

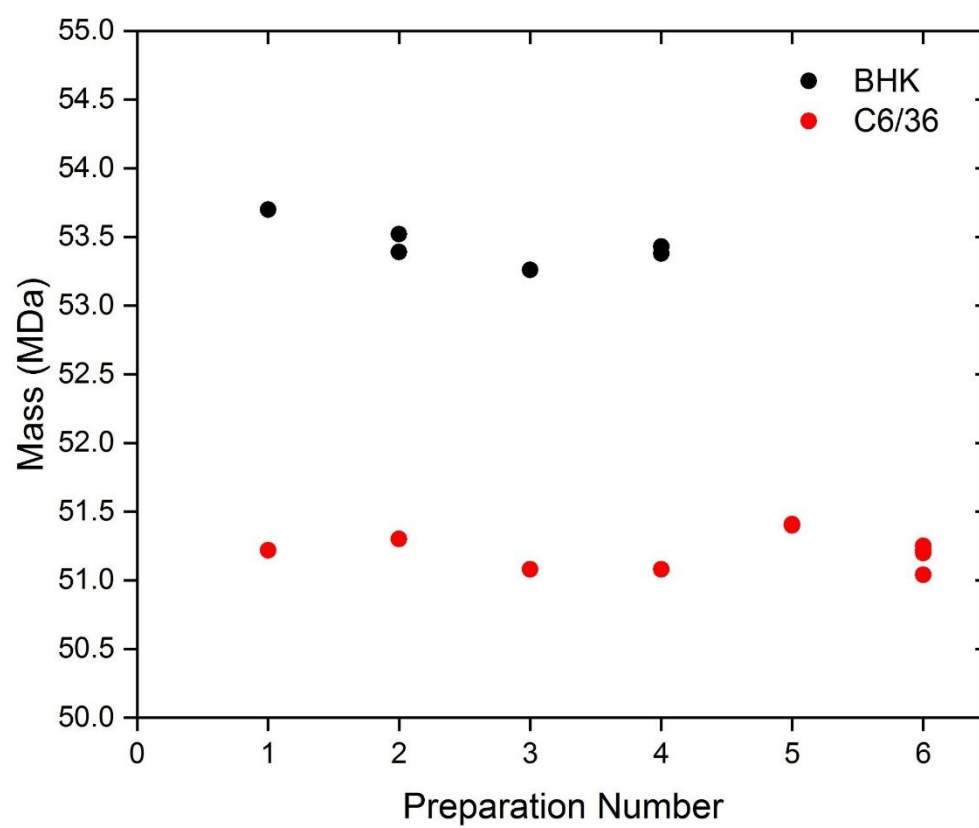
Dissecting the Components of Sindbis Virus from Arthropod and Vertebrate Hosts: Implications for Infectivity Differences

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Supporting Information

Seven pages including one figure and four tables.



Supplementary Figure 1: Mass measured versus preparation number for SINV from BHK (vertebrate) cells and C6/36 (mosquito) cells.

Supplementary Table 1. Glycan assignments for C6/36-derived SINV. The peptide sequences for each group of glycopeptides are listed in the table. The theoretical peptide mass is given in parenthesis. The number of copies of each sugar subunit, N-Acetylglucosamine (GlcNAc), Mannose (Man), Fucose (Fuc) and Sialic Acid (SA), are listed for each glycopeptide. The copy numbers are used to calculate the theoretical mass of the glycan. The mass deviation of the glycopeptide is the sum of the theoretical peptide and glycan masses minus the measured glycopeptide mass.

Glycosylation Site	Measured Glycopeptide mass (Da)	Relative Intensity	Charge	Glycan Mass (Da)	Mass Deviation (Da)	Sugar Subunits			
						GlcNAc	Man	Fuc	SA
E1 139	IVYGNTTSFLDVYVNGVTPGTSK (2430.2 Da)								
	3486.5	0.572	3	1056.4	+0.1	2	4	0	0
	3810.6	0.428	3	1380.5	+0.1	2	6	0	0
E1 245	NNSGRPLQETAPFGCK (1775.8 Da)								
	2668.1	0.240	3	892.3	0.0	2	3	0	0
	2992.3	0.198	3	1216.4	0.0	2	5	0	0
	3154.3	0.127	3	1378.5	0.0	2	6	0	0
	3478.4	0.281	3	1702.6	0.0	2	8	0	0
	3640.5	0.017	3	1864.7	0.0	2	9	0	0
E1 245	NVHVPYQTQASSGFEMWKNNSGRPLQETAPFGCK (3735.8 Da)								
	4792.1	0.024	5	1056.4	+0.1	2	4	0	0
	5116.2	0.021	5	1380.5	+0.1	2	6	0	0
	5278.3	0.031	5	1542.6	+0.1	2	7	0	0
	5440.3	0.060	5	1704.7	+0.1	2	8	0	0
E2 196	NITYECKCGDYK (1435.6 Da)								
	2985.3	0.434	4	1549.6	-0.1	2	7	0	0
	3148.4	0.566	4	1712.7	-0.1	2	8	0	0
E2 318	NFTVDRDGLLEYIWGNHEPVR (2415.1 Da)								
	3309.5	0.011	3	894.4	0.0	2	3	0	0
	3471.5	0.045	3	1056.4	0.0	2	4	0	0
	3633.6	0.130	3	1218.5	0.0	2	5	0	0
	3795.6	0.135	3	1380.5	0.0	2	6	0	0
	3957.7	0.137	3	1542.6	0.0	2	7	0	0
	3795.6	0.141	4	1380.5	0.0	2	6	0	0
	3957.7	0.152	4	1542.6	0.0	2	7	0	0
	4119.7	0.225	4	1704.7	0.0	2	8	0	0
	4281.8	0.023	4	1866.7	0.0	2	9	0	0

Supplementary Table 2. Glycan assignments for BHK-derived SINV. The peptide sequences for each group of glycopeptides are listed in the table. The theoretical peptide mass is given in parenthesis. The number of copies of each sugar subunit, N-Acetylglucosamine (GlcNAc), Mannose (Man), Fucose (Fuc) and Sialic Acid (SA), are listed for each glycopeptide. The copy numbers are used to calculate the theoretical mass of the glycan. The mass deviation of the glycopeptide is the sum of the theoretical peptide and glycan masses minus the measured glycopeptide mass.

Glycosylation Site	Glycopeptide Mass (Da)	Relative Intensity	Charge	Glycan Mass (Da)	Mass Deviation (Da)	Sugar Subunits			
						GlcNAc	Man	Fuc	SA
E1 139	IVYGNTTSFLDVYVNGVTPGTSKDLKVIAGPISASFTPFDHK (4,455.3 Da)								
	5350.0	0.160	4	894.4	−0.2	2	3	0	0
	5511.9	0.120	4	1056.4	−0.3	2	4	0	0
E1 139	VHTAAMKVGLRIVYGNTTSFLDVYVNGVTPGTSKDLK (3,947.1 Da)								
	5814.5	0.479	4	1866.7	−0.7	2	9	0	0
	5652.2	0.242	5	1704.7	−0.4	2	8	0	0
E1 245	NVHVPYTQASSGFEMWKNNRPLQETAPFGCK (3,736.7 Da)								
	5117.2	0.026	4	1380.5	0.0	2	6	0	0
	5279.3	0.008	4	1542.6	0.0	2	7	0	0
E1 245	NNSGRPLQETAPFGCK (Cys_CAM) (1,774.8)								
	2993.6	0.004	3	1218.5	−0.3	2	5	0	0
	3155.7	0.006	3	1380.5	−0.4	2	6	0	0
	3641.5	0.002	3	1866.7	0.0	2	9	0	0
	3768.6	0.172	3	1992.8	−1.0	5	6	0	0
	3914.7	0.064	3	2139.9	0.0	5	6	1	0
	3930.7	0.084	3	2154.9	−1.0	5	7	0	0
	4076.8	0.051	3	2301.9	−0.1	5	7	1	0
	3914.7	0.064	4	2139.9	0.0	5	6	1	0
	4076.8	0.051	4	2301.9	−0.1	5	7	1	0
	4134.7	0.004	4	2359.0	−0.9	6	7	0	0
E1 245	NNSGRPLQETAPFGCK (1,717.8 Da)								
	3695.5	0.105	4	1977.8	+0.1	5	5	1	0
	3857.5	0.109	4	2139.9	+0.2	5	6	1	0
	4019.6	0.249	4	2301.9	+0.1	5	7	1	0
E2 196	NITYECKCGDYKTGTVSTR (2,138.0 Da)								
	3341.6	0.007	3	1203.5	−0.1	2	4	1	0
	3503.6	0.014	3	1365.5	−0.1	2	5	1	0
	3665.7	0.035	3	1527.6	−0.1	2	6	1	0

E2 196		VYAKPPSGKNITYECKGDY (2,420.1 Da)							
	3461.5	0.001	3	1041.4	0.0	2	3	1	0
	3623.6	0.002	3	1203.5	0.0	2	4	1	0
	3785.6	0.001	3	1365.5	0.0	2	5	1	0
	3623.5	0.009	4	1203.5	+0.1	2	4	1	0
	3795.6	0.108	4	1375.5	0.0	3	2	1	1
	3827.7	0.000	4	1407.6	0.0	3	4	1	0
	3957.7	0.154	4	1537.6	0.0	3	3	1	1
	4119.7	0.111	4	1699.7	+0.1	3	4	1	1
E2 196		NITYECK (926.4 Da)							
	2830.2	0.006	2	1903.7	-0.1	4	4	1	1
	2992.3	0.108	2	2065.8	-0.1	4	5	1	1
	3154.3	0.098	2	2227.9	0.0	4	6	1	1
	3316.4	0.054	2	2389.9	-0.1	4	7	1	1
	2950.3	0.001	3	2023.8	-0.1	3	6	1	1
	3112.4	0.000	3	2185.8	-0.2	3	7	1	1
E2 196		NITYECKGDYK (1,435.6 Da)							
	2885.2	0.002	3	1449.6	0.0	4	3	1	0
	3047.3	0.004	3	1611.7	0.0	4	4	1	0
	3209.3	0.006	3	1773.7	0.0	4	5	1	0
E2 196		VYAKPPSGKNITYECKGDY (2,477.2 Da)							
	3695.5	0.023	3	1218.5	+0.2	2	5	0	0
	3857.5	0.024	3	1380.5	+0.2	2	6	0	0
	4019.6	0.054	3	1542.6	+0.2	2	7	0	0
	4181.6	0.039	3	1704.7	+0.3	2	8	0	0
	3695.5	0.023	4	1218.5	+0.2	2	5	0	0
	3857.5	0.024	4	1380.5	+0.2	2	6	0	0
	4019.6	0.054	4	1542.6	+0.2	2	7	0	0
	4181.6	0.039	4	1704.7	+0.3	2	8	0	0
E2 318		RLGANPEPTTEWIVGKTVRNFTVDR (2,871.4 Da)							
	5062.0	0.191	4	2190.8	+0.2	2	11	0	0
	5386.1	0.405	4	2515.0	+0.3	2	13	0	0
	5386.1	0.405	5	2515.0	+0.3	2	13	0	0

Supplementary Table 3. The screening library used for the lipidomics analysis. Lipid species are listed with the molecular weight of the parent mass and fragment mass used for MS/MS analysis.

<i>N</i> -acyl alanine	[M-H] ⁻	Fragment	<i>N</i> -acyl proline	[M-H] ⁻	Fragment
<i>N</i> -palmitoyl alanine	326.5	88.09	<i>N</i> -palmitoyl proline	352.53	114.12
<i>N</i> -stearoyl alanine	354.55	88.09	<i>N</i> -stearoyl proline	380.59	114.12
<i>N</i> -oleoyl alanine	352.53	88.09	<i>N</i> -oleoyl proline	378.31	114.12
<i>N</i> -linoleoyl alanine	350.52	88.09	<i>N</i> -linoleoyl proline	376.56	114.12
<i>N</i> -arachidonoyl alanine	374.5	88.09	<i>N</i> -arachidonoyl proline	400.58	114.12
<i>N</i> -docosahexaenoyl alanine	398.56	88.09	<i>N</i> -docosahexaenoyl proline	424.6	114.12
<i>N</i> -acyl ethanolamine	[M+H] ⁺	Fragment	<i>N</i> -acyl serine	[M-H] ⁻	Fragment
<i>N</i> -palmitoyl ethanolamine	300.29	62.1	<i>N</i> -palmitoyl serine	342.3	74
<i>N</i> -stearoyl ethanolamine	328.3	62.1	<i>N</i> -stearoyl serine	370.3	74
<i>N</i> -oleoyl ethanolamine	326.3	62.1	<i>N</i> -oleoyl serine	368.3	74
<i>N</i> -linoleoyl ethanolamine	324.3	62.1	<i>N</i> -linoleoyl serine	366.27	74
<i>N</i> -arachidonoyl ethanolamine	348.29	62.1	<i>N</i> -arachidonoyl serine	390.3	74
<i>N</i> -docosahexaenoyl ethanolamine	372.6	62.1	<i>N</i> -docosahexaenoyl serine	414.3	74
<i>N</i> -acyl GABA	[M-H] ⁻	Fragment	<i>N</i> -acyl taurine	[M-H] ⁻	Fragment
<i>N</i> -palmitoyl GABA	340.54	102.1	<i>N</i> -palmitoyl taurine	362.6	124
<i>N</i> -stearoyl GABA	368.58	102.1	<i>N</i> -stearoyl taurine	390.6	124
<i>N</i> -oleoyl GABA	366.57	102.1	<i>N</i> -oleoyl taurine	388.6	124
<i>N</i> -linoleoyl GABA	364.54	102.1	<i>N</i> -arachidonoyl taurine	410.6	124
<i>N</i> -arachidonoyl GABA	388.57	102.1	<i>N</i> -acyl tryptophan	[M-H] ⁻	Fragment
<i>N</i> -docosahexaenoyl GABA	412.59	102.1	<i>N</i> -palmitoyl tryptophan	441.63	203.1
<i>N</i> -acyl glycine	[M-H] ⁻	Fragment	<i>N</i> -stearoyl tryptophan	469.68	203.1
<i>N</i> -palmitoyl glycine	312.26	74.2	<i>N</i> -oleoyl tryptophan	467.67	203.1
<i>N</i> -stearoyl glycine	340.3	74.2	<i>N</i> -linoleoyl tryptophan	465.65	203.1
<i>N</i> -oleoyl glycine	338.3	74.2	<i>N</i> -arachidonoyl tryptophan	489.67	203.1
<i>N</i> -linoleoyl glycine	336.3	74.2	<i>N</i> -docosahexaenoyl tryptophan	513.69	203.1
<i>N</i> -arachidonoyl glycine	360.3	74.2	<i>N</i> -acyl tyrosine	[M-H] ⁻	Fragment
<i>N</i> -docosahexaenoyl glycine	384.3	74.2	<i>N</i> -palmitoyl tyrosine	418.59	180.18
<i>N</i> -acyl leucine	[M-H] ⁻	Fragment	<i>N</i> -stearoyl tyrosine	446.65	180.18
<i>N</i> -palmitoyl leucine	368.58	130.1	<i>N</i> -oleoyl tyrosine	444.63	180.18
<i>N</i> -stearoyl leucine	396.63	130.1	<i>N</i> -linoleoyl tyrosine	442.61	180.18
<i>N</i> -oleoyl leucine	394.61	130.1	<i>N</i> -arachidonoyl tyrosine	466	180.18
<i>N</i> -linoleoyl leucine	392.6	130.1	<i>N</i> -docosahexaenoyl tyrosine	490.66	180.18
<i>N</i> -docosahexaenoyl leucine	440.64	130.1	<i>N</i> -acyl valine	[M-H] ⁻	Fragment
<i>N</i> -acyl methionine	[M-H] ⁻	Fragment	<i>N</i> -palmitoyl valine	354.31	116.31
<i>N</i> -palmitoyl methionine	386.62	148.2	<i>N</i> -stearoyl valine	382.6	116.14
<i>N</i> -stearoyl methionine	414.64	148.2	<i>N</i> -oleoyl valine	380.59	116.14
<i>N</i> -oleoyl methionine	412.65	148.2	<i>N</i> -linoleoyl valine	378.58	116.14
<i>N</i> -linoleoyl methionine	410.64	148.2	<i>N</i> -docosahexaenoyl valine	426.62	116.14
<i>N</i> -arachidonoyl methionine	434.66	148.2	Free Fatty Acids	[M-H] ⁻	Fragment
<i>N</i> -docosahexaenoyl methionine	458.68	148.2	Oleic acid	281.5	263
<i>N</i> -acyl phenylalanine	[M-H] ⁻	Fragment	Linoleic acid	279.5	261
<i>N</i> -palmitoyl phenylalanine	402.59	164.1	Arachidonic acid	303.5	285
<i>N</i> -stearoyl phenylalanine	430.65	164.1	PhosphoLEA	[M-H] ⁻	Fragment
<i>N</i> -oleoyl phenylalanine	428.63	164.1	phosphoLEA	403.5	58.5
<i>N</i> -linoleoyl phenylalanine	426.61	164.1	2-acyl glycerol	[M+H] ⁺	Fragment
<i>N</i> -arachidonoyl phenylalanine	450.64	164.1	2-arachidonoyl glycerol	379.3	287.5
<i>N</i> -docosahexaenoyl phenylalanine	474.66	164.1	2-linoleoyl glycerol	355.5	245
			2-oleoyl glycerol	357.5	265.2
			2-palmitoyl glycerol	331.5	239.5
			Prostaglandins	[M-H] ⁻	Fragment
			PGE ₂	351.2	315
			PGF _{2α}	353.3	309.2
			6-ketoPGF _{1α}	369.3	206.9

Supplementary Table 4. The 25 lipids that showed a significant difference in concentration

between SINV from BHK when and SINV from C6/36. Concentrations are listed in moles lipid species/gram viral particles.

Lipid	Formula	BHK		C6/36		Difference (BHK/C6)
		Avg Conc.	Std Dev	Avg Conc.	Std Dev	
N-oleoyl ethanolamine	$C_{20}H_{39}NO_2$	2.58E-08	5.67E-09	4.28E-11	3.55E-11	603
N-linoleoyl ethanolamine	$C_{20}H_{37}NO_2$	1.59E-10	7.92E-11	1.13E-11	1.79E-12	14.1
N-oleoyl tyrosine	$C_{27}H_{43}NO_4$	3.60E-10	1.17E-10	4.86E-11	3.24E-11	7.4
Arachidonic Acid	$C_{20}H_{32}O_2$	6.50E-08	6.42E-09	1.17E-08	3.23E-09	5.6
N-stearoyl tyrosine	$C_{27}H_{45}NO_4$	3.57E-11	1.22E-11	6.59E-12	2.19E-12	5.4
N-palmitoyl tyrosine	$C_{25}H_{41}NO_4$	1.75E-10	4.04E-11	3.37E-11	2.15E-11	5.2
N-oleoyl leucine	$C_{24}H_{45}NO_3$	1.01E-09	3.88E-10	2.01E-10	1.95E-12	5.0
N-oleoyl phenylalanine	$C_{27}H_{43}NO_3$	1.58E-09	4.21E-10	3.21E-10	5.95E-11	4.9
2-arachidonoyl glycerol	$C_{23}H_{38}O_4$	1.35E-08	2.37E-09	3.20E-09	9.10E-10	4.2
N-stearoyl ethanolamine	$C_{20}H_{41}NO_2$	5.01E-09	1.13E-09	1.28E-09	1.71E-10	3.9
Eicosapentaenoic Acid	$C_{20}H_{30}O_2$	1.12E-08	3.12E-09	3.56E-09	1.20E-09	3.2
N-oleoyl valine	$C_{23}H_{43}NO_3$	3.08E-10	1.29E-10	9.80E-11	5.84E-11	3.1
N-stearoyl phenylalanine	$C_{27}H_{45}NO_3$	9.43E-10	9.18E-11	3.39E-10	2.90E-11	2.8
N-palmitoyl phenylalanine	$C_{25}H_{41}NO_3$	8.69E-10	5.16E-11	3.23E-10	1.53E-10	2.7
2-oleoyl glycerol	$C_{21}H_{40}O_4$	1.32E-07	1.63E-08	4.97E-08	4.12E-09	2.7
N-palmitoyl ethanolamine	$C_{18}H_{37}NO_2$	7.26E-09	1.83E-09	2.76E-09	5.40E-10	2.6
N-stearoyl leucine	$C_{24}H_{47}NO_3$	7.88E-10	8.36E-11	3.13E-10	7.90E-11	2.5
Linoleic Acid	$C_{18}H_{32}O_2$	5.85E-08	7.37E-09	2.79E-08	3.96E-09	2.1
N-arachidonoyl ethanolamine	$C_{22}H_{37}NO_2$	3.48E-10	6.15E-11	1.68E-10	3.81E-11	2.1
Oleic Acid	$C_{18}H_{34}O_2$	1.12E-07	1.30E-08	5.61E-08	2.90E-09	2.0
N-stearoyl valine	$C_{23}H_{45}NO_3$	3.10E-10	5.96E-11	1.66E-10	7.19E-11	1.87
N-palmitoyl leucine	$C_{22}H_{43}NO_3$	5.81E-10	6.76E-11	3.22E-10	8.88E-11	1.80
2-linoleoyl glycerol	$C_{21}H_{38}O_4$	1.25E-08	2.55E-09	7.48E-09	7.67E-10	1.67
2-palmitoyl glycerol	$C_{19}H_{38}O_4$	2.53E-07	3.25E-08	1.73E-07	1.64E-08	1.46
N-palmitoyl GABA	$C_{20}H_{39}NO_3$	4.31E-10	1.93E-11	6.84E-10	5.53E-11	0.63