P4.25 Structural basis for the interactions of DUX4 with Med25 and CBP/p300 Moriya Slavin1, Clothilde Claus2, Keren Zohar1, Karimatou Bah2, Tsiona Eliyahu1, Michal Linial1, Frédérique Coppée2, Nir Kalisman1 1The Hebrew University of Jerusalem, Israel 2University of Mons, Belgium The interactions of DUX4 with transcriptional activators drive its toxicity. We have previously found that the activation domain of DUX4 interacts not only with CBP/p300, but also with subunit 25 of the Mediator complex. We ventured to establish the structural basis for these interactions using the experimental approach of chemical cross-linking and mass spectrometry, together with atomic structure prediction based on the AlphaFold platform. The crosslinks identified between DUX4 and Med25 indicate that its activation domain has at least two structurally distinct binding conformations with Med25. However, one conformation is considerably more dominant and singly explains nearly all the high-intensity crosslinks. This conformation has an extensive inter-protein contact area and involves more than half of the circumference of the Med25 domain. Both canonical binding sites of Med25 are occupied by the two TAD elements from the start and end of the DUX4 activation domain. Crosslinking between DUX4 and CBP has identified the KIX domain of the latter to be the site of interaction, but follow-up mutation experiments strongly indicated that additional domain(s) of CBP/p300 are involved. Further characterization of this interaction is ongoing. Interestingly, we observed that immunodetection of p300 in human muscle cells showed a punctate nuclear or perinuclear staining in the majority of cells. Yet, following DUX4 6h-induction, nucleoplasmic staining became more pronounced.