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Supplemental Information

Structural Basis for PI(4)P-Specific Membrane Recruitment of the *Legionella pneumophila* Effector DrrA/SidM

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Supplemental Figures

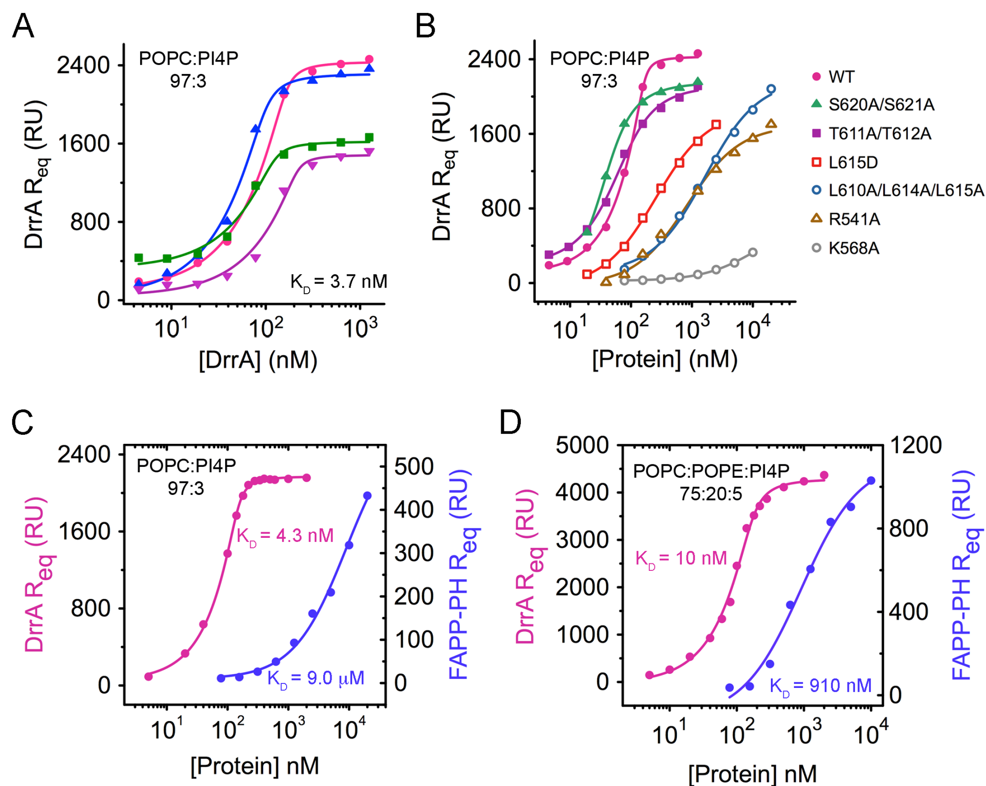

Figure S1 related to Figure 1 and 4. Analysis of equilibrium SPR responses for binding to PI(4)P-containing liposomes as a function of protein concentration. (A) Global fit of the quadratic binding model for four independent experiments with DrrA WT. Solid lines represent quadratic binding models with K_D treated as a global parameter and [PI4P], Rmax, and Rmin treated as local parameters (see also Figure 1B). (B) Fit of the quadratic binding model to WT and tight binding mutants (K_D< 50 nM, filled symbols) and of the Langmuir binding model to weak binding mutants (K_D > 200 nM, open symbols), K_D values for these mutants are included in Table S2. (C - D) Equilibrium responses and K_D values for DrrA and the FAPP1 PH domain (FAPP-PH; residues 1-98) fit with the quadratic and Langmuir binding models, respectively, under two different lipid compositions.

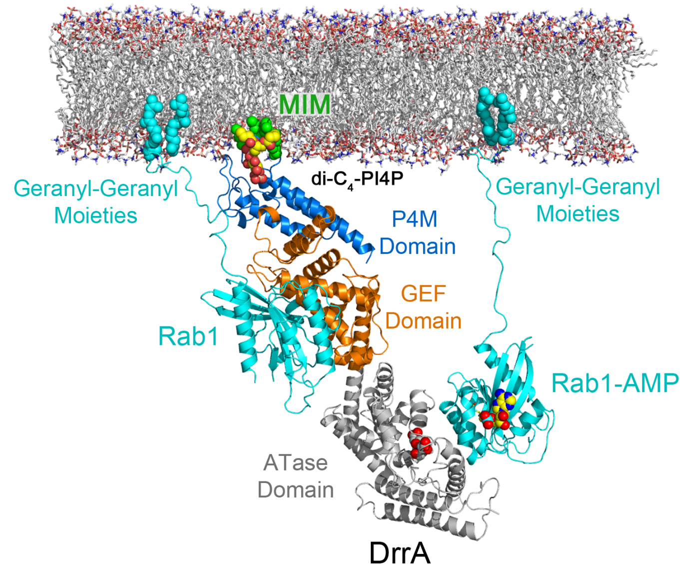

Figure S2 related to Figure 6. Structure-based membrane targeting model for full length DrrA. A composite model for full length DrrA with nucleotide free Rab1 was constructed and docked with a simulated POPC bilayer as described in the discussion. Rab1-AMP was modeled in a hypothetical orientation in which the AMPylated tyrosine is near the catalytic residues in the ATase domain. The hypervariable C-termini of Rab1 and Rab1-AMP were modeled in arbitrary conformations compatible with membrane insertion of the prenyl groups.

Table S1 related to Figure 1. Dissociation constant (K_D) for binding of $DrrA_{321-647}$ and eukaryotic PI(4)P-binding domains to PI(4)P-containing liposomes determined by SPR

Protein	K_D (μM)	Reference
DrrA	0.0038 ± 0.0027	This study
Bem1p-PX	1.2 ± 0.10	(Stahelin et al., 2007)
OSBP-PH	0.10 ± 0.020	(Stahelin et al., 2007)
GOLPH3	2.6 ± 0.20	(Wood et al., 2009)
Vps74	8.9 ± 0.30	(Wood et al., 2009)
FAPP1-PH	0.46 ± 0.080	(He et al., 2011)

Table S2 related to Figure 4. Dissociation constant (K_D) for binding of $DrrA_{321-647}$ wild type (WT) and mutants to POPC liposomes containing 3% PI(4)P determined by SPR (See also Figure S1B)

$DrrA_{321-647}$	K_D (μM)*	K_D (arb. units)	Fitted binding model
WT	0.0038 ± 0.0027	1.0	Quadratic
K568A	9.8 ± 2.5	2600	Langmuir
R541A	0.77 ± 0.086	210	Langmuir
Y532A	0.55 ± 0.16	150	Langmuir
Q608R	1.04 ± 0.022	280	Langmuir
S620A/S621A	0.0095 ± 0.0019	2.5	Quadratic
T611A/T612A	0.031 ± 0.0090	8.3	Quadratic
L610A	0.014 ± 0.0023	3.6	Quadratic
L617A	0.023 ± 0.0028	6.1	Quadratic
L614A/L615A	0.34 ± 0.058	91	Langmuir
L610A/L614A/L615A	1.7 ± 0.29	440	Langmuir
L610D	0.23 ± 0.047	62	Langmuir
L614D/L615D	6.2 ± 0.16	1600	Langmuir

* Values are mean ± standard deviation for 2-4 independent experiments.

Table S3 related to Figure 4. Thermodynamic analysis of $DrrA_{321\text{-}647}$ wild type (WT) and mutants binding to the soluble analogs di-C_4-PI(4)P and Ins(1,4)P_2 determined by ITC[§]

Protein	K_D (µM)	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)	K_D (arb. units)
		di-C_4-PI(4)P			
WT	0.056 ± 0.011	-31 ± 1.2	-10 ± 0.12	21 ± 1.2	1
S620A/S621A	5.8 ± 0.57	-17 ± 1.0	-7.1 ± 0.057	10 ± 0.93	104
T611A/T612A	0.93 ± 0.042	-25 ± 1.4	-8.1 ± 0.027	17 ± 1.4	17
L610A	0.064 ± 0.0057	-34 ± 0.71	-10 ± 0.052	24 ± 0.76	1
L614A/L615A	0.80 ± 0.11	-28 ± 1.5	-8.2 ± 0.076	20 ± 1.5	14
L610A/L614A/L615A	0.60 ± 0.092	-31 ± 2.1	-8.4 ± 0.091	22 ± 2.0	11
L610D	0.25 ± 0.014	-32 ± 3.5	-8.9 ± 0.033	23 ± 3.5	4
L614D/L615D	9.5 ± 1.4	-33 ± 4.2	-6.8 ± 0.088	26 ± 4.2	170
L610D/L614D/L615D	15 ± 3.6	-25 ± 0.00	-6.5 ± 0.14	19 ± 0.14	276
		Ins(1,4)P_2			
WT	2.1 ± 0.31	-28 ± 4.6	-7.7 ± 0.12	20 ± 4.7	1
T611A/T612A	140 ± 30	-28 ± 0.71	-5.2 ± 0.12	22 ± 0.83	69
L610A	2.1 ± 0.064	-28 ± 0.00	-7.7 ± 0.017	20 ± 0.017	1
L614A/L615A	18 ± 0.71	-28 ± 0.71	-6.4 ± 0.024	21 ± 0.73	9
L610A/L614A/L615A	13 ± 0.85	-29 ± 0.71	-6.6 ± 0 .038	22 ± 0.67	6
L610D	9.7 ± 2.1	-25 ± 3.5	-6.8 ± 0.13	18 ± 3.7	5

[§] Values are mean ± standard deviation 2-3 independent measurement. No heat was detected for binding of dibutyl-PI(4)P to K568A, Y532A, and R541A.

Supplemental Experimental Procedures

Materials

Phospholipids, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) were from Avanti, di-C_{16}-phophatidyl-inositol-polyphosphates from Cell Signals, dibutanoyl-phosphatidyl-inositol-4-phosphate (dibutyl PI(4)P) and D-*myo*-inositol 1,4-bisphosphate ($Ins(1,4)P_2$) from Echelon. For membrane insertion studies, natural $PI(4,5)P_2$ (porcine brain L-α-phosphatidyl-inositol-4,5-bisphosphate) and neutral phospholipids such as DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) and Rho-DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)) were from Avanti. Phosphatidylinositol-4-phosphate diC16 (PI(4)P diC16) was from Echelon Biosciences. The concentrations of unlabeled lipid stock solutions were routinely monitored by a phosphorus assay (Rouser et al., 1970). Subphase reagents 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), ethylenediamine-tetraacetic acid (EDTA), $CaCl_2$, and KCl were from Fisher Scientific.

Constructs, Expression and Purification

Constructs were amplified with Vent polymerase and ligated into a modified pET15b vector incorporating an N-terminal 6×His tag (MGHHHHHHGS). Site-specific mutants were generated with the QuickChange II XL kit (Stratagene). Wild type and mutated constructs were confirmed by sequencing. Constructs were expressed in BL21(DE3)RIPL cells (Stratagene) cultured in 2×YT-amp (16 g tryptone, 10 g yeast extract, 5 g NaCl, and 100 mg ampicillin per liter) at 37°C to an OD_{600} of 0.2, then at

21°C to an OD_{600} of 0.4, and induced with 50 mM IPTG for 16 h. Cells resuspended in lysis buffer (50 mM Tris, pH 8.0, 0.1M NaCl, 0.1% 2-mercaptoethanol) were disrupted by sonication in the presence of 0.1 mM PMSF, 0.2 mg/ml lysozyme, and 0.01 mg/ml protease free DNase I (Worthington). Lysates were supplemented with 0.5% Triton X-100 and centrifuged at 35,000×g for 1 h. Supernatants were added to Ni-NTA-Sepharose (GE Healthcare) equilibrated with lysis buffer and nutated for 15 min at 4°C. The beads were washed extensively with buffer containing 50 mM Tris, pH 8.0, 50 mM imidazole, 0.5 M NaCl, and 0.1% 2-mercaptoethanol. Proteins were eluted with 300 mM imidazole and further purified by ion exchange on HiTrap S or Q (GE Health Care) with gradients of 0-1M NaCl, followed by gel filtration on Superdex-75 (GE Health Care).

Supplemental References

Rouser, G., Fleische.S, and Yamamoto, A. (1970). Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids *5*, 494-6.