

 <p><b>UniSR</b> Università Vita-Salute San Raffaele</p>	<p><b>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</b></p>	<p><b>MO 20-5</b> ed. 01 del 21/02/2025 PO 20 Page 4 of 8</p>
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**PROJECT**

**Supervisor:** \_\_\_\_\_Giovanna Musco\_\_\_\_\_

Title: *Rational design of drug-like peptides blocking aberrant DUX4 in FSHD*

Curriculum: \_\_\_\_\_ Biologia Cellulare e Molecolare  
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Link to the personal page of the University or relevant hospital site website: \_\_\_<https://research.hsr.it/en/divisions/genetics-and-cell-biology/biomolecular-nuclear-magnetic-resonance/giovanna-musco.htm>\_\_\_\_\_

**Description of the Project (max 3,000 characters including spaces)**

**Background/gap of knowledge**

Aberrant activation in skeletal muscles of the transcription factor DUX4 and of the related pathways is associated to Facioscapulohumeral muscular dystrophy (FSHD), thus inhibition of DUX4 transcriptional activity constitutes a therapeutic opportunity against this severe genetic muscle disorder. Our collaborator Davide Gabellini at IRCCS San Raffaele has unveiled that MATR3 functions as a natural inhibitor of DUX4. Specifically, a fragment of MATR3 directly binds to DUX4 DNA-binding domains (DUX4dbd), preventing the activation of DUX4 target genes (1). As a result, myogenic and apoptosis defects observed in FSHD muscle cells are rescued. These data hold promise for the development of innovative MATR3-based therapeutics (e.g. mimicking peptides, mPeps) able to recapitulate the interfering activity of MATR3, herewith blocking the detrimental function of DUX4 in FSHD.

**Rationale and hypothesis**

The central hypothesis of this project posits that leveraging the MATR3-DUX4 interaction could inform the development of targeted therapies for FSHD, offering a natural and safe template for

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designing drug-like molecules that target the DUX4 protein to selectively inhibit both its aberrant expression and its toxic activity.

### **Objectives and specific aims**

The main goal of this proposal is the rational design of MATR3-mPeps inhibiting aberrant DUX4 in FSHD. To this aim we will:

- 1 Structurally and thermodynamically characterize the complex between recombinant purified MATR3\_frag and DUX4dbd by biophysical techniques (NMR, BLI, ITC, SAXS, AUC) and computational methods (docking, alphafold) herewith identifying key-residues for the interaction. The complex structure will be validated by mutagenesis.
- 2 Rationally design MATR3-derived mPeps able to interact with DUX4dbd based on MATR3\_FRAG/DUX4dbd complex using the Schroedinger Suite
- 3 Evaluate MATR3-mPeps ability to inhibit DUX4 regulated gene expression and apoptosis in FSHD cellular models (in collaboration with Dr. D Gabellini, who has a longlasting experience in cellular and murine models of FSHD).

### **Expected outcomes**

This project will not only offer molecular insights into the MATR3/DUX4 interaction, but will provide a proof of concept for a drug-like approach to inhibit DUX4 activity. As such, it will pave the way for the development of MATR3-based peptidomimetics for the treatment of FSHD.

### **Skills that the student should acquire** (max. 600 characters including spaces):

The student will learn: techniques for protein expression (in E. Coli) and purification, biophysical techniques (2) for structural and thermodynamic characterization of protein-ligand interactions (Nuclear Magnetic Resonance, Isothermal titration calorimetry, microscale thermophoresis; Analytical ultracentrifugation), Computational modelling; and biochemical and cellular assays for the cellular delivery of peptides, apoptosis assays, RNA extraction, reverse transcription, and quantitative real-time PCR.

### **References** (max. 15)

1. Runfola,V., Giambruno,R., Caronni,C., Pannese,M., Andolfo,A. and Gabellini,D. (2023) MATR3 is an endogenous inhibitor of DUX4 in FSHD muscular dystrophy. *Cell Rep*, **42**, 113120.
2. Mantonico MV, De Leo F, Quilici G, Colley SL, De Marchis F, Crippa M, Mezzapelle R, Schulte T, Zucchelli C, Pastorello, *et al.* (2024) The acidic intrinsically disordered region of the inflammatory mediator HMGB1 mediates fuzzy interactions with CXCL12. *Nat Commun*, **15**, 1–18.