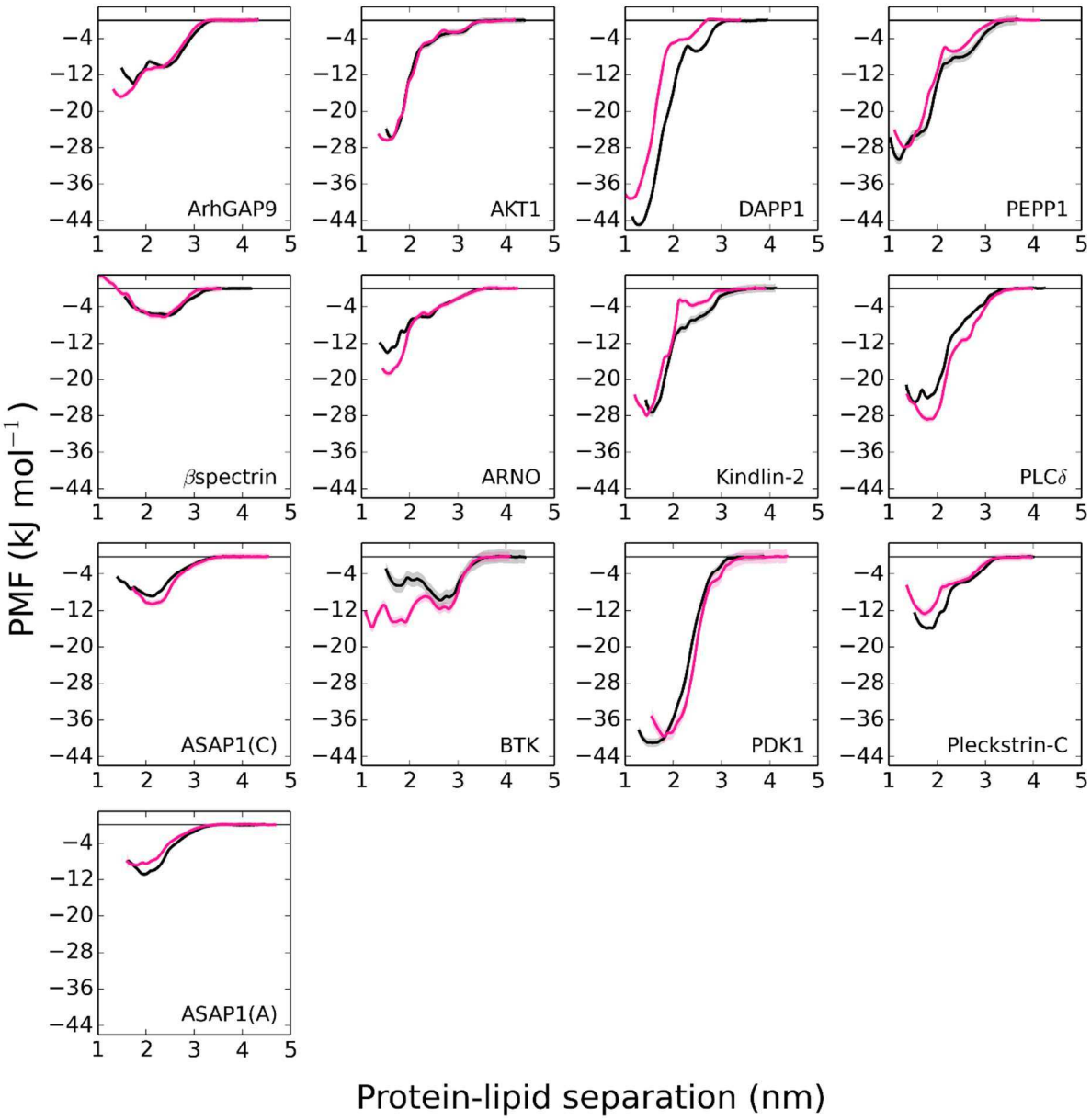
Article:
Naughton, FN, Kalli, AC orcid.org/0000-0001-7156-9403 and Sansom, MSP (2018) Modes of Interaction of Pleckstrin Homology Domains with Membranes: Toward a Computational Biochemistry of Membrane Recognition. Journal of Molecular Biology, 430 (3). pp. 372-388. ISSN 0022-2836

https://doi.org/10.1016/j.jmb.2017.12.011

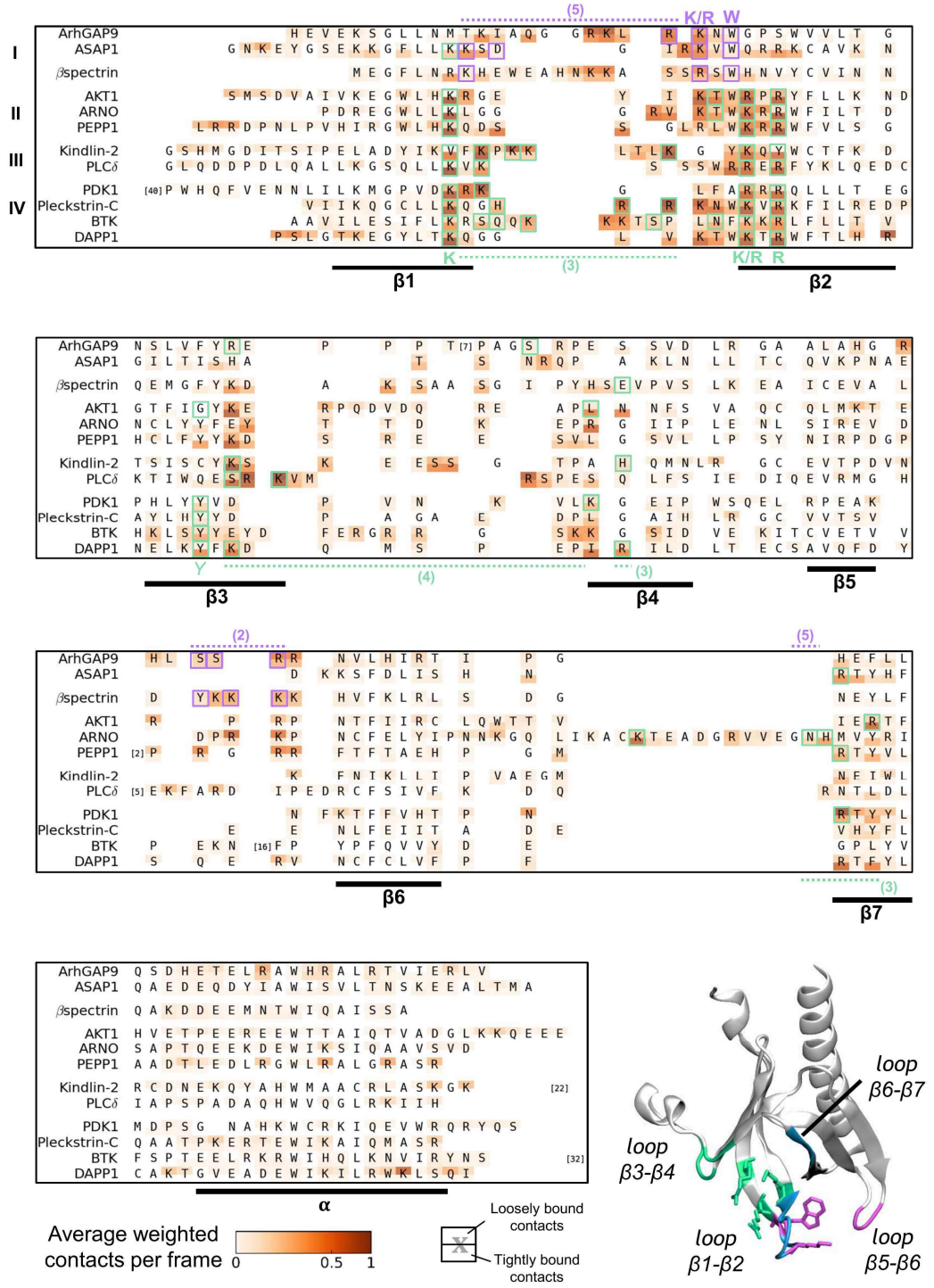
Supporting Information for:

Modes of interaction of pleckstrin homology domains with membranes: towards a computational biochemistry of membrane recognition.

Fiona B. Naughton, Antreas C. Kalli & Mark S.P. Sansom



Supplementary Figure S1 – Potential of Mean Force (PMF) profiles calculated for each of the studied PH domains binding to PIP_3, using the starting structures from directly following the PDB structure (magenta) or taken following simulation (black).



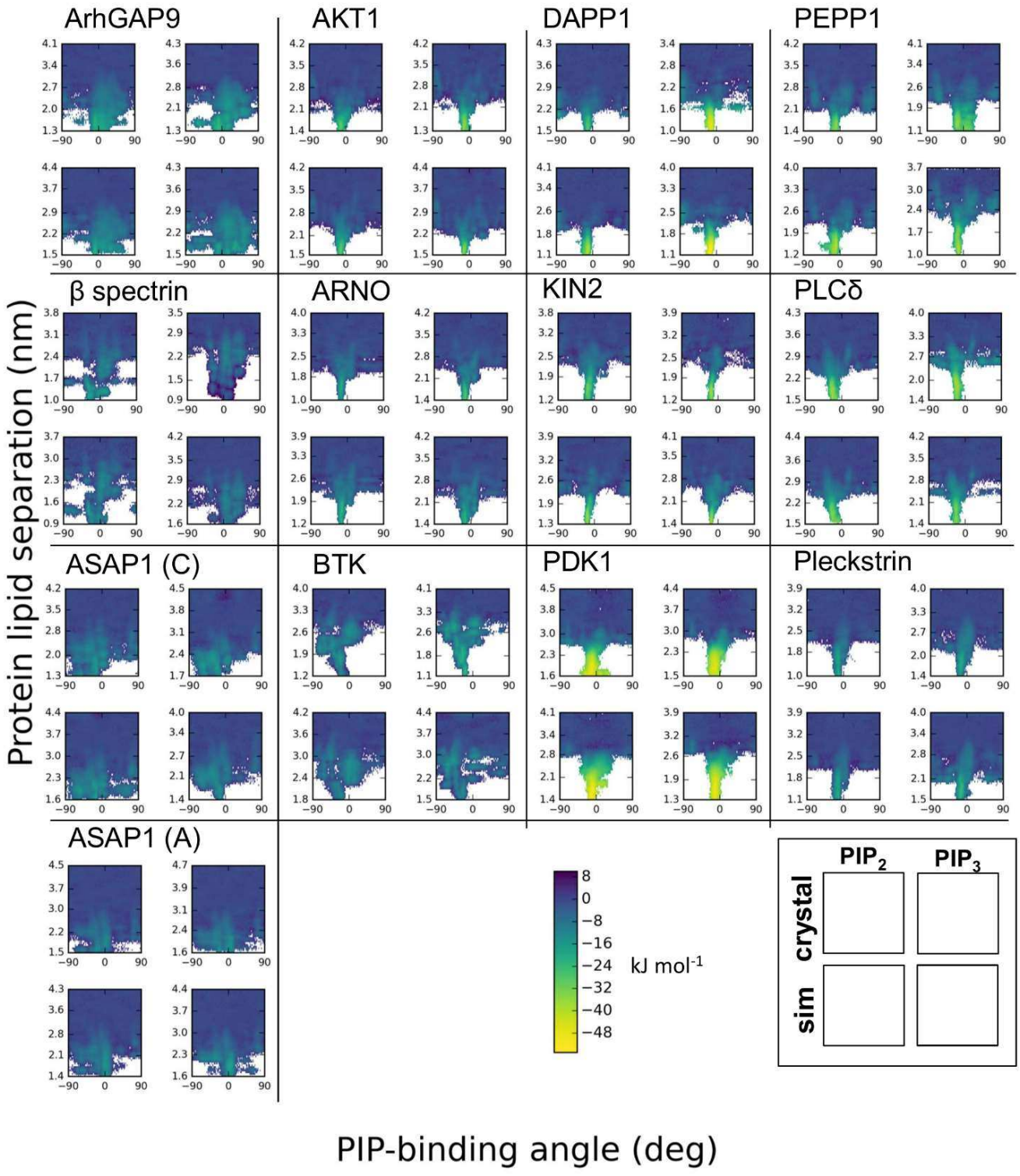
Supplementary Figure S2 – Structure-based sequence alignment of PH domains used in this study. The fraction of frames in which each residue is observed to make sidechain contacts (using a 0.6 nm cutoff with the PIP phosphate) with PIP, reweighted to account for umbrella sampling restraints, are indicated in orange for 'tightly' (down) and 'loosely' (up) bound regions. Specific residues and the loop regions characteristic of A-site or C-site binding, as determined from simulations, are indicated above or below the sequence in purple and green, respectively. Where increased positive/polar residues in a given region correspond to a particular binding orientation, the mode number is indicated (see main text Figure 7). Residues observed to contact PIP-analogues in the PDB structure are indicated by purple (A-site) or green (C-site) boxes, using the following structures from the PDB: 2POD (ArhGAP9), 1BTN (β-spectrin), 5C79 (ASAP1), 1UNQ (AKT1), 1U27 (ARNO), 2Z0P (BTK), 1FAO (DAPP1), 2LKO (Kindlin-2), 1W1D (PDK1), 1UPR (PEPP1), 1MAI (PLCδ1), 215C (Pleckstrin-C). Conserved secondary structure elements are indicated below the sequence in black. For clarity, sequence regions with few contacts and poor alignment are not shown; the number of residues omitted is indicated in square brackets.

Supplementary Table S3.

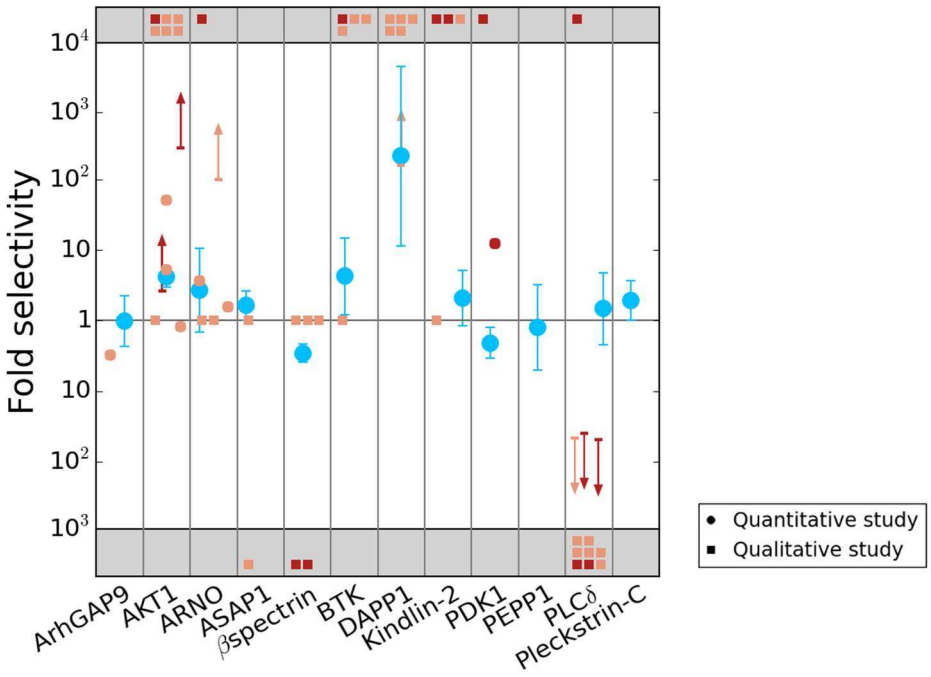
Dissociation constants (K_D) and selectivities from experimental studies for each of the 12 PH domains investigated in this study for $PI(4,5)P_2$ (or its analogues) and PIP_3 (or its analogues). The selectivity is given as K_D^{PIP2}/K_D^{PIP3}, i.e. the PH domain is PIP_3 selective if this ratio is > 1. Studies which were performed in a membrane environment are indicated by grey backgrounds. Values obtained in the same study with different conditions are listed in the same cell. Results of qualitative studies are indicated by fold selectivities '>1' (PIP_3 selective), '< 1' (PIP_2 selective) or '≈' (non-selective).

Protein	Method (reference)	PIP_2/analogue K_D (μM)	PIP_3/analogue K_D (μM)	Fold selectivity PIP_3/PIP_2
AG9	Fluorescence[1]	0.1	0.3	0.3
AKT	SPR[2]	> 0.1	0.035	> 3
	SPR[3]		0.59	
	FRET[4]	> 10	0.023	> 430
	SDS-Page[5]			> 1
	Fluorescence[6]	1.2, 2.5	1.5, 0.5	0.8, 5
	Fluorescence[1]	9.1	0.13	70
	Radiolabel[7]			> 1
	Radiolabel[8]			> 1
	Overlay[9]			> 1
	Overlay[10]			> 1
	Overlay[11]			≈
	Microarray[12]			> 1
ARNO	SPR[3]		1.7	
	SDS-Page[13]			> 1
	ITC[14]	4.15, 2.66	1.02, 1.64	4, 1.6
	Radiolabel[15]	> 10	0.0693	> 1
	Radiolabel[16]		1.6	≈
	Overlay[11]			≈
ASAP1	Co-sedimentation[17]	7		
	ITC[17]	> 100		
	ITC[18]	75		
	Overlay[11]			≈
	Overlay[19]			< 1
BTK	SPR[20]			> 1
	SPR[3]		0.08	
	ITC[21]		0.04	
	Radiolabel		0.015	> 1
	Radiolabel[22]		0.8	> 1
	Radiolabel[23]		0.04	> 1
	Overlay[11]			≈
β-spectrin	SPR[24]	0.125		< 1
	SDS-Page[25]			< 1
	CD shift[26]	40		≈
	Microarray[27]	0.006		
	Overlay[9]			≈

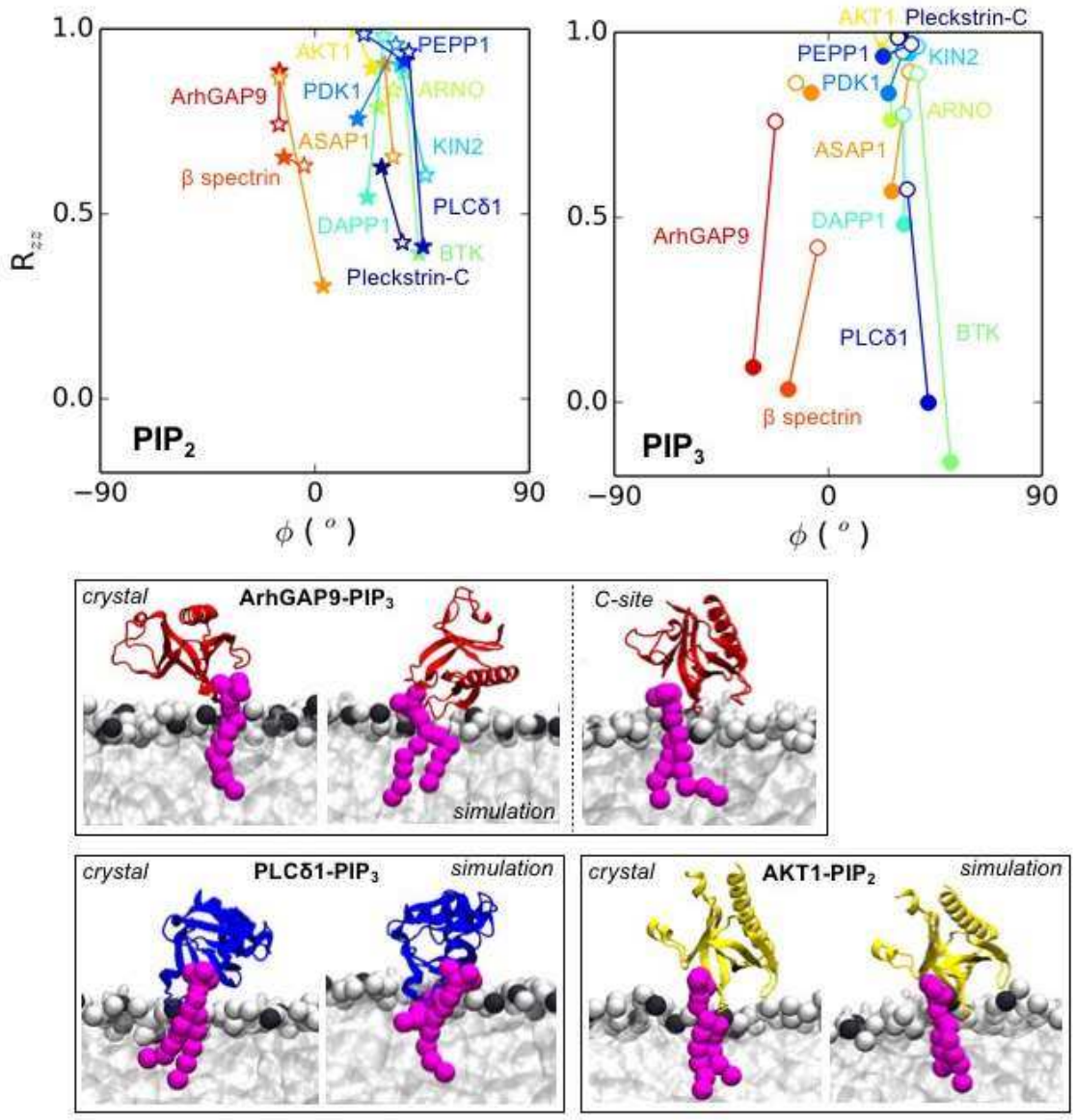
	Overlay[25]			≈
DAPP1	SPR[10]		0.003	
	SPR[3]		0.67	
	ITC[9]	> 10	0.043	> 130
	ITC[28]	0.049		
	Radiolabel[8]			> 1
	Overlay[10]			> 1
	Overlay[29]			> 1
	Overlay[30]			> 1
	Overlay[11]			> 1
Kindlin-2	SPR[31]		2.12	> 1
	SPR[32]		159	
	Cosedimentation[33]			> 1
	Overlay[34]			> 1
	Overlay[11]			≈
PDK1	SPR[2]	0.024	0.0016	15
	SPR[10]		0.06	
	SPR[35]		0.052, 0.03	> 1
	FRET[36]		0.012	> 1
	Radiolabel[8]			> 1
	Overlay[10]			> 1
	Overlay[36]			> 1
PEPP1	-			
PLCδ1	SPR[37]	2.1		
	SPR[38]	6.6		
	SPR[39]	0.8		
	FRET[4]	0.19	> 10	< 0.019
	FRET[40]	0.15	> 10	< 0.015
	ITC[41]	0.2		
	SDS-Page[42]	1.36		
	Co-sedimentation[17]	1.7		
	Co-sedimentation[43]			> 1
	Overlay[38]			< 1
	SPR[38]	0.104		
	ITC[44]	0.21, 1.66	> 13	< 0.016
	ITC[17]	0.18		
	Radiolabel[45]	0.3		< 1
	Overlay[46]			< 1
	Overlay[9]			< 1
	Overlay[29]			< 1
	Overlay[11]			< 1
Pleckstrin-C	-			



Supplementary Figure S4 – Energy landscapes generated from umbrella sampling simulations showing favourable PIP-binding angles as protein-lipid separation is varied from each of the umbrella sampling simulations performed.

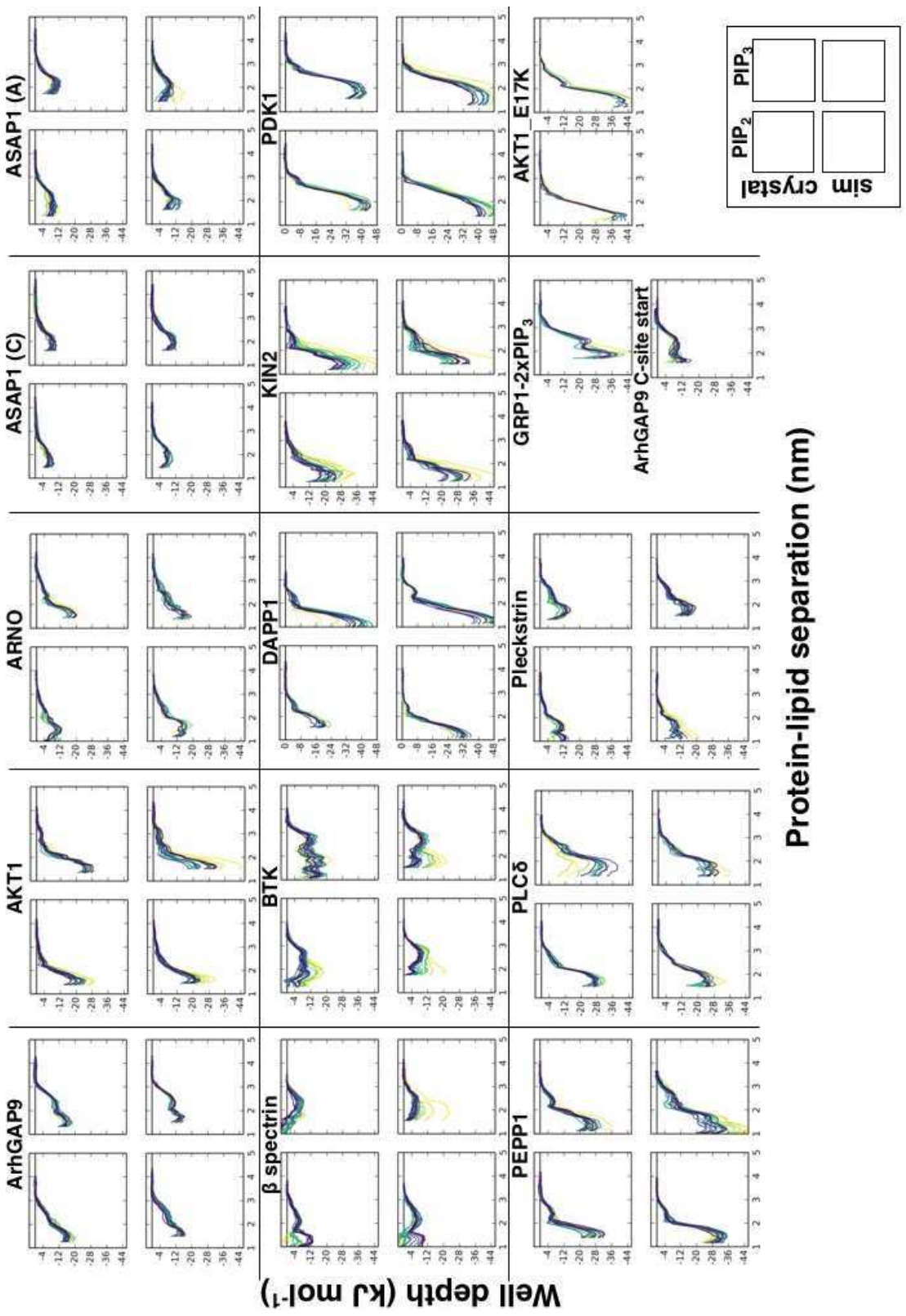


Supplementary Figure S5 – Comparison of PIP_2/PIP_3 selectivities as obtained in this simulation study (blue) and in reported experimental values (red/pink; see also Supplementary Table S3). Results from experimental studies utilising a membrane environment are shown in red; other studies are shown in pink. The quantitative selectivity is shown as the value of the ratio K_D^{PIP2}/K_D^{PIP3}, i.e. the PH domain is PIP_3 selective if this ratio is > 1. Quantitative results are represented by circles; arrows indicate upper/lower bounds determined where binding was not observed within the detectable range of the experiment. Qualitative selectivity results are represented by squares placed above (PIP_3 selective), below (PIP_2 selective) or along the centre line (non-selective) of the selectivity plot. Errors are standard deviations.



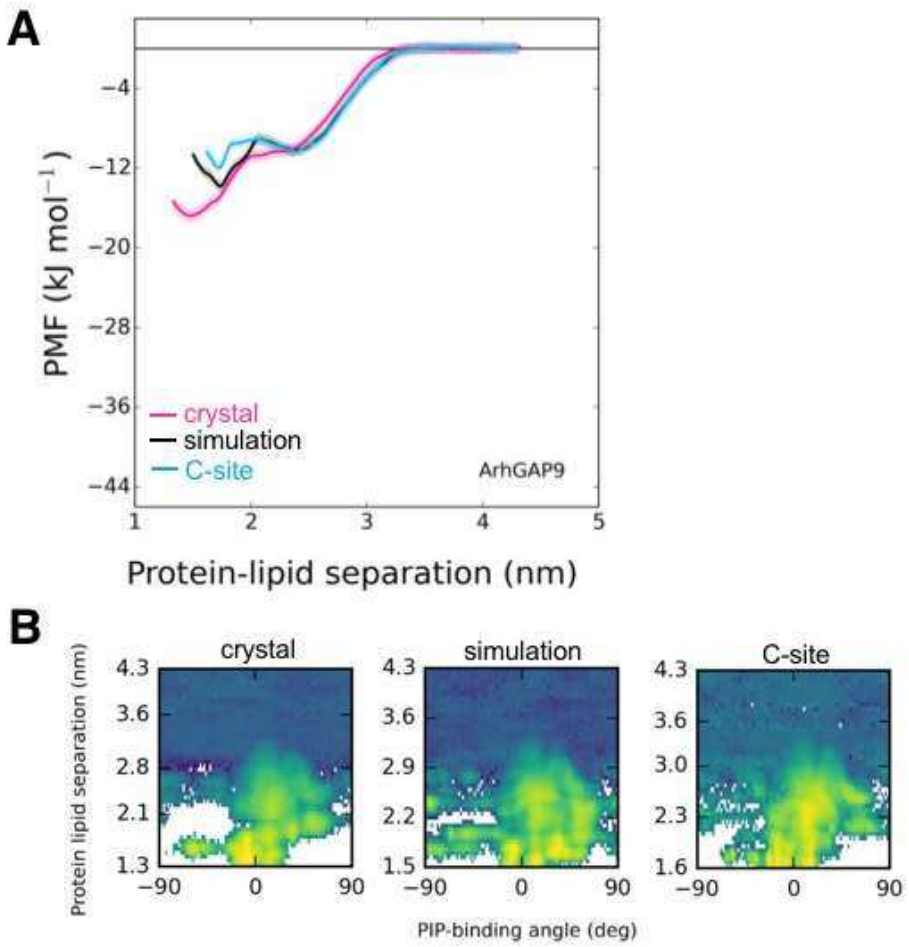
Supplementary Figure S6

The PIP-binding angle and R_{zz} component of the rotational matrix is shown for all PH systems. The 'crystal' and 'simulation' structures for each PH-PIP system (empty and filled in respectively) are linked by a line to highlight the difference in initial configurations in each system. Selected example snapshots are shown below, including the additional ArhGAP9 initial structure starting with a PIP molecule in the C-site, which was generated by aligning the ArhGAP9 PH domain with the AKT1 crystal initial structure.



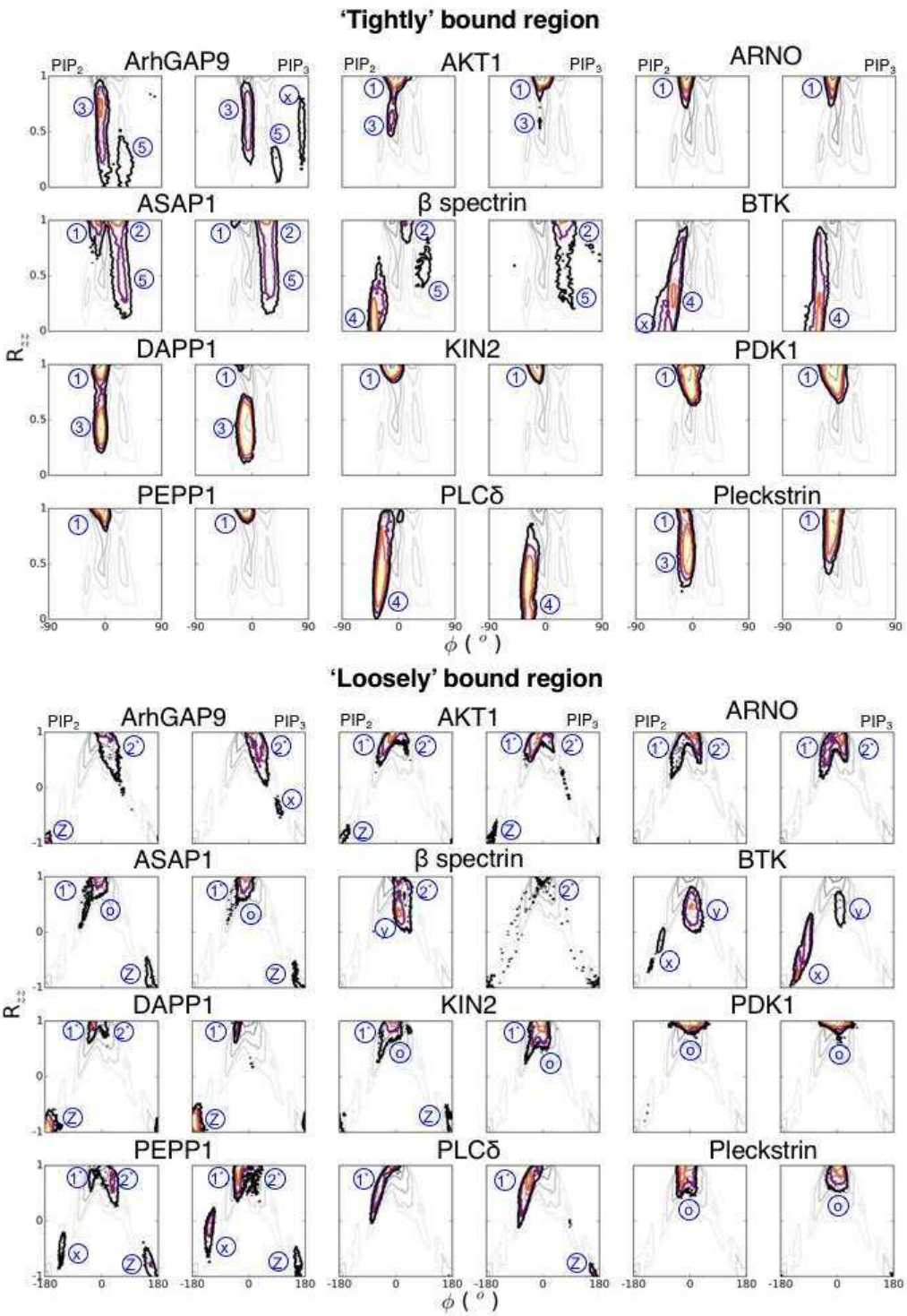
Supplementary Figure S7

Convergence analysis. PMF profiles at increasing time intervals are shown for all simulations, showing convergence. Profiles were calculated for 400 ns slices with start times at 200 ns intervals and coloured from yellow (start of simulation) to blue. In the simulation systems that the total simulation time was not divisible by 200, the final profile is shown for 300 ns intervals rather than 400 ns.



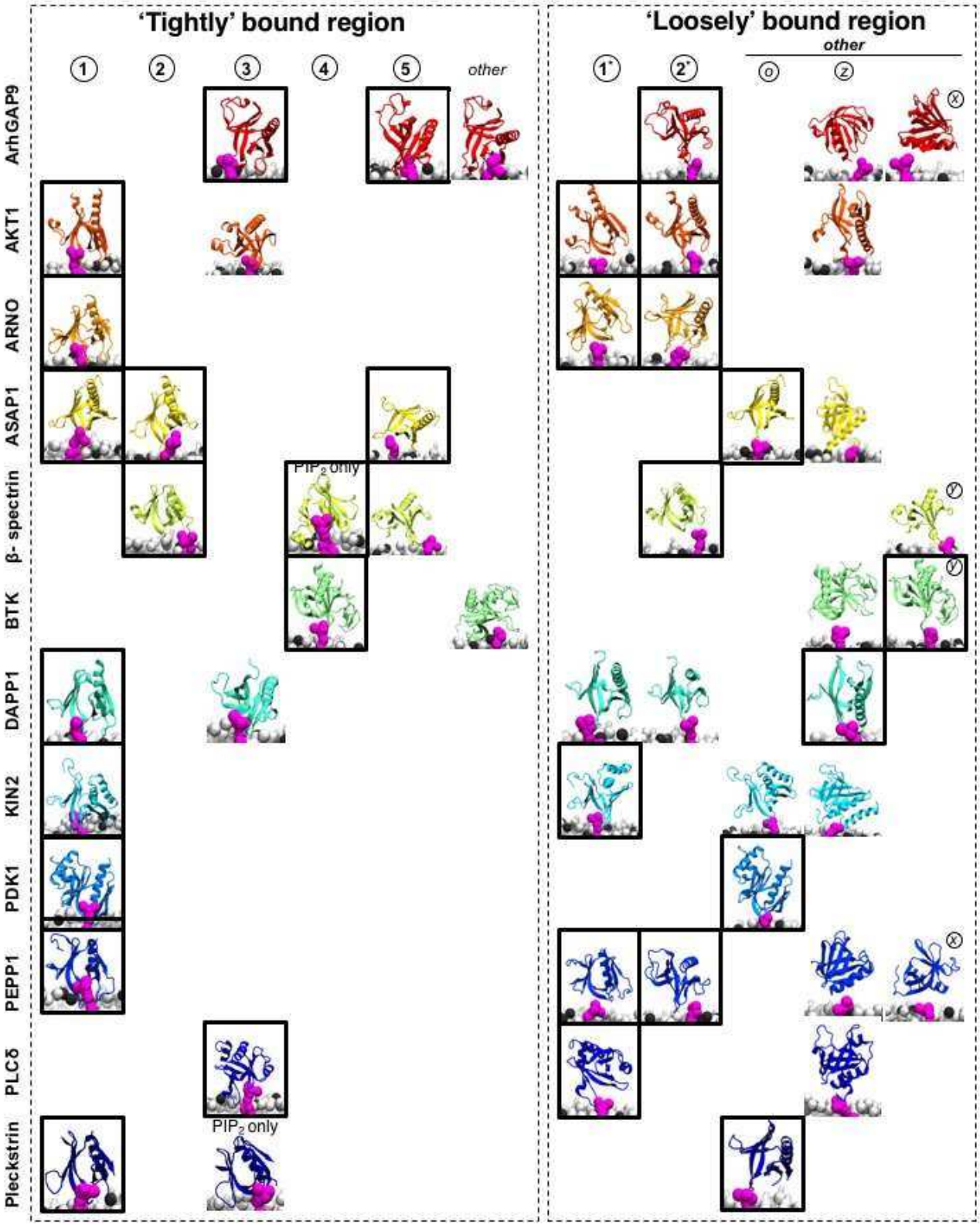
Supplementary Figure S8

The PMF profile (**A**) and PIP-binding angle relative to the protein lipid separation distance (**B**) are shown for ArhGAP9 PH simulations which started with the PIP bound in the A-site ('crystal' and 'simulation') and the C-site of the ArGAP9 PH.



Supplementary Figure S9

Contour maps showing the binding modes of all PH domains in the 'tight' and 'loosely' bound regions. The combined landscape for all systems is shown in grey in the background in each plot. The blue circles represent binding modes that are shown in the Supplementary Figure S10. Note that the plots for the 'tightly' bound region are shown only of R_{zz} values from 0 to 1 because no binding modes were observed for R_{zz} values in the from 0 to -1 region.



Supplementary Figure S10

All of the PH/PIP binding modes that have been identified in our simulations. In all cases the PIP molecule is shown in magenta. The binding modes correspond to the minima identified in our analysis in Supplementary Figure S9. Structures in boxes correspond to the lowest minima.

Table S11: Summary of umbrella sampling simulations. (c) denotes crystal and (s) simulation starting configurations.

Protein	PIP	Initial	No. windows	Window length (ns)	Converge time (ns)	Separation covered (nm)	Location of well (nm)
ArhGAP9	PIP2	c	25	3500	1000	1.25 - 4.05	1.44
		s	26	3000	600	1.53 - 4.38	1.62
	PIP3	c	26	3000	400	1.32 - 4.31	1.48
		s	24	3000	600	1.50 - 4.30	1.73
AKT1	PIP2	c	25	4000	600	1.39 - 4.19	1.56
		s	24	4000	600	1.46 - 4.15	1.61
	PIP3	c	24	3500	200	1.35 - 4.17	1.53
		s	24	4000	200	1.51 - 4.37	1.62
ARNO	PIP2	c	27	2500	400	1.01 - 4.02	1.50
		s	25	2500	600	1.20 - 4.01	1.68
	PIP3	c	24	2500	400	1.44 - 4.23	1.57
		s	24	2500	400	1.37 - 4.17	1.54
ASAP1	PIP2	c	24	3500	800	1.34 - 4.15	1.77
C-site		s	24	3500	400	1.62 - 4.41	2.05
	PIP3	c	24	3500	600	1.72 - 4.52	2.12
		s	23	3500	600	1.41 - 4.00	2.12
ASAP1	PIP2	c	26	4000	600	1.45 - 4.46	1.63
A-site		s	24	4000	200	1.44 - 4.25	1.82
	PIP3	c	26	4000	400	1.62 - 4.67	1.79
		s	24	4000	200	1.63 - 4.43	1.97
β-spectrin	PIP2	c	24	4000	1800	1.02 - 3.83	1.25
		s	24	4000	2400	0.92 - 3.72	1.31
	PIP3	c	24	4000	600	0.85 - 3.64	2.36
		s	23	4000	1000	1.56 - 4.17	2.48
BTK	PIP2	c	24	4000	1000	1.25 - 4.06	1.85
		s	24	4000	1400	1.75 - 4.45	2.74
	PIP3	c	27	4000	200	1.07 - 4.07	1.23
		s	26	4000	1200	1.51 - 4.49	2.64
DAPP1	PIP2	c	24	2500	600	1.53 - 4.33	1.64
		s	26	2500	600	1.09 - 4.09	1.19
	PIP3	c	23	3000	800	0.78 - 3.38	1.11
		s	24	3000	200	1.15 - 3.94	1.29
KIN2	PIP2	c	23	4000	400	1.22 - 3.82	1.60
		s	23	4000	600	1.26 - 3.86	1.42
	PIP3	c	24	4000	2400	1.19 - 3.89	1.45
		s	23	4000	400	1.46 - 4.12	1.55
PDK1	PIP2	c	24	2500	400	1.62 - 4.46	1.85
		s	24	2500	1000	1.37 - 4.11	1.48
	PIP3	c	24	3000	200	1.55 - 4.36	1.80
		s	23	3000	600	1.29 - 3.88	1.58
PEPP1	PIP2	c	24	3500	200	1.37 - 4.17	1.53
		s	24	3500	200	1.16 - 3.95	1.42
	PIP3	c	26	3500	200	1.11 - 4.11	1.32
		s	23	3500	1200	1.02 - 3.63	1.22
PLCδ	PIP2	c	24	2500	200	1.48 - 4.28	1.68
		s	24	2500	200	1.52 - 4.34	1.61
	PIP3	c	24	2500	600	1.37 - 3.97	1.79
		s	24	2500	600	1.39 - 4.23	1.54
Plecstrin	PIP2	c	25	3000	1200	1.04 - 3.93	1.10
		s	24	3000	600	1.10 - 3.91	1.26
	PIP3	c	23	3000	200	1.37 - 3.96	1.74
		s	23	4000	600	1.53 - 4.02	1.91
AKT1-E17K	PIP2	-	26	1500	200	1.20 – 4.30	1.42
	PIP3	-	26	1500	600	1.29 – 4.27	1.50
GRP1	2xPIP3	-	24	2000	600	1.74 – 4.53	1.86
AG9	PIP3	C start	24	3000	1000	1.61 – 4.33	1.72

Table S12

Average number of interactions between the PIP molecules and the sidechain of residues in the 'A site', 'C site' or any residue. A/C-site residues are defined based on the crystal structure of each PH domain and our structural based alignment (Supplementary Figure S2). Multiple contacts between a residue/PIP phosphate are only counted once, and backbone interactions are not considered. Errors are based on the two repeats (crystal/simulation) for each system.

		'Tightly' bound region			**'Loosely' bound region**		
Protein	**PIP**	**A site**	**C site**	**Total**	**A site**	**C site**	**Total**
ArhGAP9	PIP2	2.1 ± 0.1	1.6 ± 0.1	4.9 ± 0.3	1.0 ± 0.2	0.4 ± 0.1	2.4 ± 0.3
13	PIP3	2.0 ± 0.3	1.2 ± 0.9	5.2 ± 0.3	1.3 ± 0.1	0.4 ± 0.1	2.8 ± 0.3
AKT1	PIP2	1.4 ± 0.1	5.0 ± 0.2	6.8 ± 0.3	0.6 ± 0.1	0.7 ± 0.1	2.2 ± 0.1
10	PIP3	1.1 ± 0.01	6.1 ± 0.1	7.7 ± 0.1	0.6 ± 0.2	0.8 ± 0.4	2.6 ± 0.6
ARNO	PIP2	1.1 ± 0.03	5.0 ± 0.02	7.0 ± 0.01	1.0 ± 0.1	0.3 ± 0.1	2.1 ± 0.2
	PIP3	1.5 ± 0.2	5.6 ± 0.8	8.2 ± 03	1.4 ± 0.2	0.5 ± 01	3.0 ± 0.4
ASAP1	PIP2	1.4 ± 0.3	2.0 ± 0.4	5.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	1.0 ± 0.3
	PIP3	1.1 ± 0.4	1.5 ± 0.1	4.7 ± 0.3	0.2 ± 0.04	0.3 ± 0.1	0.8 ± 0.1
BSPEC	PIP2	0.7 ± 0.3	2.5 ± 0.1	5.8 ± 0.2	1.8 ± 0.2	0.5 ± 0.2	3.1 ± 0.2
	PIP3	1.6 ± 0.1	0.4 ± 0.2	2.6 ± 0.1	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01
BTK	PIP2	0.1 ± 0.2	1.8 ± 1	4.3 ± 2	0.0 ± 0.01	0.5 ± 0.7	1.5 ± 2
	PIP3	0.1 ± 0.2	3.8 ± 2	6.6 ± 2	0.0 ± 0.04	1.3 ± 1	2.9 ± 2
DAPP1	PIP2	0.8 ± 0.1	5.7 ± 0.5	7.0 ± 0.5	0.3 ± 0.04	0.4 ± 0.3	2.6 ± 0.9
	PIP3	0.8 ± 0.01	7.3 ± 0.5	8.5 ± 0.5	0.3 ± 0.04	0.3 ± 0.04	3.8 ± 0.8
KIN2	PIP2	0.2 ± 0.1	5.3 ± 0.5	7.2 ± 0.5	0.1 ± 0.01	1.2 ± 0.4	2.9 ± 0.3
	PIP3	0.4 ± 0.01	5.5 ± 0.1	7.8 ± 0.1	0.1 ± 0.01	2.4 ± 0.02	3.5 ± 0.4
PDK1	PIP2	0.0 ± 0.02	4.9 ± 0.01	5.4 ± 0.01	0.1 ± 0.01	1.6 ± 0.5	2.6 ± 0.3
	PIP3	0.0 ± 0.02	4.0 ± 0.4	4.3 ± 0.4	0.0 ± 0.01	1.5 ± 0.1	2.3 ± 0.2
PEPP1	PIP2	0.1 ± 0.04	5.1 ± 0.1	6.0 ± 0.3	0.6 ± 0.01	0.3 ± 0.2	2.5 ± 0.1
	PIP3	0.3 ± 0.3	6.1 ± 1	7.5 ± 0.7	0.7 ± 0.1	1.0 ± 1	3.1 ± 0.3
PLCD	PIP2	0.0 ± 0.02	5.3 ± 0.7	7.3 ± 0.5	0.1 ± 0.1	1.3 ± 0.3	2.6 ± 0.3
	PIP3	0.1 ± 0.03	5.4 ± 0.1	7.8 ± 0.1	0.1 ± 0.1	1.6 ± 0.4	2.9 ± 0.6

PLEC	PIP2	0.7 ± 0.04	5.2 ± 0.04	6.1 ± 0.02	1.8 ± 0.5	0.6 ± 0.1	3.0 ± 0.4
	PIP3	0.8 ± 0.1	4.6 ± 0.3	5.7 ± 0.2	1.0 ± 0.2	0.3 ± 0.1	1.5 ± 0.3

Table S13

Identification of binding as being 'A' and/or 'C' site, from contact analysis, PIP binding angle analysis, and the final overall orientation – showing the general agreement between these different analyses. A protein/lipid system was considered to have an A/C binding mode if the fraction of total sidechain contacts with corresponding residues ('from contacts'; based on Table S12) or the (unbiased) fraction of frames with PIP-binding angles in the corresponding ranged ('from angle') was greater than 0.33 (after averaging between the two repeats (crystal/simulation) for a system); in brackets indicates the fraction was greater than 0.2. Angle ranges were -5 to 50 for A and -60 to -5 for C. [a] indicates that the analyses suggest different binding sites.

		'Tightly' bound region			'Loosely' bound region		
Protein	**PIP**	**From contacts**	**From angle**	**Overall**	**From contacts**	**From angle**	**Overall**
ArhGAP9	PIP2	A C	A C	A C	A	A	A
	PIP3	A (C)	(A C)	A C	A	A	A
AKT1	PIP2	(A) C	C	C	(A C)	(A C)	A C
	PIP3	C	C	C	(A C)	(A C)	A C
ARNO	PIP2	C	C	C	A	(A C)	A
	PIP3	C	C	C	A	(A C)	A
ASAP1	PIP2	(A) C	(A) C	A C	(A C)	(C)	C
	PIP3	(A C)	(A C)	A C	(A C)	(C)	C
BSPEC	PIP2	C	(A) C	C	A	A	A
	PIP3	A	A	A	(A)	-	-
BTK	PIP2	C	C	C	(C)	A (C)	-[a]
	PIP3	C	C	C	C	(A)	-[a]
DAPP1	PIP2	C	C	C	-	-	-
	PIP3	C	C	C	-	-	-
KIN2	PIP2	C	C	C	C	(A C)	C
	PIP3	C	C	C	C	(A) C	C
PDK1	PIP2	C	C	C	C	A (C)	-[a]
	PIP3	C	C	C	C	A (C)	-[a]

PEPP1	PIP2	C	C	C	(A)	(A)	A
	PIP3	C	(A) C	C	(A C)	(A C)	A C
PLCD	PIP2	C	C	C	C	C	C
	PIP3	C	C	C	C	C	C
PLEC	PIP2	C	C	C	A	(C)	-[a]
	PIP3	C	C	C	A	(C)	-[a]

Table S14: Biological roles of the PH domains studied.

Protein	Function	Role of PH domain	PDB id
ArhGAP9	GAP activity at inner plasma; involved in adhesion	May recruit/binding to plasma membrane	*2POD*
AKT1	Kinase activity as part of many signalling pathways including apoptosis, proliferation, migration, glucose metabolism	Recruit to the inner plasma membrane in response upstream signalling (changing PIP concentration) PIP binding causes conformational change that enables activation Localise to membrane, alongside activator and targets	*1UNQ*
ARNO	GEF activity at inner plasma membrane; involved in cytoskeletal remodelling, adhesion, migration	Assist in recruiting protein to membrane PIP binding activates enzyme	*1U27*
ASAP1	GAP activity at inner plasma membrane; involved in cytoskeletal remodelling	May assist in anchoring protein to membrane PIP binding increases enzyme activity	*2C79*
β-spectrin	Structural protein (cytoskeleton)	Assist in anchoring to membrane	*1BTN*
BTK	Kinase activity as part of B-lymphocyte development and signalling pathways	Recruit to the inner plasma membrane in response upstream signalling (changing PIP concentration) Localise to membrane, alongside activator	*2Z0P*
DAPP1	Adaptor protein – assists the assembly of protein complexes; involved in adhesion	Recruit to the inner plasma membrane in response upstream signalling (changing PIP concentration) Localise to membrane, alongside targets	*1FAO*
KIN2	Activation of integrins; involved in cell adhesion	Assist in recruiting to plasma membrane	*2LKO*
PDK1	Kinase activity in signalling pathways including cell survival, proliferation, adhesion, glucose metabolism	Recruit to the inner plasma membrane in response upstream signalling (changing PIP concentration) Localise to membrane, alongside targets	*1W1D*
PEPP1	Not known		*1UPR*
PLCδ	Phospholipase activity, producing second messengers IP_3 and DAG	Recruit to membrane where substrate is located	*1MAI*
Pleckstrin	Actin organisation	Localise to membrane, where it is activated	*215C*

References

(1) Ceccarelli, D. F. J.; Blasutig, I. M.; Goudreault, M.; Li, Z.; Ruston, J.; Pawson, T.; Sicheri, F. *J. Biol. Chem.* **2007**, *282* (18), 13864.

(2) Currie, R. A.; Walker, K. S.; Gray, A.; Deak, M.; Casamayor, A.; Downes, C. P.; Cohen, P.; Alessi, D. R.; Lucocq, J. *Biochem. J.* **1999**, *337* (3), 575.

(3) Manna, D.; Albanese, A.; Park, W. S.; Cho, W. *J. Biol. Chem.* **2007**, *282* (44), 32093.

(4) Landgraf, K. E.; Pilling, C.; Falke, J. J. *Biochemistry* **2008**, *47* (47), 12260.

(5) Stephens, L.; Anderson, K.; Stokoe, D.; Erdjument-Bromage, H.; Painter, G. F.; Holmes, A. B.; Gaffney, P. R.; Reese, C. B.; McCormick, F.; Tempst, P.; Coadwell, J.; Hawkins, P. T. *Science* **1998**, *279* (5351), 710.

(6) Frech, M.; Andjelkovic, M.; Ingley, E.; Reddy, K. K.; Falck, J. R.; Hemmings, B. A. *J. Biol. Chem.* **1997**, *272* (13), 8474.

(7) Franke, T. F.; Kaplan, D. R.; Cantley, L. C.; Toker, A. *Science* **1997**, *275* (5300), 665.

(8) Rao, V. R.; Corradetti, M. N.; Chen, J.; Peng, J.; Yuan, J.; Prestwich, G. D.; Brugge, J. S. *J. Biol. Chem.* **1999**, *274* (53), 37893.

(9) Kavran, J. M.; Klein, D. E.; Lee, A.; Falasca, M.; Isakoff, S. J.; Skolnik, E. Y.; Lemmon, M. A. *J. Biol. Chem.* **1998**, *273* (46), 30497.

(10) Dowler, S.; Currie, R. A.; Downes, C. P.; Alessi, D. R. *Biochem. J.* **1999**, *342* (1), 7.

(11) Park, W. S.; Heo, W. Do; Whalen, J. H.; O'Rourke, N. A.; Bryan, H. M.; Meyer, T.; Teruel, M. N. *Mol. Cell* **2008**, *30* (3), 381.

(12) Rowland, M. M.; Gong, D.; Bostic, H. E.; Lucas, N.; Cho, W.; Best, M. D. *Chem. Phys. Lipids* **2012**, *165* (2), 207.

(13) Macia, E.; Paris, S.; Chabre, M. *Biochemistry* **2000**, *39* (19), 5893.

(14) Cronin, T. C.; DiNitto, J. P.; Czech, M. P.; Lambright, D. G. *EMBO J.* **2004**, *23* (19), 3711.

(15) Venkateswarlu, K.; Oatey, P. B.; Tavaré, J. M.; Cullen, P. J. *Curr. Biol.* **1998**, *8* (8), 463.

(16) Klarlund, J. K.; Tsiaras, W.; Holik, J. J.; Chawla, A.; Czech, M. P. *J. Biol. Chem.* **2000**, *275* (42), 32816.

(17) Jian, X.; Tang, W. K.; Zhai, P.; Roy, N. S.; Luo, R.; Gruschus, J. M.; Yohe, M. E.; Chen, P. W.; Li, Y.; Byrd, R. A.; Xia, D.; Randazzo, P. A. *Structure* **2015**, *23* (11), 1977.

(18) Luo, R.; Miller Jenkins, L. M.; Randazzo, P. A.; Gruschus, J. *Cell. Signal.* **2008**, *20* (11), 1968.

(19) Kruljac-Letunic, A.; Moelleken, J.; Kallin, A.; Wieland, F.; Blaukat, A. *J. Biol. Chem.* **2003**, *278* (32), 29560.

(20) Salim, K.; Bottomley, M.; Querfurth, E. *EMBO J.* **1996**, *15* (22), 6241.

(21) Baraldi, E.; Carugo, K. D.; Hyvönen, M.; Surdo, P. Lo; Riley, A. M.; Potter, B. V.; O'Brien, R.; Ladbury, J. E.; Saraste, M. *Structure* **1999**, *7* (4), 449.

(22) Rameh, L. E.; Arvidsson, A. -k.; Carraway, K. L.; Couvillon, A. D.; Rathbun, G.; Crompton, A.; VanRenterghem, B.; Czech, M. P.; Ravichandran, K. S.; Burakoff, S. J.; Wang, D.-S.; Chen, C.-S.; Cantley, L. C. *J. Biol. Chem.* **1997**, *272* (35), 22059.

(23) Fukuda, M.; Kojima, T.; Kabayama, H.; Mikoshiba, K. *J. Biol. Chem.* **1996**, *271* (48), 30303.

(24) Das, A.; Base, C.; Manna, D.; Cho, W.; Dubreuil, R. R. *J. Biol. Chem.* **2008**, *283* (18), 12643.

(25) He, M.; Abdi, K. M.; Bennett, V. *J. Cell Biol.* **2014**, *206* (2), 273.

(26) Hyvönen, M.; Macias, M. J.; Nilges, M.; Oschkinat, H.; Saraste, M.; Wilmanns, M. *EMBO J.* **1995**, *14* (19), 4676.

(27) Gong, D.; Smith, M. D.; Manna, D.; Bostic, H. E.; Cho, W.; Best, M. D. *Bioconjug. Chem.* **2009**, *20* (2), 310.

(28) Ferguson, K. M.; Kavran, J. M.; Sankaran, V. G.; Fournier, E.; Isakoff, S. J.; Skolnik, E. Y.;

Lemmon, M. A. *Mol. Cell* **2000**, *6* (2), 373.
(29) Dowler, S.; Currie, R. A.; Campbell, D. G.; Deak, M.; Kular, G.; Downes, C. P.; Alessi, D. R. *Biochem. J.* **2000**, *351* (1), 19.
(30) Thomas, C. C.; Dowler, S.; Deak, M.; Alessi, D. R.; van Aalten, D. M. *Biochem. J.* **2001**, *358* (Pt 2), 287.
(31) Liu, J.; Fukuda, K.; Xu, Z.; Ma, Y.-Q.; Hirbawi, J.; Mao, X.; Wu, C.; Plow, E. F.; Qin, J. *J. Biol. Chem.* **2011**, *286* (50), 43334.
(32) Yates, L. A.; Lumb, C. N.; Brahme, N. N.; Zalyte, R.; Bird, L. E.; De Colibus, L.; Owens, R. J.; Calderwood, D. A.; Sansom, M. S. P.; Gilbert, R. J. C. *J. Biol. Chem.* **2012**, *287* (52), 43246.
(33) Legate, K. R.; Takahashi, S.; Bonakdar, N.; Fabry, B.; Boettiger, D.; Zent, R.; Fässler, R. *EMBO J.* **2011**, *30* (22), 4539.
(34) Qu, H.; Tu, Y.; Shi, X.; Larjava, H.; Saleem, M. A.; Shattil, S. J.; Fukuda, K.; Qin, J.; Kretzler, M.; Wu, C. *J. Cell Sci.* **2011**, *124* (Pt 6), 879.
(35) Lucas, N.; Cho, W. *J. Biol. Chem.* **2011**, *286* (48), 41265.
(36) Komander, D.; Fairservice, A.; Deak, M.; Kular, G. S.; Prescott, A. R.; Peter Downes, C.; Safrany, S. T.; Alessi, D. R.; van Aalten, D. M. F. *EMBO J.* **2004**, *23* (20), 3918.
(37) Hirose, K.; Kadowaki, S.; Tanabe, M.; Takeshima, H.; Iino, M. *Science* **1999**, *284* (5419), 1527.
(38) Ferguson, C. G.; James, R. D.; Bigman, C. S.; Shepard, D. A.; Abdiche, Y.; Katsamba, P. S.; Myszka, D. G.; Prestwich, G. D. *Bioconjug. Chem.* **2005**, *16* (6), 1475.
(39) Flesch, F. M.; Yu, J. W.; Lemmon, M. A.; Burger, K. N. J. *Biochem. J.* **2005**, *389* (Pt 2), 435.
(40) Pilling, C.; Landgraf, K. E.; Falke, J. J. *Biochemistry* **2011**, *50* (45), 9845.
(41) Gallego, O.; Betts, M. J.; Gvozdenovic-Jeremic, J.; Maeda, K.; Matetzki, C.; Aguilar-Gurrieri, C.; Beltran-Alvarez, P.; Bonn, S.; Fernández-Tornero, C.; Jensen, L. J.; Kuhn, M.; Trott, J.; Rybin, V.; Müller, C. W.; Bork, P.; Kaksonen, M.; Russell, R. B.; Gavin, A.-C. *Mol. Syst. Biol.* **2010**, *6*, 430.
(42) Uekama, N.; Sugita, T.; Okada, M.; Yagisawa, H.; Tuzi, S. *FEBS J.* **2007**, *274* (1), 177.
(43) Garcia, P.; Gupta, R.; Shah, S.; Morris, A. J.; Rudge, S. A.; Scarlata, S.; Petrova, V.; McLaughlin, S.; Rebecchi, M. J. *Biochemistry* **1995**, *34* (49), 16228.
(44) Lemmon, M. A.; Ferguson, K. M.; O'Brien, R.; Sigler, P. B.; Schlessinger, J. *Proc. Natl. Acad. Sci.* **1995**, *92* (23), 10472.
(45) Tall, E.; Dormán, G.; Garcia, P.; Runnels, L.; Shah, S.; Chen, J.; Profit, A.; Gu, Q. M.; Chaudhary, A.; Prestwich, G. D.; Rebecchi, M. J. *Biochemistry* **1997**, *36* (23), 7239.
(46) Várnai, P.; Lin, X.; Lee, S. B.; Tuymetova, G.; Bondeva, T.; Spät, A.; Rhee, S. G.; Hajnóczky, G.; Balla, T. *J. Biol. Chem.* **2002**, *277* (30), 27412.