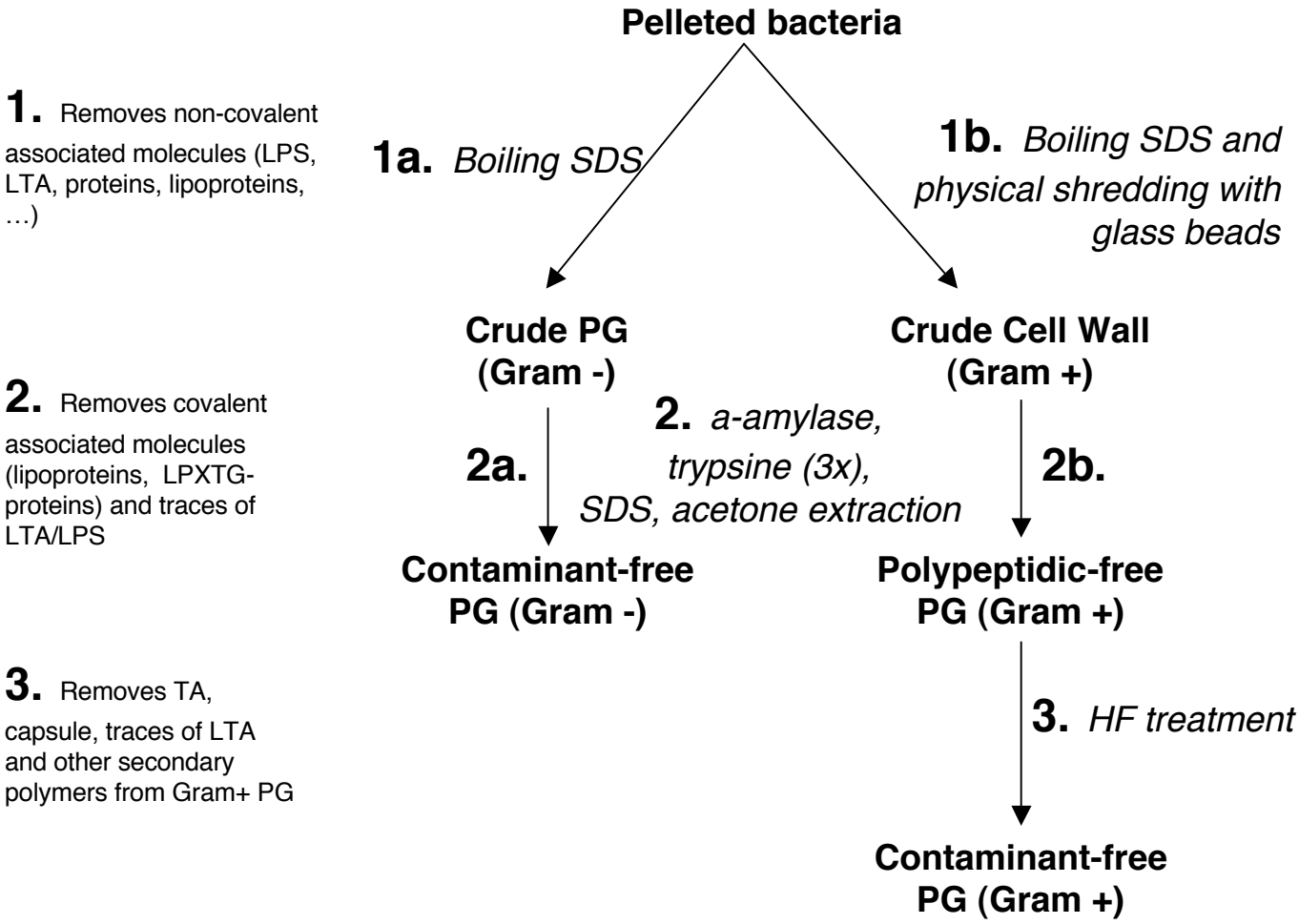
Supplementary Figure 1



Schematic representation of the different key purification steps to obtain PG according to the two different cell wall architectures. Note that the purification procedure is radically different between Gram-negative and Gram-positive bacteria due to their distinct cell wall architecture. Hence, Gram-negative bacteria have a fine peptidoglycan layer rapidly extractable with SDS as an insoluble polymer removing membranes and associated molecules such as lipopolysaccharide (LPS), lipotecichoic acid (LTA) and non-covalently PG-linked lipoproteins (step 1a). Removal of physically linked lipoproteins such as Lpp is achieved by enzymatic degradation and solvent extraction to obtained highly purified Gram-negative bacteria PG (step 2a). In contrast, PG purification from Gram-positive bacteria is more complex due to the thicker nature of its peptidoglycan layer and the presence of a variety of secondary polysaccharides such as wall teichoic acids (TA) or capsules. Therefore, step 1b allows to obtain crude cell walls of Gram-positive bacteria after physically breaking the bacteria with glass beads. Since we also treat the cells with SDS, this step allows to remove most of the lipophylic molecules such as LTA. Purified cell walls are obtained by removing contaminating lipoproteins or covalently linked proteins such as those carrying a LPXTG-anchoring motif (step 2b). Finally, highly purified PG (step 3) is obtained after hydrofluoric acid (HF) treatment of cell walls, which allows removal of secondary polysaccharides which are anchored to the PG via phosphodiester bonds and complete hydrolysis of LTA traces .