Orientation of the N-terminal Lobe of the Myosin Regulatory Light Chain in Skeletal Muscle Fibers

Daniela Romano,[†] Birgit D. Brandmeier,[†‡] Yin-Biao Sun,[‡] David R. Trentham,[†] and Malcolm Irving[‡]

[†]Medical Research Council, National Institute for Medical Research, London, United Kingdom; and
[‡]Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom

Supporting Material

Materials and Methods

Preparation of skRLCs

The pMW172 vector was used to direct RLC synthesis (21,22). The preparation of *E. coli* inclusion bodies was as for smRLCs (16), but the pellets after centrifugation were small and the supernatants contained large quantities of skRLC. Accordingly the protein suspension was treated as follows to remove some of the contaminants. The suspension was incubated with 6% chloroacetic acid and stirred for 20 min at 4^{o}C, centrifuged, and the pellet re-suspended in 2 M Tris and stirred for 15 min at 22^{o}C. The solubilized skRLC was dialysed against 0.5 mM dithiothreitol, 2 mM $MgCl_2$ and 25 mM Tris/HCl at pH 7.5 and 4^{o}C and any precipitate removed by centrifugation.

skRLCs were purified in several steps, first by DEAE cellulose chromatography using a 50-175 mM NaCl gradient in 6 M urea. Solvents in this and all subsequent purification steps contained 1 mM dithiothreitol, 1 mM $MgCl_2$ and 25 mM Tris/HCl at pH 7.5. The purest samples as identified by SDS PAGE were concentrated to 34 mg/mL. Further purification was achieved through Sephacryl S-200 HR gel chromatography in 2M urea and concentrating the protein to ~6 mg/mL. This was followed by using a SP Sepharose cation exchange column with a 0-0.4 M NaCl gradient in 2 M urea. Purest fractions were concentrated to 2 mg/mL. The urea was removed by dialysis and the skRLC concentrated to 9 mg/mL by placing the dialysis bag in solid sucrose. The skRLCs were snap frozen in liquid N_2. Electro-spray mass spectrometry showed an extra Ala as a minority species in each case; measured mass in Daltons for skRLC-D was 18551 (calculated mass 18549) and +Ala 18618 (18620); for skRLC-E 18572 (18565) and +Ala 18639 (18636).

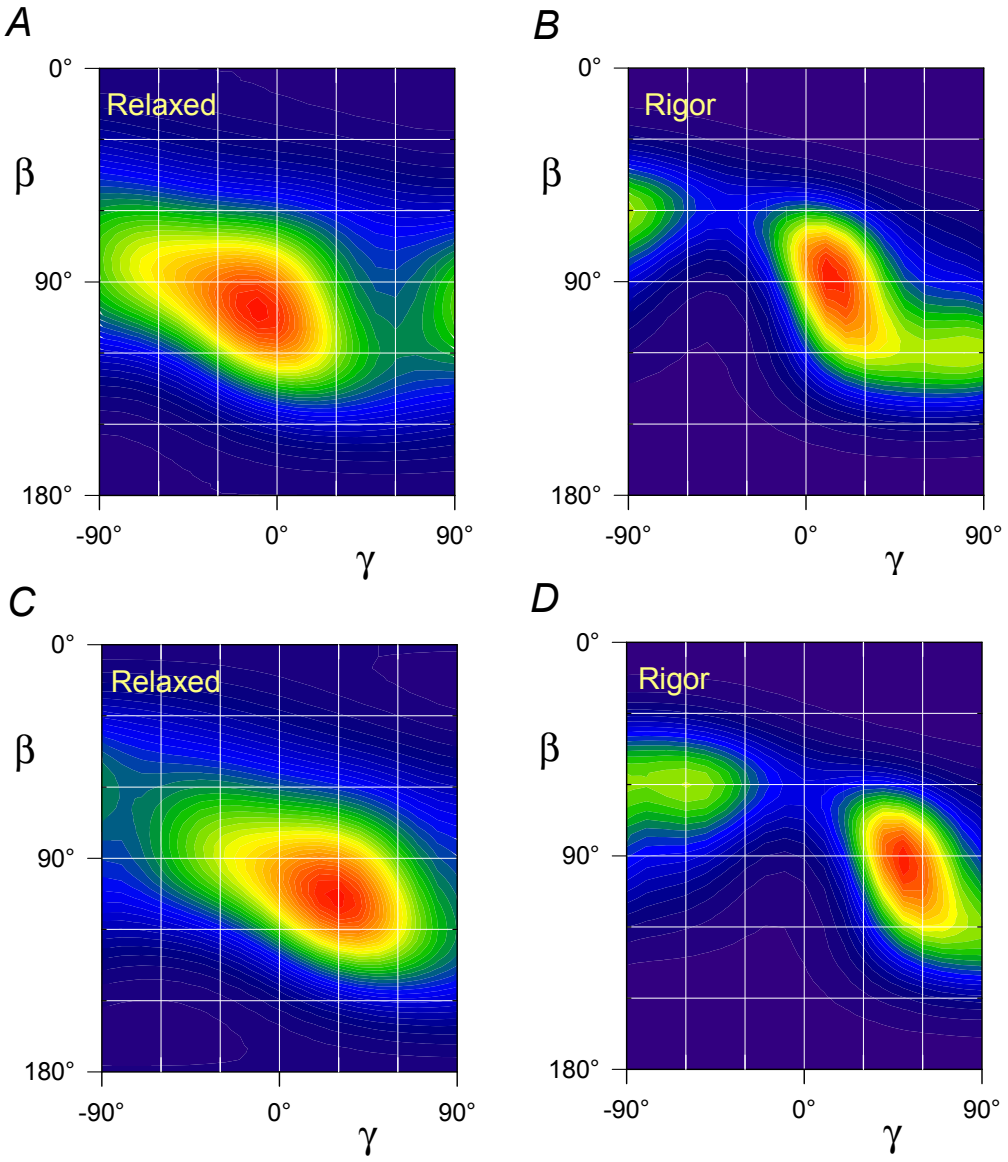

Figure S1 Maximum entropy distributions of the orientation of the light chain domain of the myosin heads in relaxation (*A,C*) and rigor (*B,D*) calculated from the orientation of BR probes on the C-lobe of the RLC (16) using the 'molecule 1' (*A,B*) and 'molecule 2' (*C,D*) structures of the light chain domain of myosin from scallop catch muscle (9).

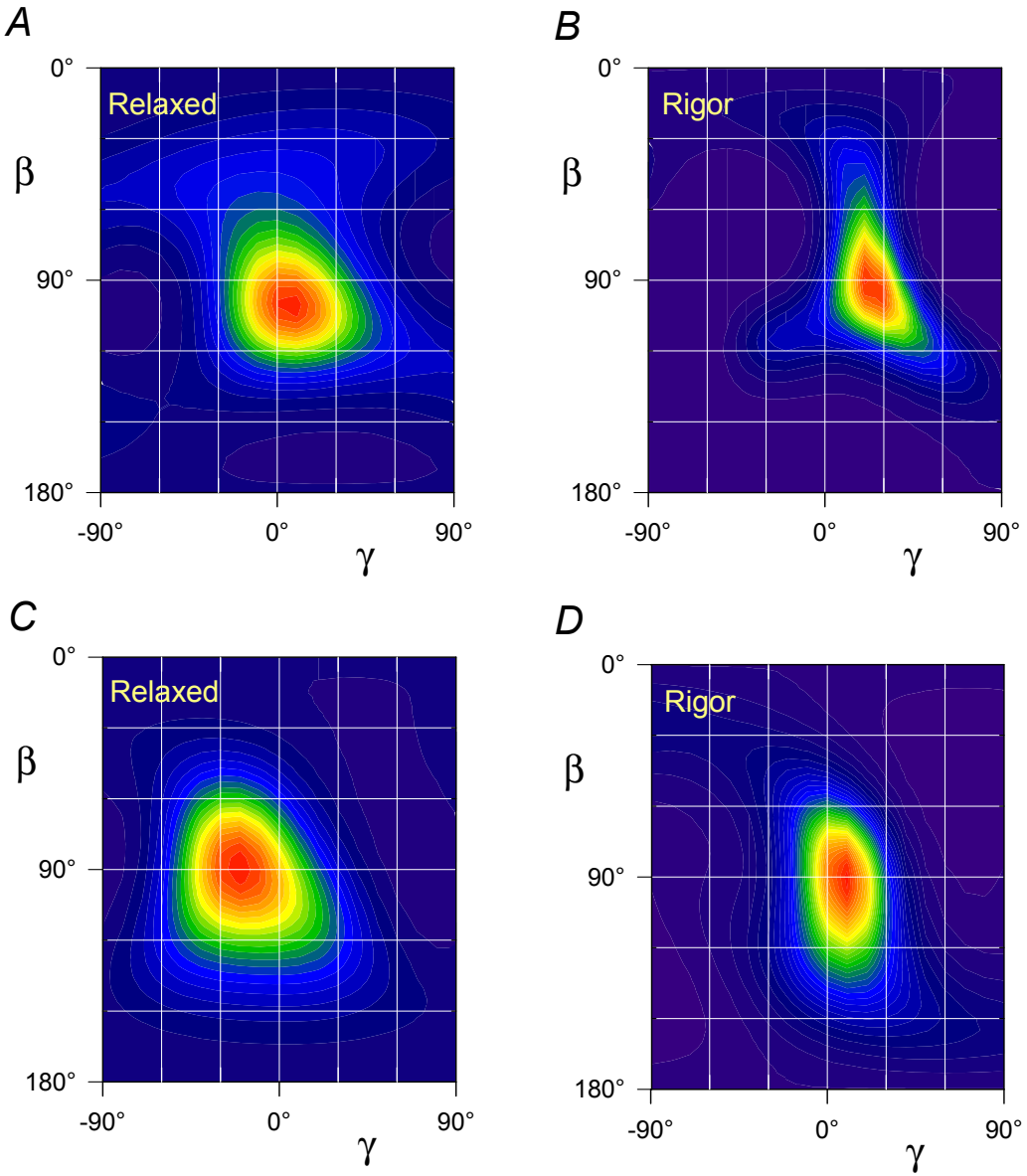

Figure S2 Maximum entropy distributions of the orientation of the light chain domain of the myosin heads in relaxation (*A,C*) and rigor (*B,D*) calculated from the orientation of BR probes on the N-lobe (*A,B*) and C-lobe (*C,D*) of the RLC using the structure of the light chain domain of squid myosin in the rigor-like state (35).