

Towards a proteome-scale map of the human protein-protein interaction network

Jean-François Rual^{1*}, Kavitha Venkatesan^{1*}, Tong Hao¹, Tomoko Hirozane-Kishikawa¹, Amélie Dricot¹, Ning Li¹, Gabriel F. Berriz², Francis D. Gibbons², Matija Dreze^{1,3}, Nono Ayivi-Guedehoussou¹, Niels Klitgaard¹, Christophe Simon¹, Mike Boxem¹, Stuart Milstein¹, Jennifer Rosenberg¹, Debra S. Goldberg², Lan V. Zhang², Sharyl L. Wong², Giovanni Franklin², Siming Li^{1†}, Joanna S. Albalá^{1†}, Janghoo Lim⁴, Carlene Fraughton¹, Estelle Llamosas¹, Sebiha Cevik¹, Camille Bex¹, Philippe Lamesch^{1,3}, Robert S. Sikorski⁵, Jean Vandenhoute³, Huda Y. Zoghbi⁴, Alex Smolyar¹, Stephanie Bosak⁶, Reynaldo Sequerra⁶, Lynn Doucette-Stamm⁶, Michael E. Cusick¹, David E. Hill¹, Frederick P. Roth² & Marc Vidal¹

Systematic mapping of protein-protein interactions, or ‘interactome’ mapping, was initiated in model organisms, starting with defined biological processes^{1,2} and then expanding to the scale of the proteome^{3–7}. Although far from complete, such maps have revealed global topological and dynamic features of interactome networks that relate to known biological properties^{8,9}, suggesting that a human interactome map will provide insight into development and disease mechanisms at a systems level. Here we describe an initial version of a proteome-scale map of human binary protein-protein interactions. Using a stringent, high-throughput yeast two-hybrid system, we tested pairwise interactions among the products of ~8,100 currently available Gateway-cloned open reading frames and detected ~2,800 interactions. This data set, called CCSB-HI1, has a verification rate of ~78% as revealed by an independent co-affinity purification assay, and correlates significantly with other biological attributes. The CCSB-HI1 data set increases by ~70% the set of available binary interactions within the tested space and reveals more than 300 new connections to over 100 disease-associated proteins. This work represents an important step towards a systematic and comprehensive human interactome project.

Our working definition of a human interactome map is the complete collection of binary protein-protein interactions detectable in one or more exogenous assay. This definition excludes dynamic and functional properties of these interactions (Supplementary Data I). Thus, we treat interactome maps as ‘scaffold’ information, from which increasingly detailed and reliable biological models can be generated by integrating other functional genomic and proteomic data sets¹⁰ (Supplementary Data II).

The currently available information on the human interactome network originates from either literature-curated (LC) interactions^{11–15}, or from ‘interologs’ (that is, potential interactions predicted from interactome data available for model organisms given evolutionary conservation of two known partners)^{2,16}. This information needs to be complemented by systematic experimental mapping approaches that are: (1) not biased towards any particular biological interest (that is, without ‘inspection bias’), as is the case for

LC data sets; (2) more complete; and (3) supported by experiments rather than predictions. We are mapping the human interactome network systematically in successive versions, with each version defined by the availability of recombinationally cloned open reading frames (ORFs) in the human ‘ORFeome’¹⁷.

In this initial version, we use human ORFeome v1.1 (ref. 17), a resource containing ~8,100 Gateway-cloned ORFs (generated using the Mammalian Gene Collection, as previously described¹⁷) that correspond to ~7,200 distinct protein-coding genes (Supplementary Table S1). Thus, our initial ‘search space’ (Space-I) encompasses protein pairs encoded by a 7,200 × 7,200 matrix of genes. Future interactome versions can be generated by successively increasing the search space as additional versions of the human ORFeome become available (Supplementary Data III). Accepting a total of ~22,000 protein-coding genes in the human genome¹⁸ and excluding polymorphic and splice variants, Space-I corresponds to ~10% of the total search space for a comprehensive human interactome map (Fig. 1a). Currently, 4,067 binary LC interactions are available in Space-I (LCI interactions; Supplementary Table S2).

Our high-throughput yeast two-hybrid system is highly specific, benefiting from the following features, which were not uniformly present in earlier large-scale studies: relatively low levels of expression of both Gal4 DNA-binding domain (DB) and Gal4 activation domain (AD) hybrid proteins (DB-X and AD-Y, or DB-ORF and AD-ORF); three different yeast two-hybrid inducible reporter genes; and a plasmid-shuffling counter selection to eliminate systematically *de novo* auto-activators¹⁹ (Supplementary Data IV). We tested each of the ~8,100 individual DB-X proteins against 45 mini-libraries, each containing a pool of 188 AD-Y fusion proteins (AD-188Ys), by yeast-mating in a 96-well format (Fig. 1a). Such small pools offer high sensitivity, because positive clones are less likely to be masked by other AD-Y clones within the same pool. Indeed, our overall reproducibility rate was ~55%, close to that observed in proteome-scale affinity purification followed by mass spectrometry experiments²⁰ (Supplementary Data V).

In our Space-I yeast two-hybrid matrix, we identified ~65,000 primary positive colonies, of which 12,251 scored positive after

¹Center for Cancer Systems Biology and Department of Cancer Biology, Dana-Farber Cancer Institute and Department of Genetics, Harvard Medical School, 44 Binney Street, Boston, Massachusetts 02115, USA. ²Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 250 Longwood Ave, Boston, Massachusetts 02115, USA. ³Unité de Recherche en Biologie Moléculaire, Facultés Notre-Dame de la Paix, 61 Rue de Bruxelles, 5000 Namur, Belgium. ⁴Howard Hughes Medical Institute, and Departments of Pediatrics, Neurology, Neuroscience, and Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA. ⁵Arcbay, Inc., 6 Whittier Place, Suite 7J, Boston, Massachusetts 01915, USA. ⁶Agencourt Bioscience Corporation, 500 Cummings Center, Suite 2450, Beverly, Massachusetts 01915, USA. †Present addresses: ArQule, Inc., 19 Presidential Way, Woburn, Massachusetts 01081, USA (S.L.); Departments of Cancer Biology, and Otolaryngology, Head and Neck Surgery, University of California Davis, 2521 Stockton Blvd, Suite 7200, Sacramento, California 95817, USA (J.S.A.).

*These authors contributed equally to this work.

the yeast two-hybrid methodology. Estimating biological false positive interactions, which are genuinely observed in one or more assay but do not occur *in vivo*, is more difficult. We partially addressed this by examining the correlation of CCSB-HI1 data with other biological information (see below).

To measure the sensitivity of CCSB-HI1, we selected two high-confidence subsets from among all 4,067 LCI direct binary interactions. LCI-core contains 624 interactions supported by at least two PubMed entries. LCI-hypercore contains 275 interactions supported by at least two PubMed entries and present in at least two curated databases (Supplementary Table S2). Overall, the fractions of LCI, LCI-core and LCI-hypercore interactions found in CCSB-HI1 are 2.3%, 4.6% and 8.4%, respectively (Fig. 2a). These overlaps are larger than expected by chance ($P < 6 \times 10^{-56}$) and are similar to those found for interactome maps in *Caenorhabditis elegans* and *Drosophila melanogaster*^{7,21}. That the fraction of CCSB-HI1 interactions increases markedly with increasingly confident subsets of LCI suggests that literature-derived interactions are variable in quality and should not necessarily be interpreted as a 'gold standard'. Because Space-I represents ~10% of the human network (without accounting for alternative splice variants), and because we detected ~10% of LCI-hypercore interactions, we conclude that the CCSB-HI1 data set contains ~1% of the human interactome (Supplementary Data X).

We represented the union of all CCSB-HI1 and LCI interactions in a network graph in which nodes are proteins and edges are interactions. The main component of this network contains 2,784 nodes and 6,438 edges (Fig. 2b), and shows interactions largely segregated into two neighbourhoods: one enriched for CCSB-HI1 interactions (red edges) and the other enriched for LCI interactions (blue edges). To explore this hypothesis, we calculated, for each node, the fraction of yeast two-hybrid edges within paths of length 1, 2 and 3 (that is, within '1-hop', '2-hop' and '3-hop' neighbourhoods). The distribution of this fraction (Fig. 2c; see also Supplementary Fig. S2) confirms the evidence-type segregation apparent in Fig. 2b. One explanation for this phenomenon is that different biases exist in the CCSB-HI1 and LCI data sets. For example, certain protein classes (such as those involved in cancer) are studied more extensively than others, resulting in an inherent inspection bias in LC data (Supplementary Table S4). Furthermore, the methodologies used to detect interactions (including yeast two-hybrid) each have different biases for example, under-representation of membrane proteins (Supplementary Data X).

The novelty of CCSB-HI1 interactions was evaluated by systematically searching the PubMed and Google Scholar literature databases for co-occurrence of the corresponding gene symbols. More than 85% of the CCSB-HI1 pairs (as compared with only 25% of

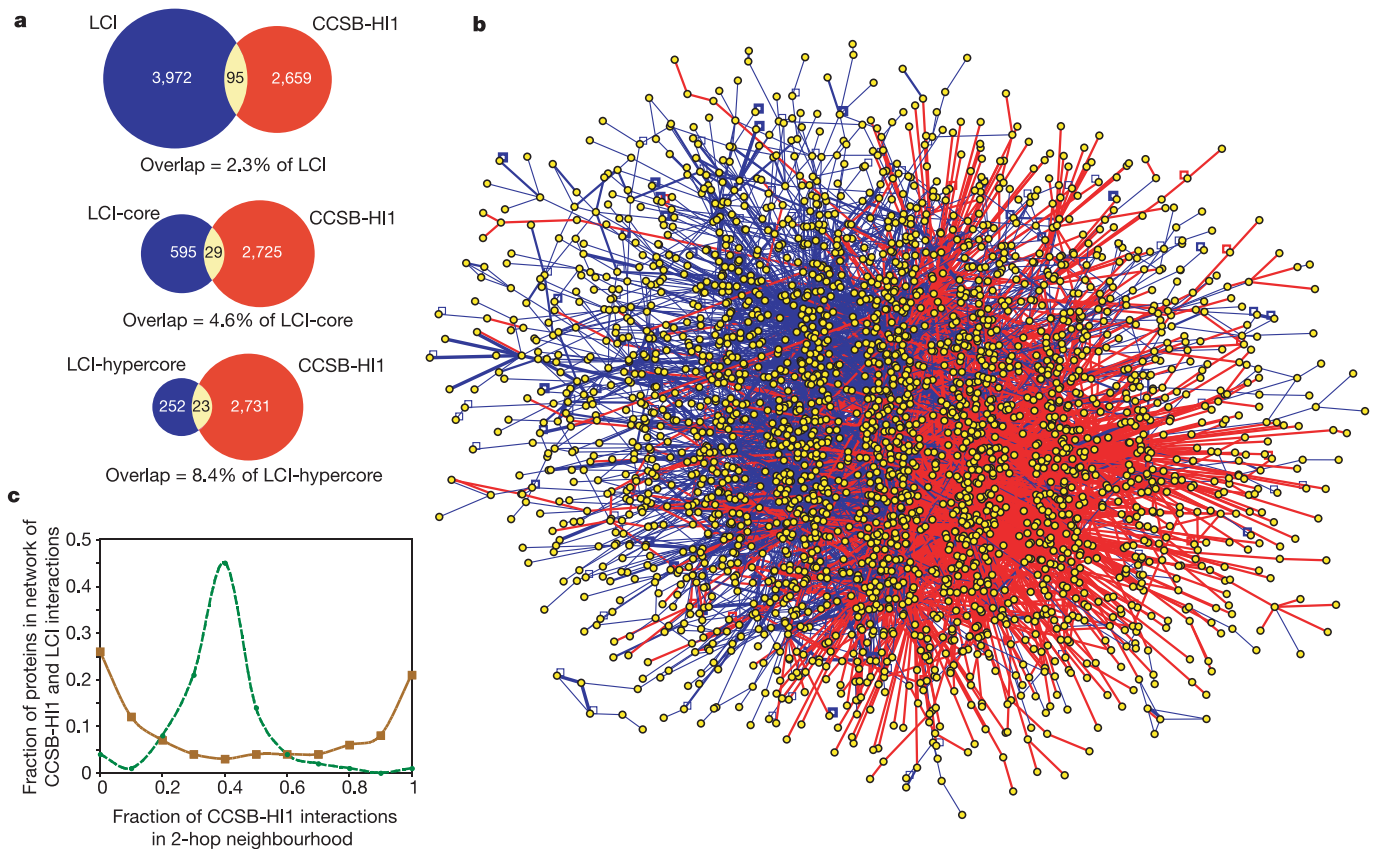


Figure 2 | Overlap of CCSB-HI1 with existing literature-curated (LC) data. a, Overlap between CCSB-HI1 and LC interactions in Space-I (LCI). The top, middle and bottom panels represent the overlap between CCSB-HI1 and LCI, LCI-core and LCI-hypercore, respectively. **b**, Network graph of the union of all CCSB-HI1 and LCI interactions. Proteins are shown as yellow nodes and CCSB-HI1 and LCI interactions are shown as red and blue edges, respectively. Blue edges with increasing thickness indicate LCI-non-core, LCI-core and LCI-hypercore, respectively. The apparent banding pattern of the yellow nodes is an artefact of the graph layout algorithm (Supplementary Data). Importantly, the layout algorithm was not informed by type of supporting evidence and therefore does not explain the

evident separation of blue and red edges. **c**, Bias in 2-hop network neighbourhood for either CCSB-HI1 or LCI interactions. The frequency of nodes with a given proportion of CCSB-HI1 interactions in their 2-hop neighbourhood is depicted for the interactome network graph in **b** (solid curve) and for a network in which the types of supporting evidence (CCSB-HI1 or LCI) are randomly permuted among edges (dashed curve). The solid curve indicates that most of the proteins in the network of **b** have either only CCSB-HI1 or only LCI interactions in their 2-hop neighbourhood. In contrast, neighbourhoods are well mixed when evidence labels are randomly permuted among edges.

Table 1 | Overlap of protein interactions with other gene- or protein-pair characteristics

Protein pairs	Share mouse phenotype		Share upstream motif		Have correlated expression	
	F(C)	P-value	F(C)	P-value	F(C)	P-value
All possible within Space-I	0.128	NA	0.086	NA	0.063	NA
CCSB-HI1	0.257	2.53×10^{-3}	0.115	1.14×10^{-4}	0.130	2.14×10^{-7}
LCI	0.336	4.91×10^{-43}	0.146	9.05×10^{-20}	0.204	5.45×10^{-56}
LCI-core	0.471	7.53×10^{-20}	0.137	3.77×10^{-3}	0.243	3.57×10^{-12}
LCI-non-core	0.306	2.54×10^{-27}	0.147	3.65×10^{-18}	0.198	7.78×10^{-46}

Protein pairs	Share GO component		Share GO function		Share GO process	
	F(C)	P-value	F(C)	P-value	F(C)	P-value
All possible within Space-I	0.059	NA	0.021	NA	0.036	NA
CCSB-HI1	0.488	1.49×10^{-28}	0.250	5.49×10^{-20}	0.233	2.68×10^{-28}
LCI	0.656	6.47×10^{-139}	0.228	5.72×10^{-120}	0.410	1.74×10^{-405}
LCI-core	0.870	5.90×10^{-43}	0.270	1.70×10^{-32}	0.616	3.40×10^{-137}
LCI-non-core	0.610	3.84×10^{-100}	0.218	1.33×10^{-89}	0.368	4.03×10^{-280}

F(C) represents the fraction of gene- or protein-pairs (defined for each row) the given characteristic C. Assessed characteristics include shared mouse phenotype, shared upstream motif, correlated expression³⁰ and shared Gene Ontology annotation. 'All possible within Space-I' represents all possible gene- or protein-pairs in Space-I for which information regarding C is available. For each analysis of a shared characteristic, only gene- or protein-pairs for which both members had some annotation for that characteristic were considered. For analysis of correlated expression, only gene pairs with expression measurements for both genes were considered.



Figure 3 | Interaction network of disease-associated CCSB-HI1 proteins. The network has 121 OMIM disease-associated proteins (green nodes) and 424 CCSB-HI1 interactions involving them (red edges), along with known LC interactions (solid blue edges represent binary LCI interactions and dashed blue edges represent non-binary interactions).

Proteins without an OMIM disease association are depicted as yellow nodes, and blue edges with increasing thickness indicate LCI-non-core, LCI-core and LCI-hypercore interactions, respectively. We note that 94 out of the 424 CCSB-HI1 interactions involve the Ewing sarcoma related protein (EWSR1; also known as EWS).

LCI pairs) showed no linkage of the corresponding gene symbols (Supplementary Fig. S3). These results indicate that most of our yeast two-hybrid interactions are novel.

To determine whether messenger RNAs corresponding to interacting protein pairs are likely to be co-expressed, we used Pearson correlation coefficients of the corresponding gene pairs in the CCSB-HI1 and LCI data sets from four expression studies in diverse human and mouse tissues (Supplementary Data XI). LCI pairs were enriched for correlated expression in all four cases ($P < 3 \times 10^{-17}$) and CCSB-HI1 pairs were enriched in three of the four cases ($P < 3 \times 10^{-5}$) (Table 1; see also Supplementary Fig. S4a and Supplementary Tables S2 and S5). In addition, CCSB-HI1 interactions are more enriched than would be expected by chance for (1) presence of a common upstream DNA sequence that is conserved across human, mouse, rat and dog genomes²² ($P = 1 \times 10^{-4}$), (2) orthologous genes in mouse having a specific phenotype in common²³ ($P = 3 \times 10^{-3}$), and (3) annotation with the same Gene Ontology (GO) terms²⁴ ($P < 6 \times 10^{-20}$ for all three GO branches) (Table 1; see also Supplementary Tables S2 and S5 and Supplementary Data XI).

The higher likelihood of LCI interactions to share other biological attributes is not surprising given inspection bias and potential circularity where functional annotation has been derived from an LCI interaction. That the CCSB-HI1 interaction pairs (which do not have such biases) yield statistically significant correlation supports their biological relevance. In all, 357 CCSB-HI1 interactions are supported by at least one additional characteristic and thus represent particularly appealing hypotheses of functional relatedness (Supplementary Fig. S4b). Lack of additional biological evidence is not an argument against any interaction (Supplementary Data XII). Importantly, complementary information from the interactome and other functional genomic data can be integrated to formulate biological models²⁵.

The CCSB-HI1 network has topological properties that are similar to other sampled interactome networks, such as an approximately power-law degree distribution, hierarchical organization and a tendency for highly connected (hub) proteins to interact with less highly connected proteins (Supplementary Data XIII and Supplementary Fig. S5a–d). Surprisingly, although the CCSB-HI1 network has a small characteristic path length, it does not exhibit high clustering, seemingly contradicting findings from the sparsely sampled networks of other organisms that protein interaction networks are ‘small world’^{6,7,26} (that is, have a short characteristic path length and a high clustering coefficient). Possible explanations for this apparent discrepancy are discussed in Supplementary Data XIII.

To gain an insight into the evolution of the interactome, we classified proteins in the CCSB-HI1 network as ‘eukaryotic’, ‘metazoan’, ‘mammalian’ or ‘human’, and asked whether proteins specific to different evolutionary classes tend to interact with one another. The CCSB-HI1 network appears to be enriched for interactions between proteins of the same evolutionary class but not for interactions between proteins from two different evolutionary classes (Supplementary Table S6). This suggests that the human interactome has evolved through the preferential addition of interactions between lineage-specific proteins. Further investigation may provide hypotheses for mechanisms underlying interactome evolution.

To detect densely connected subgraphs potentially representing biological modules, we applied the MCODE graph clustering algorithm²⁷ to the CCSB-HI1 and to the combined CCSB-HI1–LCI and CCSB-HI1–LC networks (Supplementary Fig. S6, Supplementary Table S7 and Supplementary Data XIV). We identified functionally enriched MCODE complexes using FuncAssociate²⁸. Out of 172 complexes (Supplementary Table S7) containing at least one CCSB-HI1 interaction, we identified 102 in which at least one GO term was significantly over-represented ($P \leq 0.05$), ten times the number expected by chance alone. The enriched functional terms we identified may also apply to unannotated proteins present in the complex (‘guilt by association’ predictions).

CCSB-HI1 represents a repository of novel biological hypotheses for genes implicated in human diseases. We compared all CCSB-HI1 proteins to the list of genes associated with human diseases in the Online Mendelian Inheritance in Man (OMIM) database and identified 424 interacting pairs for which at least one partner had been previously associated with a human disease (Supplementary Table S8). In a query of PubMed and Google Scholar, searching for gene symbols, 352 of the 424 interaction pairs appeared to be new based on the absence of any hit in either database. Along with 79 LC interactions (including LCI and non-binary LC interactions) among proteins in this space, the resulting network contains 484 interactions among 417 proteins (Fig. 3).

In one example, we found an interaction between RTN4, a neurite outgrowth inhibitor, and SPG21, the spastic paraplegia 21 protein. Mutations in SPG21 cause an autosomal recessive motor disorder called Mast syndrome. SPG21 protein localizes to intracellular endosomal/trans-Golgi transportation vesicles and is thought to function in protein transport and sorting. Although the function of its interacting partner, RNT4, remains elusive, RNT4 belongs to a family of proteins that localize to the endoplasmic reticulum and are markers of neuroendocrine differentiation²⁹. In addition, RNT4 shares two regulatory motifs with SPG21 that are conserved across mammalian genomes²² and may have a role in Mast syndrome. This and other examples (Supplementary Data XV) suggest that CCSB-HI1 can be used to connect biological processes in order to understand further network and disease relationships.

Although the CCSB-HI1 data set is far from comprehensive, and incomplete sampling limits conclusions regarding some network properties, our data provide useful hypotheses and can guide further studies of the expanded network. Currently, CCSB-HI1 is a static graph. Eventually the dynamics of the human interactome network will need to be considered to address where and when interactions take place and how they are regulated. The functional consequences of these physical interactions will also have to be studied to understand the logic of complex biological networks. Just as the first drafts of the human genome changed strategies for disease gene identification, the emerging human interactome will greatly further the understanding of human health and disease.

METHODS

The CCSB-HI1 data set was generated using a high-throughput version of the yeast two-hybrid system. First, all 8,100 cloned ORFs of the human ORFeome v1.1 were transferred from Entry clones to both AD and DB vectors by Gateway recombinational cloning. Resulting constructs were transformed in haploid yeast cells. After mating, diploids were tested on selective media for their ability to grow in an AD-Y-dependent manner. The identity of the interactors was determined after PCR amplification and sequencing of the AD and DB inserts from positive colonies. Resulting interactions were re-tested by the yeast two-hybrid system, individually, assessed for quality by co-affinity purification assays and analysed for correlation with other biological information. For a detailed description of the various methods, see Supplementary Data.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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