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  |
| Examine pore size and resealing time in electroporated gram-**negative bacteria** in a medium containing a **fluorescent dye** | 1.1.22 | 31.2.22 | 2 monthsA 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 following PEF treatment, the suspension will be diluted in brain heart infusion (BHI), a rich medium including the fluorescent dye Lucifer Yellow (LY). **LY-positive cells** will be immediately analyzed using a **flow cytometer**, which will continue until the number of LY-positive cells will be reduced to near zero. **Controls: 1-** Same conditions, but the bacteria will be suspended in ultra-pure (UP) water**; 2-** non PEF-treated ***P. putida*** will be diluted identically to the experiment and will be examined for LY-positive cells. |
| Modeling: Basic kinetic model for mass transfer into the cell | 1.1.22 | 31.6.22 | 6 monthsDevelop a basic kinetic model for mass transfer into the bacterial cell and preliminary experimental data validation.Estimate the relevant transport properties.  |
| Modeling: Basic CFD model for PEF treatment  | 1.1.22 | 31.6.22 | 6 monthsDevelop a basic CFD model for field parameters during PEF exposure.Numerical description of the PEF process. |
| Examine pore size and resealing time in electroporated gram-**negative bacteria** in a medium containing different **relative hydrophilic compounds** | 1.3.22 | 31.10.22 | 8 monthsA 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 following PEF treatment, the suspension will be diluted into the BHI nutrient-rich medium containing 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. Permeabilization rates of the hydrophilic compounds will be analyzed using High Performance Liquid Chromatography (HPLC) at different time intervals (for example, every 15 min) immediately after BHI dilution until the permeabilization rate will be reduced to zero.**Controls: 1-** Same conditions, but bacteria will be suspended in ultra-pure (UP) water**; 2-** non PEF-treated ***P. putida*** will be diluted identically to the experiment, and the permeabilization rateof **hydrophilic compounds** will be examined.  |
| Examine pore size and resealing time in electroporated gram-**negative bacteria** in a medium containing **different hydrophobic compounds** | 1.11.21 | 31.5.22 | 8 monthsA 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 PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI, containing **hydrophobic compounds** from one aromatic hydrocarbon to 10 rings, which include seven aromatic hydrocarbon and three rings of five carbons. Compounds tested 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 each examined molecule's concentration will be lower than the concentration that damages cells. The hydrophobic compounds' permeabilization rate will be analyzed using HPLC at different time intervals (for example, every 15 min) immediately after the dilution in BHI until the permeabilization rate will be reduced to zero.**Controls: 1-** Same conditions, but the bacteria will be suspended in ultra-pure (UP) water**; 2-** non PEF-treated ***P. putida*** will be diluted identically to the experiment, and the rate permeabilization of the **hydrophobic compounds** will be examined.  |
| Examine pore size and resealing time in electroporated gram-**positive bacteria** in a medium containing a **fluorescent dye** | 1.6.22 | 31.7.22 | 2 monthsA 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 PEF treatment, the suspension will be diluted into the rich brain heart infusion (BHI) medium containing the fluorescent dye, Lucifer Yellow (LY). The **LY-positive cells** will be analyzed using a **flow cytometer** immediately after dilution and continue until the number of LY-positive cells is reduced to near zero. **Controls: 1-** Same conditions, but the bacteria will be suspended in ultra-pure (UP) water**; 2-** non PEF-treated *S. aureus* will be diluted and examined for LY-positive cells identically to the experimental group.  |
| Examine 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 monthsA 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 PEF treatment, the suspension will be diluted into the rich brain heart infusion (BHI) medium containing relatively **hydrophilic compounds** such as **phenol, bisphenol A, ellagic acid, epigallocatechin gallate, procyanidin B2, and theaflavin-3-gallate** with molecular weights of 94.11- 716.604 g/mol. The permeabilization rate of the hydrophilic compounds will be analyzed using High Performance Liquid Chromatography (HPLC) at different time intervals (for example, every 15 min) immediately after dilution in BHI until the permeabilization rate will be reduced to zero.**Controls: 1-** Same conditions, but the bacteria will be suspended in ultra-pure (UP) water**; 2-** non PEF-treated ***S. aureus*** will be diluted identically to the experimental group, and the rate permeabilization of **hydrophilic compounds** will be examined.  |
| Examine pore size and resealing time in electroporated gram-**positive bacteria** in a medium containing **different hydrophobic compounds** | 1.4.23 | 31.11.23 | 8 monthsA 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 PEF treatment, the suspension will be diluted into the rich brain heart infusion (BHI) medium containing **hydrophobic compounds** from one aromatic hydrocarbon to 10 rings, including seven aromatic hydrocarbon and three rings of five carbons. Compounds 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 each molecule's concentration will be lower than the concentration, which causes cell damage. The permeabilization rate of the hydrophobic compounds will be analyzed using HPLC at different time intervals (for example, every 15 min) immediately after the dilution in BHI until the permeabilization rate is reduced to zero.**Controls: 1-** Same conditions, but the bacteria will be suspended in ultra-pure (UP) water**; 2-** non PEF-treated ***S. aureus*** will be diluted identically to the experimental group, and the rate permeabilization of **hydrophobic compounds** will be examined  |
| Modeling: 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. Identify the relevant mechanisms and drive forces on the membrane.  |
| Bacterial protoplast preparation | 1.12.23 | 31.2.24 | 3 months*P. putida* and *S. aureus* peptidoglycans will be digested using murein hydrolases and the commonly used hen egg-white lysozyme. Since the outer membrane of gram-negative bacteria prevents enzyme entry, these types of cells require pre-treatment with a chelating agent (e.g., EDTA) or detergent (e.g., Triton X-100) for outer membrane removal. |
| Examine pore size and resealing time in electroporated gram-**bacterial protoplast** in a medium containing a **fluorescent dye** | 1.3.24 | 31.5.24 | 3 monthsA suspension of ***bacterial protoplast*** in PBS with different **selected** conductivities appropriate for protoplasts: 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). After PEF treatment, the suspension will be diluted into the rich BHI medium containing the fluorescent dye, Lucifer Yellow (LY). The **LY-positive protoplast bacteria** will be immediately analyzed using a **flow cytometer** and will continue until the LY-positive protoplast cells are reduced to near zero. **Controls: 1-** non PEF-treated bacterial protoplastwill be diluted identically to the experimental group and examined for LY-positive cells.  |
| Examine 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 monthsA suspension of**bacterial protoplast** in PBS (with different **selected** conductivities 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 PEF treatment, the suspension will be diluted into the nutrient rich medium, BHI, containing **selected hydrophilic compounds (**one of the following**:** **benzene, naphthalene, anthracene, pyrene, benzo[e]pyrene, and decacyclene**, with molecular weights of 78.12- 450.5 g/mol). The permeabilization rate of the **selected** hydrophilic compound will be analyzed using HPLC at different time intervals (for example, every 15 min) immediately dilution until the permeabilization rate is reduced to zero.**Controls: 1-** non PEF-treated bacterial protoplastwill be diluted identically to the experimental group, and the permeabilization rate of the **hydrophilic compound** will be examined.  |
| Examine pore size and resealing time in electroporated **bacterial protoplast** in a medium containing a selected **hydrophobic compound** | 1.11.24 | 31.3.24 | 5 monthsA 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 PEF treatment, the suspension will be diluted into the nutrient rich BHI medium containing a **hydrophobic compound (**one of the following**:** **phenol, bisphenol A, ellagic acid, epigallocatechin gallate, procyanidin B2, and theaflavin-3-gallate** with molecular weights of 94.11- 716.604 g/mol. The permeabilization rate of the **selected** hydrophilic compound will be analyzed using HPLC at different time intervals (for example, every 15 min) immediately after the dilution in BHI until the permeabilization rate is reduced to zero.**Controls: 1-** non PEF-treated bacterial protoplastwill be diluted identically to the experimental group, and the permeabilization rate of the **hydrophilic compound** will be examined.  |
| Shed light on the electroporated recovery process of *P. putida* and *S. aureus*.The proteome of PEF-treated and untreated bacteria will be examined using mass spectrometry analysis (MS).  | 1.4.24 | 31.12.25 | 9 monthsAt selected times, electroporated bacteria and 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. Data will be quantified by label-free analysis, based on extracted ion currents (XICs) of peptides, thus enabling quantification from each LC/MS run for each peptide identified in the experiments. |
| Modeling: Full kinetic model for Pore size dynamics  | 1.1.24 | 31.12.25 | 12 monthsDevelopment of a full kinetic model for membrane destruction and recovery and experimental data validation.Simulation of cell membrane dynamics.  |
| Modeling: Full CFD model for PEF treatment and recovery stages | 1.1.24 | 31.12.25 | 12 monthsDevelopment of a full CFD model for field parameters during PEF treatment and recovery and experimental data validation.Numerical simulation of the PEF process on single cells and cell populations (field description). |