Limitations of coarse grain models of actin filament on prediction of X-ray diffraction patterns in contracting skeletal muscle

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DOI: 10.46793/ICCBIKG25.390P

Abstract: X-ray diffraction from contracting skeletal muscle provides critical structural information about myofilament organization and conformational changes during contraction. However, interpretation is challenging because observed patterns arise from the superposition of signals from multiple structural components with varying orientations and dynamics. Computational models based on explicit 3D sarcomere structures offer a way to separate these contributions and assign features in the diffraction pattern to specific molecular origins. Here, we use the MUSICO platform to generate stochastic, explicit 3D sarcomere structures and simulate X-ray diffraction patterns from all-atom and coarse-grained (CG) actin filament models with 1, 9, and 47 spheres per actin monomer. MUSICO fiber simulations were run for relaxed skeletal muscle (pCa 9), incorporating experimental values for sarcomere and interfilament spacing, temperature, actin filament length, and thin filament regulation with a 9-state crossbridge model. Comparison of simulated diffraction patterns revealed two principal CG-induced artifacts: (i) attenuation of scattering intensity due to the sphere form factor; and (ii) changes in the helical radius distribution, distorting radial intensity profiles and shifting peaks towards center. While meridional peak shapes were preserved across models, intensity differences were observed, though normalization largely removed these discrepancies. In contrast, equatorial and off-meridional profiles were more sensitive to CG level, with the 47-sphere model only valid up to the first actin meridional reflection. These findings quantify CG limitations in actin diffraction modeling and inform optimal model resolution for specific structural analyses.

Keywords: X-ray diffraction, skeletal muscle, actin filament, coarse-graining, MUSICO simulation

1. Introduction

Small-angle X-ray fiber diffraction is a powerful method for probing molecular mechanisms in muscle contraction, as it yields structural information from living tissue under physiological conditions. The highly ordered arrangement of actin and myosin filaments in the sarcomere produces rich diffraction patterns, reflecting the

superposition of scattering from multiple filament systems and regulatory proteins. While recent synchrotron facilities have enabled collection of higher-order reflections, their interpretation remains difficult because patterns combine contributions from many helically arranged proteins undergoing stochastic, nonuniform deformations.

To address this, we are using a stochastic, spatially explicit 3D sarcomere simulation platform MUSICO (MUscle SImulation COde), that tracks the coordinates of individual molecules during contraction [1]. This allows computation of diffraction patterns from realistic filament configurations, incorporating all thin and thick filament proteins.

Accurate diffraction simulations require representing each atom, but muscle filaments are massive molecular assemblies. Coarse-grained (CG) models are often used to reduce computational cost, where clusters of atoms are represented by spheres. Here, we examine the limitations of CG models for actin filament by comparing them to all-atom pattern predictions.

2. Methodology

Simulations were based on the *dom4b.pdb* actin monomer (Figure 1 E), originally provided through personal communication with K.C. Holmes, and has been used in several previous modeling studies [2].

The 1-sphere CG representation of the actin filament, where the entire monomer is replaced by a single scattering center, has been used in our earlier modeling work to predict X-ray diffraction patterns from relaxed and contracted muscle [3, 4] for meridional profile analysis despite its simplification. In addition, we selected 9-sphere and 47-sphere CG models and all-atom representations. The 9- and 47-sphere models were previously used by Koubassova et al. [5] in modeling actin and actin–S1 diffraction, where each sphere had a constant radius chosen to approximate specific resolution ranges. To more accurately evaluate the effects of CG, we adopted a variable-radius approach in which the radius of each sphere is derived from the sum of the van der Waals volumes of the atoms it contains, conserving the total filament mass.

The atomic coordinates of the monomer were partitioned into a set number of clusters (1, 9, or 47) using a *k*-means clustering algorithm [6]. Each cluster was replaced

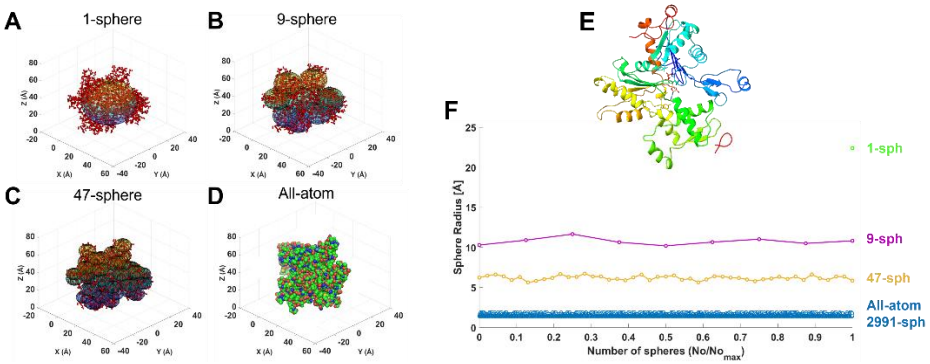

Figure 1. Structural representations of the protein model at different coarse-graining levels: (A) 1-sphere, (B) 9-sphere, (C) 47-sphere, and (D) all-atom. (E) Representation of the *dom4b.pdb*. (F) Variability of sphere radii for each CG model.

by a sphere centered at the cluster's center of mass, with radius computed from its total van der Waals volume. The resulting mean radii were: 1-sphere = 22.39 Å, 9-sphere = 10.75 ± 0.37 Å, 47-sphere = 6.20 ± 0.28 Å, and all-atom = 1.55 ± 0.13 Å (Figure 1).

Diffraction patterns were computed for actin filaments within the MUSICO platform, simulating skeletal muscle sarcomeres at very low [Ca^{2+}] (pCa = 9) to represent relaxed conditions. These simulations provided 3D coordinates of each atom (all-atom) or CG sphere. Cylindrically averaged diffraction patterns were calculated from the FT of multiple discontinuous helices, one per atom or sphere, with the finite sphere radius included as a form factor.

3. Results and Discussion

Reducing the number of spheres in the CG actin model while preserving the total filament mass changes two key features of the simulated X-ray diffraction pattern: (1) intensity attenuation due to the sphere form factor and (2) the radial peak positions and shapes. Because the total mass is conserved, fewer spheres result in larger volume and therefore a larger radius, which in turn shifts the first zero of the sphere's Fourier transform toward the pattern center. For relaxed filaments, the 1-sphere model retains intensity only up to ~31.3 Å, the 9-sphere to ~15 Å, the 47-sphere to ~8.7 Å, and the all-atom model to ~2.2 Å (Figure 2 A). The second effect comes from changes in the spatial arrangement of CG spheres centers. As the number of spheres decreases, the helical arrangement of their centers starts to diverge gradually from that of the all-atom model. This changes the interference pattern in reciprocal space, shifting the positions of peaks in radial intensity profiles, with characteristic peaks moving closer to the meridional axis and exhibiting reduced definition.

When analysis is restricted to meridional reflections, peak shapes are largely preserved across all CG levels, and normalization to each peak's own maximum effectively removes intensity differences. This means relative intensity changes between relaxed and contracted states can still be compared with the experimental observations

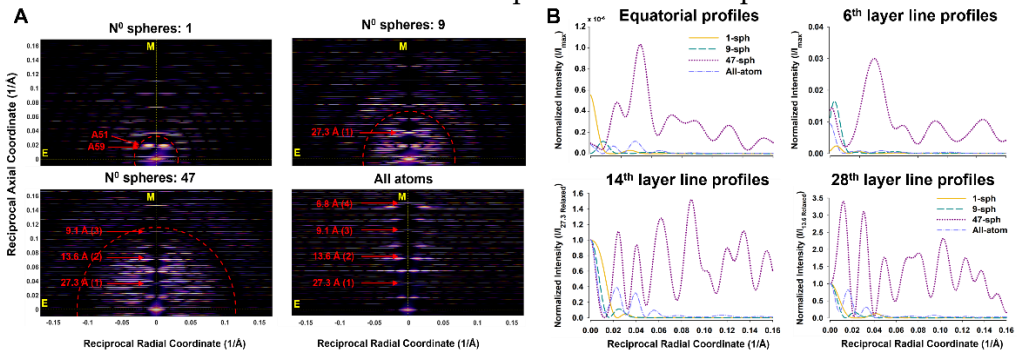

Figure 2. Comparison of simulated X-ray diffraction patterns using different model resolutions. (A) 2D diffraction patterns computed with increasing numbers of CG spheres per actin monomer (B) Corresponding equatorial and layer line intensity profiles for selected reflections.

even with highly CG models. In contrast, radial and equatorial profiles are far more sensitive to CG. Even the 47-sphere model diverges significantly from all-atom results

beyond the first actin meridional reflection (14th layer line), limiting its use in detailed radial profile analysis (Figure 2 B).

4. Conclusions

By directly comparing all-atom and CG actin filament models, we identified two main sources of error introduced by CG: attenuation of high-resolution features from the finite sphere size and distortion of radial peak positions caused by altered helical geometry. For analysis restricted to meridional reflections, even highly CG models preserved relative intensity changes between relaxed and contracted states. However, equatorial and off-meridional profiles were highly sensitive to the level of CG. These findings provide guidance for balancing accuracy and computational efficiency in future studies using CG models and indicate that higher resolution is needed (e.g., ≥260 spheres for 2991 atoms in actin monomer) for accurate radial pattern reproduction.

Acknowledgment

This research is funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants: No. 451-03-136/2025-03/200378 and the US National Institutes of Health (NIH): R01GM144555.

References

[1] S. M. Mijailovich, O. Kayser-Herold, B. Stojanovic, D. Nedic, T. C. Irving, and M. A. Geeves, "Three-dimensional stochastic model of actin-myosin binding in the sarcomere lattice," *J Gen Physiol,* vol. 148, pp. 459-488, Dec 2016.

[2] K. C. Holmes, I. Angert, F. J. Kull, W. Jahn, and R. R. Schroder, "Electron cryo-microscopy shows how strong binding of myosin to actin releases nucleotide," *Nature,* vol. 425, pp. 423-7, Sep 25 2003.

[3] M. Prodanovic, T. C. Irving, and S. M. Mijailovich, "X-ray diffraction from nonuniformly stretched helical molecules," *J Appl Crystallogr,* vol. 49, pp. 784-797, Jun 01 2016.

[4] S. M. Mijailovich, M. Prodanovic, and T. C. Irving, "Estimation of Forces on Actin Filaments in Living Muscle from X-ray Diffraction Patterns and Mechanical Data," *Int J Mol Sci,* vol. 20, Nov 30 2019.

[5] N. A. Koubassova and A. K. Tsaturyan, "Direct modeling of x-ray diffraction pattern from skeletal muscle in rigor," *Biophys J,* vol. 83, pp. 1082-97, Aug 2002.

[6] D. Arthur and S. Vassilvitskii, "k-means++: the advantages of careful seeding," presented at the Proceedings of the eighteenth annual ACM-SIAM symposium on Discrete algorithms, New Orleans, Louisiana, 2007.