Expanding the Types of Lipids Amenable to Native Mass Spectrometry of Lipoprotein Complexes

Supplementary Material

*Marius M. Kostelic, Alex M. Ryan, Deseree J. Reid, Jibriel M. Noun, and Michael T. Marty**

University of Arizona, Department of Chemistry and Biochemistry, Tucson, AZ 85721

*Address reprint request to:

Michael T. Marty

1306 E University Blvd

Tucson, AZ 85721

Email: mtmarty@email.arizona.edu

SUPPLEMENTAL FIGURES

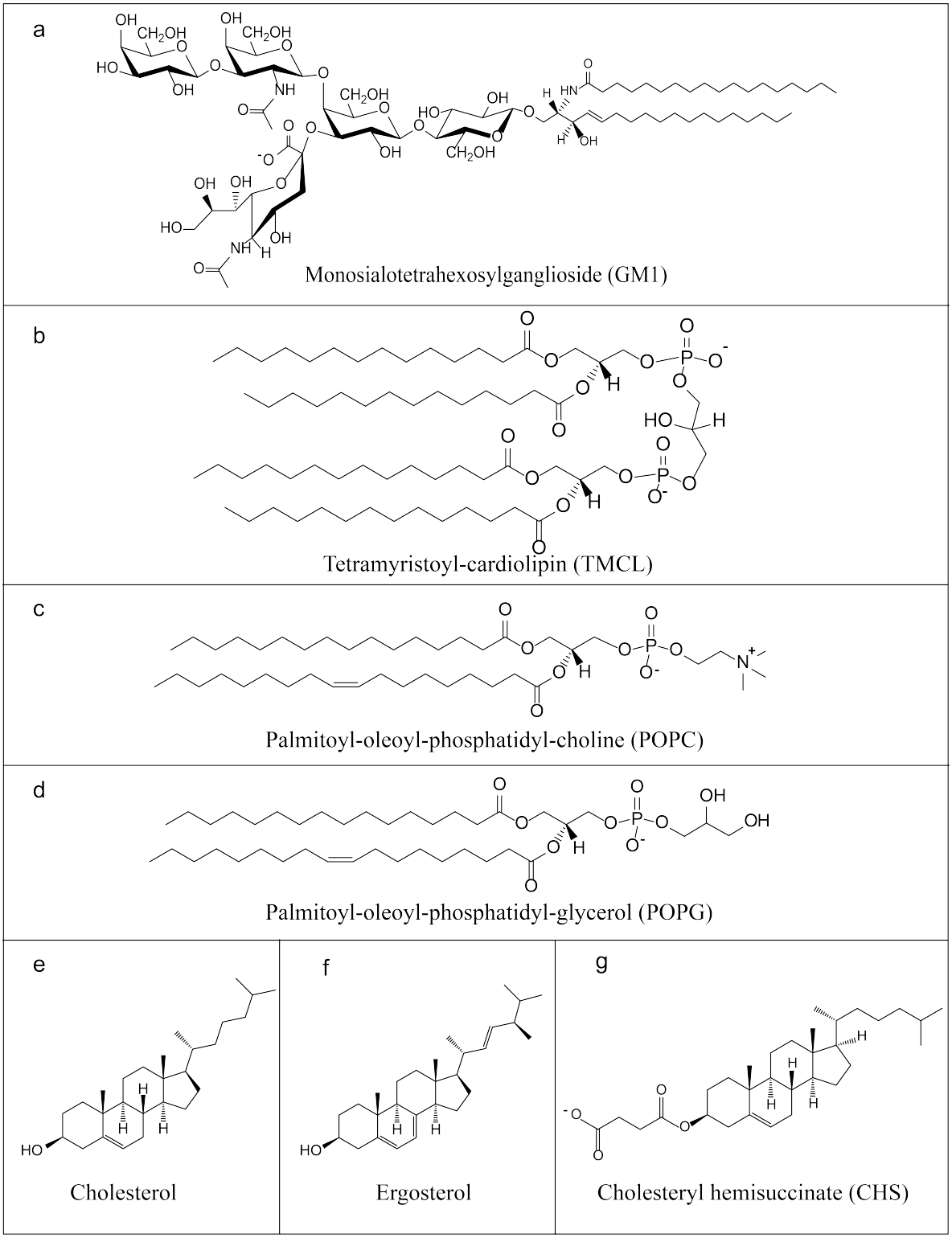

Figure S1: Structures of lipids used for lipoprotein complex formation.

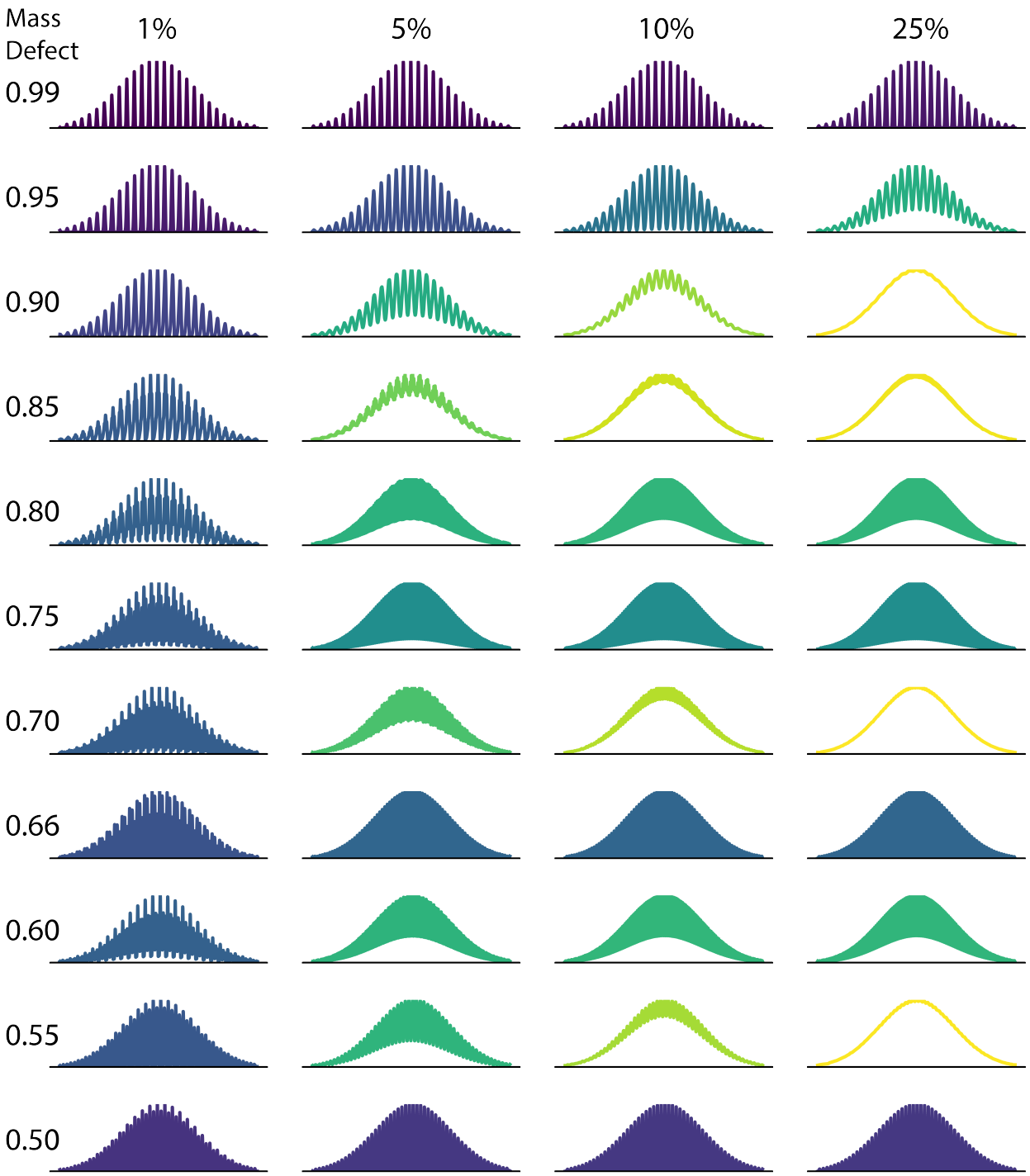

Figure S2: Simulated mass distributions for nanodiscs with a peak full width at half max of 100 Da. Nanodiscs were simulated with a Gaussian distribution in the number of total lipids centered at 140 lipids with a standard deviation of ±5 lipids. The reference lipid mass was set to the mass of POPC, 760 Da. An additional theoretical lipid with mass defects ranging from 0.5 to 0.99 (*left*)

was mixed at a ratio of 1, 5, 10, or 25% (*top*). The mass of the added lipid was calculated as the mass defect times the reference lipid mass. Because the absolute mass shifts with added lipid, the x-axis is not labelled for clarity, but each x-axis spans a uniform 20 kDa in mass. A binomial distribution was assumed for the number of each of the two lipid types. Colors show the degree of overlap between peaks ranging from no overlap (*purple*) to complete overlap (*yellow*).

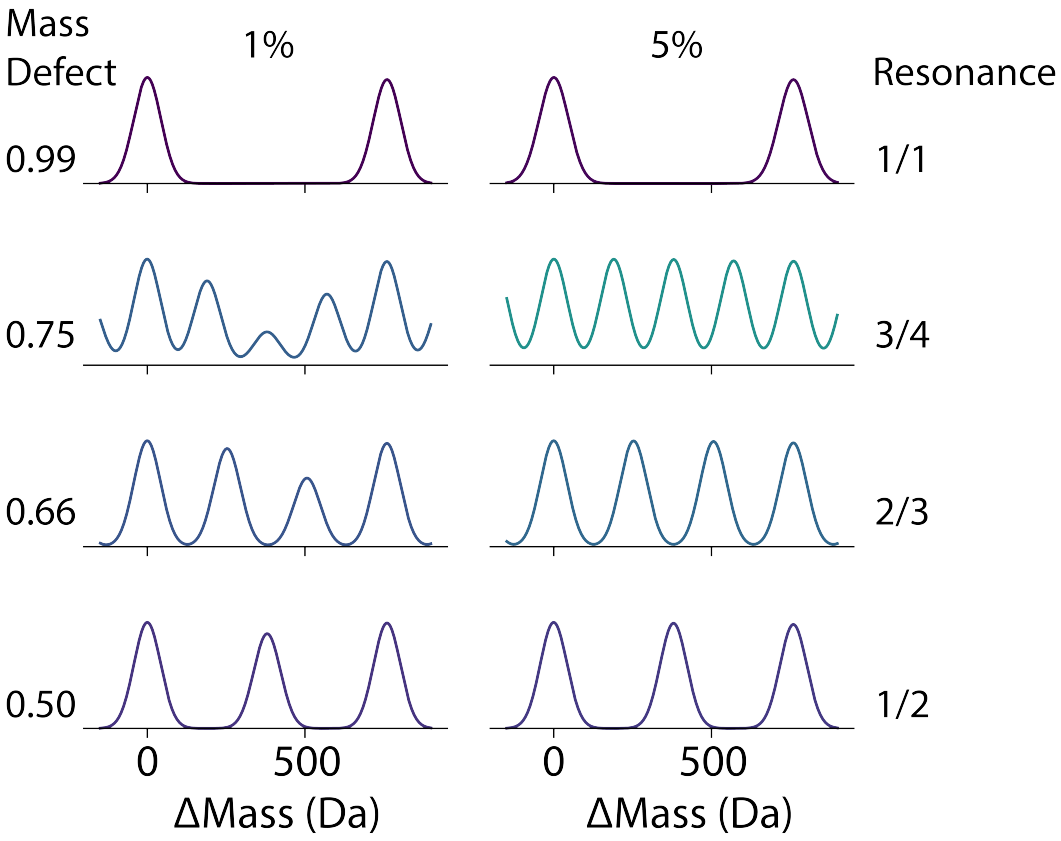

Figure S3: Zoomed simulated mass distributions for select mass defect values and mixing ratios from Figure S2. The x-axis is plotted as the change in mass from the highest peak in the distribution and focuses on only the region between the highest peak and +1 POPC molecule. The denominator of the resonance (*right*) determines how many peak series are present.

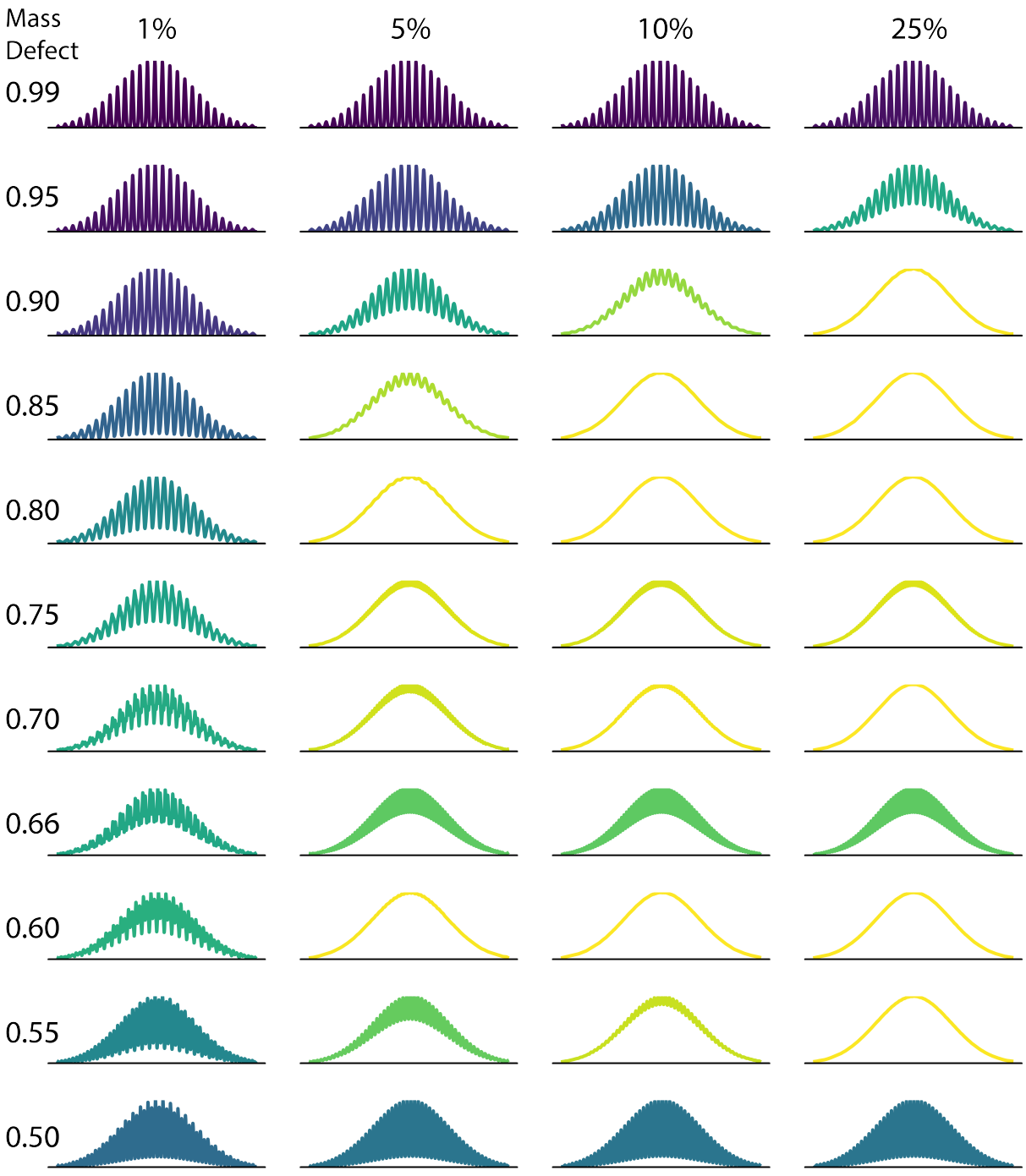

Figure S4: Simulated mass distributions for nanodiscs as described in Figure S2 except with a peak full width at half max of 200 Da.

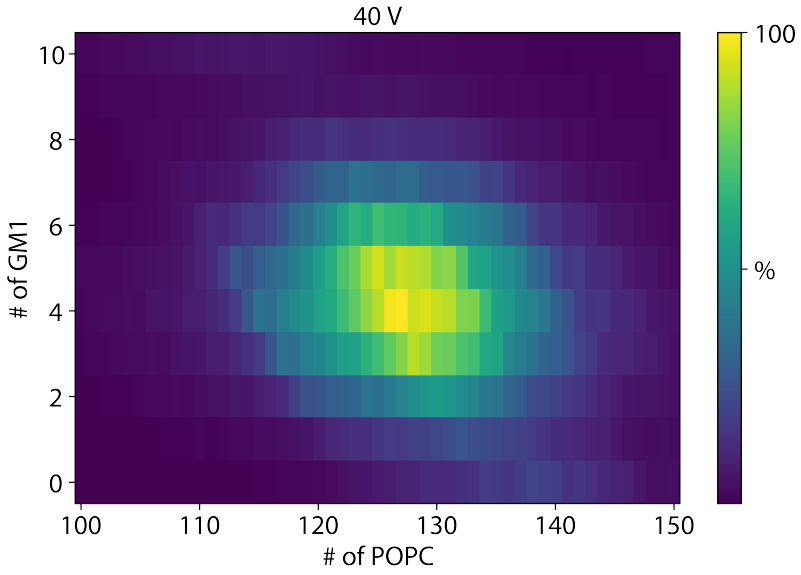

Figure S5: The extracted intensity of the mass distribution at 40 V CID for POPC/GM1 nanodiscs with two MSP1D1T1(-) belts and different numbers of POPC and GM1 per complex.

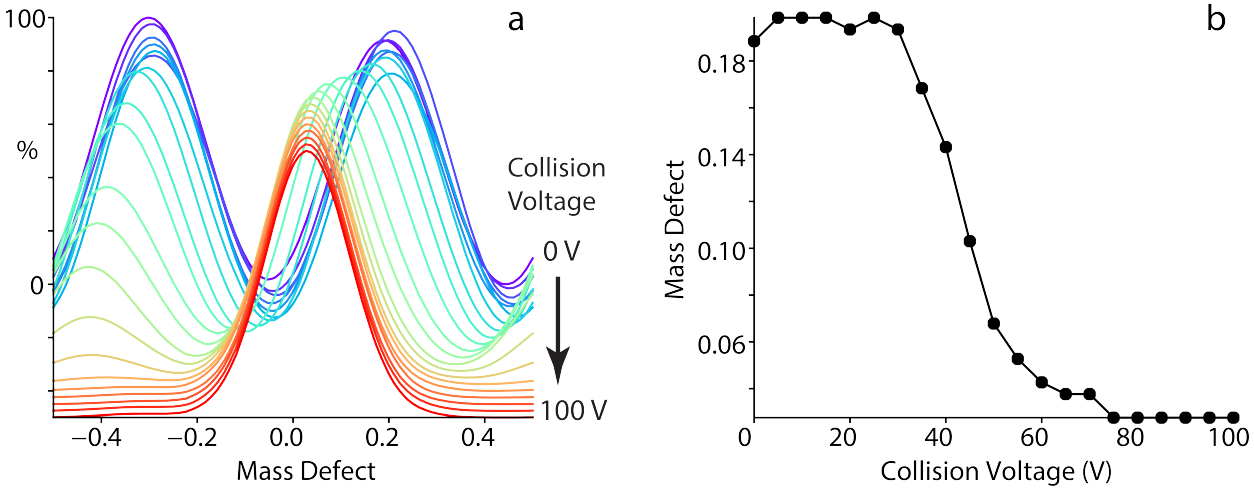

Figure S6: a) The mass defect values summed across the spectrum for POPC/cholesterol nanodiscs from 0 (*purple*) to 100 V (*red*) CID. Note: this is the same data as Figure 2b *right* but centered from -0.5 to 0.5 to clearly show the shift in the mass defect peak for the evens. b) The extracted peak position of the even mass defect series as a function of collision voltage. A clear shift is seen around 40 V due to dissociation of cholesterol from the nanodisc.

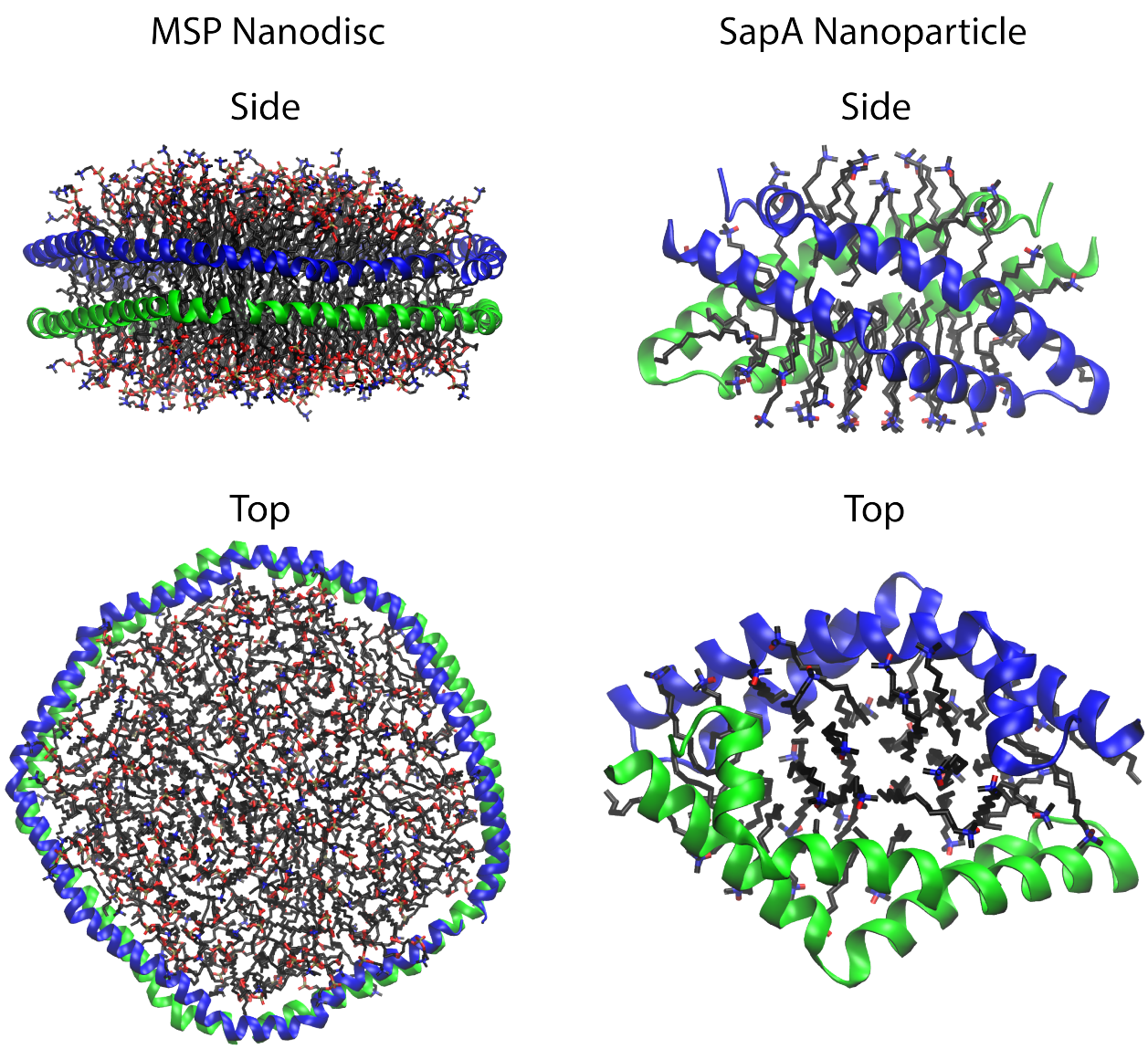

Figure S7: Schematic of MSP nanodiscs (*left*) and an example Saposin A nanoparticle (*right*), which is taken from PDB: 4DDJ in complex with lauryl-dimethylamine oxide detergent. Each complex contains two proteins, one *blue* and one *green*. MSP wraps around the lipid bilayer with one MSP per each leaflet. In contrast, each SapA contacts both leaflets but does not encircle the bilayer.

SUPPLEMENTAL TABLES

Reference Lipid	Name	Mass (Da)	Mass Defect
POPC	MSPD1(-)	22,044	0.002
	MSPD1T1(-)	22,145	0.135
	GM1	1,573.0	0.070
	Cholesterol	386.66	0.509
	Ergosterol	396.66	0.522
	CHS	486.73	0.640
	POPC	760.08	0.000
POPG	MSPD1(-)	22,044	0.431
	SapA	9,131	0.190
	TMCL	1,241.6	0.655
	POPG	749.00	0.000

Table S1: Protein and lipid analytes with corresponding masses and calculated mass defect values for either POPC (*top*) or POPG (*bottom*) reference lipids. Note: GM1 is a mixture of two masses (1,545 and 1,573 Da) but is annotated for only the most abundant.

		Number of Molecules					
Pattern	**Name**	**1**	**2**	**3**	**4**	**5**	**6**
Single	*SapA*	0.19	0.38	0.57	0.76	0.95	0.14
	GM1	0.07	0.14	0.21	0.28	0.35	0.42
Double	Cholesterol	0.51	0.02	0.53	0.04	0.55	0.05
	Ergosterol	0.52	0.04	0.57	0.09	0.61	0.13
Triple	CHS	0.64	0.28	0.92	0.56	0.20	0.93
	TMCL	0.66	0.31	0.97	0.62	0.28	0.84

Table S2: The predicted mass defect values for lipids and proteins categorized by resulting mass defect pattern. Calculated mass defects are for POPC as the reference lipid except where italicized, which denotes POPG as the reference. Note: GM1 is a mixture of two masses (1,545 and 1,573 Da) but is annotated for only the most abundant.