In Vivo Hydration Changes in Hb SS and SC Cells

Robert D. Franco, PhD. Principal Investigator
Clinton H. Joiner, MD, PhD. Co-Investigator

To develop effective treatment strategies for sickle cell disease, a clear understanding of cellular pathophysiology is required. After entering the circulation, sickle cells experience a number of changes and selection processes that profoundly influence their propensity to sickle and their survival characteristics. This proposal is focused on understanding these time-dependent changes. *In vivo* studies will track small volume of highly purified light or dense sickle cells in the circulation, utilizing a biotin label. This method, unique to our laboratory, offers a cell-by-cell analysis of multiple cellular properties during *in vivo* cell aging. One of these properties, hydration state, has been recognized to be of paramount importance. It now appears that a one-directional dehydration model is not adequate, and that sickle cells eventually become rehydrated and have high sodium content, perhaps secondary to severe membrane damage. A preliminary biotin label study has demonstrated that these sodium loaded cells have an extremely short in vivo survival. Taken together with their numbers in the circulation, these data strongly support an important role for these cells as a terminal cellular state. The proposed studies will include examinations of the mechanism of formation and survival characteristics of these older, hydrated cells. A mechanistic model of sickle cell rehydration, based on preliminary studies with the transport inhibitors DIDS and bumetanide, will be tested in the proposed studies. Related experiments will examine the remarkably high level of phosphatidylserine (PS) externalization that preliminary studies demonstrated in sickle cells that were rehydrated *in vitro*. *In vivo* and *in vitro* studies will evaluate both the low level PS exposure that is present in very young erythroid cells, both sickle and nonsickle, and the high level exposure that appears to develop as sickle cells age and become dehydrated. Patients with SC and CC disease offer an important and informative counterpoint to homozygous sickle cell disease. The dehydration kinetics and survival characteristics of SC and CC cells will be explored using established and newly developed experimental approaches. This will allow a comparison to similar investigations with SS cells, and lead to a better understanding of similarities and differences in the dehydration mechanisms for these diseases.