# A Complementary Perspective on the Evolutionary Emergence of Essential and Non-Essential Amino Acids

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**Abstract**

All animals, including humans, lack the ability to synthesize nearly half of the 20 amino acids required for protein synthesis. It has long been hypothesized that the biosynthetic pathways for these essential amino acids (EAAs) were lost due to their higher biosynthetic cost and that the remaining non-essential amino acids (NEAAs) retained their lower-cost pathways. However, the specific mechanism by which amino acid synthesis cost determines the maintenance or loss of these pathways remains unclear.

In this paper, I propose the **Differential Recycling Efficiency (DRE) Framework** as a new perspective on the emergence of EAAs and NEAAs. Specifically, (1) as organisms evolved, their bodies became increasingly compartmentalized, (2) this compartmentalization led to varying levels of resource-recycling efficiency across different compartments, (3) and, driven by selective pressures favoring metabolic optimization, proteins rich in low-cost amino acids came to be preferentially allocated to compartments where resource recovery is more difficult, (4) this preference, in turn, raised overall demand for low-cost amino acids, and (5) ultimately led to the retention of their biosynthetic pathways and their classification as NEAAs. These five propositions, taken together, form the basis of the DRE Framework. Under this framework, the commonly observed amino acid requirements among various phagotrophic organism lineages—exemplified by the essential amino acids in humans—can be considered as predetermined outcomes of resource optimization throughout evolution.

**Keywords:** Essential Amino Acids, Non-Essential Amino Acids, Metabolic Optimization, Compartmentalization, Differential Recycle Efficacy Framework

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# Introduction

All animals, including humans, lack the ability to synthesize nearly half of the 20 amino acids required for protein synthesis. These are referred to as essential amino acids (EAAs), while the remaining amino acids are designated as non-essential amino acids (NEAAs). Except for certain cases in which arginine is additionally deemed essential in specific animals, the nine EAAs recognized in humans appear to be the same across virtually all animal species. Consequently, the boundary between EAAs and NEAAs is generally consistent among animals [1−4].

Although numerous studies have investigated why animals cease to synthesize these EAAs, the explanations proposed so far have been mostly indirect or vague, and no direct framework has been firmly established [1−4]. For example, multiple studies indicate that EAAs require more energy to synthesize than NEAAs, and it has long been hypothesized that the biosynthetic pathways for these EAAs were lost due to their higher biosynthetic cost [3,4]. However, there is still no widely accepted explanation for why organisms specifically lose the capacity to synthesize higher-cost amino acids, and new hypotheses continue to emerge as debate persists on this point [4].

In the course of my own research—statistically analyzing amino acid compositions from various organism samples using publicly available datasets—I became aware of a framework that might account for this phenomenon. This framework arose from observations that EAAs and NEAAs appear to be utilized differently in constructing organismal bodies. To introduce and explain this framework, the present paper begins with a **Literature Review** of relevant findings, followed by **Empirical Findings** derived from my analyses. Building on these points, I will then present the framework itself—referred to here as the **Differential Recycling Efficiency (DRE) Framework**—and evaluate both its validity and its likely implications in the **Discussion** section.

# Literature Review

In this section, I review three strands of prior research. The first addresses how differences in the biosynthetic costs of amino acids constrain their usage in bacteria. The second examines the tendency of bacteria to allocate lower-cost amino acids to extracellular proteins. Finally, the third focuses on verifying the finding that essential amino acids carry higher biosynthetic costs than non-essential amino acids.

### 1. Biosynthetic-Cost Constraints on Whole Bacterial Proteomes

A genome-wide survey of *Escherichia coli* and *Bacillus subtilis* showed that among the 20 amino acids, each requires a distinct amount of ATP and precursor molecules to synthesize [5]. In their proteomes, highly expressed proteins were systematically depleted of the most expensive residues and enriched in cheaper ones [5]. These results suggest that metabolic efficiency shapes amino acid

usage across bacterial proteomes, with follow-up analyses in other bacteria and archaea reinforcing the role of cost as a primary constraint.

**2. Cost Minimization in Extracellular Bacterial Proteins**

Extending this pattern to cellular compartments, a study of over 1,500 secreted proteins from various microbes found that extracellular proteins contain significantly fewer high-cost residues—particularly tryptophan, phenylalanine, and methionine—than intracellular proteins [6]. Because extracellularly located proteins are less likely to be recycled, reducing their synthetic cost provides a direct energetic benefit, a phenomenon termed "economical evolution" [6].

**3. Cost and the Essential–Non-Essential Boundary in Animals**

Comparative phylogenomic analyses across more than 100 eukaryotic species revealed that the nine canonical essential amino acids in animals are consistently the most ATP-intensive to synthesize [4]. Statistical tests indicate that it is highly unlikely this pattern arose by chance [4]. Although the processes behind this remain entirely unclear, animals have nonetheless ceased synthesizing these high-cost amino acids.

**Review Summary**

In this section, I reviewed three strands of research. The first strand demonstrates that the disparity in biosynthetic cost among the 20 proteinogenic amino acids is a key factor influencing bacterial amino acid usage. The second strand shows that bacteria tend to allocate proteins composed of relatively low-cost amino acids to extracellular regions, where the risk of protein loss is high. The third strand highlights that essential amino acids, commonly found across animals, have significantly higher biosynthetic costs than non-essential amino acids, suggesting that this pattern is non-random.

How might these three findings contribute to a new framework? In the next section, **Preliminary Findings**, I present new statistical analyses that shed light on how low-cost amino acids may also be favored in the extracellular regions of multicellular species. In doing so, I introduce the missing link that bridges the three strands discussed here to the DRE Framework I will ultimately propose.

# Preliminary Findings

In this section, I explain the results of two statistical analyses. Both analyses use publicly available data, but the examination methods and their interpretation are my own. The first analysis involves principal component analysis (PCA) of amino acid composition data from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) food database [7]. The second compares intracellular and extracellular amino acid compositions in chicken skeletal muscles [8].

## 1. Principal Component Analysis of Amino Acid Composition in a Food Database

I performed a principal component analysis (PCA) on amino acid composition data from the publicly available MEXT food database, which encompasses roughly 2,000 foods and ingredients [7]. The original database quantifies each food item's total amino acid composition by listing the mass (per 100 g) of each amino acid after protein hydrolysis. I converted these mass values into molar amounts for all 20 proteogenic amino acids, normalized them so that each sample's total equaled 1, and then performed PCA on the resulting compositional data. Due to analytical constraints, glutamine (Gln) and glutamate (Glu) were reported together as Glu, asparagine (Asn) was reported as aspartate (Asp), and hydroxyproline (Hyp)—biosynthetically derived from proline (Pro)—was merged with Pro. These adjustments yielded a working set of 18 amino acids for analysis (Figure 1). An identical version of this figure was published in a previous preprint [9]. In addition, full details of the preprocessing workflow and further results are available in my earlier preprints [9–11].

**Figure 1** shows the PCA loadings for these 18 amino acids, with the first principal component (PC1) on the horizontal axis and the second principal component (PC2) on the vertical axis. The results indicate that essential amino acids (EAAs) and non-essential amino acids (NEAAs) cluster separately along PC1, suggesting that the first principal component distinguishes EAAs from NEAAs. Notably, nearly all the foods and food ingredients in this database are body parts of multicellular organisms or processed products derived from them, implying that the analysis effectively reflects the amino acid composition of various animal and plant tissues. However, in the observed EAA–NEAA separation, tyrosine (Tyr) and arginine (Arg) exhibited exceptional behavior. Specifically, tyrosine (commonly classified as a NEAA) appears on the "EAA side," whereas arginine (essential in some animals) appears on the "NEAA side." These exceptions will be addressed later in the Discussion section.

## 2. Comparison of Intracellular vs. Extracellular Amino Acid Composition in Chicken Muscle

For the second analysis, I used data from an older study that separately measured the intracellular and extracellular amino acid compositions of chicken skeletal muscle tissue [8]. As with the MEXT food database analysis, I focused on the same set of 18 amino acids and calculated their molar proportions in each fraction. I then computed the ratio of intracellular to extracellular concentrations and ranked the amino acids in descending order, illustrating the compositional differences between the intracellular and extracellular fractions (Table 1). A nearly identical version of this figure, along with details of the preprocessing workflow, was published in my earlier preprints [9–11].

**Table 1** reveals a clear trend: NEAAs tend to be relatively more abundant in the extracellular fraction, while EAAs are comparatively more abundant intracellularly. Although this pattern is not absolute, it nevertheless suggests that extracellular spaces are generally enriched in NEAAs, whereas intracellular compartments contain a higher proportion of EAAs. Additionally, consistent with Figure 1, tyrosine again appears on the "EAA side," while arginine appears on the "NEAA side." I will discuss these exceptions further in the Discussion section.

**Summary of Preliminary Findings**

In this section, I presented two analyses. First, the PCA of the food composition data suggests that, across the diverse organisms represented in the database, EAAs and NEAAs may be utilized differently in various tissues or body parts. Second, the comparison of intracellular and extracellular amino acid compositions in chicken muscle shows that the distinction between EAAs and NEAAs aligns with differences in intracellular versus extracellular distribution.

Taken together, these findings imply that the intracellular and extracellular compartments of multicellular organisms may exhibit a systematic bias in amino acid compositions, one that aligns with the EAA–NEAA boundary. If such a bias exists, then in conjunction with the findings from the **Literature Review**, it may help explain not only how EAAs and NEAAs become segregated but also why certain amino acids deemed non-essential continue to be synthesized.

In the next section, I introduce the **DRE Framework**, which builds on these insights and proposes a potential explanation for how the boundary between essential and non-essential amino acids emerges.

# Differential Recycle Efficacy (DRE) Framework

Building on previous research and my preliminary findings, this section proposes the **Differential Recycle Efficacy (DRE) Framework** as a conceptual model. The framework aims to explain how compartment-specific recycling efficiencies, together with metabolic cost considerations, inevitably shape the evolutionary boundary between amino acids that remain synthesized (non-essential) and those that do not (essential).

**Core Propositions of the DRE Framework**

1. **As organisms evolved, their bodies became increasingly compartmentalized.**
   Over the course of evolution, organisms transitioned from simple to more complex structural forms. In parallel, the subcellular localization of proteins diversified alongside function, leading to the formation of various protein compartments. This diversification can be seen, for example, in cytoplasmic proteins, nuclear proteins, organelle proteins, membrane proteins, and extracellular proteins.

2. **Compartmentalization gave rise to differences in resource-recycling efficiency across compartments.**
   Inevitably, having multiple protein compartments means each one may differ in how efficiently it recycles resources. For instance, extracellular proteins are often harder to degrade and recover than cytoplasmic proteins, and may be lost entirely rather than recycled.

3. **Selective pressures for metabolic optimization favor the use of low-cost amino acid residues in compartments with limited resource recovery.**
   If recycling efficiencies vary among compartments, minimizing metabolic expenditure where proteins are difficult to recycle confers a selective advantage. Consequently, proteins in compartments with lower recycling efficacy become enriched in amino acids that are cheaper to synthesize, thereby reducing overall energetic costs. A prime example in humans is type I collagen, whose genes encode protein sequences composed largely of lower-cost glycine and proline residues—together accounting for more than half of its amino acid composition.

4. **Increased reliance on low-cost amino acids elevates their overall demand.**
   As these lower-cost residues are preferentially used in compartments where recycling is challenging, their total consumption would increase relative to that of more expensive amino acids. Although this specific increase has not been directly observed, it follows logically from the preceding propositions.

5. **The constant need to synthesize lower-cost amino acids preserved their biosynthetic pathways, rendering them "non-essential."**
   Driven by the increased demand for extracellular proteins, retaining biosynthetic pathways for lower-cost amino acids became more advantageous, prompting organisms to continue producing them—thereby classifying them as "non-essential." Meanwhile, because the selection pressure to maintain the biosynthetic capacity for the remaining higher-cost amino acids may have been relatively weak, the pathways for these now "essential" amino acids could have been lost.

By integrating insights from both the **Literature Review** and **Preliminary Findings**, I developed the **DRE Framework** outlined here. In the following **Discussion**, I will evaluate its validity and explore the broader implications, including additional phenomena it may help to explain.

# Discussion

Why animals uniformly cease synthesizing certain amino acids has long been an unresolved question [1–4]. Notably, my statistical results indicate that the intracellular and extracellular disparities in amino acid composition closely align with the EAA–NEAA boundary. Recognizing this alignment prompted the idea that such differences between intracellular and extracellular compartments might be a key factor distinguishing EAAs from NEAAs—a concept that formed the basis of the earlier **"Extracellular Protein Hypothesis"** [11]. However, while that hypothesis did suggest that intracellular–extracellular amino acid composition differences could define the boundary between essential and non-essential amino acids, it did not explain why these differences arise in a similarly consistent form across multiple evolutionary lineages.

In exploring the historical context behind the "Extracellular Protein Hypothesis," I realized that combining my hypothesis with discussions from previous research could yield a simple yet robust

framework. Consequently, by integrating my statistical findings with earlier work, I developed the **Differential Recycle Efficacy (DRE) Framework**. Each proposition within the DRE Framework is both conceptually straightforward and appears to be supported by the evidence. Indeed, the observation that intracellular–extracellular disparities match the EAA–NEAA boundary aligns well with the framework's predictions [9,10].

Meanwhile, earlier studies also noted that essential amino acids have higher biosynthetic costs than their non-essential counterparts, generally attributing this to the advantage of outsourcing higher-cost amino acids rather than producing them [3,4]. Yet, these explanations did not address why low-cost amino acids would continue to be synthesized, even though losing that capacity would presumably pose a fitness disadvantage. The outsourcing perspective remains unclear on that point. In contrast, the DRE Framework focuses on explaining not just why high-cost amino acid biosynthetic pathways might be lost, but more crucially why it is necessary to preserve the ability to synthesize low-cost amino acids. By centering on this latter issue, the DRE Framework resolves the ambiguity inherent in previous outsourcing-based explanations, thereby offering an additional rationale for its contribution as a novel hypothesis.

Nevertheless, the DRE Framework has exceptions. Tyrosine, commonly classified as non-essential, deviates from the predicted pattern, possibly because it serves additional functions beyond extracellular protein production [1,9]. Arginine, meanwhile, may represent a special case in organisms that rely heavily on the urea cycle, altering its classification as essential or non-essential depending on physiological context [1].

It is also known that "essential" amino acids—remarkably similar to those in humans—are found in organisms beyond animals, although the specific boundaries can vary [1–3,12]. This variation suggests that for amino acids whose biosynthetic costs hover near the "threshold" for cost-effectiveness, evolutionary trajectories can differ: some species may retain the relevant biosynthetic pathways, while others abandon them.

The DRE Framework itself provides an explanation as to why it was necessary to maintain the capacity to synthesize non-essential amino acids more than essential ones; however, it does not clarify why organisms could afford to stop synthesizing essential amino acids in the first place. Nevertheless, if an organism's amino acid usage and recycling mechanisms are ultimately optimized, and if EAAs are relatively abundant within cells, it is plausible that a surplus of higher-cost amino acids—arising when synthesizing extracellular proteins from intracellular resources—helps mitigate any penalty from losing the ability to synthesize these amino acids. Although this speculation goes beyond the scope of the DRE Framework, I propose it as a reason why the biosynthetic capacity for roughly half of the 20 amino acids was ultimately—and remains—lost.

In this paper, I introduced the DRE Framework, proposing that as organisms became increasingly compartmentalized, they were compelled to retain the biosynthetic capacity for lower-cost amino acids. Under this framework, the extensive collagen production observed in present-day animals can be viewed as a logical extension of "economical evolution." Furthermore, the DRE Framework

provides a new perspective on the evolutionary processes that distinguish EAAs from NEAAs, suggesting that compartment-specific resource recycling, metabolic cost considerations, and extracellular protein enrichment collectively shape the boundary between those amino acids that remain synthesized and those that do not.

# Conclusion

In this paper, I proposed the **Differential Recycle Efficacy (DRE) Framework**, which integrates previous research findings with my own analytical results. Under the DRE Framework, the widely observed amino acid requirements among phagotrophic organisms—equivalent to what humans classify as essential and non-essential amino acids—are understood not as an arbitrary occurrence but rather as a predetermined outcome of resource optimization throughout evolution.

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# Figure & Table

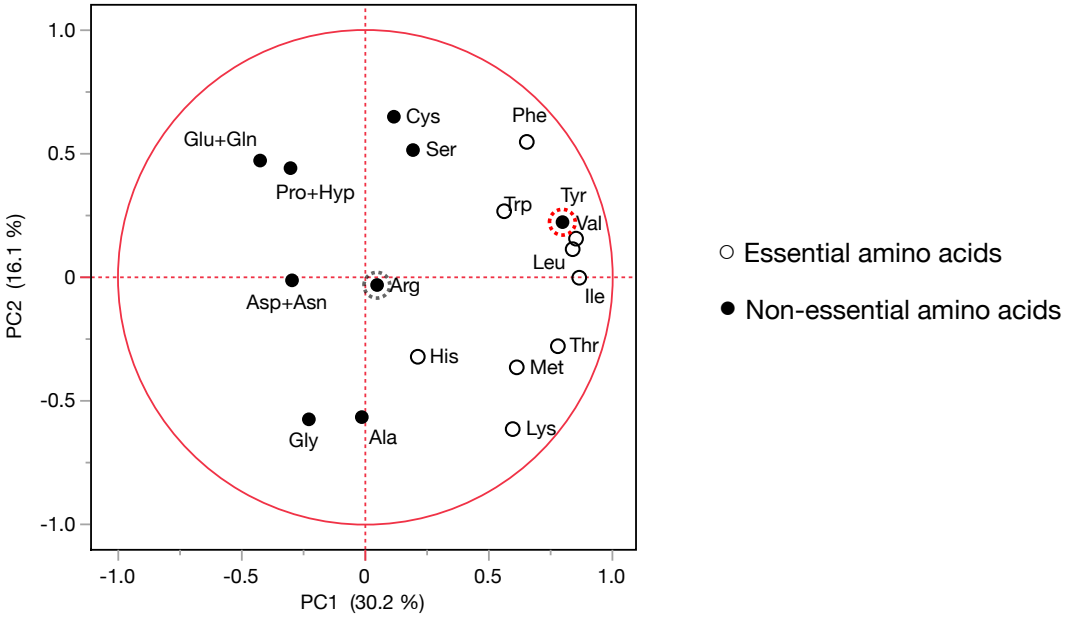


**Figure 1. Eigenvector Plot of Food Amino Acid Composition (Principal Component Analysis)**

Eigenvector plot of the first two principal components (PC1 and PC2) derived from the amino acid composition of all 1,954 food items in the latest official Japanese food composition database [7], following the methodology described in previous reports [10,11]. A similar figure has also appeared in my earlier preprint [9]. Open circles represent essential amino acids, while filled circles represent non-essential amino acids. Although tyrosine (Tyr), highlighted with a red dotted circle, is classified as non-essential, it clusters among the essential amino acids. Furthermore, because arginine (Arg) can be essential in certain species, it is highlighted with a gray dotted circle. The percentages next to each axis label indicate the proportion of variance explained by the respective principal component.

Notably, essential amino acids tend to cluster on the positive (right-hand) side of PC1, whereas non-essential amino acids cluster on the negative (left-hand) side, indicating a clear separation along the primary axis.

| | Intracellular | | | | Extracellular | | | | LN(Extra/Intra)+0.65 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid | Leg | Breast | 6 mo | 1.2 yr | Leg (6mo-1.2yr) | Breast (6mo-1.2yr) | 6 mo (Leg-Breast) | 1.2 yr (Leg-Breast) | Leg | Breast | 6 mo | 1.2 yr | Average (II) | NEAA |
| Pro+Hyp | 0.0429 | 0.0410 | 0.0425 | 0.0413 | 0.2090 | 0.2042 | 0.2070 | 0.2062 | 2.235 | 2.256 | 2.234 | 2.258 | 2.246 | ● |
| Gly | 0.0559 | 0.0561 | 0.0572 | 0.0547 | 0.2745 | 0.2695 | 0.2670 | 0.2770 | 2.241 | 2.220 | 2.191 | 2.271 | 2.231 | ● |
| Ala | 0.0763 | 0.0785 | 0.0771 | 0.0776 | 0.0989 | 0.0960 | 0.0973 | 0.0976 | 0.909 | 0.852 | 0.883 | 0.879 | 0.881 | ● |
| Arg | 0.0537 | 0.0544 | 0.0545 | 0.0535 | 0.0555 | 0.0514 | 0.0539 | 0.0529 | 0.682 | 0.593 | 0.639 | 0.639 | 0.638 | (●) |
| Ser | 0.0491 | 0.0500 | 0.0492 | 0.0499 | 0.0343 | 0.0389 | 0.0364 | 0.0367 | 0.290 | 0.398 | 0.351 | 0.343 | 0.345 | ● |
| Glu+Gln | 0.1422 | 0.1384 | 0.1401 | 0.1404 | 0.0902 | 0.0933 | 0.0908 | 0.0926 | 0.194 | 0.255 | 0.216 | 0.234 | 0.225 | ● |
| Asp+Asn | 0.0964 | 0.0982 | 0.0985 | 0.0970 | 0.0568 | 0.0583 | 0.0572 | 0.0578 | 0.121 | 0.129 | 0.108 | 0.133 | 0.123 | ● |
| Cys | 0.0111 | 0.0067 | 0.0068 | 0.0110 | 0.0046 | 0.0056 | 0.0065 | 0.0038 | 0.228 | 0.474 | 0.597 | 0.420 | 0.106 | ● |
| Thr | 0.0521 | 0.0517 | 0.0515 | 0.0522 | 0.0227 | 0.0257 | 0.0243 | 0.0241 | 0.182 | 0.047 | 0.101 | 0.123 | 0.113 | |
| Phe | 0.0344 | 0.0330 | 0.0339 | 0.0336 | 0.0159 | 0.0155 | 0.0164 | 0.0150 | 0.124 | 0.106 | 0.075 | 0.158 | 0.116 | |
| Lys | 0.0872 | 0.0874 | 0.0872 | 0.0873 | 0.0386 | 0.0372 | 0.0368 | 0.0391 | 0.164 | 0.204 | 0.213 | 0.154 | 0.184 | |
| Val | 0.0629 | 0.0675 | 0.0659 | 0.0644 | 0.0259 | 0.0282 | 0.0292 | 0.0248 | 0.239 | 0.222 | 0.164 | 0.302 | 0.232 | |
| Leu | 0.0900 | 0.0898 | 0.0896 | 0.0900 | 0.0329 | 0.0348 | 0.0349 | 0.0327 | 0.356 | 0.299 | 0.293 | 0.362 | 0.327 | |
| Ile | 0.0555 | 0.0563 | 0.0557 | 0.0560 | 0.0172 | 0.0184 | 0.0188 | 0.0168 | 0.522 | 0.466 | 0.437 | 0.552 | 0.495 | |
| Met | 0.0262 | 0.0267 | 0.0260 | 0.0269 | 0.0078 | 0.0075 | 0.0074 | 0.0079 | 0.562 | 0.616 | 0.606 | 0.572 | 0.589 | |
| Tyr | 0.0300 | 0.0291 | 0.0302 | 0.0288 | 0.0082 | 0.0081 | 0.0085 | 0.0078 | 0.645 | 0.627 | 0.619 | 0.654 | 0.637 | ● |
| His | 0.0261 | 0.0280 | 0.0271 | 0.0269 | 0.0072 | 0.0074 | 0.0075 | 0.0071 | 0.641 | 0.679 | 0.641 | 0.680 | 0.660 | |
| Trp | 0.0081 | 0.0074 | 0.0071 | 0.0084 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 | |

**Table 1. Intracellular vs. Extracellular Amino Acid Composition in Chicken Skeletal Muscle**

This table presents the molar compositions of amino acids in the intracellular and extracellular compartments of four chicken muscle categories—an unconventional and overlapping classification (leg, breast, and samples at 6 months and 1.2 years of age) as defined in the original study [8]. A similar table appeared in my previous preprints [9−11]. Here, I converted the original mass-based values into molar quantities and normalized each compartment so that its total amino acid content equals one. The columns labeled “LN(Extra/Intra)” show the natural logarithm of the ratio of extracellular to intracellular abundance, highlighting distribution differences between the two compartments. Amino acids are arranged according to their average logarithmic ratios across all four tissue types. To facilitate visualization, each logarithmic value is depicted with a color bar—scaled somewhat arbitrarily but offset by a constant to align with the boundary between essential and non-essential amino acids. The rightmost column indicates amino acids classified as non-essential in humans.

Overall, the data suggest a tendency for non-essential amino acids to be relatively more abundant in the extracellular compartment, whereas essential amino acids predominate intracellularly. However, tyrosine—despite being more abundant intracellularly—is conventionally considered non-essential, and arginine—often deemed essential in certain species—appears relatively higher in the extracellular fraction. These two cases represent exceptions to the otherwise consistent correlation between intracellular–extracellular differences and the essential–non-essential classification.