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Database issue

MultifacetedProtDB: a database of human proteins with multiple functions

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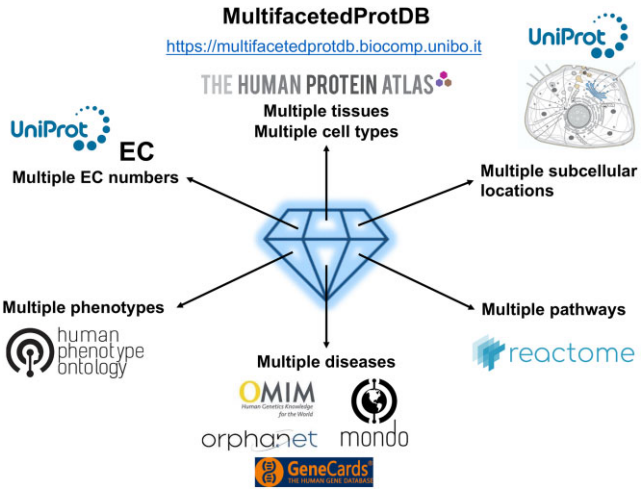
Abstract

MultifacetedProtDB is a database of multifunctional human proteins deriving information from other databases, including UniProt, GeneCards, Human Protein Atlas (HPA), Human Phenotype Ontology (HPO) and MONDO. It collects under the label 'multifaceted' multitasking proteins addressed in literature as pleiotropic, multidomain, promiscuous (in relation to enzymes catalysing multiple substrates) and moonlighting (with two or more molecular functions), and difficult to be retrieved with a direct search in existing non-specific databases. The study of multifunctional proteins is an expanding research area aiming to elucidate the complexities of biological processes, particularly in humans, where multifunctional proteins play roles in various processes, including signal transduction, metabolism, gene regulation and cellular communication, and are often involved in disease insurgence and progression. The webserver allows searching by gene, protein and any associated structural and functional information, like available structures from PDB, structural models and interactors, using multiple filters. Protein entries are supplemented with comprehensive annotations including EC number, GO terms (biological pathways, molecular functions, and cellular components), pathways from Reactome, subcellular localization from UniProt, tissue and cell type expression from HPA, and associated diseases following MONDO, Orphanet and OMIM classification. **MultiFacetedProtDB** is freely available as a web server at: https://multifacetedprotdb.biocomp.unibo.it/.

Graphical abstract



Introduction

Characterising multifunctional proteins is an expanding research area aiming to elucidate the complexities of biological processes (1–5). Proteins endowed with remarkable versatility play roles in various processes, including signal transduction, metabolism, gene regulation, and cellular communication, and are often involved in disease insurgence and progression. Advances in structural biology techniques facilitate the understanding of multiple molecular mechanisms and recent research identified and characterized new multifunctional proteins, uncovering their roles in disease development and progression, which in turn is the key to understand their potential as therapeutic targets and eventually to delve into the outcomes related to diseases comorbidity (6–12).

Originally lens crystallins were recognised as previously known metabolic enzymes (13,14). The term 'moonlighting' was put forward by Constance Jeffery (15) while Joran Piatigorsky proposed 'gene sharing' (16). Since then, different types of 'moonlighting' have been described (17–20) and functionally/structurally characterized in relation to their

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multiple functions (21). Multitasking proteins can perform different functions, mostly related to cell type and location, oligomeric state, concentration of cellular ligands, substrates, and cofactors (22,23). In literature (see (7), among others) multifunctional proteins are addressed as pleiotropic, multidomain, promiscuous (in relation to enzymes catalysing multiple substrates) and moonlighting (with two or more molecular functions). For this reason, we introduce here the term 'multifaceted', hoping to comprise the different categories, and focus on the human reference proteome, its link to diseases and known drugs, when available.

Databases like UniProt (https://uniprot.org, (24)), NCBI Protein (https://www.ncbi.nlm.nih.gov/protein/), and PDB (https://www.rcsb.org/, (25)) lack explicit labelling of multifunctional proteins. Specific resources are then needed to focus on this important and increasing subset of proteins, whose characterisation will help unravelling the mechanisms of cell complexity. To date, three resources collecting experimental data are available: MoonDB (http://moondb.hb.univ-amu.fr/, (26,27)), MoonProt (http://www.moonlightingproteins.org/, (28)) and MultitaskProtDB II (http://wallace.uab.es/multitaskII/, (29)). When restricted to humans, these databases collect 47, 103 and 185 proteins, respectively. Noticeably, the overlap among the three databases is limited (only 15 human proteins are shared among them) and, when merged, they provide information on 241 human proteins.

We hereby introduce MultifacetedProtDB, an integrated and manually curated database providing a comprehensive collection of multifunctional human proteins. MultifacetedProtDB has been built by: (i) merging the above-mentioned datasets; (ii) searching new multifunctional proteins reported in the recent literature; (iii) collecting enzymes endowed with multiple EC codes, emphasizing the differences at the digit-level codes.

The final dataset contains 1103 multifaceted proteins, of which 812 are enzymes. Therefore, MultifacetedProtDB increases by more than 4 times the number of multifunctional proteins reported in currently available resources.

MultifacetedProtDB links directly to GeneCards (https://www.genecards.org/, (30)) and to UniProt/Swiss-Prot for functional and structural features, providing access to protein structures from PDB, and AlphaFold models (AlphaFold Protein Structure Database, https://alphafold.ebi.ac.uk/, (31)), when available. Furthermore, evidence of protein family classification is provided by InterPro and Pfam (InterPro https://www.ebi.ac.uk/interpro/), (32). Catalytic reactions and EC numbers, when it is the case, GO terms (33), including biological pathways, molecular functions, and cellular components, subcellular location and protein-protein interactions are derived from UniProt. Biological pathways link directly to Reactome (https://reactome.org/, (34)). The database enables easy access to protein variants (UniProt variant viewer, https://www.uniprot.org/help/disease_phenotypes_variants_section). Entries in MultifacetedProtDB are associated to diseases, deriving from UniProt, Humsavar (https://ftp.uniprot.org/pub/databases/uniprot/knowledgebase/complete/docs/humsavar.txt), Monarch (https://monarchinitiative.org/, (35)) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/, (36)). Diseases are categorized according to MONDO (https://mondo.monarchinitiative.org/, (37)), ICD10 (https://www.cdc.gov/nchs/icd/icd10.htm), Orphanet (https://www.orpha.net/consor/cgi-bin/index.php) and OMIM (https://www.omim.org/, (38)). Links are provided to HPO phenotypes (https://hpo.jax.org/, (39)) along with valuable insights into tissue and cell type expression patterns, sourced from the Human Protein Atlas (https://www.proteinatlas.org/). We also include information, when available, on specific drugs, by linking to GeneCards. Links to quoted literature is preserved and/or included (publications, https://www.uniprot.org/help/return_fields_databases).

We offer an advanced search option with which the user can browse the dataset including up to four conditions, which can be selected from a drop-down menu, and are connected by logical operators (AND, OR, NOT). The information available in the database offers a comprehensive, richly annotated resource for investigating the role of multifunctional proteins within the complex realm of cellular processes, in particular in relation to the development of diseases.

Web server implementation

The database is stored on a PostgreSQL DBMS (v.13; https://www.postgresql.org). For the backend, we adopted the Django Python application server (v.4.1, https://www.djangoproject.com). The look-and-feel of the website graphical interface has been implemented focusing on simplicity, ease of use and optimization of the interaction with the user. The frontend web pages are designed using the Bootstrap framework (v5.3.0, https://getbootstrap.com). JQuery (v3.7, https://jquery.com) implements interactivity. DataTables (v1.13.4, https://datatables.net) was adopted for visualizing tabular data. To improve web server interoperability and cross-referencing, all the identifiers of external databases were linked to the original resources.

Content of MultifacetedProtDB

The overall content of MultifacetedProtDB is summarized in Table 1. The database contains information on 1103 multifaceted proteins, 812 of which are endowed with at least one EC number. For the remaining 291, no catalytic function has been reported so far. Proteins cluster into 913 groups when their sequences are aligned with MMSeq2 (40) at 50% sequence identity with a sequence coverage higher than 80%.

The three-dimensional structures of 743 proteins have been resolved, at least partially, and are reported in 13 147 PDB entries. 630 proteins are endowed with more than one resolved structure; among them, carbonic anhydrase 2 (UniProt: P00918) is the most extreme case, being associated with 1031 different PDB entries.

Some 86% of all proteins (698 enzymes and 252 non enzymes) participate in Reactome pathways that are part of all the 27 main Reactome roots (excluding 'Disease' and 'Drug ADME'). All proteins are endowed with InterPro annotations and only 9 (of which, 5 enzymes) lack annotations in Pfam. Collectively, proteins in MultifacetedProtDB are covered by 2626 and 1045 InterPro and Pfam domains, respectively.

The most represented Pfam domains are: PF00782 (Dual specificity phosphatase, catalytic domain, 33 proteins), PF00069 (Protein kinase domain, 32 proteins), PF00067 (Cytochrome P450, 21 proteins), PF00029 (Connexin, 21 proteins), PF00501 (AMP-binding enzyme, 19 proteins), PF00106 (short chain dehydrogenase, 17 proteins), PF13193 (AMP-binding enzyme C-terminal domain, 15 proteins), PF00856 (SET domain, 14 proteins), PF00005 (ABC

Table 1. Statistics of MultifacetedProtDB

	Proteins (#)	PDB (#, # *proteins*)	Reactome paths (# leaves, # internal nodes*, # *proteins*)[a]	Reactome roots (#)[a]	InterPro (#, # *proteins*)	Pfam (#, # *proteins*)	Diseases (# MONDO, # ICD10 categories and chapters, # *proteins*)
Enzymes	812	10 469 entries *520 proteins*	662 leaf nodes 163 internal nodes *698 proteins*	27	1943 entries *812 proteins*	761 entries *807 proteins*	619 MONDO diseases 172 ICD10 categories 16 ICD10 chapters *321 proteins*
Non-enzymes	291	2840 entries *223 proteins*	465 leaf nodes 106 internal nodes *252 proteins*	25	784 entries *291 proteins*	314 entries *287 proteins*	309 MONDO diseases 117 ICD10 categories 13 ICD10 chapters *110 proteins*
All proteins	1103	13 147 entries *743 proteins*	849 leaf nodes 198 internal nodes *950 proteins*	27	2626 entries *1103 proteins*	1045 entries *1094 proteins*	895 MONDO diseases 213 ICD10 categories 17 ICD10 chapters *431 proteins*

#: number of
*: "internal nodes" represent the intermediate nodes within the Reactome hierarchy.
[a]: Reactome entries related to "Disease" and "Drug ADME" are not considered when counting the number of Reactome pathways and roots.

transporter, 14 proteins), PF02798 (Glutathione S-transferase, N-terminal domain, 13 proteins) and PF00030 (Beta/Gamma crystallin, 13 proteins).

We collected associations among proteins and diseases merging the information reported in UniProt, Humsavar, Monarch initiative and ClinVar and reported the disease nomenclatures provided by the MONDO ontology, the OMIM and Orphanet catalogs, and the ICD10 classification scheme.

Some 30% of proteins in our database (321 enzymes and 110 non enzymes) are associated with 895 MONDO diseases classified into 213 ICD10 categories and into 17 (out of the 19) ICD10 main chapters, after excluding chapters not describing diseases with a genetic component (namely, XX: 'External causes of morbidity and mortality', XXI: 'Factors influencing health status and contact with health services', and XXII: 'Codes for special purposes'). The most represented chapter is 'XVII: Congenital malformations, deformations and chromosomal abnormalities' accounting for 226 diseases associated with 135 multifaceted proteins.

Of the 895 diseases, 323 are included in the Orphanet catalog of rare diseases. Overall, when considering the phenotypic characterization of diseases, 430 proteins are linked to terms out of the Human Phenotype Ontology (HPO).

The proteomic distribution of multifaceted proteins in our database is characterized in terms of subcellular location, tissues and tissue cell types. The subcellular location has been extracted from the Swiss-Prot curated annotation: 1005 proteins are distributed among 168 locations, out of the 561 defined in the UniProt controlled vocabulary (https://www.uniprot.org/locations). Of the 1005 proteins, 411 are annotated in only one location, while 594 are endowed with multiple locations. The most multilocalised protein is Annexin A1 (P04083), endowed with 16 different locations.

As to the distribution among tissues and tissues cell types, data have been downloaded from the Human Protein Atlas (HPA): 804 proteins are distributed among 58 tissues and 127 cell types, out of the 64 tissues and 145 cell types defined in HPA. Only 27 proteins are specific for a single tissue. The remaining proteins are expressed in at least two tissues and, in most cases, they have a very low specificity. 549 proteins are found in at least 30 different tissues; the extreme case is represented by 120 proteins detected in 49 tissues.

Enzymes in MultifacetedProtDB

Over 73% of our database includes enzymes. Enzymes can be multifunctional when their catalytic activity is performed independently of other activities in the cell, or when, depending on the environment, they change their catalytic activity at different extents (22). For enzymes, the Enzyme Commission (EC) number is a four-level traditional code, referring to the catalysed biochemical reaction and following the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) (https://iubmb.qmul.ac.uk/enzyme/). EC numbers describe the catalysed biochemical reaction (1st digit), and the relationship among protein activity (2nd digit), substrates and products (3rd and 4th digits). Presently EC codes include seven major classes: (i) oxidoreductases, (ii) transferases, (iii) hydrolases, (iv) lyases, (v) isomerases, (vi) ligases, (vii) translocases (28).

For sake of clarity, Table 2 groups multifunctional enzymes into three sets. The first set includes enzymes that are annotated only with one EC number in UniProt, and can perform other functions (Table 2, second row); the second set includes enzymes that are characterized by EC numbers belonging to different classes as indicated by the change of the first digit (Table 2, third row). The third group includes all the enzymes in which the first digit is conserved while the remaining three may change at the second, third and/or fourth level (Table 2, fourth row). In each group, enzymes can be associated to diseases (Table 2, rightmost column). It is evident that 39.5% of the multifunctional enzymes are disease associated. A recent estimate reports that some 1494 enzymes in humans are involved in diseases (41,42). Therefore, it turns out that presently about 21% of enzymes involved in diseases are multifunctional.

Table 2. Statistics of enzymes in MultifacetedProtDB

	Enzymes (#)	PDB (#, # *enzymes*)	Reactome paths (# leaves, # internal nodes*, # *enzymes*)[a]	Reactome roots (#)[a]	InterPro (#, # *enzymes*)	Pfam (#, # *enzymes*)	Diseases (# MONDO, # ICD10 categories and chapters, # *enzymes*)
Enzymes	812	10 469 entries *520 enzymes*	662 leaf nodes 163 internal nodes *698 enzymes*	27	1943 entries *812 enzymes*	761 entries *807 enzymes*	619 MONDO diseases 172 ICD10 categories 16 ICD10 chapters *321 enzymes*
Enzymes with only 1 EC	144	3315 entries *120 enzymes*	287 leaf nodes 82 int. nodes *135 enzymes*	23	623 entries *144 enzymes*	213 entries *142 enzymes*	173 MONDO diseases 89 ICD10 categories 13 ICD10 chapters *82 enzymes*
Enzymes with Multiple ECs with different 1st levels	136	1472 entries *88 proteins*	149 leaf nodes 27 int. nodes *112 enzymes*	18	527 entries *136 enzymes*	200 entries *136 enzymes*	123 MONDO diseases 74 ICD10 categories 12 ICD10 chapters *56 enzymes*
Enzymes with Multiple ECs with different figures other than the 1st	532	5750 entries *312 enzymes*	488 leaf nodes 107 int. nodes *451 enzymes*	27	1110 entries *532 enzymes*	440 entries *529 enzymes*	349 MONDO diseases 161 ICD10 categories 15 ICD10 chapters *183 enzymes*

#: number of
*: 'internal nodes' indicates the intermediate nodes within the Reactome hierarchy.
[a]: Reactome entries related to 'Disease' and 'Drug ADME' are not considered when counting the number of Reactome pathways and roots.

One example for each group in Table 2 is described in the following.

An example of a multifaceted protein enzyme associated with a single EC number (row 'Enzymes with only 1 EC' in Table 2) is Hexokinase-1 (UniProt: P19367, gene: HK1). Its catalytic activity is described by EC: 2.7.1.1 (Hexokinase, Transferase class); as a second function it recognises bacterial peptidoglycans, playing a role in innate immunity and inflammation. It is a multilocalized protein and it is associated with the mitochondrial outer membrane when acting as an enzyme, while it dissociates to the cytoplasm when interacting with N-acetyl-D-glucosamine, a component of peptidoglycans (3). It is a housekeeping gene, showing a low specific expression pattern, being detected in 90 tissue cell types out of 46 different tissues. Variants of HK1 have been related to 5 different diseases classified in ICD10 chapters III (Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism), VI (Diseases of the nervous system) and VII (Diseases of the eye and adnexa).

As a second example we select one multiclass enzyme (row 'Enzymes with Multiple ECs with different 1st levels' in Table 2). The cytoplasmic C-1-tetrahydrofolate synthase (UniProt: P11586, gene MTHFD1) is a trifunctional enzyme that catalyzes the interconversion of three forms of one-carbon-substituted tetrahydrofolate. The three catalytic activities are described by the following codes: EC: 1.5.1.5 (Methylenetetrahydrofolate dehydrogenase (NADP+), Oxidoreductase class); EC: 3.5.4.9 (Methenyltetrahydrofolate cyclohydrolase, Hydrolase class); and EC: 6.3.4.3 (formate-tetrahydrofolate ligase, Ligase class). The enzyme is monolocalised (cytoplasm) and involved in a single Reactome pathway (Metabolism of folate and pterines, R-HSA-196757). Three-dimensional structures are available for the N-terminal 306-residue long domain, performing both the oxidoreductase and hydrolase activities, while the ligase activity is predicted to be performed in a separate domain, identified by the Pfam domain PF01268 (formate-tetrahydrofolate ligase, residues 319–695). It shows a low specific expression pattern, being detected in 80 tissue cell types out of 49 different tissues (HPA). Variants of MTHFD1 are associated with three diseases: one disease is unclassified by ICD10 and the remaining two belong to ICD10 chapters II (neoplasm) and XVII (congenital malformations, deformations and chromosomal abnormalities) (43).

An example of third group in Table 2 (Enzymes with Multiple ECs with different digits other than the 1st) is the mitochondrial α-aminoadipic semialdehyde synthase (UniProt: Q9UDR5, Gene: AAS), a bifunctional enzyme catalysing the first two steps in lysine degradation, with EC: 1.5.1.8 (saccharopine dehydrogenase (NADP+, L-lysine-forming), oxidoreductase class) and EC: 1.5.1.9 (saccharopine dehydrogenase (NADP+, L-glutamate-forming), oxidoreductase class). It is monolocalised (mitochondrion) and involved in a single Reactome pathway (lysine catabolism, R-HSA-71064). Similarly to the cytoplasmic C-1-tetrahydrofolate synthase, it shows a low specific expression pattern, being detected in 61 tissue cell types out of 46 different tissues. Variants of AAS are associated with three diseases classified in ICD10 chapters IV

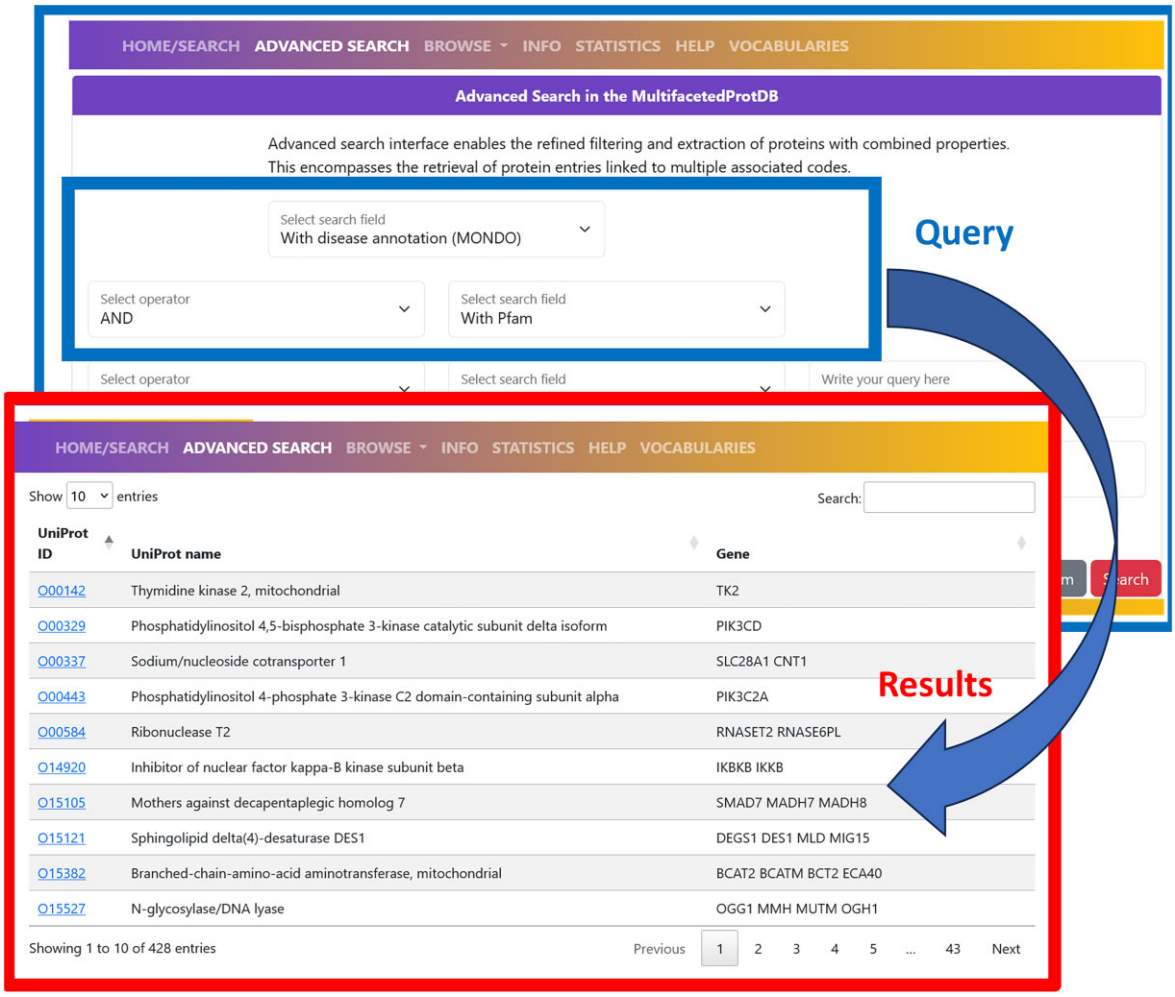

Figure 1. Example of advanced search in MultifacetedProtDB. Complex search can be performed by combining different searches with Boolean connectors AND, OR, NOT. In the case illustrated in the figure, proteins endowed with a Pfam annotation and associated with MONDO diseases are retrieved. The search shows a list of 428 entries that can be inspected for obtaining detailed information.

(Endocrine, nutritional and metabolic diseases) and VI (diseases of the nervous system) (44).

Retrieving from MultifacetedProtein DB

The database can be searched for any of the information fields it includes. 'ADVANCED SEARCH' interface can be used to combine different searches, connected with the 'AND', 'OR', 'NOT' boolean operators. The clauses 'With' and 'Multiple' in the search fields allow to restrict the search to entries endowed with at least one or multiple annotations for the field, respectively.

For example, is it possible to search for multifunctional proteins associated to MONDO diseases and endowed with Pfam annotations, obtaining as a result a list of 428 entries that can be explored in detail (Figure 1). Searching for specific Pfam domains highlights those mostly associated with diseases. These are listed in Table 3. Specifically, the Protein kinase domain (PF00069), the cytochrome P450 domain (PF00067), the Connexin domain (PF00029) and the β/γ crystallin domain (PF00030) are most frequently associated with diseases in multifunctional proteins. By inspecting the entries obtained with the advanced search, it is possible to retrieve and integrate information on the diseases, their classification and the structural and functional features of the highlighted proteins including their participation to Reactome pathways.

Conclusions and perspectives

The current version of MultifacetedProtDB increases four folds the number of multifunctional proteins with respect to others specialized databases available for the same subject. The database is useful when searching for a multitasking protein in relation to its involvement in cell molecular complexity and possibly in diseases. A list of drugs is provided, when available. The actual content of 1103 proteins, inclusive of 812 enzymes, is a possible underestimation of the total number of multitasking proteins that characterizes the human reference proteome, most likely due to lack of knowledge that future researches will fill, particularly in relation to different isoforms and their involvement in disease (45). Presently, data in our DB indicate that multifunctionality is not exclusively related to multiple subcellular locations, and/or association to diseases. Proteins with specific domains (Figure 1 and Table 3) seems more involved than others in diseases. However, data may change as more experimental knowledge will be acquired. For this reason, we will update the database every year,

Table 3. Protein domains mostly related to disease

Pfam domain	Proteins in DB (#)	Proteins related to diseases (#)	Diseases (#)	ICD10 chapters	Reactome pathways (#)[a]	Reactome roots[a]
PF00069 Protein kinase domain	32	13	24	II: Neoplasm III: Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism IV: Endocrine, nutritional and metabolic diseases V: Mental and behavioural disorders VI: Diseases of the nervous system IX: Diseases of the circulatory system XIII: Diseases of the musculoskeletal system and connective tissue XVII: Congenital malformations, deformations and chromosomal abnormalities	104	Cell cycle Cellular responses to stimuli Circadian clock Developmental biology Gene expression Hemostasis Immune system Metabolism Metabolism of RNA Metabolism of proteins Neuronal system Organelle biogenesis and maintenance Programmed cell death Signal transduction
PF00067 Cytochrome P450	21	11	19	IV: Endocrine, nutritional and metabolic diseases VI: Diseases of the nervous system VII: Diseases of the eye and adnexa IX: Diseases of the circulatory system XI: Diseases of the digestive system XVII: Congenital malformations, deformations and chromosomal abnormalities	25	Metabolism Sensory perception
PF00029 Connexin	21	10	49	IV: Endocrine, nutritional and metabolic diseases VI: Diseases of the nervous system VIII: Diseases of the ear and mastoid process IX: Diseases of the circulatory system XVII: Congenital malformations, deformations and chromosomal abnormalities	10	Vesicle-mediated transport Neural system Signal transduction
PF00030 β/γ crystallin	13	10	21	VII: Diseases of the eye and adnexa XVII: Congenital malformations, deformations and chromosomal abnormalities	0	-

[#]: number of
[a]: Reactome pathways associated to disease related proteins, excluding pathways collected under the roots 'Disease' and 'Drug ADME'.

integrating literature and new UniProt and other main database releases.

Data availability

The data discussed in this paper are publicly available at https://multifacetedprotdb.biocomp.unibo.it/.

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Conflict of interest statement

None declared.

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