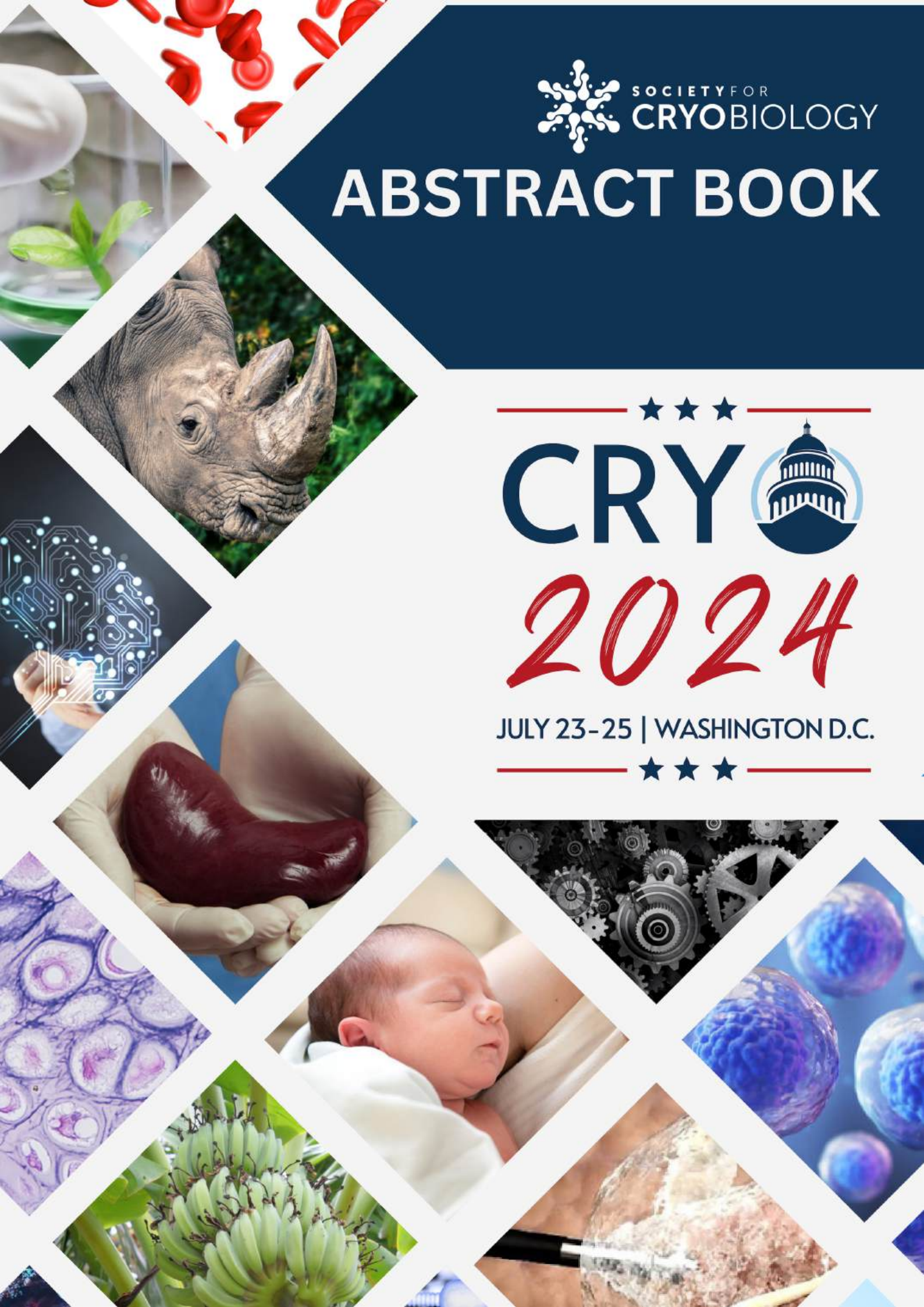

SOCIETY FOR CRYOBIOLOGY

ABSTRACT BOOK

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In this presentation, we reformulate the Kleinhans two-parameter formalism for cryoprotectant loading under the constraint of constant cell volume. We then derive simple, concise, exact analytic solutions to these equations, showing the transient extracellular permeating and nonpermeating cryoprotectant concentrations required to load a cell at constant volume, thus eliminating osmotic stresses during cryoprotectant loading. Additionally, we show analytic approximations of both ramp (linear) as well as stepwise loading and how one can use the hydraulic conductivity L_p, membrane permeability P_s, cell volume V_o, and osmotically inactive fraction to derive cryoprotectant loading protocols that minimize or eliminate osmotic stress.

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S24 POSTHYPERTONIC LYSIS OF ERYTHROCYTES WHEN USING AMPHIPHILES AND VARYING TEMPERATURE

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The research is dedicated to study the effect of the temperature on human erythrocytes, preserved after combined action of posthypertonic shock (PHS) and amphiphiles. Sodium decyl sulfate (C10), trifluoperazine (TFP) and decyl-β,D-glucopyranoside (DGP) were used. Posthypertonic shock was simulated by transferring erythrocytes from dehydration medium (1.65 mol/L NaCl) to rehydration one (0.15 mol/L NaCl) at 37 and 0°C. Amphiphiles were added into rehydration medium prior to cells transfer. The hemolysis level of erythrocytes was determined spectrophotometrically (λ = 543 nm). The distribution of phosphatidylserine in the erythrocyte membrane was assessed by flow cytometry using the Annexin V-FITC-kit. It was established that all amphiphiles reduced the posthypertonic lysis level of erythrocytes at 0°C (in contrast to at 37°C) and showed high antihemolytic activity (60–70%). During the experiment, the preserved after PHS effect erythrocytes as well as relevant amphiphile (0°C) were heated and kept for 30 min at a certain temperature (from 0 to 37°C). The findings testify that human erythrocytes, preserved after the combined action of posthypertonic shock (0°C) and C10 (400 μmol/L) are resistant to further heating (up to 37°C), and after usage of DGP (600 μmol/L) and TFP (150 μmol/L) they are sensitive to temperature rise. The method of flow cytometry showed that the amount of annexin-labeled cells depended on the concentration of C10. Under PHS conditions of erythrocytes at concentration of C10 (180 μmol/L) percentage of annexin-labeled cells was 5.77%, while the usage of C10 (400 μmol/L) led to an increased number of annexin-labeled cells 38.3%. Hence, it`s feasible to choose such a concentration of the amphiphilic compound at which it would show significant antihemolytic activity and not affect to phosphatidylserine transmembrane redistribution in the membrane.

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S25 COMBATING HEMOGLOBIN OXIDATION DURING DRYING