Widespread Recruitment of Ancient Domain Structures in Modern Enzymes during Metabolic Evolution

Hee Shin Kim[1,*], Jay E. Mittenthal[2], Gustavo Caetano-Anollés[1,†]

[1]Evolutionary Bioinformatics Laboratory, Department of Crop Sciences and [2]Department of Cell and Developmental Biology, University of Illinois, Urbana, IL 61801, USA, http://manet.illinois.edu

Summary

Protein domains sometime combine to form multidomain proteins and are acquired or lost in lineages of organisms. These processes are ubiquitous in modern metabolism. To sort out evolutionary patterns of domain recruitment, we developed an algorithm that derives the most plausible ancestry of an enzyme from structural and evolutionary annotations in the MANET database. We applied this algorithm to the analysis of 1,163 enzymes with structural assignments. We then counted the number of enzymes along a time series and analyzed enzyme distribution in organisms belonging to superkingdoms Archaea, Bacteria, and Eukarya. The generated timelines described the evolution of modern metabolic networks and showed an early build-up of metabolic activities associated with metabolism of nucleotides, cofactors, and vitamins, followed by enzymes involved in carbohydrate and amino acid metabolism. More importantly, we find that existing domain structures were pervasively co-opted to perform more modern enzymatic tasks, either singly or in combination with other domains. This occurred differentially in lineages of the superkingdoms as the world diversified and organisms adapted to various environments. Our results highlight the important role of recruitment and domain organization in metabolic evolution.

1 Introduction

Recruitment represents a widespread phenomenon in biology that occurs when a component, ensemble, repertoire, or a more complex system adapts (coopts) an existing feature for a new purpose and within a different context, such as enzyme cooption [16] or exaptation of complex traits [14]. In the molecular world, proteins that perform a particular function in one biological context can be brought to perform a related or different function in a different context. One fundamental question in biology is how protein molecules are recruited in biological networks when patterns and processes of recruitment unfold in evolution. Here we explore recruitment in the networks of cellular metabolism.

Metabolism can be defined as a complex collection of enzymatic reaction and transport processes that are driven by enzymes, proteins responsible for chemical catalysis in cellular systems [7]. These reactions transform and transport small organic molecules (metabolites) and in doing so establish a complex network of products and intermediates of the enzymatic reactions. A core ensemble of reactions of the metabolic network is common to cellular life, supporting the existence of strong evolutionary and thermodynamic constraint in network structure [3,24,25,30]. This core is composed of protein catalysts that harbor only few three-dimensional (3D) fold structures [5,7].

[*] Current address: Duke Center for Human Genome Variation, Duke University, Durham, NC 27708, USA

[†] To whom correspondence should be addressed. Email: gca@illinois.edu

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The active catalytic sites of enzymes are nested in the structures of protein domains. Domains are 3D arrangements of elements of secondary structure that fold autonomously [34], are compact [28], and are evolutionary conserved [15,29]. Domains combine with others in multidomain proteins to establish 'architectures' that link patterns of domain structure and organization. Since domains in these architectures behave as modular and evolutionary units, the principle of evolutionary continuity dictates that the thousands of domain structures of the cell must appear progressively in time, as they engage in domain combinatorics. Consequently, the time of origin of individual domains establishes their relative evolutionary age (their ancestry). Supported by this *domain-centric* framework, we have reconstructed phylogenetic histories of domains based on a genomic census of structures in hundreds of organisms with genomes that have been fully sequenced [4]. In these studies, structures are defined at fold (F), fold superfamily (FSFs), and fold family (FF) levels of the STRUCTURAL CLASSIFICATION OF PROTEINS (SCOP) [1,23] taxonomical hierarchy and their genomic abundances are used to build phylogenetic trees that describe the evolution of the protein world (reviewed in [6]). These evolutionary statements can be used to determine the relative age of individual domain structures, generally in a relative zero-to-one scale, with zero representing the origin of proteins. Moreover, since the appearance of new domain structures follows a 'molecular clock' [33], the universal recurrence results in timelines of domain innovation that correlate approximately linearly with geological time scales. Thus, timelines assign ages to enzymes and uncover patterns of origin, recruitment and evolution in metabolic networks [5,7,9].

In this study, we focus on enzymes with predicted F and FSF structural assignments in the MOLECULAR ANCESTRY NETWORK (MANET) database [19]. MANET integrates ancestries of SCOP domain structures and enzymatic activities in metabolic pathways of the KYOTO ENCYCLOPEDIA OF GENES AND GENOMES (KEGG) database [17]. Using MANET we now engage in a novel *enzyme-centric* phylogenomic study that traces the distribution of domains in metabolic networks and compares the age of enzymes belonging to organisms in cellular superkingdoms. The study unfolds levels of domain cooption in single and multidomain enzymes as these evolve in lineages of organisms.

2 Methods

2.1 Phylogenomic analysis of enzymatic activities in metabolic networks

We used MANET 2.0 (July 2007) [19] (http//manet.illinois.edu) to trace the evolution of enzymes in metabolic pathways of KEGG [17]. MANET assigns protein domain structures defined by 7,238 PDB to 1,450 enzymes in 11 mesonetworks and 136 subnetworks of KEGG. The age of domains (*nd*) derived from a structural phylogenomic census of 784 F domain structures defined by SCOP 1.67 (May 2004) in the proteomes of 174 organisms (19 Archaea, 117 Bacteria and 36 Eukarya) is then traced in metabolic enzymes [18]. Enzymatic activities were described using Enzyme Commission (EC) nomenclature. SCOP domains were defined with *concise classification strings* (*ccs*) [1,22].

2.2 Deriving the age of enzymes with one or more protein domains in superkingdoms

A total of 856,988 amino acid sequences were retrieved directly from the gene catalog file of KEGG. FSF structures of these sequences were predicted using linear hidden Markov models (HMMs) of structural recognition in SUPERFAMILY [13] as previously described [19]. We kept track of lineages and superkingdom distribution. A total of 1,163 enzymes defined by EC numbers and belonging to organisms with genomes that have been fully sequenced (generally

defined at species level in KEGG) were retained. Since enzymes harbored a wide range of domain structures and organization, we developed an algorithm that calculates the age of an enzyme that is present in an organism. For a given enzyme, the algorithm examines alternative structural assignments, identifies FSFs that have the highest organismal occurrence in the superkingdom, and assigns the age of the F structure that corresponds to the FSF of the enzyme. In the case that the most frequent assignment is a domain combination, the algorithm chooses the age of the youngest F structure. In the case of equal assignment frequencies, the age of the enzyme is that of the most ancient F. The algorithm also provides information about the number of organisms that was analyzed, structural assignments for each enzyme and their frequency in organisms, and the range of ancestries derived from these assignments. Its pseudocode and its rationale are described in Figure 1. The algorithmic implementation assumes that: (i) protein evolution unfolds through duplication, amplification, mutational change, and recombination of genes [32], though gene amplification and de novo generation of genes have been recently shown to be common phenomena [10]; and (ii) domain accretion occurs fundamentally by fusions of old or new domains to already functional structural units. The algorithm does not consider the role of fission processes by which enzymatic units are built from structures that were split from other proteins. While this may appear problematic, a careful mechanistic study of domain fusion and fission in proteins of hundreds of proteomes showed fissions occurred relatively late in protein evolution [32]. Since we show that the most significant recruitment event occur early in evolution, the role of fission processes in domain organization should be considered negligible in the early stages of metabolic evolution and should not affect the main conclusions of this study.

```
Hash tmpStructures;   //key:structural assignment value:ancestry
Hash Count;           //key:structural assignment value:occurrence
For each enzyme in enzymes in metabolic network{
        For each species in the enzyme{
                if (new structural assignment){
                        if (assignment == combination){
                                Count{assignment} += 1;
                                tmpStructures{assignment} = younger ancestry value
                        } else{
                                Count{assignment} = 1;
                                tmpStructures{assignment} =  ancestry value
                        }
                }else
                        Count{assignment} += 1;
        }
        if (the most frequent assignment exist){
                Structure = key of max(Count);
                Ancestry = tempStructures{Structure};
                Range = From min(tempStructures) to max(tempStructures);
        }else{
                Ancestry = min(tempStructures)
                Range = From min(tempStructures) to max(tempStructures);
        }
}
```

Figure 1: Pseudocode for the calculation of the most plausible ancestry of an enzyme. The algorithm assumes that the most important mechanisms of protein evolution involve duplication, amplification, mutational change, and recombination of genes and that these processes result in gradual buildup of new structures in protein architectures. Consequently, a domain combination in an enzyme that harbors multiple F structures probably arose by either an older structure recruiting a younger structure or vice versa. In both cases, the recruitment had to occur once the younger F structure appeared in evolution and the ancestry of the combination must be the ancestry of the youngest F. Using the algorithm we constructed a table that describes each individual enzyme, number of species analyzed, assignment of structures to enzymes in all organisms, a range of ancestry values derived from these assignments, and the most plausible enzymatic ancestry (http://manet.illinois.edu/download.php).

2.3 Graph representations of metabolic recruitment in metabolic network evolution

Graph representations helped unfold global pathways of enzymatic recruitment in metabolism by representing mesonetworks as vertices and shared enzymes as edges at 7 evolutionary time points. En each case, an adjacency matrix was constructed in which elements signal the existence of edges between vertices. The matrix was used as input for PAJEK (http://vlado.fmf.uni-lj.si/pub/networks/pajek/), a software application for large network analysis [2]. This allowed display of connectivity relationships among mesonetworks.

3 Results and Discussion

3.1 Algorithmic analysis of evolution of domain structure and organization

The patchy distribution of ancestries in enzymes of the metabolic networks of MANET [19] provides strong phylogenomic support to widespread enzymatic recruitment [31] in evolution of modern metabolism. Under a recruitment scenario and as enzymatic functions unfold [11], new and old domain structures fulfill biological functions either singly or in combination with other domains. It is likely that this process manifests in different lineages and at different levels as life diversifies and organisms adapt to various environments. In order to test this premise we explored collective recruitment patterns in organisms belonging to the three superkingdoms as first approximation to finding evolutionary patterns linked to metabolic networks of individual species.

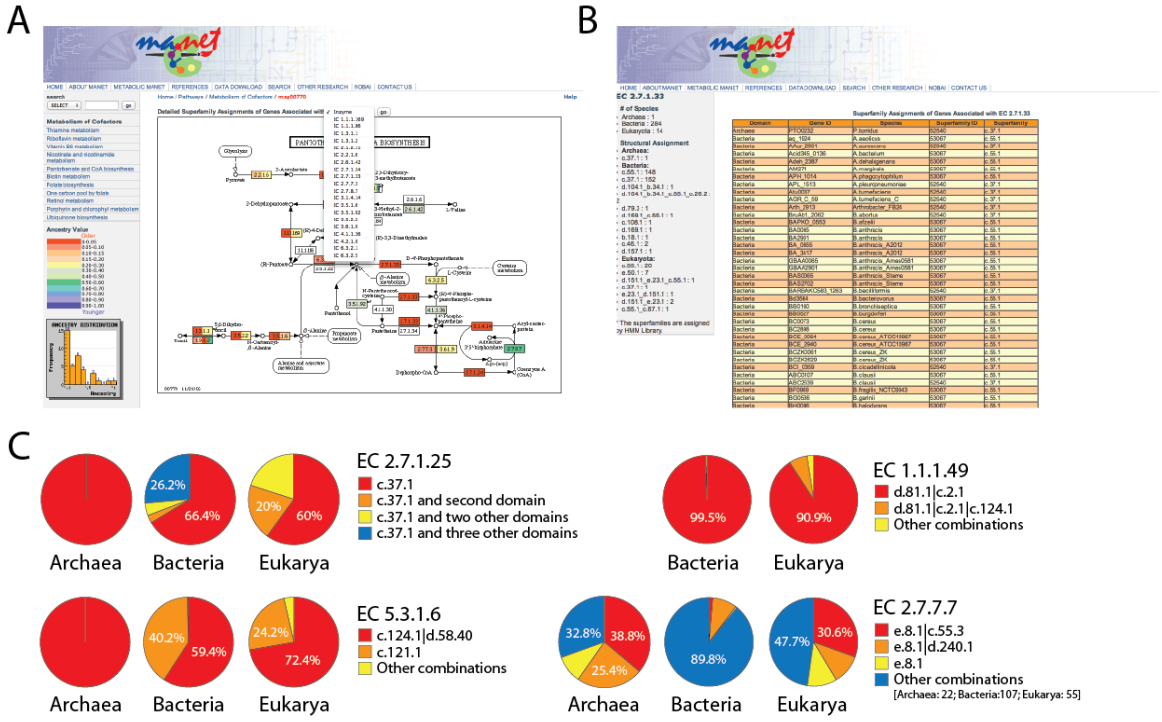

Figure 2: Analyzing enzymes in MANET. A. Subnetwork of MANET with pull-down menu. B. Structural annotations in species and related statistics obtained by clicking enzyme EC 2.7.1.33 in the subnetwork pull-down menu (only the top segment of the scroll-down list is shown). C. Examples of the enzymatic distribution of domain architectures in superkingdoms (see text).

In MANET, SCOP and KEGG database entries are mapped onto each other using knowledge from crystallographic structural models of the Protein Data Bank (PDB entries) and structural predictions [19]. However, this mapping does not dissect how structural annotations vary

among species. We therefore selected amino acid sequences of enzymes from the gene catalog file of KEGG and predicted FSF structures from these sequences with HMMs of structural recognition [13], keeping track of lineages and superkingdom distribution. We incorporated these results into the MANET database, building for each metabolic subnetwork pull-down menus that access structural annotations in individual organisms (Figure 2). We find that 1,163 enzymes in MANET had predicted structural assignments across surveyed organisms. Their architectures portray the diversity of domain structures and organization of enzymatic biological units that exists in organisms. For example:

(i) Several enzymes harbor a single domain and in few organisms domain combinations that include that domain. For example, the adenylyl-sulfate kinase enzyme (EC. 2.7.1.25) has in most organisms the P-loop containing nucleoside triphosphate hydrolase FSF (c.37.1) and in few cases several other domains that are combined with this structure (Figure 2). While all archaeal genomes contain only single-domain enzymes, 33.6% and 40% of bacterial and eukaryal species, respectively, contain multi-domain variants. More specifically, the enzyme of *Brucella anthracis* embeds only the c.37.1 FSF but a related species, *B. abortus*, holds the domain combination c.37.1|c.37.1|b.44.1|b.43.3 (verti-bars indicate domain linkers).

(ii) In other cases, enzymes carry out a same enzymatic activity with different domains in different lineages, sometimes in combination with one or more additional domains. For example, the function of ribose-5-phosphate isomerase (EC 5.3.1.6) generally involves the D-ribose-5-phosphate isomerase (RpiA)-lid domain (d.58.40) in organisms from the three superkingdoms (Figure 2). However, bacterial and eukaryal species sometimes use the ribose/galactose isomerase RpiB/AlsB motif (c.121.1) for the same enzymatic reaction.

(iii) Enzymes can be made almost exclusively of domain combinations. For example, the multi-domain glucose-6-phosphate dehydrogenase (EC 1.1.1.49) enzyme contains the glyceraldehyde-3-phosphate dehydrogenase-like C-terminal (d.81.1) and N-terminal (c.2.1) domains in the vast majority of Bacteria and Eukarya (Figure 2). The enzyme was absent in Archaea.

(iv) Enzyme makeup can be remarkably diverse and in some cases still provide a backbone of one or more domains present consistently in many organisms. For example, the complex DNA polymerase enzyme (EC 2.7.7.7) shares the DNA/RNA polymerase motif (e.8.1) and the ribonuclease H-like motif (c.55.3) in 217 species of Bacteria and Eukarya (Figure 2). However, the e.8.1|c.55.3 combination is present in 38.8%, 1.6% and 30.6% of archaeal, bacterial and eukaryotic species, respectively. However, these domains appear combined with many others in Bacteria in different arrangements.

In order to explore recruitment patterns in metabolic evolution, we developed a simple algorithm that derives the most plausible ancestry of an enzyme at FSF level of abstraction from domain structures that are most frequently found in organisms. We applied the algorithm to the analysis of the newly developed dataset. The analysis returned domain composition of enzymes, number of organisms associated with them, range of ancestries of domains, and the most plausible ancestry for each enzyme. This information can be retrieved with pull down menus in MANET. Essentially, the algorithm provides a 'corrected' upper-bound estimate of how old is an enzyme given domain structures and organization in each superkingdom. The age of enzymes reveal that the interplay between architectural discovery, recruitment, and replacement accounts for most patterns of metabolic evolution.

3.2 Timeline of enzyme evolution

The appearance of enzymes along a timeline that spans ~3.8 billion years of evolution (Figure 3) shows that enzyme accumulation was rapid at first (*nd* = 0-0.22 accounts for 50%

entries) but then decreased considerably, especially after *nd* = 0.6 (Figure 3A). This pattern matches the reported rapid accumulation of enzymatic activities that occurred very early in metabolic evolution, which was followed by a slowdown in structural and functional innovation defined using EC and SCOP definitions [5,7].

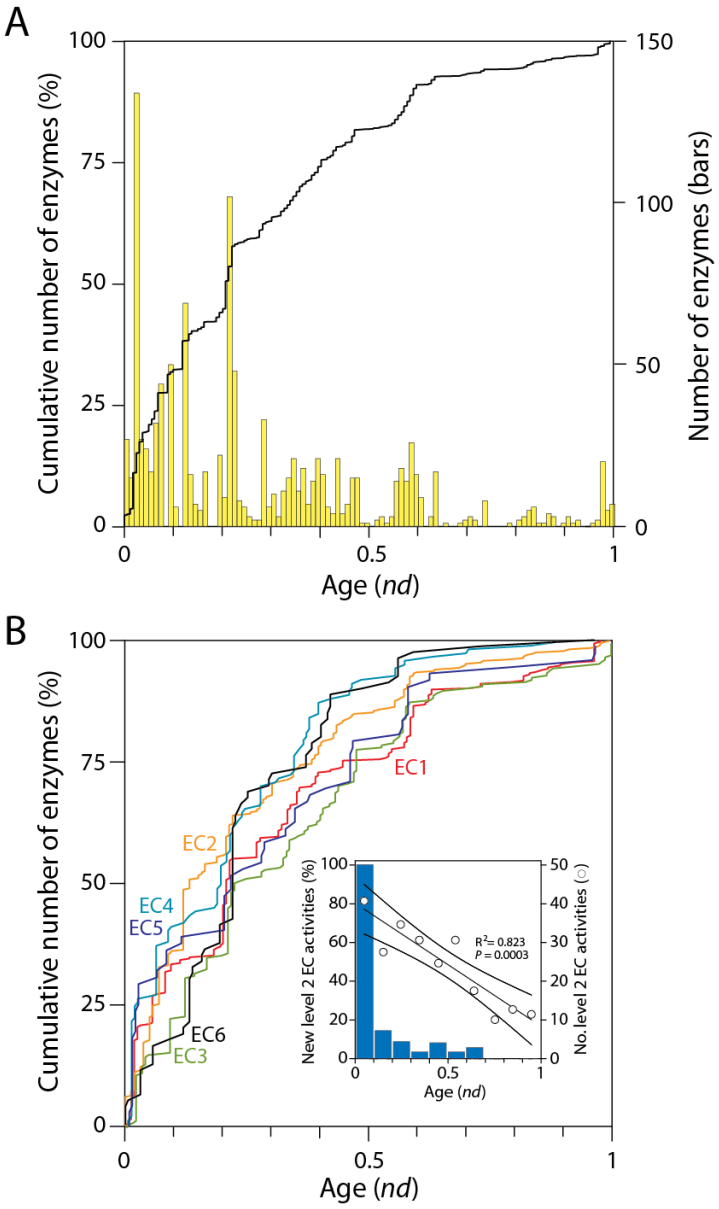

Figure 3: Timeline of enzyme evolution. A. Appearance (bar plot) and accumulation of enzymes in evolution. The age of enzymes was derived from ancestries of protein domain constituents (*nd*). These ancestries define enzyme age, with *nd* = 0 representing the origin of enzymes and *nd* = 1 the present. A molecular clock of folds shows the entire timeline spans ~3.8 billion years of evolution and that *nd* is linearly proportional to geological time [33]. B. Accumulation of enzymes belonging to major enzymatic classes defined by the first level of EC classification. The inset shows appearance of level 2 EC activities and percentage of those that are new.

There were however notable bursts of enzymatic innovation, especially early in protein evolution. The intensity of these bursts decreased with time. Dissection of enzyme accumulation in the 6 major enzymatic classes (first level of EC classification) show that enzymes in these groups appeared almost concurrently in evolution but accumulated at different pace (Figure 3B). The plot reveals the very early appearance of transferases (EC 2) and ligases (EC 6) (*nd* = 0.02) and a first major peak of enzyme discovery (*nd* = 0.02) mostly associated with oxidoreductases (EC 1), lyases (EC 4), and isomerases (EC 5). This was

followed by smaller bursts of oxidoreductases and transferases (*nd* = 0.9) and then transferases, ligases, and hydrolases (EC 3)(*nd* = 0.12), and later, by an additional and substantial burst of mostly hydrolases and ligases (*nd* = 0.21). As enzyme innovation decreased in time, there were later minor bursts involving mostly oxidoreductases, hydrolases and isomerases (*nd* = 0.58).

The early accumulation of enzymes in the timeline is consistent with the observation that most enzymatic activities appeared very early in time and were associated with the nine most ancient and widely distributed F domain structures [7]. In fact, the very early and massive appearance of transferases and ligases (Figure 3B) is congruent with bursts of enzymatic diversification of transferases transferring phosphorus-containing groups and ligases forming C-N bonds harboring the P-loop containing nucleoside triphosphate hydrolase F (c.37), the most ancient structure of the protein world [4,7]. Interestingly, many of the transferases with P-loop hydrolase folds were involved in interconversion, chemical energy storage and recycling, and terminal production of nucleotides and cofactors associated with purine and pyrimidine metabolism, which are point of origin of modern metabolism. The timelines also show that the diversity of enzymatic activities defined at different levels of EC classification decreased in time. For example, while the 243 level 2 (subclass) activities decreased monotonically with ancestry, only 53 of these were unique and most of these (77.4%) appeared at the beginning of the timeline (*nd* = 0-0.1) (Figure 3B, inset). This initial remarkable diversity can be explained by catalytic promiscuity of the primordial enzymes. Since substrate ambiguity in modern enzymes enhances the diversification potential of enzymes and chemistries [18], promiscuity most likely benefitted cooption of primitive enzymes into varying roles [26]. Indeed, most level 2 (subclass) activities (78%) were mostly drawn from the initial burst of enzymatic innovation and were used and reused throughout the timeline, suggesting old activities are pervasively recruited into younger proteins. Our findings are also compatible with computer simulations that show metabolic networks with highly specialized enzymes evolve from a few multifunctional enzymes [27]. Remarkably, the simulations show group transfer reactions were fundamental for the emergence of hubs in metabolic networks.

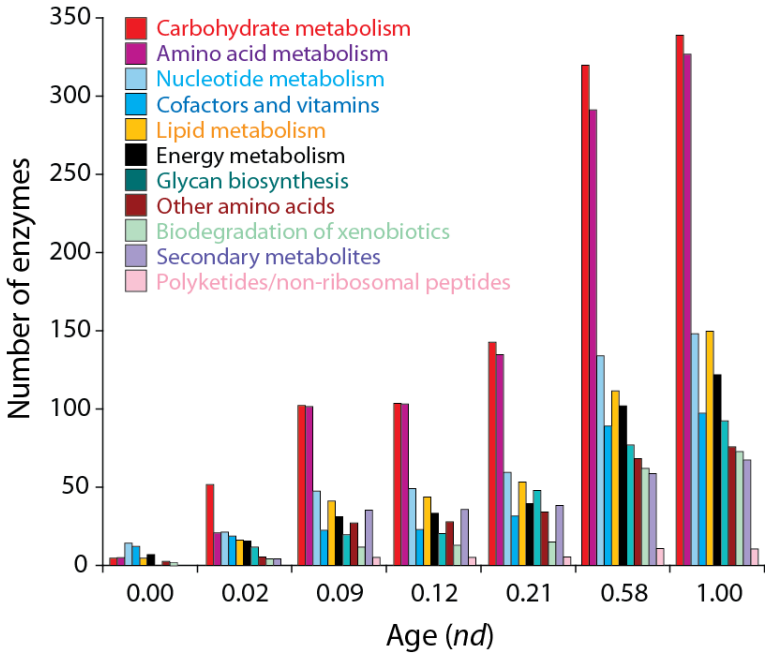

Figure 4: Evolutionary accumulation of enzymes in mesonetworks of KEGG. The plot shows the cumulative number of core mesonetwork enzymes appearing in the timeline, with age suitably binned in *nd* intervals.

3.3 Build-up and sharing of enzymatic activities in metabolic evolution

Timelines describing the evolution of metabolic networks, suitably binned in *nd* intervals that highlight the initial burst of enzymatic diversity (Figure 3), showed an early build-up of metabolic activities of metabolism of nucleotides, cofactors, and vitamins, followed by enzymes involved in carbohydrate and amino acid metabolism (Figure 4). A graph approach helped visualize enzymatic build up and global pathways of enzymatic recruitment in the 11 mesonetworks of KEGG (Figure 5). Mesonetworks group subnetworks with pathways that are functionally related. These distinct units of metabolic function include metabolisms of carbohydrates (CAR), energy (NRG), lipids (LIP), nucleotides (NUC), amino acids (AAC), other amino acids (AA2), cofactors and vitamins (COF), glycans (GLY), and secondary metabolites (SEC), the biosynthesis of polyketides and nonribosomal peptides (POL), and the biodegradation of xenobiotics (XEN). In the graphs, mesonetworks are represented as vertices (nodes) and enzymes shared between them as edges, with sizes of nodes and edges proportional to number and sharing of enzymes, respectively. Changes in node size show the same patterns of enzymatic expansion described in previous figures but atomized into metabolic mesonetworks. For example, if EC 1.1.1.1 belongs to CAR, LIP, and AAC, edges are placed connecting nodes representing these three mesonetworks. We found that 249 out of the 1,163 enzymes that had structural assignments were shared between the mesonetworks of MANET. However, clear historical patterns of recruitment were only evident when enzyme sharing was displayed along the time series. Recruitment (graph connectivity) was proportional to enzyme appearance during the first half of enzyme history (data not shown). Initial connectivity was restricted to NUC, NRG and COF, which were also the most populated mesonetworks (Figure 5, *nd* = 0). They donated enzymes to AAC, AA2, LIP, XEN and CAR. This supports the early origin of NUC and COF recruitment gateways inferred previously using a domain-centric phylogenomic approach [5,7]. CAR, AAC, LIP and COF became initial hubs very quickly in evolution, with CAR dominating connectivity at *nd* = 0.02. While the primacy of CAR continues throughout the timeline, AAC became the primary hub at *nd* = 0.09 and remained so until the present, both in number and sharing of enzymes. During this time we note: (i) the marked connectivity between AAC and SEC, which coincides with the development of the non-ribosomal protein biosynthesis and translation machineries that makes use of metabolites of these mesonetworks [8], and (ii) important recruitment patterns involving NUC responsible for fully functional nucleotide biosynthesis pathways, which coincide with the rise of the ribosomal machinery [9]. Most patterns of enzyme sharing were established at *nd* = 0.58, and these patterns did not change much in the second half of enzyme history, matching the metabolic slow down in structural and functional innovation described earlier (Figure 3). Today, patterns of sharing are clearly dominated by subnetworks in AAC, CAR, LIP and NRG (Figure 5). These patterns are made evident in a graph that shows enzyme recruitments patterns between subnetworks, which show the tight connection of AAC, CAR and NRG that unfolds in the timeline already at *nd* = 0.02 (see Figure 3 in ref. [7]).

The progressions of enzyme innovation and sharing are consistent with the conclusions of a study of similarities of F distribution in subnetworks that describes subnetworks as vectors in a space of F abundance [7]. Results also match the proposal that an initial energy amphiphile shell of prebiotic pathways preceded the rise of amino acid pathways [22]. Thus, enzyme growth in metabolic networks appears a palimpsest of archaic prebiotic metabolic cycles that were slowly replaced by modern biochemistries. This scenario is compatible with a recent evolutionary study of purine metabolic pathways that shows that the nucleotide interconversion pathway benefited most parsimoniously from the prebiotic formation of adenine nucleotides and that pathways of nucleotide biosynthesis, catabolism and salvage developed much later through concerted enzymatic recruitments [9].

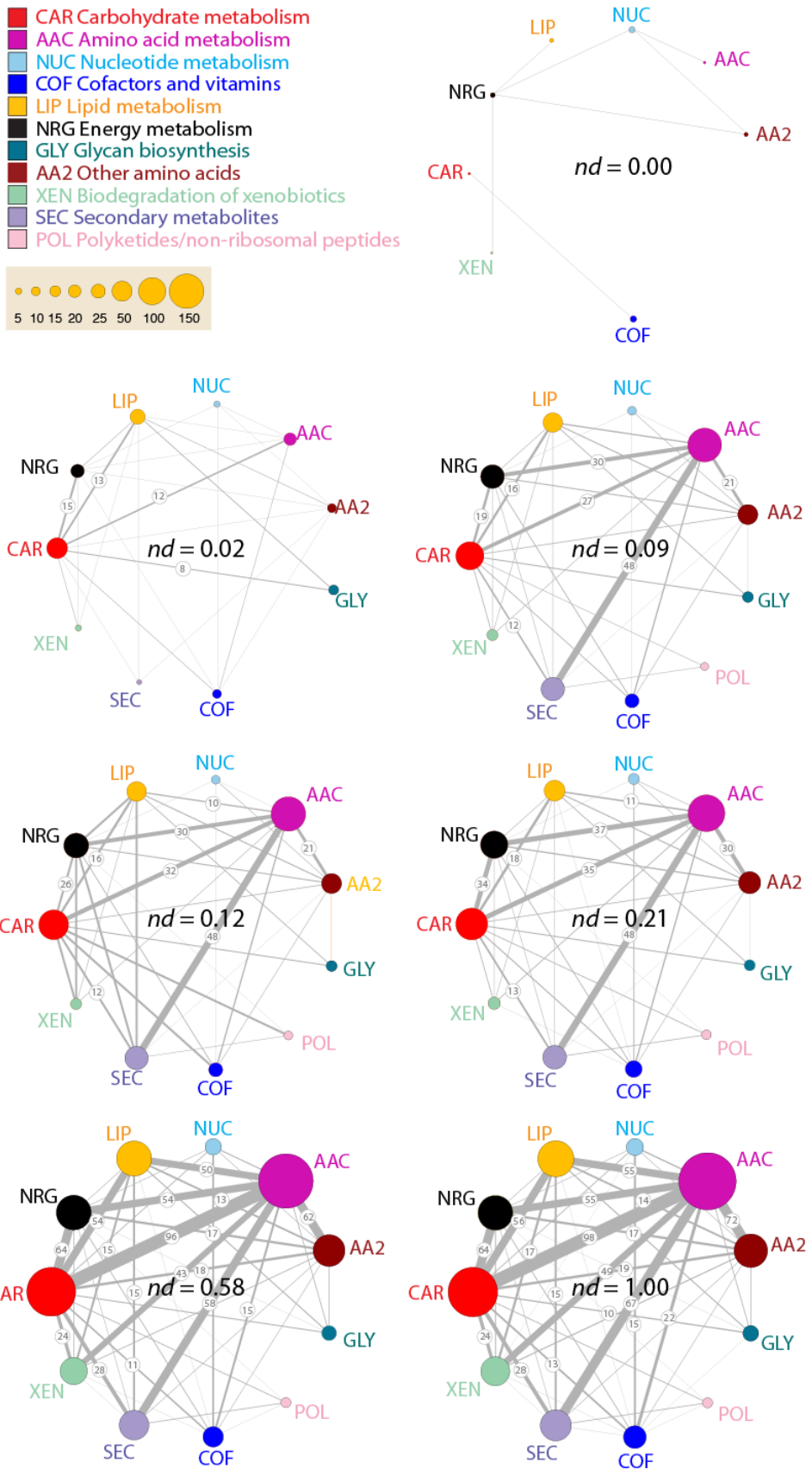

Figure 5: Graph representations describing the evolutionary build-up and sharing of enzymatic activities in metabolic evolution. The area of vertices is proportional to the number of enzymes and the width of edges is proportional to the number of shared enzymatic activities between mesonetworks. Consequently, the graphs display both the evolutionary accumulation of enzymes (see Figures 3 and 4) and the build-up of patterns of recruitments between mesonetwork.

3.4 Sharing of enzymes among superkingdoms

Enzymes and their domains are not equally distributed in the proteomes of Archaea (A), Bacteria (B) and Eukarya (E). Enzymes exist that are uniquely present (groups A, B or E) or are shared by two (BE, AB, or AE) or all superkingdoms (ABE). These patterns are also observed in domains (e.g. [21,32]) and molecular functions derived from (GO) annotations [20](A. Nasir, K.M. Kim and G. Caetano-Anollés, ms. submitted) and their biases illustrate the evolutionary history of superkingdoms. Venn diagrams describe the distribution of the enzymes and domains of this study in these taxonomic groups (Figure 6). Superkingdoms share most enzymes (61%) and Fs (91%), suggesting the existence of an ancient enzymatic core made of universal domain structures. A recent study has made this suggestion explicit for enzymes shared by organisms, pathways and structures [33]. The ancient core is also supported by the ancestrality of most enzymes in this study. The ABE group is the most populated with 393 enzymes and 506 F structures. The BE group is the second largest with 208 enzymes and 176 Fs. The Bacteria-specific (B) and Eukarya-specific (E) groups are the third largest in terms of enzymes (256) and Fs (52), respectively, showcasing domain combination in bacterial enzymes and domain innovation in Eukarya. Similar evolutionary patterns have been observed in global phylogenomic studies of FSF and FF domains and their corresponding molecular functions [6,20,21,32].

Since domain make up in enzymatic taxonomic groups is specific to each organism, and collectively, specific to each superkingdom, we plotted the age of enzymes of each superkingdom for individual taxonomic groups (Figure 6). Cumulative and scatter plots of ancestries consistently reveal that archaeal enzymes are older than those of Bacteria, and Eukarya, in that order. This pattern is consistent with the evolutionary origin of superkingdoms and the very early appearance of archaeal microbes made explicit by the evolutionary analysis of FF domain structures [21]. Reference (black) curves in cumulative plots also show that enzyme age is on average substantially older than the age of F structures of corresponding taxonomic groups (Figure 6). In itself, this observation strongly supports the pervasive cooption of ancient domains in new enzymes.

Scatter plots show that domain organization in superkingdoms did not affect the age of most enzymes (Figure 6). They fell in straight lines with *nd* values equal for individual superkingdoms. However, many others scattered throughout ancestry space, demonstrating idiosyncratic recruitment histories. Enzymes accumulated continuously until an ancestry of *nd* = 0.6-0.7 was reached. After this point, enzymatic innovation and recruitments decreased considerably. It is clear that most of the catalytic toolkit was developed in the first half of enzymatic history. The only exception is the Eukarya-specific enzymes (E), which accumulate throughout evolution. This is probably due to larger genomes and the evolutionary development of a larger set of Eukarya-specific F architectures, which continue to unfold novel enzymatic diversity and new schemes of domain organization until the present (*nd* = 1) [32].

4 Conclusions

The atomic structure of domains and the organization of domains in proteins carry significant historical information that we here mine in enzymes of metabolic networks. We note that the conservation of domain structure and sequence is not necessarily correlated. This is because sequences saturate quickly by accumulation of mutations and this process erases ancient historical information [6]. With the exception of selected sequence motifs that are constrained by structure and function, mutational saturation is pervasive and generates problems when reconstructing phylogenetic relationships of gene sequences that are historically deep.

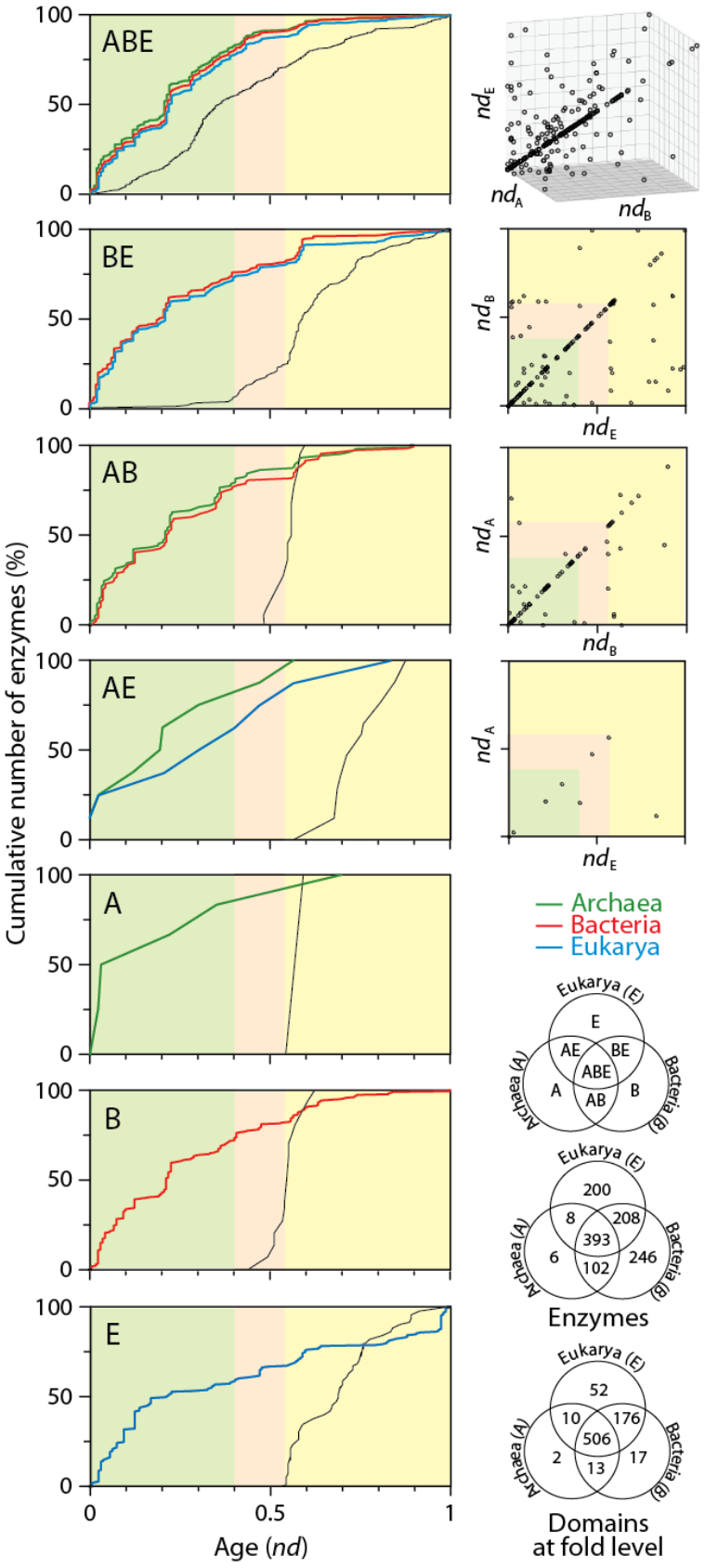

Figure 6: Timelines of enzyme evolution. Cumulative (left) and scatter (right) plots of ancestries are provided for each taxonomic group of enzymes described in the text. Reference curves in black show the accumulation of F domain structures for each taxonomic group. Venn diagrams describe the distribution of enzymes and domains in superkingdoms.

In contrast, conservation of molecular structure involves higher order features of structure and function that carry durable historical signatures [6]. These features include fold topologies (such as those defined in SCOP [1,23]) or their associated molecular functions (such as those defined by the GO hierarchy [20]). In particular, the structural cores of proteins are generally orders of magnitude more conserved than corresponding sequences and act as 'living fossils' for phylogenomic reconstruction.

Here we developed an algorithm that derives the most plausible ancestry of an enzyme from history in the structure and organization of protein domains. The algorithm places enzymes in an evolutionary timeline and is implemented in the MANET database [19]. Timelines of enzyme appearance revealed the evolutionary build-up of metabolic networks. This build-up was massive very early in protein history and supported a proposed metabolic 'big bang' responsible for major gateways of enzymatic recruitment [5,7]. Remarkably, we find that structural and functional innovation slowed down in evolution in a process driven by the pervasive cooption of domain structures that were recruited to perform more modern enzymatic tasks. This process occurred differentially in lineages of the superkingdoms of life. This global tendency of cooption of domains from core metabolic pathways is probably responsible for the structural and historical patchy distribution of enzymes in metabolic networks [7,19,31] that is also observed in the most ancient metabolic pathways [9].

Acknowledgements

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References

[1] A. Andreeva, D. Howorth, J.-M. Chandonia, S.E. Brenner, T.J.P. Hubbard, C. Chothia and A.G. Murzin. Data growth and its impact on the SCOP database: new developments. *Nucleic Acids Research,* 36:D419-D425, 2008.

[2] V. Batagelj and A. Mrvar. Analysis and visualization of large networks. In M. Jünger and P. Mutzel (editors), *Graph Drawing Software,* pp. 77-103. Springer, Berlin, 2003.

[3] R. Braakman and E. Smith, The compositional and evolutionary logic of metabolism. *Physical Biology,* 10:011001, 2013.

[4] G. Caetano-Anollés and D. Caetano-Anollés. An evolutionarily structured universe of protein architecture. *Genome Research,* 13:1563-1571, 2003.

[5] G. Caetano-Anollés, H.S. Kim and J.E. Mittenthal, The origins of modern metabolic networks inferred from phylogenomic analysis of protein structure. *Proceedings of the National Academy of Sciences USA,* 104:9358-9363 (2007).

[6] G. Caetano-Anollés, M. Wang, D. Caetano-Anollés and J.E. Mittenthal. The origin, evolution and structure of the protein world. *Biochemical Journal,* 417:621-637, 2009.

[7] G. Caetano-Anollés, L.S. Yafremava, H. Gee, D. Caetano-Anollés and J.E. Mittenthal. The origin and evolution of modern metabolism. *International Journal of Biochemistry and Cell Biology,* 41:285-297, 2009.

[8] G. Caetano-Anollés, K.M. Kim and D. Caetano-Anollés. The phylogenomic roots of modern biochemistry: Origins of proteins, cofactors and protein biosynthesis. *Journal of Molecular Evolution,* 74:1-34, 2012.

[9] K. Caetano-Anollés and G. Caetano-Anollés. Structural phylogenomics reveals gradual evolutionary replacement of abiotic chemistries by protein enzymes in purine metabolism. *PLoS ONE*. (in press)
[10] A.-R. Carvunis, R. Rolland, I. Wapinski, M.A. Calderwood, M.A. Yildirim, N. Simonis, B. Charloteaux, C.A. Hidalgo, J. Barbette, B. Santhanam, G.A. Brar, J.S. Weissman, A. Regev, N. Thierry-Mieg, M.E. Cusick and M. Vidal. Proto-genes and *de novo* gene birth. *Nature*, 487:370-374, 2012.
[11] N. Furnham, I. Sillitoe, G.L. Holliday, A.L. Cuff, R.A. Laskowski, C.A. Orengo and J.M. Thornton. Exploring the evolution of novel enzyme functions within structurally defined protein superfamilies. *PLoS Computational Biology*, 8:e1002403, 2012.
[12] A.D. Goldman, J.A. Baross and R. Samudrala. The enzymatic and metabolic capabilities of early life. *PLoS ONE*, 7:e39912, 2012.
[13] J. Gough, K. Karplus, R. Hughey and C. Chothia. Assignment of homology to genome sequences using a library of hidden Markov models that represent all proteins of known structure. *Journal of Molecular Biology*, 31:903-919, 2001.
[14] S.J. Gould and E.S. Vrba. Exaptation – a missing term in the science of form. *Paleobiology*, 8:4-15. 1982.
[15] J. Janin and S.J. Wodak. Structural domains in proteins and their role in the dynamics of protein function. *Progress in Biophysics and Molecular Biology*, 42:21-78, 1983.
[16] R.A. Jensen. Enzyme recruitment in evolution of new function. *Annual Review of Microbiology*, 30:409–25, 1976.
[17] M. Kanehisa, S. Goto, Y. Sato, M. Furumichi and M. Tanabe. KEGG for integration and interpretation of large-scale molecular datasets. *Nucleic Acids Research*, 40:D109-D114, 2012.
[18] O. Khersonsky and D.S. Tawfik. Enzyme promiscuity: A mechanistic and evolutionary perspective. *Annual Review of Biochemistry*, 79:11.1-11.35, 2010.
[19] H.S. Kim, J.E. Mittenthal and G. Caetano-Anollés. MANET: tracing evolution of protein architecture in metabolic networks. *BMC Bioinformatics*, 7:351, 2006.
[20] K.M. Kim and G. Caetano-Anollés. Emergence and evolution of modern molecular functions inferred from phylogenomic analysis of ontological data. *Molecular Biology and Evolution*, 27:1710-1733, 2010.
[21] K.M. Kim and G. Caetano-Anollés. The evolutionary history of protein fold families and proteomes confirms that the archaeal ancestor is more ancient than the ancestors of other superkingdoms. *BMC Evolutionary Biology*, 12:13, 2013.
[22] H. Morowitz. A theory of biochemical organization, metabolic pathways, and evolution. *Complexity*, 4:39-53, 1999.
[23] A. Murzin, S.E. Brenner, T. Hubbar and C. Clothia. SCOP: a structural classification of proteins for the investigation of sequences and structures. *Journal of Molecular Biology*, 247:536-540, 1995.
[24] J.M. Peregrín-Alvarez, S. Tsoka and C.A. Ouzounis. The phylogenetic extent of metabolic enzymes and pathways. *Genome Research*, 13:422-427, 2003.
[25] J.M. Peregrín-Alvarez, C. Sanford and J. Parkinson. The conservation and evolutionary modularity of metabolism. *Genome Biology*, 10:R63, 2009.
[26] J. Pereto. Out of fuzzy chemistry: from prebiotic chemistry to metabolic networks. *Chemical Society Reviews*, 41:5394-5403, 2012.
[27] T. Pfeiffer, O.S. Soyer and S. Bonhoeffer. The evolution of connectivity in metabolic networks. *PLoS Biology*, 3:1269–75, 2005.
[28] J.S. Richardson. The anatomy and taxonomy of protein structure, *Advances in Protein Chemistry*, 34:167-339, 1981.

[29] M. Riley and B. Labedan. Protein evolution viewed through *Escherichia coli* protein sequences: Introducing the notion of a structural segment of homology, the module. *Journal of Molecular Biology*, 268:857-868, 1997.
[30] E. Smith and H.J. Morowitz. Universality in intermediate metabolism. *Proceedings of the National Academy of Sciences USA*, 101:13168-13173, 2004.
[31] S.A. Teichmann, S.C.G. Rison, J.M. Thornton, M. Riley, J. Gough and C. Chothia. Small-molecule metabolism: an enzyme mosaic. *Trends in Biotechnology,* 19:482-486, 2001.
[32] M. Wang and G. Caetano-Anollés, The evolutionary mechanics of domain organization in proteomes and the rise of modularity in the protein world. *Structure*, 17:66-78, 2009.
[33] M. Wang, Y.-Y. Jiang, K.M. Kim, G. Qu, H-F. Ji, H-Y. Zhang and G. Caetano-Anollés. A molecular clock of protein folds and its power in tracing the early history of aerobic metabolism and planet oxygenation. *Molecular Biology and Evolution,* 28:567-582, 2011.
[34] D.B. Wetlaufer. Nucleation, rapid folding, and globular intrachain regions in proteins. *Proceedings of the National Academy of Sciences USA*, 70:697-701, 1973.

Supplementary Data

EC number	Age (nd)
EC 1.1.1.1	0.220
EC 1.1.1.100	0.025
EC 1.1.1.102	0.025
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EC 1.5.1.7	0.207
EC 1.5.1.8	0.207

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EC 1.6.2.2	0.578	EC 2.3.1.12	0.584	EC 2.4.1.38	0.119	EC 2.6.1.57	0.069
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EC 2.1.1.2	0.050	EC 2.4.1.101	0.119	EC 2.5.1.18	0.584	EC 2.7.1.4	0.207
EC 2.1.1.20	0.050	EC 2.4.1.11	0.119	EC 2.5.1.19	0.257	EC 2.7.1.40	0.446
EC 2.1.1.28	0.050	EC 2.4.1.117	0.119	EC 2.5.1.2	0.031	EC 2.7.1.41	0.119
EC 2.1.1.37	0.050	EC 2.4.1.12	0.119	EC 2.5.1.21	0.301	EC 2.7.1.45	0.207
EC 2.1.1.4	0.050	EC 2.4.1.122	0.981	EC 2.5.1.26	0.339	EC 2.7.1.47	0.037
EC 2.1.1.43	0.433	EC 2.4.1.129	0.553	EC 2.5.1.29	0.301	EC 2.7.1.49	0.207
EC 2.1.1.45	0.522	EC 2.4.1.13	0.119	EC 2.5.1.3	0.018	EC 2.7.1.5	0.037
EC 2.1.1.49	0.050	EC 2.4.1.130	0.037	EC 2.5.1.30	0.301	EC 2.7.1.50	0.207
EC 2.1.1.5	0.018	EC 2.4.1.132	0.119	EC 2.5.1.31	0.396	EC 2.7.1.51	0.037
EC 2.1.1.53	0.050	EC 2.4.1.133	0.119	EC 2.5.1.32	0.301	EC 2.7.1.52	0.132
EC 2.1.1.6	0.050	EC 2.4.1.134	0.088	EC 2.5.1.33	0.301	EC 2.7.1.53	0.037
EC 2.1.1.64	0.050	EC 2.4.1.135	0.119	EC 2.5.1.44	0.207	EC 2.7.1.56	0.207
EC 2.1.1.71	0.050	EC 2.4.1.14	0.119	EC 2.5.1.48	0.069	EC 2.7.1.58	0.037
EC 2.1.1.76	0.050	EC 2.4.1.142	0.119	EC 2.5.1.49	0.069	EC 2.7.1.59	0.037
EC 2.1.1.8	0.050	EC 2.4.1.143	0.836	EC 2.5.1.54	0.018	EC 2.7.1.60	0.037
EC 2.1.1.86	0.018	EC 2.4.1.144	0.911	EC 2.5.1.56	0.433	EC 2.7.1.63	0.037
EC 2.1.2.1	0.069	EC 2.4.1.145	0.408	EC 2.5.1.6	0.433	EC 2.7.1.66	0.698
EC 2.1.2.10	0.402	EC 2.4.1.149	0.119	EC 2.5.1.61	0.421	EC 2.7.1.67	0.163
EC 2.1.2.11	0.018	EC 2.4.1.15	0.119	EC 2.5.1.7	0.257	EC 2.7.1.68	0.402
EC 2.1.2.2	0.352	EC 2.4.1.152	0.069	EC 2.5.1.9	0.433	EC 2.7.1.69	0.358
EC 2.1.2.3	0.427	EC 2.4.1.157	0.119	EC 2.6.1.1	0.069	EC 2.7.1.71	0.000
EC 2.1.2.5	0.012	EC 2.4.1.16	0.452	EC 2.6.1.11	0.069	EC 2.7.1.73	0.207
EC 2.1.2.9	0.572	EC 2.4.1.17	0.119	EC 2.6.1.13	0.069	EC 2.7.1.74	0.000
EC 2.1.3.1	0.018	EC 2.4.1.18	0.572	EC 2.6.1.16	0.283	EC 2.7.1.76	0.000
EC 2.1.3.2	0.289	EC 2.4.1.182	0.119	EC 2.6.1.17	0.069	EC 2.7.1.82	0.163
EC 2.1.3.3	0.289	EC 2.4.1.20	0.465	EC 2.6.1.18	0.069	EC 2.7.1.90	0.509
EC 2.1.4.1	0.440	EC 2.4.1.21	0.119	EC 2.6.1.2	0.069	EC 2.7.1.91	0.119

EC 2.7.2.1	0.037	EC 3.1.1.32	0.622	EC 3.2.1.31	0.993	EC 3.5.3.11	0.566
EC 2.7.2.11	0.308	EC 3.1.1.34	0.874	EC 3.2.1.33	0.465	EC 3.5.3.13	0.207
EC 2.7.2.2	0.276	EC 3.1.1.4	0.635	EC 3.2.1.39	0.018	EC 3.5.3.19	0.088
EC 2.7.2.3	0.459	EC 3.1.1.41	0.088	EC 3.2.1.4	0.119	EC 3.5.3.3	0.402
EC 2.7.2.4	0.276	EC 3.1.1.45	0.088	EC 3.2.1.45	0.018	EC 3.5.3.8	0.566
EC 2.7.2.7	0.037	EC 3.1.1.47	0.031	EC 3.2.1.46	0.018	EC 3.5.4.1	0.207
EC 2.7.2.8	0.276	EC 3.1.1.5	0.088	EC 3.2.1.48	0.817	EC 3.5.4.10	0.427
EC 2.7.3.2	0.817	EC 3.1.1.7	0.088	EC 3.2.1.49	0.572	EC 3.5.4.12	0.427
EC 2.7.3.3	0.817	EC 3.1.2.1	0.213	EC 3.2.1.50	0.018	EC 3.5.4.13	0.433
EC 2.7.4.1	0.427	EC 3.1.2.12	0.088	EC 3.2.1.52	0.018	EC 3.5.4.16	0.314
EC 2.7.4.10	0.150	EC 3.1.2.14	0.213	EC 3.2.1.54	0.018	EC 3.5.4.19	0.867
EC 2.7.4.14	0.000	EC 3.1.2.23	0.213	EC 3.2.1.58	0.018	EC 3.5.4.2	0.427
EC 2.7.4.16	0.301	EC 3.1.2.4	0.220	EC 3.2.1.65	0.471	EC 3.5.4.21	0.207
EC 2.7.4.2	0.132	EC 3.1.2.6	0.138	EC 3.2.1.67	0.320	EC 3.5.4.25	0.345
EC 2.7.4.3	0.150	EC 3.1.3.1	0.207	EC 3.2.1.76	0.018	EC 3.5.4.26	0.427
EC 2.7.4.6	0.012	EC 3.1.3.11	0.333	EC 3.2.1.78	0.018	EC 3.5.4.27	0.691
EC 2.7.4.7	0.207	EC 3.1.3.12	0.119	EC 3.2.1.80	0.993	EC 3.5.4.3	0.207
EC 2.7.4.8	0.000	EC 3.1.3.13	0.471	EC 3.2.1.84	0.018	EC 3.5.4.4	0.018
EC 2.7.4.9	0.000	EC 3.1.3.15	0.276	EC 3.2.1.85	0.018	EC 3.5.4.5	0.427
EC 2.7.6.1	0.213	EC 3.1.3.18	0.119	EC 3.2.1.86	0.018	EC 3.5.4.6	0.018
EC 2.7.6.2	0.352	EC 3.1.3.2	0.433	EC 3.2.1.91	0.993	EC 3.5.4.9	0.276
EC 2.7.6.3	0.012	EC 3.1.3.25	0.333	EC 3.2.1.93	0.572	EC 3.5.5.1	0.327
EC 2.7.6.5	0.433	EC 3.1.3.26	0.471	EC 3.2.1.96	0.559	EC 3.5.5.7	0.327
EC 2.7.7.1	0.056	EC 3.1.3.27	0.566	EC 3.2.2.1	0.628	EC 3.5.99.3	0.207
EC 2.7.7.10	0.396	EC 3.1.3.29	0.119	EC 3.2.2.16	0.119	EC 3.5.99.6	0.283
EC 2.7.7.12	0.396	EC 3.1.3.3	0.119	EC 3.2.2.3	0.628	EC 3.5.99.7	0.194
EC 2.7.7.13	0.119	EC 3.1.3.37	0.333	EC 3.2.2.4	0.119	EC 3.6.1.1	0.044
EC 2.7.7.14	0.056	EC 3.1.3.4	0.566	EC 3.2.2.5	0.729	EC 3.6.1.11	0.037
EC 2.7.7.15	0.056	EC 3.1.3.45	0.119	EC 3.2.2.9	0.119	EC 3.6.1.13	0.213
EC 2.7.7.18	0.056	EC 3.1.3.46	0.471	EC 3.3.1.1	0.031	EC 3.6.1.14	0.245
EC 2.7.7.2	0.132	EC 3.1.3.5	0.578	EC 3.3.2.1	0.389	EC 3.6.1.15	0.220
EC 2.7.7.22	0.119	EC 3.1.3.56	0.572	EC 3.3.2.3	0.088	EC 3.6.1.17	0.396
EC 2.7.7.23	0.188	EC 3.1.3.57	0.333	EC 3.3.2.6	0.547	EC 3.6.1.22	0.213
EC 2.7.7.24	0.119	EC 3.1.3.66	0.974	EC 3.4.11.2	0.547	EC 3.6.1.23	0.433
EC 2.7.7.27	0.119	EC 3.1.3.67	0.981	EC 3.4.11.5	0.088	EC 3.6.1.26	0.396
EC 2.7.7.3	0.056	EC 3.1.3.7	0.333	EC 3.4.13.20	0.119	EC 3.6.1.29	0.396
EC 2.7.7.30	0.119	EC 3.1.3.73	0.471	EC 3.4.13.3	0.119	EC 3.6.1.3	0.000
EC 2.7.7.33	0.119	EC 3.1.3.8	0.471	EC 3.4.19.9	0.031	EC 3.6.1.31	0.213
EC 2.7.7.38	0.119	EC 3.1.3.9	0.566	EC 3.4.21.89	0.320	EC 3.6.1.40	0.037
EC 2.7.7.39	0.056	EC 3.1.4.11	0.981	EC 3.4.23.36	0.553	EC 3.6.1.41	0.138
EC 2.7.7.4	0.056	EC 3.1.4.12	0.572	EC 3.4.23.43	0.647	EC 3.6.1.6	0.163
EC 2.7.7.41	0.823	EC 3.1.4.14	0.031	EC 3.4.25.1	0.163	EC 3.6.1.7	0.012
EC 2.7.7.43	0.119	EC 3.1.4.16	0.578	EC 3.5.1.1	0.415	EC 3.6.1.8	0.213
EC 2.7.7.5	0.396	EC 3.1.4.17	0.830	EC 3.5.1.10	0.352	EC 3.6.1.9	0.207
EC 2.7.7.53	0.396	EC 3.1.4.3	0.207	EC 3.5.1.11	0.163	EC 3.6.3.10	0.924
EC 2.7.7.58	0.220	EC 3.1.4.4	0.968	EC 3.5.1.12	0.327	EC 3.6.3.14	0.452
EC 2.7.7.6	0.383	EC 3.1.4.46	0.018	EC 3.5.1.15	0.119	EC 3.6.3.6	0.471
EC 2.7.7.62	0.000	EC 3.1.5.1	0.830	EC 3.5.1.16	0.119	EC 3.6.4.1	0.000
EC 2.7.7.7	0.037	EC 3.1.6.1	0.207	EC 3.5.1.18	0.119	EC 3.7.1.2	0.402
EC 2.7.7.8	0.446	EC 3.1.6.12	0.207	EC 3.5.1.19	0.389	EC 3.7.1.5	0.402
EC 2.7.7.9	0.119	EC 3.1.6.13	0.207	EC 3.5.1.2	0.220	EC 3.8.1.3	0.119
EC 2.7.8.1	0.044	EC 3.1.6.14	0.207	EC 3.5.1.23	0.163	EC 3.8.1.5	0.088
EC 2.7.8.13	0.213	EC 3.1.6.4	0.207	EC 3.5.1.24	0.163	EC 3.8.1.8	0.207
EC 2.7.8.15	0.591	EC 3.1.6.8	0.207	EC 3.5.1.25	0.207	EC 4.1.1.1	0.201
EC 2.7.8.20	0.465	EC 3.1.8.1	0.471	EC 3.5.1.26	0.163	EC 4.1.1.11	0.402
EC 2.7.8.26	0.433	EC 3.11.1.1	0.119	EC 3.5.1.27	0.522	EC 4.1.1.15	0.069
EC 2.7.8.3	0.566	EC 3.2.1.1	0.572	EC 3.5.1.28	0.559	EC 4.1.1.17	0.245
EC 2.7.8.5	0.427	EC 3.2.1.10	0.572	EC 3.5.1.31	0.522	EC 4.1.1.19	0.018
EC 2.7.8.7	0.572	EC 3.2.1.106	0.465	EC 3.5.1.32	0.119	EC 4.1.1.20	0.245
EC 2.7.8.8	0.427	EC 3.2.1.108	0.018	EC 3.5.1.38	0.415	EC 4.1.1.21	0.031
EC 2.7.9.1	0.150	EC 3.2.1.113	0.465	EC 3.5.1.4	0.371	EC 4.1.1.22	0.069
EC 2.7.9.2	0.119	EC 3.2.1.114	0.861	EC 3.5.1.41	0.276	EC 4.1.1.23	0.018
EC 2.7.9.3	0.301	EC 3.2.1.122	0.364	EC 3.5.1.49	0.150	EC 4.1.1.28	0.069
EC 2.8.2.11	0.000	EC 3.2.1.132	0.232	EC 3.5.1.5	0.207	EC 4.1.1.29	0.069
EC 2.8.2.2	0.000	EC 3.2.1.14	0.018	EC 3.5.1.54	0.371	EC 4.1.1.3	0.371
EC 2.8.2.23	0.000	EC 3.2.1.147	0.018	EC 3.5.1.56	0.993	EC 4.1.1.31	0.018
EC 2.8.2.4	0.000	EC 3.2.1.15	0.320	EC 3.5.1.68	0.119	EC 4.1.1.32	0.578
EC 2.8.4.1	0.698	EC 3.2.1.18	0.471	EC 3.5.1.78	0.729	EC 4.1.1.33	0.132
EC 2.9.1.1	0.069	EC 3.2.1.2	0.018	EC 3.5.1.9	0.088	EC 4.1.1.36	0.371
EC 3.1.1.1	0.088	EC 3.2.1.20	0.018	EC 3.5.2.14	0.037	EC 4.1.1.37	0.018
EC 3.1.1.11	0.320	EC 3.2.1.21	0.018	EC 3.5.2.2	0.207	EC 4.1.1.39	0.018
EC 3.1.1.13	0.088	EC 3.2.1.22	0.364	EC 3.5.2.3	0.207	EC 4.1.1.4	0.018
EC 3.1.1.17	0.471	EC 3.2.1.23	0.993	EC 3.5.2.5	0.207	EC 4.1.1.41	0.094
EC 3.1.1.22	0.088	EC 3.2.1.24	0.861	EC 3.5.2.6	0.220	EC 4.1.1.44	0.635
EC 3.1.1.23	0.088	EC 3.2.1.25	0.993	EC 3.5.2.7	0.207	EC 4.1.1.45	0.018
EC 3.1.1.24	0.088	EC 3.2.1.28	0.465	EC 3.5.2.9	0.037	EC 4.1.1.47	0.201
EC 3.1.1.3	0.088	EC 3.2.1.3	0.465	EC 3.5.3.1	0.566	EC 4.1.1.48	0.018

EC 4.1.1.49	0.578	EC 4.2.3.19	0.465	EC 5.4.2.2	0.207	EC 6.3.4.1	0.132
EC 4.1.1.5	0.220	EC 4.2.3.4	0.358	EC 5.4.2.3	0.207	EC 6.3.4.10	0.132
EC 4.1.1.50	0.138	EC 4.3.1.1	0.283	EC 5.4.2.4	0.471	EC 6.3.4.11	0.132
EC 4.1.1.55	0.094	EC 4.3.1.12	0.025	EC 5.4.2.6	0.119	EC 6.3.4.13	0.194
EC 4.1.1.65	0.094	EC 4.3.1.18	0.194	EC 5.4.2.7	0.207	EC 6.3.4.14	0.194
EC 4.1.1.68	0.402	EC 4.3.1.19	0.194	EC 5.4.2.8	0.207	EC 6.3.4.15	0.157
EC 4.1.1.7	0.201	EC 4.3.1.2	0.352	EC 5.4.2.9	0.018	EC 6.3.4.16	0.402
EC 4.1.1.71	0.201	EC 4.3.1.3	0.283	EC 5.4.3.2	0.012	EC 6.3.4.2	0.031
EC 4.1.1.74	0.201	EC 4.3.1.5	0.283	EC 5.4.3.3	0.031	EC 6.3.4.3	0.000
EC 4.1.1.77	0.402	EC 4.3.2.1	0.283	EC 5.4.3.8	0.069	EC 6.3.4.4	0.000
EC 4.1.1.8	0.201	EC 4.3.2.2	0.283	EC 5.4.4.2	0.383	EC 6.3.4.5	0.415
EC 4.1.1.81	0.069	EC 4.3.3.2	0.471	EC 5.4.99.1	0.031	EC 6.3.4.6	0.371
EC 4.1.2.13	0.018	EC 4.4.1.1	0.069	EC 5.4.99.16	0.572	EC 6.3.4.9	0.132
EC 4.1.2.14	0.018	EC 4.4.1.11	0.069	EC 5.4.99.2	0.031	EC 6.3.5.1	0.056
EC 4.1.2.17	0.377	EC 4.4.1.14	0.069	EC 5.4.99.5	0.415	EC 6.3.5.10	0.031
EC 4.1.2.19	0.377	EC 4.4.1.15	0.194	EC 5.4.99.7	0.465	EC 6.3.5.2	0.232
EC 4.1.2.20	0.018	EC 4.4.1.16	0.069	EC 5.4.99.8	0.465	EC 6.3.5.4	0.163
EC 4.1.2.21	0.018	EC 4.4.1.17	0.490	EC 5.5.1.1	0.352	EC 6.3.5.5	0.402
EC 4.1.2.27	0.069	EC 4.4.1.5	0.220	EC 5.5.1.13	0.465	EC 6.3.5.7	0.371
EC 4.1.2.4	0.018	EC 4.4.1.8	0.069	EC 5.5.1.4	0.207	EC 6.3.5.8	0.383
EC 4.1.2.40	0.251	EC 4.6.1.1	0.012	EC 5.5.1.6	0.805	EC 6.3.5.9	0.031
EC 4.1.2.5	0.069	EC 4.6.1.12	0.125	EC 5.5.1.7	0.352	EC 6.4.1.2	0.220
EC 4.1.2.9	0.100	EC 4.6.1.13	0.018	EC 6.1.1.1	0.238	EC 6.4.1.3	0.220
EC 4.1.3.1	0.018	EC 4.6.1.2	0.232	EC 6.1.1.10	0.226	EC 6.4.1.4	0.220
EC 4.1.3.16	0.018	EC 4.99.1.1	0.226	EC 6.1.1.11	0.138	EC 6.6.1.1	0.000
EC 4.1.3.27	0.383	EC 4.99.1.3	0.226	EC 6.1.1.12	0.415	EC 6.6.1.2	0.591
EC 4.1.3.3	0.018	EC 4.99.1.4	0.345	EC 6.1.1.13	0.220		
EC 4.1.3.30	0.018	EC 5.1.1.1	0.245	EC 6.1.1.14	0.132		
EC 4.1.3.34	0.018	EC 5.1.1.13	0.289	EC 6.1.1.15	0.540		
EC 4.1.3.38	0.383	EC 5.1.1.17	0.069	EC 6.1.1.16	0.226		
EC 4.1.3.4	0.018	EC 5.1.1.3	0.289	EC 6.1.1.17	0.484		
EC 4.1.3.6	0.018	EC 5.1.1.4	0.333	EC 6.1.1.18	0.383		
EC 4.1.99.1	0.069	EC 5.1.1.7	0.333	EC 6.1.1.19	0.251		
EC 4.1.99.2	0.069	EC 5.1.2.2	0.352	EC 6.1.1.2	0.056		
EC 4.2.1.1	0.559	EC 5.1.2.3	0.220	EC 6.1.1.20	0.138		
EC 4.2.1.10	0.031	EC 5.1.3.1	0.018	EC 6.1.1.21	0.220		
EC 4.2.1.104	0.710	EC 5.1.3.12	0.025	EC 6.1.1.22	0.132		
EC 4.2.1.11	0.352	EC 5.1.3.13	0.088	EC 6.1.1.3	0.251		
EC 4.2.1.12	0.018	EC 5.1.3.14	0.119	EC 6.1.1.4	0.421		
EC 4.2.1.17	0.220	EC 5.1.3.15	0.283	EC 6.1.1.5	0.421		
EC 4.2.1.18	0.220	EC 5.1.3.17	0.465	EC 6.1.1.6	0.132		
EC 4.2.1.2	0.283	EC 5.1.3.2	0.025	EC 6.1.1.9	0.421		
EC 4.2.1.20	0.194	EC 5.1.3.20	0.025	EC 6.2.1.1	0.220		
EC 4.2.1.22	0.194	EC 5.1.3.3	0.572	EC 6.2.1.12	0.220		
EC 4.2.1.24	0.018	EC 5.1.3.4	0.377	EC 6.2.1.13	0.119		
EC 4.2.1.28	0.188	EC 5.1.3.8	0.465	EC 6.2.1.14	0.088		
EC 4.2.1.3	0.383	EC 5.1.3.9	0.018	EC 6.2.1.16	0.220		
EC 4.2.1.30	0.836	EC 5.1.99.1	0.220	EC 6.2.1.17	0.220		
EC 4.2.1.32	0.559	EC 5.2.1.2	0.584	EC 6.2.1.2	0.220		
EC 4.2.1.33	0.150	EC 5.2.1.4	0.584	EC 6.2.1.20	0.220		
EC 4.2.1.36	0.383	EC 5.3.1.1	0.018	EC 6.2.1.25	0.220		
EC 4.2.1.39	0.352	EC 5.3.1.12	0.018	EC 6.2.1.26	0.220		
EC 4.2.1.40	0.352	EC 5.3.1.14	0.018	EC 6.2.1.27	0.220		
EC 4.2.1.41	0.018	EC 5.3.1.16	0.018	EC 6.2.1.3	0.220		
EC 4.2.1.42	0.025	EC 5.3.1.17	0.088	EC 6.2.1.30	0.220		
EC 4.2.1.46	0.025	EC 5.3.1.22	0.018	EC 6.2.1.4	0.031		
EC 4.2.1.47	0.025	EC 5.3.1.24	0.018	EC 6.2.1.5	0.031		
EC 4.2.1.49	0.893	EC 5.3.1.25	0.628	EC 6.3.1.1	0.132		
EC 4.2.1.51	0.012	EC 5.3.1.26	0.584	EC 6.3.1.10	0.006		
EC 4.2.1.52	0.018	EC 5.3.1.4	0.628	EC 6.3.1.2	0.371		
EC 4.2.1.55	0.220	EC 5.3.1.5	0.018	EC 6.3.1.5	0.056		
EC 4.2.1.58	0.213	EC 5.3.1.6	0.012	EC 6.3.1.8	0.729		
EC 4.2.1.6	0.352	EC 5.3.1.8	0.088	EC 6.3.2.1	0.056		
EC 4.2.1.60	0.213	EC 5.3.1.9	0.283	EC 6.3.2.10	0.559		
EC 4.2.1.61	0.213	EC 5.3.3.1	0.025	EC 6.3.2.12	0.295		
EC 4.2.1.7	0.559	EC 5.3.3.10	0.566	EC 6.3.2.13	0.559		
EC 4.2.1.70	0.238	EC 5.3.3.12	0.962	EC 6.3.2.17	0.295		
EC 4.2.1.75	0.427	EC 5.3.3.2	0.018	EC 6.3.2.19	0.962		
EC 4.2.1.8	0.018	EC 5.3.3.4	0.012	EC 6.3.2.2	0.119		
EC 4.2.1.80	0.402	EC 5.3.3.8	0.220	EC 6.3.2.3	0.194		
EC 4.2.1.84	0.704	EC 5.3.99.2	0.584	EC 6.3.2.4	0.194		
EC 4.2.1.9	0.364	EC 5.3.99.3	0.584	EC 6.3.2.6	0.402		
EC 4.2.1.91	0.094	EC 5.3.99.4	0.968	EC 6.3.2.8	0.559		
EC 4.2.2.2	0.320	EC 5.3.99.5	0.968	EC 6.3.2.9	0.559		
EC 4.2.2.3	0.465	EC 5.4.1.2	0.031	EC 6.3.3.1	0.301		
EC 4.2.2.6	0.471	EC 5.4.2.1	0.471	EC 6.3.3.2	0.157		
EC 4.2.3.12	0.314	EC 5.4.2.10	0.207	EC 6.3.3.3	0.000		