

Docking assays of Musashi-1 allosteric RNA-binding sites



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FICHA DEL TRABAJO FINAL

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Resumen del Trabajo

Máximo 250 palabras, con la finalidad, contexto de aplicación, metodología, resultados y conclusiones del trabajo

Abstract

A maximum of 250 words, detailing the purpose, context of application, methodology, results and conclusions of the work

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1 Introduction

1.1 Context and justification

The Musashi-1 (MSI-1) protein belongs to a family of neural RNA-binding proteins (RBPs) that play a fundamental role in neural development in both vertebrates and invertebrates [1, 2, 3, 4]. In particular, the mouse MSI-1 consists of 362 aminoacids with a molecular mass of 39 kDa [2], and contains 2 sequences of around 80 to 90 aminoacids that interact with RNA (RRMs; short for RNA-recognition motifs) following the consensus sequence $RU_nAGU^{1,2}$ [4, 6].

Despite multiple efforts, the complete structure of any of the MSI-1 protein homologs remains a mystery. The most notable successes have only been able to completely elucidate MSI-1's RRM1 and RRM2 domains [7, 8, 9, 10], see for example **Figure 1** for the 3D structure of RRM1.

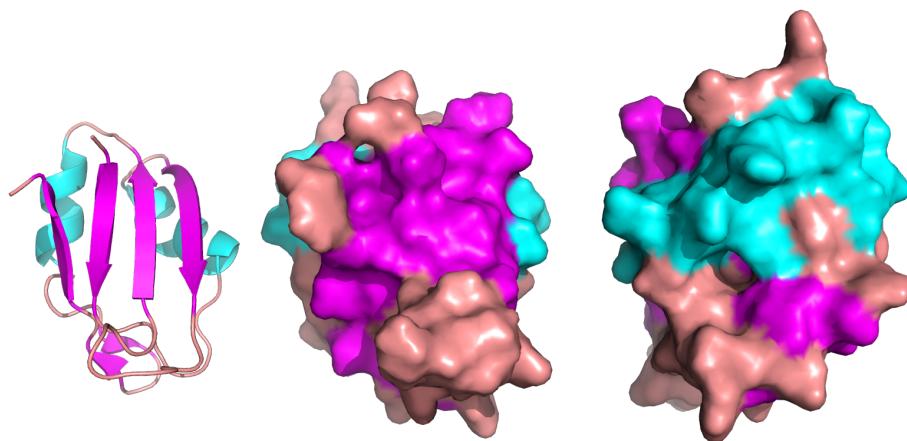


Figure 1: 3D RRM1 structure of the MSI-1 protein constructed by means of protein crystalization and RMN. From left to right: RRM1 in *cartoon* setting, RRM1 in *surface* setting and RRM1 in *surface* setting turned 180°. Colors represent the secondary structures found within the protein: cyan corresponds to α -helices, magenta corresponds to β -sheets and salmon corresponds to *random coil*. The model was retrieved from [Uniprot entry Q61474](#). Visualized through [Pymol](#).

¹Following the IUPAC standard for degenerate base symbols, R represents both an Adenine (A) and a Guanine (G). Refer to [5] for the complete IUPAC standard.

² U_n stands for repeating U within the consensus sequence n times. In most cases, $n \in \{1, 2, 3\}$ [4].

At the moment, the available complete MSI-1 structures only originate from computational predictions, such as the one shown in **Figure 2** which is an aminoacid-sequence-based prediction yielded by [AlphaFold](#) [11].

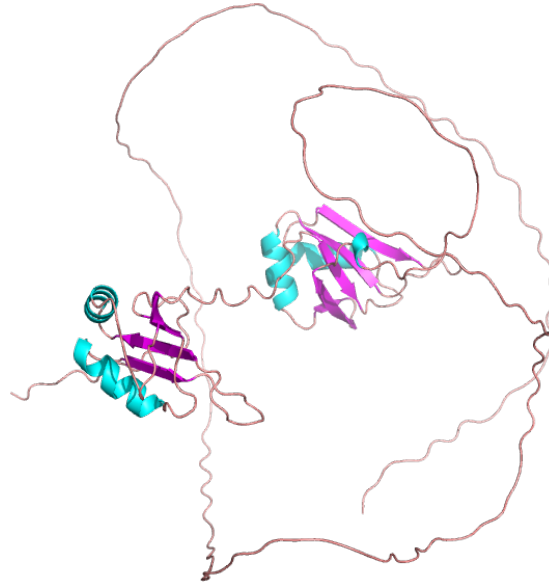


Figure 2: 3D mouse MSI-1 structure as predicted by [AlphaFold](#), in *cartoon* setting. Colors represent the secondary structures found within the protein: cyan corresponds to α -helices, magenta corresponds to β -sheets and salmon corresponds to *random coil*. The model was retrieved from [Uniprot entry Q61474](#). Visualized through [Pymol](#).

With the surge of synthetic biology during the past two decades, RBPs such as MSI-1 have gained a special interest for their applicability as post-transcriptional regulators of gene expression in synthetic gene circuits [12, 13, 14]. In particular, Dolcemascolo and colleagues pioneered by designing a synthetic genetic circuit employing MSI-1 as post-transcriptional regulator [15]. In addition, they also exploited MSI-1's particularity of binding to fatty acids in addition to RNA, out of which the *cis*-9-Octadecenoic acid (also known as oleic acid) presents a higher binding affinity. The latter interaction weakens the MSI-1 protein's RNA-binding affinity, resulting in a dissociation between MSI-1 and the RNA sequence [15, 16]. In fact, the pocket in which the fatty acids interact with the RMM1 domain of MSI1 can be easily seen in the rightmost protein in **Figure 1**.

Therefore, in order to confidently add MSI-1 as an allosteric regulator to the synthetic biologist's toolbox, there is a need to understand how the underlying interaction mechanisms work. Clingman and colleagues were the first

who performed docking simulations with the MSI-1 core RNA-binding-motif and several fatty acids [16]. Nevertheless, these results only hold for the aforementioned RNA-motif: it is expected that any mutation on the RNA-motif would drastically impact the manner in which MSI-1 interacts with the RNA.

1.2 Objectives

This Master's Thesis aims to extend the knowledge behind the allosteric interactions occurring in the MSI-1 proteins by performing docking simulations between MSI-1's RRM1 domain, several RNA motifs and fatty acids, with the objective of obtaining a model for each of the combinatorial cases.

This general objective breaks down into the following specific objectives:

1. *In silico* Generate the 3D structures of 5 mutant MSI-1 RNA-binding motifs used in [15]. The 3D structures of the motifs will depend on the sequence-based-prediction of their secondary structures.
2. Perform docking simulations between MSI-1 and each of the mutants.
3. Perform docking simulations between MSI-1 and several fatty acids of interest: oleic acid, linoleic acid, palmitoleic acid, arachidonic acid and stearic acid.
4. Keep the top 2 fatty acids whose interaction with MSI-1 is the clearest.
5. Repeat the docking simulations of the mutant RNA-motifs in presence of each of the selected fatty acids.

1.3 Impact on sustainability, social-ethical and diversity

1.3.1 Sustainability

The result of this Master's Thesis has had a negligible but negative impact on sustainability. This impact originates on the energy consumption during the following steps:

1. Design and debugging of the docking pipeline followed in this work.
2. Computation of the different models.

This impact can be considered negligible because the net amount of energy consumed during the computations is very small. Nevertheless, it is important to highlight this aspect as there is room for improvement: the source code of the docking software employed in this work is mainly composed of two programming languages: C (72%) and Python (28%).

Pereira and colleagues developed a benchmark to order programming languages with respect of their energy consumption when executing well-known computer science algorithms and data structures [17]. Their finding was that the least energy consuming programming language was C, while Python was one of the most energy consuming.

It can be discussed that, in order to reach minimal energy consumption, the programming language to be used in software that is going to be distributed and executed multiple times should be exclusively C. The disadvantages of C are that it takes more time to write a full program than with higher level languages (such as Python), it is more difficult to maintain a codebase written in C and that C is not a particularly accessible programming language for non-computer scientists.

There is a trade-off that has to be considered. Let L be a programming language, E_{W_L} be the energy consumed by a computer during the crafting of a software written in L , E_{E_L} be the energy consumed by a computer during one execution of the software written in L (in this particular case, one docking simulation with some fixed parameters) and n be the number of times the software is executed, then it is possible to approximate $E_{T_L}(n)$ (the total energy consumed by the software written in L at the moment n) as follows:

$$E_{T_L}(n) \approx E_{W_L} + n \times E_{E_L} \quad (1)$$

For the pair Python and C, we have that $E_{W_{\text{Python}}} \lll E_{W_C}$ and $E_{E_{\text{Python}}} \ggg E_{E_C}$. So the larger n becomes (the number of times the software is executed), by **Equation 1**, Python becomes less and less energy efficient when compared to C.

Nevertheless, as mentioned previously, the codebase of the software used is in its majority written in C, the energy consumption of a personal computer is low and the number of executions n in this work is small (less than 10^2). Therefore this negative impact on energy consumption can be considered negligible.

1.3.2 Social and ethical responsibility

The technical nature of this work separates it from any positive nor negative impacts on socio-ethical aspects. There are no conflicts of interests with the results, and this work's material (models and source codes) are freely available to anyone through a public `GitHub` repository.

In addition, this work doesn't have any socio-ethical motivations, as the main objective is to gather more technical knowledge for the field of molecular biology.

1.3.3 Diversity, gender and human rights

Again, the technical nature of this work separates it from any positive nor negative impacts on diversity, gender and human rights. The references and materials employed within this work were used without any gender nor race bias.

Furhermore, this work doesn't have any diversity, gender nor human rights motivations. However, this work attempts to be accessible to as many people as possible, in the spirit of the universal right for knowledge and education. Moreover, this work exclusively employed license-free and open sourced software so that all the dependencies are accessible to anyone.

1.4 Approach and methods

This work aims to extend the work of Dolcemascolo and colleagues [15] where they designed a synthetic genetic circuit that employed MSI-1 as post-transcriptional regulator. Thanks to the plasticity of RNA, several mutants of MSI-1's RNA-binding core motif were designed, and it was shown that gene regulation (which is tightly dependent on the MSI-1-RNA interaction) was altered for all mutants: some of them displayed an increase in regulation fold change while others displayed a decrease. In addition, the effects of MSI-1 induced regulation were partially nullified in presence of oleic acid.

The initial step of this work will consist in the retrieval of several 3D structures from the appropriate databases:

- the 3D structure of mouse MSI-1's RMM1 is retrieved from [UniProt](#).
- the 3D structures of several fatty acids (oleic acid among them) are retrieved from [chemspider](#).

Next, some of the RNA sequences employed in [15] are retrieved and their 3D model structures are computed by means of [NUPACK](#) and [RNAComposer](#) web server.

Then, all the docking simulations are performed:

1. Between each RNA sequence and MSI-1 RMM1.
2. Between each fatty acid and MSI-1 RMM1.
3. Between the models obtained in the simulations of step 1 and the fatty acids.

The docking software will be [LightDock](#) because it is license-free and open source. The best models will be selected based on the chosen scoring function. In addition, all the models will be visualized with [Pymol](#).

1.5 Work plan

This work is divided in the following tasks (and respective subtasks):

1. Perform docking simulations of MSI-1 and a collection of mutants of the MSI-1 RNA-binding-motif.
 - (a) Generate in silico a collection of up to 5 interesting mutant MSI-1 RNA-binding motifs.
 - (b) Perform docking simulations between MSI-1 and each of the mutants.
 - (c) Discard those mutants whose interaction with MSI-1 is poor.
2. Perform docking simulations of MSI-1 and a collection of fatty acids.
 - (a) Perform docking simulations between MSI-1 and several fatty acids of interest: oleic acid, linoleic acid, palmitoleic acid, arachidonic acid and stearic acid.
 - (b) Keep the top 2 fatty acids whose resulting model is the best.
3. Perform docking simulations of MSI-1 and a subset of mutants of the MSI-1 RNA-binding-motifs in presence of fatty acids.

4. Defense preparation

- (a) Manuscript
- (b) Presentation

The landmarks of this work are distributed among the following continuous assessment tests:

- Continuous Assessment Test 1: Thesis Definition and Work Plan.
- Continuous Assessment Test 2: Work Development (Phase 1).
Landmarks:
 - Generate in silico a collection of up to 5 interesting mutant MSI-1 RNA-binding motifs.
 - Perform docking simulations between MSI-1 and each of the mutants (*partially*).
- Continuous Assessment Test 3: Work Development (Phase 2).
 - Perform docking simulations between MSI-1 and each of the mutants.
 - Discard those mutants whose interaction with MSI-1 is poor.
 - Perform docking simulations between MSI-1 and several fatty acids of interest: oleic acid, linoleic acid, palmitoleic acid, arachidonic acid and stearic acid (*partially*).
- Continuous Assessment Test 4: Thesis Closure and Presentation.
 - Perform docking simulations between MSI-1 and several fatty acids of interest: oleic acid, linoleic acid, palmitoleic acid, arachidonic acid and stearic acid.
 - Keep the top 2 fatty acids whose resulting model is the best.
 - Manuscript closure.
 - Presentation closure.

The work plan is summarized in **Figure 3**:

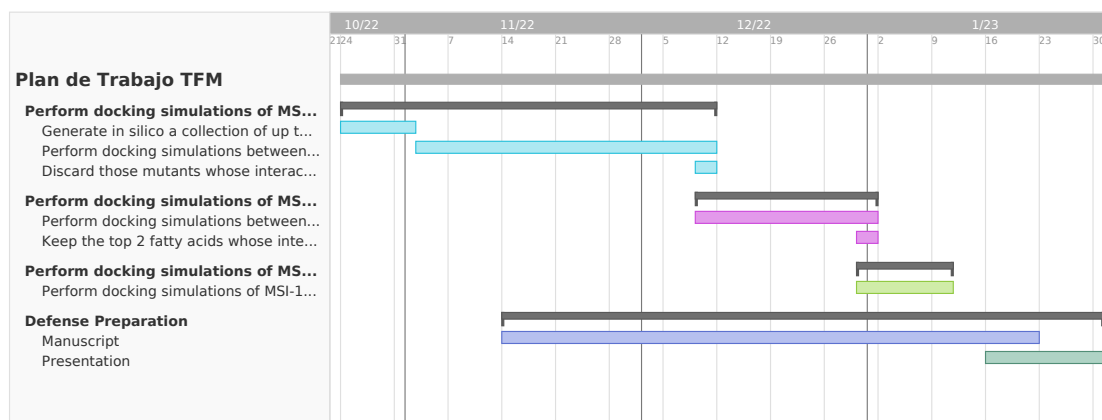


Figure 3: Work plan summarized in a Gnatt chart.

1.6 Summary of obtained products

The main products of this work are:

1. The current Master's Thesis manuscript.
2. 3D structures of 5 MSI-1 RNA-motifs used in [15].
3. *los modelos o lo que salga*
4. *los scripts del pipeline*
5. [A step-by-step tutorial on how to perform a simple protein-RNA docking with LightDock.](#)

Resulting products are available in different repositories:

1. The direct products of this work are present in [this GitHub repository](#).
2. The step-by-step tutorial on how to perform a simple protein-RNA docking with LightDock is available at [the LightDock GitHub repository](#), and soon on the [LightDock tutorials page](#).

1.7 Summary of other sections of this report

The remaining chapters of this manuscript are:

- **State of the art**
- **Materials and methods**
- **Results**
- **Conclusions and further work**
- **Glossary**
- **References**
- **Appendix**

2 State of the art

3 Materials and methods

4 Results

5 Conclusions and further work

6 Glossary

MSI-1

RBP

RRM

NMR

7 References

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8 Appendix