

SUPPORTING INFORMATION

LEGENDS TO SUPPLEMENTAL FIGURES

Figure S-1. Schematic representation of the reaction catalyzed by LRAT. The proposed mechanism of this reaction involves acyl-enzyme intermediate formation (1) prior to transfer of the acyl group to retinoid (2).

Figure S-2. Graphic representation of GST-tLRAT fusion protein. Color code of the primary GST-tLRAT sequence indicates GST (black letters), thrombin recognition site (red), and LRAT (green). The C-terminal sequence of EFIVTD is part of the pGEX_2T vector cloning site. Cleavage of the fusion protein with thrombin (marked with an arrow) liberates two peptide fragments shown with white and yellow backgrounds and calculated masses of 26,167 Da (GST) and 18,523 Da (tLRAT), respectively.

Figure S-3. Zoomed MS spectrum shows a group of selected multiply-charged protein ions for unmodified and acylated GST-tLRAT upon incubation with 0.3 mM of 8:0 PC. The protein became covalently modified as indicated by an additional peak at the higher m/z values marked by asterisks.

Figure S-4. Thrombin digest of GST-tLRAT reveals the acyl modification site located within the tLRAT sequence. The fusion protein was cleaved with thrombin. Intact proteins in the reaction mixture were separated on a C8 HPLC column and analyzed by MS. A. Chromatogram represents the elution profile of tLRAT and GST. B. MS spectra of unmodified tLRAT intact protein (18,522.4 Da) (left panel) and the liberated GST tag (26,164.0 Da) (right panel). C. Preincubation with 7:0 PC modified tLRAT is noted by the appearance of an additional series of peaks (masses shown in red) corresponding to the 18,635.6 Da protein (a 113 ± 2 Da change compared to unmodified tLRAT).

Figure S-5. Acylation of tLRAT as a function of 7:0 PC concentration. Water-soluble lipid serves as a substrate at concentrations below its CMC when only monomeric lecithin molecules are present. Concentrations above the 7:0 PC (CMC 1.4 mM) did not further increase the number of modified protein molecules.

Figure S-1

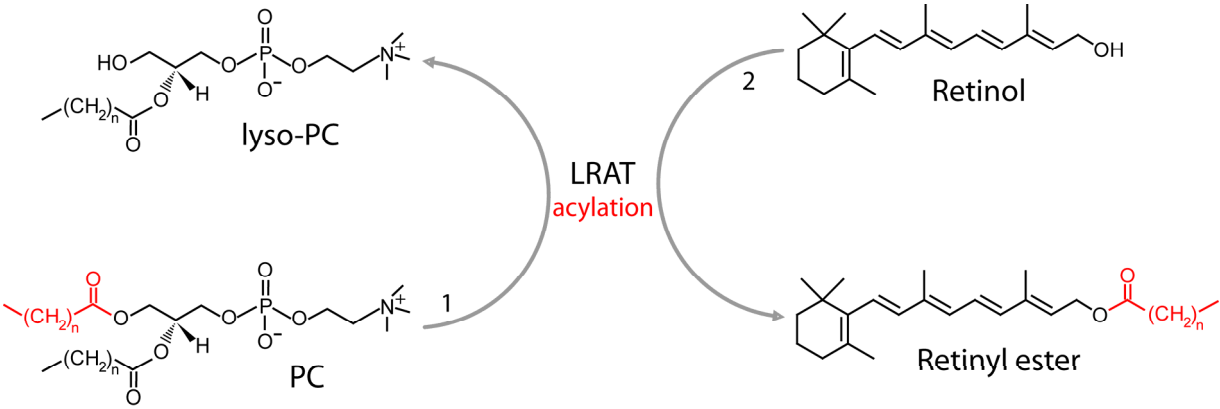


Figure S-2



MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGG

CPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPM

CLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSGGGTGKNRPYEISSFVRGDVLEVSRT

HFIHYGIYLGENRVAHLMPDILLALTNDKERTQKVVSNKRLLLGVICKVASIRVDTVEDFAYGADILVNHLDGTLKKKSLNEE

VARRAEQQLGLTPYSLWNNCEHFVITYCRYGSRISPQAEKFDYTVKEFIVTD

Total mass - 44,672 Da

26,167 Da

18,523 Da

Figure S-3

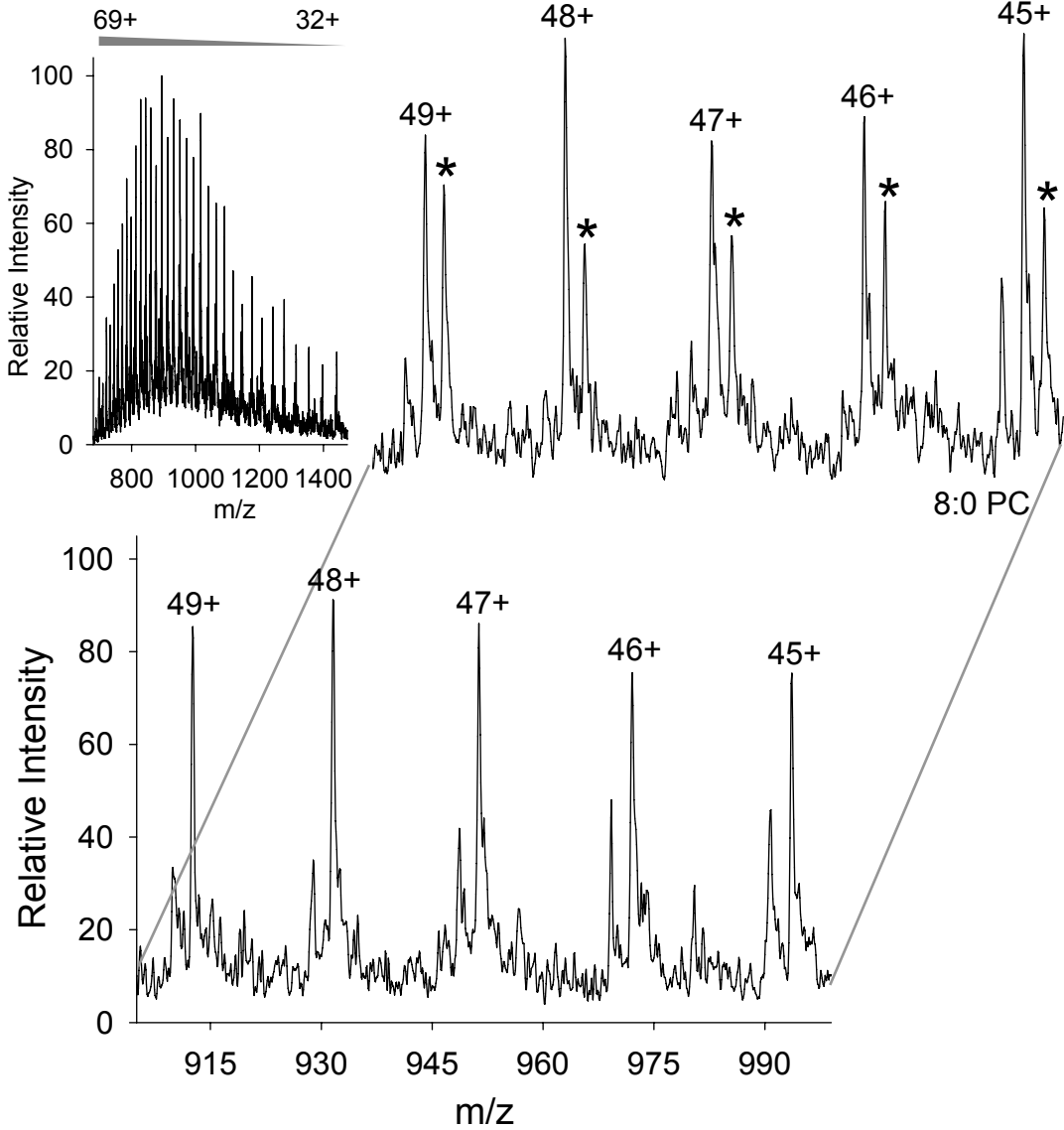


Figure S-4

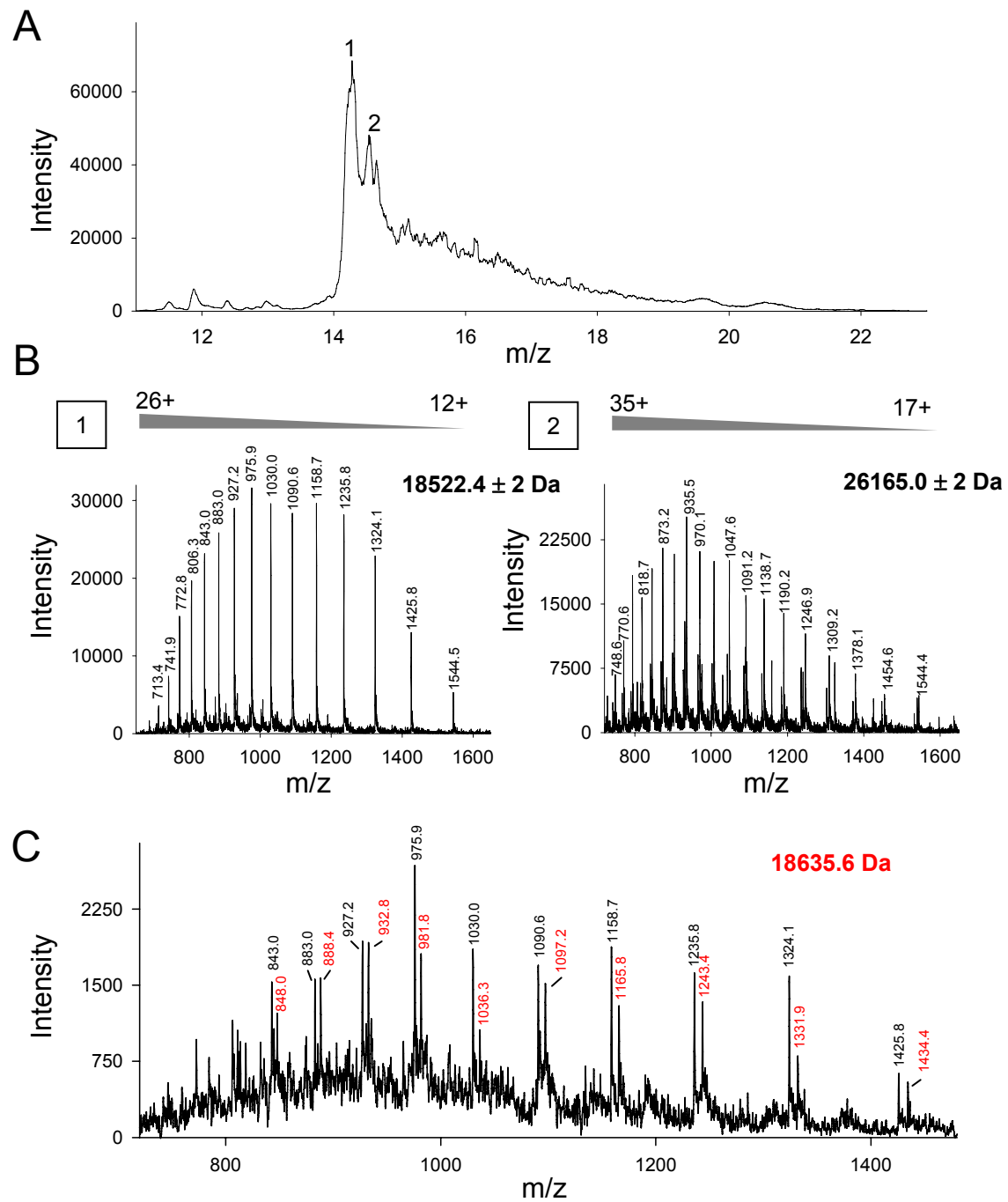


Figure S-5

