Supporting Material

Native ultrastructure of the red cell cytoskeleton by cryo-electron tomography

Andrea Nans[†‡], Narla Mohandas[§], and David L. Stokes[†¶*]

[†] Skirball Institute of Biomolecular Medicine, Department of Cell Biology, New York University School of Medicine, New York, New York 10016

[‡] Structural Biology Graduate Program, New York University School of Medicine, New York, New York 10016

[§] The Red Cell Physiology Laboratory, The New York Blood Center, New York, NY 10065

[¶] New York Structural Biology Center, New York, NY 10027

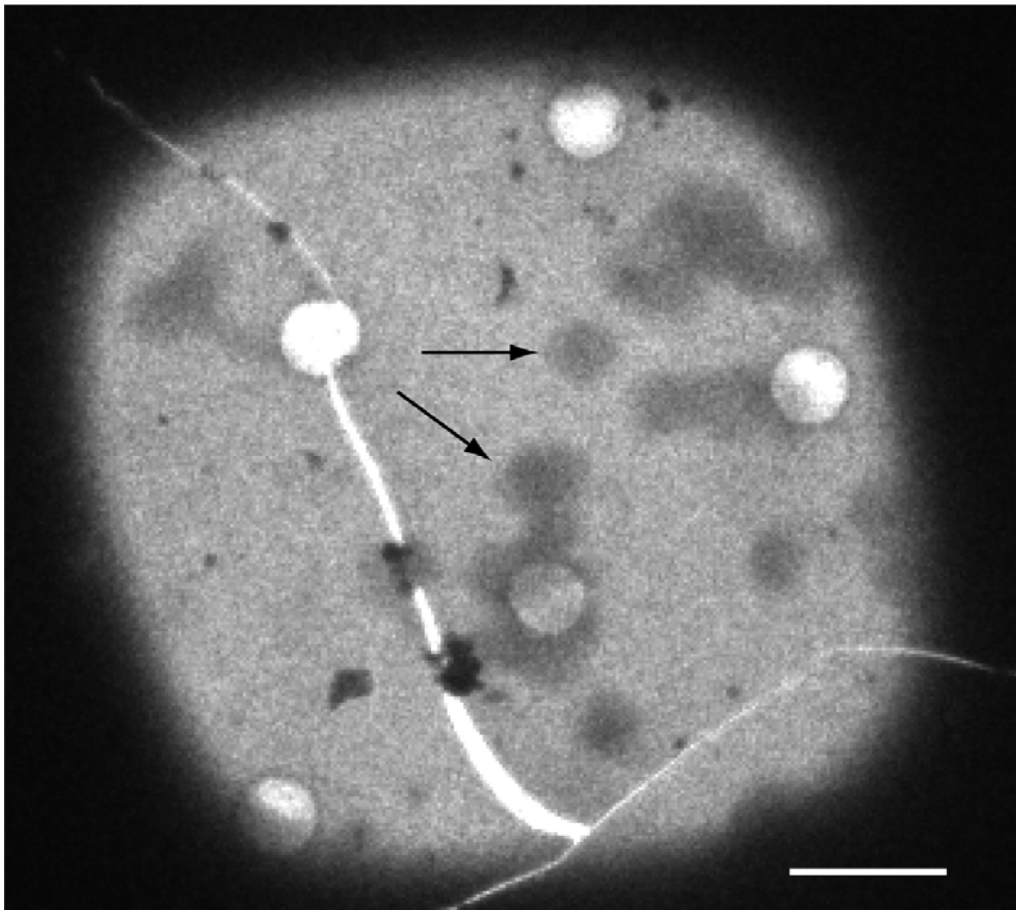

Figure S1: Low magnification image of frozen hydrated skeletons. A cryo-electron micrograph of a single square from an electron microscope grid shows the perforated carbon support film with several skeletons (black arrows). Lighter circles correspond to holes in the carbon film and only skeletons suspended over these holes were imaged. Cracks in the film of vitreous ice are also evident as long, lighter regions. Scale bar, 10 μm.

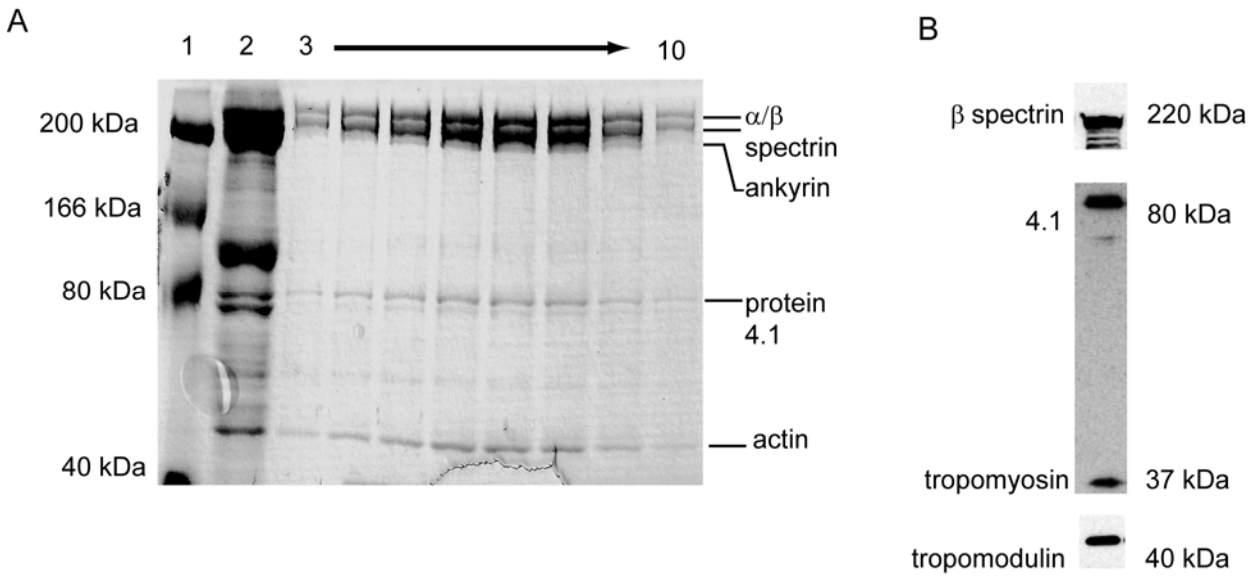

Figure S2: Biochemical characterization of red cell skeletons used for electron microscopy. (A) Coomassie-stained SDS gel showing the composition of ghost membranes and of detergent-extracted membrane skeletons. Lane 1: molecular weight standards. Lane 2: membrane ghosts. Lane 3-10: fractions from the sucrose density gradient. α/β spectrin, protein 4.1, and actin run at approximately 220kDa, 80 kDa, and 45 kDa respectively. Additionally, ghost membranes contain large amounts of the integral membrane protein Band 3 (100 kDa) and protein 4.2 (70 kDa). (B) Western blot of skeletal samples demonstrate the presence of F-actin-binding proteins, tropomyosin and tropomodulin, in addition to β-spectrin and protein 4.1. These bands were all derived from a same skeletal preparation, reacted with HRP-coupled antibodies, and developed with a chemiluminescence substrate under the same conditions. The bands for protein 4.1 and tropomyosin were derived from the same gel, exposed for the same length of time, and scanned from the same film, illustrating that tropomyosin produced a robust signal.

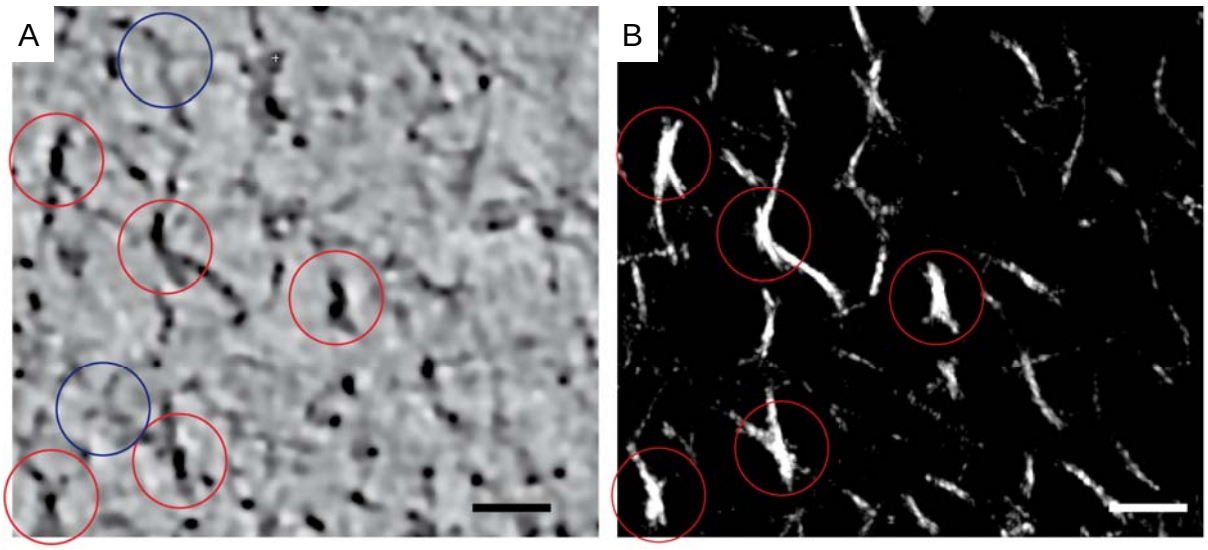

Figure S3: Validation of Junctional Complexes in Cryo-electron Tomograms
(A) 7.3 Å-thick denoised slice from a tomogram of a membrane skeleton. Putative junctional complexes (red circles) and a spectrin hexamer and octamer (blue circles) are indicated. (B) Cross-correlation function with a 12 monomer F-actin filament reference structure based on the raw volume from (A). High cross-correlation peaks are observed at the sites of junctional complexes (red) but not at the spectrin hexamers or octamers, indicating that junctional complexes can be distinguished from other features in the 3-D volume. Regions of high correlation are occasionally also found along the length of spectrin filaments. Scale bars are 30 nm.

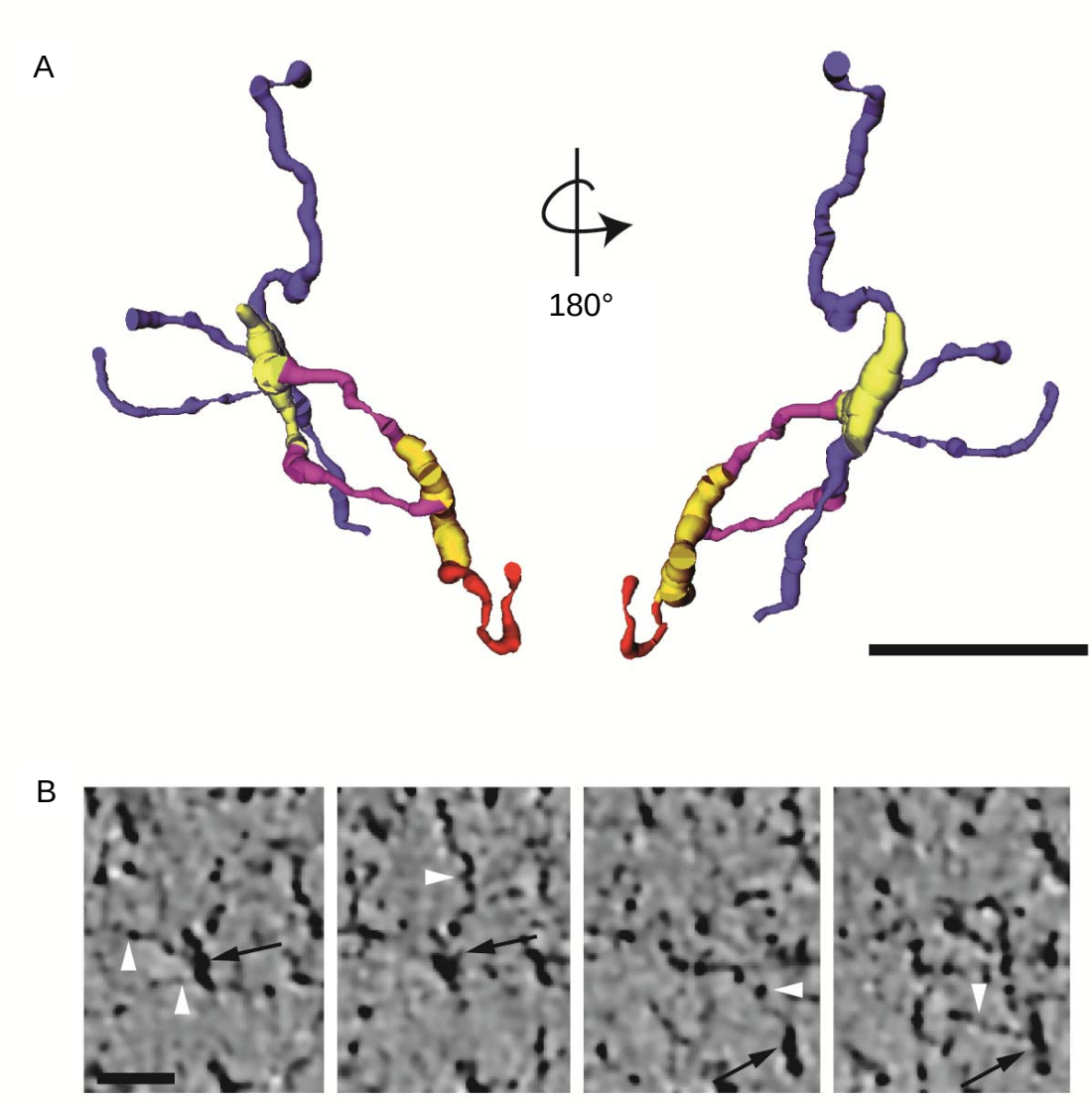

Figure S4: Three-dimensional topology of the membrane skeleton. (A) Skeletonized, volume-rendering of two junctional complexes (yellow) with their associated spectrin filaments (red and blue). Spectrin molecules connecting the two junctional complexes are highlighted in magenta. The two views are related by a 180° rotation. (B) Serial tomographic slices of these same junctional complexes at 9 nm intervals. Junctional complexes are indicated by black arrows and spectrin filaments by white arrowheads. Scale bars are 50 nm in (A) and 35 nm in (B).

Supporting Movies

Movie S1:
This movie shows an electron tomogram of the frozen-hydrated membrane skeleton shown in Fig 3.

Movie S2:
This movie shows an electron tomogram of the frozen-hydrated membrane skeleton in Fig. 3 with the corresponding volume-rendering (shown in red). The membrane skeletons are organized into a convoluted network of thin spectrin filaments that are interspersed by globular junctional complexes.

Movie S3:
This movie displays both the tomographic densities and the volume-rendering of two junctional complexes joined by a spectrin tetramer from Fig. 5C. As evidenced from this reconstruction, the connecting spectrin tetramer is considerably shorter than the full contour length of the molecule (average contour length was 46 ± 15 nm vs. 194 nm).

Movie S4:
This movie shows both the tomographic densities and the volume-rendering of a series of junctional complexes connected by a combination of spectrin tetramers, hexamers, and octamers from Fig. 7C. The mixed oligomeric state of spectrin in the intact membrane skeleton may be an important determinant in the versatility of the red cell membrane.