Table I: Top five nodes in each protein by degree and closeness

Degree and closeness are given in parentheses for each residue.

Protein	Top 5 Nodes by					
anthranilate synthase	degree	L136(12)	S135(10)	H134(9)	H107(8)	R257(8)
	closeness	G485(0.20)	K357(0.20)	K502(0.20)	D489(0.19)	R469(0.19)
ATCase	degree	T79(11)	Y240(9)	R41(8)	A245(7)	S238(7)
	closeness	E10(0.30)	V9(0.28)	A11(0.28)	R41(0.27)	D4(0.26)
ATP sulfurylase	degree	L229(8)	R572(6)	M332(6)	T230(6)	Q331(6)
	closeness	R515(1.0)	D111(0.66)	F330(0.21)	A213(0.20)	M332(0.20)
ATP-PRT	degree	R184(8)	I55(7)	S59(6)	R160(6)	E61(5)
	closeness	Q164(0.17)	K32(0.17)	R160(0.17)	D33(0.17)	D30(0.16)
DAHP synthase	degree	K97(8)	K105(7)	E96(6)	C61(3)	D326(3)
	closeness	K105(0.46)	K97(0.41)	Q170(0.38)	E96(0.37)	R99(0.34)
FBPase-1	degree	Y57 (12)	A51(12)	K71(11)	I59(10)	A54(9)
	closeness	M18(0.11)	R15(0.11)	Q32(0.11)	S88(0.11)	A60(0.11)
glcN-6-P deaminase	degree	E148(7)	L153(5)	K160(5)	S151(5)	T225(5)
	closeness	K208(0.5)	T41(0.5)	G43(0.42)	F173(0.42)	G42(0.42)
GTP cyclo-hydrolase I	degree	V209(9)	K230(8)	E119(7)	Q210(7)	R226(6)
	closeness	K230(0.09)	E233(0.08)	R226(0.08)	P229(0.08)	R232(0.08)
glycogen phosphorylase	degree	R269(10)	Y262(10)	F252(9)	V266(8)	L254(7)
	closeness	Y75(0.32)	S314(0.29)	R310(0.28)	D42(0.26)	G317(0.26)
lactate DH	degree	T236(10)	P60(9)	R156(8)	I229(8)	I230(8)
	closeness	K170(0.10)	P60(0.10)	H54(0.10)	F159(0.10)	I229(0.10)
NAD-malic enzyme	degree	Y112(9)	N421(6)	F68(6)	E314(5)	N467(5)
	closeness	N421(0.41)	N467(0.41)	L167(0.41)	N466(0.41)	D279(0.39)
phosphofructokinase	degree	A157(14)	H160(11)	T158(10)	R162(10)	S159(9)
	closeness	A157(0.43)	T158(0.41)	K214(0.41)	R162(0.41)	H160(0.40)
phosphoglycerate DH	degree	L142(5)	Q298(3)	R60(3)	A143(3)	K141(3)
	closeness	G18(0.54)	H292(0.4)	P212(0.4)	A238(0.4)	S216(0.38)
PTB1B	degree	W291(10)	F182(6)	S295(6)	N193(6)	E297(6)
	closeness	F182(0.48)	R221(0.43)	Q262(0.41)	D181(0.40)	N193(0.37)
uracil PRT	degree	R80(11)	P114(9)	L79(8)	F215(7)	Y123 (5)
	closeness	E87(0.18)	R37(0.17)	Q98(0.17)	V83(0.17)	R97(0.16)

Table II: list of allostery-altering mutations for three proteins

1) phosphofructokinase (PFK)
In WT, GDP counters PEP inhibition but has no effect by itself.

ref	mutation	effect
[1]	D59A, D59M	mild decrease in PEP inhib
	R25A, D211A	severe reduction (~100fold) of PEP inhib
[2]	R252A	large increase in PEP inhib
[3]	G212V	3x weaker PEP inhib than WT
		insensitive to GDP activation
[4]	E161Q	~10x weaker PEP inhib, ~6x stronger GDP act.
	E161A	~2x weaker PEP inhib, ~4x stronger GDP act.
[5]	E161A	coupling ΔG (PEP-fru6P) similar to WT
	R162A, E161A+R162A	coupling ΔG ~2/3 of WT

Summary of key positions (those used for testing of allosteric networks):
reduce PEP inhib: E161Q (A), R162A, G212V, R25A, D211A
increase PEP inhib: R252A

2) fructose bisphosphatase (FBPase)

ref	mutation	effect
[6]	R140A, T31S	<10-fold –AMP inhib
	A24F	10-100-fold –AMP inhib
	T31A, Y113F	>1000-fold –AMP inhib
[7]	R22A	10-fold –AMP inhib
[8]	E98Q	abolish coop of AMP inhib
[9]	R22K	10-100 fold –AMP inhib
	N9D	<10-fold –AMP inhib
	T27A, M18R	>1000-fold –AMP inhib
[10]	K42A	>1000-fold AMP required for full inhib
		cooperative AMP inhib disrupted
[11]	D187A	<10-fold –AMP inhib
	E92Q, E92A	>100-fold AMP required for full inhib
		cooperative AMP inhib disrupted
[12]	K71A	~10-fold –AMP inhib
	K71M+K72M	~150-fold –AMP inhib
	D74E, N64A	abolish AMP cooperativity
[13]	K50A, K50Q	abolish AMP cooperativity
	R49A, R49Q	abolish AMP cooperativity +
		>1000-fold –AMP inhib
[14]	Y57W	<10-fold –AMP inhib
	A51P	100-1000-fold –AMP inhib
	K50P	>1000-fold –AMP inhib
[15]	K112Q, Y113F	>1000-fold –AMP inhib

key mutants: A24, T31, Y113, R22, T27, M18, K42, E92, K72, R49, A51, K50, K112
weak or unclear mutants: R140, E98, N9, D187, K71, D74, N64, Y57

3) Aspartate transcarbamoylase (ATCase)

ref	mutation	effect
[16]	R-K94Q	no ATP activation
		weakened CTP inhib
[17]	C-Q108Y, R-N113G	slight alteration in ATP activation
		slight (~1.5) weakening of CTP inhib
[18]	R-N111A	no ATP act or CTP inhib
[19]	R-K56A	no ATP act, weakened CTP inhib
[20]	C-E50[A or D]	weakened ATP act & CTP inhib
[21]	C-Q231L	weakened ATP act & CTP inhib
	C-R167Q	no effect on ATP or CTP
[22]	C-(K164E, E239K)	no discernible ATP act or CTP inhib
[23]	R-Y77F	ATP activation -> inhibition!
[24]	R-K60H	no discernible ATP act or CTP inhib
	K60R/Q	no effect
	K60A	lost CTP inhib
	R-K94H	very slight weakening of ATP act, CTP inhib
[25]	C-D160A	no discernible ATP act or CTP inhib
[26]	R-(C109H, E119D)	lost CTP inhib (pH 7)
[27]	R-(V106W, L76A, L151Q)	weakened ATP act
[28]	R-(F145W, S146E, S146A, N148D, N148A, V149A, N153G)	weaken or abolish ATP act
[29]	C-E50A	weaken ATP act
	C-S171A	weaken CTP inhib
[30]	R-E162A	strengthen ATP act, CTP inhib
	R-I12A	weaken CTP inhib, no ATP act
[31]	R-V106L	weakened ATP act & CTP inhib
	R-D104G	lost ATP act
	I103T	ATP act -> inhib
[32]	R-T82A	stronger ATP act, weaker CTP inhib
[33]	C-D162A	weakend ATP act
[34]	C-D236A	no ATP act or CTP inhib

summary of mutants with significant effects:
catalytic chain (A, C, …): Q108, E50, Q231, K164, E239, D160, S171, D162, D236
regulatory chain (B, D, …): K94, N113, N111, K56, Y77, K60, C109, E119, V106, L76, L151, F145, S146, N148, V149, N153, E162, I12, D104, I103, T82

References for Table II:

1. Valdez, B.C., S.H. Chang, and E.S. Younathan, *Site-directed mutagenesis at the regulatory site of fructose 6-phosphate-1-kinase from Bacillus stearothermophilus.* Biochem Biophys Res Commun, 1988. **156**(1): p. 537-42.
2. Valdez, B.C., et al., *Site-directed mutagenesis in Bacillus stearothermophilus fructose-6-phosphate 1-kinase. Mutation at the substrate-binding site affects allosteric behavior.* J Biol Chem, 1989. **264**(1): p. 131-5.
3. Zhu, X., et al., *Role of glycine 212 in the allosteric behavior of phosphofructokinase from Bacillus stearothermophilus.* Biochemistry, 1995. **34**(8): p. 2560-5.
4. Auzat, I., et al., *Role of residue 161 in the allosteric transitions of two bacterial phosphofructokinases.* Biochemistry, 1995. **34**(21): p. 7062-8.
5. Kimmel, J.L. and G.D. Reinhart, *Reevaluation of the accepted allosteric mechanism of phosphofructokinase from Bacillus stearothermophilus.* Proc Natl Acad Sci U S A, 2000. **97**(8): p. 3844-9.
6. Gidh-Jain, M., et al., *The allosteric site of human liver fructose-1,6-bisphosphatase. Analysis of six AMP site mutants based on the crystal structure.* J Biol Chem, 1994. **269**(44): p. 27732-8.
7. Lu, G., et al., *Fructose-1,6-bisphosphatase: arginine-22 is involved in stabilization of the T allosteric state.* Biochemistry, 1995. **34**(41): p. 13272-7.
8. Kelley, N., et al., *Glutamic acid residue 98 is critical for catalysis in pig kidney fructose-1,6-bisphosphatase.* Biochem Biophys Res Commun, 1996. **219**(3): p. 848-52.
9. Shyur, L.F., A.E. Aleshin, and H.J. Fromm, *A study of subunit interface residues of fructose-1,6-bisphosphatase by site-directed mutagenesis: effects on AMP and Mg2+ affinities.* Biochemistry, 1996. **35**(23): p. 7492-8.
10. Lu, G., et al., *Evidence for an active T-state pig kidney fructose 1,6-bisphosphatase: interface residue Lys-42 is important for allosteric inhibition and AMP cooperativity.* Protein Sci, 1996. **5**(11): p. 2333-42.
11. Lu, G., E.L. Giroux, and E.R. Kantrowitz, *Importance of the dimer-dimer interface for allosteric signal transduction and AMP cooperativity of pig kidney fructose-1,6-bisphosphatase. Site-specific mutagenesis studies of Glu-192 and Asp-187 residues on the 190's loop.* J Biol Chem, 1997. **272**(8): p. 5076-81.
12. Kurbanov, F.T., et al., *Directed mutations in the poorly defined region of porcine liver fructose-1,6-bisphosphatase significantly affect catalysis and the mechanism of AMP inhibition.* J Biol Chem, 1998. **273**(28): p. 17511-6.
13. Carcamo, J.G., et al., *The C1-C2 interface residue lysine 50 of pig kidney fructose-1, 6-bisphosphatase has a crucial role in the cooperative signal transmission of the AMP inhibition.* Eur J Biochem, 2000. **267**(8): p. 2242-51.
14. Nelson, S.W., et al., *Mutations in the hinge of a dynamic loop broadly influence functional properties of fructose-1,6-bisphosphatase.* J Biol Chem, 2000. **275**(39): p. 29986-92.
15. Kelley-Loughnane, N. and E.R. Kantrowitz, *AMP inhibition of pig kidney fructose-1,6-bisphosphatase.* Biochim Biophys Acta, 2001. **1548**(1): p. 66-71.

16. Zhang, Y., M.M. Ladjimi, and E.R. Kantrowitz, *Site-directed mutagenesis of a residue located in the regulatory site of Escherichia coli aspartate transcarbamoylase. Involvement of lysine 94 in effector binding and the allosteric mechanism.* J Biol Chem, 1988. **263**(3): p. 1320-4.
17. Xu, W., et al., *Propagation of allosteric changes through the catalytic-regulatory interface of Escherichia coli aspartate transcarbamylase.* Biochemistry, 1988. **27**(15): p. 5507-15.
18. Eisenstein, E., D.W. Markby, and H.K. Schachman, *Changes in stability and allosteric properties of aspartate transcarbamoylase resulting from amino acid substitutions in the zinc-binding domain of the regulatory chains.* Proc Natl Acad Sci U S A, 1989. **86**(9): p. 3094-8.
19. Corder, T.S. and J.R. Wild, *Discrimination between nucleotide effector responses of aspartate transcarbamoylase due to a single site substitution in the allosteric binding site.* J Biol Chem, 1989. **264**(13): p. 7425-30.
20. Newton, C.J. and E.R. Kantrowitz, *Importance of domain closure for homotropic cooperativity in Escherichia coli aspartate transcarbamylase.* Biochemistry, 1990. **29**(6): p. 1444-51.
21. Stebbins, J.W., Y. Zhang, and E.R. Kantrowitz, *Importance of residues Arg-167 and Gln-231 in both the allosteric and catalytic mechanisms of Escherichia coli aspartate transcarbamoylase.* Biochemistry, 1990. **29**(16): p. 3821-7.
22. Newell, J.O. and H.K. Schachman, *Amino acid substitutions which stabilize aspartate transcarbamoylase in the R state disrupt both homotropic and heterotropic effects.* Biophys Chem, 1990. **37**(1-3): p. 183-96.
23. Van Vliet, F., et al., *Heterotropic interactions in aspartate transcarbamoylase: turning allosteric ATP activation into inhibition as a consequence of a single tyrosine to phenylalanine mutation.* Proc Natl Acad Sci U S A, 1991. **88**(20): p. 9180-3.
24. Wente, S.R. and H.K. Schachman, *Different amino acid substitutions at the same position in the nucleotide-binding site of aspartate transcarbamoylase have diverse effects on the allosteric properties of the enzyme.* J Biol Chem, 1991. **266**(31): p. 20833-9.
25. Newton, C.J., R.C. Stevens, and E.R. Kantrowitz, *Importance of a conserved residue, aspartate-162, for the function of Escherichia coli aspartate transcarbamoylase.* Biochemistry, 1992. **31**(11): p. 3026-32.
26. Strang, C.J., et al., *Site-directed alterations to the geometry of the aspartate transcarbamoylase zinc domain: selective alteration to regulation by heterotropic ligands, isoelectric point, and stability in urea.* Biochemistry, 1993. **32**(16): p. 4156-67.
27. Xi, X.G., et al., *The activation of Escherichia coli aspartate transcarbamylase by ATP. Specific involvement of helix H2' at the hydrophobic interface between the two domains of the regulatory chains.* J Mol Biol, 1994. **242**(2): p. 139-49.
28. De Staercke, C., et al., *Intramolecular transmission of the ATP regulatory signal in Escherichia coli aspartate transcarbamylase: specific involvement of a clustered set of amino acid interactions at an interface between regulatory and catalytic subunits.* J Mol Biol, 1995. **246**(1): p. 132-43.

29. Baker, D.P., et al., *The allosteric activator ATP induces a substrate-dependent alteration of the quaternary structure of a mutant aspartate transcarbamoylase impaired in active site closure.* Protein Sci, 1996. **5**(11): p. 2276-86.
30. Dutta, M. and E.R. Kantrowitz, *The influence of the regulatory chain amino acids Glu-62 and IIe-12 on the heterotropic properties of Escherichia coli aspartate transcarbamoylase.* Biochemistry, 1998. **37**(24): p. 8653-8.
31. Rastogi, V.K., et al., *Role of allosteric: zinc interdomain region of the regulatory subunit in the allosteric regulation of aspartate transcarbamoylase from Escherichia coli.* Arch Biochem Biophys, 1998. **354**(2): p. 215-24.
32. Williams, M.K. and E.R. Kantrowitz, *Threonine 82 in the regulatory chain is important for nucleotide affinity and for the allosteric stabilization of Escherichia coli aspartate transcarbamoylase.* Biochim Biophys Acta, 1998. **1429**(1): p. 249-58.
33. Fetler, L., et al., *Replacement of Asp-162 by Ala prevents the cooperative transition by the substrates while enhancing the effect of the allosteric activator ATP on E. coli aspartate transcarbamoylase.* Protein Sci, 2002. **11**(5): p. 1074-81.
34. Chan, R.S., et al., *The role of intersubunit interactions for the stabilization of the T state of Escherichia coli aspartate transcarbamoylase.* J Biol Chem, 2002. **277**(51): p. 49755-60.

Table III: Expected and actual occurrences of allostery-perturbing mutants in allosteric networks for three proteins

For each mutant in each protein, expected is the number of monomers containing that residue, actual is the number of times that residue occurs in the allosteric network, and hit rate = actual / expected.

A: phosphofructokinase

residue	occurences expected	occurences actual	hit rate
E161	4	4	1.00
R162	4	4	1.00
G212	4	2	0.50
R25	4	4	1.00
D211	4	2	0.50
R252	4	4	1.00
total	24	20	0.83

B: fructose bisphosphatase

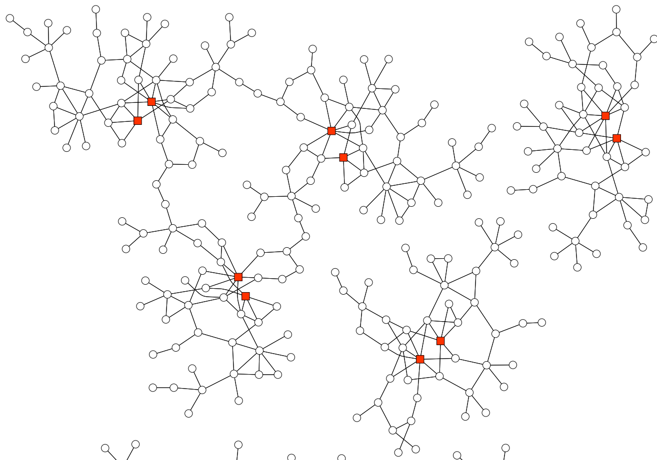
residue	occurences expected	occurences actual	hit rate
A24	4	4	1.00
T31	4	4	1.00
Y113	4	0	0.00
R22	4	4	1.00
T27	4	4	1.00
M18	4	4	1.00
K42	4	0	0.00
E92	4	0	0.00
K72	4	4	1.00
R49	4	0	0.00
A51	4	4	1.00
K50	4	4	1.00
K112	4	0	0.00
total	52	32	0.62

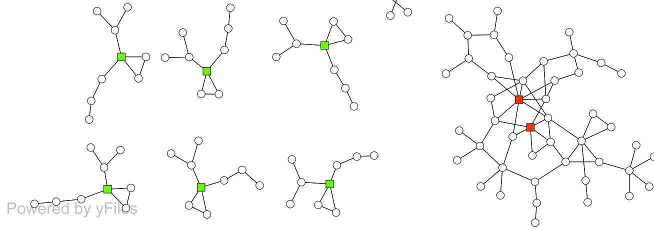
C: aspartate transcarbamoylase

The mutants in the top block are catalytic chain residues;
those in the bottom block are regulatory chain residues.

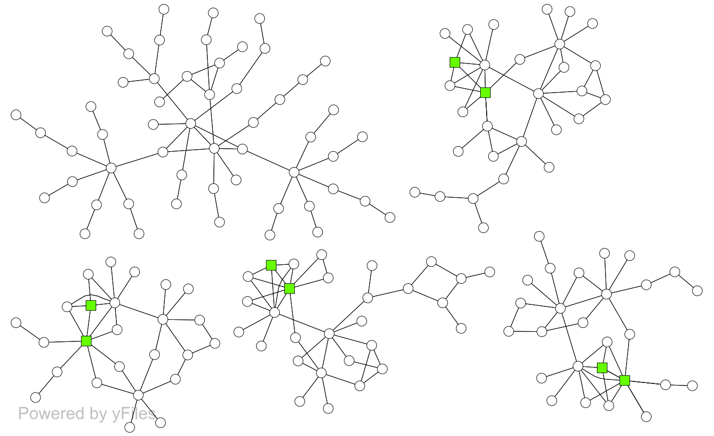
residue	occurences expected	occurences actual	hit rate
C-Q108	6	0	0.00
E50	6	6	1.00
Q231	6	6	1.00
K164	6	6	1.00
E239	6	6	1.00
D160	6	0	0.00
S171	6	4	0.67

D162	6	3	0.50
D236	6	6	1.00
R-K94	6	6	1.00
N113	6	3	0.50
N111	6	6	1.00
K56	6	3	0.50
Y77	6	6	1.00
K60	6	6	1.00
C109	6	0	0.00
E119	6	0	0.00
V106	6	6	1.00
L76	6	6	1.00
L151	6	3	0.50
F145	6	6	1.00
S146	6	3	0.50
N148	6	3	0.50
V149	6	6	1.00
N153	6	6	1.00
E162	6	0	0.00
I12	6	3	0.50
D104	6	3	0.50
I103	6	3	0.50
T82	6	6	1.00
total	180	121	0.67

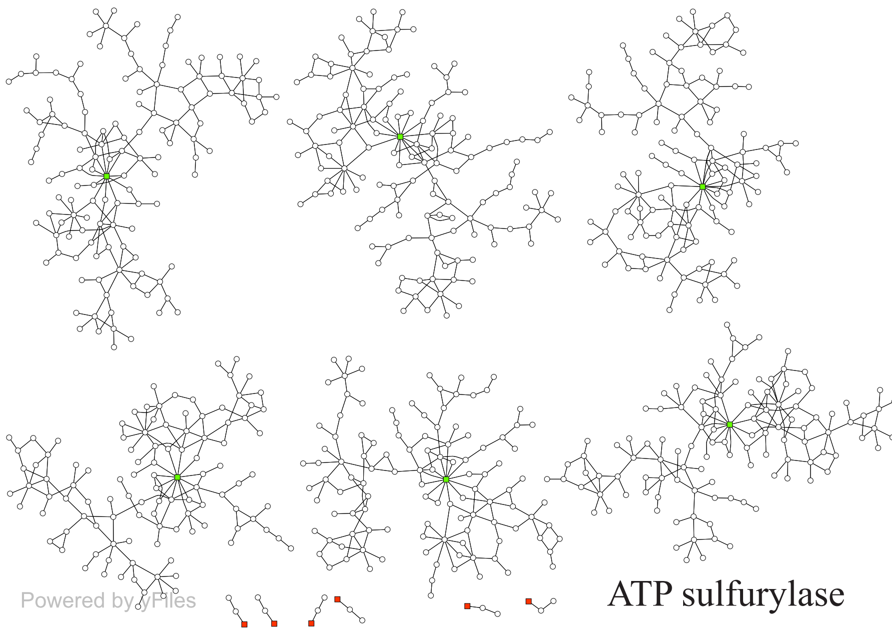




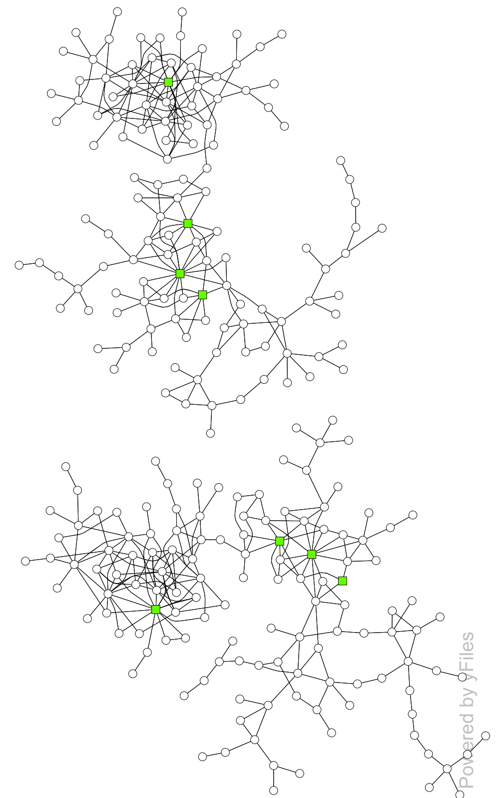
glucosamine-6-P deaminase



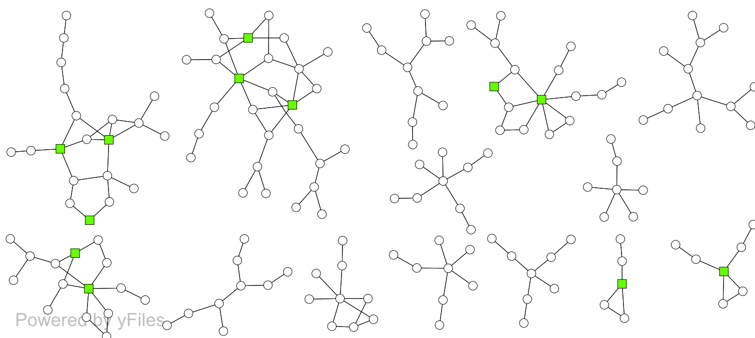
DAHP synthase



ATP sulfurylase

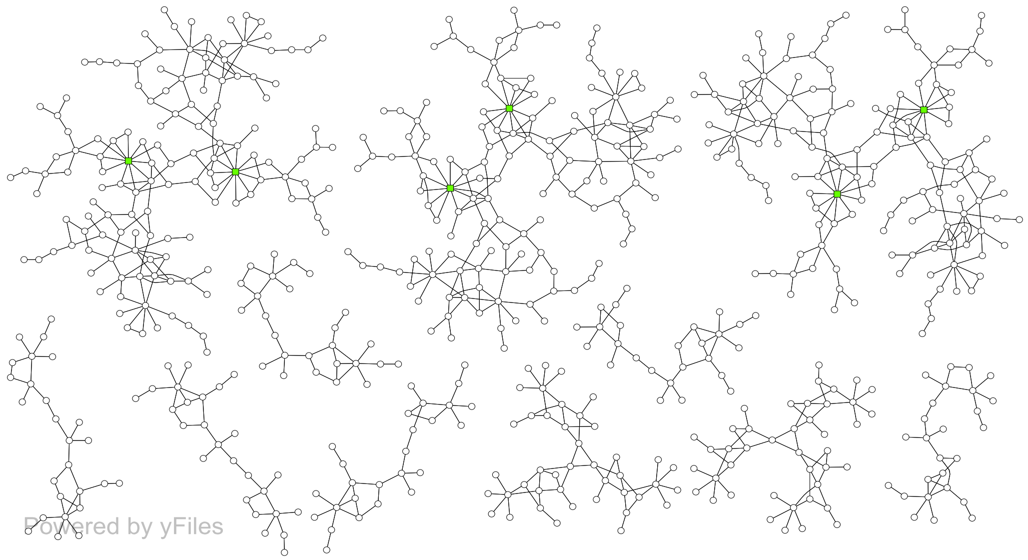


anthranilate synthase

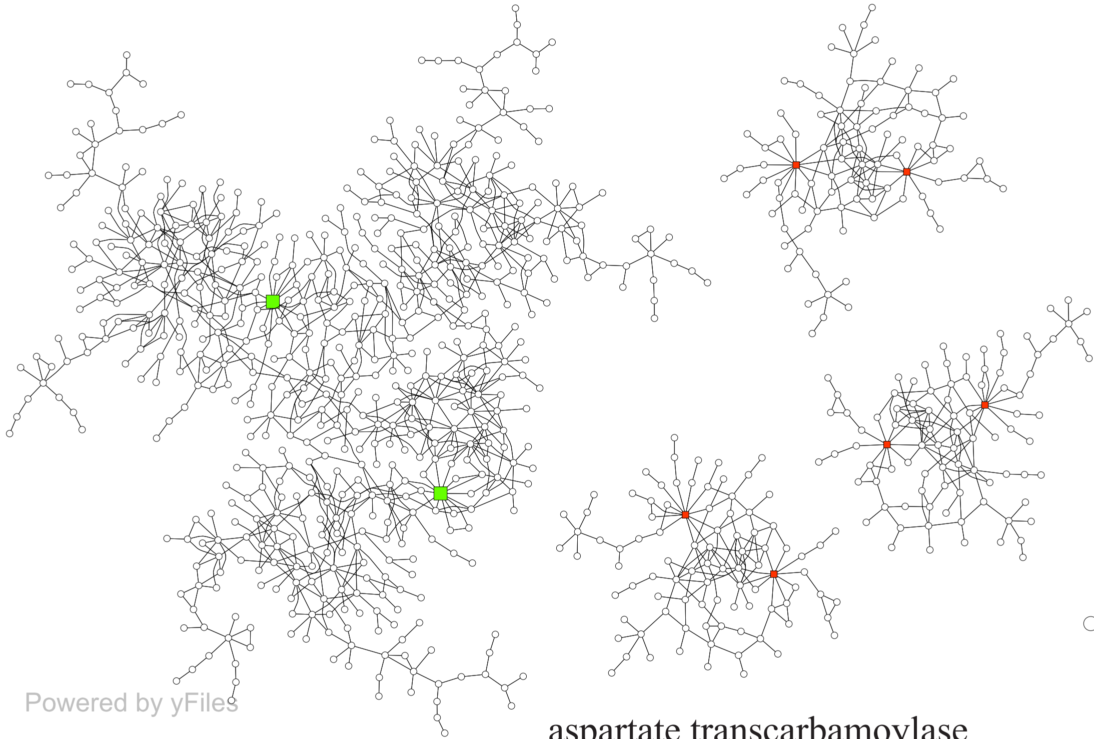


phosphoglycerate dehydrogenase

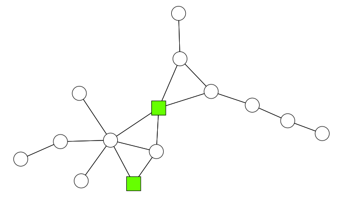
Figure 1: Additional contact rearrangement networks
Formatted as in Figure 2 of the main text. Continued on next page.



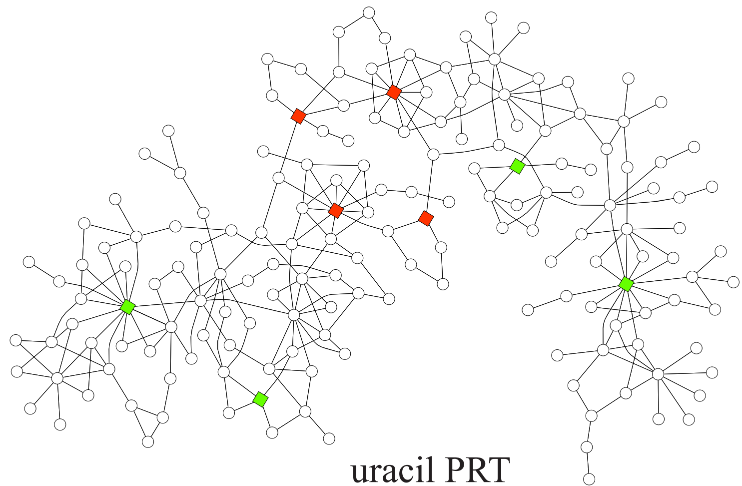
ATP-phosphoribosyltransferase



aspartate transcarbamoylase



PTP1B



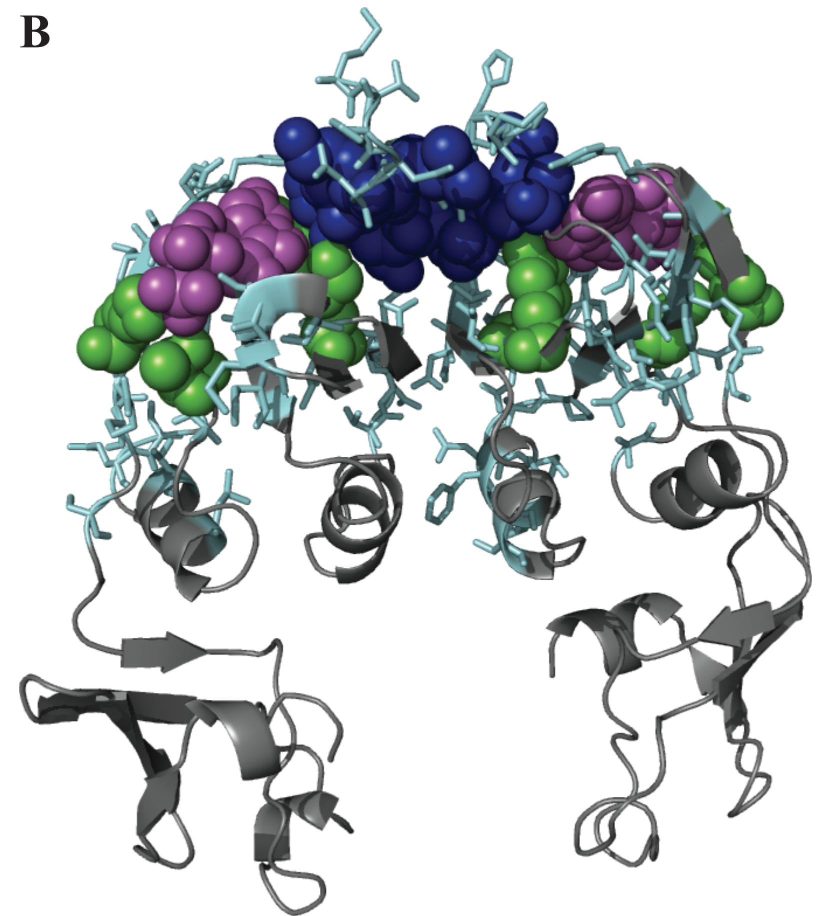
uracil PRT

Figure 1 (cont)

Figure 2: Allostery-perturbing mutants and closest residues in two clusters of ATCase

A

A: One half of ATCase (1RAC.pdb, I structure), including one catalytic trimer (white) and parts of one monomer from each of the regulatory dimers (gray). Cyan: residues in cluster 1; green: allostery-perturbing mutants in cluster 1; blue: closest residues in cluster 1 (10 per catalytic subunit). The allostery-perturbing mutants and the closest residues do not overlap.



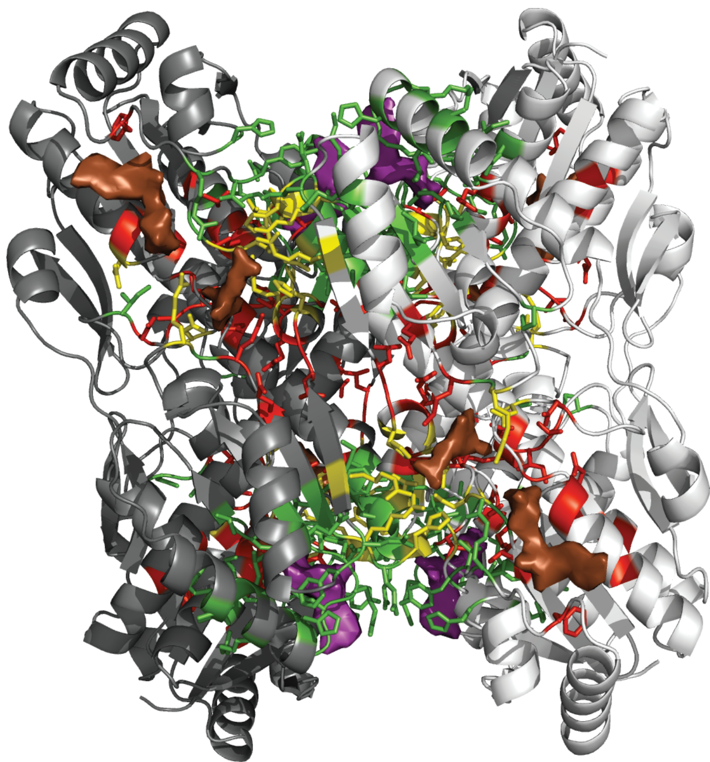
B: one regulatory dimer from ATCase. Cyan: residues in cluster 2; green: allostery-perturbing mutants in cluster 2; blue: closest residues in cluster 2 (5 per regulatory subunit); purple: inhibitor (CTP) molecules. The allostery-perturbing mutants and the closest residues do not overlap.

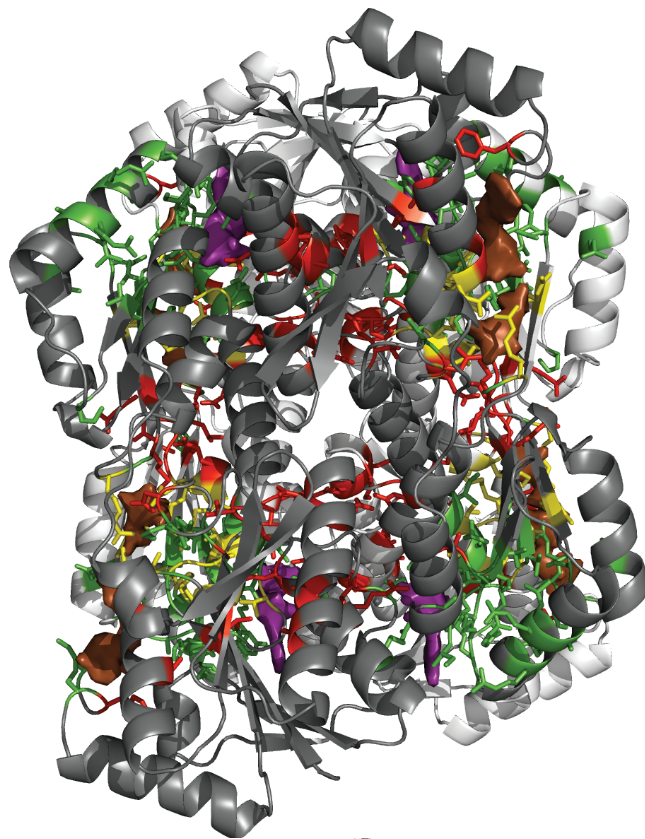
Continued on next page

Figure 2A shows that cluster 1 is located in the catalytic trimers and in the C-terminal domains of the regulatory subunits. Most known allostery-perturbing mutants are located near the substrate-binding sites (not shown) near the centers of the catalytic chains or at the interfaces between the two domains of the regulatory chains at the periphery of this large cluster. On the other hand, the 60 residues with highest closeness in this cluster (10 per subunit) lie near the central axis of the catalytic trimer in the region where the two catalytic trimers interact, which is close to the substrate-binding site but far from the regulatory chain. Thus, while closeness fails to illuminate the regions of this cluster where it is most natural to expect that there would be residues important to allostery, it may well have identified a new, previously untested region as being important to allostery in this protein. This central region might mediate communication among the catalytic subunits. ATCase clusters 2-4, which are respectively located in the three regulatory dimers in the N-terminal domain of each subunit, show similar situations (Figure 2B). In cluster 2, most known allostery-perturbing mutations surround the effector binding sites, while the 10 residues with highest closeness (5 per subunit) lie at the dimer interface, a region which might be important for communication within the dimer.

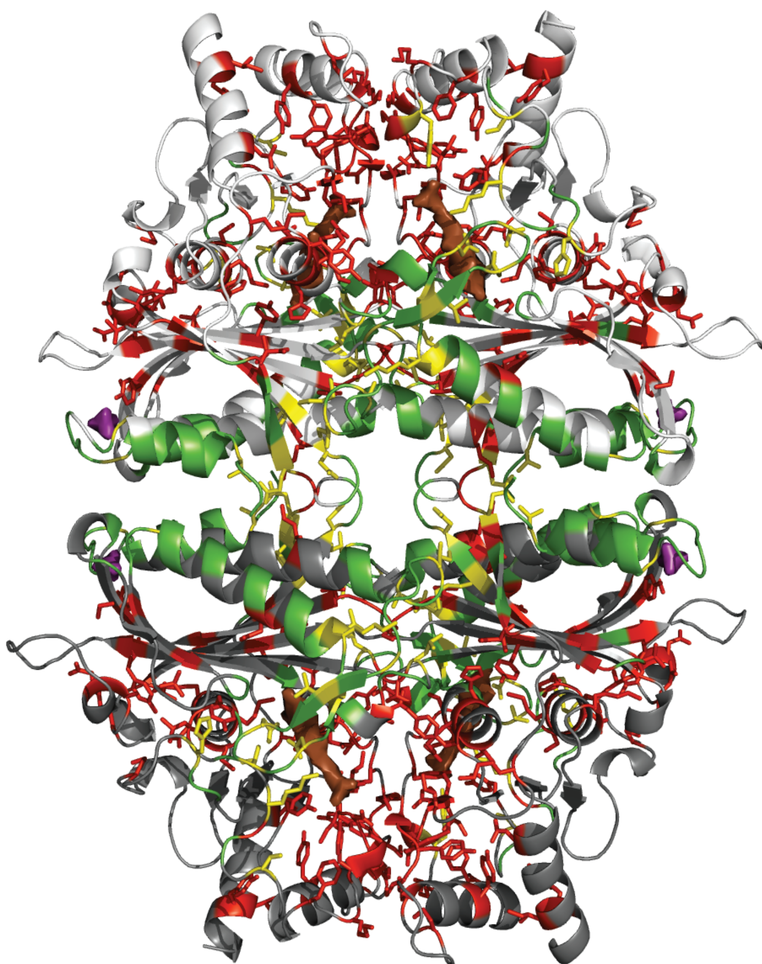
Figure 3: CRN residues vs. SCA residues for two proteins

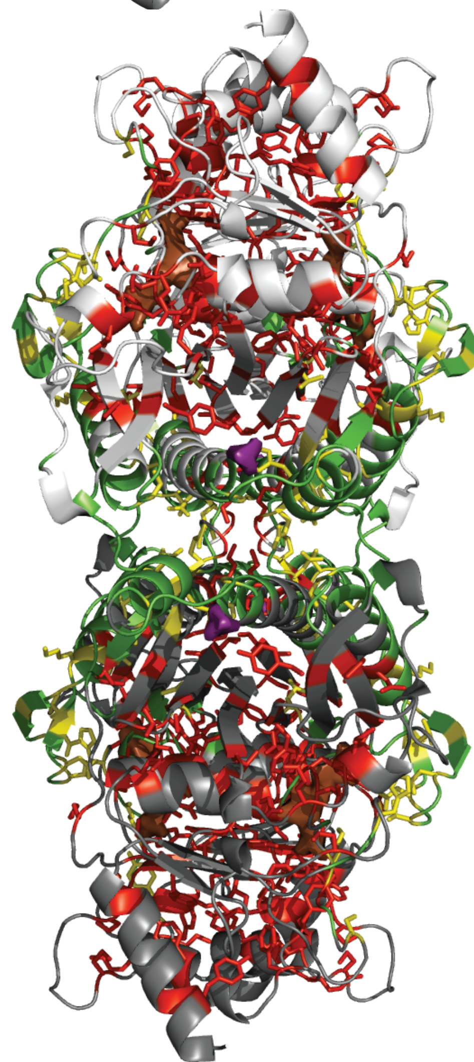
phosphofructokinase



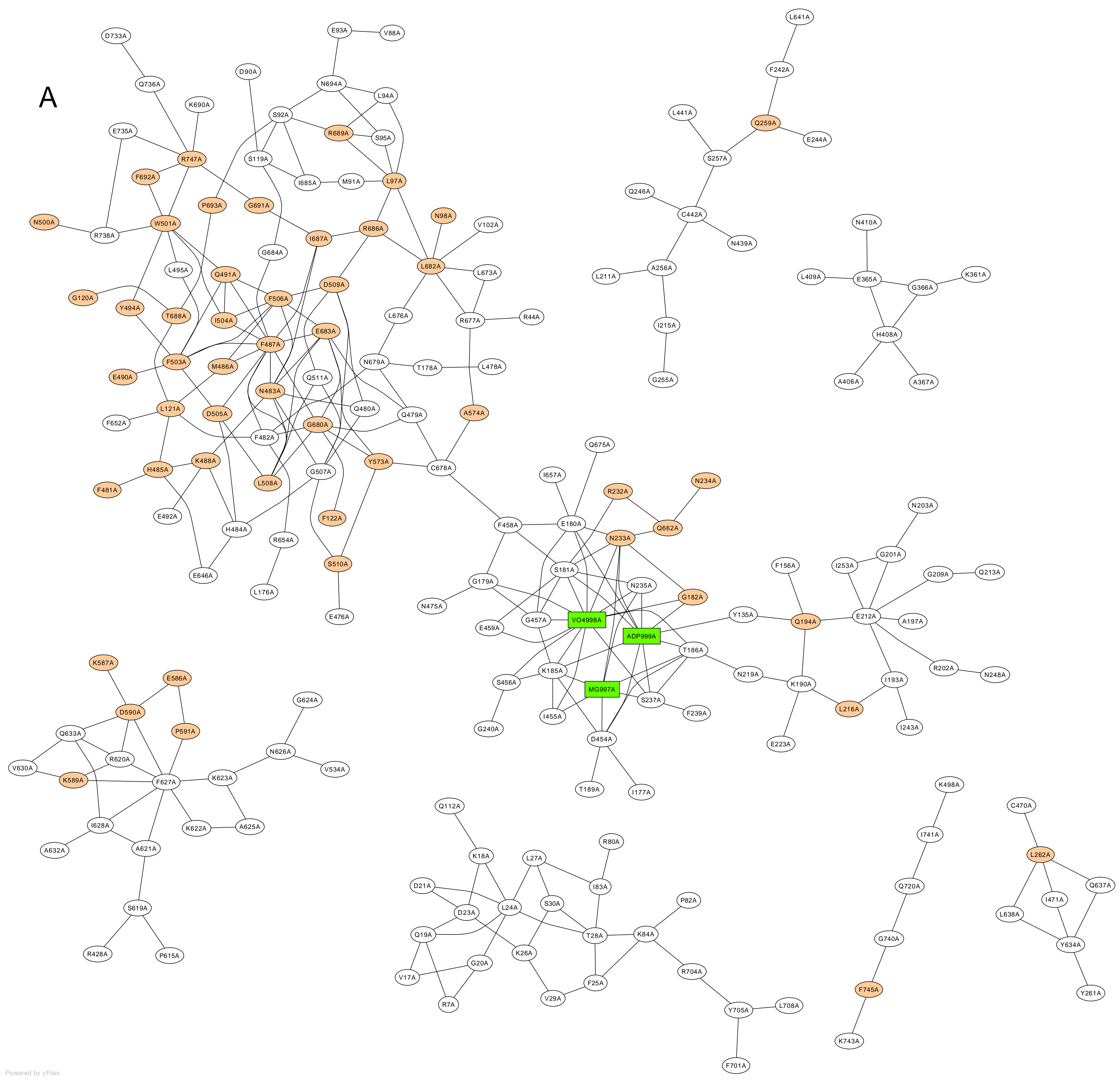


frcutose bisphosphatase





4PFK is shown for PFK and 1EYI is shown for FBPase. Views in the right column are rotated 90° about the y axis relative to the views in the left column. Red: statistically coupled (SCA) residues according to the algorithm of Suel et al. (2003); green: contact rearrangement network (CRN) residues; yellow: residues identified by both methods; purple: effector molecules; brown: substrate molecules.



A
D733A
E93A
V88A
D90A
N694A
Q736A
K690A
L94A
S92A
E735A
R689A
S95A
R747A
S119A
F692A
I685A
M91A
L97A
P693A
G691A
N98A
N500A
W501A
R738A
R686A
I687A
V102A
G684A
L682A
L495A
Q491A
L673A
D509A
G120A
F506A
Y494A
T688A
I504A
L676A
R677A
R44A
E683A
F487A
F503A
M486A
N679A
T178A
L478A
E490A
Q511A
N483A
Q480A
L121A
D505A
Q479A
A574A
F652A
F482A
G680A
K488A
H485A
G507A
Y573A
C678A
L508A
F481A
E492A
F122A
H484A
R654A
S510A
E646A
L176A
E476A
L641A
F242A
L441A
Q259A
E244A
S257A
Q246A
C442A
N439A
A256A
L211A
I215A
G255A
N410A
L409A
E365A
G366A
K361A
H408A
A406A
A367A
Q675A
I657A
R232A
N234A
E180A
Q662A
F458A
N233A
N203A
S181A
F156A
I253A
G201A
G209A
Q213A
G179A
N235A
G182A
N475A
Y135A
E212A
Q194A
A197A
G457A
VO4998A
E459A
ADP999A
T186A
R202A
N248A
K185A
N219A
S456A
MG997A
K190A
I193A
S237A
F239A
I455A
L216A
G240A
I243A
D454A
E223A
T189A
I177A
K587A
E586A
G624A
D590A
P591A
Q633A
N626A
R620A
V630A
V534A
K589A
F627A
K623A
I628A
K622A
A625A
A632A
A621A
S619A
R428A
P615A
Q112A
R80A
K18A
L27A
D21A
I83A
L24A
S30A
D23A
P82A
Q19A
T28A
K84A
K26A
G20A
V17A
F25A
R704A
V29A
R7A
Y705A
L708A
F701A
K498A
I741A
Q720A
G740A
F745A
K743A
C470A
L262A
Q637A
I471A
L638A
Y634A
Y261A
Powered by yFiles

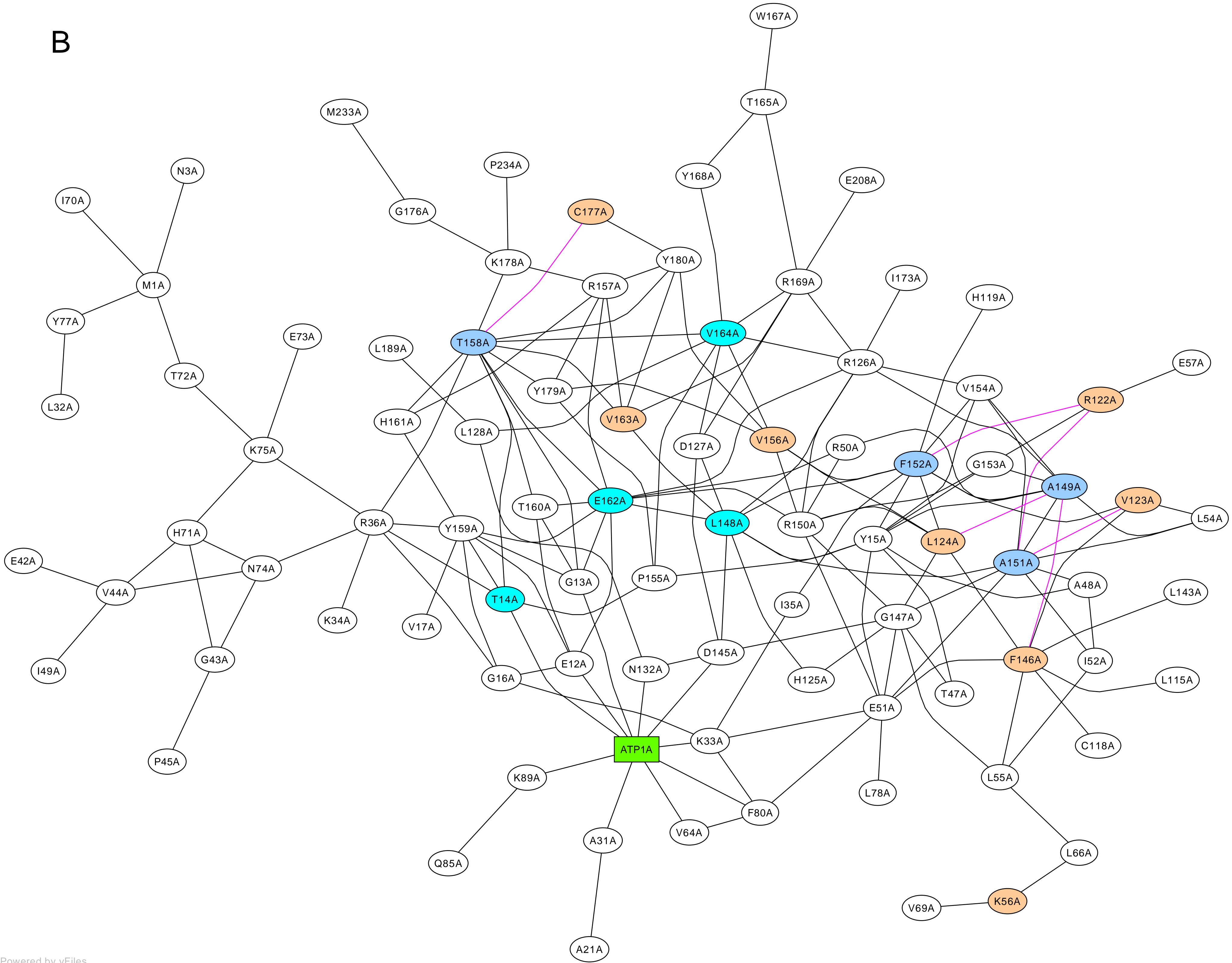

B
W167A
T165A
M233A
P234A
Y168A
E208A
N3A
I70A
G176A
C177A
Y180A
K178A
R157A
R169A
I173A
H119A
M1A
Y77A
E73A
T158A
V164A
L189A
R126A
E57A
T72A
Y179A
V154A
R122A
L32A
H161A
L128A
V163A
V156A
K75A
D127A
R50A
F152A
G153A
A149A
V123A
T160A
E162A
L54A
R36A
Y159A
L148A
R150A
Y15A
L124A
H71A
E42A
A151A
N74A
V44A
G13A
P155A
A48A
L143A
T14A
I35A
K34A
V17A
G147A
G43A
D145A
I49A
E12A
N132A
H125A
F146A
I52A
L115A
G16A
T47A
E51A
C118A
ATP1A
K33A
K89A
L55A
L78A
F80A
V64A
A31A
Q85A
L66A
K56A
V69A
A21A
Powered by yFiles

Figure 4: Comparison of contact rearrangement network results to two normal mode analysis-based studies

Graphs are formatted as in figure 4 of main text.

A: Comparison of CRN for myosin (1Q5G vs. 1VOM) to the top 10% dynamically correlated residues of the structure 1VOM as calculated by Zheng and Brooks (2005). Salmon: top 10 % dynamically correlated residues captured by top 7 clusters.

B: Comparison of CRN for cyclin A binding transition (1HCK vs. 1FIN) to PIVET analysis by Gu & Bourne (2007). Salmon and light blue: residues in the 10 pairs with the greatest influence on global fluctuation according to PIVET which also appear in the network. Light blue: CRN key residues (in the top 5 by degree or closeness) which are also in the top 10 PIVET pairs. Cyan: remaining CRN key residues. Magenta: top 10 PIVET interactions captured by the CRN.