Bee Venom Melittin Disintegrates Respiration of Mitochondria in Healthy and Leukemia Lymphocytes and Induces Formation of Non-bilayer Structures in Model Inner Mitochondrial Membranes

Edward Gasanoff, Yipeng Liu, Feng Li, Paul Hanlon, and Győző Garab

Supplementary materials

Table S1. Summary of the charged and polar groups of amino acid residues in the melittin binding sites that interact with polar head groups of truncated CL. Hypothetical binding sites in melittin that bind to the charged and polar groups of CL polar head were determined by AutoDock modeling. Designation of CL polar head groups is shown in Figure S1. Pb in $NH^{\delta+}{}_{pb}$ and $C{=}O^{\delta-}{}_{pb}$ denotes a peptide bond.

Binding site # and Affinity Energy Values	CL Polar Head Groups	Melittin a. a. Residues Interacting with CL Head	Bond Type
Binding site 1	a-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
Affinity: –4.1 kcal/mol	b-PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
	b-PO_4^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
	$CO^{\delta-}H$	R22 ($CNH_2^{\delta+}$)	hydrogen
	$CO^{\delta-}H$	R22 ($CNH^{\delta+}C$)	hydrogen
	I-$C{=}O^{\delta-}$	R22($C{=}N^+H_2$)	ion-polar
	I-$C{=}O^{\delta-}$	S18($C{-}OH^{\delta+}$)	hydrogen
	IV-$C{=}O^{\delta-}$	Q25 ($CNH_2^{\delta+}$)	hydrogen
	4-I-$CO^{\delta-}C$	Q26 ($CNH_2^{\delta+}$)	hydrogen
Binding site 2	b-PO_4^-	R22($C{=}N^+H_2$)	ionic
Affinity: –4.0 kcal/mol	a-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	a-PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
	b-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	$CO^{\delta-}H$	R22 ($CNH_2^{\delta+}$)	hydrogen
	I-$C{=}O^{\delta-}$	Q26 ($CNH_2^{\delta+}$)	hydrogen
Binding site 3	a-PO_4^-	W19 ($CNH^{\delta+}C$)	ion-polar
Affinity: –4.0 kcal/mol	b-PO_4^-	W19 ($CNH^{\delta+}C$)	ion-polar
	b-PO_4^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
	b-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	b-PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
	2-$CO^{\delta-}C$	R22 ($CNH_2^{\delta+}$)	hydrogen
	II-$C{=}O^{\delta-}$	R22($C{=}N^+H_2$)	ion-polar
	4-I-$CO^{\delta-}C$	Q26 ($CNH_2^{\delta+}$)	hydrogen
Binding site 4	II-$C{=}O^{\delta-}$	T11 ($C{-}OH^{\delta+}$)	hydrogen
Affinity:	IV-$C{=}O^{\delta-}$	W19 ($CNH^{\delta+}C$)	hydrogen

–3.9 kcal/mol	CL head hydro-phobic surface	12G,13L,14P, 15A,16L,17I	hydrophobic
Binding site 5	a-PO_4^-	W19 ($CNH^{\delta+}C$)	ion-polar
Affinity: –3.9 kcal/mol	b-PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
	b-PO_4^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
	I-$C{=}O^{\delta-}$	R22 ($CNH_2^{\delta+}$)	hydrogen
	II-$C{=}O^{\delta-}$	R22 ($CNH_2^{\delta+}$)	hydrogen
	$CO^{\delta-}H$	R22 ($CNH_2^{\delta+}$)	hydrogen
	$CO^{\delta-}H$	R22 ($CNH^{\delta+}C$)	hydrogen
	4-$CO^{\delta-}C$	Q25 ($CNH_2^{\delta+}$)	hydrogen
Binding site 6	a-PO_4^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
Affinity: –3.9 kcal/mol	a-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	a-PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
	a-PO_4^-	W19 ($CNH^{\delta+}C$)	ion-polar
	1-$CO^{\delta-}C$	W19 ($CNH^{\delta+}C$)	hydrogen
	$CO^{\delta-}H$	R22 ($CNH_2^{\delta+}$)	hydrogen
	b-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
Binding site 7	a-PO_4^-	R24 ($C{=}N^+H_2$)	ionic
Affinity: –3.9 kcal/mol	a-PO_4^-	R24 ($CNH_2^{\delta+}$)	ion-polar
	II-$C{=}O^{\delta-}$	R24 ($CNH_2^{\delta+}$)	hydrogen
	$COH^{\delta+}$	R24 ($C{=}O^{\delta-}{}_{pb}$)	hydrogen
	b-PO_4^-	R24 ($C{=}N^+H_2$)	ionic
Binding site 8	b-PO_4^-	R22 ($C{=}N^+H_2$)	ionic
Affinity: –3.9 kcal/mol	b-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	b-PO_4^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
	3-$CO^{\delta-}C$	Q26 ($CNH_2^{\delta+}$)	hydrogen
	$CO^{\delta-}H$	W19 ($CNH^{\delta+}C$)	hydrogen
	II-$C{=}O^{\delta-}$	R22 ($C{=}N^+H_2$)	ion-polar
	II-$C{=}O^{\delta-}$	R22 ($CNH_2^{\delta+}$)	hydrogen
Binding site 9	a-PO_4^-	R22 ($C{=}N^+H_2$)	ionic
Affinity: –3.9 kcal/mol	a-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	b-PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	b-PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
	3-$CO^{\delta-}C$	R22 ($CNH^{\delta+}C$)	hydrogen
	3-$CO^{\delta-}C$	Q26 ($CNH_2^{\delta+}$)	hydrogen
	III-$C{=}O^{\delta-}$	Q26 ($CNH_2^{\delta+}$)	hydrogen
	IV-$C{=}O^{\delta-}$	Q26 ($CNH_2^{\delta+}$)	hydrogen

Table S2. Summary of the charged and polar groups of amino acid residues in the melittin binding sites that interact with polar head groups of truncated PS. Hypothetical binding sites in melittin that bind to the charged and polar groups of PS polar head were determined by AutoDock modeling. Designation of PS polar head groups is shown in Figure S1. Pb in $NH^{\delta+}{}_{pb}$ denotes a peptide bond.

Binding Site # and Affinity Energy Values	**PS Polar Head Groups**	**Melittin a. a. Residues Interacting with PS Head**	**Bond Type**
Binding site 1	PO_4^-	R22 ($CNH_2^{\delta+}$)	ion-polar
Affinity: –3.8 kcal/mol	I-C=$O^{\delta-}$	R22 ($C=N^+H_2$)	ionic
	I-C=$O^{\delta-}$	R22 ($CNH_2^{\delta+}$)	ion-polar
	II-C=$O^{\delta-}$	A15 ($NH^{\delta+}{}_{pb}$)	ion-polar
	COO^-	R22 ($CNH^{\delta+}C$)	ion-polar
	COO^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
Binding site 2	PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
Affinity: –3.7 kcal/mol	PO_4^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
	COO^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	COO^-	R22 ($CNH^{\delta+}C$)	ion-polar
	COO^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
	I-C=$O^{\delta-}$	Q25 ($CNH_2^{\delta+}$)	hydrogen
Binding site 3	PO_4^-	L16 ($NH^{\delta+}{}_{pb}$)	ion-polar
Affinity: –3.6 kcal/mol	COO^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	COO^-	W19 ($NH^{\delta+}{}_{pb}$)	ion-polar
	II-C=$O^{\delta-}$	L13 ($NH^{\delta+}{}_{pb}$)	hydrogen
Binding site 4	COO^-	R22 ($C=N^+H_2$)	ionic
Affinity: –3.6 kcal/mol	COO^-	R22 ($CNH_2^{\delta+}$)	ion-polar
	I-C=$O^{\delta-}$	L13 ($NH^{\delta+}{}_{pb}$)	hydrogen
Binding site 5	PO_4^-	Q25 ($CNH_2^{\delta+}$)	ion-polar
Affinity: –3.6 kcal/mol	COO^-	R22 ($C=N^+H_2$)	ionic
	II-C=$O^{\delta-}$	Q26 ($CNH_2^{\delta+}$)	hydrogen
	2-$CO^{\delta-}$C	Q26 ($CNH_2^{\delta+}$)	hydrogen
Binding site 6	PO_4^-	K21 (CN^+H_3)	ionic
Affinity: –3.6 kcal/mol	I-C=$O^{\delta-}$	K21 (CN^+H_3)	ion-polar
	N^+H_3	S18 ($CO^{\delta-}H$)	ion-polar
	COO^-	Q25 ($CNH_2^{\delta+}$)	ion-polar
Binding site 7	PO_4^-	R22 ($C=N^+H_2$)	ionic
Affinity: –3.6 kcal/mol	PO_4^-	S18 ($COH^{\delta+}$)	ion-polar
	1-$CO^{\delta-}$C	R22 ($C=N^+H_2$)	ion-polar
	1-$CO^{\delta-}$C	S18 ($COH^{\delta+}$)	hydrogen
	I-C=$O^{\delta-}$	R22 ($CNH_2^{\delta+}$)	hydrogen

	2-$CO^{\delta-}C$	S18 ($COH^{\delta+}$)	hydrogen
Binding site 8	COO^-	K7 (CN^+H_3)	ionic
Affinity: –3.5 kcal/mol	N^+H_3	T11 ($CO^{\delta-}H$)	ion-polar
Binding site 9	COO^-	L13 ($NH^{\delta+}{}_{pb}$)	ion-polar
Affinity: –3.5 kcal/mol	II-$C=O^{\delta-}$	R22 ($CNH_2{}^{\delta+}$)	hydrogen
	II-$C=O^{\delta-}$	R22 ($C=N^+H_2$)	ion-polar

Table S3. Summary of the charged and polar groups of amino acid residues in the melittin binding sites that interact with polar head groups of truncated PC. Hypothetical binding sites in melittin that bind to the charged and polar groups of PC polar head were determined by AutoDock modeling. Designation of PC polar head groups is shown in Figure S1. Pb in $NH^{\delta+}{}_{pb}$ denotes a peptide bond.

Binding Site # and Affinity Energy Values	**PC Polar Head Groups**	**Melittin a. a. Residues Interacting with PC Head**	**Bond Type**
Binding site 1	PO_4^-	W19 ($CNH^{\delta+}C$)	ion-polar
Affinity: –3.3 kcal/mol	PO_4^-	R22($CNH_2^{\delta+}$)	ion-polar
	PO_4^-	R22 ($CNH^{\delta+}C$)	ion-polar
	PO_4^-	Q26 ($CNH_2^{\delta+}$)	ion-polar
	1-$CO^{\delta-}C$	Q26 ($CNH_2^{\delta+}$)	hydrogen
	2-$CO^{\delta-}C$	Q26 ($CNH_2^{\delta+}$)	hydrogen
Binding site 2	PO_4^-	R22 ($C=N^+H_2$)	ionic
Affinity: –3.2 kcal/mol	PO_4^-	R22($CNH_2^{\delta+}$)	ion-polar
	PO_4^-	S18 ($COH^{\delta+}$)	ion-polar
Binding site 3	II-$C=O^{\delta-}$	W19 ($CNH^{\delta+}C$)	hydrogen
Affinity: –3.2 kcal/mol	PC head hydro-phobic surface	V8, L13, A14, L16	hydrophobic
Binding site 4	II-$C=O^{\delta-}$	G12 ($NH^{\delta+}{}_{pb}$)	hydrogen
Affinity: –3.2 kcal/mol	PO_4^-	L16 ($NH^{\delta+}{}_{pb}$)	ion-polar
	PC head hydro-phobic surface	V8, W19	hydrophobic
Binding site 5	PO_4^-	R22(CN^+H_2)	ionic
Affinity: –3.1 kcal/mol	PO_4^-	R22($CNH_2^{\delta+}$)	ion-polar
	II-$C=O^{\delta-}$	W19 ($CNH^{\delta+}C$)	hydrogen
Binding site 6	II-$C=O^{\delta-}$	K21 (N^+H_3)	ion-polar
Affinity: –3.1 kcal/mol	PC head hydro-phobic surface	L6, L9, L13	hydrophobic
Binding site 7	I-$C=O^{\delta-}$	R22($C=N^+H_2$)	ion-polar

Affinity: –3.1 kcal/mol	II-C=$O^{\delta-}$ PC head hydro-phobic surface	W19 ($CNH^{\delta+}C$) V8, L13, L16	hydrogen hydrophobic
Binding site 8 Affinity: –3.1 kcal/mol	I-C=$O^{\delta-}$ II-C=$O^{\delta-}$ II-C=$O^{\delta-}$ PC head hydro-phobic surface	W19 ($CNH^{\delta+}C$) R22($C=N^{+}H_2$) R22($CNH_2^{\delta+}$) V8, L13, L16	hydrogen ion-polar hydrogen hydrophobic
Binding site 9 Affinity: –3.1 kcal/mol	PO_4^- PO_4^- PO_4^- 1-$CO^{\delta-}$C 2-$CO^{\delta-}$C	R22($CNH_2^{\delta+}$) R22 ($CNH^{\delta+}C$) Q26 ($CNH_2^{\delta+}$) Q26 ($CNH_2^{\delta+}$) Q26 ($CNH_2^{\delta+}$)	ion-polar ion-polar ion-polar hydrogen hydrogen

Table S4. Summary of the charged and polar groups of amino acid residues in the melittin binding sites that interact with polar head groups of truncated PA. Hypothetical binding sites in melittin that bind to the charged and polar groups of PA polar head were determined by AutoDock modeling. Designation of PA polar head groups is shown in Figure S1. Pb in $NH^{\delta+}{}_{pb}$ denotes a peptide bond.

Binding Site # and Affinity Energy Values	**PA Polar Head Groups**	**Melittin a. a. Residues Interacting with PA Head**	**Bond Type**
Binding site 1 Affinity: –3.5 kcal/mol	PO_4^- PO_4^- I-C=$O^{\delta-}$	R22 ($C=N^{+}H_2$) R22 ($CNH_2^{\delta+}$) W19 ($CNH^{\delta+}C$)	ionic ion-polar hydrogen
Binding site 2 Affinity: –3.4 kcal/mol	PO_4^- PO_4^-	R22 ($C=N^{+}H_2$) R22 ($CNH_2^{\delta+}$)	ionic ion-polar
Binding site 3 Affinity: –3.4 kcal/mol	PO_4^- II-C=$O^{\delta-}$ II-C=$O^{\delta-}$	L16 ($NH^{\delta+}{}_{pb}$) R22 ($C=N^{+}H_2$) R22 ($CNH_2^{\delta+}$)	ion-polar ion-polar hydrogen
Binding site 4 Affinity: –3.3 kcal/mol	PO_4^- PO_4^- 1-$CO^{\delta-}$C	R22 ($C=N^{+}H_2$) R22 ($CNH_2^{\delta+}$) R22 ($CNH_2^{\delta+}$)	ionic ion-polar hydrogen
Binding site 5 Affinity: –3.3 kcal/mol	PO_4^- PO_4^- PO_4^- 1-$CO^{\delta-}$C 2-$CO^{\delta-}$C	R22 ($CNH_2^{\delta+}$) R22 ($CNH^{\delta+}C$) Q26 ($CNH_2^{\delta+}$) Q26 ($CNH_2^{\delta+}$) Q26 ($CNH_2^{\delta+}$)	ion-polar ion-polar ion-polar hydrogen hydrogen

Binding site 6 Affinity: –3.3 kcal/mol	PO_4^- PO_4^- PO_4^-	R22 ($C=N^+H_2$) R22 ($CNH_2^{\delta+}$) S18 ($COH^{\delta+}$)	ionic ion-polar ion-polar
Binding site 7 Affinity: –3.2 kcal/mol	PO_4^- 1-$CO^{\delta-}$C	R22 ($CNH_2^{\delta+}$) W19 ($CNH^{\delta+}$C)	ion-polar hydrogen
Binding site 8 Affinity: –3.2 kcal/mol	PO_4^- I-C=$O^{\delta-}$ II-C=$O^{\delta-}$	K21 (CN^+H_3) Q25 ($CNH_2^{\delta+}$) S18 ($COH^{\delta+}$)	ion-polar hydrogen hydrogen
Binding site 9 Affinity: –3.2 kcal/mol	I-C=$O^{\delta-}$ II-C=$O^{\delta-}$ PO_4^-	R22 ($CNH_2^{\delta+}$) W19 ($CNH^{\delta+}$C) L16 ($NH^{\delta+}{}_{pb}$)	hydrogen hydrogen ion-polar

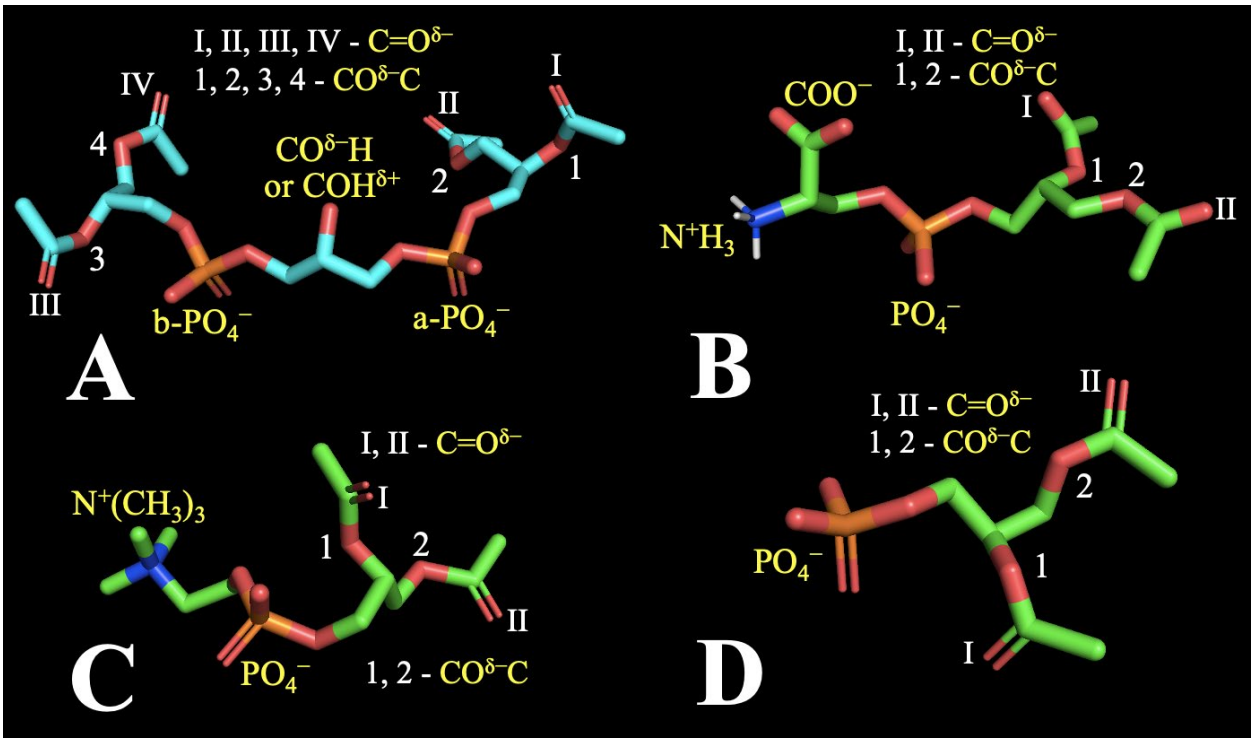

Figure S1. Designation of polar head groups (charged and dipolar) of cardiolipin (**A**), phosphatidylserine (**B**), phosphatidylcholine (**C**), and phosphatidic acid (**D**).