**Appendice 9**

# A *Unique* Method, based on the Maximum Ordinality Principle, for Skipping *any* Exon in Duchenne Muscular Dystrophy (DMD)

Corrado Giannantoni

Ex ENEA's Researcher and Consultant of
Duchenne Parent Project Onlus
Rome, Italy
email: c.giannantoni@parentproject.it, corrado.giannantoni@tin.it

***Abstract* – The present article aims at showing the possibility of a *Unique Method* finalized to skip any Exon, in the case of Duchene Muscular Dystrophy (DMD), when the process is modeled by adopting the Maximum Ordinality Principle (MOP) as the basic reference criterion.**

***Keywords- Exon-Skipping, Duchene Muscular Dystrophy (DMD), Antisense Oligo-Nucleotides (AONs), Maximum Ordinality Principle (MOP)***

## I. INTRODUCTION

The research for a *Unique Method* aimed at skipping *any* type of Exon in the case of DMD, is strictly related to the fact that, although the various AONs apt to skip every Exon have already been designed [1], the subsequent research for an effective therapy is usually focused on a *limited number* of Exons. The latter in fact generally correspond to the most frequent cases of DMD, in the population of children affected by such a severe pathology.

This limitation is essentially due to two fundamental reasons: i) the expected *return* on related investments on behalf of various Research Institutes and Pharmaceutical Companies; ii) the very rigid authorization procedures and associated conditions required by FDA and EMA, especially as far as the *wide number* of cases positively tested, as a solid base from a statistical point of view.

Both these reasons are evidently impossible to be satisfied in the case of very rare Exons, such as, for instance, Exon 39 (e.g., two sole cases in all Italy).

The research for a Unique Method, vice versa, could potentially overcome the two above-mentioned conditions, with some further additional advantages, as it will be shown in the conclusions of this article.

## II. THE RATIONAL OF THE METHOD

The Method here proposed is nothing but the transposition of the same method already adopted in the case of Protein-Protein Interaction (PPI) [2], whose explicit solution was obtained, in a *fast* and *reliable* way, as the *formal solution to an N-body interaction problem*, precisely because the process was modeled on the basis of the Maximum Ordinality Principle (MOP).

In fact, after having obtained the solution to the "Three-body Problem" in terms of *fractional incipient derivatives* [3], previously introduced in [4][5], the extension to the case of N bodies was obtained in the contest of the mathematical formulation of the MOP [6].

This result immediately suggested its application to Protein Folding [7][8] and, immediately after, to the previously recalled case of PPI.

Now, as this article will clearly show, the same method can be adopted for skipping any Exon in DMD, where the interacting systems are made up of Bases.

In this respect, Section 3 will preliminary present the input/output of the mathematical model adopted. Section 4 will illustrate the solution process through an ostensive example. The general applicability of the method and its informatics advantages will be discussed in Section 5 and 6, respectively. Section 7, in turn, will deal with the general validity of the method in the light of the MOP, while Section 8 (devoted to the conclusions) will show how the *Unique Method* here proposed is potentially able to overcome the two "limiting" conditions previously mentioned in the Introduction, with some further additional advantages.

## III. INPUT/OUPUT OF THE MATHEMATICAL MODEL

The formal enunciation of the MOP, with specific reference to biological problems, was first presented in [7][8], while the explicit Solution, structured in the form of *Harmony Relationships*, is given in [9][10].

On the basis of such a solution, in order to get the reference topological configuration of any given Exon, it is sufficient:

i) to know the *total number* of its Bases (*N1*);

ii) to assign three parameters $(\Sigma_{12}, \Phi_{12}, \Theta_{12})_{N1}$ that define, in polar coordinates, the reciprocal positions of two *arbitrary* Bases (conventionally termed as "12"), understood as being *one sole* "*isolated" entity*. This is also the reason why the latter is topologically referred to its own internal reference system;

iii) to assign, in addition, six appropriate parameters $(\varepsilon_1, \varepsilon_2, \varepsilon_3, \psi_1, \psi_2, \psi_3)_{N1}$ that define the internal *Relation Space* (RS) of the Exon analyzed.

More specifically: $(\varepsilon_1, \varepsilon_2, \varepsilon_3)$ characterize the spatial orientation of the Exon (understood as a Whole), with respect to its internal reference axes; whereas $(\psi_1, \psi_2, \psi_3)$ define the periodicities (along the three basic axes) of the mathematical solutions which "emerge" from the MOP.

These solutions are precisely those that give the positions of all the Bases with respect to the internal axes of the considered Exon. In this way, the afore-mentioned solutions characterize any considered Exon as a *unique*, *specific* and *irreducible* entity.

Under such conditions, each Exon, precisely because modeled as a "self-organizing" system of *ordinal nature* (see Section 5), is also characterized by its own specific *self-organizing capacity*, whose *activity* can faithfully be represented by its associated "virtual work", defined (in polar coordinates) as

$$W = \sum_{j=2}^{N} \{(\rho_{1j}) + (\rho_{1j}\varphi_{1j}) + (\rho_{1j}\vartheta_{1j})\} \qquad (1),$$

where the subscript 1j indicates the couples of Bases successively considered in the sum.

The same procedure can also be adopted to get the topological configuration of *any* AON (Antisense Oligo-Nucleotide), or *other biological Systems*, specifically conceived to interact with the Exon under consideration To this purpose, in fact, it is sufficient:

i) to know the *total number* of Bases of the AON (*N2*);

ii) to assign three parameters $(\Sigma_{12}, \Phi_{12}, \Theta_{12})_{N2}$ that define, in polar coordinates, the reciprocal positions of two *arbitrary* Bases of the considered AON, always understood as being *one sole* "*isolated" entity*;

iii) to assign, as in the previous case, six appropriate parameters $(\varepsilon_1, \varepsilon_2, \varepsilon_3, \psi_1, \psi_2, \psi_3)_{N2}$ , that define the internal *Relation Space* (RS) of the AON analyzed, with the same meaning previously specified. This means that, also in this case, the corresponding solution characterizes the considered AON as a *unique*, *specific* and *irreducible* entity.

On the basis of such a characterization, the Model is able to furnish the formal solution to the Interaction Process between any given Exon and the related specific AON each time considered.

Such a solution can easily be obtained, in a fast and reliable way, by means of an appropriate Simulator termed as EQS (Emerging Quality Simulator).

The latter in fact is conceptually structured in three parts: the first part is devoted to the Exon under consideration; the second one to the associated AON analyzed; while the third part is specifically referred to the final compound that "emerges" from the Interaction Process between the two.

## IV. AN OSTENSIVE EXAMPLE

The example adopted to illustrate the Methodology under consideration refers to the choice of the most appropriate AON finalized to the Skipping of Exon 39.

The Method is articulated in four successive phases:

**i) First Phase: Reconfiguration of Exon 39**

By choosing the appropriate values of the parameters presented in Section III, and by adopting them as input to the EQS section devoted to the Exon, it is possible to obtain the topological reconfiguration of Exon 39, so that it can directly be compared with the corresponding configuration available in Literature.

More explicitly, in our case it is sufficient to specify the *total number* of Bases of Exon 39 (*N1 = 138*); to assign the coordinates $(\Sigma_{12}, \Phi_{12}, \Theta_{12})_{N1}$ of the reference couple and the six parameters $(\varepsilon_1, \varepsilon_2, \varepsilon_3, \psi_1, \psi_2, \psi_3)_{N1}$ that define the *Relation Space* (RS) of Exon 39, so as to get the representation of Exon 39 which is maximally corresponding to that already available in Literature.

Fig. 1 shows the reconfiguration of Exon 39 obtained on the basis of the MOP and its explicit Emerging Solutions, when the latter are structured in the form of *Harmony Relationships* [9][10].

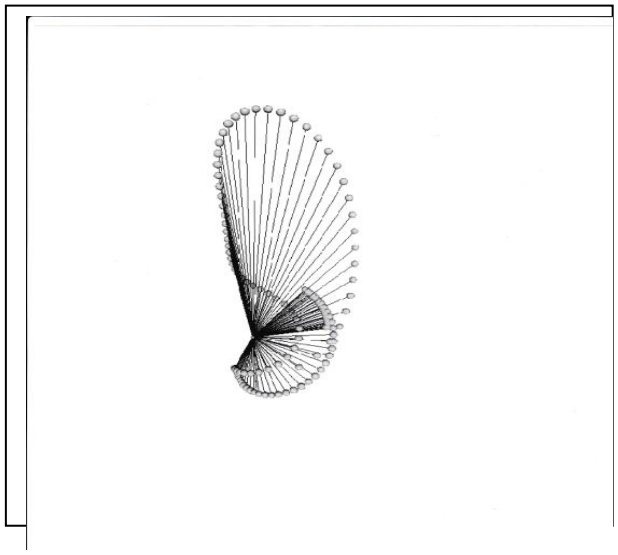


**Fig. 1 – Reconfiguration of Exon 39**

By comparing such a resulting reconfiguration with the structure of Exon 39 already available in Literature, some slight differences may sometimes appear. These, however, are in general due to the fact that, in our case, the considered Exon is always reconfigured *in the space*, on the basis of the MOP Harmony Relationships (that is in Ordinal terms), whereas all the corresponding structures available in Literature are generally drawn *on a plane*, and in terms of functional relationships (ib.).

In all cases, such slight differences do not affect the final results and the corresponding evaluations.

**ii) Second Phase: the choice of the basic AON**

The choice of the most appropriate AON to skip each given Exon is the result of an *iterative procedure* that will now be illustrated in the case of Exon 39.

The first step consists in assuming a starting reference AON characterized by an (integer) number of Bases that best approximates the ratio N1/10.

In our case:

$$N2 = N1 /10 = 138 /10 = 13.8 \quad (2)$$

a value that is consequently rounded to N2 = 14.

At this stage, in order maximize the reciprocal "electivity" between the selected AON and the Exon to be skipped, so as to avoid any "mis-targeting" process, the above-mentioned parameters $(\Sigma_{12},\Phi_{12},\Theta_{12})_{N2}$ and $(\varepsilon_1,\varepsilon_2,\varepsilon_3,\psi_1,\psi_2,\psi_3)_{N2}$, pertaining to the AON, are assumed exactly equal to the corresponding parameters of the chosen Exon. That is

$$(\Sigma_{12},\Phi_{12},\Theta_{12})_{N2} = (\Sigma_{12},\Phi_{12},\Theta_{12})_{N1} \quad (3)$$

$$(\varepsilon_1,\varepsilon_2,\varepsilon_3,\psi_1,\psi_2,\psi_3)_{N2} = (\varepsilon_1,\varepsilon_2,\varepsilon_3,\psi_1,\psi_2,\psi_3)_{N1} \quad (4).$$

**iii) Third Phase: the choice of the optimal AON**

By adopting such values as input to the third section of EQS, it is possible to see, on the screen of the computer, the topological configuration of the final compound, as the result of the Interaction between Exon 39 and the basic AON previously selected. At the same time, the third section of EQS presents several Indicators that enable us to choose the optimal AON.

The fundamental Indicator is given by the ratio

$$(\delta W)_r = \{W_3 - (W_1 + W_2)\}/(W_1 + W_2) \quad (5),$$

that is: the excess of the Virtual Work of the final compound, with respect to the sum of the Virtual Works of the initial Exon and related AON, when such an excess is referred to the latter sum.

Indicator (5) has a "comprehensive" meaning, because it accounts for two different aspects: the "deformation" (in length and/or in width) of the final compound and, at the same time, the specific typology of its "spatial re-conformation".

The optimal AON is precisely that which is characterized by a number of Bases corresponding to the maximum negative value of ratio (5). That is

$$\max\ (\delta W)_r < 0 \quad (6).$$

Such a condition is also confirmed by the variation of $(\delta W)_r$ due to a variation of $(\Sigma_{12},\Phi_{12},\Theta_{12})_{N1}$, as a consequence of the related topological reconfiguration.

Consequently, the optimal number of Bases can be found by means a simple iterative procedure. In fact, through successive steps, by adopting as an input to EQS both a progressively increasing and decreasing number of Basis of the considered AON (in our case: 15, 16, 17, etc., as well as 13, 12, 11, etc.), it is possible to find the value of N2 that satisfies condition (6).

In the case of Exon 39 the optimal value of N2 is equal to 18, because this number of Bases leads to

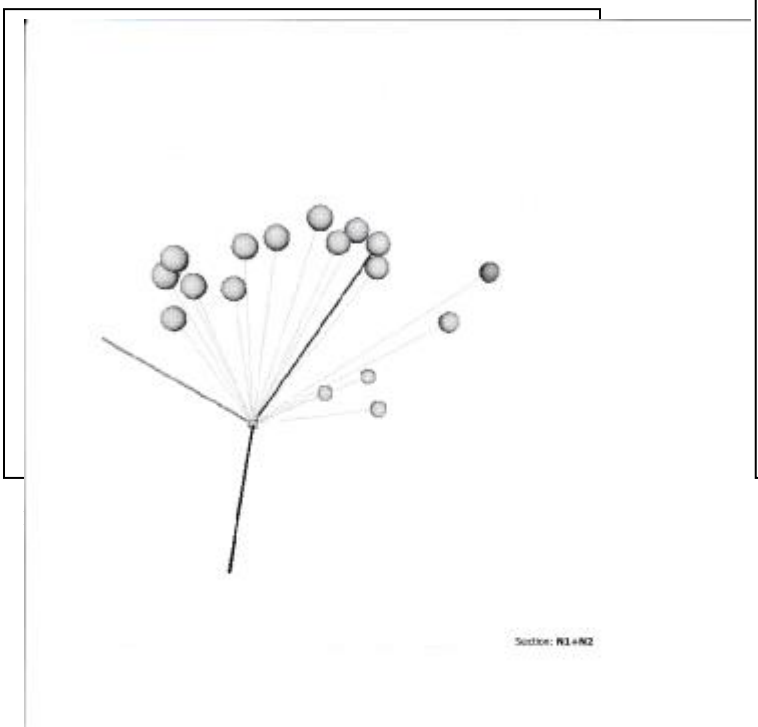
$$\max (\delta W)_r = - 0.056 \quad (6.1).$$

The corresponding spatial configuration of such an AON, made up of 18 Bases, and also characterized by the initial values (3) and (4), is represented in Fig. 2

The configuration so obtained can sometimes result as being similar to any AON already known in Literature.

For example, the AON represented in Fig. 3 (made up of 18 Bases) is somewhat similar to the optimal AON represented in Fig. 2.

The major differences between the two, in fact, as anticipated in the case of the Exon, generally depend on the fact that the AON in Fig. 2 is represented in the *space*, as a solution to the MOP in the form of Harmony Relationships (that is, in Ordinal terms). The AON in Fig. 3, on the contrary, apart from its mirror-like configuration, is given on *a plane*, in the sole respect of functional relationships and geometrical symmetries.



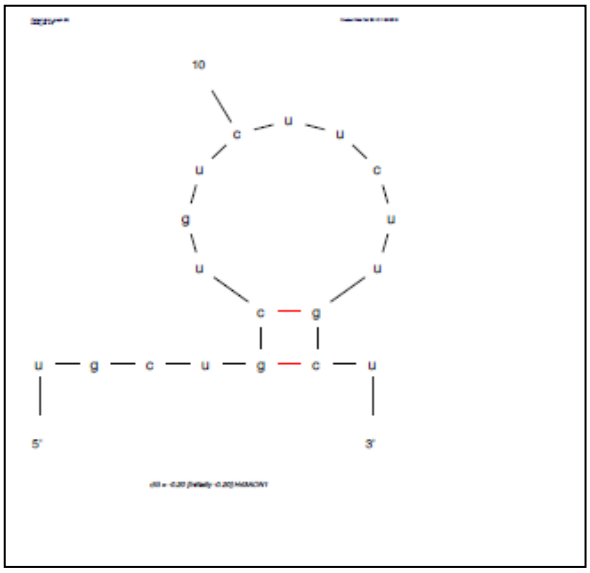



**Fig. 3 – An AON of 18 Bases already available in Literature**

Such considerations, however, only represent a simple procedural suggestion, in the sense that: if there exists in Literature an AON which is really similar to the optimal AON previously obtained, the former can be considered as being already available for the Exon-Skipping Process. Otherwise, the optimal AON, that is the one which is properly apt to Exon-Skipping, has to be realized ex novo, in laboratory, in a strict adherence and conformity to the specific output obtained from the Simulator (such as that in Fig. 2).

This is because the latter is the *unique* and *sole* AON that satisfies the reciprocal "electivity" conditions (3) and (4), and, at the same time, condition (6).

**iv) Forth Phase: the final Compound**

On the basis of the previous steps, the third part of EQS Simulator is able to furnish, in a few seconds, the topological configuration of the resulting compound, as a consequence of the Interaction Process between the Exon and AON *as previously characterized*.

At the same time, a wide set of Indicators, calculated by this section of EQS, facilitates the interpretation of the entire interaction process.

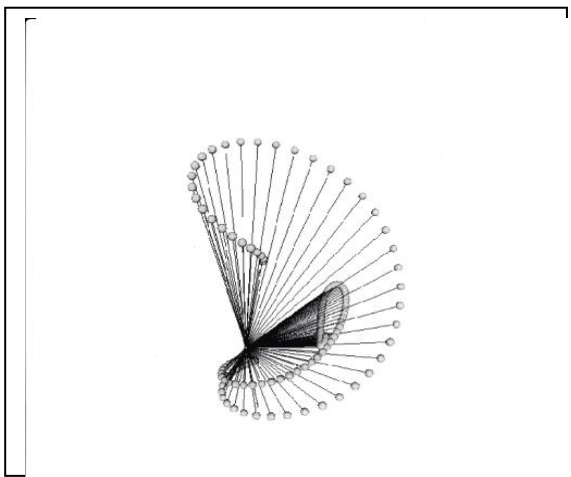
As an example, let us consider the topological configuration of the final compound shown in Fig. 4.

The spatial coordinates $(\rho_{1j}, \varphi_{1j}, \theta_{1j})$ pertaining to each Basis, as explicitly furnished by the Simulator EQS and, at the same, the topological reconfiguration of the final compound (represented in Fig. 4), enable us to calculate the *longitudinal* and *transversal* ratios of the spatial dimensions of the final compound with respect to those of the initial Exon.

In our case, such ratios are equal to

$(d_3 / d_1)_l = 0.94$ (7.1) and $(d_3 / d_1)_t = 1.15$ (7.2),

where *l* stands for *longitudinal* and *t* for *transversal*, while the values (7.1) and (7.2) already take into account for the different scales adopted in Fig. 1 and in Fig. 4, respectively.



**Fig**

These values indicate that the final compound has become a *little shorter* and, at the same time, a *little larger*.

What's more, Fig. 4 shows a very particular aspect: the final compound has *radically* modified its original configuration, in terms of increased *internal chirality*. This is particular evident with specific reference to the "open branch" which "appears" in its final topological configuration.

As already anticipated, both these two aspects are taken into account by the same "comprehensive" value of Indicator (6).

However, for their particular importance, such two above-mentioned aspects will be reconsidered in more detail later on. This is because, on the basis of both such topological characteristics, it is possible to formulate a valid hypotheses of an *actual* and *effective* Exon-Skipping process.

Nonetheless, before drawing such a conclusion, it is worth considering some further properties of the Method here proposed.

## V. INFORMATICS ADVANTAGES

The informatics advantages of the present Method are directly referable to the *formal properties* that are intrinsic to the mathematical models adopted. In fact, any system modeled on the basis of the MOP, always presents *explicit solutions* in terms of *Incipient Differential Calculus* (see [4] and [5]).

This means that the Method has the *capacity* of *predicting the 3D structure of the resulting compound* essentially because the latter is understood as a Self-Organizing System of *ordinal nature*, and thus as being *intrinsically* "*irreducible*" to functional relationships between its parts [9][10].

This correlatively also means: i) *a reduced number of computations*; ii) *a reduced need* of *High Performance Computing* (HPC); iii) *a reduced incidence* of *special numerical methods* to be adopted to get the corresponding solution.

What's more, the explicit solutions so obtained can also be termed as "*emerging solutions*" ((ib.) and [11]), because *they always show an ordinal information content which is much higher than the corresponding content of the initial formulation of the problem*.

This is because the MOP is specifically finalized to describe "Self-Organizing" Systems according to a *holistic approach*, in which, as is well-known, "*the whole is much more than the sum of its parts*".

In such a perspective, all the biological processes can always be modeled as being "Self-Organizing Systems" in the light of the MOP [10]. Such an assertion, of course, is also valid, for the same Exon-Skipping Process under consideration.

## VI. GENERAL VALIDITY OF THE METHOD IN THE LIGHT OF THE MOP

The Method previously illustrated, already adopted in the case of Protein-Protein Interaction [2], is thus applicable to the majority of biological problems usually dealt with through informatics methods.

In this sense, PPI only represents the first and, probably, the most important ostensive example.

The same method, in fact, can also be adopted in the case of Molecular Docking and Drug Design. This is because it enables us to choose the *optimal ligand*, that is the one resulting as being characterized by the most appropriate "electivity", as already shown in the case of Exon-Skipping in DMD.

Consequently, the general validity of the Method here proposed appears much more evident when the same Method is situated in its appropriate general context, that is, *The Maximum Ordinality Principle*.

In fact, by adopting the MOP as a basic reference criterion, it is possible to realize mathematical models of *all the biological Systems*, with very significant related advantages [9][10].

These considerations can also represent, at the same time, the most valid introduction to the next Section.

## VII. GENERAL APPLICABILITY OF THE METHOD TO SKIPP ALL THE EXONS IN DMD

As a consequence of the previous considerations, the Method illustrated in the case of Exon 39 can be considered as having a general applicability to all the other Exons of Dystrophyn.

In other words, it can be adopted to skip *any chosen Exon* in the case of DMD.

The Method, in fact, is able to show that:

i) the majority of Exons, by starting from their initial conditions (that is between their flanking Introns), when interact with an appropriate AON (specifically selected according to such a Method), may become *a little shorter* and**,** at the same time, *a little larger*, with respect to its initial topological dimensions;

ii) a reduced number of Exons, vice versa, may present a corresponding trend that is exactly the opposite. In fact, by starting from the same above-mentioned initial conditions, some Exons, when interacting with an appropriate AON (ad hoc selected, according to the procedure previously illustrated), may become *a little longer* and, contextually, *a little thinner* with respect to their initial topological dimensions;

iii) such a trend, however, does not represent the most important aspect. In fact, the *dominating aspect* able to induce a correlative Exon-Skipping is represented by *the internal topological reconfiguration of the Exon, as a consequence of the wide variation of its chirality* due to the interacting process (see [12][13]).

This represents, in fact, the fundamental evolving process, which ends up by radically "modifying" any considered Exons and, consequently, the one which "guides" its related successive evolution.

Under such radically modified conditions, it is expected that the Exon will not be "recognized" by the spliceosome, because it results as being profoundly modified in its topological *internal* conformation.

Consequently, the considered Exon should be spliced out together with its associated flanking introns and, correspondently, the Exon should result as being skipped.

## VIII. CONCLUSIONS

The Method here proposed seems to be able to give a significant contribution to DMD therapy. This is precisely because the results previously shown indicate that, apart from some other innovative aspects, the Method is able to overcome the two "limiting" initial conditions. In fact:

i) the extremely simple procedure to select the most appropriate AON for each Exon to be skipped reduces the theoretical research costs usually devoted to this purpose;

ii) the associated experimental costs are also reduced, because the tests can be focused on *one sole* AON per *each* Exon. In fact, additional tests of different AONs (although always possible) are not, strictly speaking, necessary;

iii) in the end, after having shown the efficacy of the AONs so selected with respect to the most frequent cases of DMD, and after having also obtained the pertinent authorization from FDA and EMA;

iv) it should result as being much easier to obtain the same authorization also for the extremely rare Exons, because the corresponding AONs are always selected on the basis of the *same* and *unique* method, already shown as being valid for the most frequent cases;

v) this is especially because conditions (3) and (4), concerning the reciprocal "electivity" between such rare Exon and the corresponding AON adopted, prevent from any possible "mis-targeting" in each operative Exon-Skipping Process;

vi) without forgetting that the *unique* Method describes the dynamic evolutions of any Exon-Skipping process on the basis of the *Maximum Ordinality Principle* [14], which, as previously shown, has a very general validity (that is, is not limited to the sole case of Exon-Skipping in DMD);

vii) in addition, when the Exon-Skipping process is modeled by means an appropriate simulator based on the MOP, that model can be run on a simple personal computer, in a computation time of a few minutes;

viii) this also means that, by adopting the afore-mentioned Method, any researcher would be able to analyze the dynamic behavior of any selected Exon-

Skipping process by means of his/her own PC, by simply sitting at his/her own desk.

ix) This represents, among others, one of the most important aspects that reduces the theoretical research investments in order to find the best AON to be adopted for each Exon to be skipped.

The factual validity of the Method, of course, has to be confirmed on the basis of experimental tests, which could also suggests some further procedural improvements of the same.

Nonetheless, the present work seems already apt to show the plausibility of a *unique* Method for the Exon-Skipping process in DMD.

## REFERENCES


[1] S.D. Wilton, A.M. Fall, P.L. Harding, G. Mc Clorey, C. Coleman, S. Fletcher, "Antisense Oligonucleotide-induced Exon Skipping Across the Human Dystrophin Gene Transcript". Molecular Therapy (2007) 15 7, 1288-1296.

[2] C. Giannantoni, "Protein-Protein Interaction in the light of the Maximum Ordinality Principle". Proceedings of the 7th International Conference on Bioinformatics, Bio-computational Systems and Biotechnologies. *BIOTECHNO 2015*. May 24 - 29, 2015, Rome, Italy.

[3] C. Giannantoni, "From Transformity to Ordinality, or better: from Generative Transformity to Ordinal Generativity". Proceedings of the 5th Emergy Conference. Gainesville, Florida, USA, January 31-February 2, 2008, pp. 581-598.

[4] C. Giannantoni, "The Problem of the Initial Conditions and Their Physical Meaning in Linear Differential Equations of Fractional Order". Applied Mathematics and Computation 141, 2003, pp. 87-102.

[5] C. Giannantoni, "Mathematics for Generative Processes: Living and Non-Living Systems". Applied Mathematics and Computation 189, 2006, pp. 324-340.

[6] C. Giannantoni, "The Maximum Ordinality Principle. A Harmonious Dissonance". Proceedings of the 6th Emergy Conference. Gainesville, USA, January 14-16, 2010, pp. 55-72.

[7] C. Giannantoni, "Protein Folding, Molecular Docking, Drug Design. The Role of the Derivative "Drift" in Complex Systems Dynamics". Proceedings of the Third International Conference on Bioinformatics. Valencia, Spain, January 20-24, 2010, pp. 193-199.

[8] C. Giannantoni, "Bio-Informatics in the Light of the Maximum Ordinality Principle. The Case of Duchenne Muscular Dystrophy". Proceedings of the 4th International Conference on Bioinformatics. Rome, January 26-29, 2011, pp. 244-250.

[9] C. Giannantoni, Toward One Sole Reference Principle Generating "Emerging Solutions" of progressively ascending Ordinality. Proceedings of the 8th Biennial Emergy Research Conference. University of Florida, Gainesville (USA), January 16-18, 2014.

[10] C. Giannatoni, "The "Emerging Quality" of Self-Organizing Systems, when modeled according to the Maximum Ordinality Principle, offers a radically New Perspective to Modern Science". Proceedings of the 9th Biennial Emergy Research Conference. University of Florida, Gainesville (USA), January 6-7, 2016.

[11] C. Giannantoni, "The Relevance of Emerging Solutions for Thinking, Decision Making and Acting. The case of Smart Grids". Ecological Modelling 271, 2014, pp. 62-71.

[12] A. Aartsma-Rus, W. E. Kaman, R. Weij, J. T. Den Dunnen, G. J. van Ommen, J. C. van Deutekom, "Exploring the Frontiers of Therapeutic Exon Skipping for Duchenne Muscular Dystrophy by Double Targeting within One or Multiple Exons". Molecular Therapy, Vol. 14, 2006, pp. 401-407.

[13] A. Aartsma-Rus, Gert-Jan B. van Ommen, "Antisense-mediated exon skipping: A versatile tool with therapeutic and research applications". RNA. 2007 Oct; 13(10): 1609-1624.

[14] www.ordinality.org: author's website that presents a general framework about the M.O.P, from the Mathematical Formulation of the Maximum Em-Power Principle up to the Mathematical Formulation of the M.O.P., together with some Ostensive Examples mentioned in this paper.