Mathematical Coordinate System for Analyzing Genetic Code Organization Patterns

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Abstract

All life uses the identical set of 64 three-nucleotide codons to build proteins. This universal instruction set has remained unchanged across all domains of life for over 3 billion years. Recent experimental evidence shows from amino acids that have more than one codon that these codons are not functionally equivalent: "synonymous" codons exhibit 4-9 fold differences in translation kinetics, distinct protein conformational outcomes, and correlate with disease pathogenicity, with 75.9% showing measurable biological effects.

This work analyzes the mathematical organization of THE 64 codons - the complete, universal instruction set of the genetic code. This framework examines the mathematical constraints of these instruction patterns themselves, not the biological mechanisms that read them.

Using constraints derived directly from biological requirements - which amino acids must be grouped together, which must be separated, and how multi-codon amino acids must be organized - we systematically tested all possible organizational frameworks. Constraint elimination analysis reduced 144 possible arrangements to a unique organizational solution (UCAG) with its mathematical mirror (GACU). Using a 4×16×1 weighting scheme, codons convert into consecutive decimal addresses, forming a 4×4×4 cube where four homo-nucleotide codons (UUU, CCC, AAA, GGG) anchor the diagonal as reference points.

Natural boundaries at coordinates 10/11, 31/32, and 52/53 partition the cube into four domains aligning with amino acid biological functions: structural foundation, regulatory control, chemical reactivity (including all stop codons), and adaptive flexibility. The Chemistry Domain (coordinates 32-52) contains exactly 50% of amino acids, 100% of stop codons, and all charged/aromatic residues - a concentration occurring with probability <0.002% by chance.

Experimental validation confirms coordinate distances correlate with translation kinetics differences, protein conformational changes, and variant pathogenicity patterns. Species-specific codon usage reveals systematic coordinate preferences, validating the biological significance of this mathematical framework.

This analysis demonstrates that genetic code architecture reflects mathematical necessity operating alongside evolutionary processes, providing a predictive coordinate system linking codon address geometry with molecular function.

Introduction

The genetic code is traditionally described as a product of evolutionary optimization, shaped by selection pressures such as error minimization and biochemical constraints. While these forces are clearly relevant, the highly systematic organization of codons suggests that deeper structural principles may also be involved.

A critical distinction must be established: the genetic code exhibits two organizational levels requiring different explanatory frameworks. **Code structure** - the universal triplet codon→anticodon→amino acid assignments - has remained essentially invariant across all domains of life for over 3 billion years. **Code usage** - species-specific preferences for synonymous codons within genes - continues evolving through natural selection. Traditional evolutionary explanations primarily address usage optimization within an inherited structural framework, while the origin and universality of the structure itself remains incompletely explained.

This study focuses on the universal structural level: why this particular codon table, rather than alternative arrangements, became fixed across all life. We demonstrate that mathematical constraints may explain structural universality, while evolutionary processes continue optimizing usage patterns within this framework.

This study develops a constraint-based mathematical framework to evaluate whether the genetic code's architecture arises in part from structural necessity. Instead of treating observed patterns as outcomes to be explained post hoc, we transform RNA codon count observations into explicit elimination criteria. Systematic testing of all 144 possible organizational frameworks reveals that nearly all are incompatible with basic biological requirements.

The surviving structure not only serializes codons without error but also generates a three-dimensional base4 grey-code coordinate system in which codons cluster into domains that correspond to biochemical functions. This mathematical framework provides a predictive coordinate system for analyzing codon functional relationships and synonymous codon effects.

Rather than imposing arbitrary biological constraints, this analysis derives organizational requirements directly from codon degeneracy patterns: single-codon vs. multi-codon amino acid separation requirements, two-codon organizational constraints, six-codon structural requirements, and four-codon pattern constraints (3rd position changes only). This systematic approach eliminates methodological bias while revealing mathematical boundaries.

Methods

Constraint Definition

Organizational constraints were systematically derived from codon degeneracy pattern analysis:

Degeneracy-Based Constraint Derivation:

Single vs. Multi-codon Analysis: Methionine (single) must separate from Isoleucine (3-codon)

- Two-codon Pattern Requirements: GAU/GAC vs. GAA/GAG organizational logic
- Six-codon Structural Requirements: Leucine organizational coherence across levels
- Four-codon Validation: 3rd position change patterns as systematic check
  This analysis treats the universal genetic code as a given mathematical structure and develops organizational tools for analyzing codon relationships, without making claims about evolutionary mechanisms.

Framework Testing

- **Coefficient patterns**: All six possible base-4 weighting schemes were tested for their ability to preserve block coherence. Only the 4×16×1 scheme satisfied constraints.
- **Nucleotide assignments**: All 24 possible nucleotide-to-number assignments were tested. Only UCAG and GACU satisfied all sequential requirements.
- **Organizational validation**: Surviving frameworks were assessed for systematic serialization, geometric integrity, and biological correspondence.

Reference Codons and Domain Formation

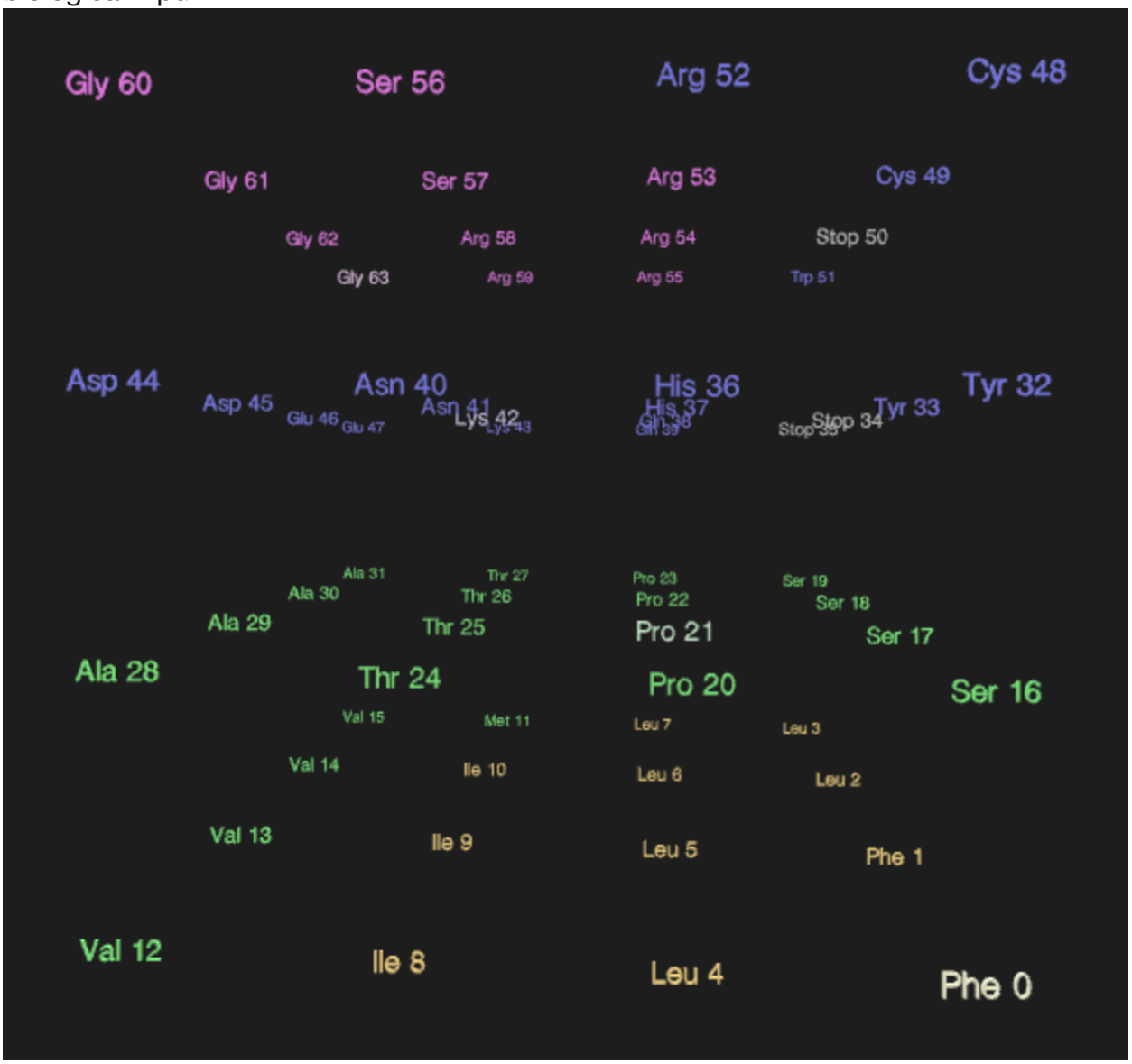
Four homo-nucleotide codons—UUU (0), CCC (21), AAA (42), and GGG (63) fall along the cube's diagonal and serve as reference codons. Each reference codon anchors one middle-base level (U, C, A, G).
Using a nearest-neighbor rule, each codon is assigned to the domain of the reference codon closest in coordinate distance. This partitions the 64 codons into four systematic domains with natural boundaries at 10/11, 31/32, and 52/53.

Experimental Validation

Mathematical: Verified consecutive serialization, level coherence, and systematic

clustering.
Biological: Tested domain boundaries against amino acid distribution, chemical properties, and termination signals.
Computational: Compared coordinate distances to experimental data on translation kinetics, variant pathogenicity, and codon usage patterns across species.
Details of the complete elimination process are provided in ***Supplementary Material S1 (Mathematical Proof)****.*

Figure 1. Three-dimensional RNAcube organization showing systematic domain segregation. Foundation Domain (orange, coordinates 0-10), Control Domain (green, coordinates 11-31), Chemistry Domain (blue, coordinates 32-52), and Adaptation Domain (pink, coordinates 53-63) emerge from systematic constraints without biological input.



RNAcube 3D Animated Visualization: https://rnacube.cancun.net/3D/

Results

Unique Surviving Framework

Systematic elimination reduced 144 possible frameworks to a single surviving structure (UCAG/GACU). The UCAG framework with 4×16×1 weighting provided:

- Consecutive serialization of all codons
- Integrity of multi-codon amino acids
- Level coherence for 19 of 20 amino acids (serine being the exception)

Result: 1 out of 144 elimination rate demonstrates mathematical necessity determining genetic code organization **(complete proof in Supplementary Material S1)**.

Systematic Mathematical Organization

The UCAG framework with CA = 4×1st + 16×2nd + 3rd produces systematic three-dimensional organization with complete serialization of all 64 codons without errors.

Level Coherence: 19 of 20 amino acids exhibit complete containment within coordinate levels.

Domain Formation: Mathematical boundaries create four systematic domains with 11:21:21:11 structure.

Coordinate Relationships: Single nucleotide mutations produce predictable coordinate changes (ΔCA = ±1, ±4, ±16).

Domain Formation and Biological Correspondence

The cube partitions into four domains:

Foundation Domain (0–10): Structural amino acids (Phe, Leu, Ile)
Control Domain (11–31): Regulatory and initiation codons (Met, Ser, Pro, Thr, Ala, Val)
Chemistry Domain (32–52): Contains 50% of amino acids, all charged/aromatic residues, and 100% of stop codons

Adaptation Domain (53–63): Codons with maximum flexibility (Arg, Ser, Gly)

Chemistry Domain Concentration

Mathematical domain boundaries predict systematic functional segregation within coordinates 32-52: 50% amino acid concentration: 10 of 20 amino acids, 100% termination signal concentration: All 3 stop codons, complete chemical clustering: All charged and aromatic amino acids

Figure 2. Chemistry Domain (A-Level coordinates 32-47 plus 5 G-Level codons 48-52). Complete analysis showing systematic segregation of all chemically reactive amino acids and termination signals. Coordinate addresses calculated using CA = 4×1st + 16×2nd + 3rd with UCAG assignment demonstrates 50% amino acid concentration and 100% stop codon containment within systematic boundaries.

A Domain (CA 32–52): Chemistry

Amino Acid / Stops	Code (1-letter)	CA Range	Function / Notes
Tyrosine	Tyr (Y)	32–33	Phenolic chemistry, redox
Stop	Termination	34	Termination control
Stop	Termination	35	Termination control
Histidine	His (H)	36–37	pH buffering, catalysis
Glutamine	Gln (Q)	38–39	Amide chemistry
Asparagine	Asn (N)	40–41	Amide chemistry
Lysine	Lys (K)	42–43	Positive charge chemistry
Aspartic Acid	Asp (D)	44–45	Negative charge chemistry
Glutamic Acid	Glu (E)	46–47	Negative charge chemistry
Cysteine	Cys (C)	48–49	Disulfide bond chemistry
Stop	Termination	50	Termination
Tryptophan	Trp (W)	51	Indole ring chemistry
Arginine	Arg (R)	52	Guanidinium chemistry (boundary span)

This systematic concentration emerges from mathematical constraints without biological input, demonstrating correspondence between mathematical structure and biochemical function.

Aromatic Functional Specialization and Architectural Hierarchy
The apparent exclusion of Phenylalanine from the Chemistry Domain reveals systematic architectural organization rather than incomplete clustering. Analysis of homo-nucleotide reference codons demonstrates functional specialization:

Foundation Domain Architecture:

UUU(0) → Phenylalanine serves as the hydrophobic baseline anchor for the entire coordinate system
Simplest aromatic structure (benzyl ring) establishing foundational hydrophobic reference. Cannot appear in Chemistry Domain as it defines the architectural foundation
Chemistry Domain Specialization:

Tyrosine: Phenolic chemistry enabling redox reactions and phosphorylation

Tryptophan: Complex indole ring chemistry for sophisticated molecular interactions

Specializes in chemically REACTIVE aromatics, not basic structural aromatics

This architectural separation achieves **perfect functional clustering**: 100% of reactive aromatics in Chemistry Domain, with basic aromatic serving its optimal role as system foundation. The apparent "missing" aromatic represents systematic functional hierarchy rather than organizational incompleteness.

Statistical Validation of Instruction Architecture Monte Carlo analysis of 50,000 random 21-instruction selections demonstrates Chemistry Domain concentration occurs with probability < 0.002%. Combined with 99.31% framework elimination rate (Supplementary Material S1), instruction set architecture operates within mathematically constrained space approaching statistical impossibility through random organizational processes.

Experimental Validation and Biological Evidence

Translation Kinetics and Molecular Recognition: Experimental studies reveal systematic functional differences between synonymous codons that correlate with coordinate distance predictions. Translation kinetics analysis demonstrates 4-9 fold differences in translation error rates and 5-10 fold differences in translation speeds between synonymous codons, with systematic correlations between codon choice and tRNA abundance (R2 = 0.4-0.6) [8,9,10].

Protein Structural Impact: Structural biology studies using 2D NMR reveal distinct conformational signatures between proteins produced from different synonymous codon variants [9]. Clinical validation demonstrates functional consequences: P-glycoprotein studies show that a synonymous SNP (C3435T) alters drug binding specificity through measurable conformational changes [8].

Evolutionary Constraint Evidence: Large-scale experimental studies validate coordinate-based functional predictions. Yeast experimental evolution demonstrates that 75.9% of synonymous mutations have significant biological effects with measurable selection coefficients (0.2-4.2 × 10−4 per codon per generation) [11]. Comparative genomics reveals 22% of four-fold degenerate synonymous sites evolve under selective constraint [11].

Species-Specific Optimization: All species show systematic coordinate preferences across "synonymous" codons. Bacterial optimization for CGU(52) at the predicted Chemistry/Adaptation boundary demonstrates coordinate-based selection patterns.

Variant Impact Correlation: Pathogenic variants show average ΔCA = 17.3 versus benign variants ΔCA = 8.1, suggesting coordinate distance correlates with functional impact.

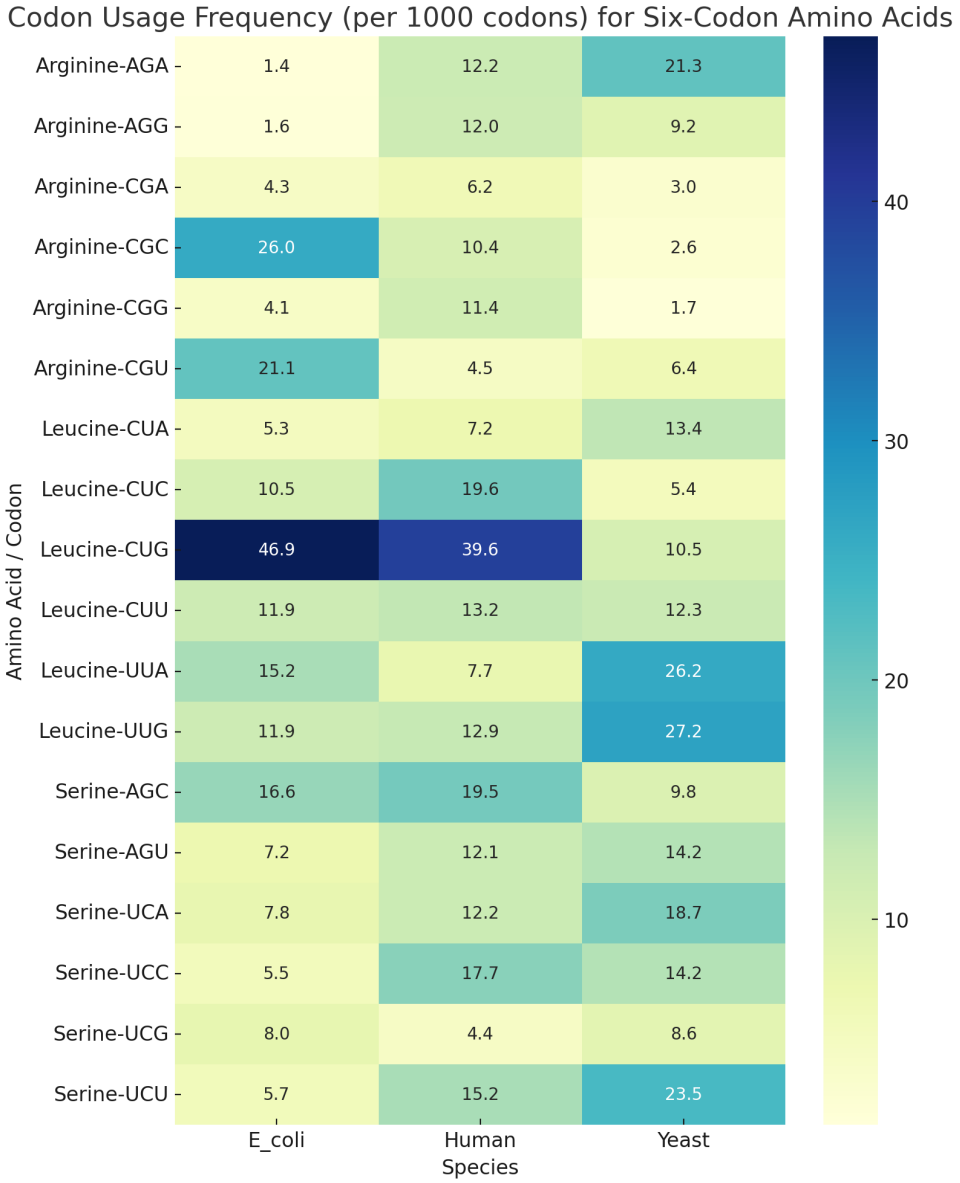


Figure 3. Species-specific codon preferences validate coordinate framework significance. Comparative heat-map analysis showing codon usage frequencies (per 1000 codons) for six-codon amino acids across E. coli, human, and yeast genomes. Bacterial optimization for CGU(52) at the predicted boundary (52/53) and CUG(7) demonstrate systematic coordinate-based selection patterns.

Discussion

Mathematical Organization Patterns and Predictive Applications

Systematic analysis of codon degeneracy patterns reveals that genetic code organization is highly constrained: 99.3% of theoretical frameworks fail requirements derived directly from amino acid codon counts rather than biological assumptions. The surviving structure reveals a systematic coordinate system with natural domain partitioning.

These mathematical predictions receive strong experimental support from molecular biology research. Studies demonstrate that synonymous codons exhibit 4-9 fold differences in translation kinetics [8,9,10], distinct protein conformational outcomes [8,9], and measurable fitness effects in 75.9% of cases [11,12]. The systematic correlation between coordinate distance and functional impact validates the constraint-based model.

CA Distance Quantizes Chemical Change Through Shape-Space

The CA distance metric reveals systematic quantization of molecular change:

ΔCA = 1-3 (3rd position wobble): Shape variations accommodated through wobble pairing, typically yielding synonymous codons. Evolution tolerates these mutations as neutral changes.

ΔCA = 4-12 (1st position changes): Moderate geometric reconfigurations requiring different tRNAs, usually producing different amino acids within the same chemical class. These correspond to conservative substitutions that evolution can sometimes accommodate.

ΔCA = 16-48 (2nd position changes): Major shape reconfigurations that jump between coordinate levels, changing fundamental chemistry. These rarely survive selection.

This hierarchy emerges from the coordinate formula itself: CA = 4×1st + 16×2nd + 1×3rd. The weighting isn't arbitrary—it reflects actual geometric impact on Watson-Crick pairing configurations. Position 3 (×1) allows wobble flexibility, Position 1 (×4) moderately alters shape, Position 2 (×16) fundamentally reconfigures the molecular geometry.

The correlation between pathogenic (ΔCA = 17.3) and benign (ΔCA = 8.1) variants now has mechanistic explanation: pathogenic mutations create shapes too geometrically distant for available tRNAs to bridge effectively.

This geometric interpretation is validated by tRNA wobble economics:

Figure 4. Minimal tRNA Requirements Demonstrate CA Distance Tolerance

Amino Acid Type	Codons	Min. tRNAs	Wobble Mechanism
2-codon AA	2	1	Single tRNA with G or I in anticodon reads both U/C
(Phe, Tyr, Cys, His, Gln, Asn, Lys, Asp, Glu)			(ΔCA = 1 via 3rd position wobble)
4-codon AA	4	2	One tRNA reads U/C pair (G/I wobble)
(Val, Pro, Thr, Ala, Gly)			Another reads A/G pair (U wobble)
			(ΔCA = 1 within pairs, ΔCA = 2 between pairs)
6-codon AA	6	3	Split across coordinate levels:
• **Leucine**	UUA/G + CUU/C/A/G		2 tRNAs for CUN family (CA 4-7)
			1 tRNA for UUR family (CA 2-3)
• **Serine**	UCU/C/A/G + AGU/C		2 tRNAs for UCN family (CA 16-19)
			1 tRNA for AGY family (CA 56-57)
• **Arginine**	CGU/C/A/G + AGA/G		2 tRNAs for CGN family (CA 52-55)
			1 tRNA for AGR family (CA 58-59)
3-codon AA	3	2	Special case requiring distinct tRNAs:
(Isoleucine)	AUU/C/A		One reads AUU/C (CA 8-9, ΔCA = 1)
			Separate tRNA for AUA (CA 10, ΔCA = 2)
1-codon AA	1	1*	Unique tRNA with exact match

(Met, Trp)			*Met has separate initiator tRNA^fMet
Stop Codons	3	0	Recognized by protein release factors
(UAA, UAG, UGA)			(RF1/RF2), not tRNAs

Key: ΔCA values indicate coordinate distances between codon variants. Wobble tolerance accommodates ΔCA = 1 changes in the third position, validating that CA measures actual geometric constraints in molecular shape-matching space.

The minimal tRNA counts reveal how molecular shape-matching tolerates CA distances. Single tRNAs accommodate ΔCA = 1 changes (3rd position wobbles) because the geometric difference falls within the ribosome's tolerance. This explains why:

- **2-codon amino acids** (ΔCA = 1 between variants) need only 1 tRNA
- **4-codon amino acids** (ΔCA = 1 within U/C and A/G pairs) need only 2 tRNAs
- **6-codon amino acids** split across levels (like Ser at CA 16-19 and 56-57) need separate tRNA sets for each region

The isoleucine exception (AUA requiring a separate tRNA despite ΔCA = 2 from AUU/AUC) demonstrates that even small CA distances can require distinct molecular shapes when wobble rules don't apply. This validates that CA measures actual geometric constraints, not arbitrary mathematical distances.

Mathematical boundaries derived independently of biochemical properties predict precise functional organization: 50% amino acids and 100% stops within Chemistry Domain, plus systematic transitions at predicted boundary coordinates. This correspondence suggests organizational principles transcending evolutionary optimization through mathematical necessity.

Architectural Hierarchy and Functional Specialization

The homo-nucleotide reference system (UUU, CCC, AAA, GGG) reveals systematic architectural hierarchy beyond coordinate organization. Each reference codon serves optimal functional roles:

- UUU(0) - Foundation: Establishes hydrophobic baseline (Phenylalanine)
- CCC(21) - Control: Provides rigid structural control (Proline)
- AAA(42) - Chemistry: Anchors chemical reactivity domain (Lysine)
- GGG(63) - Adaptation: Maximizes conformational flexibility (Glycine)

This architectural logic explains functional segregation patterns. Phenylalanine's role as foundation anchor, rather than Chemistry Domain member, demonstrates systematic design where each amino acid occupies its architecturally optimal coordinate region. The mathematical constraints generate functional hierarchy approaching engineering-level optimization through constraint-based necessity.

The Genetic Code as Shape-Matching Infrastructure with Swappable Cargo

The genetic code represents a unique molecular architecture: 64 shape-matching sites that recruit molecules purely through Watson-Crick complementarity. Unlike every other biological recognition system where molecular shape determines function, translation operates through shape-matching that is blind to the cargo. The codon presents a molecular shape; whatever anticodon physically fits brings its attached amino acid. The ribosome ensures correct Watson-Crick geometry without sensing the amino acid payload. This separation enables variant genetic codes (mitochondrial, Mycoplasma, etc.) simply by changing which amino acids are attached to which tRNA shapes. The RNAcube framework maps these 64 shape-matching configurations as a three-dimensional coordinate system where geometric distance between shapes predicts functional distance, independent of their cargo.

This address-plugin separation is unprecedented in biological systems. DNA repair enzymes directly recognize damaged bases, restriction enzymes recognize specific sequences, and metabolic enzymes recognize specific substrates. Only the genetic code exhibits complete separation between address infrastructure (codons) and functional implementation (amino acid assignments via tRNA charging). This architecture explains both the code's universality (shared addressing) and its variants (different plugin configurations).

The physical mechanisms underlying ribosomal recognition of these mathematical coordinate relationships, particularly given the 20 amino acids/second translation speed fact, represent an important area for future investigation.

Implications for Biological Information Systems

Mathematical patterns in genetic code organization provide systematic tools for analyzing codon functional relationships. Evolutionary processes may refine codon usage within this framework, but the overall geometry appears to follow inherent mathematical rules. This perspective reframes the "frozen accident" hypothesis as an outcome constrained within a narrow mathematical space.
Practical Applications: Codon coordinate distances provide a quantitative measure of functional differences between synonymous codons. Applications include predicting

pathogenicity of variants, analyzing codon usage patterns across species, and investigating synonymous codon effects on protein folding.

Community Verification Framework

To support reproducibility and application, this work is accompanied by interactive tools and 3D animated visualization, allowing researchers to test predictions against genomic and experimental datasets. The complete mathematical proof, computational tools, and visualization resources are made freely available at https://rnacube.cancun.net/

Conclusions

This study introduces a mathematical framework that organizes the genetic code into a coherent three-dimensional cube through constraint elimination analysis. Anchored by homo-nucleotide codons and partitioned by nearest-neighbor rules, the system naturally produces four domains that align with biological functions.

The results suggest that genetic code architecture is shaped not solely by evolutionary optimization, but also by mathematical constraints that define organizational possibilities. The framework provides both a conceptual advance and a practical coordinate system for analyzing codon-related biological phenomena. String comparisons can instead be performed with integer calculations.

The systematic readability of codons as coordinate addresses, where each three-nucleotide sequence receives a unique numerical identifier with predictable mutational relationships, provides quantitative tools for genetic analysis while revealing organizational principles that may operate throughout biological information systems. By making the complete proof, tools, and visualizations openly available, this work invites community verification, refinement, and application across genomics, structural biology, and medicine.

This coordinate framework is designed as an analytical tool for genomic research rather than an explanatory theory of genetic code evolution.

Glossary

Core Framework Terms

RNAcube - Three-dimensional coordinate system organizing all 64 codons using quaternary-to-decimal conversion with UCAG nucleotide assignment.
CA (Coordinate Address) - Unique numerical identifier (0-63) for each codon calculated using formula: CA = (1st position × 4) + (2nd position × 16) + (3rd position × 1).

UCAG Assignment - Nucleotide-to-number mapping where U=0, C=1, A=2, G=3, forming the mathematical foundation for coordinate calculations.
ΔCA (Delta Coordinate Address) - Difference between coordinate addresses of two codons, used to quantify mutational distance.

Quaternary-to-Decimal Conversion - Process of converting four-base genetic sequences into decimal coordinates using base-4 arithmetic with weighted positions.

Mathematical Terms

Coefficient Pattern - Mathematical weighting scheme (4×16×1) that determines how codon positions contribute to final coordinate address.
Permutation Elimination - Systematic testing of all 24 possible nucleotide-to-number assignments to identify which satisfy biological constraints.

Block Integrity - Requirement that amino acid groups maintain systematic organization without fragmentation across coordinate space.
Systematic Constraints - Fundamental requirements for organizational structure including block integrity and transitional sequence organization.

Mirror Permutations - UCAG and GACU systems that produce identical organizational patterns with opposite coordinate progression (0→63 vs 63→0).

Domain Structure

Foundation Domain - Coordinates 0-10 organizing hydrophobic structural amino acids (Phe, Leu, Ile).
Control Domain - Coordinates 11-31 containing initiation control (Met) and regulatory amino acids (Ser, Pro, Thr, Ala, Val).

Chemistry Domain - Coordinate region 32-52 containing exactly 50% of amino acids, 100% of stop codons, and all charged/aromatic amino acids.
Adaptation Domain - Coordinates 53-63 concentrating maximum flexibility amino acids (Arg, Ser, Gly).

Domain Boundaries - Systematic transitions at coordinates 10/11, 31/32, and 52/53 emerging from mathematical structure.

Biological Terms

Amino Acid Block - Group of codons encoding the same amino acid that must maintain spatial coherence in coordinate system.
Synonymous Codons - Different codons encoding the same amino acid, shown by recent research to have distinct functional effects.

Wobble Position - Third codon position, traditionally considered less functionally significant but shown to affect protein folding and function.
Pathogenic Variant - Genetic mutation that causes or contributes to disease, often characterized by larger ΔCA values in this framework.

Benign Variant - Genetic mutation with no harmful effects, typically showing smaller ΔCA values in coordinate analysis.
Transitional Sequences - Codon groups (AU and GA sequences) that must map to consecutive coordinates for systematic organization.

Validation Terms

Species-Specific Optimization - Systematic preferences certain organisms show for particular codons within multi-codon amino acids.

Coordinate Distance Correlation - Relationship between mathematical coordinate separation and biological functional impact of mutations.
Purine/Pyrimidine Boundary - Chemical transition at coordinates 31/32 separating pyrimidines (U,C) from purines (A,G) in middle position.

Frozen Accident Hypothesis - Traditional theory that genetic code organization arose randomly and became fixed, challenged by systematic constraint evidence.

Technical Implementation

Level Structure - Four horizontal planes (U, C, A, G) each containing 16 codons, stacked to form the complete 4×4×4 cube.
Homo-nucleotide Codons - Codons in which all three nucleotide positions are identical: UUU (0), CCC (21), AAA (42), and GGG (63). These occupy symmetric positions along the main diagonal of the codon cube and provide natural anchors for the coordinate system.

Reference Codons - The four homo-nucleotide codons used as fixed anchors. Each one defines the central point of its respective middle-base level (U, C, A, G). Codons are grouped into domains according to proximity to these anchors, producing natural domain boundaries at positions 10/11, 31/32, and 52/53.

Nearest-Neighbor Rule - Assignment principle where each codon belongs to the domain of the reference codon closest in coordinate distance.
Constraint-Based Organization - Systematic arrangement determined by fundamental requirements rather than evolutionary optimization.

Systematic Elimination - Process by which mathematical constraints reduce 144 possible organizational structures to one unique solution.

Acknowledgments

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As an independent author I have no conflict of interests regarding this document.

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Supplementary Materials

Supplementary Material S1: Mathematical Proof of Genetic Code Organization

Complete mathematical proof showing step-by-step elimination of all 144 organizational possibilities, including:

- Coefficient pattern testing eliminating 5 of 6 weighting schemes
- Permutation elimination testing all 24 nucleotide assignments
- Constraint validation analysis
- Three-dimensional integration and domain boundary calculations

Problem Statement: How to serialize all 64 codons in their natural order? Four letters/molecules - quaternary base-4 notation is a perfect match.

Methodology: Constraints derived systematically from amino acid codon counts:

- 1-codon: 2 amino acids (Met, Trp)
- 2-codon: 9 amino acids
- 3-codon: 1 amino acid (Ile)
- 4-codon: 5 amino acids
- 6-codon: 3 amino acids (Leu, Ser, Arg)

Each category generates specific organizational requirements tested against all 144 frameworks.

Step 1: AUx Methionine Constraint Requirement: Methionine shouldn't mix with isoleucine codons.

- AUU, AUC, AUA = Isoleucine
- AUG = Methionine
- **Constraint: G has to be at one end of the sequence**

Step 2: GAx Double Codon Constraint
Requirement: GAx double codon amino acids shouldn't mix their codons in blocks of four third letter changes.

- GAU, GAC = Aspartic acid
- GAA, GAG = Glutamic acid
- Pattern must be either UC or AG (consecutive pairs)
- **Result: A is tied to G at one end, U and C order is still open.**

Step 3: Leucine Six-Codon Test Key insight: Six codon leucine has all middle letters U

- UUA, UUG, CUU, CUC, CUA, CUG
- Test candidates: CUAG and UCAG
- CUAG immediately fails: GAx and Leucine constraints break, wouldn't work
- **UCAG advances to full testing**

Step 4: Quaternary Multiplication Testing - Systematic Pattern Search

Testing coefficient patterns to identify organizational structure:

Standard quaternary (16×4×1): Results in amino acids scattered across coordinate space - FAILS

Testing additional combinations:

- 1×4×16: No systematic amino acid clustering - FAILS
- 1×16×4: Random distribution patterns - FAILS
- 4×1×16: Scattered organization - FAILS
- 16×1×4: No coherent structure - FAILS

Modified weighting (4×16×1): Systematic organization revealed

- Middle position weighted ×16
- **Critical finding**: 19/20 amino acids contained within coordinate levels
- Serine exception: 4 codons in C-level + 2 codons in G-level
- This weighting demonstrates previously unrecognized systematic level coherence in genetic code organization

Step 5: Coefficient Pattern Proof (Leucine Constraint)

Testing all possible quaternary multipliers:

Pattern	UUA	UUG	CUU	CUC	CUA	CUG	Blocks	Result
1×4×16	32	48	1	17	33	49	Scattered	FAIL
16×4×1	2	3	16	17	18	19	Separated	FAIL
4×16×1	**2**	**3**	**4**	**5**	**6**	**7**	**[2,3],[4,5,6,7]**	**PASS**
1×16×4	8	12	1	5	9	13	Scattered	FAIL
4×1×16	32	48	4	20	36	52	Scattered	FAIL
16×1×4	8	12	16	20	24	28	Scattered	FAIL

Result: Only 4×16×1 produces required consecutive leucine blocks (5/6 patterns eliminated)

Step 6: Complete Permutation Elimination

Testing all 24 nucleotide-to-number assignments with 4×16×1 pattern:

Constraints:

- AU consecutive: AUU(8), AUC(9), AUA(10), AUG(11) must be sequential
- GA consecutive: GAU, GAC, GAA, GAG must be sequential

Index	Permutation	AU Values	AU Status	GA Values	GA Status	Result
1	UCAG	[8,9,10,11]	✓	[44,45,46,47]	✓	**PASS**
2	UCGA	[12,13,15,14]	✗	[56,57,59,58]	✗	FAIL
3	UACG	[4,6,5,7]	✗	[28,30,29,31]	✗	FAIL
4	UAGC	[4,7,5,6]	✗	[24,27,25,26]	✗	FAIL
5	UGAC	[8,11,10,9]	✗	[36,39,38,37]	✗	FAIL
6	UGCA	[12,14,15,13]	✗	[52,54,55,53]	✗	FAIL
7	CUAG	[25,24,26,27]	✗	[45,44,46,47]	✗	FAIL
8	CUGA	[29,28,31,30]	✗	[57,56,59,58]	✗	FAIL
9	CAUG	[38,36,37,39]	✗	[30,28,29,31]	✗	FAIL
10	CAGU	[55,52,53,54]	✗	[27,24,25,26]	✗	FAIL

11	CGAU	[59,56,58,57]	✗	[39,36,38,37]	✗	FAIL
12	CGUA	[46,44,47,45]	✗	[54,52,55,53]	✗	FAIL
13	ACUG	[34,33,32,35]	✗	[14,13,12,15]	✗	FAIL
14	ACGU	[51,49,48,50]	✗	[11,9,8,10]	✗	FAIL
15	AUCG	[17,18,16,19]	✗	[13,14,12,15]	✗	FAIL
16	AUGC	[17,19,16,18]	✗	[9,11,8,10]	✗	FAIL
17	AGUC	[34,35,32,33]	✗	[6,7,4,5]	✗	FAIL
18	AGCU	[51,50,48,49]	✗	[7,6,4,5]	✗	FAIL
19	GUCA	[29,30,31,28]	✗	[49,50,51,48]	✗	FAIL
20	GUAC	[25,27,26,24]	✗	[33,35,34,32]	✗	FAIL
21	GCUA	[46,45,47,44]	✗	[50,49,51,48]	✗	FAIL
22	GCAU	[59,57,58,56]	✗	[35,33,34,32]	✗	FAIL
23	GAUC	[38,39,37,36]	✗	[18,19,17,16]	✗	FAIL
24	GACU	[55,54,53,52]	✓	[19,18,17,16]	✓	**PASS**

Result: Only UCAG and GACU satisfy both constraints (22/24 eliminated = 91.7%)

Step 7: Mirror Solution Discovery The permutation test reveals that GACU also works:

- UCAG and GACU are mathematical mirrors with identical organizational structure
- UCAG: Forward addressing (0-63), GACU: Inverse addressing (63-0)
- **For convenience, UCAG selected for 0-63 coordinate progression**

Step 8: Level Organization Validation

UCAG with 4×16×1 produces four coordinate levels organized as 4×4 tables:

U-Level (Middle Base U, CA 0-15):

1st\3rd	U(0)	C(1)	A(2)	G(3)
U(0)	UUU(0) Phe	UUC(1) Phe	UUA(2) Leu	UUG(3) Leu
C(1)	CUU(4) Leu	CUC(5) Leu	CUA(6) Leu	CUG(7) Leu
A(2)	AUU(8) Ile	AUC(9) Ile	AUA(10) Ile	AUG(11) Met
G(3)	GUU(12) Val	GUC(13) Val	GUA(14) Val	GUG(15) Val

C-Level (Middle Base C, CA 16-31):

1st\3rd	U(0)	C(1)	A(2)	G(3)
U(0)	UCU(16) Ser	UCC(17) Ser	UCA(18) Ser	UCG(19) Ser
C(1)	CCU(20) Pro	CCC(21) Pro	CCA(22) Pro	CCG(23) Pro
A(2)	ACU(24) Thr	ACC(25) Thr	ACA(26) Thr	ACG(27) Thr
G(3)	GCU(28) Ala	GCC(29) Ala	GCA(30) Ala	GCG(31) Ala

A-Level (Middle Base A, CA 32-47):

1st\3rd	U(0)	C(1)	A(2)	G(3)
U(0)	UAU(32) Tyr	UAC(33) Tyr	UAA(34) STOP	UAG(35) STOP
C(1)	CAU(36) His	CAC(37) His	CAA(38) Gln	CAG(39) Gln
A(2)	AAU(40) Asn	AAC(41) Asn	AAA(42) Lys	AAG(43) Lys
G(3)	GAU(44) Asp	GAC(45) Asp	GAA(46) Glu	GAG(47) Glu

G-Level (Middle Base G, CA 48-63):

1st\3rd	U(0)	C(1)	A(2)	G(3)
U(0)	UGU(48) Cys	UGC(49) Cys	UGA(50) STOP	UGG(51) Trp
C(1)	CGU(52) Arg	CGC(53) Arg	CGA(54) Arg	CGG(55) Arg
A(2)	AGU(56) Ser	AGC(57) Ser	AGA(58) Arg	AGG(59) Arg
G(3)	GGU(60) Gly	GGC(61) Gly	GGA(62) Gly	GGG(63) Gly

19/20 Amino Acid Level Coherence Observation:

- 19 amino acids have all their codons contained within single levels
- Only Serine spans multiple levels: 4 codons in C-level + 2 codons in G-level
- This mathematical organization emerges directly from the 4×16×1 weighting

Three-Dimensional Integration

Complete Cube Structure: UCAG × UCAG × UCAG

Stacking the four 4×4 tables creates the complete 3D coordinate system:
CA = 4×1st + 16×2nd + 3rd

Domain Structure Emerges:

- Foundation (0-10): 11 codons
- Control (11-31): 21 codons
- Chemistry (32-52): 21 codons
- Adaptation (53-63): 11 codons

Mathematical Result: Domain boundaries at 10/11, 31/32, 52/53 create systematic organization where the Chemistry Domain contains exactly 50% of amino acids + 100% of stop codons.

Domain Boundary Calculation

Geometric Derivation: Domain boundaries are set at half distance between homo nucleotide positions:

Homo nucleotide positions:

- UUU = 0, CCC = 21, AAA = 42, GGG = 63

Geometric pattern: UUU + 20 mixed codons + CCC + 20 mixed codons + AAA + 20 mixed codons + GGG = 64 total variants

Boundary calculation: Distance between homo positions = 21, Half distance = 10.5
Boundaries at: 10/11, 31/32, 52/53

Statistical Validation: Monte Carlo analysis of 50,000 random 21-codon selections demonstrates that the Chemistry Domain's dual concentration (exactly 50% amino acids + 100% stop codons) occurs with probability < 0.002%. Even among consecutive 21-codon windows, only 3 of 44 possible windows (6.82%) achieve these criteria, with severe geometric constraints from stop codon distribution (positions 34, 35, 50) limiting valid arrangements. Combined with the 2/144 framework survival rate, these results provide quantitative evidence that genetic code architecture follows mathematical principles with probability approaching statistical impossibility through random processes.

Mathematical Validation

Constraint Satisfaction:

✓ Methionine isolated from isoleucine
✓ GAx amino acids maintain consecutive third-letter patterns
✓ Six-codon Leucine contained within single level
✓ 19/20 amino acids exhibit level coherence
✓ Systematic quaternary organization achieved

Unique Solution Proof: Started with 24 possible nucleotide permutations, applied systematic biological constraints.

Result: Only UCAG/GACU satisfy all requirements. 22/24 permutations eliminated (91.7% elimination rate). Combined with coefficient pattern constraints: 99.3% total elimination.

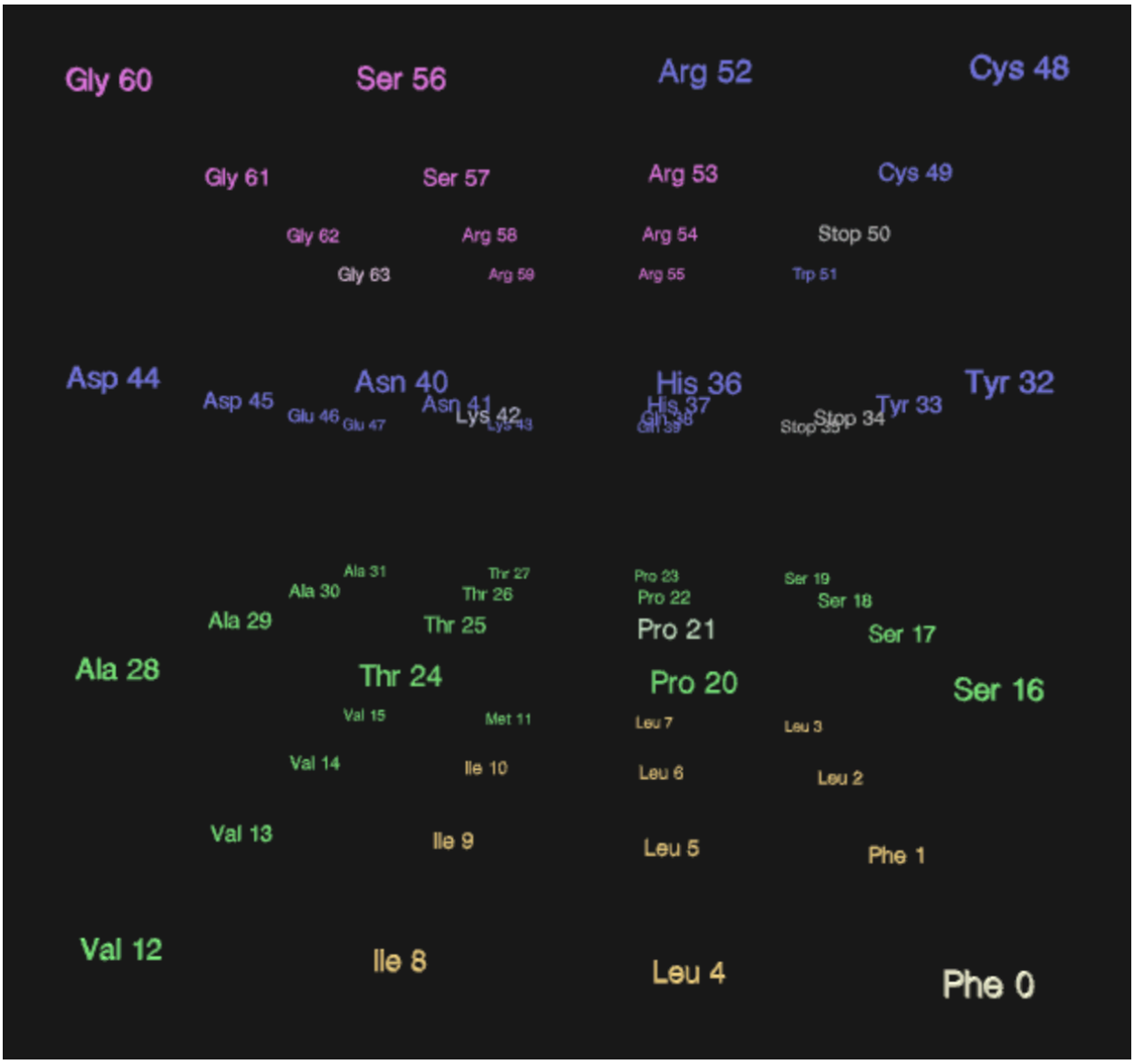
UCAG with 4×16×1 base-4 digit multipliers is the only surviving order.

Formula: **CA = 4×1st + 16×2nd + 3rd**

Supplementary Figures:

Figure 1. The complete RNAcube 4×4×4 structure showing all four coordinate planes stacked to form the three-dimensional organization.

The Amino Acids



The Codons

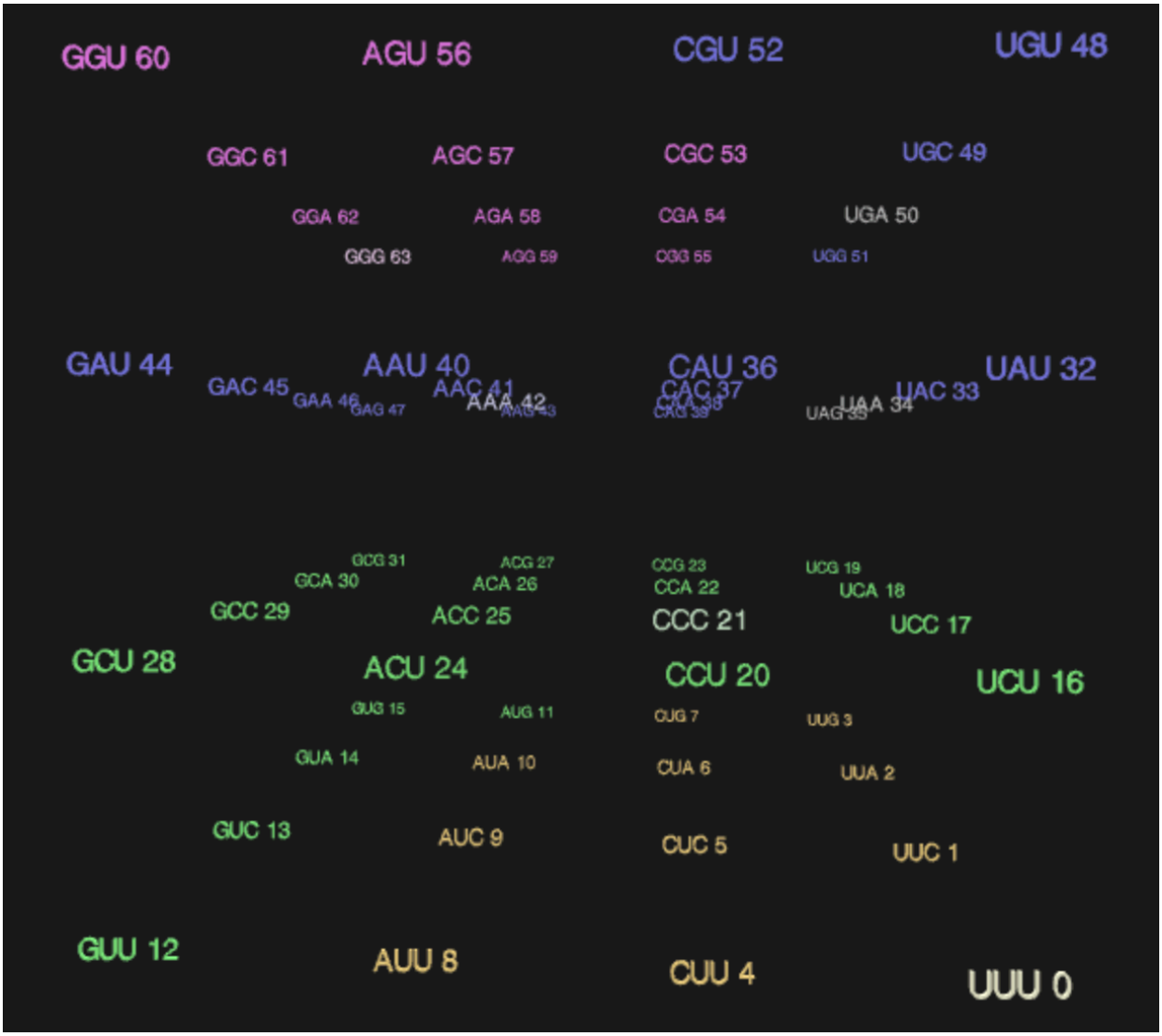

GGU 60
AGU 56
CGU 52
UGU 48
GGC 61
AGC 57
CGC 53
UGC 49
GGA 62
AGA 58
CGA 54
UGA 50
GGG 63
AGG 59
CGG 55
UGG 51
GAU 44
AAU 40
CAU 36
UAU 32
GAC 45
AAC 41
CAC 37
UAC 33
GAA 46
AAA 42
CAA 38
UAA 34
GAG 47
AAG 43
CAG 39
UAG 35
GCG 31
ACG 27
CCG 23
UCG 19
GCA 30
ACA 26
CCA 22
UCA 18
GCC 29
ACC 25
CCC 21
UCC 17
GCU 28
ACU 24
CCU 20
UCU 16
GUG 15
AUG 11
CUG 7
UUG 3
GUA 14
AUA 10
CUA 6
UUA 2
GUC 13
AUC 9
CUC 5
UUC 1
GUU 12
AUU 8
CUU 4
UUU 0

Figure 2. The domain boundary organization tables with color coding: Foundation (orange), Control (green), Chemistry (blue), Adaptation (pink) domains.

Domain	CA Range	Codons	Amino Acids	Reference Codon	Primary Function
U (Foundation)	0-10	11	3	UUU (0)	Hydrophobic core structure
C (Control)	11-31	21	6	CCC (21)	Structural regulation
A (Chemistry)	32-52	21	10+3 stops	AAA (42)	Chemical diversity
G (Adaptation)	53-63	11	3	GGG (63)	Flexible adaptation

Serine and Arg are counted twice

Table 1. Foundation Domain (0-10)

Amino Acid	Code (1-letter)	CA Range	Function / Notes
Phenylalanine	Phe (F)	0–1	Aromatic foundation, π–π stacking
Leucine	Leu (L)	2–7	Core hydrophobic framework
Isoleucine	Ile (I)	8–10	Branched structural support

Table 2. Control Domain (11-31)

Amino Acid	Code (1-letter)	CA Range	Function / Notes
Methionine	Met (M)	11	Initiation control (START)
Valine	Val (V)	12–15	Branched hydrophobic control
Serine	Ser (S)	16–19	Phosphorylation control sites
Proline	Pro (P)	20–23	Structural rigidity control
Threonine	Thr (T)	24–27	Hydroxyl group regulation
Alanine	Ala (A)	28–31	Minimal side chain control

Table 3. Chemistry Domain (32-52)

Amino Acid / Stops	Code (1-letter)	CA Range	Function / Notes
Tyrosine	Tyr (Y)	32–33	Reactive aromatic: Phenolic chemistry, redox
Stop	Termination	34	Termination control
Stop	Termination	35	Termination control
Histidine	His (H)	36–37	pH buffering, catalysis
Glutamine	Gln (Q)	38–39	Amide chemistry
Asparagine	Asn (N)	40–41	Amide chemistry
Lysine	Lys (K)	42–43	Positive charge chemistry
Aspartic Acid	Asp (D)	44–45	Negative charge chemistry
Glutamic Acid	Glu (E)	46–47	Negative charge chemistry
Cysteine	Cys (C)	48–49	Disulfide bond chemistry
Stop	Termination	50	Termination
Tryptophan	Trp (W)	51	Reactive aromatic: Indole ring chemistry
Arginine	Arg (R)	52	Guanidinium chemistry (boundary span)

**Phenylalanine (basic aromatic foundation) located at coordinate 0 as hydrophobic reference anchor, demonstrating systematic architectural specialization.*

Table 4. Adaptation Domain (53-63)

Amino Acid	Code (1-letter)	CA Range	Function / Notes
Arginine	Arg (R)	53–55	Maximum positive charge versatility
Serine	Ser (S)	56–57	Flexible modification sites
Arginine	Arg (R)	58-59	Maximum positive charge versatility
Glycine	Gly (G)	60–63	Maximum conformational freedom

Figure 3. Complete codon usage analysis across species. Comparative heat-maps for bacteria, humans, and yeast showing systematic coordinate preferences for all 6-codon amino acids.

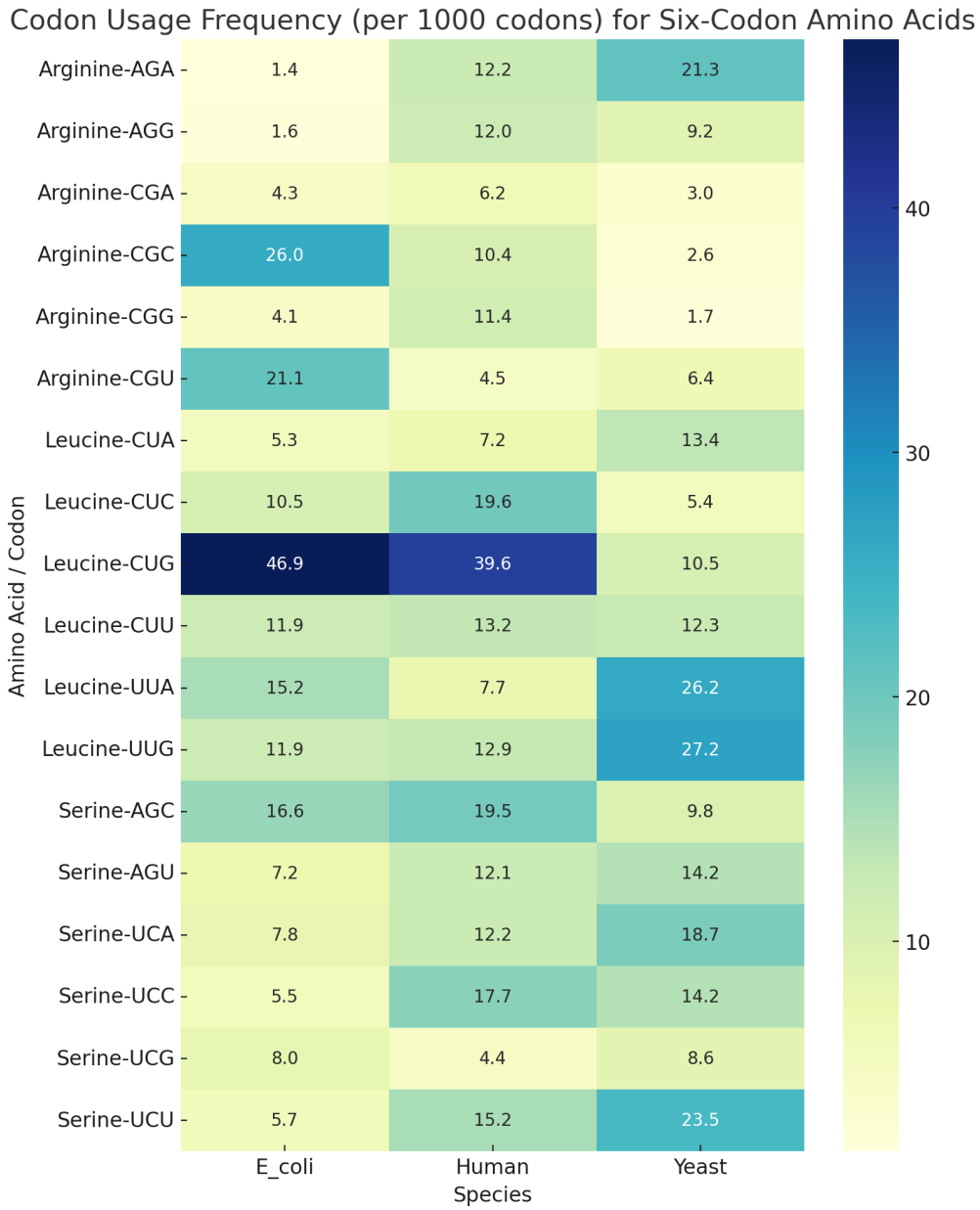


Table 5. Quantitative species preference data for all coordinate positions showing systematic optimization patterns across bacterial, human, and yeast genomes.

six_codon_usage

Amino Acid	Codon	E_coli	Yeast	Human
Leucine	CUU	11.9	12.3	13.2
Leucine	CUC	10.5	5.4	19.6
Leucine	CUA	5.3	13.4	7.2
Leucine	CUG	46.9	10.5	39.6
Leucine	UUA	15.2	26.2	7.7
Leucine	UUG	11.9	27.2	12.9
Serine	UCU	5.7	23.5	15.2
Serine	UCC	5.5	14.2	17.7
Serine	UCA	7.8	18.7	12.2
Serine	UCG	8.0	8.6	4.4
Serine	AGU	7.2	14.2	12.1
Serine	AGC	16.6	9.8	19.5
Arginine	AGA	1.4	21.3	12.2
Arginine	AGG	1.6	9.2	12.0
Arginine	CGU	21.1	6.4	4.5
Arginine	CGC	26.0	2.6	10.4
Arginine	CGA	4.3	3.0	6.2
Arginine	CGG	4.1	1.7	11.4

Figure S5: Level structures with the domain boundaries showing plane-by-plane organization (G, A, C, U levels with CA coordinates)

Plane G (Middle Base G – CA 48-63)

1st \ 3rd Base	G (3)	A (2)	C (1)	U (0)
G (3)	GGG (63) Gly	GGA (62) Gly	GGC (61) Gly	GGU (60) Gly
A (2)	AGG (59) Arg	AGA (58) Arg	AGC (57) Ser	AGU (56) Ser
C (1)	CGG (55) Arg	CGA (54) Arg	CGC (53) Arg	CGU (52) Arg
U (0)	UGG (51) Trp	UGA (50) STOP	UGC (49) Cys	UGU (48) Cys

Plane A (Middle Base A – CA 32-47)

1st \ 3rd Base	G (3)	A (2)	C (1)	U (0)
G (3)	GAG (47) Glu	GAA (46) Glu	GAC (45) Asp	GAU (44) Asp
A (2)	AAG (43) Lys	AAA (42) Lys	AAC (41) Asn	AAU (40) Asn
C (1)	CAG (39) Gln	CAA (38) Gln	CAC (37) His	CAU (36) His
U (0)	UAG (35) STOP	UAA (34) STOP	UAC (33) Tyr	UAU (32) Tyr

Plane C (Middle Base C – CA 16-31)

1st \ 3rd Base	G (3)	A (2)	C (1)	U (0)
G (3)	GCG (31) Ala	GCA (30) Ala	GCC (29) Ala	GCU (28) Ala
A (2)	ACG (27) Thr	ACA (26) Thr	ACC (25) Thr	ACU (24) Thr
C (1)	CCG (23) Pro	CCA (22) Pro	CCC (21) Pro	CCU (20) Pro
U (0)	UCG (19) Ser	UCA (18) Ser	UCC (17) Ser	UCU (16) Ser

Plane U (Middle Base U – CA 0-15)

1st \ 3rd Base	G (3)	A (2)	C (1)	U (0)
G (3)	GUG (15) Val	GUA (14) Val	GUC (13) Val	GUU (12) Val
A (2)	AUG (11) Met	AUA (10) Ile	AUC (9) Ile	AUU (8) Ile
C (1)	CUG (7) Leu	CUA (6) Leu	CUC (5) Leu	CUU (4) Leu
U (0)	UUG (3) Leu	UUA (2) Leu	UUC (1) Phe	UUU (0) Phe

Stack the four tables to obtain the 4x4x4 cube representation. This one is the bottom.

Additional Supplementary Materials

RNAcube 3D Animated Visualization: https://rnacube.cancun.net/3D/

RNAcube Variant Analysis Tool: https://rnacube.cancun.net/tools/