

Supplementary Information for

On the evolution of chaperones and co-chaperones and the expansion of proteomes across the Tree of Life

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Supplementary Methods

Constructing the Tree of Life

To construct a representative Tree of life, we used the TimeTree database (1) and the NCBI taxonomy database (2). For all major clades of bacterial, archaeal, and eukaryotic domains, non-redundant representative species were selected, ensuring that (i) the minimum splitting time for any pair of taxa is ≥ 65 million years, (ii) their annotated proteome sequences are available in NCBI genome database (3) and (iii) proteome-scale domain assignment data are available in Pfam (4). This analysis rendered 188 representative organisms covering 56 major bacterial, archaeal, and eukaryotic clades (**Data S1**). Note that phylogenetic analyses often assign parasitic and symbiotic organisms that have experienced reductive evolution as the earliest diverging clades of their corresponding kingdoms of life. Examples include *Nanoarchaeum equitans*, an obligate symbiont, assigned as the earliest diverging archaea (5-7), and parasitic Excavates assigned as one of the earliest diverging eukaryotes (8, 9). While these organisms were included in our representative set of organisms, the expansion of proteomes and chaperones has been analyzed for free-living organisms only.

The TimeTree Database comprises phylogenetic relationships, as well as literature-based annotations of predicted emergence times (Million Years Ago, MYA, from now) for > 50,000 species. Using this database, a Tree of Life (ToL) was constructed in which leaves represent the extant clades (e.g., Mammalia, which comprises three representative organisms: human, cat, and mouse). The tree's root comprises the Last Universal Common Ancestor (LUCA), and the nodes represent the hypothetical ancestors. Branch lengths represent the divergence time of the different clades. One representative species from each of our 56 phylogenetic clades was chosen, and this species set was submitted to the TimeTree Database to extract a 'core-tree' for their original tree. The obtained tree topology was manually adjusted to depict the emergence of eukaryotes from Asgard archaea and *Alphaproteobacteria* by an endosymbiosis event. The branch lengths represent the evolutionary divergence times as documented in TimeTree, and were used as proxies of the order of divergence to plot the proteome parameters.

Capturing proteome expansion

Proteome size and median protein length in the proteome. Annotated proteome sequences of the 188 representative organisms were obtained as FASTA-format files from the NCBI genome database (3). For each organism, we used Biopython v1.75 package to compute the total number of proteins in the respective proteome, and the length of each protein. These protein lengths were used to derive the median protein length for each organism (**Data S2**).

Multi-domain proteins in the proteome. Proteome-scale domain annotations of each of the 188 representative organisms were obtained from Pfam (4). In Pfam, the established profile HMMs of known domain families (Hidden Markov Models, probabilistic models used for the statistical inference of homology) are searched against the protein sequences, to find all instances of that domain. A statistical significance score (the probability that the prediction is a random hit) is assigned to each predicted instance based on the sequence similarity with the profile HMM. Any domain assigned with $p < 10^{-5}$ significance were considered for further analysis. Overall, in these 188 representative organisms, we identified 7694 domain families, which amounts to roughly 43% of all annotated domain families (17836 families) in Pfam 32.0, September 2018 release. For each species, the number of proteins comprising <3 and those comprising ≥ 3 Pfam-annotated domains were counted (**Data S2**).

Number of unique fold types and fold-combinations. In general, sequence homologies between different domain families show that they can be clustered into independently evolved lineages (meaning, members of different lineages do not exhibit any detectable sequence homology). Two databases, Pfam (10) and ECOD (11) perform this clustering considering sequence and structural similarities among domain families as the benchmark. In Pfam, these independently evolved super-families are remarked as Clans (10). In ECOD, this clustering is hierarchical (from bottom to top: F-group, T-group, X-group, and top hierarchy) and the independently evolved lineages are termed as the X-groups (11). For each of the 188 proteomes, we mapped the Pfam-assigned domains to the Pfam- Clans as well as to the ECOD X-groups. The 7694 domain families were mapped to 554 unique Clans, and to 976 X-groups, covering 88% of all annotated Clans (629 Clans in Pfam 32.0), and 42% of all annotated ECOD X-groups (2316 X-groups, ECOD v20191115). The numbers of unique Clans, or X-groups, identified in a given proteome were considered as a measure of the number of unique fold types present in a given organism (**Data S3**).

This analysis further allowed us to count the vectorial combinations ($AB \neq BA$) of Clans / X-groups, from N- to C-terminal, present in a given protein. The total number of Clan / X-group combinations present in the proteome represents the total number of unique fold-combinations present in the respective organism. The 554 Clans and 976 X-groups included in our work yielded to 15463 X-group combinations and 16538 Clan combinations respectively (**Data S4, S5**).

Proportion of beta-superfolds in the proteome. To capture the proportion of beta-structures in the proteome, for each representative organism, we identified how many proteins in the proteome are annotated as belonging to all-beta folds, and what fraction of the entire proteome they represent. All beta structures assigned under ECOD top hierarchies beta-barrel, beta meander, beta-sandwich, beta duplicates or obligate multimers, and beta complex topology were considered in the analysis (**Data S6**).

Repeated sequences in the proteome. To capture the abundance of repeated sequences in the proteome, the 188 representative proteomes were scanned by T-REKS repeat-identifier program (12). All repeats that are larger than a 'foldon' unit (~20 aa) (13), and exhibit $\geq 90\%$ sequence similarity were considered for further analysis (**Data S7**). Summing the lengths of all the identified repeats, we derived the total length of repeated sequences in each proteome, and what fraction of the total proteome length it covers (total repeat length normalized by the sum of all protein lengths, **Data S2**).

Expansion of domain lengths. To examine the expansion of domain lengths, we first identified 38 distinct folds that are conserved across the ToL, including parasites and symbionts (**Data S3**). Proteins harboring these X-groups were detected in the representative organisms and the respective domain lengths, as annotated in Pfam, were computed. For each organism, these lengths were pulled together and the average domain length was derived (**Data S2**).

Expansion of domain-flanking regions. For each protein harboring an X-group conserved across the ToL, we used the Pfam-annotated location of the protein domain in the primary sequence to measure the lengths of C- and N-termini segments, and inter-domain linkers. For each organism, the lengths of these domain-flanking regions were pulled together to obtain the average (**Data S2**).

Predicted proportion of aggregation-prone proteins in the proteome. To capture the proportion of aggregation-prone proteins in the proteome, for each representative organism, we identified how many proteins in the proteome comprise $\geq n$ 'aggregation hotspots', where n is an integer. An 'aggregation hotspot' was defined as a 'poorly soluble' protein segment of ≥ 5 aa length, with solubility predicted from the protein's sequence using CamSol v2.1 (14). The CamSol method yields the solubility profile of a protein: a solubility score is assigned to each amino acid in a way that regions with scores > 1 denote 'highly soluble' regions, while scores < -1 reflect 'poorly soluble' ones. For each of the 188 representative organisms, we computed the percent of proteins in the proteome that comprises $\geq n$ 'aggregation hotspots', for $2 \leq n \leq 20$ (**Data S8**). Note that the CamSol method does not take structural information into account, which means at least for globular proteins, the predicted poorly soluble regions might reside in the hydrophobic core of globular proteins (14).

Intrinsically disordered regions in the proteome. To capture the expansion of intrinsically disordered segments, we identified the disordered segments of each protein in the proteomes of our species set by IUPred2A that exploits the idea that in disordered regions amino acid residues form fewer energetically favorable contacts than residues in ordered regions (15). IUPred2A does not rely on any information besides the amino acid sequence and is therefore suitable for predicting disorder in large protein datasets. The disorder status of each amino acid site is represented by a

probability score; any amino acid site associated with > 0.5 probability score was assigned as intrinsically disordered. Disorder is, however, a property of segments rather than residues, and thus segments of ≥ 20 aa long were analyzed. Presented here are the results for ≥ 100 aa segments (**Data S9**) yet other thresholds yielded similar results. The percent of proteome length that is disordered was subsequently derived (sum of the lengths of all ≥ 100 aa disordered segments multiplied by 100, divided by the sum of lengths of all proteins).

Capturing chaperones in the proteome

To determine the evolutionary appearance and expansion of the core-chaperone (HSP20, HSP60, HSP70, HSP90, and HSP100), and co-chaperone families (HSP10/GroES, JDP/HSP40, HSP110, GrpE, Bag, Fes, Hip, Hop, Chip, Tom70, Cyp40, FKBP52/51, PP5, Unc45, Cdc37, P23, Aha1, Sgt1, Pih1, Tah1, and Tsc1/2 heterodimer) we identified their occurrences in the 188 representative organisms, using two complementary methods.

Identifying chaperones by BLAST-search. Chaperone family proteins were manually curated from the UniProt (16) annotated proteomes of model organisms (*Escherichia coli* and *Saccharomyces cerevisiae*). These protein sequences were then used as queries to find orthologous sequences in the other organisms by a comprehensive protein-protein BLAST (17). BLAST hits associated with 50% sequence coverage and $\leq 10^{-5}$ e-values (18) were manually inspected to extract the 'true-positive' chaperone family members.

Identifying chaperones by characteristic domain combinations. The second method involved manually looking into the Pfam-assigned domain combinations of annotated chaperones. For instance, bacterial HSP20s (e.g., IbpA protein in *E. coli*) predominantly comprise a single HSP20 domain, whereas chordate HSP20s (e.g., HSPB6 protein in *H. sapiens*) often comprise an additional N-terminal alpha-crystallin domain. For each chaperone family, we investigated their domain organization in Pfam and constructed a library of domain combinations (occurrence of the domain of interest with other domains in annotated proteomes). This library comprised any combination that Pfam reports in at least 10 different sequences. We searched these combinations in the 188 representative proteomes. Any protein comprising any of these domain combinations was assigned to be a member of that core-chaperone family. This analysis excluded eukaryote specific HSP110 co-chaperone family that is composed of two HSP70 domains, and thus cannot be distinguished from HSP70s that comprise two HSP70 domains. In this case, specific hallmark sequences in the linker and the more variable C-terminal domain of HSP110 that differ from HSP70 were used in BLAST searches.

The two complementary methods rendered an identical number of chaperone gene copy-numbers in each organism, reflecting the robustness of the overall approach (**Data S10**).

Phylogenetic analysis

Multiple sequence alignments (MSAs) were generated using Muscle v3.8.31 (19). The alignments were manually curated and gap-majority columns were trimmed using trimAl v1.330 (20). Only for HSP20, alignments were collected from EggNOG database (21), and were manually curated. These alignments were filtered by removing all sequences that are more than 80% identical (usually redundant sequences) and those that are less than 30% identical (usually interferes with rooting by creating extremely long branches) were removed using T-coffee (22). Maximum likelihood phylogenetic trees were generated by MEGA X (23), using JTT distance matrix and NJ/BioNJ initial tree. Phylogenetic trees are provided in **Table S1**. To date the emergence of individual chaperone families, the protein trees were manually compared with the ToL to assign the node of emergence and possible HGT events.

Relative mRNA abundance of core- and co-chaperones

To quantify the variation in chaperone gene-expression across the ToL, genome-scale RNA-seq data of prokaryotic/eukaryotic cells/tissues were collected from various resources. Only samples not subjected to stress or genetic modifications, and processed expression data, were considered (where the abundance of mRNA transcripts is provided as TPMs, transcripts per million). Data for metazoans, chordates, plants, and green alga were collected from Bgee (24) and Expression Atlas (25). Data for free-living prokaryotes and pathogenic bacteria were collected from a comparative transcriptomic resource (26) and Pathogenex database (27). Data for *S. pombe* were collected from YeastTSS database (28). For *S. cerevisiae*, single-cell RNA-seq expression data were collected (29). In total, data from 3287 non-redundant RNA-seq experiments were analyzed in our work, comprising 14 eukaryotes, 13 free-living bacteria, 3 free-living archaea, and 18 pathogenic bacteria. For each experiment, the sum of the provided TPM values of the core- and the co-chaperones were divided by the sum of TPMs of ribosomal proteins. The average and standard deviation over all experiments per given organism were computed (**Data S11**) and were plotted as columns in **Fig. 3C**. Note that in **Fig. 3C**, the mean chaperone expression for all bacterial pathogens is plotted alongside free-living bacteria, and the numerical values for individual species are provided as **Data S11**.

Relative protein abundance of core- and co-chaperones

The abundance of a protein simply means the number of copies of a protein molecule in a cell. Non-redundant genome-scale abundance data was collected for 11 non-extremophilic model organisms from PAXdb (30), measured under normal conditions. This database comprises whole genome protein abundance information across organisms and tissues. Dataset quality was measured by its proteome coverage and interaction consistency score (ICS). Proteome coverage

represents what fraction of the entire proteome is included in the abundance dataset. ICS is based on the assumption that proteins that contribute jointly to a shared function (such as members of a protein complex) should tend to have roughly similar protein abundance levels. For unicellular organisms, datasets that cover $\geq 40\%$ of the respective proteomes with ≥ 3.0 ICS and include $\geq 30\%$ of all core- and co-chaperones identified in the respective organism, were selected for analysis. For multicellular organisms, tissue-specific data was collected. Datasets that cover $\geq 10\%$ of the respective proteomes with ≥ 3.0 ICS and includes $\geq 30\%$ of all core- and co-chaperones identified in the respective organism, were selected for analysis. All datasets related to stress or disease-conditions were removed and the remaining 121 datasets were considered for further analysis (**Data S12**). For each abundance dataset, we classified the list of proteins into three non-overlapping groups: (i) core-chaperones, (ii) co-chaperones, and (iii) proteins that are not chaperones. For each group, the abundance values of all the proteins were summed to measure the 'total abundance'. The 'total abundance of core-chaperones', normalized by the 'total abundance of proteins that are not chaperones' represents the relative abundance of core-chaperones. Similarly, we measured the 'relative abundance of co-chaperones'. For each species, the average and standard deviations among different datasets were measured.

Visualizing the expansion of proteomes and chaperones

For each clade in our core-tree, numerical values of the proteome parameters and chaperone-copy number values obtained for its representative organisms were pulled together to derive the clade average, and the clade standard deviation. The clade average values were subsequently plotted against their predicted divergence time and were fitted into the standard exponential growth curve. The fitted exponential curves are depicted as solid black lines.

Data biases associated with the choice of representative organisms

To what extent the choice of the major clades of life and the set of representative organisms could bias the obtained results? The former is unlikely to bias the results because we considered all clades of life currently annotated in NCBI Taxonomy and TimeTree databases. However, in oppose to analyzing all reference genomes in the NCBI dataset, the set of organisms used here is 'normalized'. Consequently, clades where thousands of genomes are available and clades with only a few genomes are represented to the same extent. Nonetheless, we reexamined the data to rule out a possible bias due to the choice of the organisms that represent each clade. To this end, we re-computed the expansion of proteome size and median protein lengths for an alternate set of 181 organisms (**Data S1**) that represent the same 56 major clades shown in **Fig. 1A**. As plotted in **SI Appendix, Fig. S5**, the number of proteins in the proteome and the median protein length expanded 150-fold and 2-fold in the alternative species set (as compared to 200-fold and 2-fold in

the original set). These results indicate the robustness of our analysis in terms of the choice of organisms.

Graphics

The Tree of Life was generated using the Interactive Tree Of Life (iTOL) v4.0 (31). All the plots were generated using OriginPro software v9.1.0. Figures were compiled using Adobe Photoshop CS6 v13.0.1.

Quantification and statistical analysis

All the statistical analyses mentioned in the main text were performed using in-house Python scripts.

Supplementary Figures

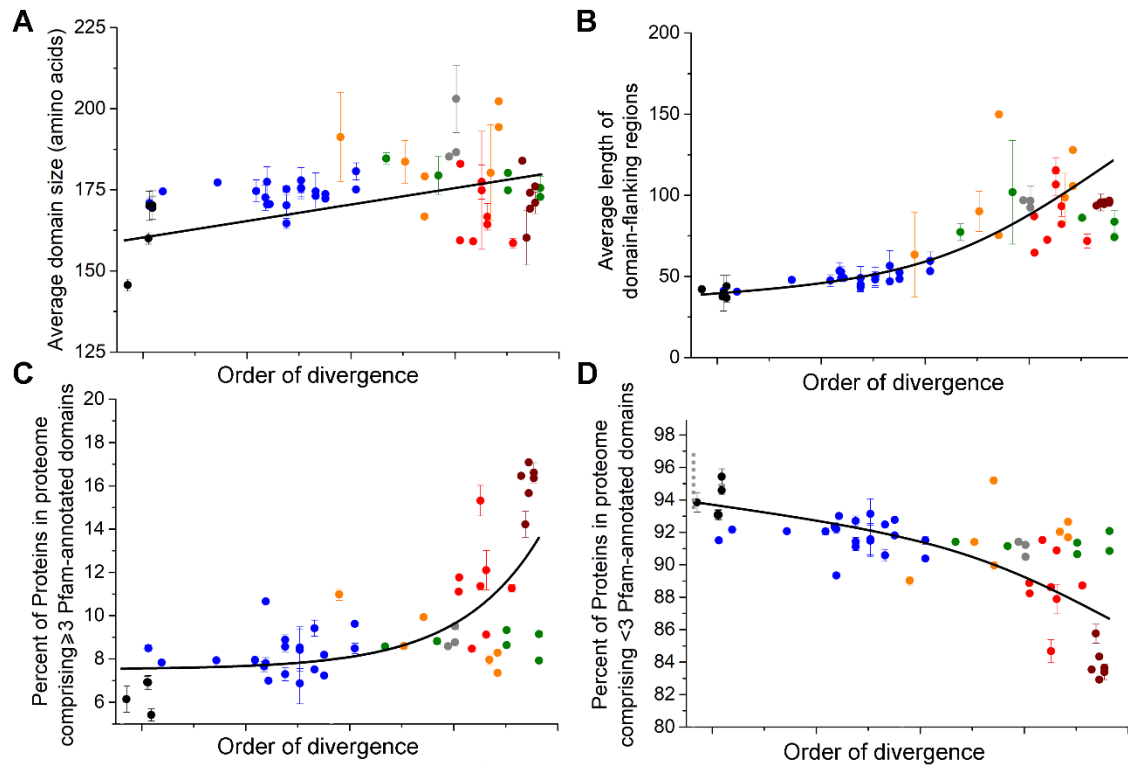


Fig. S1. Figure features follow those of **Fig. 1.** **(A)** Shown on the Y-axis (linear-scale) are the average lengths of the protein domains that belong to 38 ECOD X-groups present throughout the ToL.

(B) Same, for the length of the domain-flanking segments.

(C) Same, for the percent of proteins in the proteome comprising ≥ 3 Pfam-annotated domains.

(D) Same, for the percent of proteins in the proteome comprising < 3 Pfam-annotated domains.

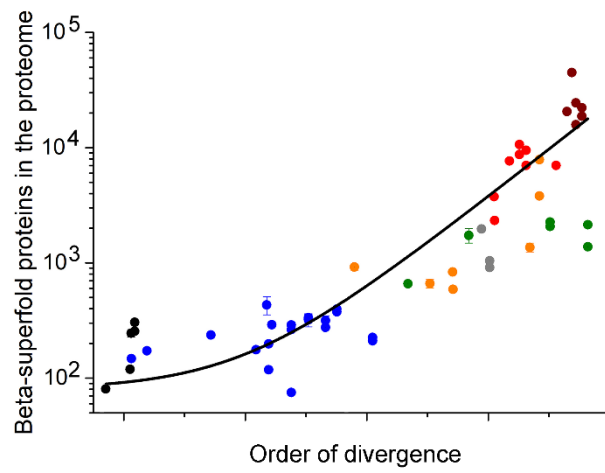


Fig. S2. Figure features follow those of **Fig. 1.** **(A)** Shown on the Y-axis (log-scale) is the number of beta-superfold proteins in the proteome.

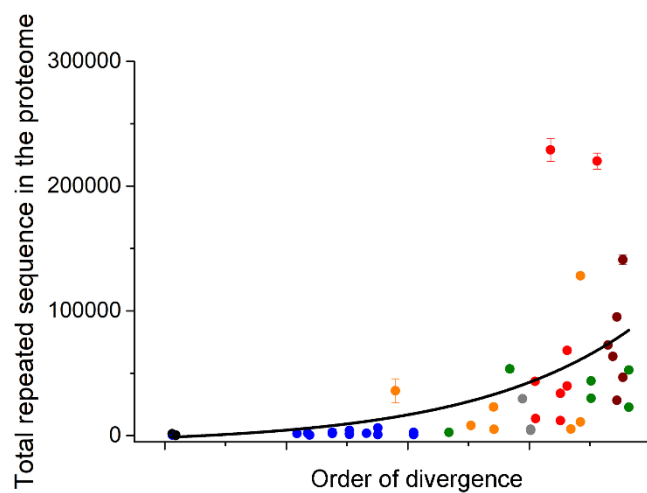


Fig. S3. Figure features follow those of **Fig. 1.** **(A)** Shown on the Y-axis (log-scale) is the total length (number of amino acids) of repeated sequences per proteome.

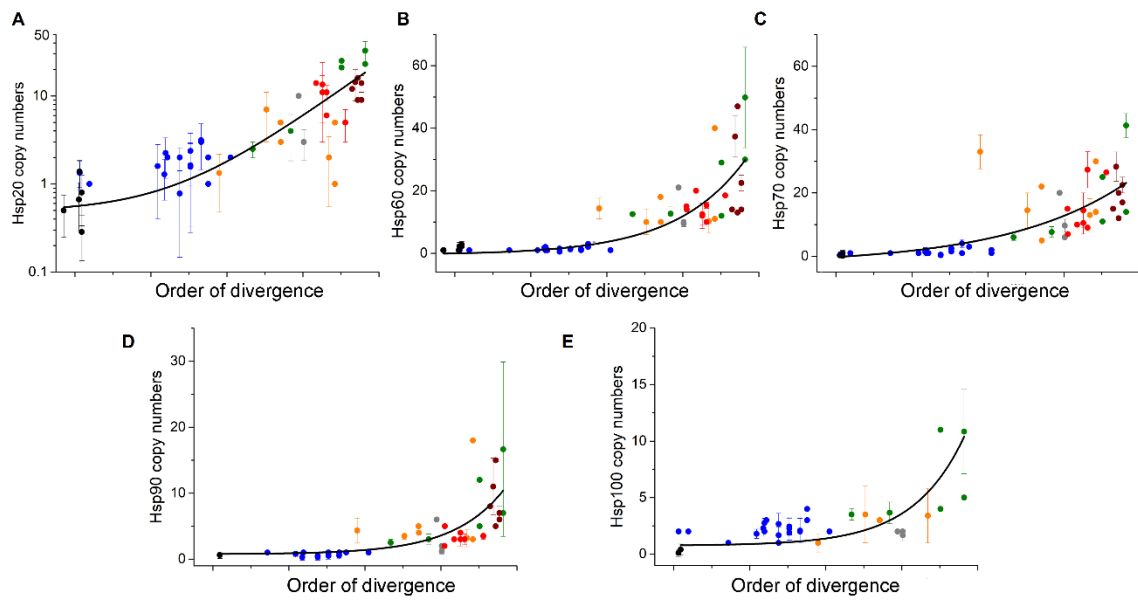


Fig. S4. Figure features follow those of **Fig. 1**. Shown on the Y-axis is the average number, per genome, of genes encoding **(A)** HSP20 (note the log-scale), **(B)** HSP60, **(C)** HSP70, **(D)** HSP90, and **(E)** HSP100.

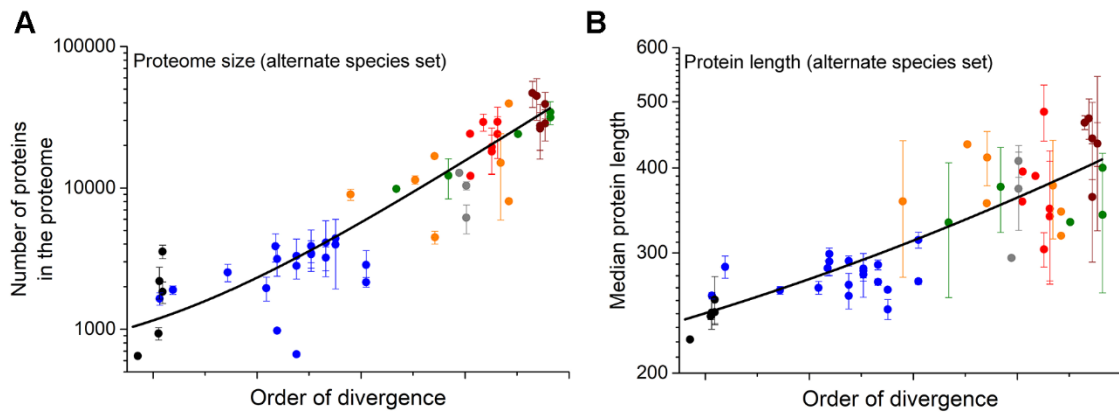


Fig. S5. Figure features follow those of **Fig. 1**. Shown on the Y-axis (linear-scale) is the #proteins per proteome (**A**), and the median protein length (**B**), for the alternative species set.

Supplementary Tables

Table S1. Protein trees (newick format) of core and co-chaperone families. These trees were inferred by using the Maximum Likelihood method. Initial trees for the heuristic search were obtained by NJ/BioNJ algorithm, using JTT distance model.

HSP20

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) :0.09712874[1.0000],((Q47LP1_Actinobacteria:0.12896991,Q82DI5_Actinobacteria:0.0971784
9):0.04770010[0.7600],(Q6NJ37_Actinobacteria:0.26034580,(Q5Z1F9_Actinobacteria:0.075722
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HSP90

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bacteria:0.20893872,(Q9CM20_Gammaproteobacteria:0.15417850,(P58480_Gammaproteobacteria:0.03429536,P0A6Z3_Gammaproteobacteria:0.02109758):0.09708175[1.0000]):0.05507428[0.8600]):0.07749593[1.0000]):0.05339411[0.6000]):0.07807216[1.0000]):0.07945529[0.6800],Q5NHT8_Gammaproteobacteria:0.39796840):0.05440426[0.4200],Q4FQZ1_Gammaproteobacteria:0.39299933):0.11091432[0.9800],(((B3Q3C5_Alphaproteobacteria:0.49147539,Q2RYB8_Alphaproteobacteria:0.31107385):0.06760929[0.3000],Q5FS51_Alphaproteobacteria:0.44185598):0.08086864[0.4400],Q3YSL9_Alphaproteobacteria:0.56234223):0.17549263[1.0000],(((D1B2V5_Epsilonproteobacteria:0.28598497,E0UTY3_Epsilonproteobacteria:0.34797126):0.06117241[0.4000],D3UH07_Epsilonproteobacteria:0.41126879):0.06884740[0.6800],(E6X0D4_Epsilonproteobacteria:0.40011154,(P61188_Spirochaetia:0.27361425,(H9UMB7_Spirochaetia:0.17682104,E1RAF0_Spirochaetia:0.19990318):0.05617327[0.9600]):0.10049339[1.0000]):0.07053906[0.5800]):0.17451768[1.0000]):0.06180512[0.4200]):0.10255859[0.5600],((Q1CZI7_Deltaproteobacteria:0.44902521,A9GDV2_Deltaproteobacteria:0.48428625):0.10950025[0.4000],(A8ZUI6_Deltaproteobacteria:0.39699631,(((Q5Z3N4_Actinobacteria:0.17778642,P9WMJ7_Actinobacteria:0.15212580):0.22159331[1.0000],D0LYJ5_Deltaproteobacteria:0.36423103):0.10764673[0.9400],(P61184_Oligoflexia:0.47618673,(A1ANS1_Deltaproteobacteria:0.11700783,B3E438_Deltaproteobacteria:0.10642882):0.19758534[1.0000]):0.06982348[0.4400]):0.05478285[0.3800]):0.09867752[0.7400]):0.06562625[0.2600]):0.06050549[0.3800],(((A1BFP7_Chlorobia:0.18153749,B4SAD1_Chlorobia:0.11997133):0.09783354[0.9600],B4S7B7_Chlorobia:0.14678316):0.39349990[1.0000]),(((A0A0F8VG15_Asgard:0.31907583,A0A1Q9N225_Asgard:0.33179790):0.29350692[1.0000],A0A1Q9P0M4_Asgard:0.42273132):0.19559655[1.0000],(F3YV29_Deltaproteobacteria:0.48214949,(Q24VT7_Clostridia:0.29004829,(A5D630_Clostridia:0.24391675,Q6ARM0_Deltaproteobacteria:0.38840911):0.08940400[0.8600]):0.22934474[1.0000]):0.09841673[0.8200]):0.08982817[0.5200]):0.14985155[0.8000]):0.08845893[0.4000],(Q8RGH4_Fusobacteria:0.35753818,(Q894P6_Clostridia:0.18459830,(B1YG43_Bacilli:0.24703828,(P46208_Bacilli:0.12122093,C0Z8X7_Bacilli:0.13982396):0.04962165[0.6600]):0.11459662[0.9400]):0.34752554[1.0000]):0.23505273[1.0000],(Q2SLM3_Gammaproteobacteria:1.44088058,(P0C938_Bacteroidia:0.52534488,(Q7NJL8_Gloeobacteria:0.26705230,B0JQV2_Cyanobacteria:0.32501593):0.27985305[1.0000]):0.41353926[1.0000]):0.36319680[1.0000])OROOT;

HSP100

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83691[0.2200],((((B1YGS2_Bacilli:0.10293732,C0ZIE7_Bacilli:0.08846637):0.03174829[0.2400],Q5L436_Bacilli:0.03939788):0.00967876[0.1200],(P37571_Bacilli:0.05613562,A0A127VTU5_Bacilli:0.08695787):0.02476921[0.2800]):0.04467314[0.2600],Q49V34_Bacilli:0.14169326):0.05773176[0.8600],Q890L5_Clostridia:0.20132961):0.03474447[0.1400],(((Q5Z2T4_Actinobacteria:0.01646690,P9WPC9_Actinobacteria:0.03072607):0.01543023[0.5600],Q6NFB1_Actinobacteria:0.08378391):0.05846318[1.0000],((Q6ACR4_Actinobacteria:0.05990043,Q83H06_Actinobacteria:0.16001473):0.08648012[0.9200],(Q47KW3_Actinobacteria:0.06447902,(Q82EB8_Actinobacteria:0.00219985,Q82K04_Actinobacteria:0.01980852):0.03806311[0.9600]):0.02962707[0.7400]):0.02924291[0.5800]):0.18198624[1.0000]):0.02719472[0.0000]):0.04178204[0.1800]):0.03389700[0.1200]):0.03810510[0.0000]):0.03067937[0.0000],(Q9WY41_Thermotogae:0.23618009,(Q9X1B1_Thermotogae:0.27766983,(F0S2V8_Aquificae:0.17293282,(O67588_Aquificae:0.12807284,C1DUW1_Aquificae:0.17808929):0.11309990[1.0000]):0.07111204[1.0000]):0.03577325[0.2800]):0.02902050[0.0400]):0.04085108[0.0000],(F0S121_Aquificae:0.17958925,(O67325_Aquificae:0.10198609,C1DT67_Aquificae:0.11426306):0.10394569[1.0000]):0.10357485[0.9800]):0.01829911[0.0400],(A1STR4_Gammaproteobacteria:0.38267723,(Q899V4_Clostridia:0.33306261,((Q837W9_Bacilli:0.07765981,Q9CI09_Bacilli:0.11351921):0.09928382[0.9800],F2I5V6_Bacilli:0.21001803):0.09901659[1.0000],(A0A127W0Q0_Bacilli:0.18929591,(Q5L1C7_Bacilli:0.10133515,(O31673_Bacilli:0.13622672,C0ZJ35_Bacilli:0.17129359):0.05729994[0.6200]):0.03333899[0.2800]):0.10860302[1.0000]):0.14059607[1.0000]):0.13173655[0.9200]):0.04546349[0.1000]):0.04841099[0.0800],(Q2IKK0_Deltaproteobacteria:0.20230948,Q1D4S0_Deltaproteobacteria:0.19768735):0.36863196[1.0000]):0.03763243[0.2200],(F2I7G8_Bacilli:0.70119660,(Q9RVB2_Deinococci:0.42708472,(((F3YX74_Deltaproteobacteria:0.25039781,Q1D2Z9_Deltaproteobacteria:0.25985985):0.08487744[0.7600],(Q9I363_Gammaproteobacteria:0.24582641,Q9KN49_Gammaproteobacteria:0.33727242):0.08504248[0.8600]):0.04593564[0.5600],(Q9I1A8_Gammaproteobacteria:0.25989487,(Q47E21_Betaproteobacteria:0.21306279,(Q2SEG4_Gammaproteobacteria:0.29099544,(Q0KDX2_Betaproteobacteria:0.30842002,(B3Q4A0_Alphaproteobacteria:0.18744507,Q9I742_Gammaproteobacteria:0.17122376):0.05095950[0.6800]):0.07778454[0.9000]):0.04813273[0.4600]):0.04544520[0.6000]):0.08917405[0.9600]):0.35538499[1.0000]):0.04200291[0.0600]):0.07169139[0.4000],(Q9RWS7_Deinococci:0.36087740,(((H9UKD7_Spirochaetia:0.23307731,E1R5D7_Spirochaetia:0.28456257):0.07690776[0.8400],Q73KU3_Spirochaetia:0.34370887):0.07919552[0.9800],((((D3UJ95_Epsilonproteobacteria:0.51089097,E6WZH7_Epsilonproteobacteria:0.22369730):0.07753449[0.4600],E0UPY5_Epsilonproteobacteria:0.26854610):0.06487524[0.7000],D1B3H1_Epsilonproteobacteria:0.28425261):0.05302876[0.5200],B9L8R6_Epsilonproteobacteria:0.30517456):0.11370463[0.6800],(Q1MQQ5_Deltaproteobacteria:0.34580086,(((F3Z1S3_Deltaproteobacteria:0.22102943,A0LE94_Deltaproteobacteria:0.18550572):0.05540222[0.6400],A8ZW51_Deltaproteobacteria:0.24233501):0.03840985[0.5200],Q6APV8_Deltaproteobacteria:0.28304500):0.03854603[0.3200],(((Q2IP33_Deltaproteobacteria:0.18820743,Q1CZL2_Deltaproteobacteria:0.16516163):0.04621905[0.6800],D0LVD0_Deltaproteobacteria:0.24033767):0.04287414[0.6400],((Q3YS04_Alphaproteobacteria:0.37946683,Q6MNG8_Oligoflexia:0.28961312):0.09590500[0.4800],(A9F365_Deltaproteobacteria:0.30185957,(((Q5FTA9_Alphaproteobacteria:0.11135771,Q2N6G4_Alphaproteobacteria:0.17871646):0.03740196[0.5800],Q2RSI6_Alphaproteobacteria:0.09793318):0.04341758[0.7000],(Q5LNN4_Alphaproteobacteria:0.13918890,(Q9A5H9_Alphaproteobacteria:0.12764280,B3PYN2_Alphaproteobacteria:0.15750165):0.02479985[0.3800]):0.04058727[0.6800]):0.09234061[1.0000],((((Q0K794_Betaproteobacteria:0.05438026,A4G7T8_Betaproteobacteria:0.06410464):0.05173790[1.0000],Q82TY2_Betaproteobacteria:0.12307751):0.02975269[0.5200],Q47BF6_Betaproteobacteria:0.10273540):0.01901530[0.3400],Q3SJH1_Betaproteobacteria:0.08753867):0.02743485[0.4600],C1DBG3_Betaproteobacteria:0.07360267):0.06945023[0.9800],Q5F9I6_Betaproteobacteria:0.22107091):0.10051258[0.9800],(A5EV83_Gammaproteobacteria:0.26872581,Q4FRL2_Gammaproteobacteria:0.25530887):0.06858617[0.5000],((((Q3J8G5_Gammaproteobacteria:0.15258761,Q5ZXB4_Gam

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JDPs

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GroES/HSP10

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Cyp40

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Legends for Datasets

Data S1. The 188 representative organisms and their phylogenetic classifications. This file also lists the 181 alternative representative organisms.

Data S2. Numerical values of the proteome parameters.

Data S3. A list of all the unique ECOD X-groups, and Pfam clans, detected in representative proteomes of our ToL, and a list of ECOD X-groups conserved across the ToL.

Data S4. A list of all the unique ECOD X-group combinations detected in the representative proteomes of our ToL.

Data S5. Unique Pfam domain combinations, and clan combinations, detected in the representative proteomes; 'no clan' relates to Pfam families that are not assigned to a clan, and thus reflect an independent lineage.

Data S6. Expansion of beta-superfolds beyond the expansion of proteome size: for representative organisms of our ToL, shown are the percent of proteins in the proteomes that contain a domain related to one of the ECOD top hierarchies.

Data S7. Repeat segments identified in the representative proteomes.

Data S8. The percent of aggregation-prone proteins per proteome.

Data S9. The predicted disordered segments in the representative proteomes.

Data S10. Pfam-annotated domain combinations, and the UniProt identifiers, of the core and co-chaperones identified in the representative proteomes.

Data S11. mRNA abundance of core- and co-chaperones.

Data S12. Protein abundance of core- and co-chaperones.

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