Please fill in only headlines in each objective in the table, and the full description in the explanatory notes section

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| objective | Beginning  | Ending  | Note  |
| Examination of the pore size and resealing time in electroporated gram-**negative bacteria** in a medium containing a **fluorescent dye** | 1.1.22 | 31.2.22 | 2 months*Suspension of* ***Pseudomonas putida*** in PBS (with different conductivities: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into brain heart infusion (BHI), a rich medium including the fluorescent dye, Lucifer Yellow (LY). The **LY-positive cells** will be examined using **flow cytometer**, immediately after the dilution in BHI and will continue until the LY-positive cells will be reduced to near zero. **Controls: 1-** Same conditions, but the bscterial will be suspended in ultra pure (UP) water**; 2-** non PEF-treated ***P. putida*** will diluted the same as the experiment and will examined for LY-positive cells as the treated PEF-cells |
| Modeling effort: Basic kinetic model for mass transfer into the cell | 1.1.22 | 31.6.22 | 6 monthsDevelopment of basic kinetic model for mass transfer into the cell and preliminary experimental data validation.Estimation of the relevant transport properties.  |
| Modeling effort: Basic CFD model for PEF treatment stage | 1.1.22 | 31.6.22 | 6 monthsDevelopment of basic CFD model for field parameters during PEF treatment.Numerical description of the PEF process. |
| Examination of the pore size and resealing time in electroporated gram-**negative bacteria** in a medium containing relative **different hydrophilic compounds** | 1.3.22 | 31.10.22 | 8 months*Suspension of* ***P. putida*** in PBS (with different conductivities: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI including relatively **hydrophilic compounds** including **phenol, bisphenol A, ellagic acid, epigallocatechin gallate, procyanidin B2 and theaflavin-3-gallate** with molecular weights of 94.11- 716.604 g/mol. The permealization rate of the hydropilic compounds will be examined using High Performance Liquid Chromatography (HPLC) at differing time intervals (for example every 15 min) immediately after the dilution in BHI until the permeabilization rate will reduce to zero.**Controls: 1-** Same conditions, but the bscterial will be suspended in ultra pure (UP) water**; 2-** non PEF-treated ***P. putida*** will diluted the same as the experiment and the rate permalization **hydrophilic compounds** will be examined as the treated PEF-cells |
| Examination of the pore size and resealing time in electroporated gram-**negative bacteria** in a medium containing **different hydrophobic compounds** | 1.11.21 | 31.5.22 | 8 months*Suspension of* ***P. putida*** in PBS (with different conductivities: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI including **hydrophobic compounds** from one aromatic hydrocarbon to 10 rings which include seven aromatic hydrocarbon and three rings of five carbons which include **benzene, naphthalene, anthracene, pyrene, benzo[e]pyrene and decacyclene**, with molecular weights of 78.12- 450.5 g/mol. It is important to note that the concentration of each examined molecule will be under the concentration which may case a damage to the cells. The permealization rate of the hydrophobic compounds will be examined using HPLC at differing time intervals (for example every 15 min) immediately after the dilution in BHI until the permeabilization rate will reduce to zero.**Controls: 1-** Same conditions, but the bscterial will be suspended in ultra pure (UP) water**; 2-** non PEF-treated ***P. putida*** will diluted the same as the experiment and the rate permalization of the **hydrophobic compounds** will be examined as the treated PEF-cells |
| Examination of the pore size and resealing time in electroporated gram-**positive bacteria** in a medium containing a **fluorescent dye** | 1.6.22 | 31.7.22 | 2 months*Suspension of* ***Staphylococcus* *aureus*** in PBS (with different conductivities: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into brain heart infusion (BHI), a rich medium including the fluorescent dye, Lucifer Yellow (LY). The **LY-positive cells** will be examined using **flow cytometer**, immediately after the dilution in BHI and will continue until the LY-positive cells will be reduced to near zero. **Controls: 1-** Same conditions, but the bscterial will be suspended in ultra pure (UP) water**; 2-** non PEF-treated *S. aureus* will diluted the same as the experiment and will examined for LY-positive cells as the treated PEF-cells |
| Examination of the pore size and resealing time in electroporated gram-**positive bacteria** in a medium containing relative **different hydrophilic compounds** | 1.8.22 | 31.3.23 | 8 months*Suspension of* ***S. aureus*** in PBS (with different conductivities: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI including relatively **hydrophilic compounds** including **phenol, bisphenol A, ellagic acid, epigallocatechin gallate, procyanidin B2 and theaflavin-3-gallate** with molecular weights of 94.11- 716.604 g/mol. The permealization rate of the hydropilic compounds will be examined using High Performance Liquid Chromatography (HPLC) at differing time intervals (for example every 15 min) immediately after the dilution in BHI until the permeabilization rate will reduce to zero.**Controls: 1-** Same conditions, but the bscterial will be suspended in ultra pure (UP) water**; 2-** non PEF-treated ***S. aureus*** will diluted the same as the experiment and the rate permalization **hydrophilic compounds** will be examined as the treated PEF-cells |
| Examination of the pore size and resealing time in electroporated gram-**positive bacteria** in a medium containing **different hydrophobic compounds** | 1.4.23 | 31.11.23 | 8 months*Suspension of* ***S. aureus*** in PBS (with different conductivities: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI including **hydrophobic compounds** from one aromatic hydrocarbon to 10 rings which include seven aromatic hydrocarbon and three rings of five carbons which include **benzene, naphthalene, anthracene, pyrene, benzo[e]pyrene and decacyclene**, with molecular weights of 78.12- 450.5 g/mol. It is important to note that the concentration of each examined molecule will be under the concentration which may case a damage to the cells. The permealization rate of the hydrophobic compounds will be examined using HPLC at differing time intervals (for example every 15 min) immediately after the dilution in BHI until the permeabilization rate will reduce to zero.**Controls: 1-** Same conditions, but the bscterial will be suspended in ultra pure (UP) water**; 2-** non PEF-treated ***S. aureus*** will diluted the same as the experiment and the rate permalization of the **hydrophobic compounds** will be examined as the treated PEF-cells |
| Modeling effort: Membrane dynamics study: Kinetic + CFD Modeling  | 1.7.22 | 31.12.23 | 12 monthsDevelopment of a model for cell membrane destruction and recovery with experimental data validation. For the tested cell and tracers. Identifying the relevant mechanisms and drive forces on the membrane.  |
| Bacterial protoplast preparation | 1.12.23 | 31.2.24 | 3 monthsThe peptidoglycan of *P. putida* as well as *S. aureus* will be digested using murein hydrolases where the commonly used enzyme is the hen egg white lysozyme. Since the outer membrane of gram-negative bacteria mostly prevents entry of enzymes towered the peptidoglycan, these types of cells require pre-treatment with a chelating agent (e.g. EDTA) or detergent (e.g. Triton X-100) for removing the outer membrane. |
| Examination of the pore size and resealing time in electroporated gram-**bacterial protoplast** in a medium containing a **fluorescent dye** | 1.3.24 | 31.5.24 | 3 months*Suspension of* ***bacterial protoplast*** in PBS with different **selected** conductivities which is appropriate for protoplast: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable **selected** physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into BHI, a rich medium including the fluorescent dye, Lucifer Yellow (LY). The **LY-positive protoplast bacteria** will be examined using **flow cytometer**, immediately after the dilution in BHI and will continue until the LY-positive protoplast cells will be reduced to near zero. **Controls: 1-** non PEF-treated bacterial protoplast will be diluted the same as the experiment and will examined for LY-positive cells as the treated PEF-protoplast cells |
| Examination of the pore size and resealing time in electroporated **bacterial protoplast** in a medium containing a selected relative **hydrophilic compound** | 1.6.24 | 31.10.24 | 5 months*Suspension of* **bacterial protoplast** in PBS (with different **selected** conductivities which is appropriate for protoplast: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable **selected** physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI including a relatively **selected hydrophilic compound (**one of the following compounds**:** **benzene, naphthalene, anthracene, pyrene, benzo[e]pyrene and decacyclene**, with molecular weights of 78.12- 450.5 g/mol). The permealization rate of the **selected** hydropilic compound will be examined using HPLC at differing time intervals (for example every 15 min) immediately after the dilution in BHI until the permeabilization rate will reduce to zero.**Controls: 1-** non PEF-treated **bacterial protoplast** will diluted the same as the experiment and the rate permalization of the **hydrophilic compound** will be examined as the treated PEF-protoplast cells |
| Examination of the pore size and resealing time in electroporated gram-**bacterial protoplast** in a medium containing a selected **hydrophobic compound** | 1.11.24 | 31.3.24 | 5 months*Suspension of* **bacterial protoplast** in PBS (with different **selected** conductivities which is appropriate for protoplast: 1 and 155–4058 µS cm-1) will be exposed to PEF treatment in a moderate electric field (1-4 kV cm-1) and variable **selected** physical parameters (e.g., pulse number, current density and total specific energy). Immediately after the PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI including **selected hydrophobic compound (**one of the following compounds**:** **phenol, bisphenol A, ellagic acid, epigallocatechin gallate, procyanidin B2 and theaflavin-3-gallate** with molecular weights of 94.11- 716.604 g/mol. The permealization rate of the **selected** hydropilic compound will be examined using HPLC at differing time intervals (for example every 15 min) immediately after the dilution in BHI until the permeabilization rate will reduce to zero.**Controls: 1-** non PEF-treated **bacterial protoplast** will diluted the same as the experiment and the rate permalization of the **hydrophilic compound** will be examined as the treated PEF-protoplast cells |
| To shed light on the electroporated recovary process of *P. putida* as well as *S. aureus*,the bacterial proteome of the PEF-treated and untreated will be examind using mass spectrometry analysis (MS).  | 1.4.24 | 31.12.25 | 9 monthsAt selected times, electroporated bacteria and the control, non-treated bacteria will be collected and centrifuged. The proteins from the washed sediment will be sonicated and treated with urea, ammonium bicarbonate, and DTT. Mass spectrometry analysis will be performed at the Smoler Proteomics Center at the Technion, Israel. The data will be quantified by label-free analysis using the same software, based on extracted ion currents (XICs) of peptides, thus enabling quantification from each LC/MS run for each peptide identified in the experiments. |
| Modeling effort: Full kinetic model for Pore size dynamics  | 1.1.24 | 31.12.25 | 12 monthsDevelopment full kinetic model for membrane destruction and recovery and full experimental data validation.Simulation of cell membrane dynamics  |
| Modeling effort: Full CFD model for PEF treatment stage and recuvery stage | 1.1.24 | 31.12.25 | 12 monthsDevelopment of full CFD model for field parameters during PEF treatment and recuvery. experimental data validation.Numerical symulation of the PEF process on single cell and cel population (field description). |