Supporting Information

Structural changes in cell wall and cell membrane organic materials following exposure to free nitrous acid

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Number of Figures: 19

Materials and Methods

Cell membrane materials	Category	Key function
Cellulose [S1]	Cell wall material	Cellulose is found in biofilms and cell walls. It is an important structural component in primary cell walls of algae and green plants.
Lipopolysaccharide [S2]	Cell membrane material	Contribute to the integrity of the outer membrane and protect the cell against (lipophilic) antibiotics [1]
Peptidoglycan [S3]	Cell wall material	Forms a mesh like layer in the cell wall, contributes to cell strength.
Plasmalogen [S4]	Cell membrane material	Type of phospholipid. Phospholipids form bilayers and contribute to the structure and integrity of cell membranes.
Lipoteichoic acid [S5]	Cell wall material	Crucial components of the cell envelope and provide control over the rigidity and porosity

		of the cell wall and influence the bacterium's morphology [2]

Table S1 Description of the cell envelope materials chosen to represent key cell wall and cell membrane molecules found in microbial cell envelopes. The 'category' corresponds to the location in the cell envelope (cell wall or cell membrane). The 'key function' describes the cell envelope material's unique role in the function of the cell envelope and the overall function of the cell.

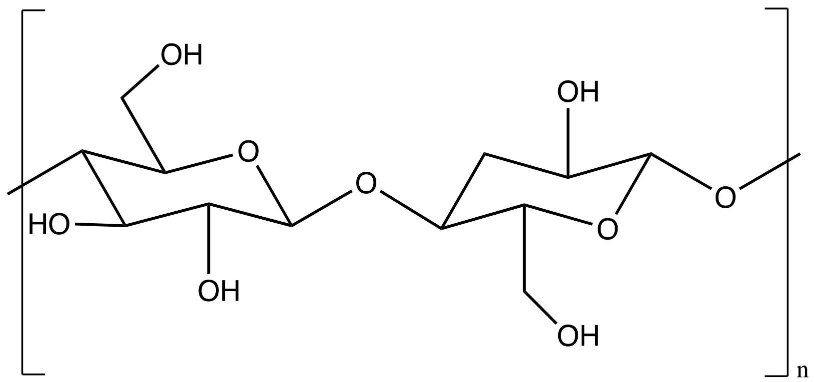

Figure S1. Cellulose molecular structure of a single cellulose unit.

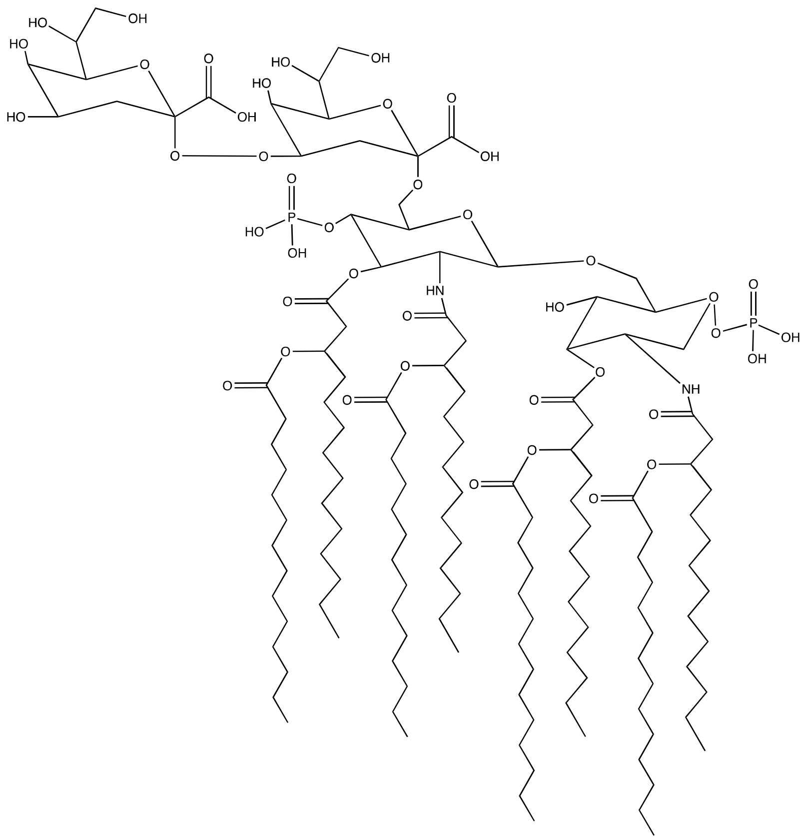

Figure S2. Lipopolysaccharide molecular structure

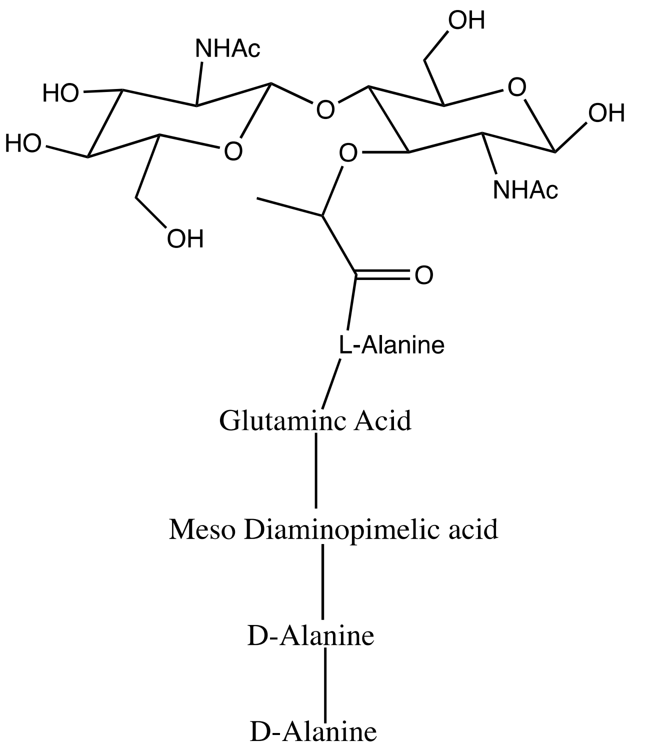

Figure S3. Peptidoglycan molecular structure

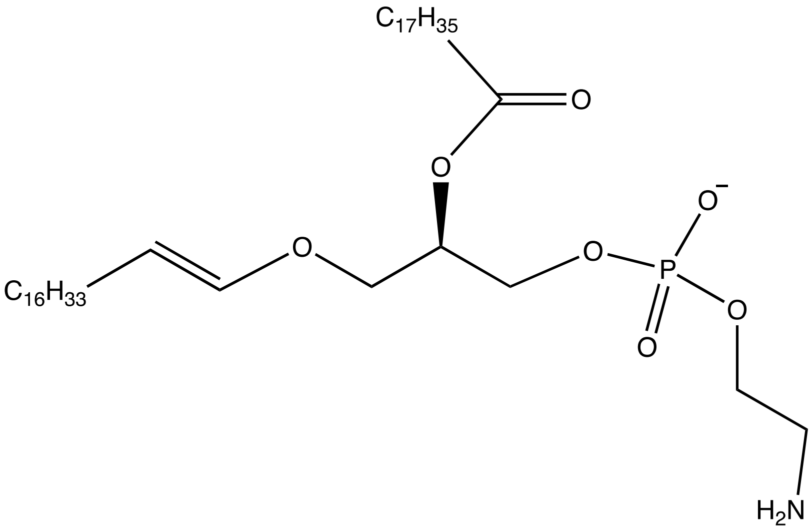

Figure S4. Plasmalogen molecular structure

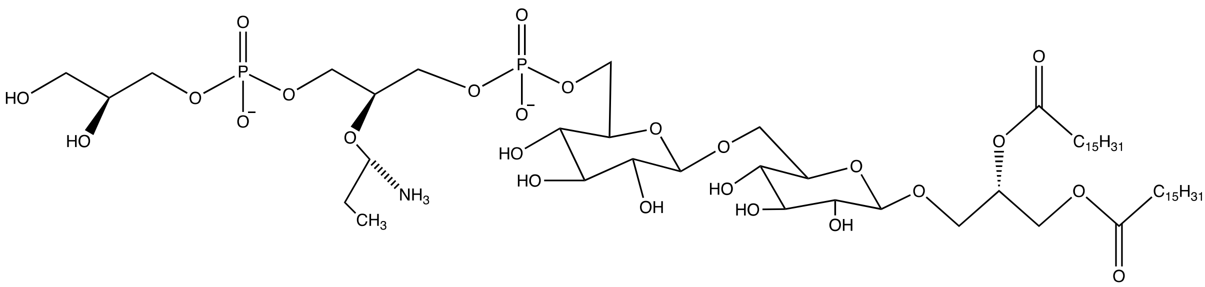

Figure S5. Lipoteichoic Acid molecular structure

Results and Discussion

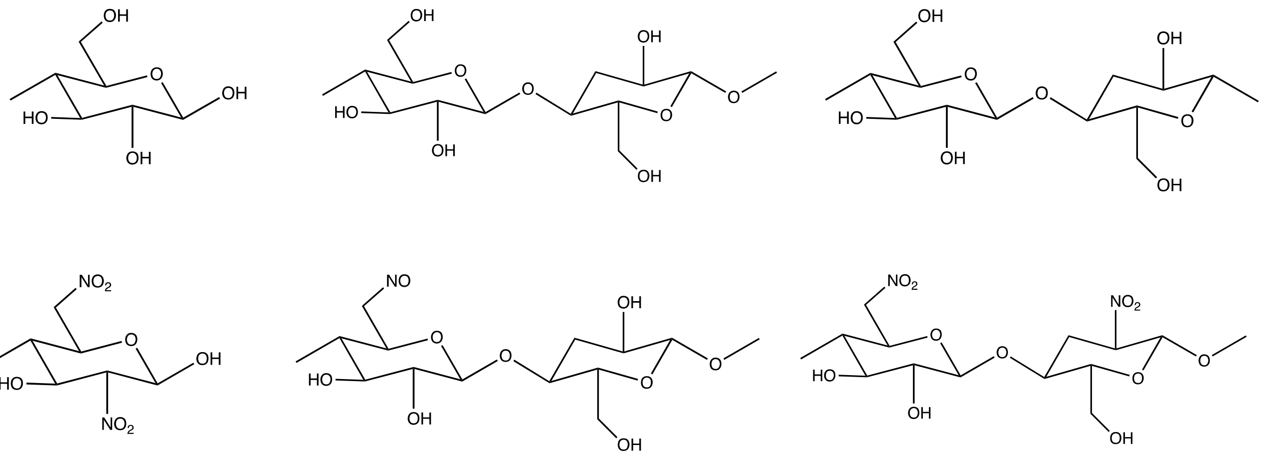

Figure S6. Cellulose breakdown products

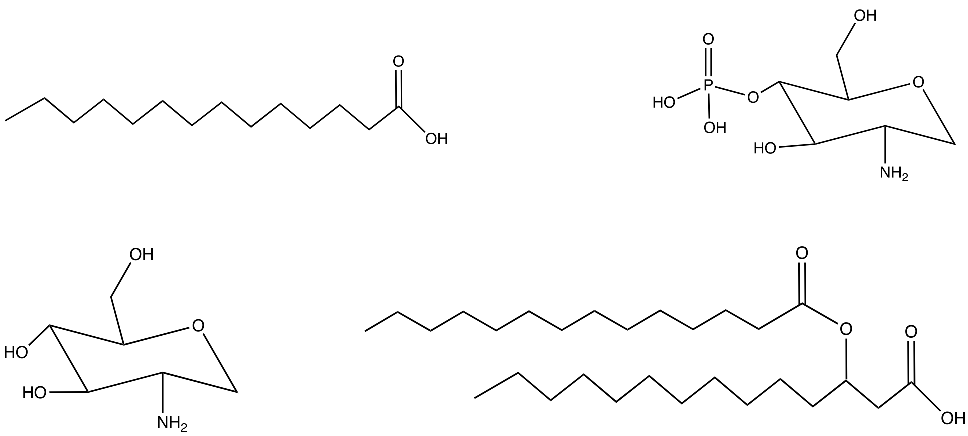

Figure S7. Lipopolysaccharide breakdown products

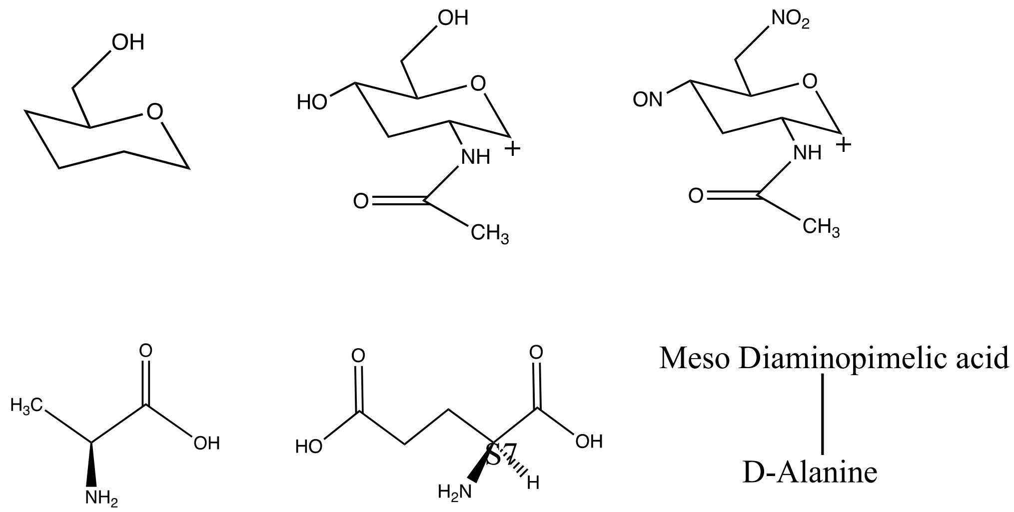

Figure S8. Peptidoglycan breakdown products

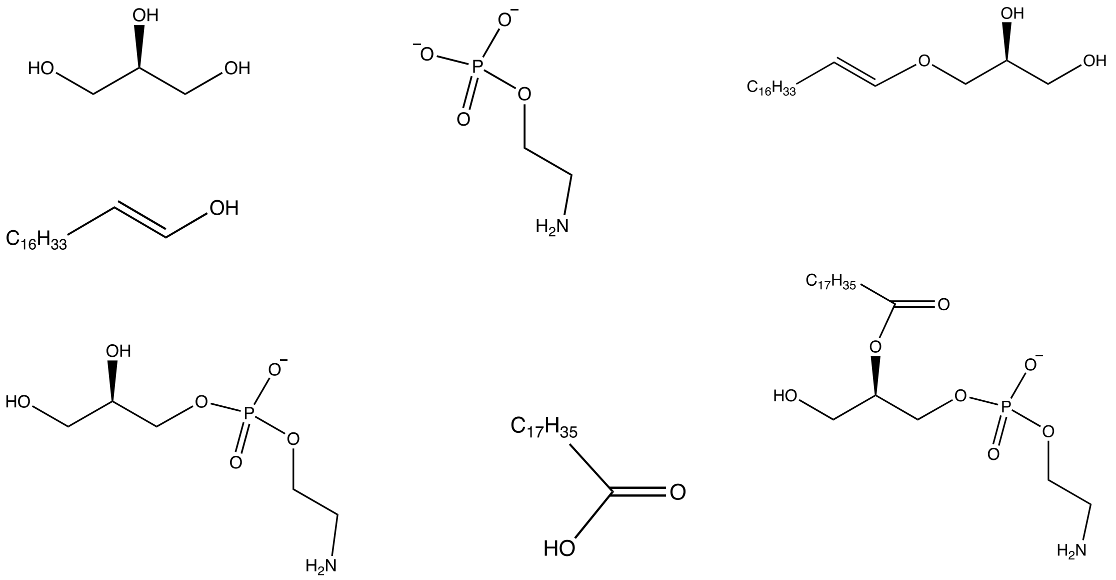

Figure S9. Plasmalogen breakdown products

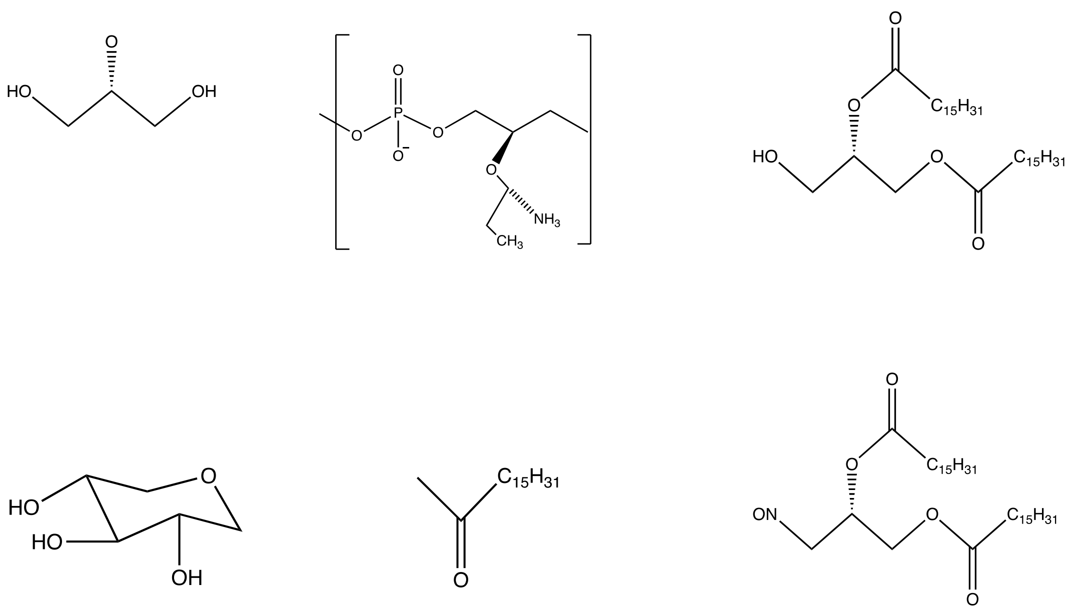

Figure S10. Lipoteichoic acid breakdown products

Equations S1-S5, Functional groups targeted by FNA

There are a number of functional groups targeted by FNA, (Eq. S1-S5) these include; carboxylic acids Eq. (1), amines Eq. (2), alcohols Eq. (3), phenols Eq. (4) and benzene rings Eq. (5).

$$HNO_2 + H_3O^+ + RCOO^- \rightleftarrows RCOONO + 2H_2O \quad [3] \qquad (1)$$

$$HNO_2 + H_3O^+ + R_2NH^- \rightleftarrows R_2N\text{-}NO + H^+ \quad [4] \qquad (2)$$

$$HNO_2 + H_3O^+ + ROH \rightleftarrows RONO + H_2O \quad [5] \qquad (3)$$

$$HNO_2 + H_3O^+ + C_2H_5OH \rightleftarrows C_2H_5OHNO + H_2O \quad [6] \qquad (4)$$

$$HNO_2 + H_3O^+ + C_2H_6 \rightleftarrows C_2H_5NO_2 + H_2O \quad [7] \qquad (5)$$

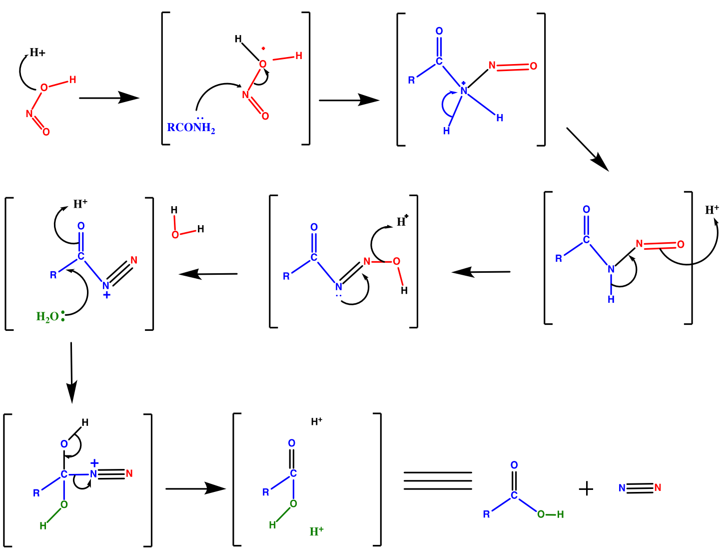

Figure S11. Reaction schematic of FNA with amides

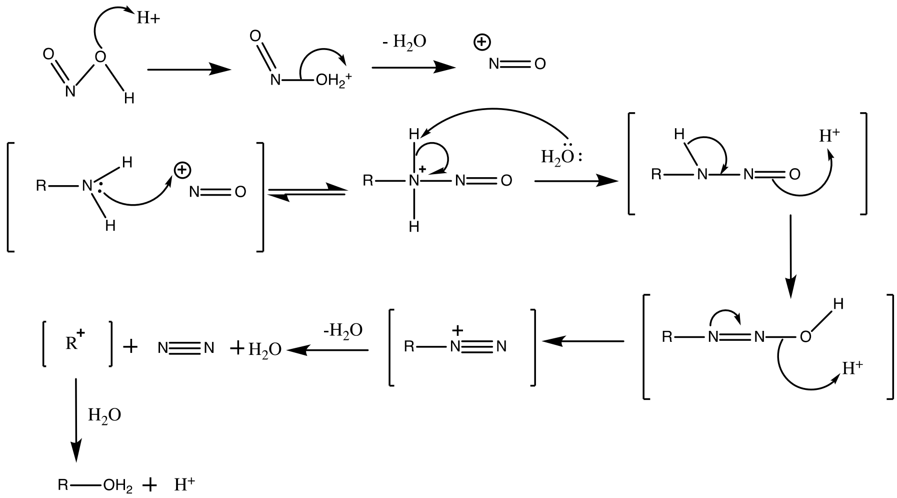

Figure S12. Reaction schematic of FNA with primary amines

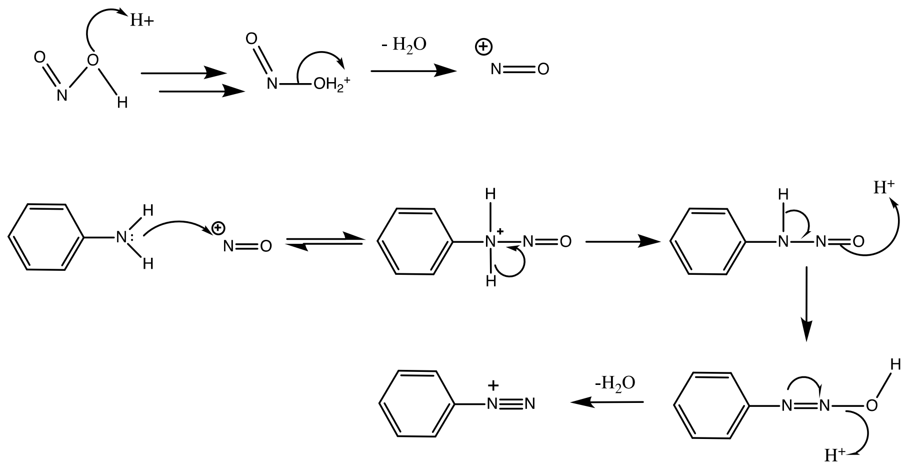

Figure S13. Reaction schematic of FNA with primary amyl amines

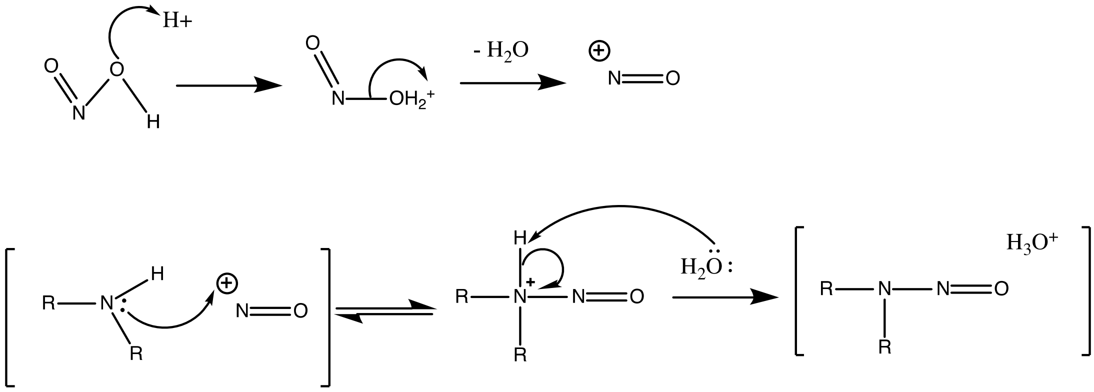

Figure S14. Reaction schematic of FNA with secondary amines

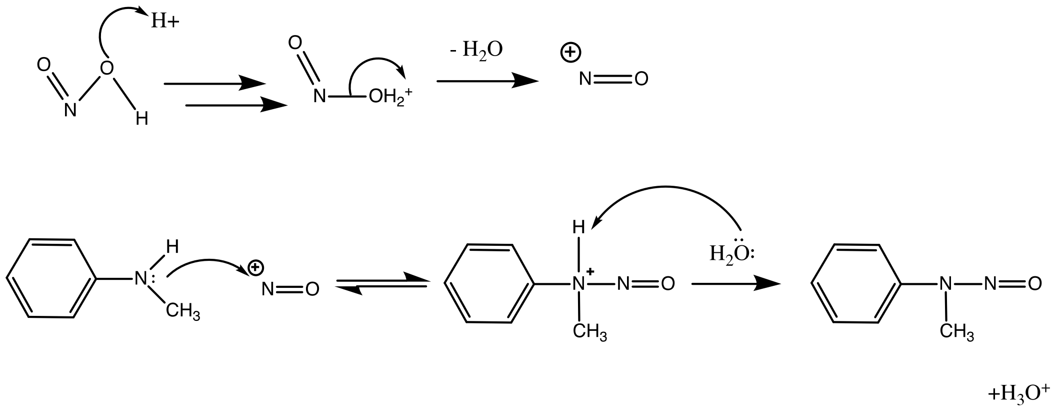

Figure S15. Reaction schematic of FNA with secondary aryl amines

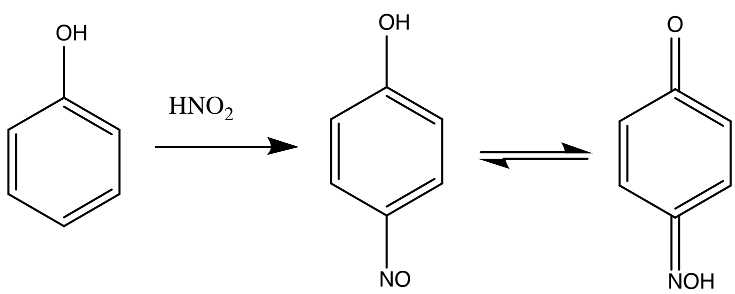

Figure S16. Reaction schematic of FNA with phenols

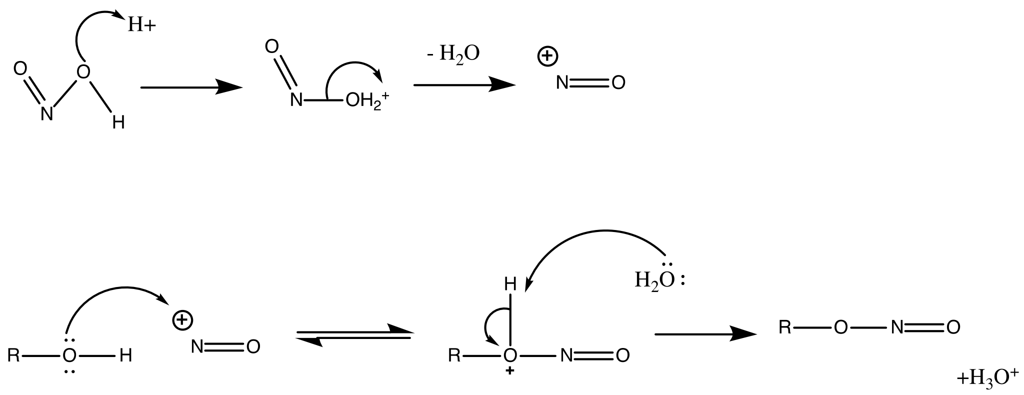

Figure S17. Reaction schematic of FNA with primary alcohols

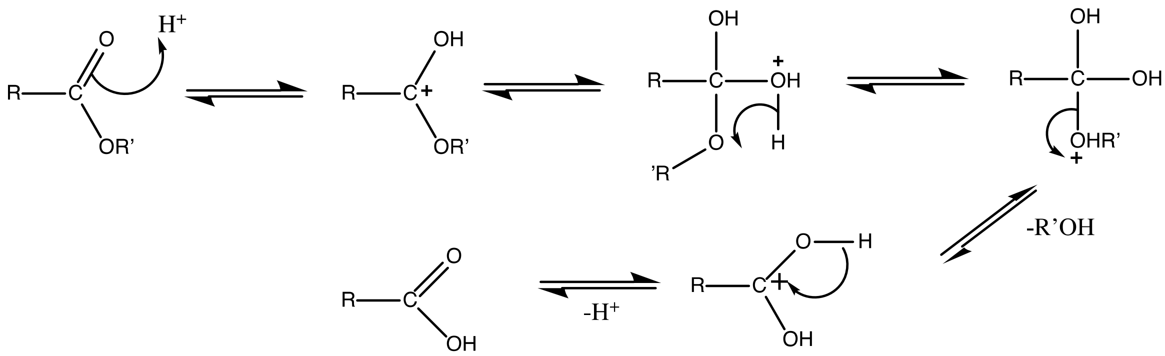

Figure S18. Reaction schematic of FNA with esters

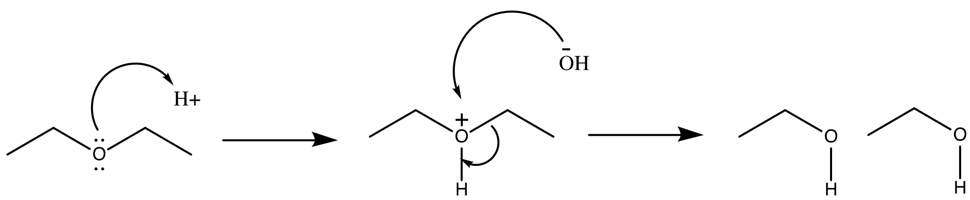

Figure S19. Reaction schematic of FNA with ethers

- **Supporting Information.**

Table S1 highlights the cell envelope materials used as representative envelope materials in this study and their function in the cell. Figure S1-S5 include the molecular structure of the cell envelope materials, highlighting the important structural bonds. In Figure S1, the R-O-R bonds represent ether linkages, which provide structural integrity to the cellulose polymer. Cellulose polymers can also bond to other cellulose polymers via hydrogen bonds between the OH groups. Figure S2 contains the chemical structure of lipopolysaccharide including the long chain lipid components, and the polysaccharide component. The polysaccharide is composed of O-antigen, outer core and inner core joined by covalent bonds; they are found in the outer membrane of Gram-negative bacteria. Figure S3 is the molecular structure of peptidoglycan from Escherichia coli. There is a pentapeptide bonded to the remaining structure via a lactyl group. Peptidoglycan is anchored to the inner membrane of Gram-negative bacteria through linkage to lipoproteins. The plasmalogen molecular structure in Figure S4 is a plasmenyl-phospholipid, which has an ether bond in position sn-1 to an alkenyl group [8]. Plasmalogens have been proposed to act as membrane antioxidants and reservoirs of polyunsaturated fatty acids as well as influence membrane dynamics [9]. LTAs molecular structure in Figure S5 has been found to have the strongest hydrophobic bonds in the entire bacteria [10]. This is because there is a presence of strong covalent ether, ester and phosphate bonding throughout the molecule. LTA is anchored to the cell membrane via a diglyceride bond [11]. It has antigenic properties being able to stimulate specific immune response.

Figure S6-S10 highlights the breakdown products that resulted from the reaction between FNA and the cell envelope materials and were identified from the ESI-MS spectra in the main text (Figure 1B-5B). Equations S1-S5 are the hypothesised equations for the reactions between FNA and the functional groups targeted by FNA that are present in the cell envelope materials.

Figure S11-19 are the reaction schematics for the reactions between FNA and the functional groups found in the cell envelope molecules.

References

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Programmed death-1–induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. *Nat. Med.* **2010,** *16*, 452-459.

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