Supplementary Project Information: Structural basis of Lrp4/Agrin/MuSK signaling at the neuromuscular junction (NMJ)

Low-density lipoprotein receptor-related protein-4 (Lrp4) plays a critical role in NMJ development and maintenance by mediating activation of Muscle-specific kinase (MuSK) in response to Agrin secreted by an approaching motor nerve terminal. Agrin does not bind directly to MuSK; instead, Agrin binds Lrp4, which forms a complex with MuSK, activating the kinase and ultimately producing the dense, stable clustering of acetylcholine receptors (AChRs) necessary for synaptic transmission.

The extracellular domain (ECD) of Lrp4 is sufficient to mediate MuSK activation in response to Agrin; however, few structural details are known for even this soluble fragment of Lrp4 (Fig. 1). I aim to determine the structure of ecto-Lrp4, alone and in complex with Agrin and MuSK, using cryo-EM. A near-atomic resolution structure of ecto-Lrp4 would represent the first such model of a LDL-receptor family member ECD at physiological pH. The flexibility of ecto-Lrp4 may preclude high-resolution structure determination, as has been observed for Lrp6. However, even intermediate-resolution (7 to 9 Å) ecto-Lrp4 structures, especially for a Agrin/Lrp4/MuSK signaling complex, would provide unprecedented insight into the mechanisms by which Agrin binds Lrp4 and how Lrp4 binds and activates MuSK.

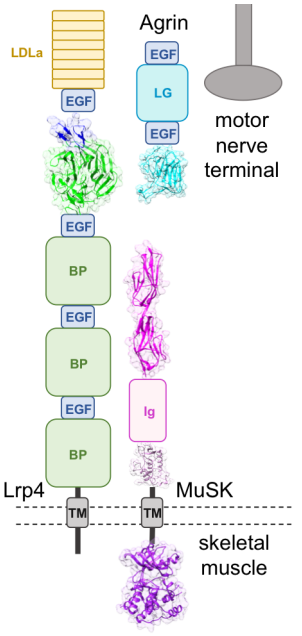

Figure 1. Lrp4/Agrin/MuSK signaling at the NMJ. Ribbons/surfaces indicate the only high-resolution structures available for Lrp4, MuSK, and Agrin. BP = β-propeller domain; EGF = EGF-like domain; LG = laminin G-like domain; LDLa = LDL-receptor class A repeat; Ig = Immunoglobulin-like domain

To this end, I have expressed and purified ecto-Lrp4 (190 kDa), ecto-MuSK (55 kDa), and a 50-kDa C-terminal fragment of Agrin from mammalian cells (in order to preserve relevant post-translational modifications). While I am currently optimizing conditions for generation of stable Agrin/ecto-MuSK/ecto-Lrp4 complexes, I attempted to determine the structure of ecto-Lrp4 alone. Purified ecto-Lrp4 appears as well-dispersed particles by both negative stain and cryo-EM (Fig. 2A-B). 2D class averaging reveals multiple views of monomeric ecto-Lrp4, including some views in which all four β-propeller domains can be easily distinguished (Fig. 2C-E). 3D reconstruction using iterative rounds of 3D classification and auto-refinement using Relion 2.1 yielded models with resolution ranging from 10 to 15 Å. To evaluate how well these density maps reconstruct the known domain structure of Lrp4, existing structures for Lrp4 β-propeller1-EGF3[1] and LDLa repeats 2-3 from the related low-density lipoprotein receptor[2] (LDLR) were fit using Chimera and fill the 3D volume reasonably well (Fig. 3). In addition to pursuing data processing strategies for improving the resolution of 3D reconstruction, I will explore the possibility of using antigen-binding fragments (Fabs) to reduce conformational heterogeneity. Ongoing efforts to capture ecto-Lrp4 in complex with ecto-MuSK and/or Agrin are also likely to yield a more homogenous, less flexible population that is more amenable to high-resolution 3D reconstruction from cryo-EM data.

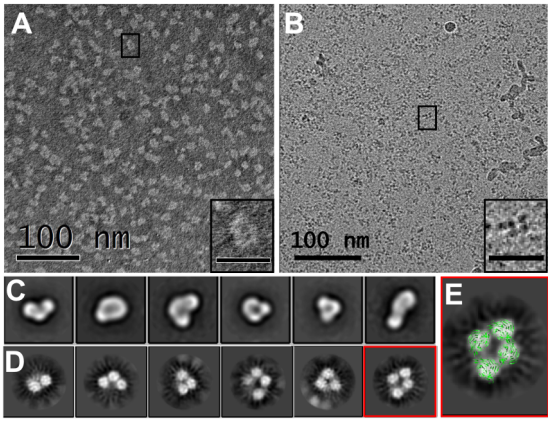

Figure 2. Well-dispersed particles of ecto-Lrp4 are visible on (A) negative stain and (B) cryo-EM micrographs. Inset scale bar = 25 nm. 2D class averages of ecto-Lrp4 from (C) negative stain and (D) cryo-EM show multiple views/conformations. (E) Overlay of Lrp4 β-propeller1 structure[1] onto a cryo-EM class average (red box from panel D).

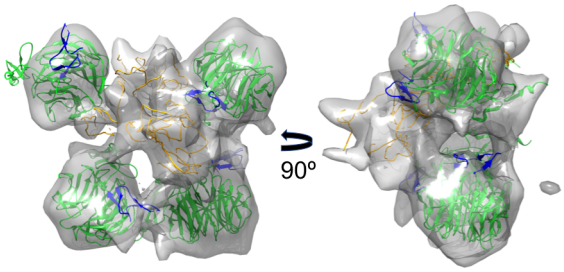

Figure 3. Fitting of atomic models into cryo-EM density map of ecto-Lrp4. Into the best 3D reconstruction of ecto-Lrp4 (10-15 Å; grey volume), the following high-resolution structures were fit using Chimera: 4 x Lrp4 β-propeller1 (green) + EGF3 (blue) from PDB ID: 3V64[1]; 4 x LDLR LDLa repeats 2-3 (yellow) from PDB ID: 1N7D[2].

1. Zong et al. (2012) Genes Dev. 26:247
2. Rudenko et al. (2002) Science 298:2353