

Abstract

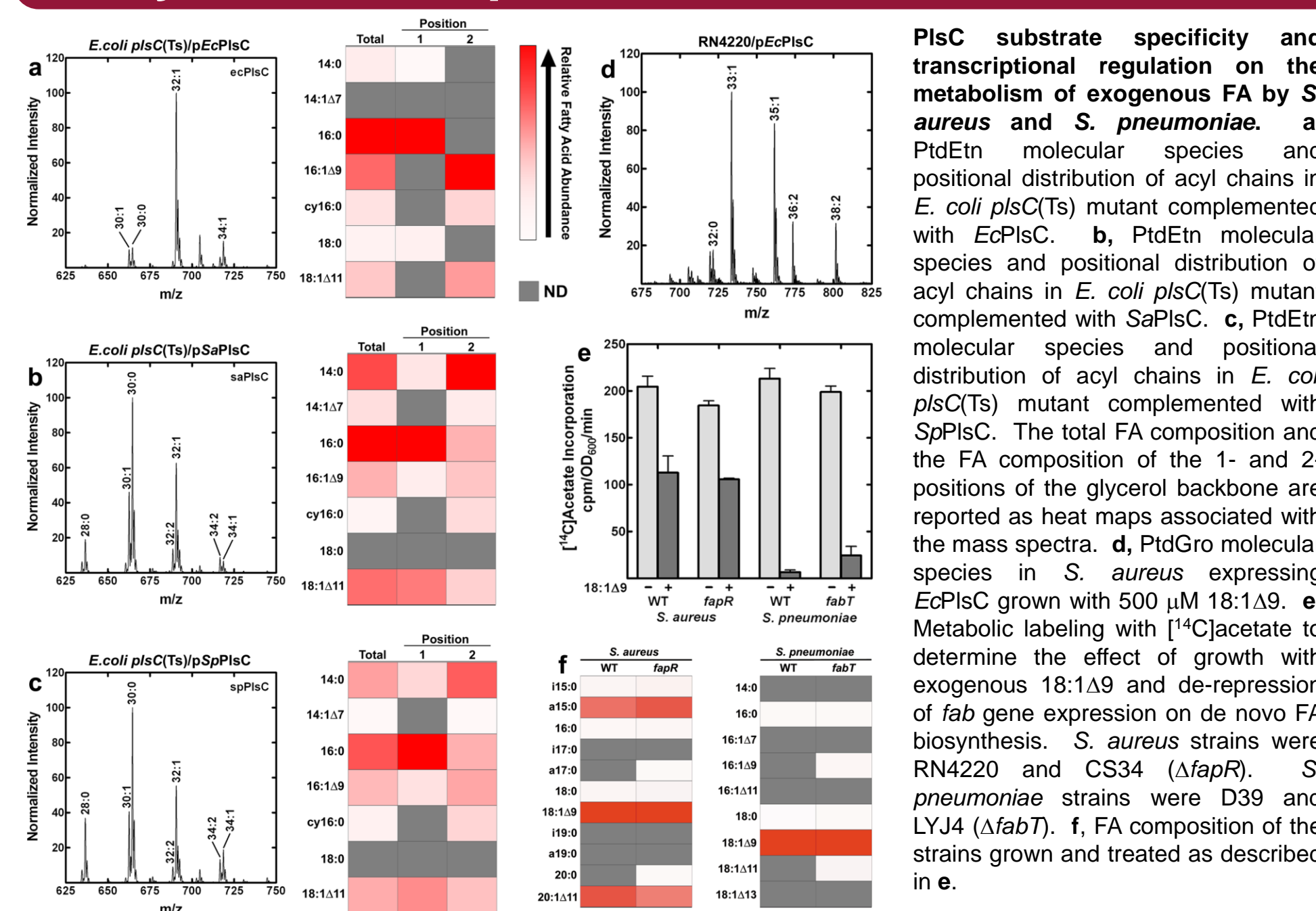
Background: Deploying fatty acid synthesis (FASII) inhibitors against Gram-positive pathogens has been questioned based on their ability to utilize exogenous fatty acids (FA). *Staphylococcus aureus* (*Sa*) and *Streptococcus pneumoniae* (*Sp*) represent the extremes of lipid metabolic diversity in Gram-positive bacteria, and our goal was to explain their different responses to extracellular FA in order to inform the development of FASII inhibitors.

Methods: Metabolic labeling, genetics, structural lipidomics and metabolomics analyses were used to determine the impact of exogenous FA on membrane biogenesis and the efficacy of FASII inhibitors.

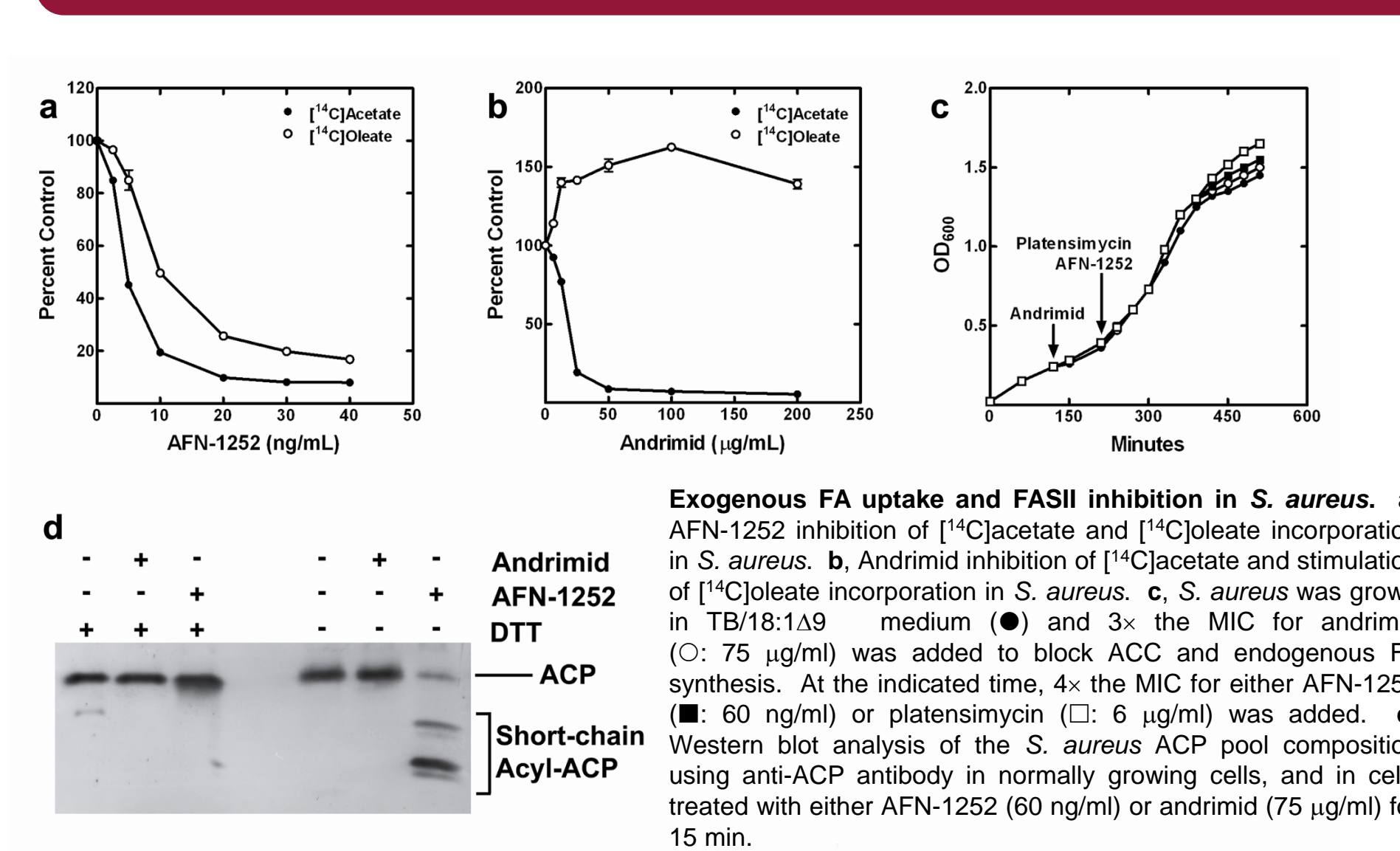
Results: *Sp* completely replaced endogenous FA with exogenous FA; however, *Sa* did not. Oleate was only incorporated into the 1-position of *Sa* phospholipids, but the substrate specificities of the Gram-positive acyltransferases were indistinguishable. *Sa* and *Sp* use different mechanisms to regulate FASII gene expression, but the pattern of FA utilization was not altered in strains with deleted transcription factors and elevated FASII enzymes. Exogenous FA overcame growth inhibition by FASII inhibitors in *Sp*. Growth inhibition of *Sa* by drugs targeting either FabI (AFN-1252) or FabF (platensimycin) was not prevented by exogenous FA; however, inhibition of acetyl-CoA carboxylase (andrimid) was overcome by a FA supplement. Exogenous FA biochemically repressed the formation of malonyl-CoA in *Sp* blocking endogenous FA formation at the acetyl-CoA carboxylase step. Repression of FASII activity by exogenous FA did not occur in *Sa*. AFN-1252 treatment led to acyl-acyl carrier protein accumulation, depleting cells of the cofactor needed to incorporate extracellular FA.

Conclusions: FASII inhibitors that target the elongation cycle cannot be overcome by providing *Sa* with exogenous FA, whereas in *Sp* they can. The ability of exogenous FA to suppress acetyl-CoA carboxylase activity in *Sp* and not *Sa* accounts for the differential response of Gram-positive bacteria to FASII inhibitors.

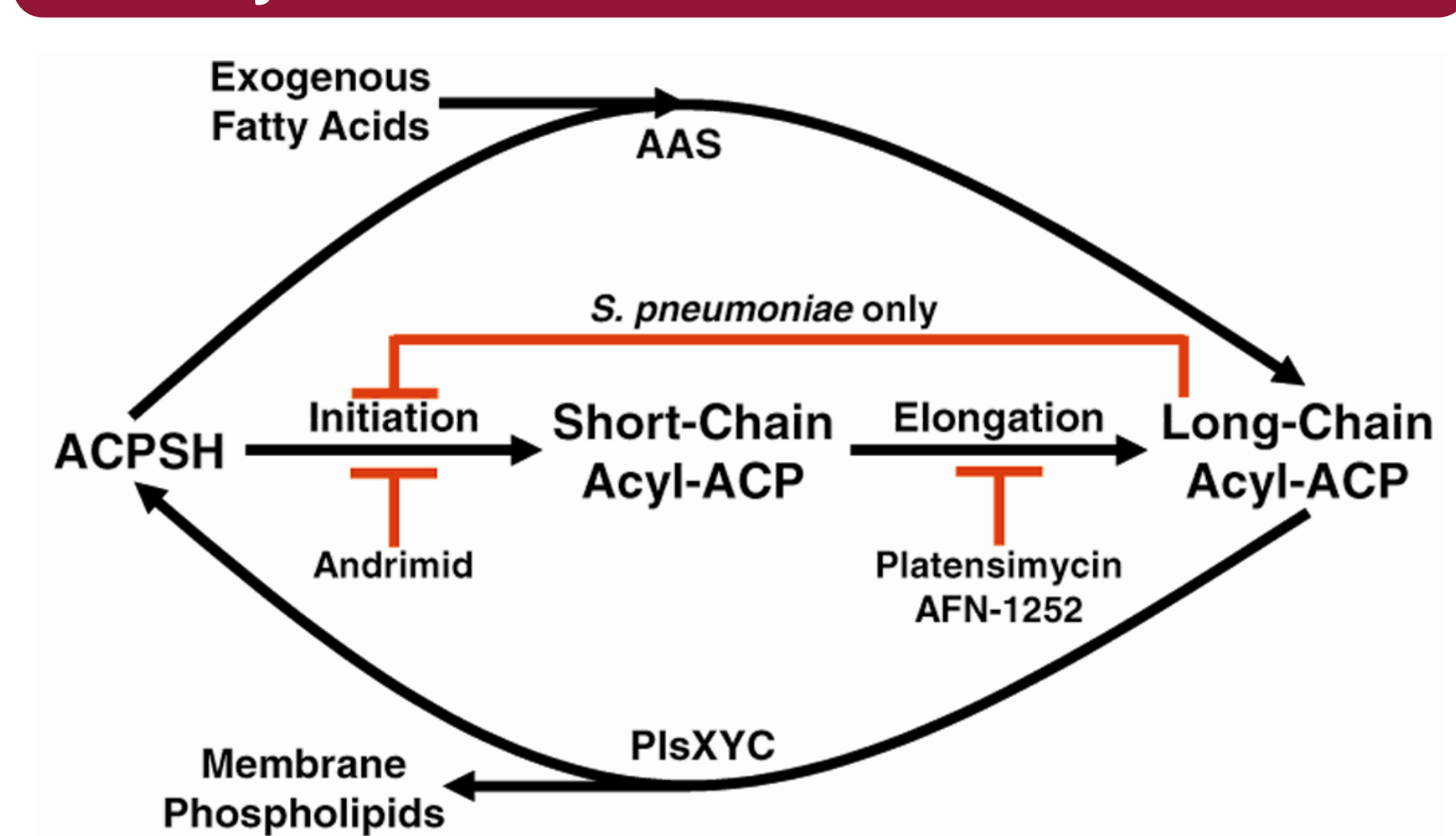
Exogenous Fatty Acids Replace Endogenous Fatty Acids in *S. pneumoniae*, but NOT *S. aureus*



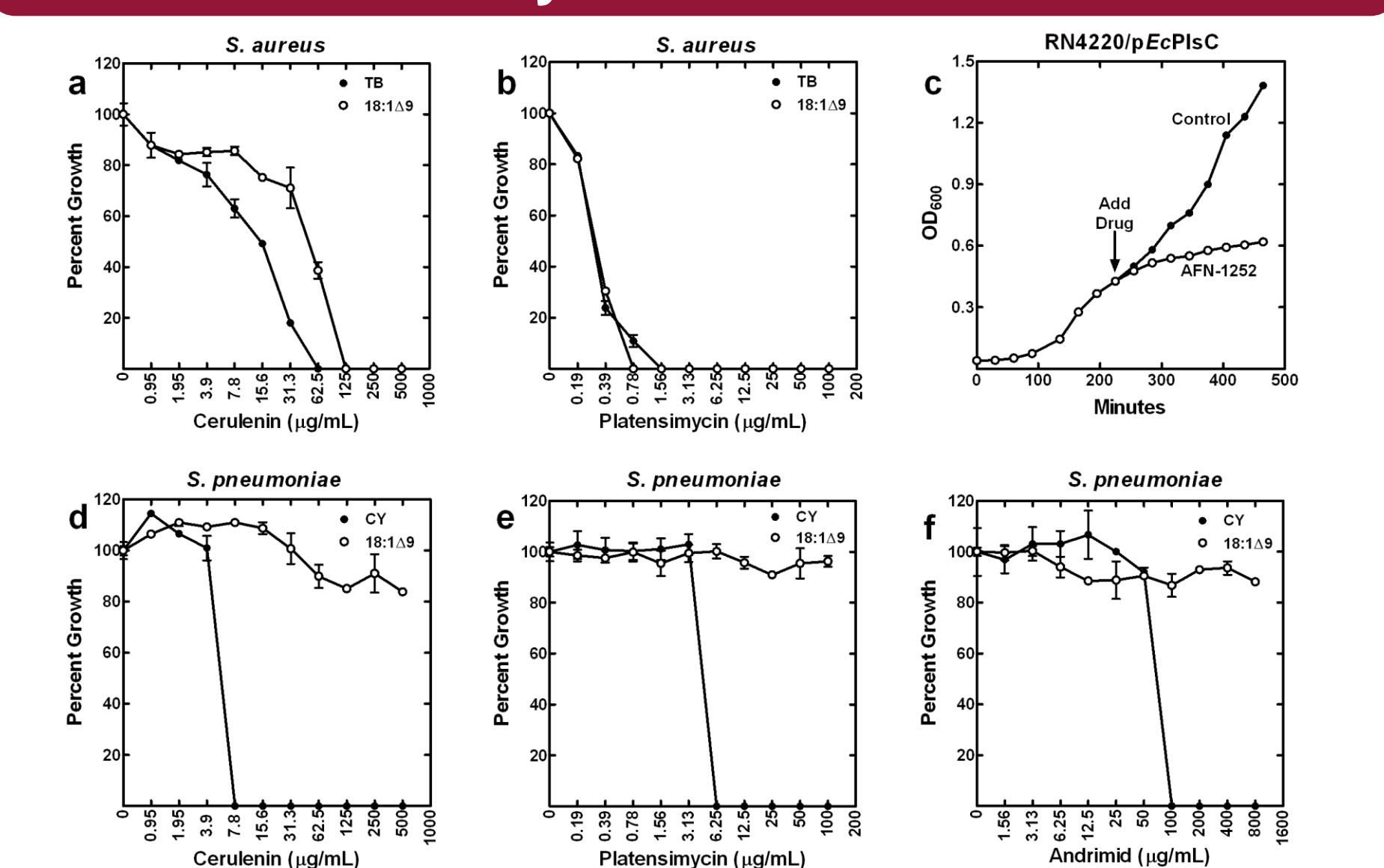
FASII Inhibitors Block Fatty Acid Incorporation in *S. aureus*



Biochemical Regulation of Fatty Acid Synthesis in Gram-Positive Bacteria



S. aureus and *S. pneumoniae* Respond Differently to FASII Inhibitors

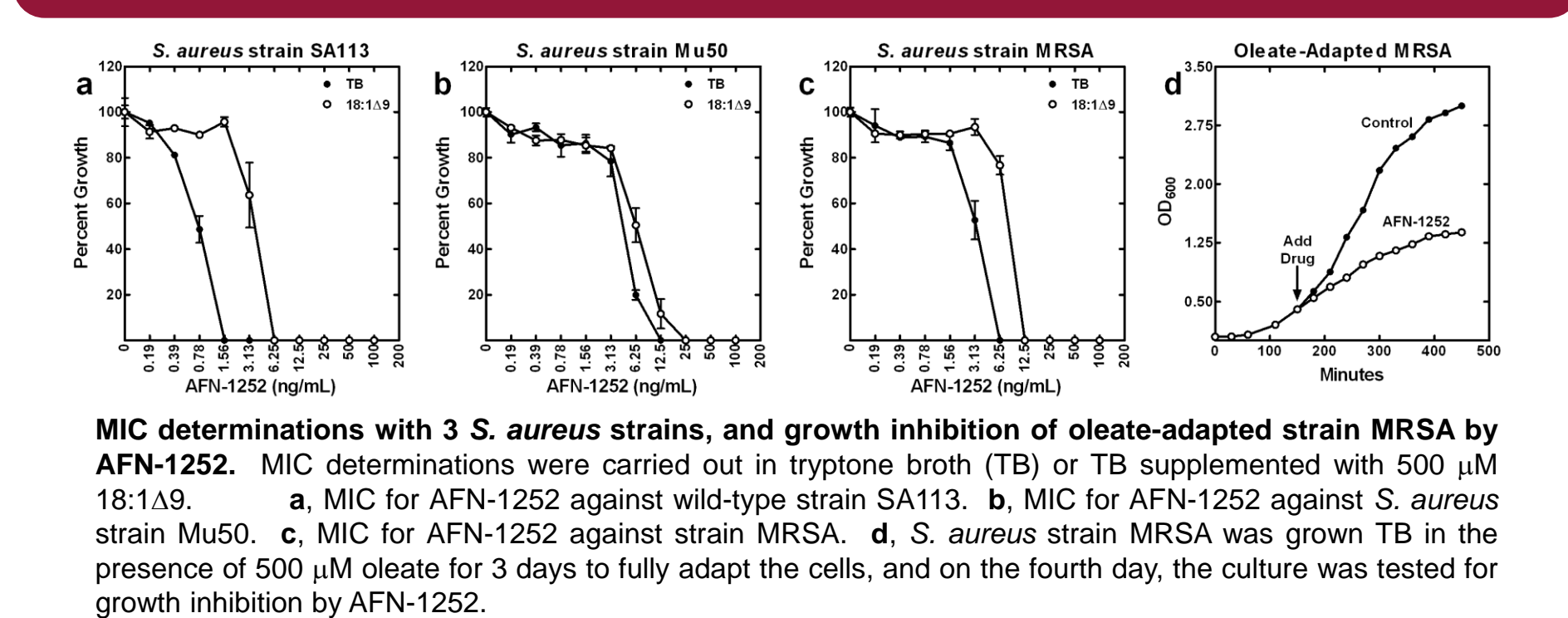


MIC determinations in the presence and absence of exogenous oleate (18:1Δ9). MIC for cerulenin (a) or platensimycin (b) against *S. aureus* strain RN4220 in the presence and absence of exogenous 18:1Δ9. c, *S. pneumoniae* strain RN4220 expression *E. coli* PlsC (pEcPlsC) was cultured in TB/18:1Δ9. At the indicated time, the culture was split and AFN-1252 (60 ng/ml) was added to one batch. MIC for cerulenin (d), platensimycin (e) or andrimid (f) against *S. pneumoniae* strain R6. TB, tryptone broth; CY, C + Y medium; 18:1Δ9, 500 μM oleate.

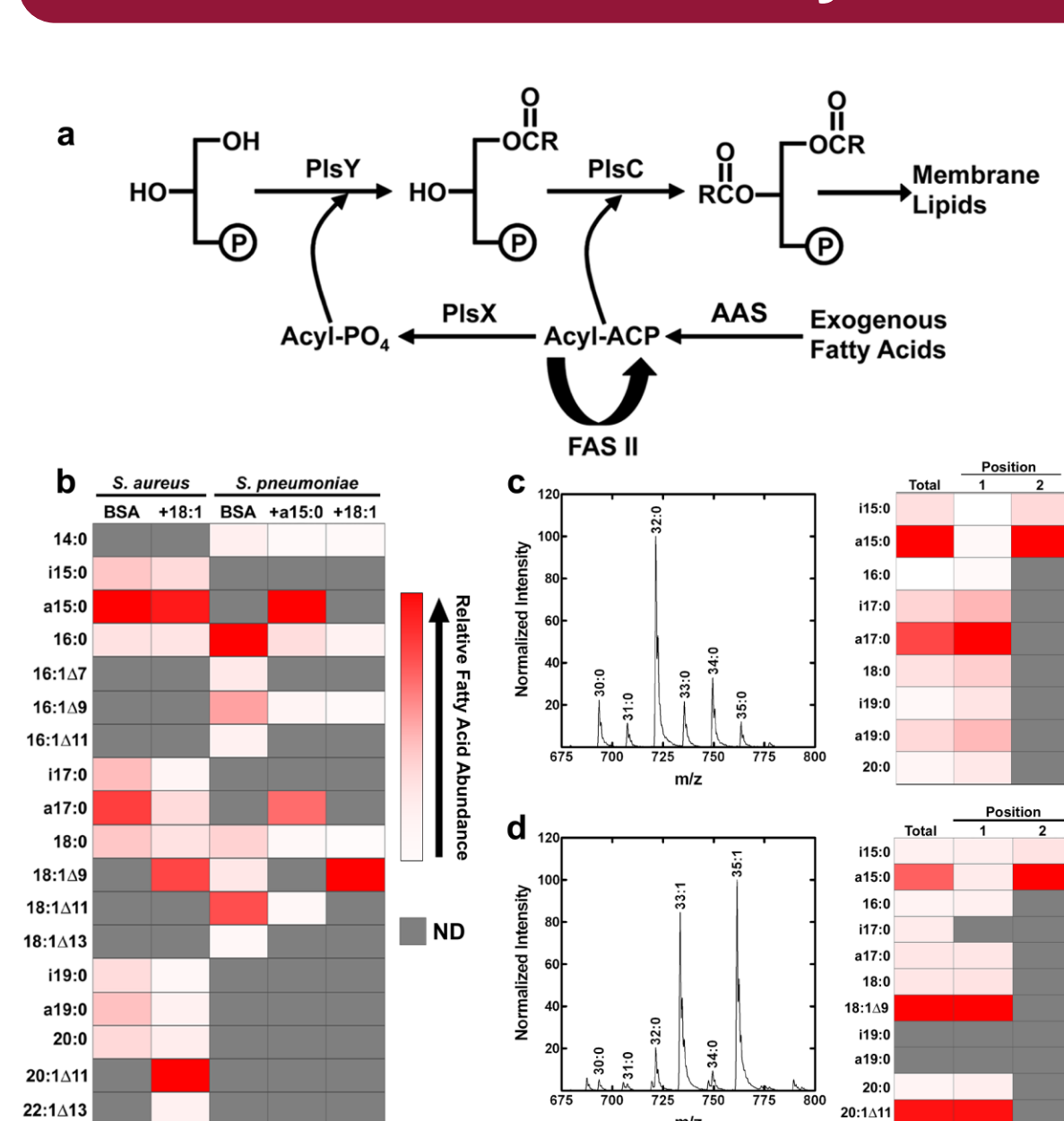
Conclusions

- Exogenous fatty acids cannot rescue *S. aureus* from FASII inhibitors.
- Fatty acid incorporation in Gram-positive bacteria goes through an acyl-ACP intermediate.
- Exogenous fatty acids strongly repress de novo fatty acid synthesis in *S. pneumoniae*, but not in *S. aureus*.
- Exogenous fatty acids rescue *S. aureus* from ACC inhibitors.
- Exogenous fatty acids rescue *S. pneumoniae* from FASII and ACC inhibitors.

Fatty Acid Cannot Rescue Multidrug Resistant *S. aureus* from FASII Inhibitors



Gram-Positive Bacteria Use the Same Enzymatic Tool Kit for Fatty Acid Metabolism



Assimilation of exogenous FA by *S. aureus* and *S. pneumoniae*. a, Pathway for the activation, metabolism and incorporation of exogenous FA into membrane phospholipids. Exogenous FA are ligated to ACP by an acyl-ACP synthetase (AAS). The acyl-ACP can use either used to acylate the 2-position by PlsC, converted to acyl-PO₄ by PlsX or elongated by FASII via the FabF condensing enzyme. b, FA composition of *S. aureus* or *S. pneumoniae* grown in the presence of exogenous FA. Relative FA abundance is indicated in the heat map. c, Analysis of PtdGro molecular species and positional distribution of acyl chains in *S. aureus* grown in the absence of exogenous FA. d, Analysis of the PtdGro molecular species and positional distribution of acyl chains in *S. pneumoniae* grown in the presence of 500 μM oleic acid (18:1Δ9). The total FA composition and the FA composition of the 1- and 2-positions of the glycerol backbone are reported as a heat map associated with the mass spectra in c and d.

Exogenous Fatty Acids Cannot Rescue *S. aureus* from FASII Inhibitors

