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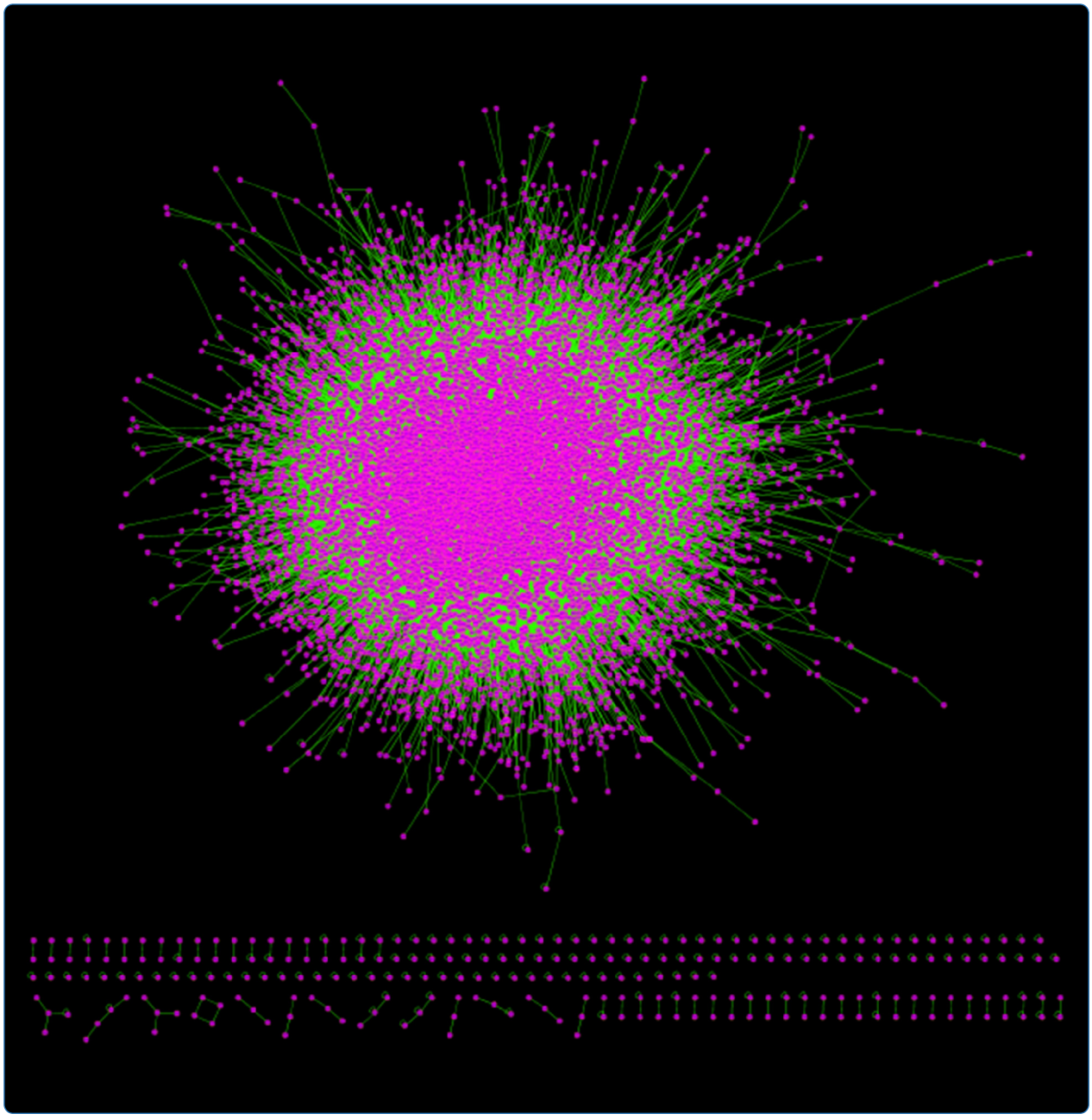

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Reconstruction of the experimentally supported human protein interactome: what can we learn?

Maria I Klapa[1], Kalliopi Tsafou[2,1], Evangelos Theodoridis[3], Athanasios Tsakalidis[3] and Nicholas K Moschonas[2*]

Abstract

Background: Understanding the topology and dynamics of the human protein-protein interaction (PPI) network will significantly contribute to biomedical research, therefore its systematic reconstruction is required. Several meta-databases integrate source PPI datasets, but the protein node sets of their networks vary depending on the PPI data combined. Due to this inherent heterogeneity, the way in which the human PPI network expands via multiple dataset integration has not been comprehensively analyzed. We aim at assembling the human interactome in a global structured way and exploring it to gain insights of biological relevance.

Results: First, we defined the UniProtKB manually reviewed human "complete" proteome as the reference protein-node set and then we mined five major source PPI datasets for direct PPIs exclusively between the reference proteins. We updated the protein and publication identifiers and normalized all PPIs to the UniProt identifier level. The reconstructed interactome covers approximately 60% of the human proteome and has a scale-free structure. No apparent differentiating gene functional classification characteristics were identified for the unrepresented proteins. The source dataset integration augments the network mainly in PPIs. Polyubiquitin emerged as the highest-degree node, but the inclusion of most of its identified PPIs may be reconsidered. The high number (>300) of connections of the subsequent fifteen proteins correlates well with their essential biological role. According to the power-law network structure, the unrepresented proteins should mainly have up to four connections with equally poorly-connected interactors.

Conclusions: Reconstructing the human interactome based on the *a priori* definition of the protein nodes enabled us to identify the currently included part of the human "complete" proteome, and discuss the role of the proteins within the network topology with respect to their function. As the network expansion has to comply with the scale-free theory, we suggest that the core of the human interactome has essentially emerged. Thus, it could be employed in systems biology and biomedical research, despite the considerable number of currently unrepresented proteins. The latter are probably involved in specialized physiological conditions, justifying the scarcity of related PPI information, and their identification can assist in designing relevant functional experiments and targeted text mining algorithms.

Keywords: Human protein interactome analysis, Human protein-protein interaction (PPI) databases, Network biology, PPI network reconstruction

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Background

Deciphering the structure and dynamics of the protein-protein interaction (PPI) networks is among the major objectives of the systems biology research in the quest for the mechanisms of life. For the human protein interactome in particular, its reconstruction and further exploration of its topology and dynamics are expected to have a significant impact in biomedical research and applications [1,2]. The number of experimentally supported PPIs has drastically increased for model organisms since 2000 [3-7] and for the human interactome since 2005 [8,9] mainly due to the gradually increasing number of high-throughput methodologies for PPI detection. The experimentally identified PPIs are mined from the literature and stored in bulk in PPI databases, most of which are repositories for many species. For the human interactome, the various source PPI databases report the protein identifiers at different molecular levels of biological information, and include protein interaction sets of limited overlap due to own literature mining criteria, differences in PPI incorporation rates from small-scale experiments, as well as differences in methods for PPI selection, curation and updating [10-14]. Therefore, several PPI meta-databases also exist, combining information from multiple source databases [15-23]. However, as each meta-database has distinct curation objectives and methods for data normalization and integration, the use of its combined PPI dataset may not be straight away comparable to the direct query on the source databases [11,12]. In addition, it is worth mentioning that the set of protein nodes of a meta-database network varies depending on the PPIs of the employed source datasets, and it may change upon updating or incorporation of new datasets. This fact creates heterogeneity between the various PPI meta-databases and hinders the direct comparison among their networks [11]. Because of this inherent heterogeneity, although there have been many studies comparing a variety of PPI datasets [10-14], the way in which the human protein interactome expands via the integration of multiple datasets has not been comprehensively explored; therefore, a global perspective of the biology emerging from the network structure is still eluding.

The objective of the present study is to reconstruct the current experimentally supported network of direct human protein interactions in a global structured way, explore it to obtain information about the fraction of the human proteome that it currently involves, discuss the biological role of proteins within the topology of the network, and identify the presently absent from the network ("orphan") proteins. To this end, we started by defining the UniProtKB manually reviewed human "complete" proteome [24] as the reference set of nodes that the human PPI network can have. Then, we mined five major source PPI databases, i.e.: HPRD [25], IntAct [26], MINT [27], DIP [28] and BioGRID [29], for direct interactions exclusively between members of the defined reference protein set. After appropriate updating of the old and filtering of the obsolete protein identifiers, the acquired PPI data were normalized to and combined at the UniProt protein identifier level. We analyzed the reconstructed network to discuss whether the revealed role of proteins based on their position in the interactome topology is supported by the currently available knowledge about their function. In addition, based on the verified scale-free structure of the PPI network in human [1,30], we predict the number of connections of the unrepresented proteins and provide a novel perspective about the presently "missing" part of the interactome.

Methods

Protein and PPI datasets

The UniProtKB/Swiss-Prot manually reviewed human "complete" proteome

From UniProtKB, the knowledgebase of the Universal Protein (UniProt) resource [24], we downloaded the tab-delimited files of: (a) the entire set of human UniProt identifiers, and (b) the manually reviewed human "complete" proteome. The latter contained 20,242 UniProt identifiers in the Dec 14 2011 release of UniProtKB downloaded on Jan 23 2012. The two tab-delimited files included all default columns augmented by the cross-references with the EMBL nucleotide, the NCBI nucleotide and the Entrez Gene databases. The text file indicating the correspondence of the secondary to the respective primary UniProt identifier(s) was downloaded too.

The Human Protein Reference Database (HPRD)

HPRD is a manually curated reference database for human protein information [25]. In this study, we used only its binary PPI dataset, which is provided in the form of interactions between HPRD identifiers. From the total 19651 HPRD identifiers in the HPRD version 9, downloaded on Jan 23 2012, 9673 were involved in at least one of the 39204 PPIs reported as binary interactions. Only the primary one-to-one correspondence of the HPRD identifiers to nucleotide sequence identifiers was considered. Any necessary updating or conversion of the nucleotide sequence identifiers to other molecular levels of biological information (i.e. gene or protein level) was carried out through cross-reference with current versions of the relevant databases.

IntAct

IntAct, a main partner of the International Molecular Exchange (IMEx) Consortium [10], is a repository of molecular interaction data for multiple organisms [26].

In the single file supplied by IntAct for external use, including interaction information from all species, PPIs are provided mainly at the UniProt protein identifier level. From the Jan 3, 2012 release downloaded on Jan 30, 2012, only the non - "spoke" PPIs between two human protein identifiers were retained, as the label "spoke" characterizes the PPIs originated from protein complex expansion.

The Molecular INTeraction database (MINT)

Similarly to IntAct, MINT [27] is a repository of literature-curated PPIs from multiple organisms and an IMEx consortium partner with PPI information provided mainly at the UniProt protein identifier level. The binary PPI file for human used in the present study was downloaded on Jan 30, 2012 (release date: Dec 8, 2011).

Database of Interacting Proteins (DIP)

DIP [28] is also a collection of experimentally supported protein interactions from multiple organisms and among the first partners of the IMEx consortium. In the downloaded on Jan 30, 2012 PPI file for human (release date: Oct 27 2011), PPIs are provided as interactions between DIP identifiers. The latter are corresponded mainly to UniProt protein identifier(s) and most to NCBI nucleotide RefSeq identifier(s), too.

The Biological General Repository for Interaction Datasets (BioGRID)

BioGRID [29] is the most recently initiated among the five source PPI databases used in this study, currently participating in the IMEx consortium as an affiliate member. The PPI file for human was downloaded from the BioGRID web site on Jan 30, 2012 (release 3.1.84 tab2 file). PPIs are provided as interactions between BioGRID identifiers, which are in one to one correspondence to Entrez Gene identifiers (GeneID). BioGRID provides extensive information about the experimental method and the nature, i.e. low- or high- throughput, of the experimental set-up used for any PPI detection; however, it does neither make a distinction between binary interaction and protein complex data nor provide a relevant filtering criterion. To avoid including PPI data expanded from protein complexes, we opted to keep (a) all physical associations identified in low-throughput setups and (b) from the physical associations detected only in high-throughput experiments, those derived from any of "protein complementation assay (PCA)", "reconstituted complex", "protein-peptide", "FRET", "two-hybrid" or "co-crystal structure" methods. Genetic interactions provided in BioGRID were *de facto* filtered out.

PPI data mining

Direct PPIs with both interactors belonging to the set of the 20,242 primary UniProt identifiers included in the manually reviewed human "complete" proteome were mined from: (a) the binary PPI dataset of HPRD, (b) all PPIs of IntAct not characterized with the term "spoke" in the "expansion" field, (c) the binary PPI dataset of MINT, (d) the DIP dataset, which is provided as containing only binary manually reviewed PPIs, and (e) all physical associations in BioGRID detected in at least one low-throughput experiment or by any of the detection methods mentioned above, if identified only in high-throughput setups.

Protein identifier normalization

Normalization of the protein identifiers to the UniProt identifier level was required for: (a) HPRD, since it reports the interactors at the nucleotide sequence level, (b) BioGRID, which reports the interactors at the gene level and (c) few cases of IntAct, MINT and DIP, for which other than the default UniProt identifier has been used.

Source PPI dataset uploading

To upload, store and handle the five PPI datasets and integrate them into the final reconstructed PPI network, the Microsoft SQL Server (MSSQL) 2008 Developer Edition platform equipped with SQL Server Integration Services (SSIS) was used under the University of Patras academic license. The source PPI dataset uploading was organized in a set of SSIS modules executed at the server side. Each module involves a series of subtasks for the filtering and updating of certain data from the source PPI dataset, along with a large number of checks to monitor and handle exceptions, avoiding thus the contamination of the final database with erroneous or ill-formatted data. Additional file 1 shows the workflow for the IntAct uploading sub-module.

The first subtask of the filtering and updating algorithm involves the extraction of the interactions between human protein identifiers. In sequence, the main interactor identifiers are retained for each PPI. For IntAct, MINT and DIP, the interactors are expected to be represented by a UniProtKB accession number. If the relevant format is not recovered from the algorithm for any of the two interactors, then the non-UniProt interactor identifier is compared against a maintained interactor identifier dictionary. If matched to a dictionary entry and identified as active, the non-UniProt interactor identifier is replaced by the corresponding primary UniProt identifier. If it has become obsolete or cannot be assigned to a UniProtKB accession number, it is removed from the finally uploaded dataset along with all associated PPIs. If active, all isoform UniProt protein identifiers are replaced by their primary UniProt

identifier(s). Any remaining non-UniProt interactor identifiers are stored in a separate table, for the curator to appropriately update the interactor identifier dictionary, so that the "patching" process is completed in a second iteration. In HPRD, the interactor identifier dictionary is used to update the nucleotide sequence identifiers to their currently active entries. Notably, among the 9673 HPRD identifiers involved in PPIs, 119 were identified to correspond to obsolete nucleotide sequence identifiers, 4 corresponded to non protein-coding RNAs, while 16 were replaced by new nucleotide sequence identifiers; due to this updating, in three cases, two HPRD identifiers were assigned to the same nucleotide sequence identifier. In BioGRID, all interactors were identified by an active Entrez GeneID, thus no updating was necessary. For the PPIs remaining after the interactor identifier patching step, the algorithm inspects the identifier of the supporting publication(s). If no publication is provided, the PPI is removed from the uploaded dataset. If a non-PubMed publication identifier is provided, this is patched based on an in-memory maintained dictionary as described for the interactor identifiers in the previous step. The utilized interactor identifier dictionary was created based on information recovered from the online UniProt converter and the online versions of all relevant databases on February 2, 2012. The Digital Object Identifier (DOI) numbers and IMEx reference identifiers were assigned to their PubMed publication identifiers based on an online converter and the online version of MINT, respectively. After uploading IntAct, MINT and DIP, their PPI data were further processed based on information from UniProtKB to include only interactions between two active primary UniProt identifiers in the human manually reviewed "complete" proteome.

Gene functional classification analysis

Gene functional classification analysis was carried out using the DAVID Bioinformatics Resources version 6.7 [31,32] by combining all available gene annotation categorizations.

Identification of network characteristics

The identification of the reconstructed PPI network characteristics was carried out using the relevant "Network Analysis" tool of the open source network visualization and analysis software Cytoscape - version 2.8 [33].

Results and discussion

Reconstructing the human protein interactome based on a well-defined set of protein nodes

The novelty of our approach regarding the PPI data integration from major literature-curated source PPI datasets compared to existing meta-databases was the *a priori* definition of the set of nodes of the human protein interactome considering the UniProtKB manually reviewed human "complete" proteome as a robust, well-defined reference set. Thus, instead of merging PPI information for any protein identifier stored in the source databases, the latter were selectively mined for PPIs exclusively between members of the as above defined reference human protein set.

For proper normalization of the source PPI datasets to the UniProt identifier level, it was also important to consider the continuous updating of biological information, since it can lead to changes in the annotation of protein identifiers and in their associations at other molecular levels. Thus, we proceeded to a careful updating of the old and filtering of the obsolete protein identifiers in the source datasets based on the current knowledge about gene annotation. UniProtKB and its cross-references with major resources at the nucleotide sequence and gene levels of molecular information (i.e. NCBI, Entrez Gene and EMBL databases) provided a valuable reference for the appropriate normalization of HPRD and BioGRID identifiers to the UniProt level, and of a small fraction of IntAct, MINT and DIP protein entries that were not provided at the default UniProt level. It is noted that during this conversion to the UniProt level, 1920 BioGRID identifiers reported as human were found to correspond to non-human UniProt identifiers (data not shown), leading thus to the exclusion of their PPIs from the final integrated PPI network.

In the normalized HPRD, IntAct, MINT, DIP and BioGRID files, only the PPIs between two active primary UniProt identifiers in the manually reviewed human "complete" proteome were retained. These datasets were combined keeping one record for each included PPI. A last source of PPI redundancy in the normalized datasets that was eliminated, concerns the double reporting of an interaction using opposite sequence of the two interactors. In some cases, such duplications may have been intentionally included by the curator of a source PPI dataset to report the experimentally supported sequence of the interactors; this type of duplications were encountered in IntAct and MINT. In most cases, however, they were just a product of the protein identifier conversions at the various stages of the PPI dataset uploading and formatting and had to be eliminated at the integration stage.

The final integrated PPI dataset will be referred to as the PICKLE (Protein InteraCtion KnowLedge BasE) dataset. Table 1 shows the number of (a) the direct PPIs in the PICKLE and the normalized source PPI datasets, (b) the UniProt identifiers in the manually reviewed human "complete" proteome covered by each of them, and (c) the publications providing experimental evidence for the PPIs. As expected, the integrated PICKLE dataset is much larger than any of the individual source datasets

Table 1 The size of the reconstructed direct PPI network for the manually reviewed human "complete" proteome

	Number of UniProt Identifiers[(1)]	Number of Direct PPIs[(2)] (x)	Number of Supporting Publications (y)	Mean Number of PPIs per Publication (x/y)
UniProtKB manually reviewed human "complete" proteome	20242	N/A	N/A	N/A
HPRD	9303	37152	19267	1.93
IntAct	6666	19425	1598	12.15
MINT	6102	16147	2398	6.73
DIP	1795	2609	1180	2.21
BioGRID	9265	42647	13818	3.08
PICKLE	**11827**	**75965**	**26689**	**2.85**

The size of the reconstructed network is compared to the size of the normalized to the UniProt identifier level source PPI datasets for the defined reference protein set. N/A: not applicable.
[(1)]members of the UniProtKB manually reviewed human "complete" proteome.
[(2)]between members of the UniProtKB manually reviewed human "complete" proteome.

with respect to the number of PPIs, of the protein interactors and of the supporting publications, verifying the value of PPI resource integration.

Reconstructing the PPI network in this global structured way:

- we resolve the issue of potential protein identifier and consequently PPI redundancy in the network originating from the combination of records of multiple databases reporting at different levels of biological information;
- we determine which protein nodes of the manually reviewed human "complete" proteome remain with no direct PPIs ("orphan" proteins) and discuss this fact in the context of the current information about these proteins;
- we comment on the proteins represented in the interactome with a high number of PPIs with respect to the importance of their function within the entire network;
- we consider the human interactome in its entirety, commenting on its future expansion to the maximum potential format in the context of the expected scale-free structure, a fundamental feature of PPI networks [30,34]. Consequently, the interactome reconstructed in the presented way can only grow in edges (PPIs) between the defined set of protein nodes, while keeping its scale-free form. In this global context, we can argue for the expected number of interactions for the "orphan" protein nodes and for the type of their interactors, suggesting a novel perspective for the currently "missing" part of the network, as it is discussed in the following sections.

The reconstructed interactome covers nearly 60% of the manually reviewed human "complete" proteome

Out of the 20,242 UniProt identifiers in the manually reviewed human "complete" proteome, 11827 (58.4%) were found to have a total number of 75965 direct interactions (Table 1). Gene functional classification analysis (see Methods section) of the proteins currently included in the reconstructed interactome compared to the "orphan" ones did not indicate any functional annotations that could differentiate the one group from the other. Thus, the presently "orphan" proteins are not associated with any apparent functional or subcellular location characteristics that could "hinder" them from binding with other proteins.

Dataset integration augments the overall network mainly with additional interactions for largely overlapping sets of proteins

HPRD and BioGRID are the main contributors of the overall human PPI network, comprising, respectively, 78.7% and 78.3% of its UniProt identifiers, and 48.9% and 56.1% of its PPIs (Table 1 and Figures 1, 2 and 3). Moreover, exclusion of the information from HPRD and BioGRID would, respectively, decrease the overall network by 20.4% and 18.9% in proteins and 33.2% and 39.1% in PPIs. These characteristics can be partially justified by the number of references used by each of these two databases, constituting 72.2% (HPRD) and 51.8% (BioGRID) of the total number of supporting references. In addition, HPRD is one of the first literature-curated databases, having though a decline in the rate of reference (and thus PPI) incorporation after 2005 (Figure 3B). BioGRID is currently the fastest growing, having also incorporated a significant part of the HPRD PPI network at the time of its creation [11,29]. This information complements the observed much higher curation overlap between HPRD and BioGRID compared to the other pairs of source PPI datasets discussed by Turinsky et al. in [12]. On the other hand, IntAct corresponds to the largest ratio of PPIs per number of references, i.e. 12.1, followed by MINT, i.e. 6.7 (Table 1), indicating that a major fraction of their datasets originates from

		UNIPROT_IDs						PPIs						REFs					
		Source of Data					PICKLE	Source of Data					PICKLE	Source of Data					PICKLE
		HPRD	IntAct	MINT	DIP	BioGRID		HPRD	IntAct	MINT	DIP	BioGRID		HPRD	IntAct	MINT	DIP	BioGRID	
Contributions from a SINGLE dataset	HPRD	942					942	14793					14793	9277					9277
	IntAct		476				476		8476				8476		556				556
	MINT			268			268			3735			3735			1092			1092
	DIP				45		45				932		932				769		769
	BioGRID					766	766					19271	19271					4650	4650
Contributions from TWO datasets	HPRD-BIOGRID	1465				1465	1465	10421				10421	10421	7792				7792	7792
	HPRD-INTACT	242	242				242	1142	1142				1142	396	396				396
	HPRD_MINT	227		227			227	1839		1839			1839	592		592			592
	HPRD-DIP	50			50		50	285			285		285	90			90		90
	IntAct-BioGRID		313			313	313		1284			1284	1284		92			92	92
	IntAct-MINT		92	92			92		1295	1295			1295		16	16			16
	IntAct-DIP		6		6		6		27		27		27		11		11		11
	MINT-BioGRID			243		243	243			3111		3111	3111			150		150	150
	DIP-BioGRID				22	22	22				283	283	283				71	71	71
	MINT-DIP			9	9		9			25	25		25			10	10		10
Contributions from THREE datasets	HPRD-IntAct-BioGRID	1006	1006			1006	1006	2317	2317			2317	2317	397	397			397	397
	HPRD-MINT-BioGRID	738		738		738	738	1290		1290		1290	1290	415		415		415	415
	HPRD-DIP-BioGRID	197			197	197	197	404			404	404	404	162			162	162	162
	HPRD-IntAct-MINT	163	163	163			163	585	585	585			585	36	36	36			36
	HPRD-IntAct-DIP	12	12		12		12	20	20		20		20	12	12		12		12
	HPRD-MINT-DIP	12		12	12		12	35		35	35		35	5		5	5		5
	IntAct-MINT-BioGRID		218	218		218	218		277	277		277	277		1	1		1	1
	IntAct-DIP-BioGRID		15		15	15	15		26		26	26	26		0		0	0	0
	MINT-DIP-BioGRID			13	13	13	13			24	24	24	24			2	2	2	2
	IntAct-MINT-DIP		4	4	4		4		32	32	32		32		1	1	1		1
Contributions from FOUR datasets	HPRD-IntAct-MINT-BioGRID	2873	2873	2873		2873	2873	3520	3520	3520		3520	3520	47	47	47		47	47
	HPRD-IntAct-DIP-BioGRID	168	168		168	168	168	137	137		137	137	137	16	16		16	16	16
	HPRD-MINT-DIP-BioGRID	164		164	164	164	164	92		92	92	92	92	14		14	14	14	14
	HPRD-IntAct-MINT-DIP	14	14	14	14		14	97	97	97	97		97	8	8	8	8		8
	IntAct-MINT-DIP-BioGRID		34	34	34	34	34		15	15	15	15	15		1	1	1	1	1
Contributions from all FIVE datasets	HPRD-IntAct-MINT-DIP-BioGRID	1030	1030	1030	1030	1030	1030	175	175	175	175	175	175	8	8	8	8	8	8
TOTAL		9303	6666	6102	1795	9265	11827	37152	19425	16147	2609	42647	75965	19267	1598	2398	1180	13818	26689

Figure 1 Source of data in the integrated PICKLE PPI dataset.

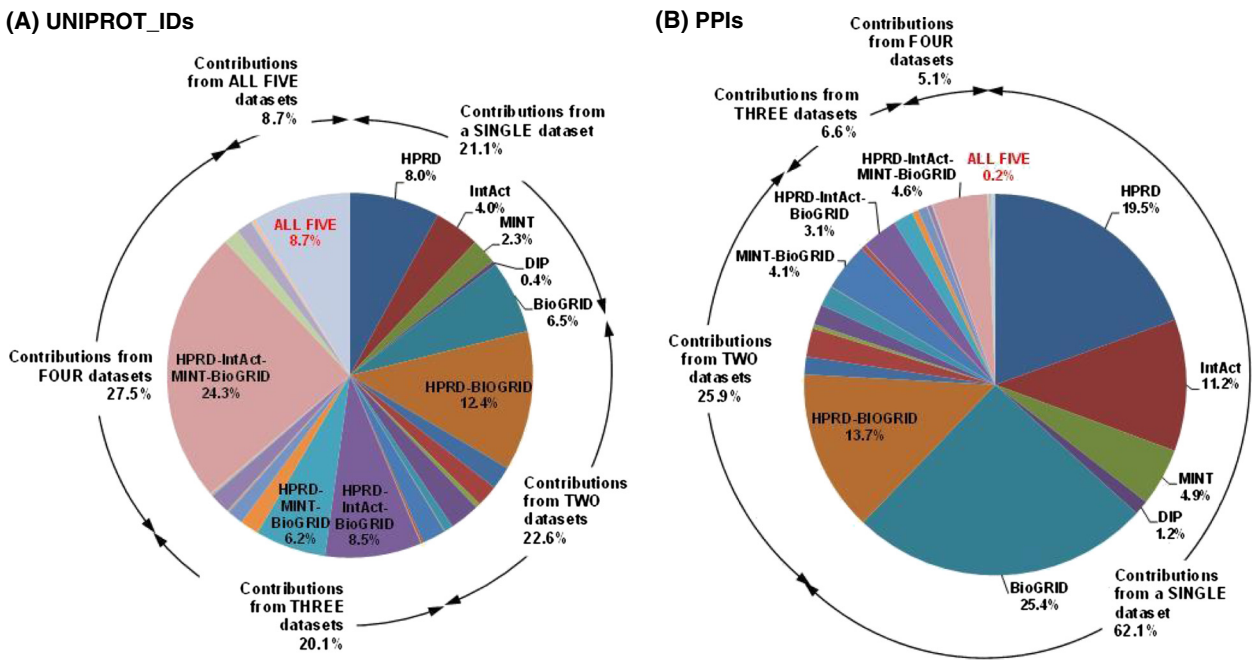

Figure 2 The fractions of PICKLE UniProt identifiers (A) and PPIs (B) contributed from combinations of source datasets. The common contributions for the nodes and the edges of the integrated PPI network from all five source datasets constitute 8.7% and 0.2% of the total, respectively. Only the values of the larger than 3% fractions are shown with the exception of the unique contributions from each individual source dataset, for which all fractions are indicated.

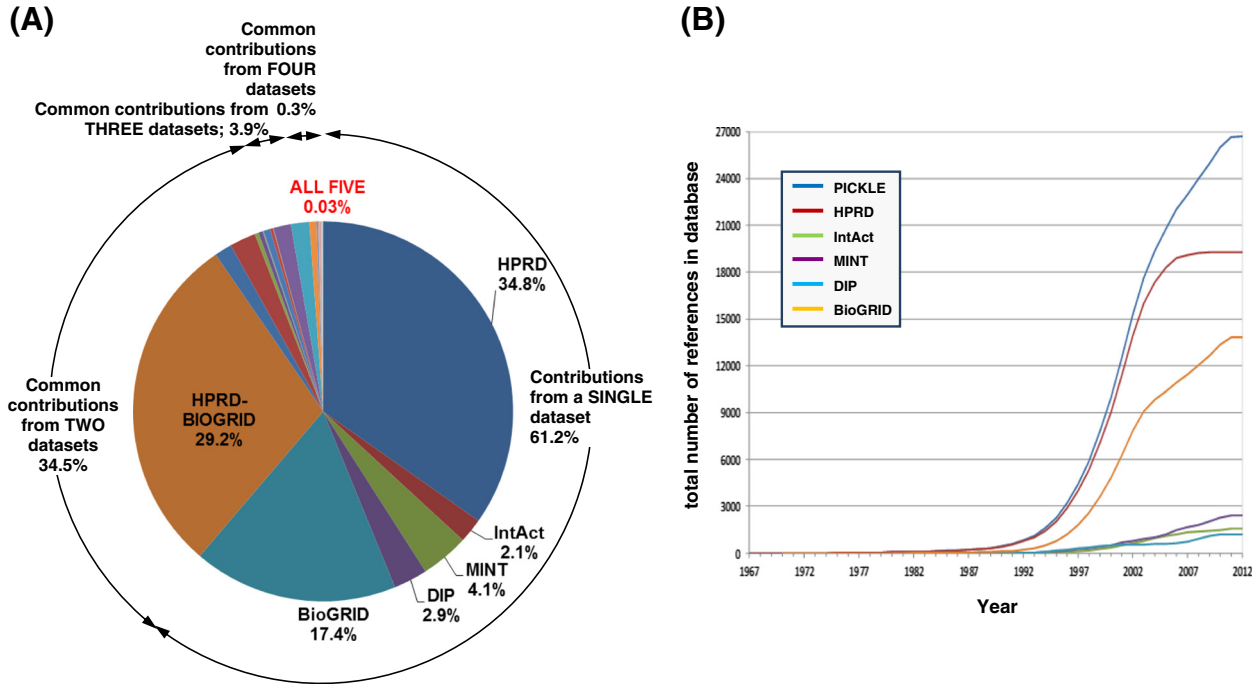

Figure 3 The PICKLE reference fraction contributed from source datasets (A) and the reference incorporation rate in the datasets (B). Only 8 common references between the five datasets were identified, confirming that they incorporate knowledge from different studies. In **(A)**, only the values of the larger than 3% fractions are shown with the exception of the unique contributions from each individual source dataset, for which all fractions are indicated.

references of high-throughput PPI experiments. Notably, the reconstructed human protein interactome is mainly supported by small-scale studies (Figure 4A); 91% of the references supporting the PICKLE PPI dataset refer to a maximum of five PPIs, and only 51 publications report more than 100 PPIs. In this aspect, PICKLE follows the characteristics of HPRD, currently the main contributor of references to the overall dataset. It is worth mentioning that 84% of the 75965 PPIs in the human interactome are supported by only one reference (Figure 4B) and just 42 PPIs by more than 20 (Additional file 2). Considering that the degree of confidence of a given PPI increases with the number of independent supporting references [35], it is evident that, apart from exploiting existing models for PPI assessment [36], further targeted experimentation is required for validating the majority of the PPI data.

A noteworthy observation of our work, revealing an interesting aspect of the literature-supported PPI data collections, is that the fraction of protein nodes that each source dataset uniquely contributes to the integrated network is much smaller than the corresponding fraction for the PPIs, even for the largest HPRD and BioGRID datasets (Figures 1 and 2). The PPI diversity between the source datasets has been discussed earlier [e.g. 10, 12] and mainly attributed to the fact that the various databases incorporate knowledge from different publications. This was recently presented for the IMEx Consortium member databases [10] and validated in the present study from the substantially small number, i.e. eight, of common references between the five employed datasets (Figure 1). Furthermore, Turinsky et al. [12] showed that the source databases exploit different curation criteria even for the shared publications. Thus, it is striking that, despite the heterogeneous text mining and data curation methods used by the various databases, the integration of multiple source PPI datasets augments mainly the interactome with different PPIs for essentially the same part of the manually reviewed human "complete" proteome.

This observation suggests that the knowledge about direct PPIs that is available in the literature and can be promptly identified through existing text mining algorithms refers mainly to the fraction, i.e. approximately 60%, of the manually reviewed human "complete" proteome already incorporated in the interactome, while evidence for PPIs for the rest 40% cannot be easily spotted. In this context, as PPI information from all high-throughput experiments has been included in at least one of the source datasets, there are two possibilities for the "orphan" proteins: either there is currently no available PPI information in the literature, or, if existing, it should concern reports of targeted small-scale functional experiments. From this kind of reports,

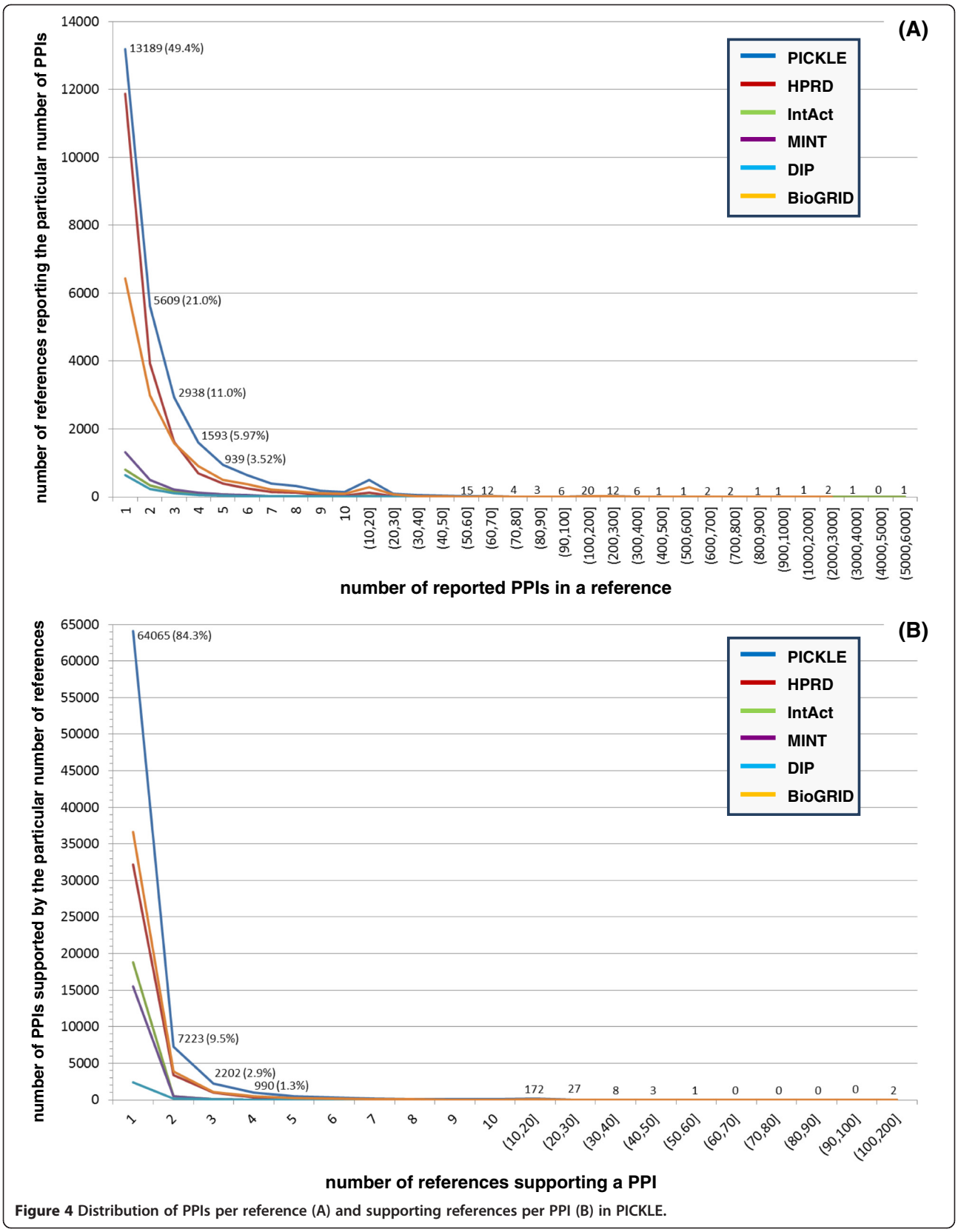

Figure 4 Distribution of PPIs per reference (A) and supporting references per PPI (B) in PICKLE.

protein interactions can be indirectly deduced, requiring thus advanced directed text mining algorithms. Furthermore, there is a higher probability for such experiments to refer to PPIs occurring under specialized and/or highly transient or rare physiological conditions, while this type of interactions cannot be easily identified in high-throughput experiments. These implied direct interaction characteristics for the "orphan" proteins support a peripheral role for most of them within the topology of the PPI network. In this context, the actual determination of the "orphan" proteins may assist in directed literature mining to extract potentially existing relevant PPI information from currently unexploited reports or promote further experimentation to verify the argument.

The proteins with a high number of interactions are involved in essential biological processes

Analysis of the integrated human PPI network characteristics indicated that 11577 out of the 11827 UniProt identifiers are connected in one component. The remaining 250 proteins are currently in separate components of up to four nodes, among which 114 homodimers and 46 heterodimers (Table 2). The vastest functional categories for these proteins as indicated by gene functional classification analysis concerned 107 glycoproteins, 64 of which are homodimers and 89 signal peptides, among which 65 glycoproteins; 68 of the signal peptides, including 39 glycoproteins, are associated with extracellular matrix. While the network diameter, i.e. the greatest distance between two protein nodes, was determined equal to 12, the characteristic path length is 3.69. This feature along with the equal to 1 radius and the high value of shortest paths metric (i.e. 95%) indicates a well-connected network, despite its low density (i.e. 0.001) (Table 2). The distribution of PPIs per protein, i.e. protein degree, indicated 53% of the proteins as having up to five interactions (Figure 5), while 16 UniProt identifiers had more than 300 PPIs each (Table 3). This pattern is consistent with the relevant "network biology" theory supported by Barabasi [30,37], according to which the human PPI network is expected to follow a scale-free structure with few protein hubs and the majority of the protein nodes having a small number of interactions. Indeed, even though it is currently incomplete and many interactions are still in need of verification, the reconstructed human protein interactome correlates well with the power law (Figure 5), implying that the degree distribution of the current PPI network already suggests the role of most proteins as high-, middle- or low-degree nodes.

Table 2 The characteristics of the integrated PPI network

Network characteristic[(1)]	Value[(2)]
Number of Nodes	11827
Isolated Nodes (homodimers)	114
Connected components	174 (i.e.: 1 cluster of 11577 nodes, 114 homodimers, 46 heterodimers,13 isolated of 3 or 4 nodes)
Number of self-loops	2715 (i.e.: 2601 nodes having interactions with other proteins as well, and 114 isolated homodimers)
Network radius	1
Network diameter	12
Characteristic Path Length	3.691
Average Number of Neighbors	12.387
Shortest Paths	95%
Clustering Coefficient	0.127
Network Density	0.001
Network Centralization	0.093
Network Heterogeneity	2.193

[(1)]Detailed description for every characteristic can be found in http://med.bioinf.mpi-inf.mpg.de/netanalyzer/help/2.6.1/index.html.
[(2)]Determined using the network analysis tool of Cytoscape (2.8.2).

The sixteen proteins determined with more than 300 PPIs (Table 3) are mainly implicated in the regulation of apoptosis (10 proteins), the MAP kinase signalling pathway (6 proteins) and the cell cycle (7 proteins). A full list of the most significant protein ontology clusters for these high-degree proteins is shown in Additional file 3. Notably, eight of them have been associated with pathways in cancer, while subsets of nine are involved in transcription regulation, covalent chromatin modification or the ubiquitin-like modifier (ubl) conjugation pathway. This information indicates that the observed central role of these proteins within the topology of the PPI network is not a mere result of them being extensively studied, i.e. "study bias", but correlates well with the current knowledge about their function, as it has also been suggested earlier for the cancer-associated proteins [38,39]. An additional fact which counter argues the "study bias" for these proteins is that, apart from various targeted small-scale experiments, many of their direct interactions have also been detected in independent high-throughput setups. For example, at least 54 interactions of the cellular tumor antigen p53 [8,40], 257 interactions of the 14-3-3 protein zeta/delta [41], 212 interactions of the Myc proto-oncogene protein [42] and 48 interactions of the TNF receptor-associated factor 6 [43] have been identified in high-throughput studies.

Polyubiquitin: a hub to be discussed

Polyubiquitin (UniProt identifier: P0CG48, UBC) was the protein identified with the largest number of interactions in the reconstructed network. It interacts with

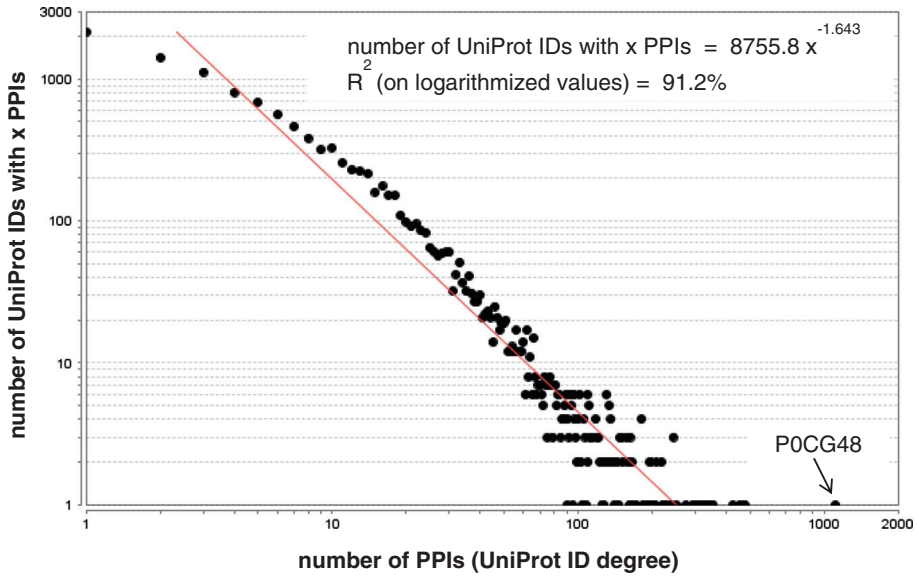

Figure 5 The distribution of direct interactions for the UniProt identifiers in the PICKLE network. The red line indicates the power-law fit (logarithmic scale); the related equation and R^2 correlation are also shown. UniProt identifier P0CG48 (Polyubiquitin, UBC) is identified with the largest number of interactions, i.e. 1112; note the deviation of this UniProt identifier degree from the power-law fit.

more than a thousand, i.e.: 1112, members of the manually reviewed human "complete" proteome, while the second ranked high-degree node, i.e.: TP53 (UniProt identifier: P04637), has 476 interactions. Notably, this much larger number of interactions for polyubiquitin compared to the other protein hubs deviates from the scale-free network structure, assigning a centralized role to a single protein (Figure 5). Querying the PICKLE dataset, we identified HPRD, IntAct, MINT, DIP and BioGRID reporting, respectively, 19 (2 unique), 5 (0 unique), 143 (48 unique), 53 (15 unique) and 1423 (909 unique) polyubiquitin PPIs. Without exhausting our search regarding polyubiquitin PPI supporting publications, we detected that our integrated dataset contains interactions from studies investigating polyubiquitin function in the context of protein degradation (e.g. [44]). Polyubiquitin can be covalently linked to a protein through an isopeptide bond and mark it for degradation at the proteasome. However, it is questionable whether this one-sided polyubiquitin action on a protein should be included in the interactome or should be considered in the post-translational modification (PTM) network [45,46]. The latter could explain why, apart from BioGRID, the other source databases used in this work have considered a limited number of polyubiquitin PPIs. In the context of the non-directional PPI network, the existence of an interaction link from one protein to another directly implies a link in the opposite direction, too. Consequently, the absence of a protein and thus its interactions will affect its neighbours and add a certain stress to the network, the extent of which depends on the network structure and dynamics. In the case of unidirectional polyubiquitination of a protein for leading it to degradation, the absence of the protein will neither affect polyubiquitin nor exert a stress to the rest of the polyubiquitin substrates. Thus, this type of actions of a protein on another should be considered as a separate category than the non-directional protein-protein interactions and modelled differently for their role in cell physiology dynamics. On the other hand, the monoubiquitination of proteins for regulatory purposes (e.g. [47]) fits into the notion of the non-directional PPI network. However, even in this case, it is questionable whether ubiquitin itself or rather the ubiquitinated proteins should be included as nodes of the network. In this context, the incorporation of ubiquitin PPIs in the interactome should be cautiously curated. Accordingly, this argument is also relevant to other proteins involved in interactions of similar type, like the small ubiquitin-related modifiers (SUMO1-4) and neddylin (NEDD8) engaged in the sumoylation and neddylation reactions, respectively.

The bulk of the proteins currently absent from the network should have up to four interactions

As shown, the reconstructed human protein interactome follows the scale-free structure with a very good correlation (Figure 5). The part of the network that contributes to the decrease in the correlation coefficient refers to the proteins with up to four interactions. The difference between the data and the power-law curve for a nearly perfect fit is

Table 3 The 16 UniProt identifiers with more than 300 interactions in the integrated PPI network

UniProt Identifier	UniProt Entry Name	Gene Symbol	Protein Name(s) (based on UniProt Naming Convention)	No of PPIs (Degree)
P0CG48	UBC_HUMAN	UBC	Polyubiquitin-C [Cleaved into: Ubiquitin]	1112
P04637	P53_HUMAN	TP53	Cellular tumor antigen p53 (Antigen NY-CO-13) (Phosphoprotein p53) (Tumor suppressor p53)	476
P63104	1433Z_HUMAN	YWHAZ	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1) (KCIP-1)	471
P01106	MYC_HUMAN	MYC	Myc proto-oncogene protein (Class E basic helix-loop-helix protein 39) (bHLHe39) (Proto-oncogene c-Myc) (Transcription factor p64)	453
Q9Y4K3	TRAF6_HUMAN	TRAF6	TNF receptor-associated factor 6 (EC 6.3.2.-) (E3 ubiquitin-protein ligase TRAF6) (Interleukin-1 signal transducer) (RING finger protein 85)	424
Q13547	HDAC1_HUMAN	HDAC1	Histone deacetylase 1 (HD1) (EC 3.5.1.98)	353
P12931	SRC_HUMAN	SRC	Proto-oncogene tyrosine-protein kinase Src (EC 2.7.10.2) (Proto-oncogene c-Src) (pp60c-src) (p60-Src)	351
P62993	GRB2_HUMAN	GRB2	Growth factor receptor-bound protein 2 (Adapter protein GRB2) (Protein Ash) (SH2/SH3 adapter GRB2)	341
Q14164	IKKE_HUMAN	IKBKE	Inhibitor of nuclear factor kappa-B kinase subunit epsilon (I-kappa-B kinase epsilon) (IKK-E) (IKK-epsilon) (IkBKE) (EC 2.7.11.10) (Inducible I kappa-B kinase) (IKK-i)	338
P61981	1433G_HUMAN	YWHAG	14-3-3 protein gamma (Protein kinase C inhibitor protein 1) (KCIP-1) [Cleaved into: 14-3-3 protein gamma, N-terminally processed]	335
Q09472	EP300_HUMAN	EP300	Histone acetyltransferase p300 (p300 HAT) (EC 2.3.1.48) (E1A-associated protein p300)	331
Q9UQL6	HDAC5_HUMAN	HDAC5	Histone deacetylase 5 (HD5) (EC 3.5.1.98) (Antigen NY-CO-9)	324
P00533	EGFR_HUMAN	EGFR	Epidermal growth factor receptor (EC 2.7.10.1) (Proto-oncogene c-ErbB-1) (Receptor tyrosine-protein kinase erbB-1)	313
P03372	ESR1_HUMAN	ESR1	Estrogen receptor (ER) (ER-alpha) (Estradiol receptor) (Nuclear receptor subfamily 3 group A member 1)	312
P27348	1433T_HUMAN	YWHAQ	14-3-3 protein theta (14-3-3 protein T-cell) (14-3-3 protein tau) (Protein HS1)	311
Q9Y6K9	NEMO_HUMAN	IKBKG	NF-kappa-B essential modulator (NEMO) (FIP-3) (IkB kinase-associated protein 1) (IKKAP1) (Inhibitor of nuclear factor kappa-B kinase subunit gamma) (I-kappa-B kinase subunit gamma) (IKK-gamma) (IKKG) (IkB kinase subunit gamma) (NF-kappa-B essential modifier)	304

calculated to be about 8300 UniProt identifiers, with more than 6500 of them corresponding to degree equal to 1. Consequently, with 8415 UniProt identifiers not currently included in the interactome ("orphan" proteins), it could be speculated that the vast majority of them should have up to four interactions with nodes in the same degree group. This anticipated network structure implies that the core of the human protein interactome has essentially been revealed and could provide a reasonable explanation for the current lack of PPI information for about 40% of the human proteome, agreeing with a specialized "peripheral" role for most of these "orphan" proteins. Indeed, with most of them expected to have a single PPI, and in general no more than four, with similarly not well-connected proteins, the probability of them being involved in specialized physiological conditions is high. This speculation further corroborates with the fact that interactions for these proteins cannot be easily confirmed in PPI identification experiments, as discussed in section C.

Conclusions

We have obtained a normalized and clean from outdated protein identifier annotations integrated set of direct PPIs referring to the well-defined UniProtKB manually reviewed human "complete" proteome. We suggest that this PPI network with the involvement of approximately 60% of the "complete" proteome represents the core of the human protein interactome. Based on a global view of the way in which the current network will have to expand to its maximum potential in accordance with the scale-free theory, we provide a novel perspective for suggesting its currently "missing" part. We envisage that the proteins not yet identified in direct PPI assays may participate in specialized biological functions interacting with a limited number of other not well-connected proteins. Now determined, this set of "orphan" proteins may trigger targeted text mining efforts or appropriately designed functional experiments for the identification of any relevant PPIs. In effect, we suggest that this reconstructed human interactome already provides a

useful tool for generating valuable working hypotheses for the investigation of important biological processes and molecular functions in the context of biomedical research and applications.

Additional files

Additional file 1: The uploading process flowchart for the IntAct PPI dataset using MS-SQL Integration Services (print screen shot).

Additional file 2: List of the 42 PPIs supported by 20 or more references in the reconstructed network.

Additional file 3: The major annotation clusters of the 16 UniProt identifiers with the largest number of PPIs. The UniProt identifier list is provided in Table 3. The clusters were determined by the functional annotation software DAVID using all relevant gene annotation categorizations.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NKM conceived and coordinated the study; MIK, AT and NKM participated in the design of the study; MIK, KT and NKM selected the source datasets; MIK supervised and KT created the dictionaries for the protein and publication identifier updating; MIK and ET designed and validated the data uploading process; ET developed the data uploading modules; MIK and NKM analyzed the reconstructed network; MIK drafted and NKM finalized the manuscript. All authors read and approved the final manuscript.

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