

## **Disregulation of the Sickle Cell Membrane Skeleton**

P.I.: Steven R. Goodman, Ph.D.

A major goal of my laboratory is to understand the mechanisms by which homozygous sickle cell (SS) red blood cells (RBCs) become irreversible sickled cells (ISCs) and dense. This was a necessary step towards devising therapeutics that block formation of ISCs and cellular dehydration. We had demonstrated that ISC membrane skeletons disassemble far more slowly at 37<sup>o</sup> C than reversible sickled cells (RSCs) and control membrane skeletons. This suggested that the reason that ISCs cannot change shape is because their membrane skeletons cannot dynamically disassemble and reassemble. Furthermore, we demonstrated that this slow disassembly was due to modifications in spectrin and actin. The modification in ISC beta-actin had been demonstrated to be a C284-C373 disulfide bridge which leads to actin filaments that disassemble slowly. We did not know, at the beginning of this funding period, the modification of ISC spectrin that leads to slower modification of the spectrin -4.1-actin ternary complex. We knew that spectrin had E2 ubiquitin conjugating activity and could target itself. We also knew that the E2 thioester site and one target site were within the alpha spectrin repeats 20/21. The ubiquitination of alpha spectrin in its DTT sensitive E2 site and insensitive target site(s) was diminished by 80-90%; probably due to glutathiolation of E2 cysteine(s). Since alpha spectrin 20/21 is the heterodimer nucleation site and is associated with the protein 4.1 and adducin binding sites on beta spectrin, we hypothesized that diminished ubiquitination of SS spectrin would lead to faster rates of heterodimer formation and higher affinity spectrin-4.1-actin and spectrin-adducin-actin ternary complexes. This would have supplied an explanation as to why the ISC skeleton dissociates far more slowly than the RSC or control membrane skeleton. To test this hypothesis we proposed three aims.

### Specific Aims:

Aim 1: to identify the cysteines and lysines involved in E2 and E3 activity and target sites in alpha spectrin 20/21 utilizing proteomics technologies.

Aims 2 and 3: to determine the role of spectrin ubiquitination in regulating heterodimer formation and spectrin-4.1 or adducin-actin ternary complex formation and disassembly, respectively.