

Article

An Analysis of Protein Crystals Grown under Microgravity Conditions

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Abstract: Microgravity has been shown to be an excellent tool for protein crystal formation. A retrospective analysis of all publicly available crystallization data, including many that have not yet been published, clearly demonstrates the value of the microgravity environment for producing superior protein crystals. The parameters in the database (the Butler Microgravity Protein Crystal Database, B μ CDB) that were evaluated pertain to both crystal morphology and diffraction quality. Success metrics were determined as improvements in size, definition, uniformity, mosaicity, diffraction quality, resolution limits, and B factor. The proteins in the databases were evaluated by molecular weight, protein type, the number of subunits, space group, and Matthew's Coefficient. Compared to ground experiments, crystals grown in a microgravity environment continue to show improvement across all metrics evaluated. General trends as well as numerical differences are included in the assessment of the B μ CDB. The microgravity environment improves crystal formation across a spectrum of metrics and the datasets utilized for this investigation are excellent tools for this evaluation.

Keywords: crystallization; protein; microgravity



Citation: Jackson, K.; Hoff, R.; Wright, H.; Wilkinson, A.; Brewer, F.; Williams, A.; Whiteside, B.; Macbeth, M.R.; Wilson, A.M. An Analysis of Protein Crystals Grown under Microgravity Conditions. *Crystals* **2024**, *14*, 652. <https://doi.org/10.3390/cryst14070652>

Academic Editor: Borislav Angelov

Received: 1 July 2024

Revised: 12 July 2024

Accepted: 14 July 2024

Published: 16 July 2024



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1. Introduction

In 1984, the results of the crystallization of β -galactosidase and lysozyme in microgravity in a Spacelab experiment in 1983 were published [1]. The proteins grown in this experiment showed crystals that were larger, more well-shaped, and of higher quality. This landmark study inspired hundreds of other experiments in microgravity from researchers across the globe. Protein crystal growth has seen important improvements over time [2], and the structures of these proteins have allowed for important medical and pharmaceutical advancements [3].

A second landmark study [4–7], the comprehensive crystal study performed on the Space Shuttle in the late 1980s and early 1990s, provided additional evidence that a wide variety of protein crystals can be grown in a microgravity environment. Since that time, a proliferation of protein crystallizations in microgravity has taken place, and several reviews detailing this work have appeared [8–11]. In addition, an analysis of protein crystallizations [12], an exploration of the number of microgravity experiments that have provided more precise structural information [13], a perspective on the outlook of protein crystallizations [14], a review of overall methodologies [15], and an account of types of diffusion techniques for macromolecular crystallization [16] have been reported.

It is understood that microgravity environments improve protein crystallization due to the reduction of gravity-driven convective effects near growing crystal surfaces [17], the sedimentation of impurities thought to inhibit crystal growth [8], and capitalization of the diffusion-limiting conditions provided by low gravity [18]. In this work, a database was generated to assess multiple parameters that define crystal quality. Using our database and the metrics therein, we directly compared the protein crystals grown in microgravity environments to those grown on Earth.

2. Materials and Methods

A subset of our database containing “organic” crystallization experiments [19] was evaluated and proteins were selected and placed into a new database: the Butler Microgravity Protein Crystal Database, B μ CDB. The Protein Data Bank (PDB) [20] also contained microgravity protein crystals that had not yet been published in a manuscript. The data were evaluated in light of the listings as of April 30, 2024. Combining the information from all sources provided 353 unique entries. All the experiments have been included in this report, including duplicate experiments (where crystals were fabricated in multiple runs of a single PI or author’s work), where experiments failed due to equipment failure and/or complications from launch or landing, where crystallization chambers failed, where crystals were unstable, and/or where syringe leaks were documented. This worst-case scenario provides an accurate representation of the overall probability for success for protein crystallization experiments under microgravity conditions.

For proteins listed in the PDB, an in-depth evaluation of resolution was performed. The resolution for each individual structure is included in the B μ CDB and this was compared to the weighted average of proteins of the same name (e.g., lysozyme; NAD⁺ synthetase; canavalin; etc.) and same enzyme/protein classification or source organism as the protein in crystallized in microgravity. The weighted average was determined by taking the number of crystals reported in a particular refinement resolution range (e.g., 2.5–3.0) and multiplying this number by the middle of the range (e.g., 2.75). This crude measure of the average resolution of each protein allowed for a comparison to the resolution reported from microgravity crystallization, even for crystals that had not appeared in a publication.

This B μ CDB database includes source material information (material crystallized, authors/Pis, year flown, mission flown, article title, journal and/or book title, DOI—if available—, sponsoring country) and specific data about the protein crystallized (molecular weight, methods, conditions, temperature, crystal shape, number of crystals, pictures if they are included in the report, crystal size, Matthew’s Coefficient, number of subunits, unit cell parameters, space group, resolution, I/sigma, B factor, flight complications, materials, etc.). An analysis of improvement over ground-based experiments (larger crystal size, increased uniformity, structural improvement, superior mosaicity, and improved resolution limit) is also provided.

A correlation study utilizing pairwise correlations to calculate the Pearson correlation coefficient for linear relationships between two variables was undertaken. A result of 1 would be a perfect positive relationship, a result of -1 would be a perfect negative relation (inverse), and 0 is no relationship. Scores ranging from 0.2 to 0.4 are considered a weak correlation, while 0.4–0.7 is a moderate correlation, and 0.7 and above is a strong correlation.

3. Results

The proteins in the database were evaluated in terms of improved crystal quality. The metrics for improvement included the following: larger volume; improved uniformity; better morphology; superior mosaicity; and better resolution limit of diffraction. A separate analysis by B factor was also performed, but not included in the combined analyses as the number of data points was relatively low ($n = 57$) compared to the other metrics. Several proteins in this report were first crystallized in microgravity. For the purposes of this study, these examples were included as “improved” for all metrics. These factors were evaluated individually, and collectively. Microgravity experiments were also evaluated utilizing five different factors: molecular weight; protein type; the number of subunits; space group; and Matthew’s Coefficient. The results of these analyses are reported below.

Of the proteins reported, 10 experiments were included from the Protein Data Bank (PDB) that had not yet been published. In addition, there were several structures included in the PDB that did not report on metrics in the reference articles. In order to assess the impact of microgravity on crystallization, an analysis of the resolution reported in the PDB

for microgravity-grown crystals was compared to the weighted average of the resolution reported for all other crystals of this type in the PDB.

3.1. Individual Metrics

The metrics of size, morphology, resolution limit, mosaicity, and uniformity were evaluated individually. The metrics may have shown improvement, remained the same, or been worse compared to ground experiments under the same conditions. The results of these comparisons are shown in Figure 1.

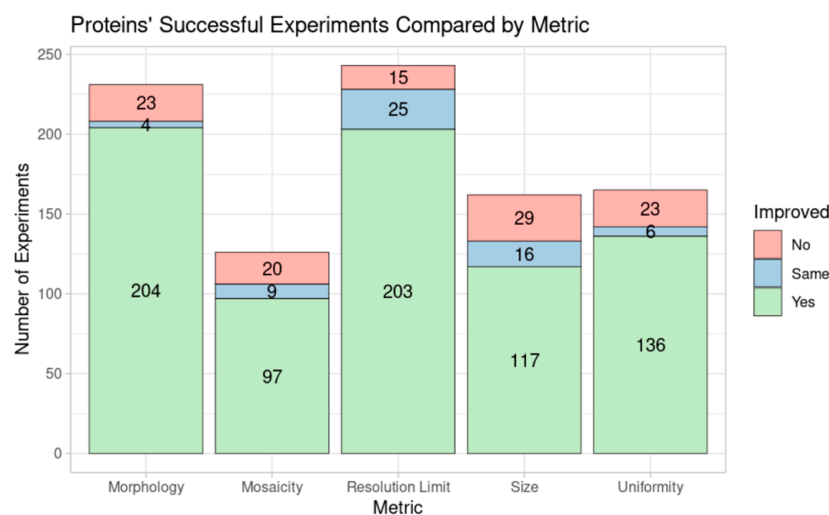


Figure 1. Protein crystal metrics compared to ground studies.

3.1.1. Size

Of the 353 individual database entries, 162 reported data on crystal size (Figure 1). Of these microgravity protein experiments, 117 (72%) were reported to be a larger size, 16 (10%) were reported to be the same size, and 29 (18%) were reported as smaller than their Earth-grown counterparts. Increasing crystal size was important in early microgravity protein crystallization experiments in order to grow crystals large enough for X-ray diffraction. As crystallography techniques have improved, the need for “large” crystals has diminished [21].

To assess how many larger crystals were in space compared to their ground counterparts, an analysis of the volumes reported in the source literature was undertaken. Many of the source photographs showed that the microgravity-grown crystals were visually larger, without reporting numerical data. Seven experiments also reported crystals grown for the first time in space without ground comparisons. A generalization about how much crystal growth can be improved by microgravity is still challenging as, even for those authors who did report volume data, this was often given for the “best” crystal in the batch. The range of size differences included ground-based crystals being ten times larger than their microgravity-grown counterparts [22], and microgravity-grown crystals being 1000 times larger than their counterpart crystals grown under the same conditions terrestrially [1].

3.1.2. Morphological Improvements

In the database, there are 231 entries that report on structural improvement, for example sharper edges, a more optically clear appearance, fewer visible flaws, etc. (Figure 1). We are relying on the authors of these studies to make these determinations. Of the microgravity-grown crystals, 204 (88%) were reported to be structurally improved, 4 (2%) were reported to be the same, and 23 (10%) were reported to be inferior to their terrestrial controls. Improvements to crystal morphology have been one of the prime motivators for protein crystallization in the microgravity environment.

3.1.3. Improved Uniformity

Uniformity data were reported for 165 of the entries in the database. Microgravity conditions provided more uniform crystals for 136 (82%) of the proteins reported. Uniformity was the same for six (4%) of the reports. The uniformity was worse for 23 (14%) of the reported proteins.

3.1.4. Resolution Limit

The quantitative metric of the resolution limit was reported for 254 of the proteins in the literature (excluding the PDB). Of these entries, 243 compared the microgravity data to ground-based experiments (Figure 1). Of these proteins grown in microgravity, 203 (84%) reported an improved resolution limit, 25 (10%) reported the same resolution limit, and 15 (6%) reported poorer resolution limits than crystals grown on the ground. As resolution limit is a key factor for the structure determination of proteins, it would be ideal to understand the impact of microgravity on this metric.

For the 195 sources that listed specific numerical differences in the resolution limit compared to ground experiments (rather than stating that it was improved or poorer), an analysis of these numerical data was made (this excluded 18 proteins that were crystallized in space that had no known ground counterpart). The average improvement in resolution was 0.30 Å with the median improvement at 0.23 Å (see Figure 2).

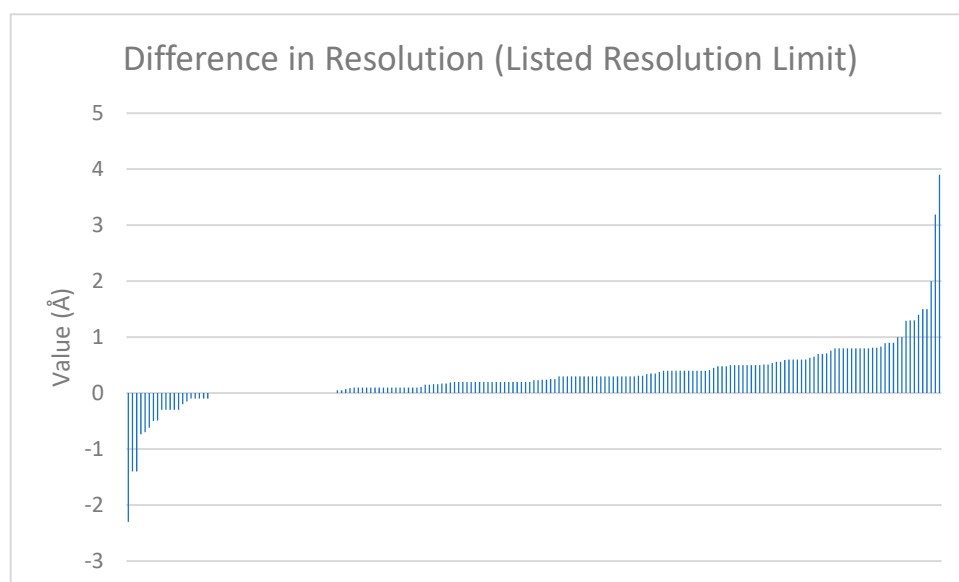


Figure 2. Difference in resolution limit listed in the database.

3.1.5. Mosaicity

Protein mosaicity, also a quantitative metric, was reported for 126 of the database entries. Mosaicity was reported to be improved for 97 (77%) of the crystals grown in microgravity. This metric was reported as the same for 9 (7%) and worse for 20 (16%) of the proteins compared to crystals grown on Earth.

For the 103 entries that listed mosaicity values specifically for ground vs. microgravity experiments, the average improvement to mosaicity was 0.093 and the median was 0.030 (see Figure 3). There were reports of improved or poorer mosaicity without comparative numerical data.

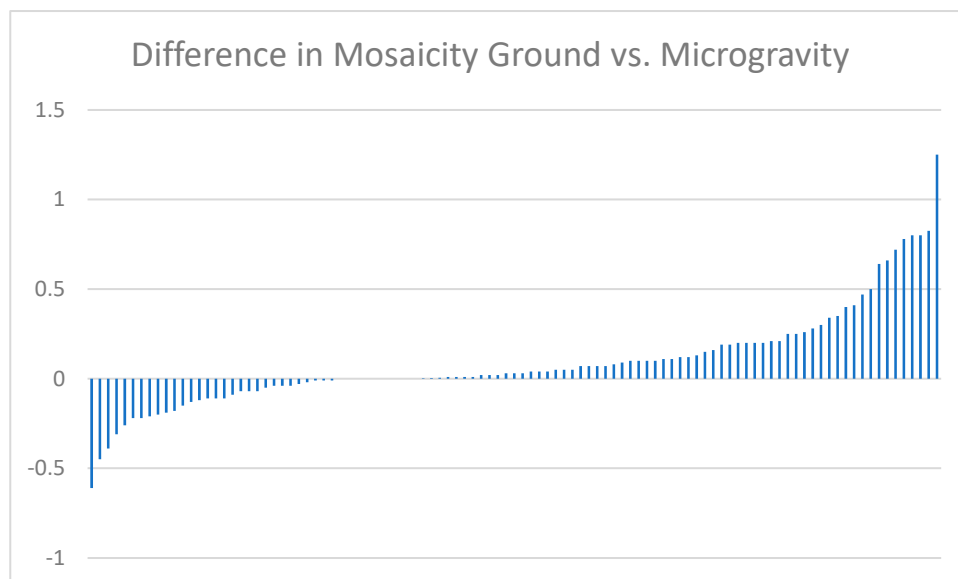


Figure 3. Difference in reported values from ground vs. microgravity experiments.

3.1.6. Improvement in at Least One Metric

Out of the 353 database entries, 307 reported data in the literature for at least one of the metrics of size, structure, uniformity, resolution limit, or mosaicity (Figure 4). Of these, 282 (91.9%) reported at least one metric with improvement. Those reporting improvement in at least two metrics numbered 220 (71.7%). Over 46% (142, 46.3%) of the microgravity protein crystals reported improvement in three or more metrics. Lastly, more than one fourth (84, 27.4%) of the proteins crystallized in microgravity reported an improvement in four or more of the key metrics.

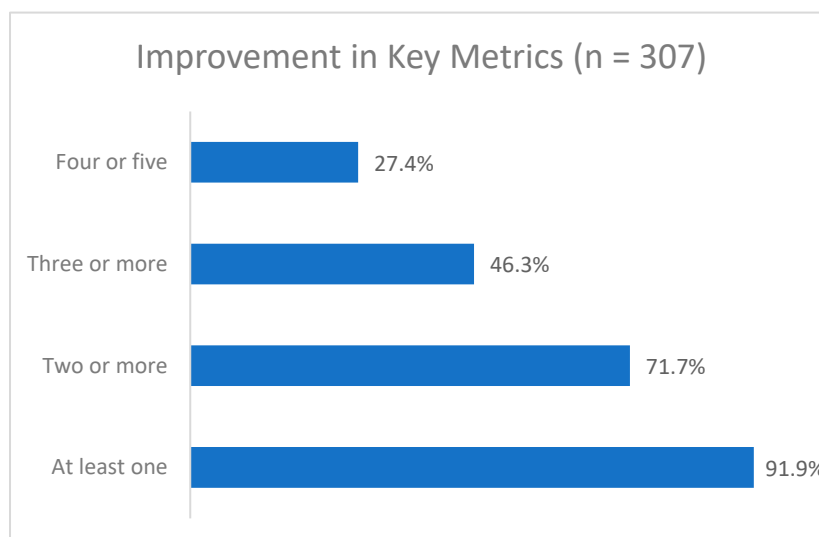


Figure 4. Improvement in key metrics under microgravity.

B factor is a measure of “dynamic disorder caused by the temperature-dependent vibration of the atoms, and static disorder” [23]. A lower number indicates an improvement in this metric. In the source material that reported changes in B factor ($n = 57$), all but one study reported a smaller number (Figure 5). As this is only 16% of the protein crystals reported in the database, it is not enough to provide a statistically relevant insight into the role of microgravity on B factor.

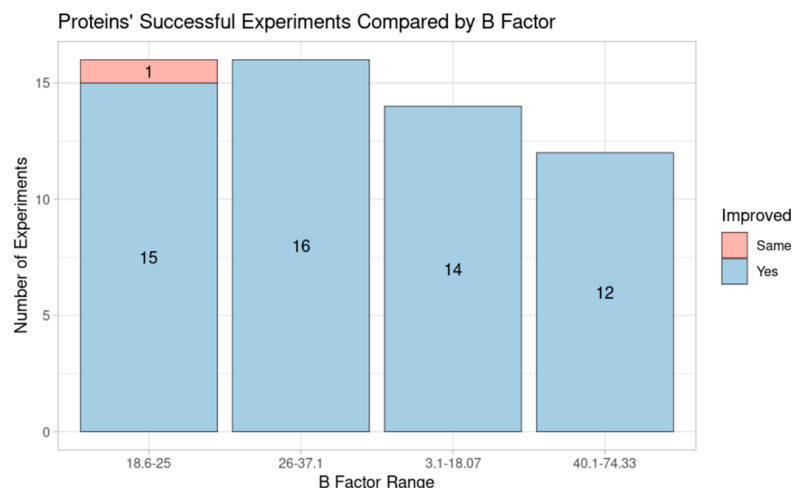


Figure 5. Improvement in B factor by B factor range.

3.2. Key Protein Characteristics

3.2.1. Molecular Weight

The molecular weight of the proteins crystallized in microgravity ranged from small proteins (e.g., Insulin, 5.8 kDa) [24], to very large proteins (e.g., Photosystem 1, 1200 kDa) [12]. Similar to a previous report of an expanded database that included small organic and inorganic materials [19], the results demonstrate that the protein crystals were improved by at least one metric across the molecular weight ranges (Figure 6). Remarkably, all “large” protein systems (501 kDa or larger) were improved compared to ground data.

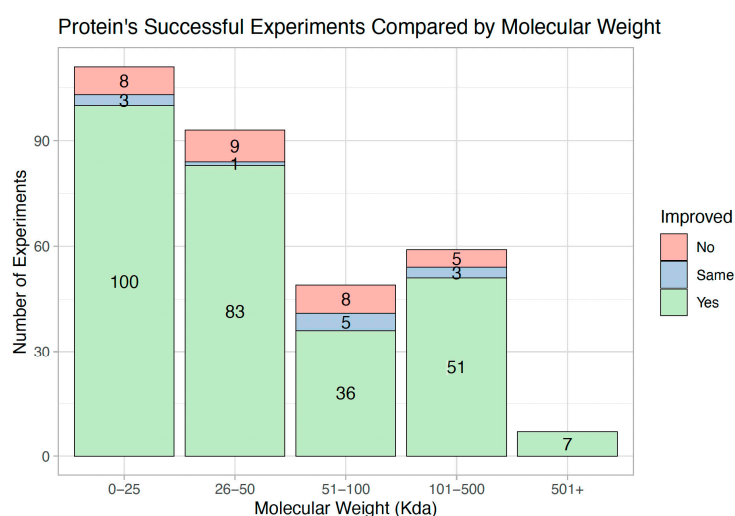


Figure 6. Improvement in at least one metric by molecular weight of protein.

3.2.2. Protein Type

The vast majority of proteins crystallized in microgravity were enzymes. This is likely due to their solubility, the relative ease of their purification, and their subunit architecture often forming symmetric oligomers. Enzymes are also models for protein folding, ligand binding, biocatalysis, and allosteric regulation. Additionally, mutations in enzymes or the misregulation of enzymatic activity are the root causes of a variety of metabolic diseases/disorders. Thus, enzymes are common subjects for structure/function studies, and understanding their structure is imperative for pharmaceutical design and functional studies. There was no significant difference in the success of the crystallization of protein types in microgravity (Figure 7).

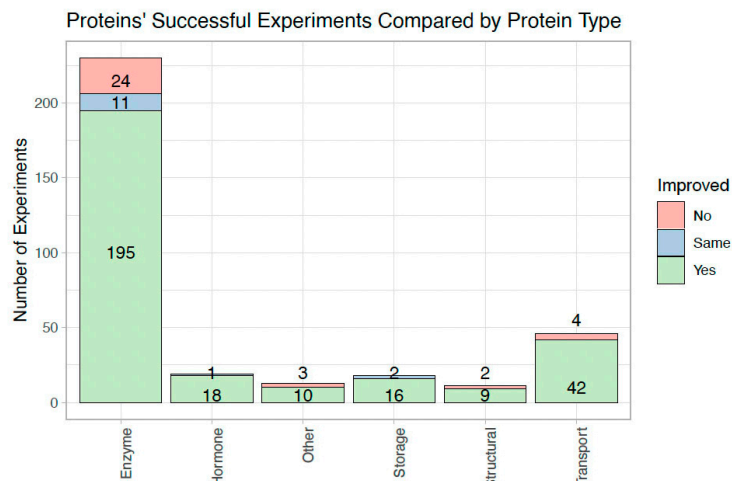


Figure 7. Improvement of at least one metric by protein function.

3.2.3. Number of Subunits

From the reported data, it is clear that most of the proteins that were crystallized at zero gravity were monomers or dimers (Figure 8), which is in correlation with the fact that most proteins in the database are also enzymes (Figures 7 and 8). All large multi-subunit complexes (9 or 12 subunits) showed improvements in at least one metric, and the number of experiments was small. While the overall improvements in crystallization are dramatic, the data indicate that when comparing percentages of crystals that show an improvement in one parameter with respect to the number of subunits, the improvements appear to be independent of the number of subunits in the complex.

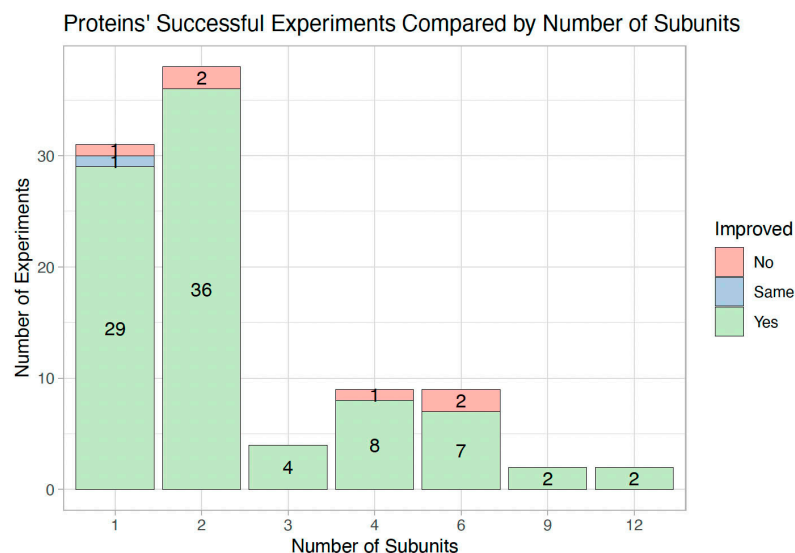


Figure 8. Success in at least one metric by the number of subunits in the unit cell.

3.2.4. Space Group

We analyzed the data to determine if improvements in crystallization observed in a zero G environment were correlated to the degree of symmetry. We observed no significant trend in improvements with respect to symmetry. For example, for those crystals with P6 symmetry, 16/18 (89%) showed improvements in at least one metric compared to 56/65 (86%) for crystals with P2 symmetry (Figure 9).

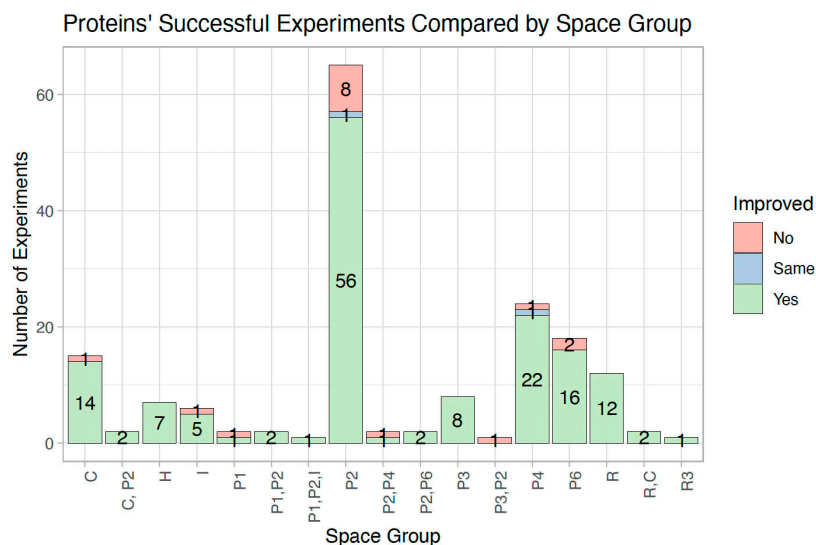


Figure 9. Success in at least one metric by space group.

3.2.5. Matthew's Coefficient

Matthew's Coefficient, or the ratio of the volume of the asymmetric unit to the molecular weight of the protein, is inversely proportional to the solvent content [25]. Working under the assumption that a driving factor for improvements in crystallization at zero gravity are due to less gravity-driven solvent convection, we asked if molecular packing in an asymmetric unit could be correlated with solvent content as quantified using Matthew's Coefficient. The results in Figure 10 indicate that there is little to no correlation between improvements in crystallization in a zero-gravity environment.

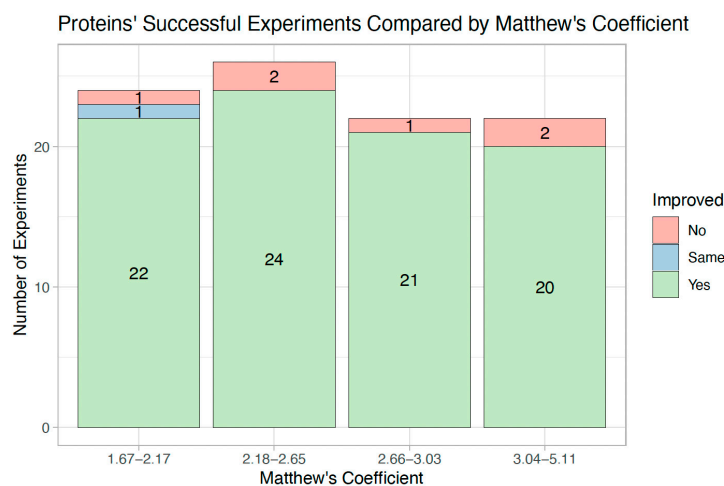


Figure 10. Success in at least one metric by Matthew's Coefficient.

3.3. Resolution Data from the Protein Data Bank

Of the protein crystals reported in the μ CDB, 106 of them appear in the Protein Data Bank (PDB). Of these, 92 were featured in peer-reviewed publications, but an improvement in resolution limit was not reported in 39 of these publications. By evaluating the resolution limit reported in the PDB against a weighted average of reported resolution limits (Figure 11) in the PDB for the same proteins, a measure of "improvement" could be determined for crystals grown in microgravity. While this is an imperfect comparison compared to a direct comparison of crystal growth under the same crystallization conditions, it does provide insight into the role that microgravity plays in crystallization conditions.

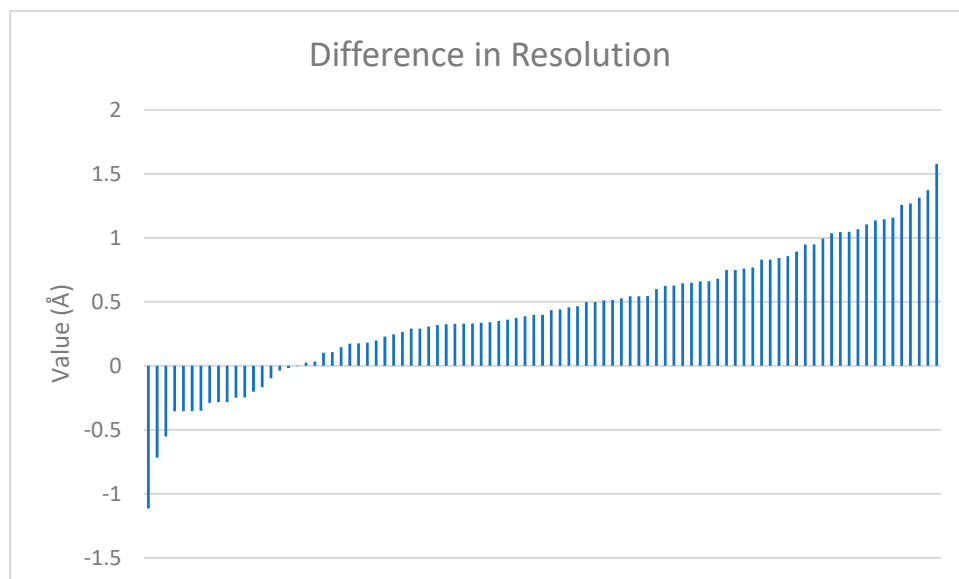


Figure 11. Difference between resolution reported for microgravity crystals and weighted average of resolution for that crystal type reported in the PDB.

Of the data reported in the PDB, 12 of the microgravity-grown crystals are the only reports of these compounds. Of the remaining 94 reports, 18 (19%) crystals had a resolution higher than the weighted average of other crystals of that type, and 76 (81%) had a resolution lower than the weighted average of other crystals of that type (Figure 11). This is consistent with the percentages of improvement seen when utilizing other types of data. The average improvement was 0.42 Å with a mean of 0.40 Å, which can represent substantial gains in structure determination [26].

3.4. Correlation Studies

From our data, none of the metrics combined (i.e., Resolution + Growth) demonstrated a relationship better than weak (Table 1). The strongest correlation was Resolution and Structure with 0.36. Several of the correlations (uniformity with growth and mosaicity, and growth and mosaicity) demonstrated no statistical correlation. None of the relationships resulted in a negative score and therefore an inverse correlation. This implies that there may be a positive correlation, and it is unlikely that there is a negative correlation between the metrics evaluated. With more data, analyses of correlations could provide greater insights.

Table 1. Correlation studies.

Metrics	Correlation
Resolution + Growth	0.24
Resolution + Structure	0.36
Resolution + Uniformity	0.255
Resolution + Mosaicity	0.21
Structure + Growth	0.25
Structure + Uniformity	0.26
Structure + Mosaicity	0.21
Uniformity + Growth	0.187
Uniformity + Mosaicity	0.18
Growth + Mosaicity	0.13

4. Discussion

Compared to other types of data in the **BμCDB**, crystal size seems to be the least significant predictor of success for crystallization in microgravity. Structure, morphology, resolution limit, and mosaicity are clearly improved for the majority (77–88%) of crystals

grown under microgravity conditions. The overall resolution limit for proteins grown in microgravity improved by 0.23–0.30 Å and the mosaicity improved by 0.030–0.093. This allowed the researchers to have a clearer understanding of the target proteins' structures. The analysis of B factor implies that the number will reduce under microgravity conditions, but there are too few reports to make a conclusive judgment.

As resolution limit is a key factor for the structure determination of proteins, this is an important metric to improve through crystallization techniques. Given the large number of crystals (203/243) that reported an improvement in this metric in the literature, similar results are demonstrated in the comparison data in the PDB (76/94); 20 proteins in the B μ CDB were crystallized for the first time in microgravity, utilizing microgravity crystallization as a tool for particularly challenging structural problems in proteins. When comparing the resolution limit's improvement in the database (0.23–0.30 Å) to the weighted average reported in the PDB (0.40–0.42 Å), it is clear that the trend holds even for underreported data.

The analysis of protein size (molecular weight), protein type, the number of subunits, space group, and Matthew's Coefficient did not provide any indicators that these factors had an impact on success for crystallization in microgravity. The most surprising of these was the lack of difference in microgravity on the number of subunits and Matthew's Coefficient. Each of these factors are dependent on the solution dynamics of the crystallizing system, as fluids behave differently in microgravity [27].

Correlation experiments between the metrics that we evaluated in this study also indicated that there was no statistical correlation between success in one metric and another, even though this appeared to be intuitively connected. This may be largely due to incomplete data (not all source articles report on all metrics), as well as the overwhelmingly positive outcomes which dominated the correlation. There were no indications that a factor other than reduced convection was observed at the surface of the crystal [17] and diffusion limitations of crystal growth [18] play a role in the remarkable improvements seen across protein crystal types.

Since our initial report [28], researchers have suggested several additional types of data be added to the database. If the metrics are available in references or in the PDB, we added those metrics. As additional techniques, like X-ray topography which is a tool for characterizing crystal defects [29,30], are routinely added to the suite of analyses performed on microgravity-grown crystals, these metrics will be incorporated into the B μ CDB. In addition, data such as distributions of the half-width of the diffraction beam and video data, will also be included. We would encourage authors to make data available on all of the metrics for protein crystals grown in microgravity. This, along with more studies, will provide more data to determine what factors or combination of factors are most influential in the success or failure of crystallization in microgravity.

5. Conclusions

Microgravity conditions have a positive impact on the structure and properties of protein crystals. However, the exact nature of the causes for this impact are not yet clear. The use of these data in the B μ CDB has provided a platform from which to analyze the factors that may cause these positive impacts. At this point, factors such as size, protein type, the number of subunits, space group, and Matthew's Coefficient do not suggest any insights into why protein crystals grown in microgravity are larger, have better morphologies, are more uniform, have a higher resolution limit, and possess an improved mosaicity. Additional data will be added to the B μ CDB in order to provide a platform where the links between the improvements and their corresponding indicators may be more thoroughly analyzed.

Author Contributions: Conceptualization, A.M.W. and M.R.M.; methodology, A.M.W.; formal analysis, K.J.; data curation, H.W., R.H., A.W. (Ashley Wilkinson), A.W. (Amari Williams), B.W. and F.B.; writing—original draft preparation, A.M.W. and M.R.M.; writing—review and editing, A.M.W. and M.R.M.; final editing, B.W.; project administration, A.M.W.; funding acquisition, A.M.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Aerospace Corporation, contract number WR 120622, and Butler University for sabbatical support of AMW.

Data Availability Statement: The database is available at: https://docs.google.com/spreadsheets/d/1mRCn0m1vNynou8Yl26Sw7__l5Jf4Ta_UXYgHPkG6mw8/edit?usp=sharing (accessed on 30 April 2024).

Conflicts of Interest: The authors declare that this study received funding from Aerospace Corporation. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

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