

Intensification: A resource for amplifying population-genetic signals with protein repeats

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Abstract

Large-scale genome sequencing holds great promise for the interpretation of protein structures through the discovery of many rare, functional variants in the human population. However, because protein-coding regions are under high selective constraints, these variants occur at low frequencies, such that there is often insufficient statistics for downstream calculations. To address this problem, we develop the Intensification approach, which uses the modular structure of repeat protein domains to amplify signals of selection from population genetics and traditional inter-species conservation. In particular, we are able to aggregate variants at the codon level to identify important positions in repeat domains that show strong conservation signals. This allows us to compare conservation over different evolutionary timescales. It also enables us to visualize population-genetic measures on protein structures. We make available the Intensification results as an online resource (<http://intensification.gersteinlab.org>) and illustrate the approach through a case study on the tetratricopeptide repeat.

Introduction

The combined efforts from large-scale human sequencing projects and clinical sequencing have given rise to an exponentially increasing number of human sequences in recent years.¹⁻³ With substantial drop in the sequencing cost and improvement in sequencing technologies and data processing capabilities, we now have the ability to generate a huge catalog of variants that exist in the human population in a fairly rapid and high-throughput fashion. One of the challenges is to provide functional annotations for these variants efficiently and accurately.

Much of the variant annotation work has been performed in the protein-coding regions. A non-synonymous mutation is considered functionally disruptive if it occurs in a region of high conservation, which are considered to be important evolutionarily.⁴ Evolutionary conservation can be observed at different levels. Inter-species comparison can pick out fixed differences between the dominant homologous sequences of the chosen species across their phylogeny over a long evolutionary time.⁵⁻⁷ At a more recent timescale, intra-species conservation (across a population) has been observed over specific sites in a few large-scale sequencing studies, by aggregating variants over a region or site within the human population.⁸⁻¹⁰ However, all protein-coding regions are, in general, under high selection pressure. As such, almost all positions in

high-impact protein domains tend to be extremely conserved, making it tricky to pinpoint specific positions. Variants also occur sparsely across the coding region and at very low frequencies within a population. Consequently, it is difficult to increase the number of variants for population analyses without increasing the pool of sequenced individuals. To this end, we devise an “intra-genome conservation” approach that is able to “amplify” the variant signal in protein-coding regions within a population.

There is a wide range of repeat protein domains (RPDs).^{11,12} Each RPD is made up of modular repeat motifs of the same class. We focus on a category of RPDs that explicitly mediates protein-protein interactions (PPI), and in which the repeat motifs in each RPD require each other to maintain their structural fold. Each repeat unit is also relatively short with length of 12-60 amino acids. Many of these classes of RPDs have been studied extensively.¹⁴⁻¹⁶ For example, tetratricopeptide repeat (TPR) domains are made up of only TPR motifs and Ankyrin repeat (ANK) domains of ANK repeat motifs. This modularity gives rise to a strategy that was first introduced in the field of protein engineering to generate protein design templates to create synthetic proteins with desired specificities and affinities.¹⁷⁻¹⁹ We adapted the strategy to build a multiple sequence alignment (MSA) profile, which we term a ‘motif-MSA’ profile, for each class of RPD. Using the TPR as an example of a class of PPI RPD, we demonstrate that the motif-MSA strategy can “amplify” variant signal by aggregating the variants from all homologous motifs for each class of RPD within the human genome. Interestingly, we note that such analyses of intra-genome conservation can only be performed using a dataset as large as those from ExAC. Our Intensification database contains our results as a resource for annotating variants in 12 PPI RPDs (see ‘Methods’ for selection criteria).

Results

Intensification database

Figure 1a shows our strategy that is used to build up the resources in our publicly available Intensification database (<http://intensification.gersteinlab.org>) that relates protein residue to genomic information in 12 RPDs, which encompass 5,508 motifs and 971 proteins in *Homo sapiens* (**Supplementary Table 1**). Our strategy first produces a motif sequence alignment profile for a class of repeat domain. We obtain every repeat motif of a given amino acid length in the human proteome (typically the length with the most number of available motifs). We then perform an MSA of all the motifs (we term ‘motif-MSA’) to obtain a residue frequency table, which shows the percentage occurrence of each amino acid at each position in the motif. This table can then be translated into a sequence logo for better visualization. For each repeat motif, we then locate its genomic positions in the human genome. Subsequently, we map SNVs onto the genomic coordinates of the repeat motifs. This allows us to obtain aggregate counts of variants at each residue positions for each class of repeat domain based on SNV allele frequencies and the functional impact, namely whether the SNV is rare (R) or common (C) in the human population and whether the SNV causes a synonymous (S) or non-synonymous (NS) change. From these statistics, we can subsequently derive more meaningful metrics such as ratio of NS-to-S-SNV profile (NS/S) and enrichment of rare variants (R/C) for interpretation of each residue position. We provide these results for the users in our Intensification database. Here, we use the 34-amino-acid TPR repeat motif as an example (see ‘Methods’ for details; **Figure 1** and **Supplementary Figure 1**).

Comparing species- and motif-MSA

An MSA is more typically performed using homologous sequences from multiple species (Figure 1b; we term ‘species-MSA’). Here, we perform species-MSA for the first three TPR motif sequences in the TPR-containing protein TTC21B, using orthologous sequences from 66 species (see ‘Methods’ for details) (Figure 2a). TTC21B contains about 16-19 TPR motifs, with almost all of them having a length of 34 amino acids and is a cilia-specific protein that is necessary for retrograde intra-flagellar transport.²⁰ Expectantly, most positions are comparably high in sequence conservation. In contrast, the motif-MSA profile exhibits substantially differential sequence conservation among the motif positions (Figure 2b). We were able to easily identify positions 8, 11, 20, 24 and 27 as more conserved within the TPR repeat motif.

Computing population genetic metrics and amplification by motif-MSA

When we focus only on the human species, variant positions in a conventional species-MSA profile are restricted to the sequence of a single human protein (since the alignment is based on orthologs). Hence even with a large catalog of human exonic variants, only three variant positions can occur for each codon (Figure 1b). As such, the variant signal is extremely tenuous for any meaningful downstream population genetics analyses. However, in the TPR motif-MSA, variants are aggregated from all 34-amino-acid TPR motifs within the human genome. This accumulation of variants amplifies the signal, thereby facilitating the computation of various population genetic metrics to investigate selective constraints in the protein domains. At this juncture, we note that even with amplification, our results were only most apparent with the ExAC dataset (60,706 exomes), but not when solely with the 1000 Genomes Project Phase 1 data (1000GP; 1,092 whole genomes)⁸ nor its combination with the Exome Sequencing Project (ESP; 6,500 exomes)¹⁰, which total more than 7,500 protein-coding exomes (Figure 2c and Supplementary Table 2). This further underscores the value of amplification, and exemplifies the fact that more genomes are necessary to yield better statistics for such analyses.

We use four evolutionary measures derived from the accumulation of genomic variants on the motif-MSA. We use the TPR domains as an example to illustrate them.

* **SIFT** – For inter-species conservation, we use the SIFT score of a non-synonymous SNV, which is directly computed from a species-MSA, such that a lower SIFT score denotes a greater likelihood of an SNV being deleterious (most likely due to high residue conservation).⁴ Since protein-coding regions are generally under high selective constraints across species, almost all positions of highly functional PPI domains tend to have very low median SIFT scores across the motif. In the TPR motif-MSA, the most highly conserved position 20 exemplified this observation (Figure 3a).

* **R/C** – As a proxy for intra-species conservation within the human population, we compute a population genetic measure used in the 1000 Genomes Project, the rare-to-common-variant ratio (R/C), where an enrichment of rare variants (or depletion of common variants) signifies high conservation over a shorter evolutionary timescale.⁸⁻¹⁰ We find high rare variant enrichments across the motif-MSA profiles of all classes of RPDs, regardless of residue or positional conservation within the repeat motifs (Supplementary Figure 2).

* **NS/S** – We further compute the NS/S for each position in the motif-MSA profile (Figure 3b). The use of NS/S has been traditionally useful in the estimation of selection pressures in the protein-coding regions typically at the gene level.²¹ Here, rather than at the gene level, the accumulation of variants enables NS/S to be calculated at the codon level (Figure 3b). We observe that most of the positions in the TPR motif with very low NS/S coincide very well with positions of high sequence conservation in the motif-MSA profile. In fact, if we arbitrarily take the top five positions with the lowest NS/S over the human population, four of them correspond to four of the most conserved positions in the TPR motif-MSA, reinforcing the utility of motif-MSA in picking out functionally important residue positions (Figure 3b).

* **Δ DAF (pop)** – The difference of derived (population) allele frequencies, or Δ DAF, has been used in the 1000 Genomes Project to quantify population differentiation and positive selection and identify highly differentiated sites between pairs of populations.^{8,22} Because the majority of the variants are rare even within sub-populations, we observe that most SNVs have low Δ DAF < 0.5. More interestingly, we were able to identify some TPR residue positions that seem to harbor more variants that are highly differentiated between populations than other positions (Figure 3f). High differentiation can be indicative of positive selection and adaptive evolution among the human populations.

Combining protein and genomic information to identify important residues

Using the motif-MSA, we are able to integrate both protein (from MSA) and genomic information (SNVs) to better pinpoint positions that might be more functionally important. By combining positions with the highest five sequence conservation in the TPR motif-MSA and the lowest five median SIFT scores and NS/S ratio, we are able to identify eight positions (out of 34 positions on the TPR motif), with four positions that fulfil at least two of the three selective constraint conditions (Figure 3c). The differences in R/C between positions within the TPR motif-MSA are too subtle to be used.

Mapping genomic information onto protein structures

Because the motif-MSA identifies important residues in the simplest unit of an RPD, we can visualize the residues in 3D structures of the same class of RPDs with any number of motifs. As an example, we use the X-ray crystal structure of a three-motif TPR domain (TPR1) from the human protein Hsp-organizing protein (HOP) bound to its cognate ligand, a short peptide sequence consisting of seven amino acids, PTIEEVD (PDB ID: 1ELW).²³ We map the eight positions derived from Figure 3c onto all three motifs of the protein structure, identifying 24 residues in total (Figure 3d). In each TPR motif, except for position 17, we find that all the other seven residue positions with high selective constraints – from either low median SIFT scores, low log (NS/S) or high motif sequence conservation – are buried residues in the PPI domain (Figure 3d), in line with a previous study.²⁴

Relating residues positions to clinically-relevant and disease-related mutation data

Using two databases, ClinVar²⁵ and the proprietary Human Gene Mutation Database (HGMD)²⁶, we found that the highly constrained positions have some of the most occurrences of clinically-relevant or disease-related mutations along the TPR motif-MSA profile, including the highest two at positions 6 and 7, which would otherwise not be detected if only motif-MSA or inter-species conservation was used (Figure 3e). In fact, mechanistic studies of a number of these

mutations show that the occurrence of certain NS mutations on these positions give rise to diseases precisely as a result of ablation of protein-protein interactions.^{27,28}

Discussion

For decades, the focus in research on PPI has typically been the investigation of protein interfaces that directly take part in the protein interaction. Most studies involved the use of 3D protein structures, for instance, to identify protein-protein interfaces,^{29,30} investigate interfacial properties^{31,32} or to predict interacting ‘hotspots’^{33–35}. While extremely useful in protein engineering and drug design, it is also very limited by the number of available protein structures. On the other hand, the amount of human sequencing data has been growing dramatically over the past decade, in particular, the number of protein-coding exome sequences.³⁶ This huge trove of sequence information should be leveraged upon for variant annotation in protein-coding regions, especially in the interpretation of protein data. Our introduction of the motif-MSA facilitates genomic analyses with protein information (and vice versa) in several ways.

Firstly, motif-MSA removes the limitation imposed by species-MSA. Thus far, the utility of protein sequences has been largely focused on the more traditional perspective of sequence conservation across multiple species based on homology.^{5,6,37} While there is independence for each aligned orthologous sequence, the functional similarity of the sequences gives rise to widespread conservation across the species-MSA. On the other hand, in motif-MSA, while aligned motif sequences can be co-dependent because they come from the same protein, the functional dissimilarity and structural similarity give rise to differential conservation across the MSA. Moreover, we can systematically aggregate variants from similar protein regions within the genome of a single species in a reasonable manner to identify important sites, regardless of whether the sites are independent of one another. This aggregation is key to achieving the variant statistics required to perform analyses that are meaningful, especially in light of the observation that even a combined set of 1000GP and ESP6500 variant data, derived from almost 7,600 exomes, was not sufficient to yield immediately-interpretable results (**Figure 2c and Supplementary Table 1**). At this point, it is also important to note that the motif-MSA contains two categories of ‘variations’, namely variations found in the repeat motif sequences of the human reference genome and genomic variant information from a representative human population. Motif sequence variation can stem from the duplication and divergence of the same class of repeat motifs within the genome, and can be of long and short evolutionary timescales (before and after speciation). In contrast, the genomic variant catalogue corresponds to the possible polymorphisms found in the human population, representing a shorter evolutionary timescale of a single species. Thus, the biological interpretation of selective constraints in metrics such as $\log(\text{NS}/\text{S})$ is a confluence of evolutionary timescales and mutation processes.

Secondly, the ability to gain statistical power from variant aggregation makes motif-MSA an extremely powerful platform in investigating evolutionary constraints using genomic information. Potentially, motif-MSA is amenable to the entire repertoire of genomic metrics. We used four metrics as examples to demonstrate how motif positions and residues that show evidence for clinical and disease relevance can be identified beyond the use of the more conventional species conservation (**Figure 3**).

Lastly, motif-MSA is also able to reflect protein structural properties and their roles in PPI. Conventional species-MSA aligns sequence orthologs that are similar in function and structure. Hence, highly conserved residues or positions are a mix of structural and functional residues. On the other hand, because the protein motifs are classified by their structural folds, sequence features in a motif-MSA are important structural features that determine the folds of the PPI domains. These features are observed as buried residues within the interior of PPI domains (Figure 3d). In addition, it has been suggested that because motifs in motif-MSA are from a myriad of proteins with diverse binding partners, positions that are low in sequence conservation, or ‘hypervariable’, are found in the binding pockets of the corresponding domains.^{24,38} We noticed few hypervariable positions harbor a large number of disease-related variants, for example, position 2 in TPR motifs, which has been identified by the Δ DAF analysis. Hence, while we cannot definitively identify interface residues that participate in protein interactions, motif-MSA does still hold potential in facilitating such an endeavor.

The motif-MSA approach provides a powerful and versatile platform to facilitate the combination of protein and genome information for use in the annotation of protein structures. It enables the leveraging of the vast amount of human sequencing data currently available. This will become increasingly more imperative and urgent in the future as human genome sequencing becomes more commonplace and personal genome interpretation takes center stage.

Methods

Intensification database

Our publicly available Intensification database (<http://intensification.gersteinlab.org>) provides data files for 12 RPDs, namely ankyrins (ANK), annexins (ANX), armadillos (ARM), cadherin repeats (CA), fibronectin type 2 domains (FN2), fibronectin type 3 domains (FN3), leucine-rich repeats (LRR_TYP), spectrin repeats (SPEC), tetratricopeptide repeats (TPR), ubiquitin-interacting motifs (UIM), WD40 repeats (WD40), and WW domains (WW). The 12 RPDs were semi-manually curated from the domains found in the SMART database for species, *Homo sapiens* (downloaded Oct 25, 2013),⁴⁰ and selected for those that are known to mediate protein-protein interactions and have at least 20 unique repeat motifs in the human genome as annotated by SMART database (Supplementary Table 1). The results for each class of RPD is a tarball, which contains residue frequency tables (to rebuild the sequence logo), the SIFT score distributions, median SIFT scores, log(NS/S), log(R/C) and values for each position along each RPD motif to allow versatile thresholding by the users. The resource and scripts used in the pipeline are freely downloadable at the database.

Multiple sequence alignment (MSA)

All protein, motif and domain information are extracted from Ensembl database version 73, and SMART database, under the ‘genomic’ mode, for species, *Homo sapiens* (downloaded Oct 25, 2013).⁴⁰ The 12 PPI repeat domains are manually selected based on their availability in the SMART database, repeat nature and involvement in PPI.

We will use the TPR domains as an example to illustrate the process of motif- and species-MSA in our study.

To obtain a motif-MSA sequence profile, (1) we first extract all TPR domains in the human proteome and break them up into its constituent motifs. (2) Here, the motif-MSA is performed based on the most representative size of the motif. Hence, in order to select the motif size, a histogram of all sizes of TPR motifs is constructed ([Supplementary Figure 1](#)) and the most common motif size is selected for motif-MSA alignment; in TPR motifs, the most common motif size is 34 amino acids. There are a total of 114 human proteins (from unique genes) with 571 unique 34-amino-acid TPR motif sequences; we only keep one motif when there are multiple with 100% sequence identity. (3) MSA is then performed on of these 571 TPR motifs with 34 amino acids, with no gaps allowed, i.e. we line up all sequences by position end to end. This ‘ungapped’ alignment allows the derivation of a 20-by- n frequency table for 20 residues and n positions on the motif profile, and subsequently, visualization, using a sequence logo constructed by WebLogo 3.2.⁴¹

The TPR species-MSA is obtained by aligning the homologous protein sequences of TTC21B from 43 species in an ‘ungapped’ fashion. Using the MEGA5 software⁴², we extracted the TPR domain from the 45-sequence alignment, based on the human TTC21B information in SMART database. There are 16 TPR motifs in TTC21B found in the SMART database. We remove two orthologs due to the existence of gaps in at least one of the 16 TPRs. Finally, we construct the sequence logo of all 16 TPRs using WebLogo 3.2.⁴¹ We show the alignment of only the first three TPR motifs of TTC21B in [Figure 2a](#).

Sequence logo visualization

All sequence logos are created by WebLogo 3.2⁴¹, using the following parameters:

```
-A protein -U bits --composition
```

```
"{'L':9.975,'A':7.013,'S':8.326,'V':5.961,'G':6.577,'K':5.723,'T':5.346,'I':4.332,'E':7.096,'P':6.316,'R':5.650,'D':4.728,'F':3.658,'Q':4.758,'N':3.586,'Y':2.653,'C':2.307,'H':2.639,'M':2.131,'W':1.216}
```

```
" -n 34 -c chemistry --stack-width 25 --errorbar no
```

For the ‘composition’ parameter (used for the relative entropy calculation), we provided manually the background distribution of the amino acids in the entire SMART database (‘genomic’ mode), in order to be in line with our input data from the SMART database; the values above are in percentages. We separately computed these values from the SMART database.

Variant information from exomes

For all the analyses in this study, we use the SNVs and their minor allele frequencies from 60,706 exomes found in the ExAC database³ (Version 0.3, downloaded February 1, 2015), after removing the variants from the sex and mitochondrial chromosomes and singletons (those variants that only occur in one chromosome in the entire ExAC dataset). This ends up with **7,202,445** autosomal SNVs. We obtained SIFT scores, and non-synonymous nature of the SNVs on the proteins using the VEP tool (Version 73) from Ensembl release 73.⁴³ DAF information is derived from 1000 Genomes Phase 1 SNVs with ancestral alleles and ExAC population frequencies. Δ DAF (pop) values are computed from the pairwise differences in population DAF for each SNV among five populations, namely Africans/African Americans (AFR), Latino (AMR), East Asians (EAS), South Asians (SAS) and Europeans (EUR), which are combined

from the Finnish (FIN) and non-Finnish European (NFE) populations in ExAC; we excluded SNVs in the ‘others’ (OTH) category. For Figure 3f, only non-synonymous SNVs are used, and in addition, any SNVs with < 100 subjects in a population that is being compared is removed, because they will skew Δ DAF.

To produce [Figure 2c](#) and [Supplementary Table 2](#), we have used a combined number of **1,328,447** unique, non-singleton, and autosomal SNVs from the 1000 Genomes Project Phase 1 (1,092 whole genomes)⁸ and Exome Sequencing Project data (6,500 exomes)¹⁰.

All coordinates are based on the human reference genome assembly version of hg19.

Relating genomic and protein information

Custom scripts are written to relate genomic to protein information. The key portion is in identifying codon coordinates. We first obtain all genomic coordinates and strand information of protein-coding exons and residue coordinates of SMART protein domains from Ensembl 73 and GENCODE 18 on the reference genome, hg19. The exon information will give us the exact genomic coordinates of the codons for each protein-coding gene, using the locations of the exon-intron junctions. This allows mapping of genomic variants to specific codons, enabling positional accumulation of variant information across a motif-MSA profile. **These scripts are part of the pipeline available for download in the Intensification database.**

Protein structure visualization

The X-ray crystal structures from Protein Data Bank (PDB) are created using Pymol 1.3.⁴⁴

Clinically-relevant and disease-related variants

Clinically-relevant and disease-related variants in GRCh37 were downloaded from ClinVar²⁵ on July 8, 2015 and the proprietary HGMD Professional Database downloaded on July 27, 2015.²⁶

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Conflicts of interest statement

The authors declare that there is no conflict of interest.

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Figure Legends

Figure 1. Our motif-MSA approach amplifies variant information as compared to species-MSA. (a) (1) We first query a database and obtain all the proteins with the desired domains or motifs. We use the TPR motifs as an example in this figure. These motifs have to be the same length. Here, we select TPR motifs that are 34 amino acids since they are the most frequently-occurring size. (2) Subsequently, we perform an ‘ungapped’ multiple sequence alignment (MSA) of the human TPR motifs by lining them up end to end, to obtain a sequence conservation profile. This motif-based MSA (black sequence logo) typically exhibits differential sequence conservation among the positions across the length of the motif. (3) The third step involves collecting genomic single nucleotide variants (SNVs) for each amino acid position of the motif-based alignment profile. In TPR domains, we obtain the specific genomic coordinates of each codon (in each motif), and then we locate all variants (black diamonds) that fall into each codon, allowing us to aggregate variants over all motifs within the human genome, thereby amplifying variant information sufficiently for further downstream analyses. (4) For each motif-MSA, we then host the results on our Intensification database. For each protein repeat domains, we build a motif-MSA, and compute corresponding SNV profiles, including residue frequency tables, $\log(\text{NS}/\text{S})$, $\log(\text{R}/\text{C})$ and SIFT score distributions. (b) For species-MSA, we align orthologous sequences across multiple species. However, because we are focusing on proteins and sequencing data only in the human species, only three variant positions can occur at each codon in a species-MSA profile. We illustrate this with the human protein, TTC21B, which contains TPR motifs.

Figure 2. Motif-MSA can uncover important domain positions missed by species-MSA. This figure uses TPR as an example. (a) We perform a species-MSA using orthologous TTC21B from 66 species (species-MSA). Here, we show the alignment profiles for the first three TPR motifs (red, blue and green sequence logos), out of the possible 16. We observe that almost all the positions are highly conserved. (b) In contrast to conventional species-MSA, there is a differential sequence conservation profile across the TPR motif-MSA (black sequence logo), which facilitates the identification of more conserved motif positions that are potentially important (five positions are highlighted in orange). (c) In order to show the utility of motif-MSA and amplification, we compare the results for $\log(\text{NS}/\text{S})$ among three variant sets, namely from 1000 Genomes Project Phase 1 (1000GP), the combined set of 1000GP and the Exome Sequencing Project (1000GP+ESP6500) and the ExAC dataset. We can see that there are only subtle differences in $\log(\text{NS}/\text{S})$ for each position along the TPR motif when using variant datasets from 1000GP to 1000GP+ESP6500. We were only able to make meaningful interpretations only when we use variant data from ExAC.

Figure 3. Using genomic variant information in the motif-MSA profile to investigate selective constraints in PPI motifs. Using SNVs from the ExAC dataset, we use various SNV properties to investigate the extent of selective constraints at each position in the motif-MSA profile. (a) For each non-synonymous SNV, a score can be computed from the SIFT tool, where a lower SIFT score means the SNV may be more deleterious. Each blue violin plot represents the distribution of SIFT scores at each position in the TPR motif, with the width of the plot showing

frequency density and the black dot denoting the median SIFT score. The distribution provides an estimation of selective constraints based on inter-species comparison. **(b)** We can also calculate the log ratio of non-synonymous versus synonymous SNVs ($\log \text{NS/S}$). A depletion of NS variants with respect to the background of S SNVs suggests a position might be functionally significant. **(c)** The five positions with the least median SIFT scores are numbered in blue according to their rank (there are four positions tied at rank 2). The five positions with the lowest $\log \text{NS/S}$ are ranked in red. The top five most conserved positions in the motif-MSA are highlighted in orange. There are eight candidate positions which fulfil at least one of the above criteria of the lowest SIFT median scores, $\log \text{NS/S}$ and motif-MSA sequence conservation, with four positions satisfying at least two. **(d)** Using the X-ray crystal structure of the human HOP TPR1 domain (PDB ID: 1ELW), it consists of three TPR motifs as shown as cartoon ribbons in the inset, colored separately in shades of grey (white represents capping helix). We can see the 24 residues (8 residues in each of three motifs) in the spatial context and observe that they are mostly buried residues. Residues 6 and 7 are identified by SIFT scores (blue in (a)). Residues 8, 11, 20, 24 and 27 are identified motif conservation (orange). Residue 17 is identified by $\log \text{NS/S}$. The ligand-binding convex profile of the TPR1 domain (the cognate ligand is represented by the green stick model) is rotated 180° to reveal the concave profile of the same TPR1 domain. **(e)** We also use two databases, ClinVar (dark purple) and HGMD (light purple) and the union of the two sets (purple), to demonstrate which TPR motif positions accumulates more clinically-relevant and disease-related SNVs. We use the same color scheme to number the residue numbers identified in (d). **(f)** The collective ΔDAF values for non-synonymous SNVs found for the TPR motif-MSA show that some motif positions, such as positions 2, 17 and 32, contain more SNVs that are highly differentiated among populations than other positions. We calculated the ΔDAF for pairwise comparisons of five populations, namely individuals with African ancestry (AFR), as well as, Latino (AMR), East Asian (EAS), European (EUR) and South Asian (SAS) ancestries. The DAFs are compared between pairs of populations.

Supplementary Figure 1. The most frequent size of the TPR motif is 34 amino acids.

Supplementary Figure 2. For each SNV, the minor allele frequency (MAF) in the human population can determine whether an SNV is rare ($\text{MAF} \leq 0.005$) or otherwise, common. The log ratio of the number of rare versus common variants ($\log \text{R/C}$) represents the enrichment/depletion of rare variants, which has been used as a metric for estimating selective constraints based on intra-species comparison. All positions have an enrichment of rare variants, with position 25 having no common variants (\log ratio with a zero denominator is undefined).

Supplementary Table 1. The lists of repeat domains that we performed the motif-MSA approach and are included in the Intensification repository, with corresponding number of motifs (regardless of size) and proteins in the human proteome.

Supplementary Table 2. The 1000 Genomes Project (1000GP) provides the least number of autosomal SNVs, followed by an approximate 6-fold increase in number of exomes in the combined set of 1000GP and Exome Sequencing Project (ESP6500); this is a corresponding ~3-fold increase in the number of autosomal SNVs. Our study uses the dataset from ExAC, with 60,706 individuals, an almost 8-fold increase from the combined set of 1000GP+ESP6500; this is a corresponding ~5-fold increase in the number of autosomal SNVs.

