

## A Substitute for Dystrophin: Why Utrophin Fails

Cyrina Ostgaard

Each year 1 in 3500 males in the United States are born with Muscular Dystrophy (MD).<sup>1</sup> In serious cases this disease is marked by heavily atrophied musculature, mental impairment, cardiomyopathy, and a shortened lifespan (~20 years).<sup>1</sup> Currently, there is no cure for this debilitating disease and treatment options remain abysmal. Two major forms of this disease exist, Duchenne's Muscular Dystrophy (DMD), a severe form, and Becker's Muscular Dystrophy (BMD), a mild form. Both disease forms are caused by mutations in a protein called dystrophin. This is a protein that's primarily found in muscle cells and functions by binding to actin to link it to the muscle cell membrane (sarcolemma).

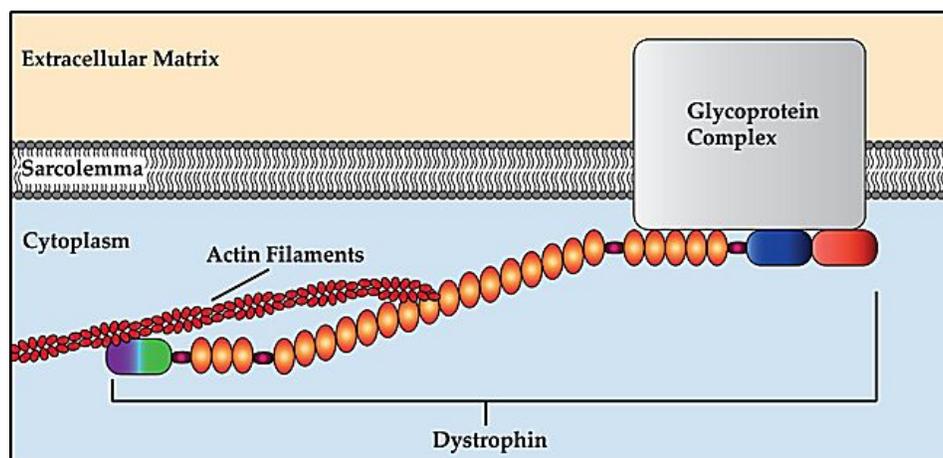
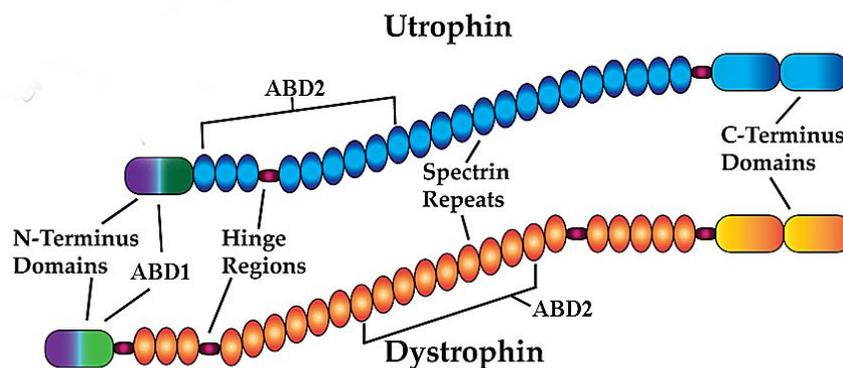


Figure 1. Dystrophin binding actin the muscle cell membrane (sarcolemma).

Dystrophin functions to maintain muscle integrity under stress. It's been proposed that by linking actin to the membrane, the muscle cell is kept intact in addition to preventing deformation of the membrane. This occurs because actin is a structural protein associated with maintaining the cytoskeleton of the cell. Another cytoskeleton protein, myosin, is used to allow for muscle contraction. This occurs upon receipt of an electrical impulse from the neuron which results in the synchronized motion of myosin pulling actin fibers past to contract the muscle. The

problem is that in people who have muscular dystrophy don't have a properly functioning form of dystrophin. Without dystrophin, the muscle cell membranes are easily deformed, have reduced stiffness, and limited ability to contract due to reduced ability for actin to link with the contractile apparatus. These effects on the muscle cells result in the myopathy (muscle disease) known as muscular dystrophy.

Finding a way to combat this disease is a major issue to be addressed. Through searching for a treatment for muscular dystrophy came utrophin. Utrophin is the fetal homolog of dystrophin and has a similar structure organization. Dystrophin is 427 kDa large whereas utrophin is 394 kDa. Both proteins contain an N-terminal actin binding domain (ABD1) and a second actin binding domain (ABD2) that is found later in the protein, albeit in different regions on the two proteins. Dystrophin has 24 spectrin like repeats on it, while utrophin only has 22. Dystrophin also has 4 hinge regions compared to the 2 that utrophin contains. Both contain a C-



terminal domain that is cysteine rich and binds to the glycoprotein complex.

Figure 2. Structure of utrophin vs. dystrophin.

It has been proposed that by upregulating the expression of utrophin, that it could serve to replace the nonfunctional dystrophin and recover function. There are some issues with this

though. Utrophin is already found to be in higher concentrations in people who have MD than in people with normal functioning dystrophin. This indicates that there is already upregulation naturally present in people with the disease. Another issue, is that previously conducted mouse studies have upregulated utrophin expression and found initial improvement in muscle function, but eventually the muscles failed in the same way as before.<sup>2</sup> This brings up the question of why does utrophin fail as replacement for dystrophin?

To answer this question requires breaking down the protein one region at a time. The reason for this is because dystrophin and utrophin are immense proteins. The average protein is 50 kDa large, whereas utrophin is almost 8 times that in size. The gene for dystrophin is one of the largest in the human body. It is because of size constraints that full length dystrophin is not able to be synthesized in a lab and introduced into a complex living system. Such a large molecule would be difficult to get past the membrane along with likely protease action that would degrade the protein before it could even reach the point where it could be used. And thus, the need to break the protein down into manageable components is needed.

The region of interest in this research is the N-terminal actin binding domain 1 (ABD1). This region of the protein possesses almost one third of the mutations found in people with MD. In addition, about 50% of disease causing missense mutations occur at this region. These observations are consistent with the idea that ABD1 is the main functional region of dystrophin. Knowing this, ABD1 is the logical starting places for analysis on why utrophin fails to serve as a viable substitute for dystrophin. My hypothesis is that utrophin ABD1 is a more stable protein and is unable to exhibit the flexible conformations needed to link actin the membrane.

ABD1 contains two Calponin Homology (CH) domains which are common structural motifs found in actin binding proteins. These two CH domains are held together by an  $\alpha$ -helical linker in ABD1.

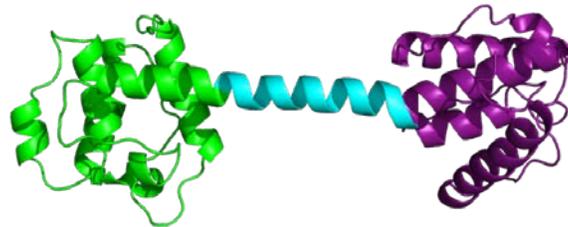
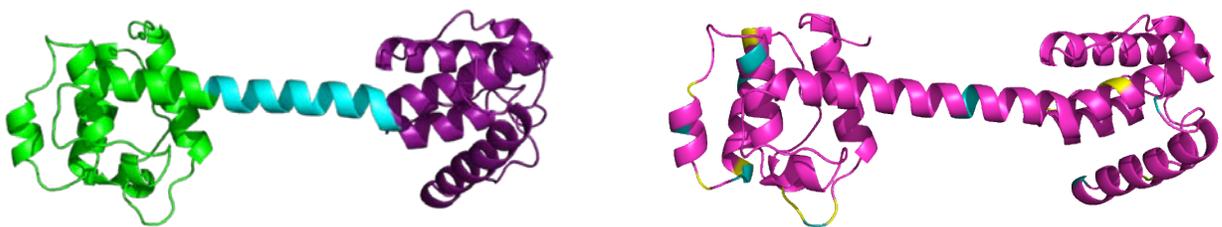


Figure 3. Dystrophin Actin Binding Domain 1 (ABD1) in where the green region is CH2, blue is the linker, and purple is the CH1 region.

This region behaves in a manner similar to teeth to bite down on the actin when it binds. Between dystrophin ABD1 and utrophin ABD1, there is 73% identity indicating that this region is highly similar between the two proteins. Sequence comparison was conducted to provide the images below that show the amino acid changes that result in hydrophobicity changes that occur



between dystrophin ABD1 and utrophin ABD1.

Figure 4. Dystrophin ABD1 (left) and the amino acid changes that result in utrophin ABD1 (right). Yellow residues correspond to hydrophilic-to-hydrophobic amino acid changes while teal residues correspond to hydrophobic-to-hydrophilic amino acid changes.

The amino acid changes that result in utrophin ABD1 can result in the following conformation of utrophin ABD1, which is only one of the many conformations that utrophin ABD1 can exhibit.

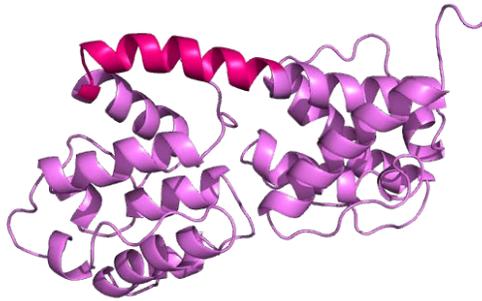


Figure 5. Structure of utrophin ABD1. Light purple region corresponds to the CH domains and the dark pink region is the linker.

Sequence comparison was also conducted for the mutated forms of dystrophin ABD1 that result in DMD and BMD.<sup>4</sup> The result of those comparisons is shown below.

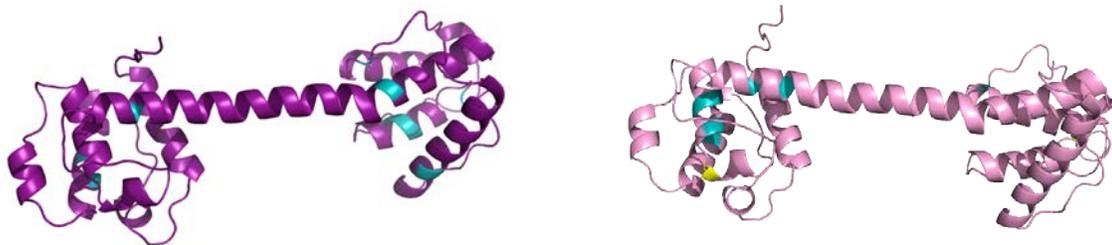


Figure 6. DMD Dystrophin ABD1 (left) and BMD Dystrophin ABD1 (right). Yellow residues correspond to hydrophilic-to-hydrophobic amino acid changes while teal residues correspond to hydrophobic-to-hydrophilic amino acid changes.

These amino acid changes resulted in charge differences compared to wild type dystrophin, the results of which are summarized in the table below.

Table 1. Overall Charges on each region of dystrophin ABD1 compared to wild type

	CH2	linker	CH1
DMD	-1	0	3
BMD	0	0	0
Utrophin	2	-2	1

dystrophin

The oppositely charged regions on DMD dystrophin ABD1 have the potential to interact and lead to irreversible binding that could cause aggregation which is consistent with the past studies that have shown DMD dystrophin to be prone to forming aggregates.<sup>3</sup> BMD dystrophin ABD1 has no alteration in charge which could allow for more functionality of the protein, which is consistent with BMD having less severe effects than DMD. Utrophin ABD1 has similar charges on CH2 and CH1 which have the potential to repel each while being attracted to the negatively charged linker. This could produce a more open conformation of the protein, but this data would need to be confirmed with molecular dynamic simulations to see what residues have the greatest potential to interact. Current observations of utrophin ABD1 are consistent with the hypothesis that it would be unable to bring the CH regions together to clamp down on actin.

The percent of residues mutated does not need to be a large amount to produce a large response. The following table illustrates the percent of mutations that result DMD, BMD, and utrophin ABD1 compared to wildtype dystrophin.

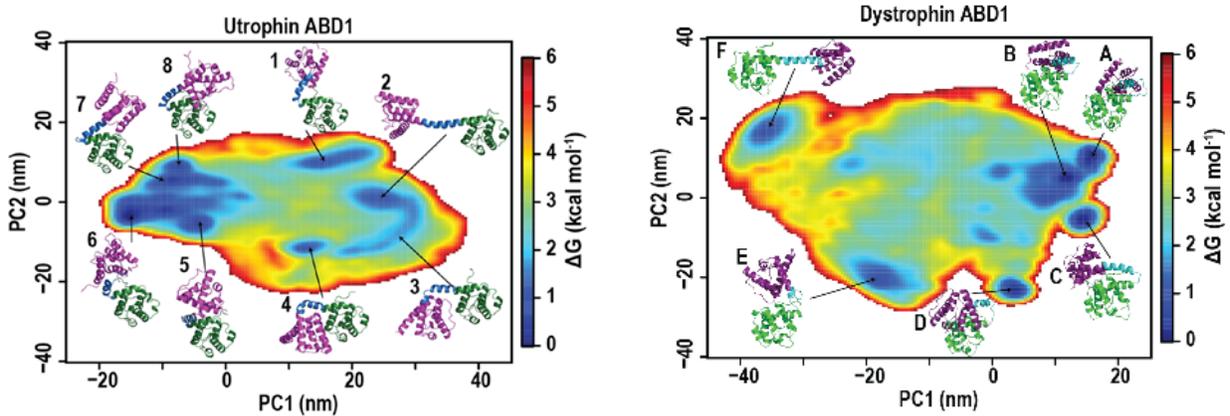
Table 2. DMD, BMD, and utrophin ABD1 percent of mutations that result in hydrophilic

	DMD	BMD	Utrophin
hydrophilic	3.66%	1.63%	3.78%
hydrophobic	0	0.81%	5.46%

residues and hydrophobic residues compared to wild type dystrophin ABD1.

DMD dystrophin ABD1 has only hydrophilic resulting mutations, while BMD dystrophin ABD1 has both types of mutations but in a fewer amount. Contrary to my original idea, utrophin ABD1 had more hydrophobic resulting amino acid changes than hydrophilic. This caused me to revise some of my thinking where the CH regions are the more important functional components, but rather that the linker where two of the hydrophilic mutations occurred is of key interest to utrophin function. Additionally, it may be less about hydrophilic vs. hydrophobic changes, but more about the opposite alteration and whether it occurs on the interior or the exterior of the CH domain.

Previous work was conducted that used molecular dynamic simulations to produce a free



energy landscapes of utrophin ABD1 and dystrophin ABD1.

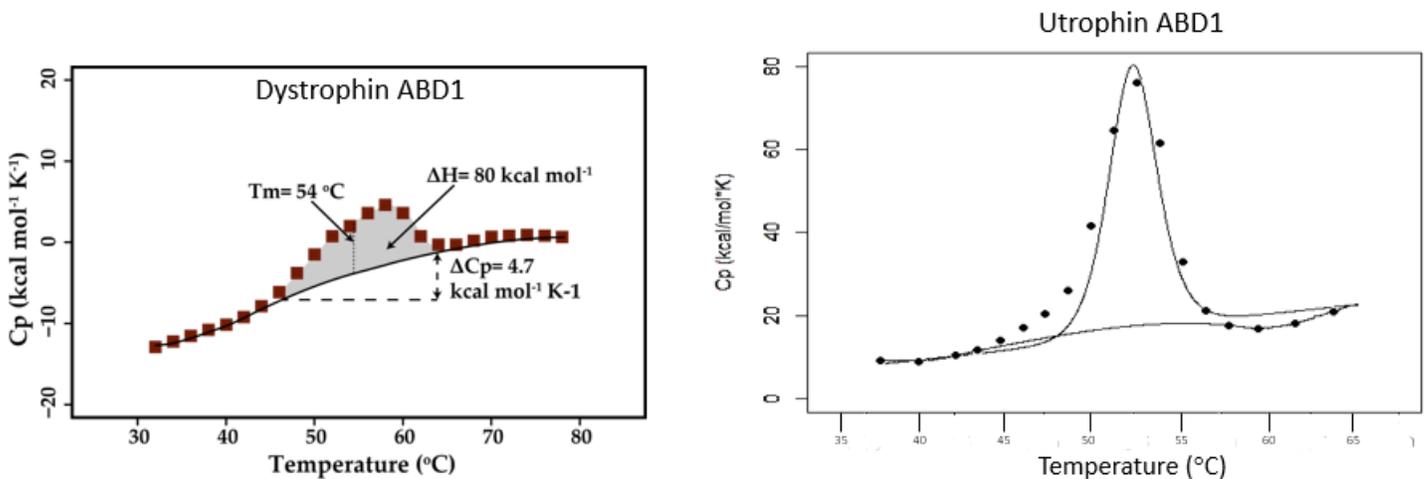
Figure 7. Utrophin ABD1 and dystrophin ABD1 free energy landscapes.

These free energy landscapes show in the dark blue low energy regions what the most stable conformations for each protein are. Dystrophin ABD1 could exhibit both open and closed conformations while utrophin ABD1 only exhibited some degree of open conformations. This consistent with what sequence comparison would predict as possible. A future direction for this project involves confirming proposed dynamics from the simulations through use of protein NMR.

Values like Instability Index can be used to predict whether a protein will be stable or unstable.<sup>5</sup> Utrophin ABD1 has an Instability Index of 33, under 40 is stable and greater than 40 is unstable, while dystrophin ABD1 has an Instability Index of 45. To confirm the stability of these proteins requires an experimental approach to the problem. Differential Scanning Calorimetry works by comparing temperature change with heat capacity. These curves produced can be fitted using the Gibb's Helmholtz equation to provide thermodynamic parameters. The equation is as

$$\text{follows: } C_p(T) = \Delta H(T) * \left( \frac{\left[ \left( \frac{\Delta H_{Tm}}{T} - \frac{\Delta C_p T_m}{T} + \Delta C_p \right) \right] K}{(K+1)^2} \right) + \left( \frac{\Delta C_p K}{K+1} \right) + FBL_{DSC} \text{ in which } K = e^{-G^\circ/RT}$$

and the folded baseline is  $FBL = m_n * T + b_n$  and the Gibbs free energy is given by  $\Delta G^\circ = \Delta H_{Tm}(1 -$



$T/T_m) + \Delta C_p [T - T_m - T(\ln(T/T_m))]$ . The following spectra were fitted with these equations.

	Dystrophin ABD1	Utrophin ABD1
T <sub>m</sub>	54 °C	52 °C
ΔH	80 kcal/mol	233 kcal/mol
ΔC <sub>p</sub>	4.7 kcal/mol*K	2.0 kcal/mol *K
ΔG (37°C)	2.0 kcal/mol	9.8 kcal/mol
Instability Index	45	33

Figure 8. DSC data for dystrophin ABD1 and Utrophin ABD1.

The parameters in the table below were produced from fitting the above parameters.

Table 3. Thermodynamic properties produced for dystrophin ABD1 and utrophin ABD1. T<sub>m</sub> is the melting temperature, ΔH is enthalpy, ΔC<sub>p</sub> is heat capacity, and ΔG is the Gibbs free energy.

With a greater ΔG at the physiological temperature utrophin ABD1 requires more energy to reach denaturation. Even with the repellant positive CH regions from the attractive linker charge would result in an overall increase in intramolecular interactions as seen in the more compact open conformation of utrophin increasing ΔH. The heat capacity change is less due to polar residues being more common on exposed residues. This is consistent with my hypothesis of utrophin ABD1 being more stable than dystrophin.

Knowing how utrophin fails to replace dystrophin has potential for introducing factors that could fix this failure. That is part of the future of where this project is headed in terms of drug design and possible implications that could be had for disease treatment. This is one of the

first projects that looks into the thermodynamics and molecular dynamics of these proteins to find how they function as little is still known about the way in which dystrophin transduces force and what role utrophin plays.

## References

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