

La familia PriA

PriA es una familia de enzimas de Actinobacteria homóloga a la familia HisA en Enterobacteria. Según las definiciones de este trabajo PriA es una familia promiscua. Se han caracterizado experimentalmente varios miembros de PriA con la capacidad de catalizar tanto la reacción correspondiente a HisA como la de TrpF. Es decir PriA participa a la vez en las rutas de síntesis de Histidina y Triptofano, al menos en varias Actinobacterias. Algunos de los primeros miembros caracterizados como promiscuos son *Streptomyces coelicolor* y XXXX. Dentro de la ruta de histidina HisA convierte ProFAR en PRFAR. Además de la isomerización de PROFAR también convierte PRA en CdRP actuando análogamente a la función TrpF en la ruta del triptófano. La mayoría de las actinobacterias han perdido el gen *trpF* en la ruta del triptofano, por lo que se cree que la promiscuidad de proFAR está extendida en un gran subconjunto de Actinobacteria.

Además, en Actinobacteria PriA ha mostrado un gradiente funcional. Esta variación divide a la familia en varias subfamilias según su funcionalidad. En la tabla section se muestra la capacidad de catálisis PriA en diferentes organismos para sus dos sustratos conocidos. Estos datos ejemplifican las subfamilias de PriA, en la tabla se encuentran por ejemplo miembros de PriB, una subfamilia con presencia en el género *Streptomyces*. PriB muestra poca actividad *trpF*. Varios *Streptomyces* con un elemento de PriB, contienen en su genoma un gen *trpF* localizado fuera del contexto genómico cercano operón de histidina. Otra subfamilia es subHisA con miembros en *Corynebacteria* y *Actinomyces*. SubHisA ha perdido totalmente la actividad *trpF*. Finalmente en *Actinomyces* encontramos hay *subTrpF* que ha perdido su actividad de HisA.

En este capítulo se explora la distribución de PriA en diversos linajes genómicos utilizando EvoMining y CORASON. Como resultado se encuentran variantes en el número de copias según el linaje. En Actinobacteria PriA es promiscua a pesar de no tener copias extra con marcas de reclutamiento al metabolismo especializado. Otro resultado es el descubrimiento de que al analizar datos de cinéticas enzimáticas de PriA, no existe una trayectoria Darwiniana para ambos sustratos que lleve a la adquisición de promiscuidad. Es decir que al menos en este ejemplo, las mutaciones puntuales que llevan a una enzima monofuncional en la ruta para adquirir promiscuidad nunca incrementan la capacidad catalítica sobre ambas reacciones.

Tabla Datos Cinéticos de subfamilias de PriA

Fuente		HisA Familia <i>in vivo</i>	TrpF <i>in vivo</i>	K_{cat}^{ProFAR} [M ⁻¹ s ⁻¹]	K_m^{ProFAR} [μM]	HisA $\frac{K_{cat}}{K_m}$	K_{cat}^{PRA} [M ⁻¹ s ⁻¹]	K_m^{PRA} [μM]	TrpF $\frac{K_{cat}}{K_m}$	Referencia
<i>Escherichia coli</i>	HisA	-	-	1.6	4.9	3.1	-	-	0	[@henn-sax_two_2002]
<i>Escherichia coli</i>	TrpF	-	-	-	-	0	12.2	34.5	2.82	[@sterner_phosphoribosyl_1
<i>Mycobacterium smegmatis</i>	PriA	*	*	2.6 ± 0.5	0.85 ± 0.04	0.33	7.9 ± 2.4	3.1 ± 0.43	0.39	Verduzco [@verduzco-castro_co-occurrence_2016]
<i>Mycobacterium tuberculosis</i>	PriA	-	-	19	0.23	12	21	3.6	0.17	Due et al. (2011)
<i>Streptomyces globisporus</i>	PriA	*	*	4.2 ± 0.8	0.74 ± 0.03	0.18	11 ± 1.0	3.8 ± 0.2	0.34	Verduzco [@verduzco-castro_co-occurrence_2016]
<i>Streptomyces coelicolor</i>	PriA	-	-	3.6 ± 0.7	1.3 ± 0.2	0.4	5.0 ± 0.08	3.4 ± 0.09	0.7	Noda [@noda-garcia_identification_2010]
<i>Streptomyces coelicolor</i>	PriA	-	-	3.6 ± 0.7	1.3 ± 0.2	0.4	5.0 ± 0.08	3.4 ± 0.09	0.7	Noda [@noda-garcia_identification_2010]

Fuente		HisA	TrpF	K_{cat}^{ProFAR}	K_m^{ProFAR}	HisA	K_{cat}^{PRA}	K_m^{PRA}	TrpF	
	Familia	<i>in vivo</i>	<i>in vivo</i>	[M ⁻¹ s ⁻¹]	[μM]	$\frac{K_{cat}}{K_m}$	[M ⁻¹ s ⁻¹]	[μM]	$\frac{K_{cat}}{K_m}$	Referencia
<i>Streptomyces ipomoeae</i>	PriB	*	*	3.8 ± 0.2	0.82 ± 0.02	0.21	60.8 ± 1.1	8.25 ± 0.4	0.14	Verduzco [@verduzco- castro_co- occurrence_2016]
<i>Streptomyces Mg1</i>	PriB	*	*	13.2 ± 3.4	0.92 ± 0.19	69	129.6 ± 34	0.29 ± 0.04	0.0022	Verduzco [@verduzco- castro_co- occurrence_2016]
<i>Streptomyces sp. C</i>	PriB	*	*	11.4 ± 3.4	2.53 ± 0.74	0.22	149. 9 ± 29	1.4 ± 0.12	9	Verduzco [@verduzco- castro_co- occurrence_2016]
<i>Streptomyces sviceus</i>	PriB	*	*	3.9 ± 0.89	0.69 ± 0.04	0.18	24.5 ± 4.0	1.6 ± 0.29	67	Verduzco [@verduzco- castro_co- occurrence_2016]
<i>Corynebacterium diphtheriae</i>	subHisA	-	-	4.4 ± 0.5	2.6 ± 0.3	0.59			0	Noda [@noda- garcia_evolution_2013]
<i>Corynebacterium jeikeium</i>	PriA	-	-	2.3 ± 0.2	0.9 ± 0.08	0.39	5.1 ± 1.0	1.6 ± 0.16	0.31	Noda [@noda- garcia_evolution_2013]
<i>Corynebacterium striatum</i>	subHisA	-	-	6.9 ± 0.7	2.1 ± 0.5	0.3			0	Noda [@noda- garcia_evolution_2013]
<i>Corynebacterium diphtheriae L48L-F50L-T80S</i>	subHisA	-	-	4.5 ± 1.5	0.6 ± 0.08	0.13	133 ± 10	0.05 ± 0.01	0.0004	Noda [@noda- garcia_evolution_2013]
<i>Actinomyces urogenitalis</i> DSM 15434	PriB	*	*	2.1 ± 0.5	1.8 ± 0.2	0.9	26.3 ± 6.3	0.37 ± 0.09	14	Verduzco [@verduzco- castro_co- occurrence_2016]
<i>Actinomyces odontolyticus</i> ATCC 17982	subTrpF	*	-	-	-	0			0.02	Juarez [@juarez- vazquez_evolution_2017]
<i>Actinomyces oris K20</i> BABV01	PriA					0.02			0.01	Juarez [@juarez- vazquez_evolution_2017]
<i>Actinomyces</i> oral taxon 171 str. F0337	PriA					0.01			4	Juarez [@juarez- vazquez_evolution_2017]
<i>Actinomyces</i> oral taxon 848 str. F0332	subTrpF	*	-	-	-	0			0.0001	Juarez [@juarez- vazquez_evolution_2017]
<i>Actinomyces urogenitalis</i> DSM 15434	PriA					0.01			0.02	Juarez [@juarez- vazquez_evolution_2017]
<i>Bifidobacterium adolescentis</i> L2-32	PriA	*	*			0.2			0.1	Juarez [@juarez- vazquez_evolution_2017]

Fuente		HisA Familia <i>in vivo</i>	TrpF <i>in vivo</i>	K_{cat}^{ProFAR} [M ⁻¹ s ⁻¹]	K_m^{ProFAR} [μM]	HisA $\frac{K_{cat}}{K_m}$	K_{cat}^{PRA} [M ⁻¹ s ⁻¹]	K_m^{PRA} [μM]	TrpF $\frac{K_{cat}}{K_m}$	Referencia
<i>Bifidobacterium gallicum</i> DSM 20093	PriA	*	*			0.1			0.04	Juarez [@juarez-vazquez_evolution_2017]
<i>Bifidobacterium longum</i> ATCC 15697	PriA	*	*			0.1			0.3	Juarez [@juarez-vazquez_evolution_2017]
Camera CAM1	Metagenoma			1.7 ± 0.1	0.3 ± 0.03	0.2	40 ± 7	3.5 ± 0.04	0.09	Noda [@noda-garcia_insights_2015]
CAM1_A81G	Metagenoma			1.7 ± 0.2	0.1 ± 0.01	0.06	32.2 ± 1.7	1.9 ± 0.1	0.06	Noda [@noda-garcia_insights_2015]
CAM1_A81S	Metagenoma			4.0 ± 0.9	0.2 ± 0.03	0.04	23.5 ± 6.5	0.5 ± 0.1	0.02	Noda [@noda-garcia_insights_2015]
CAM2	Metagenoma			n.d.	n.d.	0	n.d.	n.d.	0	Noda [@noda-garcia_insights_2015]
PriA_Ancestral	Ancestral			9.4±1.6	0.3±0.009.03		4.3±0.4	0.6±0.02	0.13	Verduzco-Castro Noda-García
PriA_SubHisA	Ancestral			3.7±1.01	0.5±0.03	0.1	-	-	0	Verduzco-Castro Noda-García
SubHisA_Ancestral	Ancestral			6.3±0.7	0.15±0.03	0.02	-	-	0	Verduzco-Castro Noda-García
SubHisA_PriA	Ancestral			27.7±3.4	0.05±0.005		167.82	0.03±0.0020001		Verduzco-Castro Noda-García
<i>scabies</i>	0	0	163.6	0.1						Ernesto
<i>Avisco</i>		46	1.37	36	3.4					Ana

PriA como modelo de familia enzimática donde las expansiones no son condición necesaria para la promiscuidad.

PriA en EvoMining

Se investigaron las expansiones de la familia PriA en los linajes Actinobacteria, Cyanobacteria, Pseudomonas y Archaea. En Actinobacteria, donde se sabe que PriA es promiscua no se detectaron copias extra. EvoMining ha detectado un parólogo de PriA en Actinobacteria y una expansión conservada en un grupo de saxitoxinas en Cianobacteria [@moustafa_origin_2009].

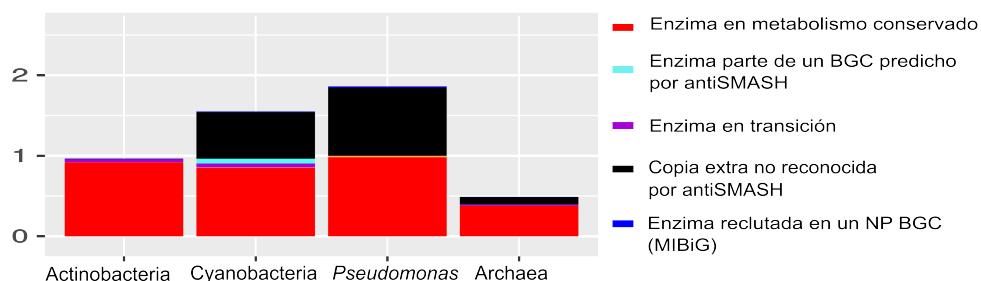


Figure 1:

Los reclutamientos que tuvieron estas expansiones están listados en la tabla Table 2

Table 2: Reclutamientos de expansiones de PriA en MIBiG

Compuesto	Actinobacteria	Cyanobacteria	Pseudomonas	Archaea	BGC origen	Clase
saxitoxin	x	x	x	x	Cyanobacteria	Alkaloid
lipopoly saccharide	x	x	x	x	Proteobacteria	Saccharide
toxin	x	x	x	x	Cyanobacteria	Other T3
2'-chloro pentostatin	x	-	-x	-	Actinobacteria	Other
2'-amino-2'- deoxy adenosine						

Finalmente, los árboles que se produjeron por EvoMining son:

Linaje	Link al árbol de EvoMining en MicroReact
Actinobacteria	7g2IGfkv9
Cyanobacteria	qF6jWRMox
PriA_Pseudomonas	ydff6DWqs
Archaea	Ig-m9Cm6f

Tabla: Árboles EvoMining de PriA en MicroReact

PriA CORASON

En Actinobacteria, se observó que todos los Streptomyces tienen el cluster de PriA parcialmente conservado con respecto al BGC de *Streptomyces coelicolor*. El único organismo de este género con una copia extra es *Streptomyces* CT34. La copia parece deberse a transferencia horizontal pues su hit en NCBI proviene de una *Lentzea*. Este fue sin embargo un homólogo lejano ya que tuvo 50% de identidad y 98% de cobertura. Otro caso interesante en Actinobacteria es XXXX

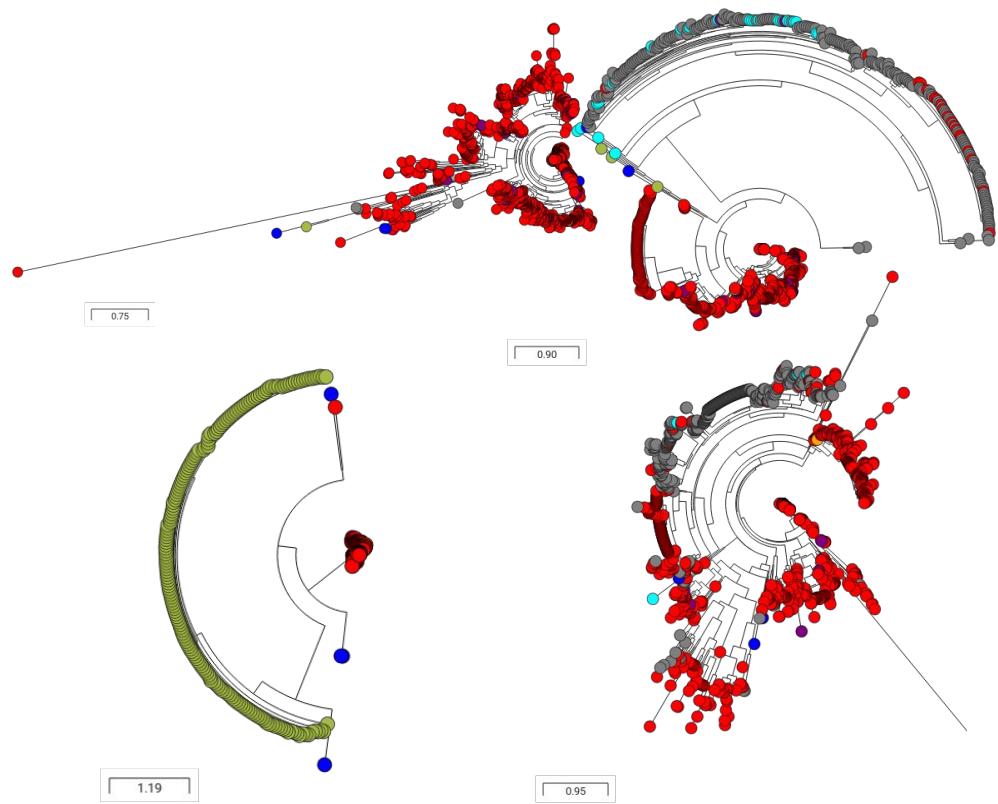


Figure 2:

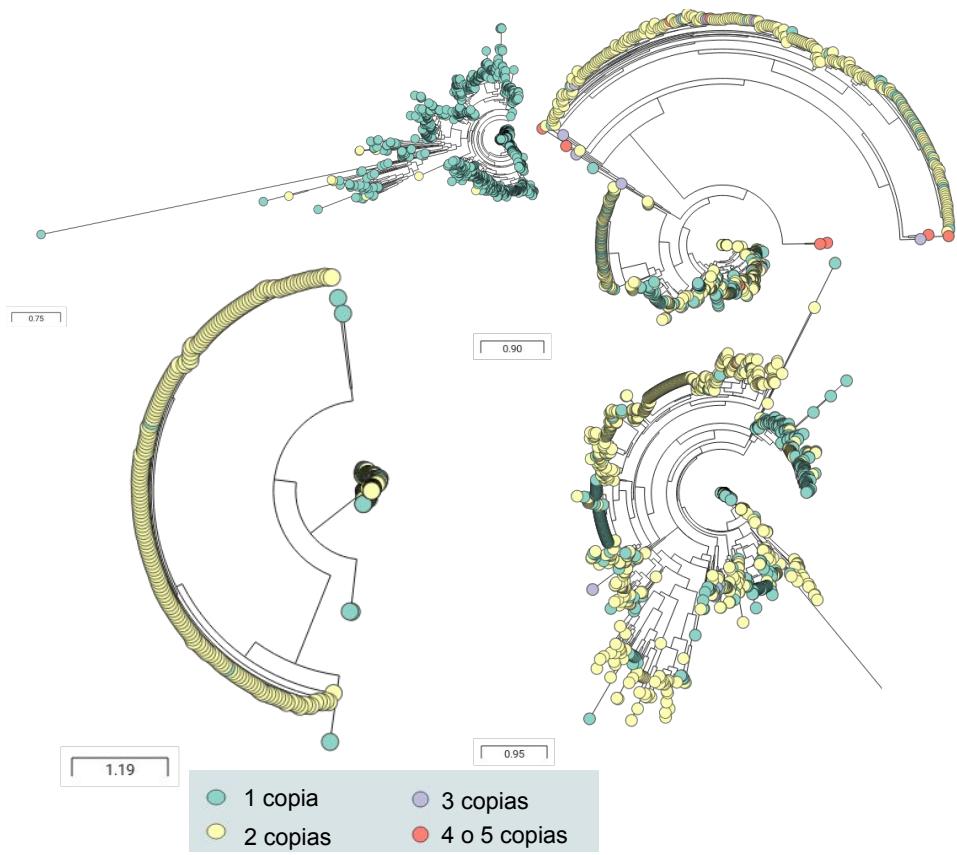


Figure 3:

No es el caso de Cyanobacteria. Copia doble Oscillatoriales cyanobacterium MTP1

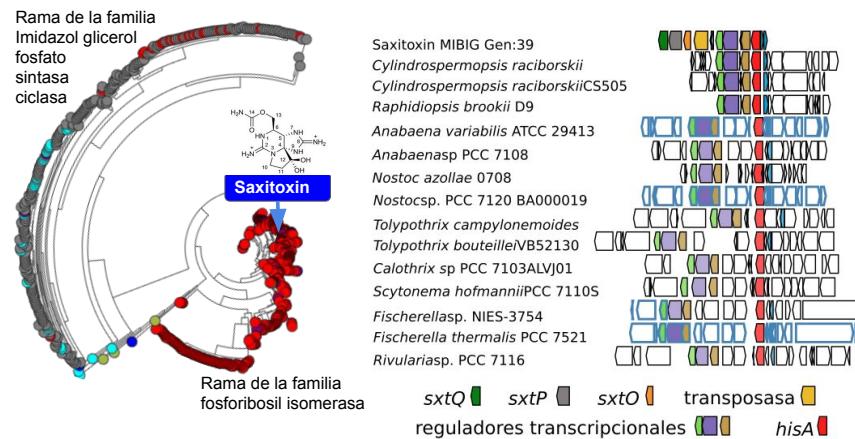


Figure 4: HisA en saxitoxin, un cluster de Cyanobacteria

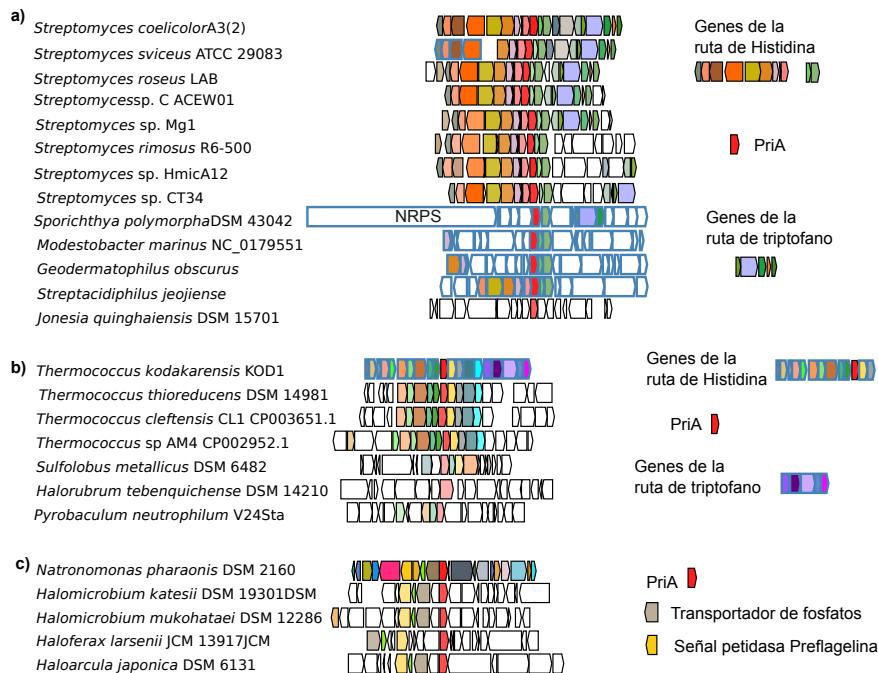


Figure 5: Contextos de PriA en Actinobacteria y Archaea

TrpF1 TrpF1 queries gave hits with TrpC enzyme present on every Streptomyces, additionally *S. rimosus*, *S. coelicolor*, *S. venezuelae* and *S. NRRL S-1813* had an extra copy. *S rimosus* TrpC vicinity has PKS and siderophore genes.

TrpF2 Conserved cluster with NRPS sequences flanking TrpF2

TrpF3 Non conserved cluster

TrpF4 purpleofuscus and *S. bikiniensis*

PriA a nivel de aminoácidos

Al transformar una subHisA en una PRiA mediante mutaciones no se observó ninguna trayectoria creciente para ambos sustratos (darwiniana)

En esta sección analizamos cómo cambia la capacidad catalítica de un sustrato mientras se transforma la del otro. Para ello se utilizaron datos de capacidad catalítica de mutantes de subHisA de *Corynebacterium diphtheriae*. Estos datos fueron obtenidos de la tesis de Lianet Noda [@noda_tesis_2012]. A partir de la secuencia original que se mostró es una subHisA se obtuvieron mutantes con el objetivo de alcanzar la promiscuidad, es decir de convertir la enzima en una PriA. Con esos datos, quedaba pendiente la exploración de las rutas, ¿cuántas son? ¿Existe alguna tendencia en ciertos momentos de la ruta sobre el incremento/decremento de alguna de las dos funciones?

Adicionando una mutación cada vez hasta la mutante con 11 mutaciones. Así pues se desarrolló un script utilizando recursividad que permitiera reconstruir todas las rutas posibles. Como resultado No existieron rutas darwinianas. Finalmente, estos aminoácidos fueron comparados con los provistos por EVCouplings, de covariación.

<i>Corynebacterium</i>	<i>Streptomyces</i>
D20V	21V
L48I	49L
F50L	51L
M66I	67I
T80S	81S
A97C	98C
D127A	128G
A129D	130D
T139L	136L
Y214L	211Y
E230A	227A

Los residuos que covarian en el registro evolutivo de PriA permiten una reconstrucción aproximada de su estructura tridimensional

Resultado se modelo PriA con evcouplings y los aminoácidos encontrados coinciden con los encontrados experimentalmente.

La estructura reconstruida es parecida, pero para obtener mejor refinamiento se debe seleccionar mejor las secuencias del alineamiento

Estandarización de protocolos experimentales de PriA en sus sustratos tradicionales

Además de la exploración gen

Afinidad de enzimas selectas por sustratos químicamente parecidos a PRA y PROFAR

Algunas de estas enzimas se seleccionaron en busca de nuevas funciones promiscuas, las reacciones catalizadas por los candidatos se investigaron mediante la exploración de sustratos químicamente similares a los nativos.

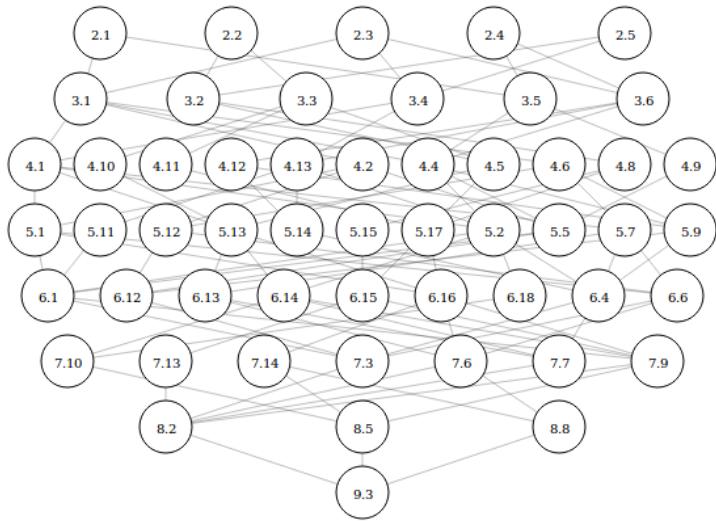


Figure 6:

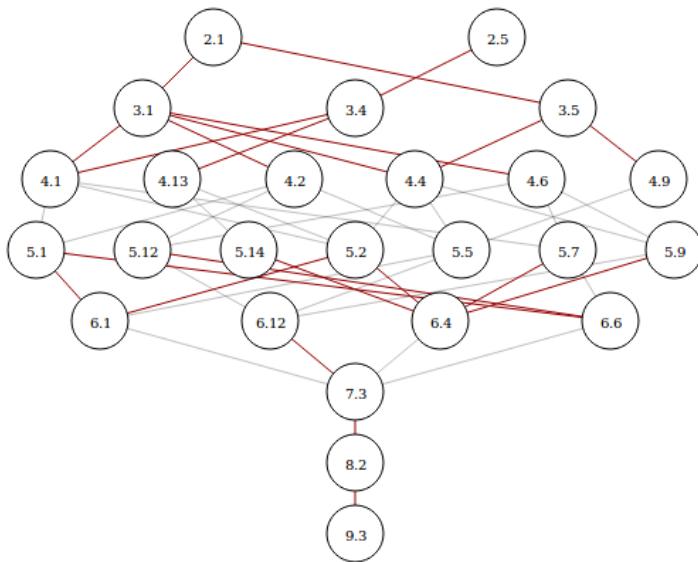


Figure 7:

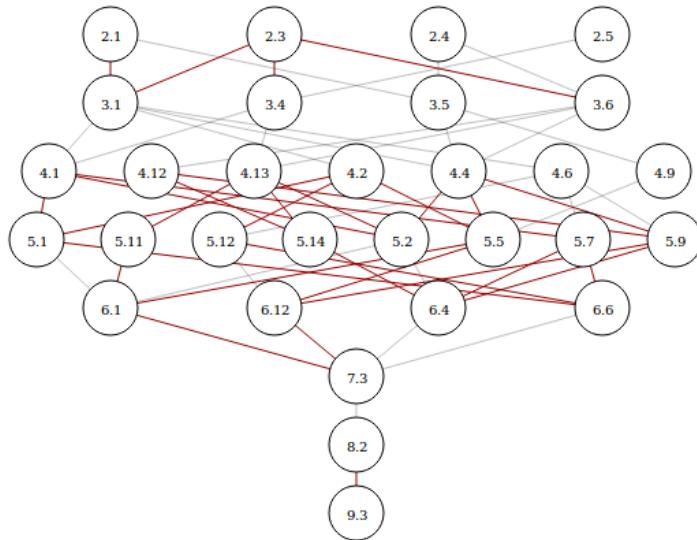


Figure 8:

Dado que en *Streptomyces* se han encontrado representantes de las familias PriA y PriB, se seleccionaron 39 secuencias homólogas para realizar el análisis del sustrato de la enzima de acoplamiento. Estas secuencias seleccionadas de PriA / PriB pertenecen a *Streptomyces* uniformemente distribuidas en un árbol de especies RpoB con diferentes condiciones sobre la presencia / ausencia de TrpF. En este estudio se incluyeron otros homólogos de PriA Actinobacterial caracterizados químicamente, y finalmente se agregaron HisA de *Escherichia coli*, *Arthrobacter Aurescens*, *Salmonella enterica* y *Acidimicrobium ferrooxidans* y Actinobacterial TrpF como controles.

Cuando existían estructuras de cristal, de lo contrario, se generaban estructuras homólogas utilizando como plantilla la enzima más cercana disponible con estructura de cristal.

Controls

HisA Enterobacteria enzymes from *Salmonella enterica* (PDB:5AHE), *Escherichia coli K12 Acidimicrobium ferrooxydans* (PDB:4WD0)

TrpF Actinobacteria *Jonesia denitrificans* and *Streptomyces sp Mg1* sequences.

Chemically characterized Actinobacterial enzymes

PriA

Mycobacterium tuberculosis (Mtub PDB:2Y88,2Y89,2Y85,3ZS4) *Streptomyces coelicolor* (Scoe PDB:2VEP,2X30,1VZW), *Streptomyces globisporus*, *Actinomyces urogenitalis* 4X2R *Corynebacterium jeikeum*

subHisA

Corynebacterium diphtheriae *Actinomyces car* (PDB:4X2R)

subTrpF

Arthrobacter aurescens (PDB:4WD0)

PriB

Streptomyces ipomoeae, *Streptomyces sviceus* (PDB:4U28,4TX9)

TrpF controls *Jonesia denitrificans* (PDB:4WUI) *Chlamidya trachomatis*, *Streptomyces sp. Mg1* TrpF and *Actinomyces odontolyticus* were included

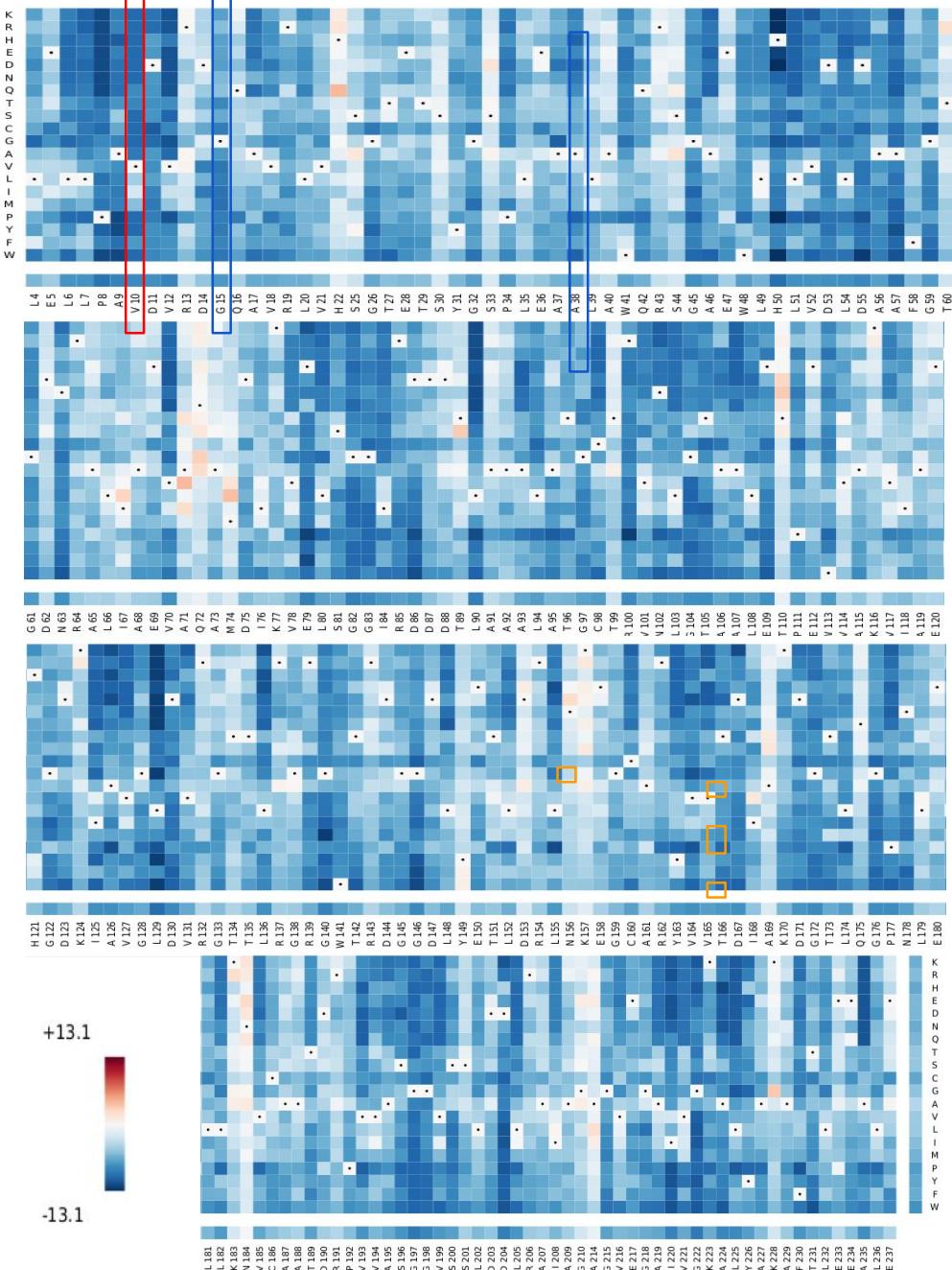


Figure 9:

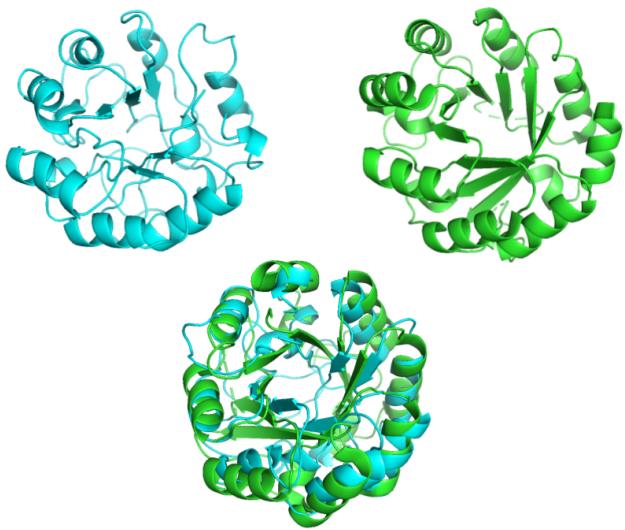
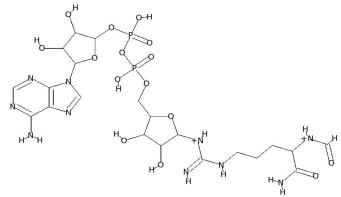


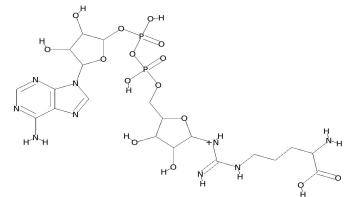
Figure 10:



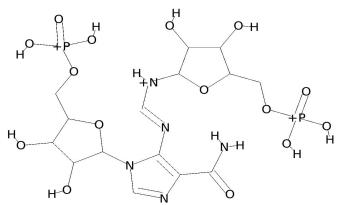
Figure 11:



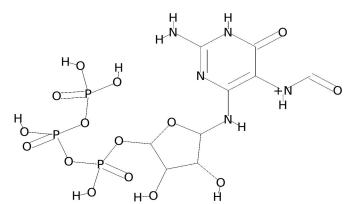
1
ADP-D-ribosyl-[dinitrogen reductase]



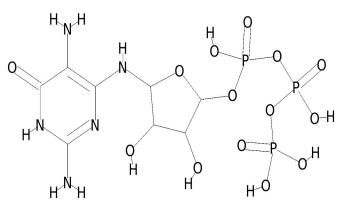
2
N(omega)-(ADP-D-ribosyl)-L-arginine



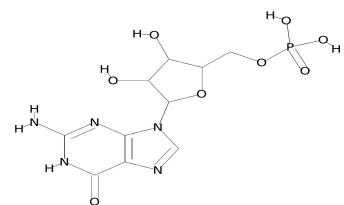
3
5-(5-Phospho-D-ribosylaminoformimino)-1-(5-phosphoribosyl)-imidazole-4-carboxamide



4
Formamidopyrimidine nucleoside triphosphate



5
2,5-diaminopyrimidine nucleoside triphosphate



6
Guanosine monophosphate

Figure 12: Substrates 1

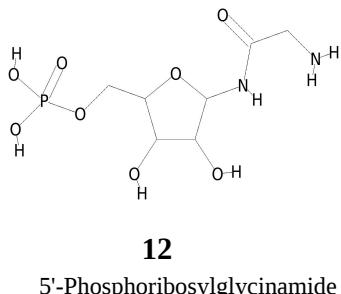
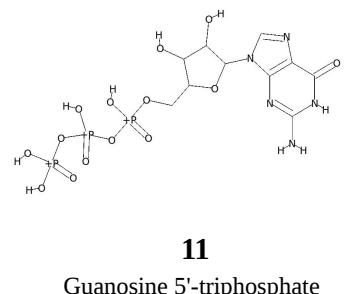
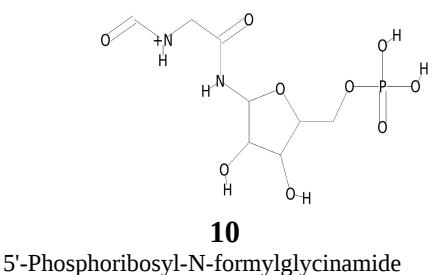
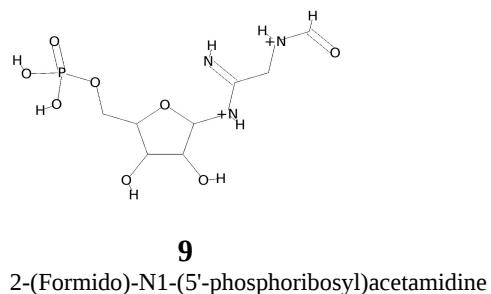
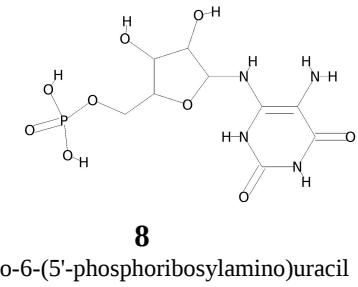
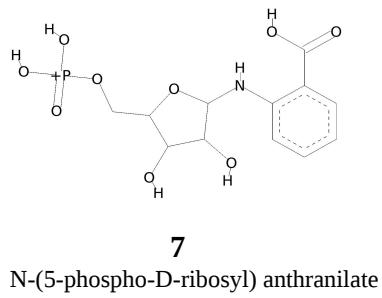
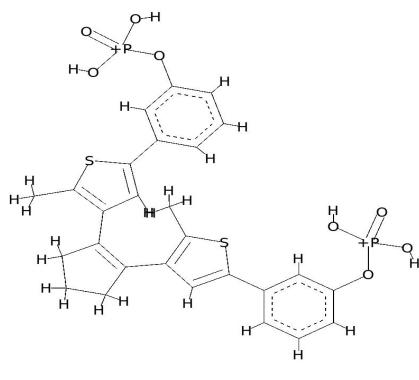
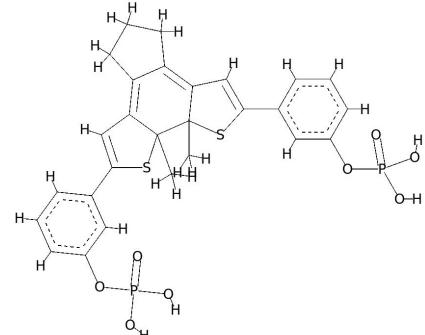


Figure 13: Substrates 2



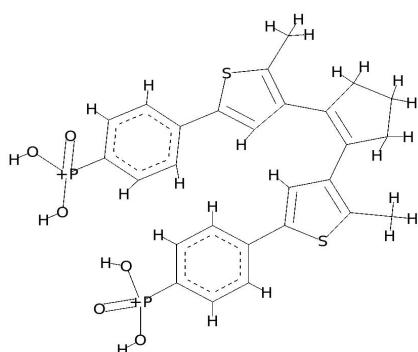
13

DTE-metaphosphate(dte6_Open form)



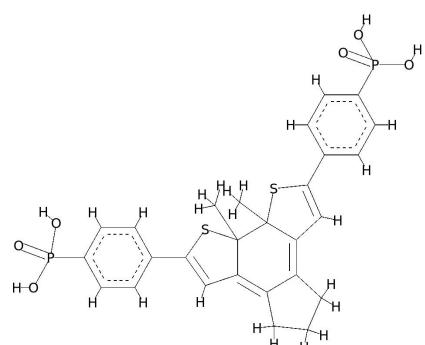
14

DTE-metaphosphate(dte6_Closed form)



15

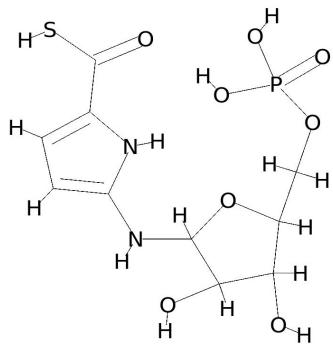
DTE-Para-Phosphonate(dte13_closed form)



16

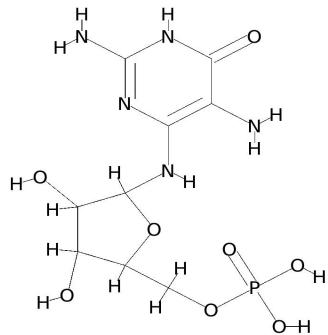
DTE-para-phosphonate(dte13_closed form)

Figure 14: Substrates 3



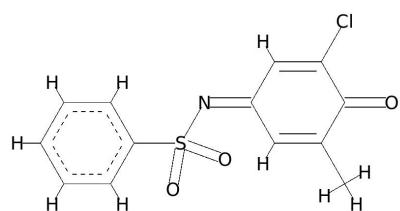
17

4N'-(5'-phosphoribosyl) 4-aminopyrrole-2-carboxilate



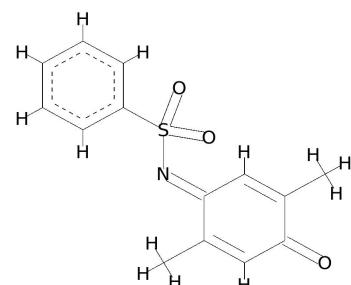
18

2,5-di-amino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate



19

(E)-N-(3-chloro-5-methyl-4-oxocyclohexa-2,5-dienylidene)benzenesulfonamide



20

2,5 dimethyl-N-(4-oxocyclohexa-2,5-dienylidene)benzenesulfonamide

Figure 15: Substrates 4

Es posible que PriA tenga actividad en GTP

Activity was measured fluorometrically in 96-well plates (Nuc 96-Well Optical Botto Plates) in a TECAN infinite M1000 plate reader (excitation at 286 nm and emission at 386 nm)

Preliminar activity essays were performed on an active PriA from *Streptomyces coelicolor* and an inactive mutant D11A.

Enzymes were cloned on coli V68 strains, overexpression were induced and protein were purified.

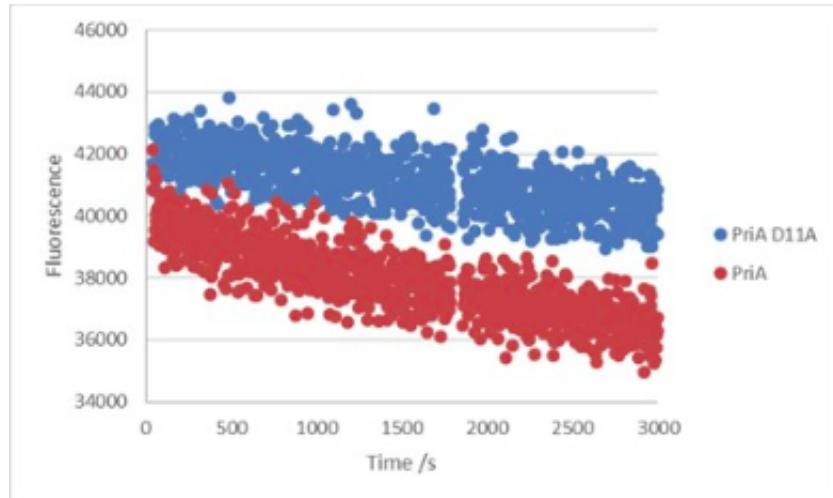


Figure 16:

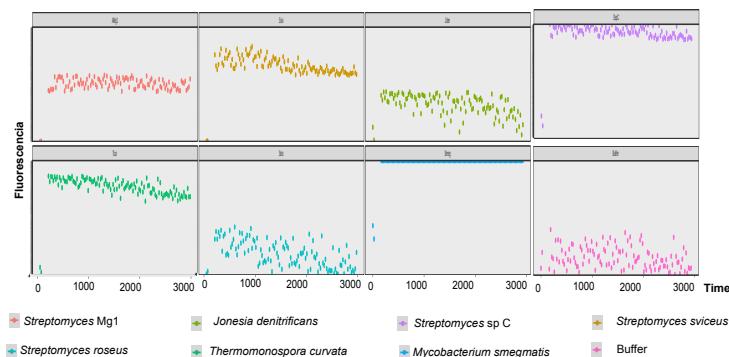


Figure 17:

las dos enzimas con actividad *Thermomonospora curvata* es una Actinobacteria termófílica de la familia *Thermomonosporaceae*, puede ser encontrada en compostas ya que participa en la degradación de celulosa [chertkov_complete_2011].

Jonesia trpF no mostró actividad *Jonesia denitrificans* está clasificada como un organismo patogénico para animales, su genoma fue originalmente aislado de from cooked ox blood [pukall_complete_2009].

```
docking <- read.csv("chapter4/SmallHeat.data", header=TRUE, sep="\t")
kable(docking,
      caption = "Enzymes docking \\label{tab:docking}",
      caption.short = "Enzymes docking ")
```

Table 5: Enzymes docking

Enzima	S13	S15	S14	S16	S10	S12	S9	S18	S5	S4	S8	S17	S7	S6	S11	S1
Srub_2VEP	-7.4	-7.3	-7.5	-7.1	-6.5	-6.2	-6.5	-7.7	-9.4	-9.3	-7.9	-7.2	-8.3	-8.6	-8.9	-9.0
Saver_2VEP	-7.4	-7.2	-7.0	-6.5	-7.3	-6.4	-7.0	-7.5	-9.6	-8.5	-7.9	-7.6	-8.4	-8.7	-9.8	-8.3
Scoe_2VEP	-7.5	-7.5	-7.9	-7.0	-7.0	-6.2	-6.5	-7.8	-8.8	-9.2	-7.8	-7.9	-8.0	-8.9	-10.3	-9.2
Scoe_2X30	-8.1	-7.4	-7.6	-6.9	-6.7	-6.8	-7.1	-7.9	-9.1	-9.0	-8.3	-8.6	-8.5	-9.0	-10.6	-10.0
Scoe_1VZW	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sfla_2VEP	-7.1	-7.6	-7.2	-7.3	-7.2	-6.8	-6.9	-8.1	-8.2	-7.2	-8.2	-7.2	-8.4	-8.3	-8.5	-7.9
Sgha_2VEP	-9.2	-8.7	-6.7	-6.7	-7.4	-7.7	-7.2	-7.5	-9.8	-8.9	-8.2	-7.8	-8.8	-8.7	-10.1	-9.1
Siak_2VEP	-6.6	-7.3	-7.0	-7.1	-7.1	-7.1	-6.8	-8.0	-9.2	-8.7	-7.8	-7.6	-8.3	-8.4	-9.1	-5.8
Sbic_2VEP	-7.2	-6.7	-6.8	-6.5	-6.2	-6.6	-5.9	-7.8	-8.5	-7.8	-7.8	-7.2	-8.2	-8.0	-9.6	-8.2
Sbot_2VEP	-9.6	-10.9	-8.9	-7.1	-6.0	-6.2	-6.7	-8.1	-9.1	-8.8	-8.3	-7.9	-8.9	-9.3	-9.4	-9.8
Sipo_2VEP	-6.6	-6.6	-7.3	-6.9	-6.5	-6.3	-6.5	-8.1	-8.6	-9.1	-8.0	-7.9	-8.0	-8.4	-8.5	-7.2
Ssvi	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ssvi_4U28	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ssvi_4TX9	-5.9	-8.2	-7.1	-6.6	-6.8	-7.0	-7.6	-7.9	-8.4	-8.3	-8.2	-8.1	-8.4	-8.7	-7.7	-8.2
Salb_2VEP	-6.3	-7.1	-7.4	-6.8	-7.7	-6.9	-6.6	-8.0	-9.6	-8.9	-8.6	-7.9	-8.6	-8.4	-9.1	-7.4
Sbik_2VEP	-7.4	-6.7	-7.4	-7.3	-7.7	-6.5	-6.8	-8.5	-9.9	-9.8	-8.3	-7.8	-8.2	-8.4	-10.5	-7.1
Stsu_2VEP	-6.9	-7.5	-7.9	-7.8	-7.5	-7.3	-7.7	-8.1	-10.0	-9.7	-8.7	-7.8	-8.8	-8.8	-10.6	-7.8
Sven_2VEP	-8.3	-7.6	-7.5	-6.8	-6.4	-7.0	-6.2	-8.2	-9.6	-9.0	-7.9	-7.4	-8.3	-8.6	-10.0	-8.2
Scal_2VEP	-7.3	-8.8	-7.5	-6.7	-7.0	-6.5	-6.5	-8.7	-10.0	-9.7	-8.8	-7.7	-8.2	-8.6	-10.5	-8.1
Sbaa_2VEP	-8.9	-6.6	-6.7	-6.8	-7.2	-7.2	-6.2	-7.7	-9.6	-9.5	-8.4	-7.4	-8.5	-8.2	-9.7	-8.5
Sglo_2VEP	-6.1	-6.8	-6.9	-6.5	-6.7	-6.3	-6.7	-7.5	-9.6	-9.6	-8.6	-7.8	-8.7	-8.7	-9.9	-6.2
Sful_2VEP	-9.0	-6.9	-7.1	-6.8	-6.3	-7.2	-6.6	-8.1	-9.8	-9.3	-7.7	-8.0	-8.7	-8.8	-10.5	-7.6
Sgri_2VEP	-6.6	-8.2	-7.1	-6.4	-7.1	-7.4	-7.1	-7.4	-9.4	-8.5	-8.6	-7.5	-8.8	-8.4	-9.6	-8.8
S34_2VEP	-7.8	-7.2	-7.6	-7.3	-6.2	-6.5	-6.9	-8.0	-8.8	-8.6	-7.7	-7.1	-7.7	-7.9	-7.2	-7.1
Srim_2VEP	-7.4	-6.8	-7.1	-7.2	-7.2	-7.4	-7.8	-8.6	-9.7	-9.3	-8.8	-7.7	-8.9	-8.7	-10.2	-6.8
Satr_2VEP	-7.6	-7.1	-7.5	-7.4	-6.2	-6.5	-6.9	-8.0	-8.9	-8.7	-7.7	-7.4	-7.7	-7.8	-6.5	-7.0
S1813_2VEP	-7.5	-6.7	-6.8	-6.6	-6.8	-7.0	-6.7	-7.9	-9.9	-9.7	-8.3	-7.7	-8.5	-8.6	-10.0	-7.6
Svar_2VEP	-6.3	-6.8	-7.0	-6.3	-6.9	-6.9	-6.6	-7.8	-9.3	-7.4	-8.5	-7.3	-8.0	-8.7	-8.5	-7.4
Sfra_2VEP	-7.3	-7.0	-6.8	-6.6	-6.5	-7.3	-6.6	-8.6	-9.1	-8.7	-8.9	-7.7	-8.9	-9.0	-8.7	-6.0
Smeg_2VEP	-8.0	-7.3	-6.9	-6.6	-7.2	-6.7	-6.1	-7.6	-9.2	-9.1	-7.8	-7.4	-8.2	-8.5	-9.6	-9.2
Ssul_2VEP	-7.5	-7.0	-6.9	-6.6	-6.6	-6.7	-6.9	-7.7	-8.4	-8.2	-8.2	-7.5	-8.1	-7.7	-8.2	-6.7
Slav_4X9S	-8.0	-7.1	-6.7	-6.9	-6.6	-6.8	-6.7	-8.2	-9.5	-9.1	-8.6	-8.0	-8.4	-8.7	-9.1	-9.5
Sery_4X9S	-7.8	-7.2	-7.6	-7.2	-7.4	-6.9	-7.4	-8.5	-9.4	-9.4	-8.6	-7.6	-8.4	-8.6	-9.1	-10.0
SspC_4X9S	-7.4	-6.9	-7.3	-6.8	-7.3	-6.6	-7.5	-8.0	-9.8	-9.3	-8.7	-7.6	-8.5	-8.4	-10.6	-9.1
Sxan_4X9S	-7.5	-6.9	-6.8	-6.8	-6.5	-6.6	-6.5	-8.3	-8.0	-8.1	-7.6	-7.4	-8.7	-8.1	-8.5	-8.5
Skat_4X9S	-6.4	-7.0	-7.1	-6.7	-7.7	-6.3	-6.2	-8.1	-8.5	-9.2	-8.3	-7.6	-9.0	-8.5	-9.7	-9.5
SMg1_4X9S	-6.5	-6.9	-7.2	-7.1	-6.5	-6.9	-6.4	-7.3	-7.8	-7.7	-8.3	-7.5	-7.9	-8.4	-9.5	-7.6
SMg1_W9T	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sniv_2VEP	-6.2	-6.8	-7.4	-6.5	-7.8	-6.8	-6.7	-9.5	-9.6	-9.4	-8.7	-8.2	-8.6	-9.1	-9.9	-5.4
Scla_2VEP	-6.4	-7.7	-7.4	-7.2	-7.6	-7.5	-7.2	-8.5	-10.1	-10.0	-8.7	-7.9	-8.8	-9.1	-10.2	-6.9

Enzima	S13	S15	S14	S16	S10	S12	S9	S18	S5	S4	S8	S17	S7	S6	S11	S1
Save_2VEP	-7.6	-6.7	-7.1	-7.2	-7.1	-6.5	-6.6	-7.8	-8.6	-10.0	-8.6	-7.5	-8.3	-8.5	-8.8	-5.3
Spur_2vEP	-6.8	-7.3	-6.9	-6.7	-8.0	-7.9	-7.7	-8.5	-9.5	-9.8	-8.1	-7.8	-8.7	-9.7	-10.0	-5.3
Scar_2Y89	-6.9	-6.8	-7.5	-7.1	-7.8	-7.3	-7.2	-8.3	-9.2	-8.3	-8.9	-8.4	-8.9	-9.3	-9.4	-5.3
Mtub_2Y88	-10.0	-7.8	-8.9	-5.4	-8.6	-7.3	-7.8	-9.5	-10.9	-10.3	-9.5	-9.0	-9.8	-9.8	-11.3	-10.1
Mtub_2Y89	-8.7	-8.6	-9.6	-9.4	-6.4	-5.9	-5.6	-7.1	-7.0	-7.5	-7.3	-6.8	-7.4	-8.4	-7.5	-8.1
Mtub_2Y85	-8.2	-7.9	-9.2	-7.4	-7.6	-7.5	-7.6	-8.4	-9.7	-9.5	-9.3	-7.8	-8.6	-8.6	-10.2	-9.8
Mtub_3ZS4	-10.0	-10.5	-11.4	-6.4	-8.2	-7.2	-7.0	-9.6	-10.2	-10.2	-9.9	-8.5	-9.3	-9.6	-10.9	-10.0
S34_3ZS4	-7.4	-8.0	-7.6	-6.4	-5.2	-5.4	-5.2	-6.1	-6.8	-6.4	-5.7	-6.4	-6.3	-6.3	-7.1	-7.4
Cdip_4AXK	-9.2	-7.8	-10.9	-7.2	-7.5	-7.6	-7.7	-8.9	-9.0	-9.8	-9.0	-8.3	-8.8	-9.2	-10.1	-9.5
Cjei_4AXK	-7.5	-6.9	-7.2	-6.5	-8.4	-8.0	-8.5	-8.7	-9.5	-9.6	-9.4	-8.8	-9.0	-9.5	-9.3	-8.1
Aaur	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Aaur_4WD0	-8.2	-8.5	-8.1	-7.9	-5.7	-5.5	-5.9	-7.0	-7.5	-7.2	-7.1	-6.7	-7.1	-7.4	-8.4	-7.2
Acar_4X2R	-10.6	-9.3	-9.3	-7.2	-7.2	-6.8	-7.3	-7.5	-9.5	-9.8	-8.0	-7.8	-9.3	-8.9	-10.3	-9.6
Auro_4X2R	-9.9	-9.1	-9.8	-8.1	-7.4	-7.1	-7.4	-7.8	-9.2	-9.8	-8.3	-7.8	-9.3	-9.0	-9.9	-9.0
Afer_4WD0	-5.7	-5.8	-6.0	-5.7	-6.7	-6.2	-5.8	-7.6	-9.2	-8.8	-7.8	-7.4	-8.3	-8.4	-9.3	-6.7
Sent_5AHE	-9.1	-5.4	-6.4	-5.2	-8.0	-7.3	-7.4	-8.8	-10.7	-10.2	-8.7	-8.7	-9.6	-9.9	-10.9	-7.8
Ecoli_K12	-9.7	-9.7	-9.2	-6.1	-7.2	-6.6	-6.8	-8.6	-9.5	-9.1	-8.6	-8.2	-9.0	-8.6	-10.2	-9.9
Jden_4WUI	-8.3	-8.8	-8.2	-6.8	-6.2	-6.1	-6.0	-6.8	-7.4	-7.6	-7.5	-6.9	-7.6	-7.5	-7.7	-7.5
Ctra	-8.2	-8.0	-7.4	-7.2	-6.1	-5.5	-5.4	-6.5	-7.1	-7.1	-7.0	-6.2	-6.8	-6.8	-6.9	-7.2
SMg1_trpF	-7.2	-8.8	-8.2	-7.3	-6.2	-5.7	-5.7	-6.9	-6.6	-6.7	-7.3	-6.7	-7.6	-6.9	-7.5	-7.1
Aodo_4X2R	-8.5	-8.6	-8.8	-7.3	-7.1	-7.1	-7.1	-7.5	-9.7	-9.4	-7.8	-7.6	-9.6	-8.8	-10.1	-8.4

Enzima	S13	S15	S14	S16	S10	S12	S9	S18	S5	S4	S8	S17	S7	S6	S11	S1
--------	-----	-----	-----	-----	-----	-----	----	-----	----	----	----	-----	----	----	-----	----

El análisis de PriA a nivel estructural sugiere que GTP es el sustrato más afín

```
table <- read.csv("chapter4/EstructurasPDB", row.names = 1,sep="\t")
kable(table, caption = "Enzyme PDB \\label{tab:Enzyme PDB}",caption.short = "Enzyme PDB ")
```

Table 6: Enzyme PDB

Organismo	Family	Observations	Resolution	Year	
4WUI	TrpF		1.09	2014	
4X9S	PriB		1.60	2014	
5DN1	PriA		1.95	2015	
1DL3	TrpF		2.70	1999	
1LBM	TrpF	RCDRP	2.80	2002	
1NSJ	TrpF		2.00	1996	
1V5X	TrpF		2.00	2003	
1VZW	PriA		1.80	2004	
2VEP	PriA		1.80	2007	
2X30	PriA	R139N	1.95	2010	
2Y85	PriA	RCDRP	2.40	2011	
2Y88	PriA	D11N PRFAR	1.33	2011	
2Y89	PriA	D11N	2.50	2011	
3ZS4	PriA	PRFAR	1.90	2012	
4AAJ	TrpF		1.75	2012	
4TX9	PriB	ProFAR	1.60	2014	
4U28	PriB		1.33	2014	
4W9T	PriB		1.57	2014	
4WD0	PriB		1.50	2014	
4X2R	Actinomyces urogenitalis		1.05	2014	
4AXK	Corynebacterium efficiens	SubHisA	2.25	2013	
5AHE	Salmonella enterica	HisA	1.70	2015	
5AB3	Salmonella enterica	HisA	D7N, D10G, dup13-15, Q24L, G102A	1.80	2016
5ABT	Salmonella enterica	HisA	D7N, G102A, V106M, D176A	1.65	2016
5AC7	Salmonella enterica	HisA	D7N, D10G, dup13-15	1.90	2016
5AC8	Salmonella enterica	HisA	D10G, dup13-15, G102A	1.70	2016
5AC6	Salmonella enterica	HisA	D7N, D10G, dup13-15, Q24L, G102A	1.99	2016
5A5W	Salmonella enterica	HisA	HisA D7N D176A with ProFAR	NA	2015
5AHF	Salmonella enterica	HisA	HisA D7N with ProFAR	NA	NA
4GJ1	Campylobacter jejuni	HisA		2.15	2012
2W79	Thermotoga maritima	HisA		1.85	2008
1QO2	Thermotoga maritima	HisA		1.85	2000
5LHE	Thermococcus kodakaraensis	TrpF		1.85	2016
5LHF	Thermococcus kodakaraensis	TrpF		1.75	2016

Similar substrates to PRA and PROFAR suggested by Tanimoto distance or previously tested

S1,S2,...S20 substrates were collected from literature and chemoinformatics predictions. S3 PRA and S7 PROFAR are native substrates, S13-S16 are light activated substrates, S17 PRAP, S18 Compound V, were found on literature, S6 GMP, S11 GTP and other were suggested by chemoinformatics.

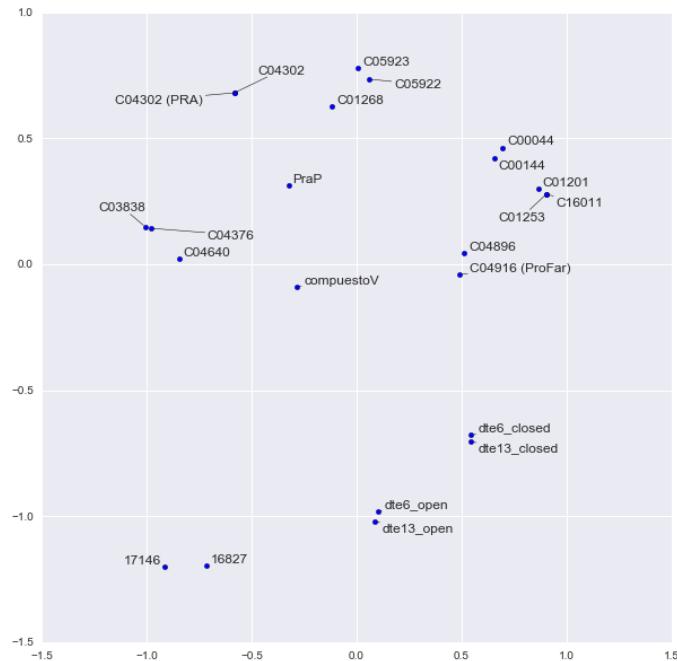


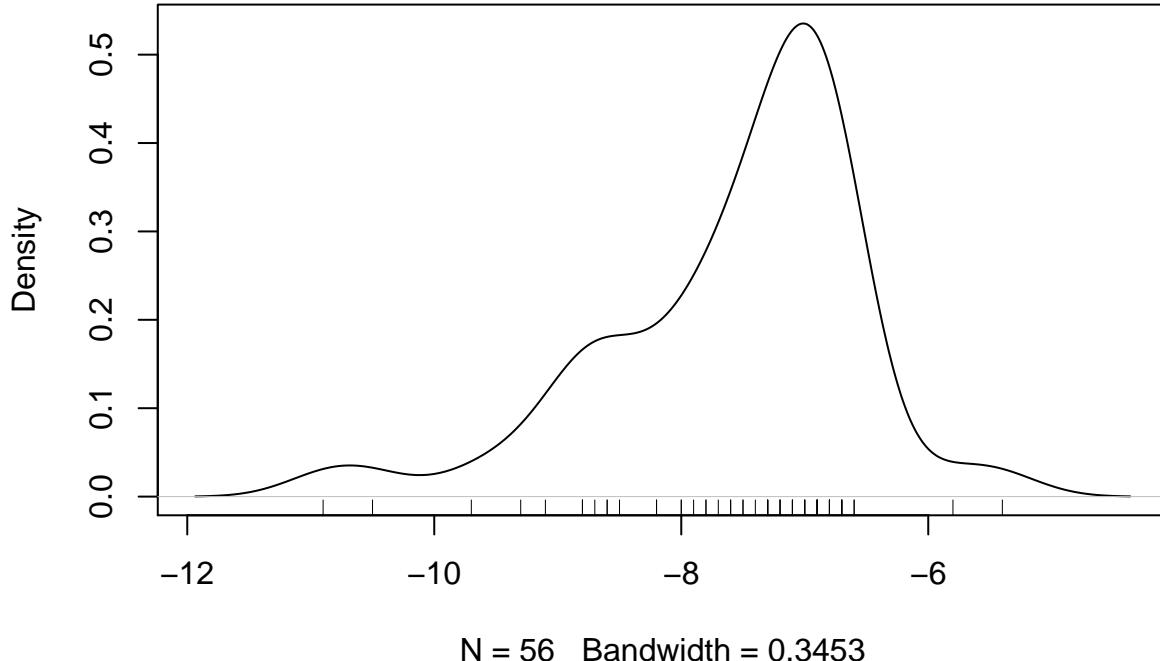
Figure 18:

On next figure we can see their chemical structures.

```
### Docking between PriA enzymes and selected substrates  
Docking simulation were calculated for PriA Streptomyces enzymes. TrpF enzymes from Streptomyces Mg1,  
Jonesia denitrificans, were added as controls
```

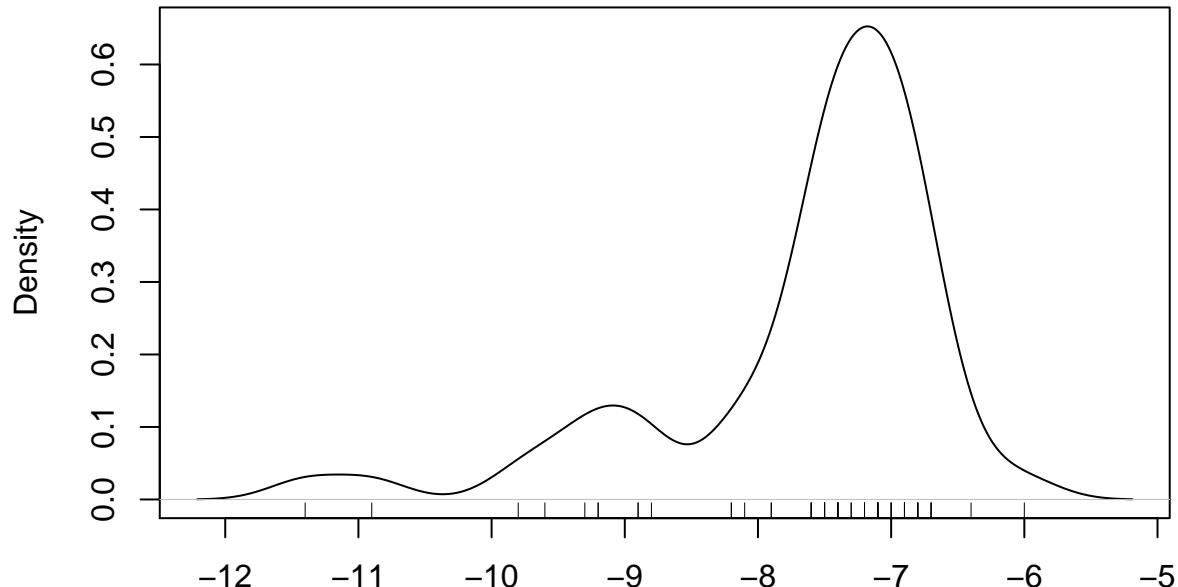
Procedures can be found at Docking Protocols

```
## Called from: eval(expr, envir, enclos) ## debug en <text>#4: plot(density(docking[,  
i], na.rm = T))  
density.default(x = docking[, i], na.rm = T)
```



```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en  
<text>#4: plot(density(docking[, i], na.rm = T))
```

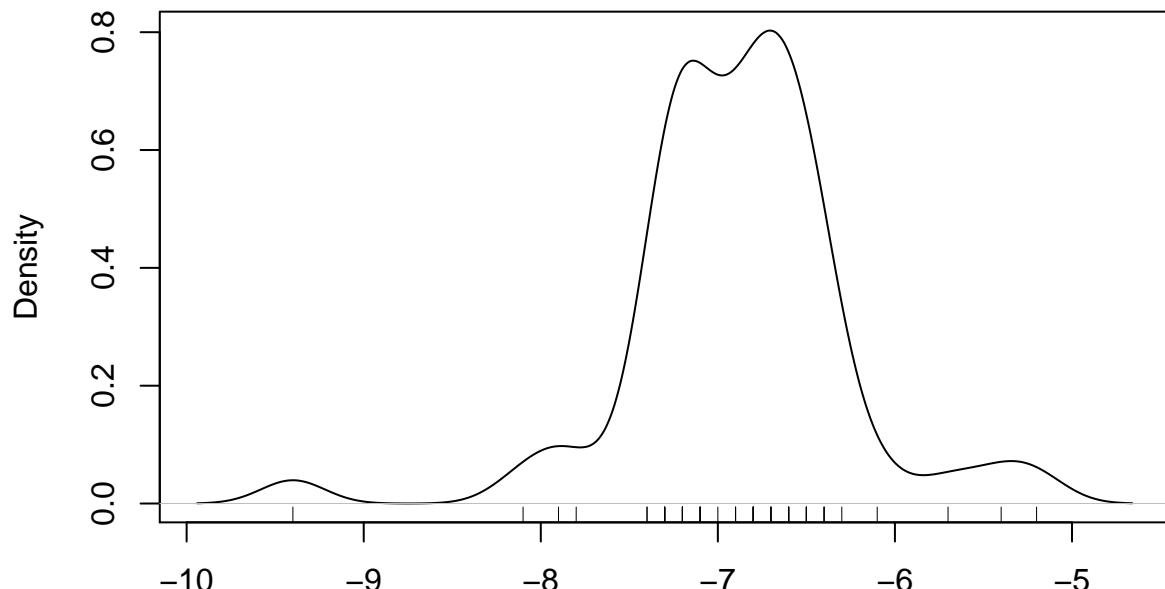
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.2702

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

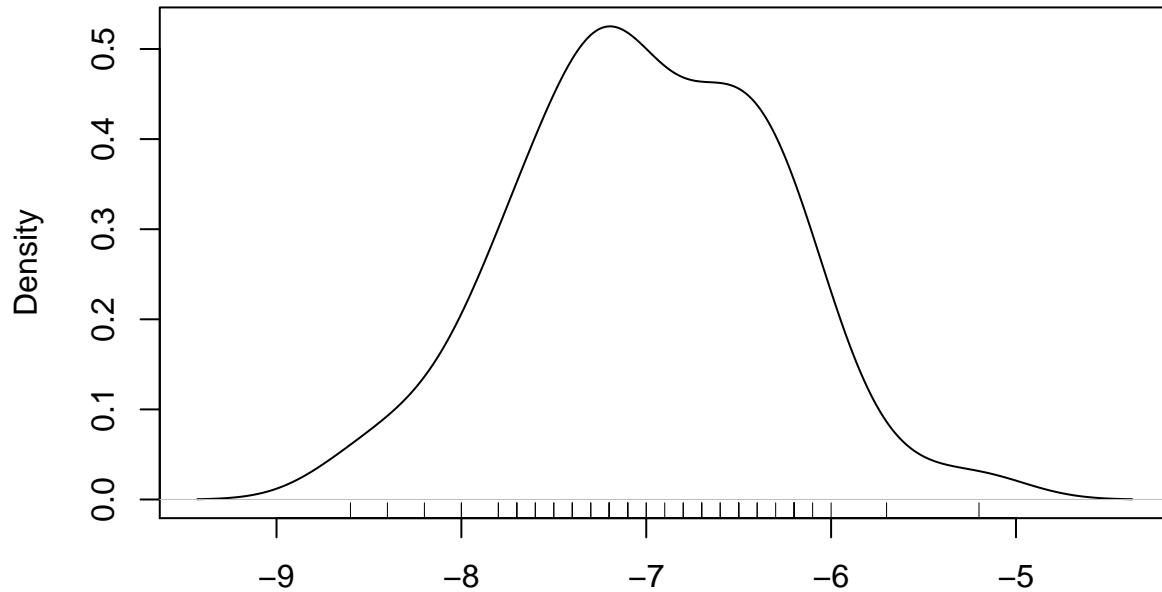
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.1802

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

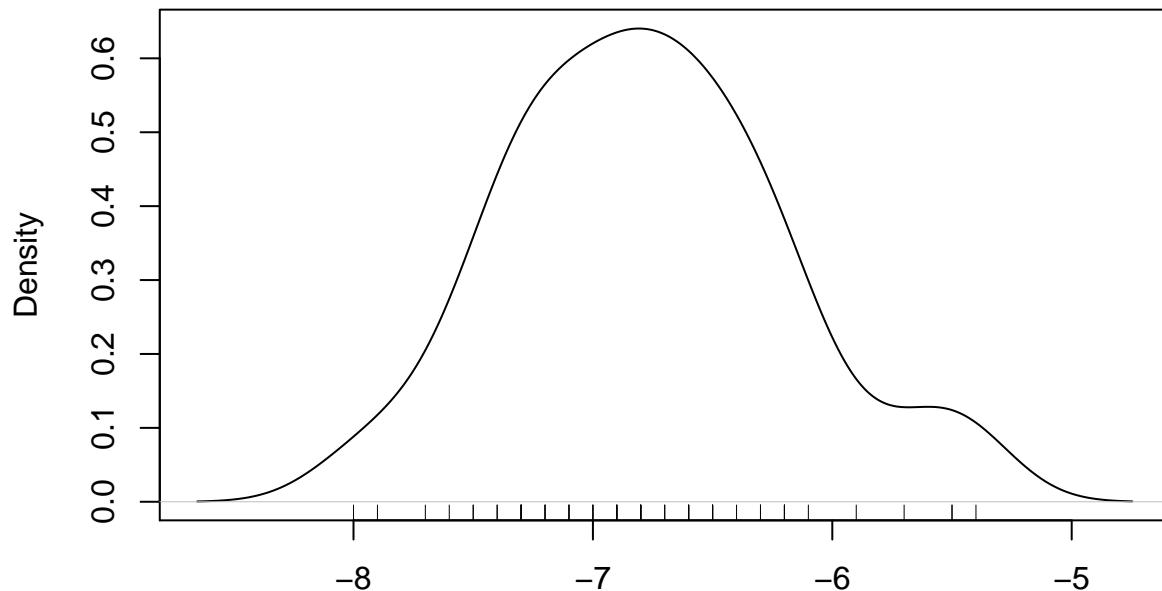
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.2764

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

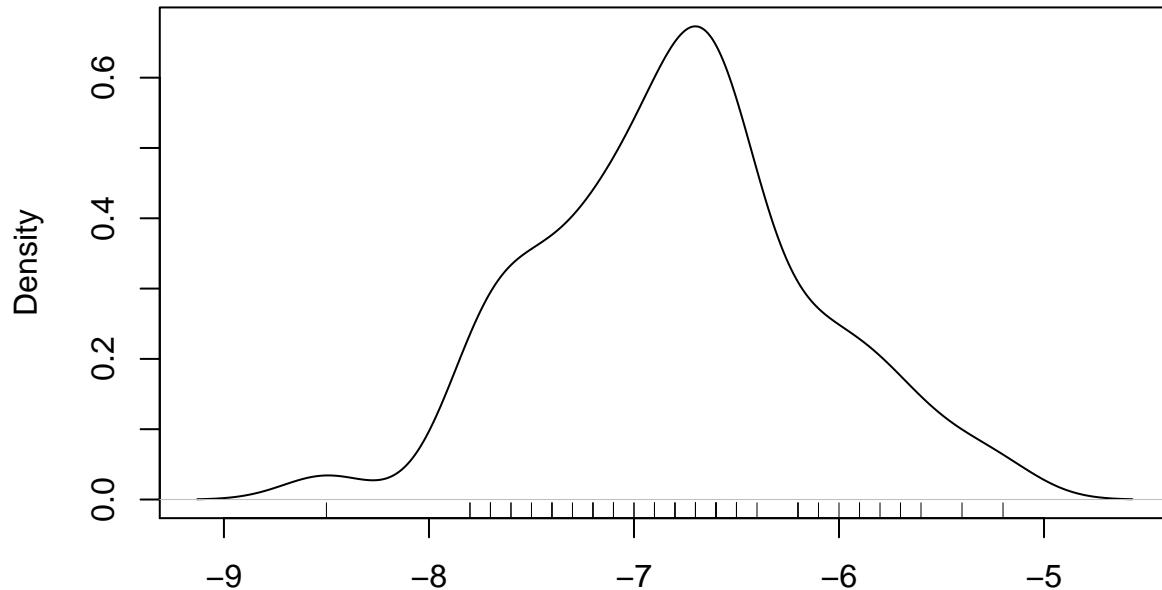
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.2177

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

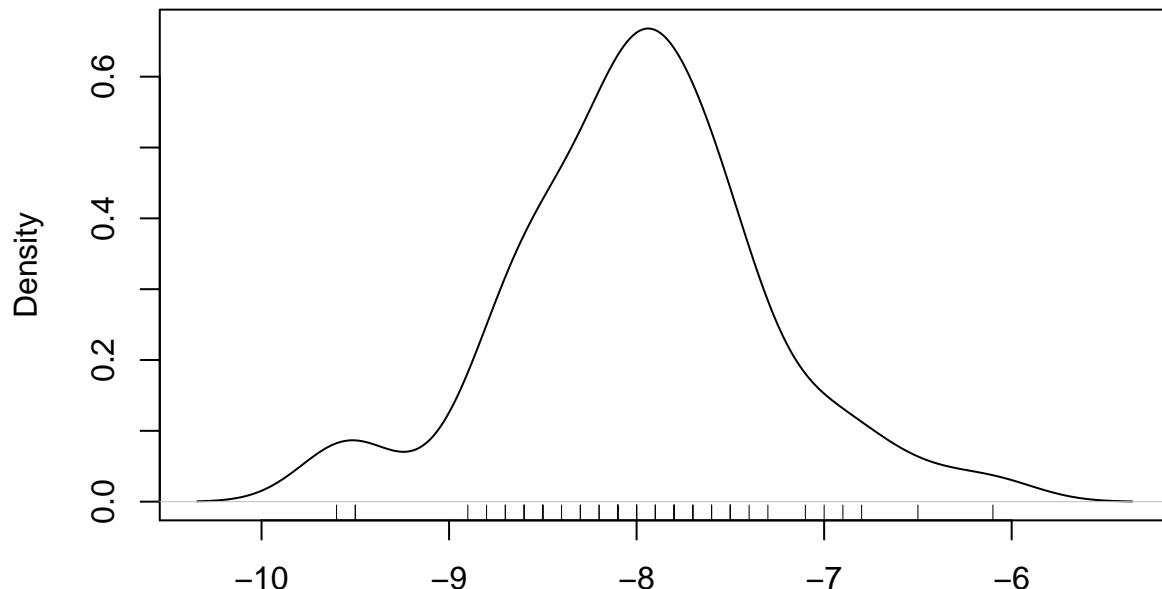
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.2102

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

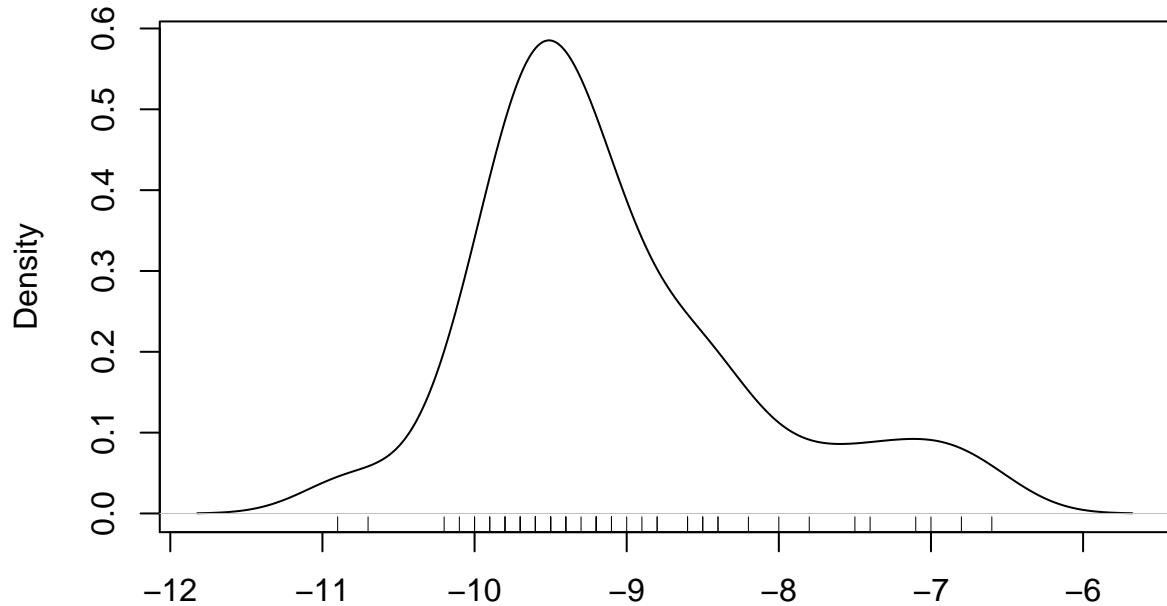
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.2477

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

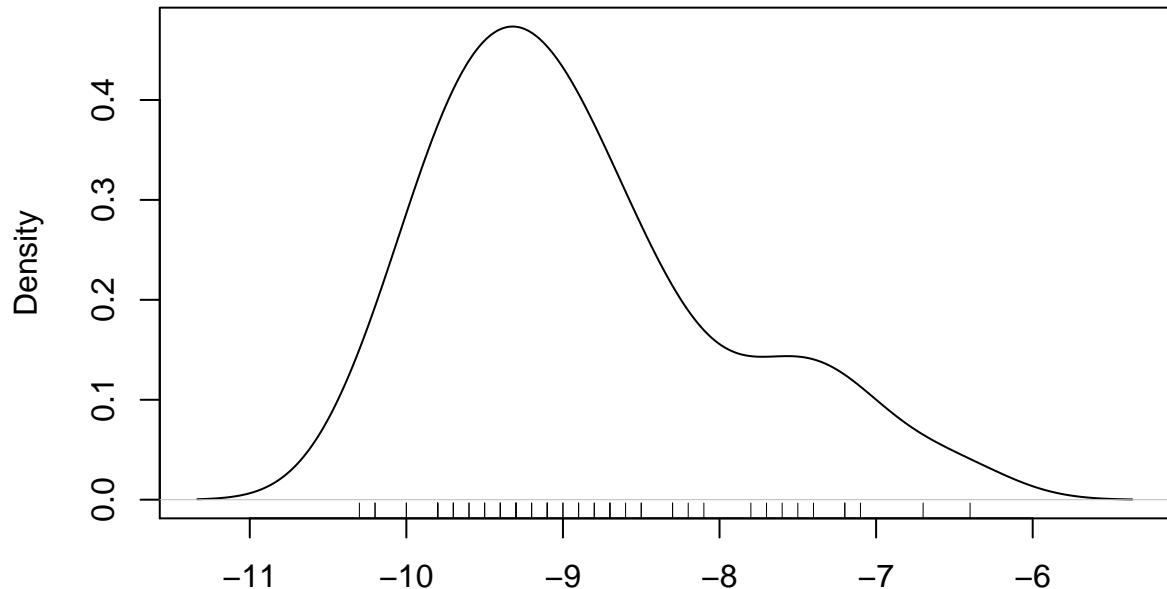
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.3078

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

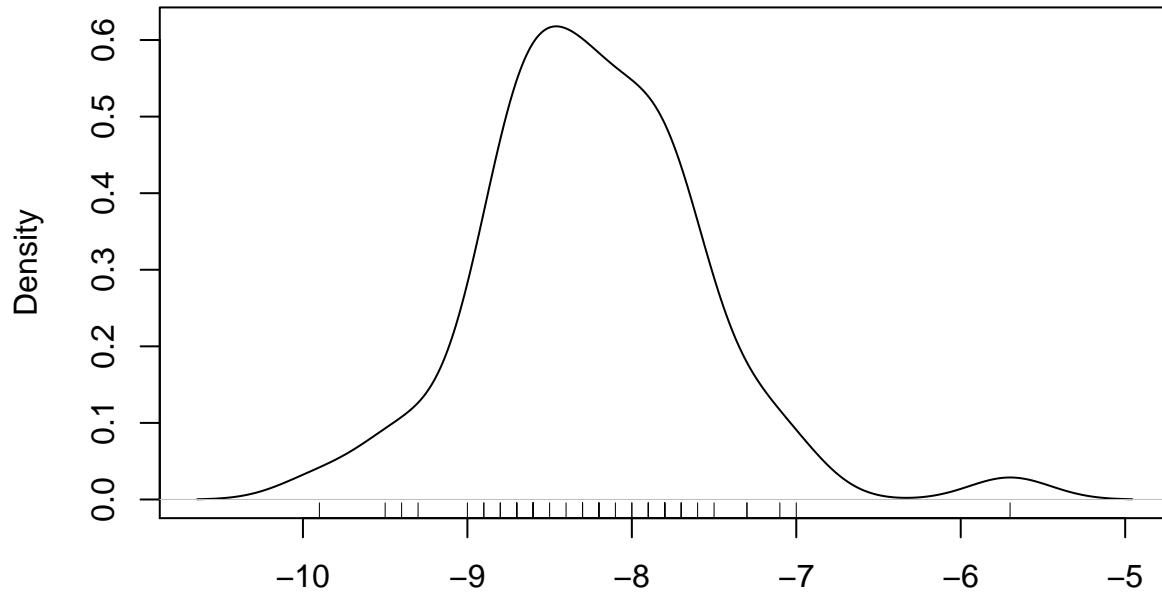
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.3453

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

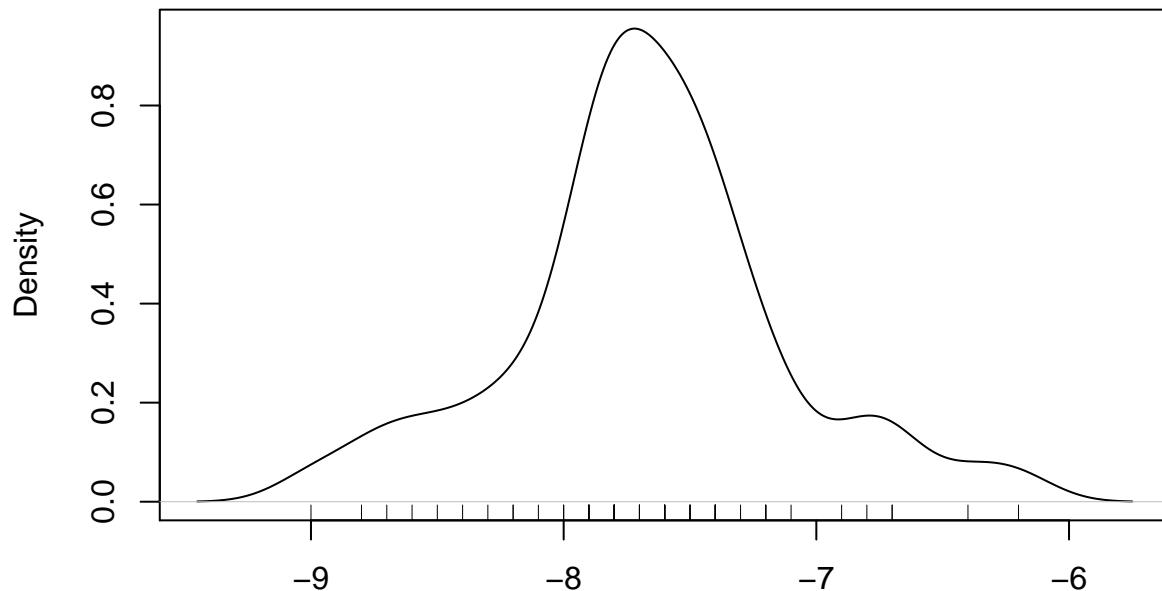
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.2477

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

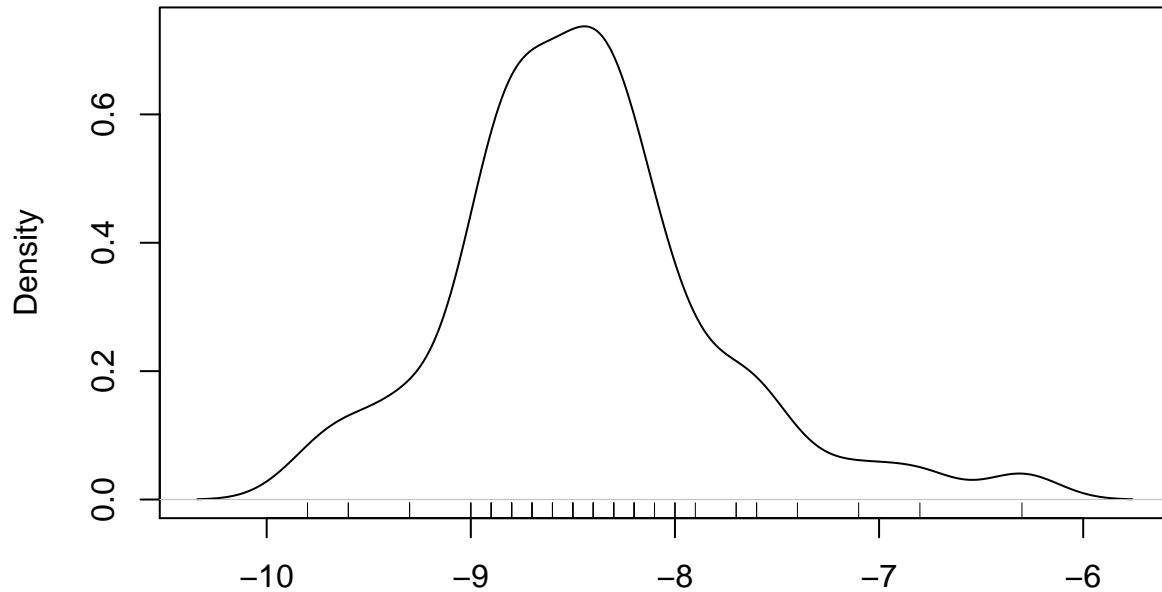
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.1501

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

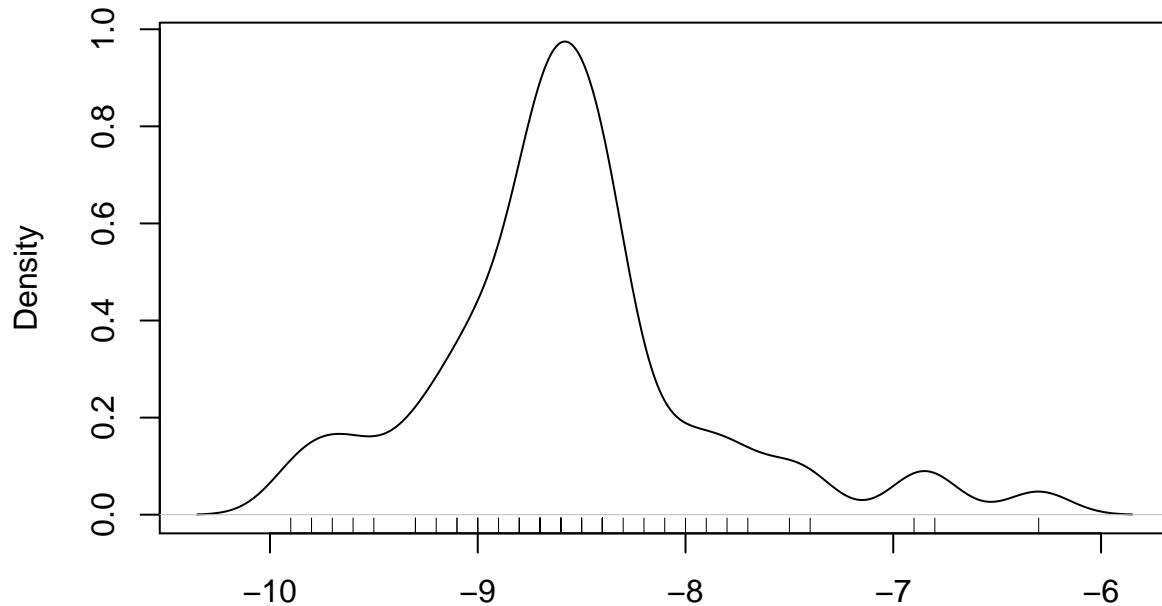
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.1802

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

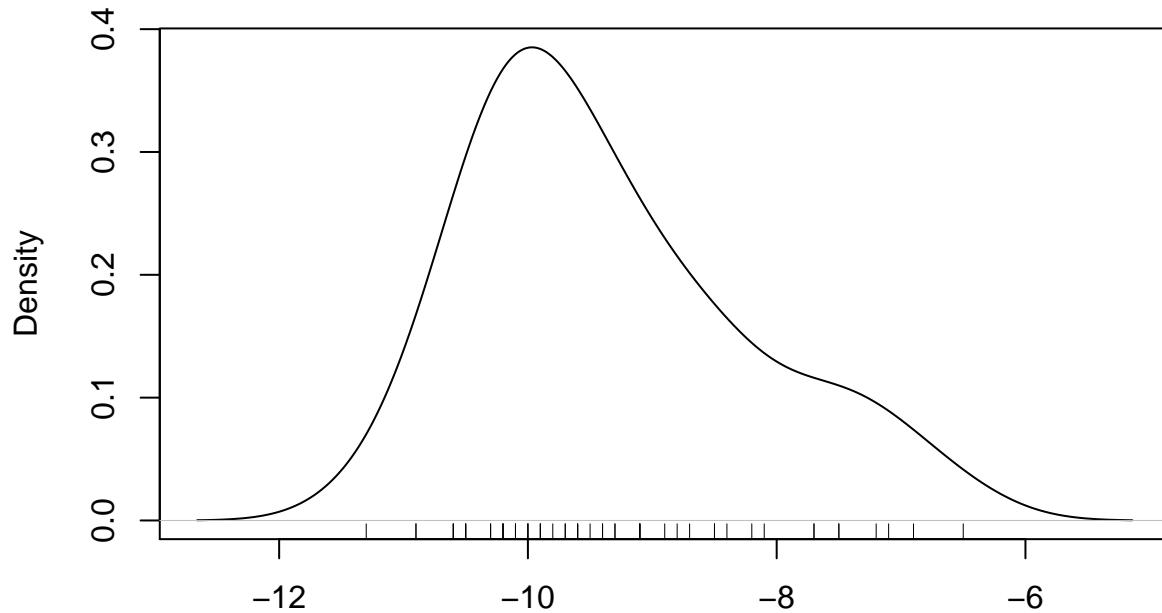
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.1501

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

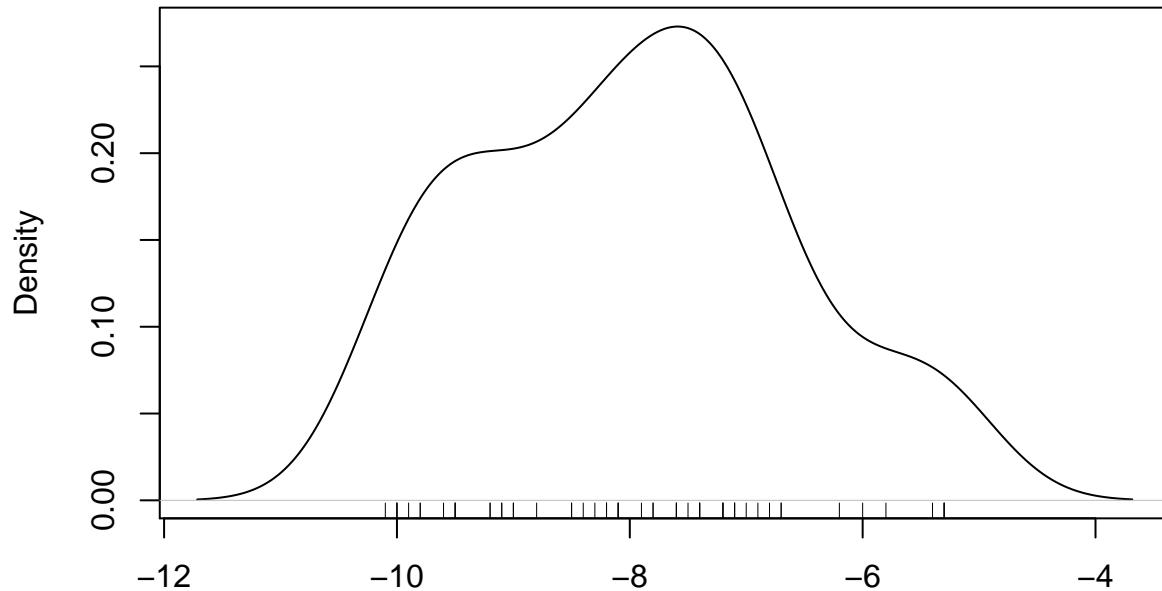
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.453

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

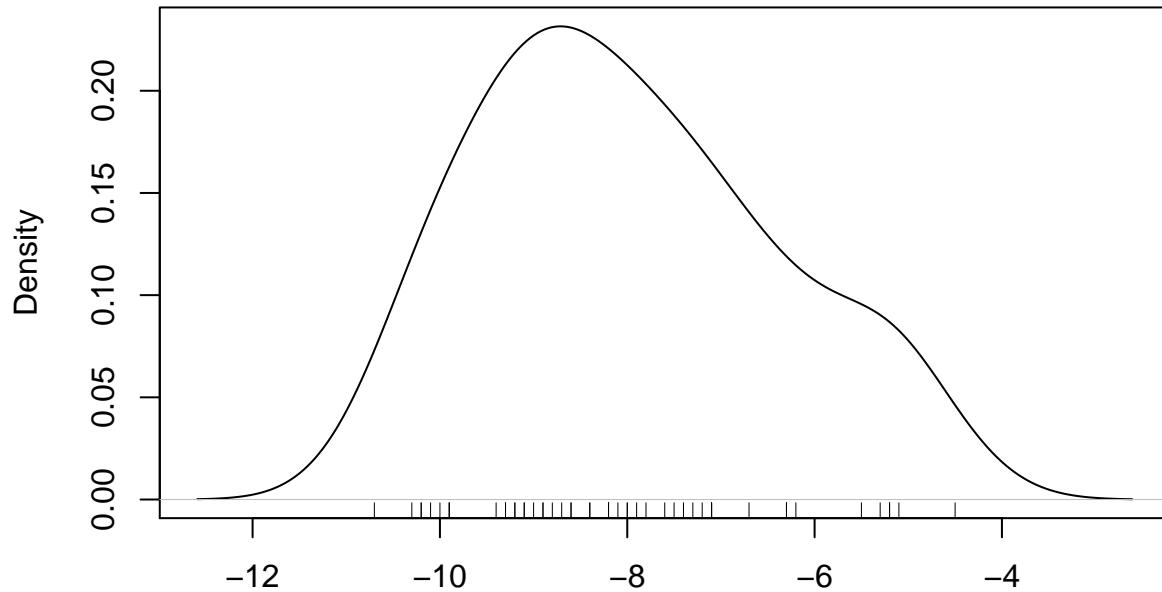
density.default(x = docking[, i], na.rm = T)



N = 56 Bandwidth = 0.5386

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

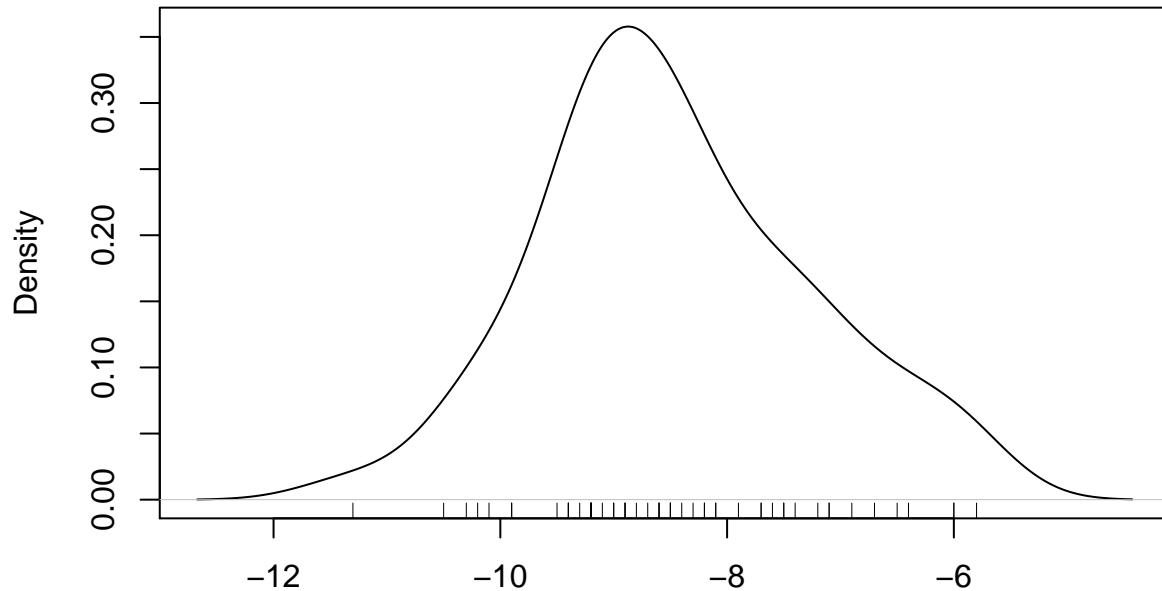
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.6305

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

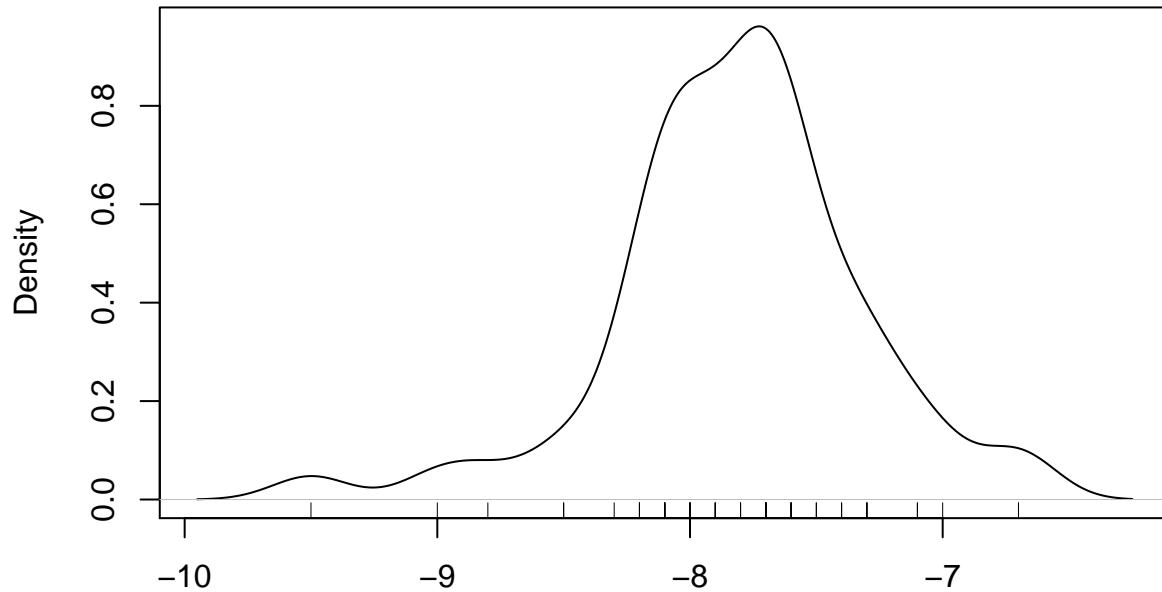
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.4579

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

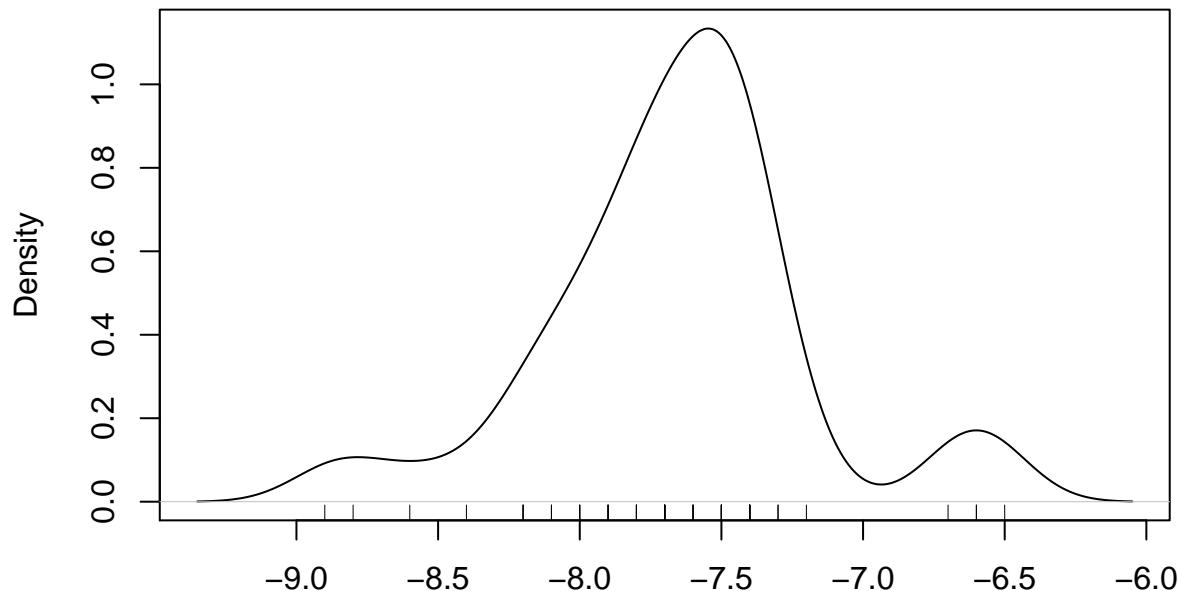
```
density.default(x = docking[, i], na.rm = T)
```



N = 56 Bandwidth = 0.1501

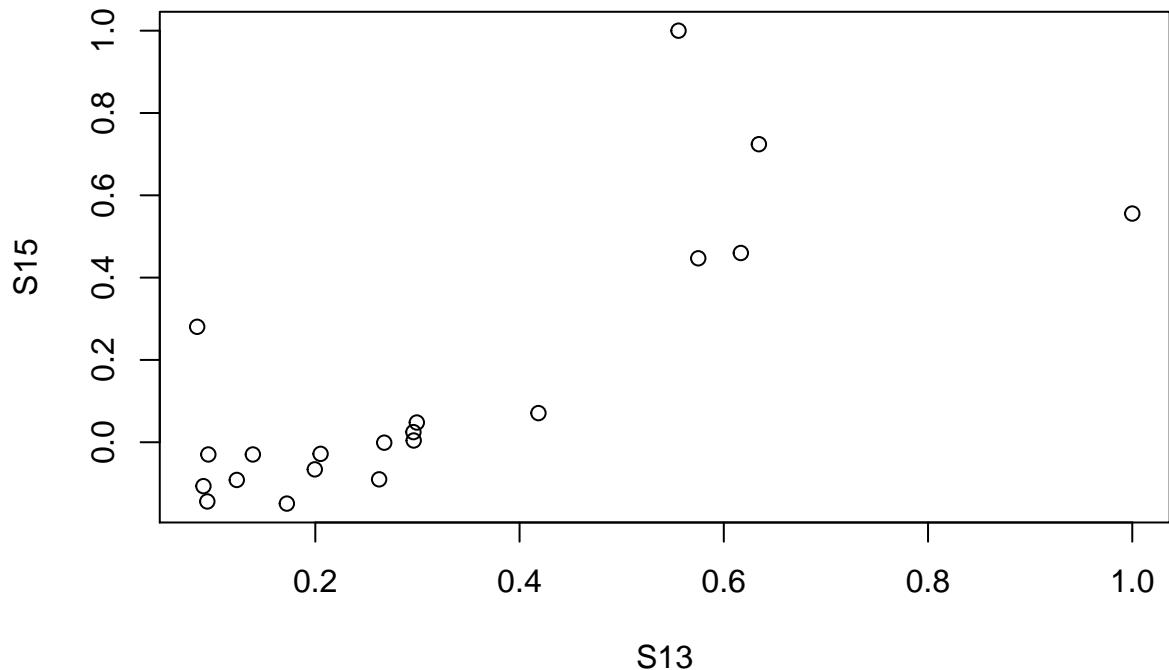
```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser() ## debug en
<text>#4: plot(density(docking[, i], na.rm = T))
```

```
density.default(x = docking[, i], na.rm = T)
```

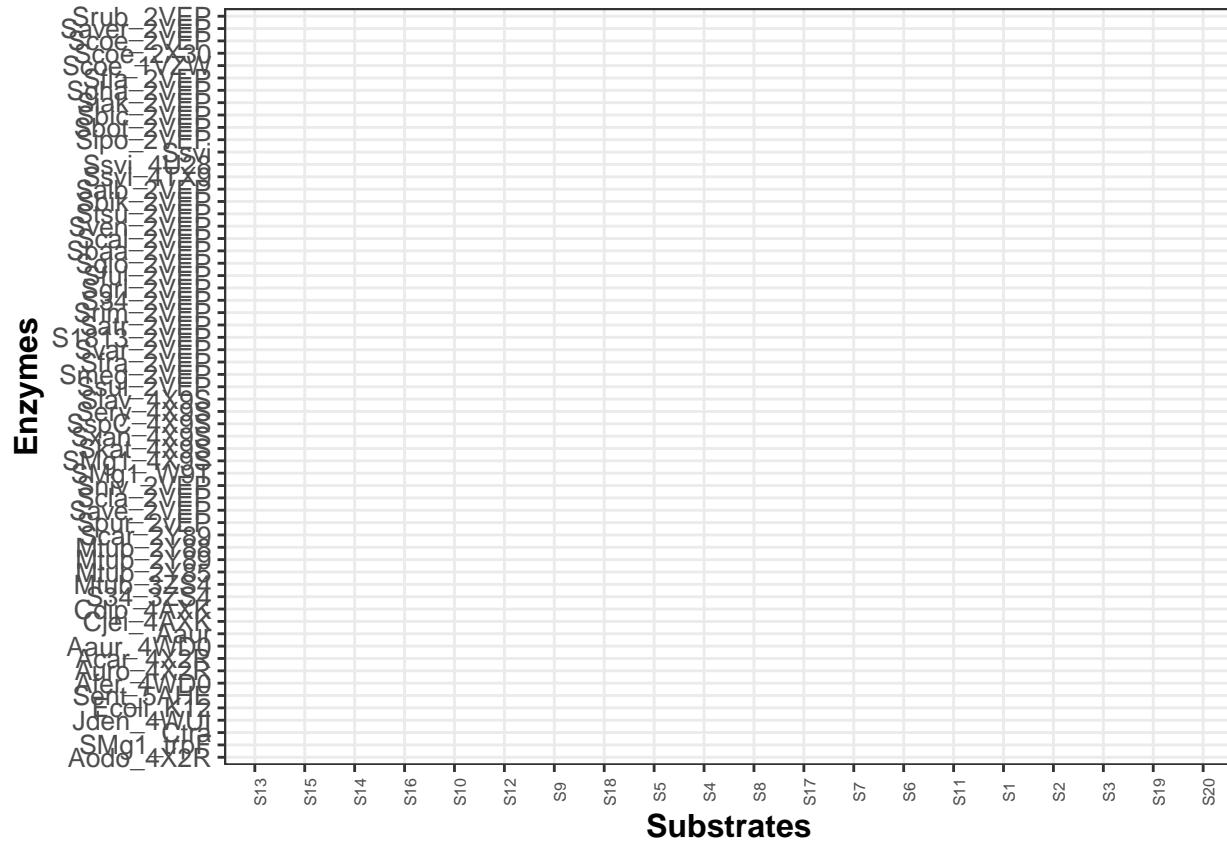


N = 56 Bandwidth = 0.1501

```
## debug en <text>#4: rug(docking[, i]) ## debug en <text>#4: browser()
r plot(cor(docking[-c(5,12,13,38,51),-1]))
```



```
r ## Leer sobre la incertidumbre del 2 y explicarla      ## Y leer el paper de Julian y
el de mauricio sobre reportes de docking -->
ggplot(docking.m, aes(x=variable, y=Enzima)) + labs(x = "Substrates", y = "Enzymes",text = element_text
```



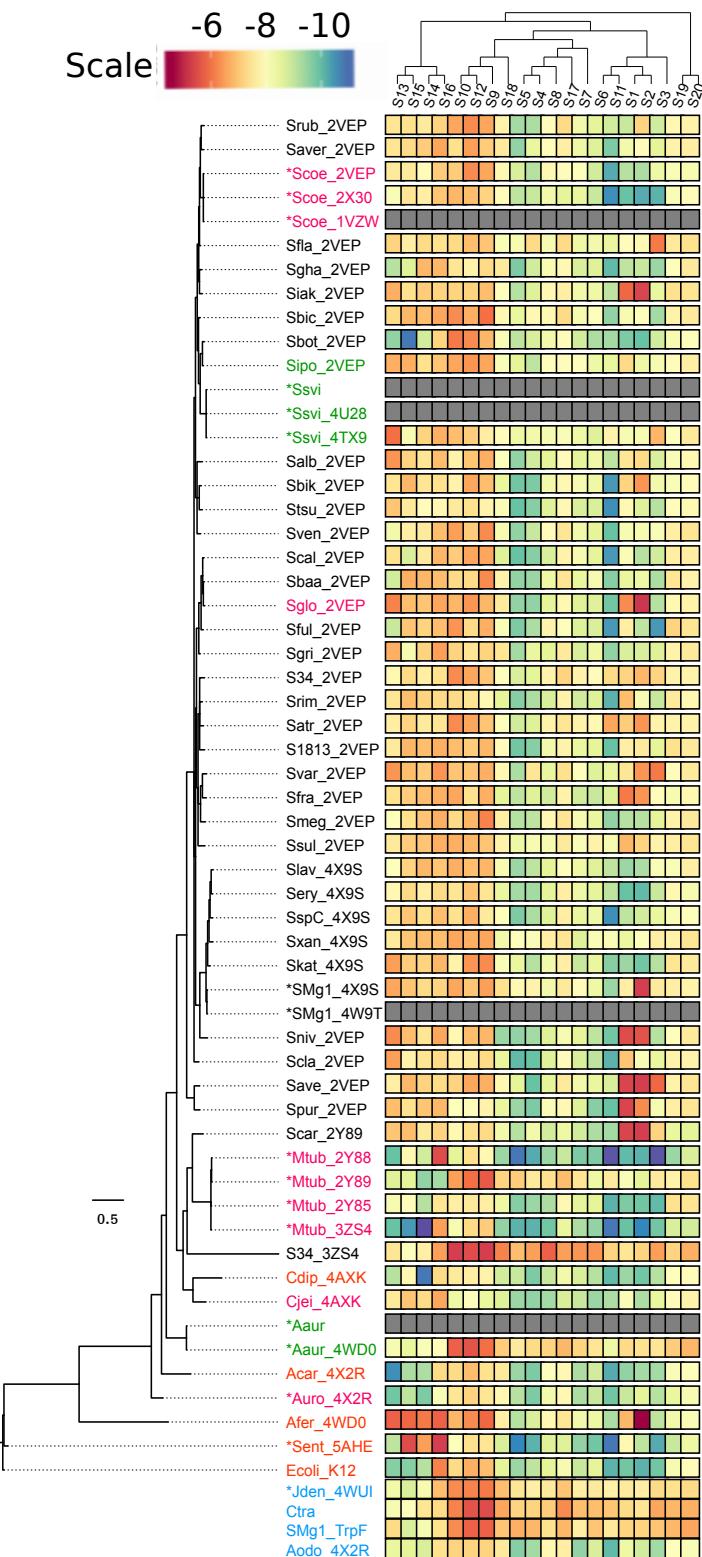


Figure 19:
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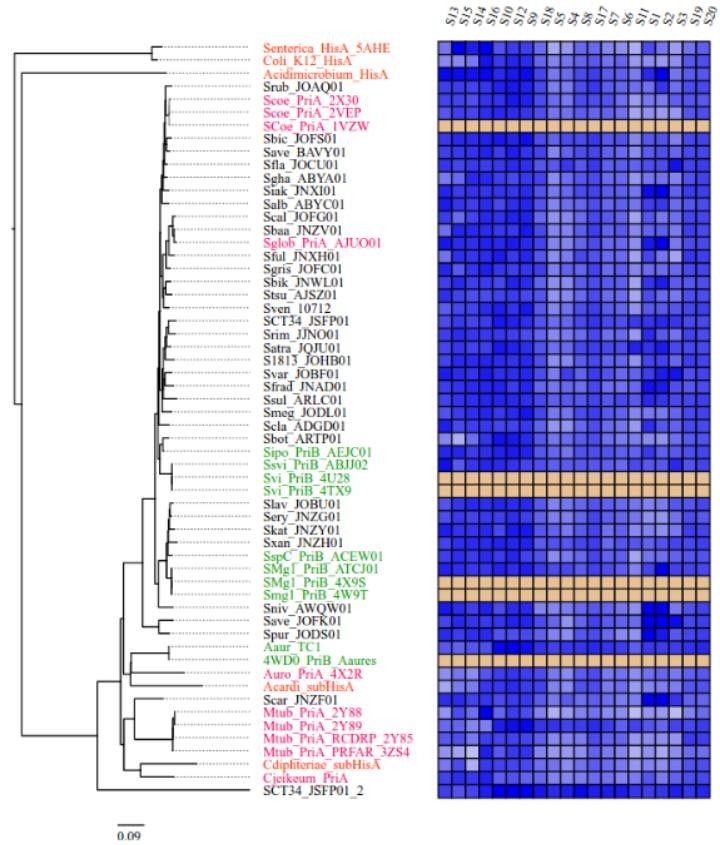
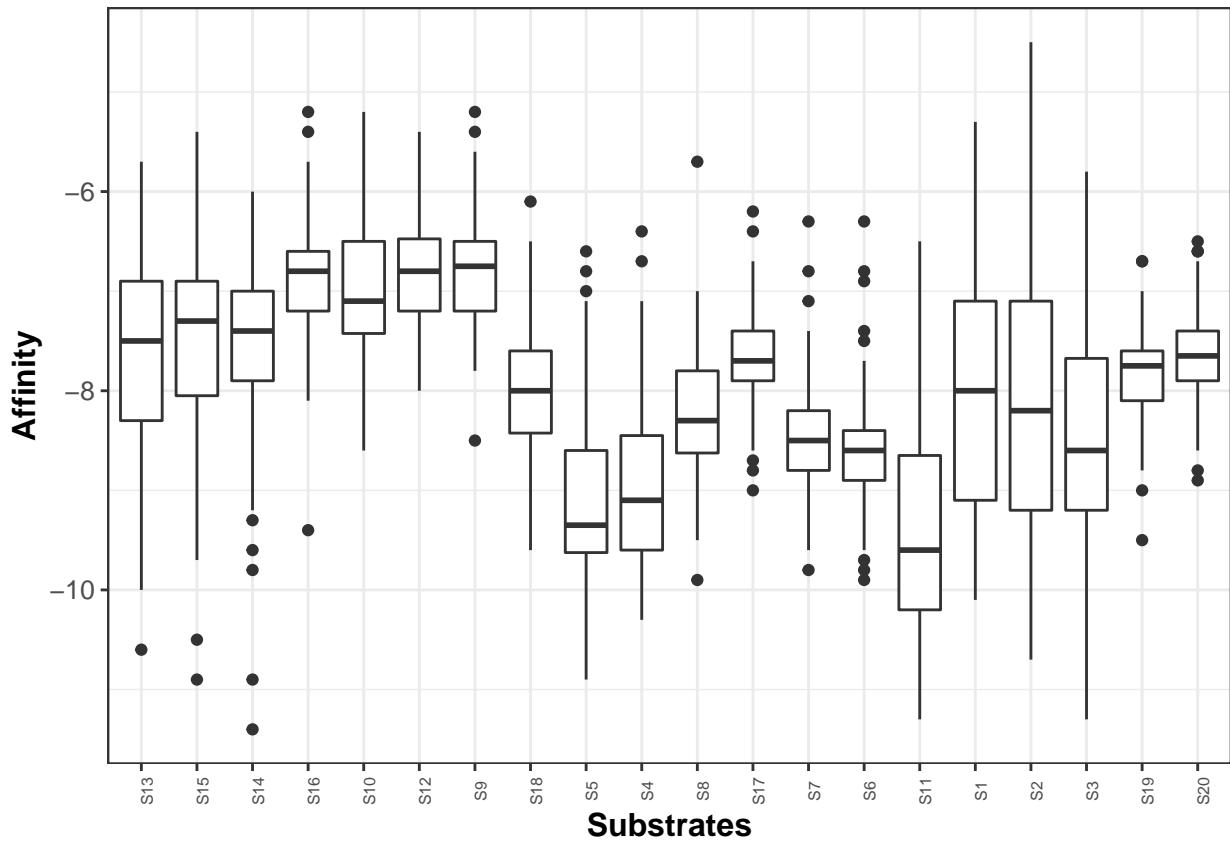


Figure 20:

GTP is the substrate with best PriA affinity

```
## boxplot de los sustratos
ggplot(docking.m, aes(x=variable, y=value)) + labs(x = "Substrates", y = "Affinity",text = element_text)

## Warning: Removed 100 rows containing non-finite values (stat_boxplot).
```



Molecular dynamics vs experimental data

PriA activity on S3 (PRA)

organism	Family	K_M	k_{cat}	$\frac{k_{cat}}{K_M}$	Pre MD	Pos MD	Reference
Afer	HisA	1.1 ± 0.2	0.05 ± 0.001	0.045	-10.1	-12.3	Noda-García L et al 2015
Ecoli	HisA	1.6	4.9	3.1	-9.9	-16	Henn-Sax et al. (2002)
Sent	HisA	17.0 ± 0.1	7.8 ± 2.4	4.5×10^5	-10.3	-20.1	Söderholm A et al (2015)
Aaur	PriB	2.1 ± 0.5	1.8 ± 0.2	0.9	-7.4		verduzco-castro 2016
Sipo	PriB	3.8 ± 0.2	0.82 ± 0.02	0.21	-8.2	-14.7	verduzco-castro 2016
SspC	PriB	11.4 ± 3.4	2.53 ± 0.74	0.22	-8.5	-12.7	verduzco-castro 2016
SMg1	PriB	13.2 ± 3.4	0.92 ± 0.19	0.069	-8	-15.2	verduzco-castro 2016
Ssvi	PriB	3.9 ± 0.89	0.69 ± 0.04	0.18	-8.2	-16.7	verduzco-castro 2016
ScOE	PriA	3.6 ± 0.7	1.3 ± 0.2	0.4	-8.4	-15	Noda-García et al (2010)
Sglob	PriA	4.2 ± 0.8	0.74 ± 0.03	0.18	-9.2	-16.7	verduzco-castro
Mtub 2Y85	priA	190.23	0.012 – 9.7				Due et al 2011
Mtub 3ZS4	priA	?	-9.9				Due et al 2011 (To be published)
Auro	priA	4.0 ± 0.9	0.2 ± 0.03	0.04	-9.2		Vazquez-Juarez (2016)
Cjei	PriA	2.3 ± 0.2	0.9 ± 0.08	0.39	-8.5		Noda-García et al (2013)
Cdip	subHisA	4.4 ± 0.5	2.6 ± 0.3	0.59	-9.2		Noda-García et al (2013)
SMg1 TrpF	TrpF3	-	-	-	-6.9	-9.6	verduzco-castro 2016
Jden	TrpF3	-	-	- -7.2	-9.4	16.8 ± 3.3	Verduzco-Castro E et al 2016
Acar	SubHisA	0.02					
Aodo	SubTrpF	-	-	-			

PriA activity on S7 (PROFAR) \$\$

organism	Family	K_M	k_{cat}	$\frac{k_{cat}}{K_M}$	Pre MD	Pos MD	Reference
Afer	HisA	-	-	-	-9.2	-9	Noda-García L et al. (2015)
Ecoli	HisA	-	-	-	-9	-11.1	Henn-Sax et al. (2002)
Sent	HisA	-	-	-	-9.6	-10.2	Söderholm A et al (2015)
Aaur	PriB	26.3 ± 6.3	0.37 ± 0.09	0.014	-7.1	-	verduzco-castro 2016
Sipo	PriB	60.8 ± 1.1	8.25 ± 0.4	0.14	-8	-8.5	verduzco-castro 2016
SspC	PriB	149.9 ± 29	1.4 ± 0.12	0.009	-8.5	-10.8	verduzco-castro 2016
SMg1	PriB	129.6 ± 34	0.29 ± 0.04	0.0022	-7.5	-11	verduzco-castro 2016
Ssvi	PriB	24.5 ± 4.0	1.6 ± 0.29	0.067	-8	-9.7	verduzco-castro 2016
Scoe	PriA	5.0 ± 0.08	3.4 ± 0.09	0.7	-8	-9.4	Noda-García et al (2010)
Sglob	PriA	11 ± 1.0	3.8 ± 0.2	0.34	-8.7	-9.4	verduzco-castro 2016
Mtub2Y85	priA	21	3.6	0.17	-8.6		Due et al 2011
Mtub3ZS4	priA				-9.3		Due et al 2011 (To be published)
Auro	priA	23 ± 6.5	0.5 ± 0.05	0.02	-9.3		Vazquez-Juarez (2016)
Cjei	PriA	5.1 ± 1.0	1.6 ± 0.16	0.31	-9		Noda-García et al (2013)
Cdip	subHisA	-	-	-	-8.8		Noda-García et al (2013)
SMg1 TrpF	TrpF3	8.4 ± 1.7	10.5 ± 2.4	1.25	-7.6	-9	verduzco-castro
Jden	TrpF3	16.8 ± 3.3	27 ± 1.6	1.6	-7.6	-7.7	verduzco-castro
Acar	SubHisA	Na	Na	0.02	Na	Na	Na
Aodo	SubTrpF	-	-	-	-	Na	Na

\$\$

Con actividad de FolE i.e activa para el compuesto V Adams et al (2014) Genome size vs Total antismash cluster coloured by order

```
#sessionInfo()
```