

**Protocol S1  
Supporting Data**

**Molecular basis for evolving modularity in the  
yeast protein interaction network**

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**I. PDB-reported intra-modular hubs in yeast class \((11111) \***

**(\*) The class \((11111) is the complement of the ancestral class (11111) of proteins with orthologs in all life domains considered, and thus contains all the nodes incorporated to the ancestral network up to the present day.**

SWISSPROT					PDB	
ID	ORF	Gene name	conn.	Code	Method	Chain
P02309	YNL030W	H4_YEAST	38	2FSB	Model	B/F=1-103
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Q02821	YNL189W	IMA1_YEAST	30	1WA5	X-ray	B=1-530
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Q02821	YNL189W	IMA1_YEAST	30	1EE5	X-ray	A=87-510
Q02821	YNL189W	IMA1_YEAST	30	1EE4	X-ray	A/B=87-509
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P02829	YPL240C	HSP82_YEAST	30	2CG9	X-ray	A/B
P02829	YPL240C	HSP82_YEAST	30	2BRE	X-ray	A/B=1-219
P02829	YPL240C	HSP82_YEAST	30	2BRC	X-ray	A=1-214
P02829	YPL240C	HSP82_YEAST	30	2AKP	X-ray	A/B=25-210
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P02829	YPL240C	HSP82_YEAST	30	1AM1	X-ray	2-214
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P52286	YDR328C	SKP1_YEAST	22	1NEX	X-ray	A/C=1-194
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P06787	YBR109C	CALM_YEAST	16	1F54	NMR	A=2-78
P39517	YDL160C	DHH1_YEAST	15	1S2M	X-ray	A=31-425
P29468	YKR002W	PAP_YEAST	15	1FA0	X-ray	A/B=1-537
P39081	YDR228C	PCF11_YEAST	14	2BF0	X-ray	X=1-138
P39081	YDR228C	PCF11_YEAST	14	1SZA	X-ray	A/B/C=1-140
P39081	YDR228C	PCF11_YEAST	14	1SZ9	X-ray	A/B/C=1-140
P32628	YEL037C	RAD23_YEAST	14	1X3Z	X-ray	B=238-309
P32628	YEL037C	RAD23_YEAST	14	1X3W	X-ray	B=238-309
P25293	YKR048C	NAP1_YEAST	14	2AYU	X-ray	A=1-417

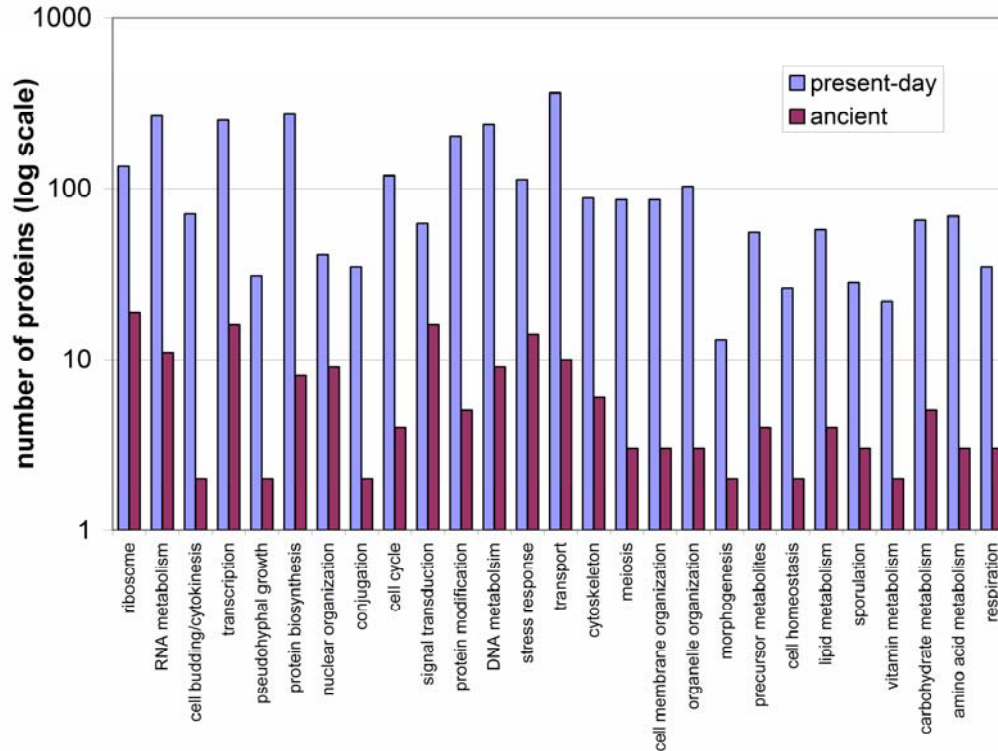
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P41832	YNL271C	BNI1_YEAST	12	1Y64	X-ray	B=1327-1769
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P22336	YAR007C	RFA1_YEAST	12	1YNX	NMR	A=181-294
P14907	YJL041W	NSP1_YEAST	12	1O6O	X-ray	D/E/F=497-608
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P06782	YDR477W	SNF1_YEAST	12	2FH9	X-ray	A=46-319
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P04147	YER165W	PABP_YEAST	12	1IFW	NMR	A=489-577
P29366	YBR200W	BEM1_YEAST	11	1IPG	NMR	A=472-551
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P10081	YJL138C	IF4A_YEAST	11	1QVA	X-ray	A=1-224
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P10081	YJL138C	IF4A_YEAST	11	1FUU	X-ray	A/B=1-395
P10081	YJL138C	IF4A_YEAST	11	1FUK	X-ray	A=231-395
P34162	YHR041C	MED20_YEAST	10	2HZS	X-ray	A/C/E/G=2-210
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P16892	YBL016W	FUS3_YEAST	10	2FA2	X-ray	A/B=1-353
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P16892	YBL016W	FUS3_YEAST	10	2B9I	X-ray	A=1-353
P16892	YBL016W	FUS3_YEAST	10	2B9H	X-ray	A=1-353
P16892	YBL016W	FUS3_YEAST	10	2B9F	X-ray	A=1-353
P54784	YML065W	ORC1_YEAST	9	1ZHI	X-ray	A=1-219
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P22216	YPL153C	RAD53_YEAST	9	1K3Q	NMR	A=14-164
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P22216	YPL153C	RAD53_YEAST	9	1K3J	NMR	A=14-164
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P17555	YNL138W	CAP_YEAST	9	1K4Z	X-ray	A/B=369-526
Q08649	YOR244W	ESA1_YEAST	8	1MJB	X-ray	A=160-435
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Q08649	YOR244W	ESA1_YEAST	8	1FY7	X-ray	A=160-435
Q08278	YOL135C	MED7_YEAST	8	1YKH	X-ray	A=102-205
Q08278	YOL135C	MED7_YEAST	8	1YKE	X-ray	A/C=102-205
P38304	YBR193C	MED8_YEAST	8	2HZS	X-ray	I/J/K/L=190-216
P34087	YDR404C	RPB7_YEAST	8	2B8K	X-ray	G=1-171
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P34087	YDR404C	RPB7_YEAST	8	1Y1Y	X-ray	G
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P25454	YER095W	RAD51_YEAST	8	1SZP	X-ray	A/B/C/D/E/F=81-400
P23561	YLR362W	STE11_YEAST	8	1X9X	NMR	A/B=37-104
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P18851	YOR212W	GBB_YEAST	8	1SCG	Model	B=1-423
P16649	YCR084C	TUP1_YEAST	8	1ERJ	X-ray	A/B/C=282-713

## II. Variation in functional composition with network evolution

To fully characterize the evolutionary trend towards increasing modularity, the functional composition of the ancient network is contrasted against the present-day network, using the genetic ontology (GO) Slim classification of open reading frames [1]. The functional distribution differences associated with evolutionary change are shown in Fig. S1. We find that the relative representation of certain functional categories persists throughout evolutionary change, in spite of the drastic reduction in number of proteins. Furthermore, dominant

functionalities that become significant in the ancient network may be identified by a representativity larger than twice the basal value 3.5% of the corresponding representativity of the functional class in the present-day proteome. The basal value corresponds to the fact that the ancient proteome contains 3.5% of present-day proteins. Thus, dominant functional classes in the ancient network are: ribosome, RNA metabolism, transcription, nuclear organization, signal transduction and stress response.



**Figure S1.** Functional composition of present-day and ancient proteome. The classification used the slim gene ontology (GO).

### III. Supplementary results on the ortholog-based trimming procedure

Our analysis of the topological evolution of modularity hinges on the validity of a trimming procedure based on the retention of nodes according to their extent of orthologous representation. This procedure is supported by recent analysis [2], yet it is somewhat at odds with the estimation by Wagner [3] that interactions in yeast are lost at a rate of  $2.2 \times 10^{-3}$ /million year. This assessment uses the extent of synonymous substitution as surrogate for evolutionary time and adopts high-throughput two-hybrid experiments to map protein interactions. The latter data is known to be unreliable and plagued by false positives [4] and would require a careful curation which was not performed at the time of the analysis. Furthermore, to assess the

loss of interactions, one would need to perform a structural analysis to verify whether indeed the protein interfaces have been modified or have coevolved through evolutionary change, which is not likely to be uniform across the protein chain. This analysis was not carried out in [3]. Most importantly, the assertion on the loss of interactions is at odds with the established high level of conservation of functionally competent protein interfaces [5, 6].

1. Prachumwat A, Li W-H (2006) Protein function, connectivity and duplicability in yeast. *Mol Biol Evol* 23: 30-39
2. Qin H, Lu H S, Wu W B, Li W-H (2003) Evolution of the yeast protein interaction network. *Proc Natl Acad Sci USA* 100: 12820-12824
3. Wagner A (2001) The yeast protein interaction network evolves rapidly and contains few redundant duplicate genes. *Mol Biol Evol* 18: 1283-1292
4. Krogan D, Cagney G, Yu H, Zhong G, Guo X, *et al.* (2006) Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 440: 637-643
5. Fernández A, Berry R S (2004) Molecular dimension explored in evolution to promote proteomic complexity. *Proc Natl Acad Sci USA* 101: 13460-13465
6. Kim P M, Lu L J, Xia Y, Guerstein M B (2006) Relating three-dimensional structures to protein networks provides evolutionary insights. *Science* 314: 1938-1941