimurium LsrK kinase. Red shading shows the identical residues. Secondary structural elements of the crystal structure were shown above the alignment.  $\alpha$ -Helices were shown as squiggles and  $\beta$ -strands as arrows, strict  $\beta$ -turns as TT and strict  $\alpha$ -turns as TTT. Regions that were not identified in the X-ray structure are highlighted with black boxes.



Figure S2. Numbering for the reference of structure of LsrK (PDBID: 5YA1)



Figure S3. Protein ( $C\alpha$ -atoms) RMSD and ligand RMSD of crystal structure (5YA1:CS-Open-ATP) during the dynamic simulations. RMSD is shown during the simulation timescale. Blue lines in the graph indicate protein RMSD and brown lines indicate ligand RMSD with respect to the protein.



Figure S4. RMSD of protein and ligand during the 500ns simulations of homology models. Protein-ligand interaction diagram of the Closed-ADP during the 500ns simulation time scale. Interactions and time of residence (%) are depicted.



Figure S5. RMSD of LsrK structure through 500 ns simulation trajectory.

Table S1. Sitemap predicted pocket parameters for the trajectory cluster centroids of ATP and substrate bound crystal structure (5YA0 or CS-Open-Apo).

Cluster Pocket Number	Size	Volume
Pocket 1	60	300.12
Pocket 2	67	187.96
Pocket 3	88	252.44
Pocket 4	69	294.98
Pocket 5	89	316.93

Table S2. Sitemap predicted pocket parameters for the trajectory cluster centroids of ATP bound crystal structure (5YA1 or CS-Open-ATP).

Cluster Pocket Number	Size	Volume
Pocket 1	40	190.70
Pocket 2	35	146.80
Pocket 3	51	243.53
Pocket 4	55	294.637

Table S3. Sitemap predicted pocket parameters for the trajectory cluster centroids of ADP bound crystal structure (5YA2 or CS-Open-ADP).

Cluster Pocket Number	Size	Volume
Pocket 1	19	112.16
Pocket 2	59	284.69
Pocket 3	44	177.67
Pocket 4	31	133.08
Pocket 5	49	227.40

Table S4. Sitemap predicted pocket parameters for the trajectory cluster centroids of HM-Open-ATP.

Cluster Pocket Number	Size	Volume
Pocket 1	31	245.24
Pocket 2	66	297.72
Pocket 3	87	332.71
Pocket 4	109	380.73
Pocket 5	98	410.22

Table S5. Sitemap predicted pocket parameters for the trajectory cluster centroids of HM-Closed-ADP model.

Cluster Pocket Number	Size	Volume
Pocket 1	84	132.05
Pocket 2	158	227.75
Pocket 3	145	209.57
Pocket 4	156	261.70