Escherichia coli glutaredoxin 3: mapping the areas of the molecule interacting with its protein ligands

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Introduction

The intracellular environment of all cells is kept reduced by the glutaredoxin (Grx) and thioredoxin systems [1]. *Escherichia coli* (*E. coli*) has four Grxs (Grx1-4). Glutaredoxin 3 from *E. coli* (EcoGrx3, 83 amino acids, 9,12 kDa) was discovered in viable null mutants for the two efficient reductors of ribonucleotide reductase Ia (RRIa), thioredoxin 1 and Grx1. RRIa is essential enzyme for the synthesis of deoxyribonucleotides, the precursors of DNA. EcoGrx3 may amount up to 0,5 % of total soluble protein but can barely reduce RRIa, implying that its function(s) may not be confined to the described weak reduction of RRIa [1,2]. Using affinity chromatography, we have previously identified the protein interactome of EcoGrx3. The present work applied *in silico* approaches to pinpoint the critical amino acid residues (contact hot spots) and greater potential surfaces for interactions of EcoGrx3 with its protein ligands.

Methods

First, PDB files were extracted from the Uniprot protein database and subjected to molecular docking using the web tool Prism which also gave the predicted interfaces and the respective docking scores [3]. Dissociation constants (K_Ds) and Gibbs free energy bindings were calculated with the web tool Prodigy. The detection of amino acid contacts and types of interactions was performed by Biovia. The number with which each amino acid of EcoGrx3 participated in the different complexes was used to calculate its relative percentage in forming complexes. This approach gave the statistically significant contact residues ("contact hot spots") [4]. The predicted thermodynamic hot spots were derived from the webtool Proton. Energy network analysis (https://bioinf.iiit.ac.in/NAPS) was applied on the contact hot spots to give the proposed interfaces of interactions (as in figure 7).

Results

1. Docking

Table 1: The 162 proteins forming *in silico* complexes with EcoGrx3 and presentation of their binding characteristics. The proteins were identified previously from affinity purification experiments. The types of interactions between EcoGrx3 and its ligands were covalent (C) confirmed by DTT elution, strong non-covalent (NC) confirmed by acid elution, and ionic, confirmed by elution with KCl.

#No.	Protein ligand ID	gene	interface ID	Docking Score (kcal)	ΔG (kcal mol^{-1})	Kd (M) at 25°C	Interaction type
1	P04335	*frsA*	1ve7AB	-26.18	-12.8	4.2e-10	sNC
2	P77425	*allC*	3k7eCD	-15.21	-9.8	6.9e-08	sNC
3	P24242	*ascG*	3hi9CD	-15.8	-10.4	2.3e-08	sNC
4	P75858	*elfG*	1yptAB	-6.94	-10.4	2.5e-08	sNC
5	P0A8Y8	*entH*	2qb7AB	-14.9	-10.3	2.8e-08	sNC
6	P0AEK4	*fabI*	2qb7AB	-39.37	-7.7	2.4e-06	sNC
7	P0ABY4	*fldB*	1r7hAB	-0.39	-9.8	6.6e-08	sNC
8	P0AAP3	*frmR*	3nr1AB	-40.38	-12.2	1.1e-09	sNC
9α	P0A6T9	*gcvH*	2qmyBC	-22.44	-11	8e-09	sNC
9β			3n10AB	-20.94	-7.9	1.5e-06	sNC
10	P27248	*gcvT*	1xsvAB	-19.29	-11	7.9e-09	sNC
11	P27305	*gluQ*	3ld7AC	-19.37	-8.3	8.1e-07	sNC
12	P0A6W9	*gshA*	3e2pBJ	-2.19	-6.7	1.3e-05	sNC
13	P60664	*hisF*	1vi6AB	-19.62	-12	1.7e-09	sNC
14	P0ACD4	*iscU*	2qlcAG	-5.01	-5.2	0.00015	sNC
15	P22939	*ispA*	1barAB	-13.94	-7.3	4.3e-06	sNC
16	P21517	*malZ*	3fxdBD	-8.16	-10.8	1.2e-08	sNC
17	P0ABU0	*menB*	3n10AB	-37.68	-8.2	9.5e-07	sNC
18	P76112	*mnaT*	2b6mAB	-11.88	-7.7	2.3e-06	sNC
19	P0A749	*murA*	2a6aAB	-49.94	-7.5	3e-06	sNC
20	P17802	*mutY*	3hi9CD	-29.63	-8.4	6.5e-07	sNC
21	P24555	*ptrB*	2qlcAG	-10.54	-8.3	8.1e-07	sNC
22	P37760	*rfbD*	1t8qBC	-22.88	-9.8	6.6e-08	sNC
23	P0AFW4	*rnk*	2iwpAB	-14.64	-6.1	3.4e-05	sNC
24	P52043	*scpC*	3n10AB	-16.37	-10.7	1.3e-08	sNC
25α	P21170	*speA*	2b7lAB	-21.31	-7.7	2.4e-06	sNC
25β			3itqAB	-18.88	-10	4.7e-08	sNC
26	Q46927	*tcdA*	3d31AB	-18.46	-9	2.4e-07	sNC
27	P77718	*thiI*	3k7eCD	-16.42	-9.9	5.6e-08	sNC
28	P76055	*ttcA*	2a6aAB	-50.89	-6.6	1.3e-05	sNC
29	P09184	*ppiA*	3hi9CD	-5.85	-9.6	8.4e-08	sNC
30	P77213	*ybdK*	3mhsAD	-21.6	-12.2	1e-09	sNC
31	P0ABU2	*ychF*	3nrdAB	-7.96	-8.6	4.8e-07	sNC
32	P76097	*ydcJ*	3rrvCD	-13.9	-11.6	3.2e-09	sNC
33	P76121	*yddH*	2b6mAB	-57.84	-8.7	3.9e-07	sNC
34α	P77588	*ydeQ*	1r1uAB	-4.03	-9	2.5e-07	sNC
34β			2iwpAB	-3.27	-10.4	2.3e-08	sNC
35	P0AG84	*yghA*	3nwcAB	-12.16	-8.7	4.2e-07	sNC
36	P45424	*nanQ*	3dieAB	-10.07	-7.4	3.6e-06	sNC
37	P39337	*yjgM*	3fpvAF	-35.62	-8.7	4.3e-07	sNC
38	P39358	*yjhG*	3dieAB	-32.08	-8.1	1.1e-06	sNC
39	P39325	*ytfQ*	1r1uAB	-4.95	-7.7	2.4e-06	sNC
40	P64596	*dolP*	3sidAB	-22.96	-8.2	1e-06	sNC
41	P06971	*fhuA*	2a6aAB	-29.42	-10.4	2.5-e08	sNC
42	P63228	*gmhB*	1i4uAB	-10.88	-5	0.00021	sNC
43	P0AC59	*grxB*	2a6aAB	-14.91	-9.1	2.1e-07	sNC
44	P0A7A5	*pcm*	1z8hAB	-6.47	-7.9	1.5e-06	sNC
45	P64483	*proXp-y*	2a6aAB	-8.1	-5.1	0.00018	sNC
46	P0AD49	*raiA*	3idfAB	-2.98	-6	4e-05	sNC
47	P0A7R5	*rpsJ*	1f9mAB	-19.04	-7.2	5e-06	sNC
48	P0AEU7	*skp*	1tqjCD	-34.57	-9.4	1.2e-07	sNC
49	P0AAS7	*ybcJ*	3f13AB	-9.46	-10.6	1.6e-08	sNC
50α	P69346	*yefM*	2a6aAB	-19.49	-8.3	8.3e-07	sNC
50β			2a6qCF	-18.39	-7.9	1.5e-06	sNC
50γ	P69346	*yefM*	3d31AB	-19.9	-9.9	5.3e-08	sNC
51	P45469	*yraR*	3n10AB	-10.89	-8.4	7.5e-07	sNC
52	P0A6A8	*acpP*	3p3bAB	-7.2	-9.7	7.4e-08	C
53	P09832	*aegA*	2qmyBC	-5.95	-9.1	2e-07	C
54	P77434	*AlaC*	1mn8AB	-8.97	-6.5	1.8e-05	C
55	P0A6E1	*aroL*	3n10AB	-41.94	-9.9	5.9e-08	C
56	P0ABQ0	*coaBC*	1xsvAB	-17.66	-8.3	7.7e-07	C
57	P0ACB4	*hemG*	2aq0AB	-22.16	-7.3	4.5e-06	C
58	P11880	*murF*	2b6mAB	-4.86	-13.7	9.4e-11	C
59	P76002	*pliG*	2a6aAB	-26.74	-9.6	8.8e-08	C
60	P60422	*rplB*	3cjxGH	-11.01	-8.7	3.9e-07	C
61	P0A7V8	*rpsD*	1t1vAB	-11.38	-11.8	2.2e-09	C
62α	P0A7R9	*rpsK*	1tqjCD	-17.86	-12.3	9.8e-10	C
62β			3fxdBD	-19.06	-10.1	3.7e-08	C
63	P0AF90	*rraB*	3nrdAB	-12.81	-8.0	1.4e-06	C
64	P60651	*speB*	1aorAB	-6.5	-7.9	1.7e-06	C
65	P0A8B5	*ybaB*	2qmyBC	-6.1	-9.1	2e-07	C
66	P37626	*yhiI*	3k7eCD	-8.7	-5.4	0.0001	C
67	P64588	*yqjI*	2qlcAG	25.56	-8.1	1.1e-06	C
68α	P0ACS5	*zntR*	1cmvAB	-0.23	-9.8	6.1e-08	C
68β			3f1rAB	-0.67	-10.8	1.2e-08	C
69	P18776	*dmsB*	3gn3AB	-0.84	-10.9	9.9e-09	C
70	P32177	*fdhD*	2fchBC	-23.75	-7.5	3.1e-06	C
71	P0AC19	*folX*	1k50BD	-12.88	-8.2	1e-06	C
72	P37052	*ychJ*	2pqaAB	-19.8	-7.7	2.1e-06	C
73	P0A9W6	*ibaG*	3b76AB	-5.44	-8.7	4.2e-07	C
74	P77348	*mppA*	2a6aAB	-12.88	-10.2	3.1e-08	C
75	P77150	*pdxY*	1t1vAB	-17.81	-8.6	4.6e-07	C
76α	P08400	*phoR*	1mn8AB	-7.77	-8.8	3.7e-07	C
76β			3fxdBD	-9.2	-8.4	6.8e-07	C
77α	P0A9K9	*slyD*	2iwpAB	-16.55	-8	1.4e-06	C
77β	P0A9K9	*slyD*	3d31AB	-10.37	-7.8	1.9e-06	C
78	P00547	*thrB*	1mn8AB	-5.77	-12.2	1.2e-09	C
79	P0AGG4	*trxC*	3dieAB	-44.56	-10.2	3.5e-08	C
80	P0AAZ7	*ycaR*	1t1vAB	-18.81	-7.7	2.3e-06	C
81	P46855	*yhhZ*	3n10AB	-12.52	-10.6	1.7e-08	C
82	P64636	*yrfG*	1vi6AB	-21.19	-8.8	3.8e-07	C
83	P25737	*lysP*	1t8qBC	-34,85	-6.8	1e-05	Ionic
84	P04846	*nlpA*	3nrdAB	-18,42	-9.2	1.8e-07	Ionic
85	P0AD01	*dcuR*	3dieAB	-6,72	-7.2	5.2e-06	Ionic
86	P38101	*eamB*	2okuAB	-30,00	-10.0	5e-08	Ionic
87	P10346	*glnQ*	3hi9CD	-3,23	-7.6	2.8e-06	Ionic
88	P0AF98	*lptF*	2qmyBC	-67,98	-9.1	2.3e-07	Ionic
89	P15977	*malQ*	1t8qBC	-27,91	-9.9	5.8e-08	Ionic
90	P41036	*nanT*	2a6aAB	-35,19	-5.7	6.5e-05	Ionic
91	P0AGL5	*ratA*	2a6aAB	-8,17	-7.5	3e-06	Ionic
92	P0A7X3	*rpsI*	1x8dAB	-29,62	-9,2	1.6e-07	Ionic
93	P69441	*adk*	2a6aAB	-6,46	-5.9	4.5e-05	Ionic
94	P00963	*asnA*	3qkwCD	-14,58	-9.0	2.6e-07	Ionic
95	P76290	*cmoA*	1tqjCD	-38,49	-10.6	1.8e-08	Ionic
96	P0AEN1	*fre*	1t1vAB	-13,47	-9.4	1.2e-07	Ionic
97	P0AAB6	*galF*	3nwcAB	-9,11	-9.4	1.2e-07	Ionic
98	P0C8J8	*gatZ*	1t1vAB	-11,43	-8.9	3.1e-07	Ionic
99	P0AFU4	*glrR*	1t8qBC	-17,46	-6.6	1.5e-05	Ionic
100	P60546	*gmk*	2a6aAB	-29,35	-9.1	2.1e-07	Ionic
101	P0AA04	*ptsH*	2a6aAB	-25,1	-8.3	8.7e-07	Ionic
102	P0AEX9	*malE*	3n10AB	-12,98	-9.7	8e-08	Ionic
103	P26365	*amiB*	2qlcAG	-12,83	-10.7	1.5e-08	Ionic
104	P0AE91	*creA*	3dtnAB	-10,6	-9.8	6.4e-08	Ionic
105	P39176	*erfK*	1r1uAB	-23,9	-8.6	4.7e-07	Ionic
106	P0A6Q3	*fabA*	3nrdAB	-0,05	-6.2	2.8e-05	Ionic
107	P24186	*folD*	2b6mAB	-31,69	-11.4	4.2e-09	Ionic
108	P25748	*galS*	2b7lAB	-25,63	-8.7	4.1e-07	Ionic
109	P25519	*hflX*	2wmcAB	-14,65	-10.2	3.5e-08	Ionic
110	P0ADE6	*kbp*	2a6aAB	-8,99	-8,4	7.2e-07	Ionic
111	P69797	*manX*	2qlcAG	-43,0	-8.1	1.2e-06	Ionic
112	P13669	*mngR*	1i4uAB	-0,54	-10.2	3.1e-08	Ionic
113	P0AFT5	*btsR*	2a6aAB	-18,34	-8.4	7.5e-07	Ionic
114	P0ABI8	*cyoB*	3nwcAB	-37,5	-8.8	3.7e-07	Ionic
115	P0CB39	*eptC*	2a6aAB	-27,55	-7.2	5.4e-06	Ionic
116	P0A9A9	*fur*	2qlcAG	-17,33	-7.9	1.5e-06	Ionic
117	P03024	*galR*	3k7eCD	-11,93	-9.3	1.4e-07	Ionic
118	P69831	*gatC*	1yxoAB	-16,79	-7.1	6.3e-06	Ionic
119	P0ACB7	*hemY*	3jz3AB	-3,81	-10.5	1.9e-08	Ionic
120	P36771	*lrhA*	3k7eCD	-24,38	-9.6	9.2e-08	Ionic
121	P08660	*lysC*	2yw3AE	-33,37	-7.9	1.5e-06	Ionic
122	P06720	*melA*	3kbqAB	-8,24	-9.1	2e-07	Ionic
123	P00811	*ampC*	3dtnAB	-21,8	-10.3	2.6e-08	Ionic
124	P30860	*artJ*	3n10AB	-16,33	-8.5	6.2e-07	Ionic
125	P0A9F3	*cysB*	1t1vAB	-4,23	-10.8	1.3e-08	Ionic
126	P33013	*dacD*	2lbuDE	-12,99	-12.5	6.7e-10	Ionic
127	P0ACZ4	*evgA*	3n10AB	-20,77	-8.4	7.5e-07	Ionic
128	P15286	*flk*	3d31AB	-12,7	-11	8.1e-09	Ionic
129	P0ACK8	*fucR*	3ibsAB	-4,01	-8,5	6.2e-07	Ionic
130	P37326	*intS*	2qlcAG	-28,76	-6.8	9.9e-06	Ionic
131	P0ACJ0	*irp*	3n10AB	-13,19	-8.3	8.6e-07	Ionic
132	P08337	*muyT*	2qmyBC	-16,47	-6.6	1.5e-05	Ionic
133	P69503	*apt*	1t1vAB	-11,14	-7.2	5.2e-06	Ionic
134	P45955	*cpoB*	2a6aAB	-12,69	-8.4	6.6e-07	Ionic
135	P0ABS1	*dksA*	3fxdBD	-8,0	-10,5	1.9e-08	Ionic
136	P0AEM0	*fkpB*	1w9aAB	-1,05	-6.4	2.1e-05	Ionic
137	P0A9L3	*fklB*	3jz3AB	-30,56	-5.8	5.7e-05	Ionic
138	P0A9M5	*gpt*	1nt2AB	-19,63	-7.1	6.5e-06	Ionic
139	P0ACE7	*hinT*	3nr1AB	-19,68	-9.1	2.3e-07	Ionic
140	P0A7B8	*hslV*	1ii7AB	-33,63	-7.6	2.7e-06	Ionic
141	P37647	*kdgK*	2a6aAB	-15,92	-8.4	6.5e-07	Ionic
142	P03817	*mioC*	2ijhBC	-0,22	-6.1	3.5e-05	Ionic
143	P46854	*aaaT*	2b6mAB	-31,76	-8.2	9.1e-07	Ionic
144	P39099	*degQ*	2iwpAB	-7,06	7.4	3.9e-06	Ionic
145	P23840	*dinD*	3kd2AC	-5,33	-8.6	5.1e-07	Ionic
146	P0ABT2	*dps*	3jz3AB	-9,65	-11.2	6e-09	Ionic
147	P75933	*flgA*	1z8hAB	-8,16	-8.6	5.3e-07	Ionic
148	P23874	*hipA*	2fchBC	-14,96	-8.3	7.5e-07	Ionic
149	P0ACF0	*hupA*	3f1rAB	-6,64	-7.3	4.1e-06	Ionic
150	P0ACF4	*hupB*	3ezqCD	-22,68	-8.6	5.1e-07	Ionic
151	P0A7G2	*rbfA*	1z8hAB	-2,62	-8.3	8.8e-07	Ionic
152	P0A7R1	*rplI*	1rd5AB	-21,76	-7.2	5.1e-06	Ionic
153	P06966	*dicA*	2qlcAG	-4,45	-7.7	2.1e-06	Ionic
154	P0A6X3	*hfq*	3f13AB	-20,26	-8.1	1.2e-06	Ionic
155	P21499	*rnr*	3f1rAB	-2,52	-10.8	1.1e-08	Ionic
156	P13445	*rpoS*	1rd5AB	-18,38	-5.6	8.4^{5}-05	Ionic
157	P0A832	*smpB*	3fxdBD	-10,12	-6.7	1.1e-05	Ionic
158	P75828	*ybjD*	3oxnAD	-35,37	-10	4.5e-08	Ionic
159	P75838	*ycaO*	1qxjAB	-13,94	-11.1	7.2e-09	Ionic
160	P0AGJ5	*yfiF*	1ve7AB	-18,56	-10.6	1.7e-08	Ionic
161	P46837	*yhgF*	2a6aAB	-7,34	-11.3	4.9e-09	Ionic
162	P0ADK8	*yibL*	2a6aAB	-2,86	-6.3	2.3e-05	Ionic

Monothiol Grx3 was from pdb 1FOV mutated *in silico* to mono thiol

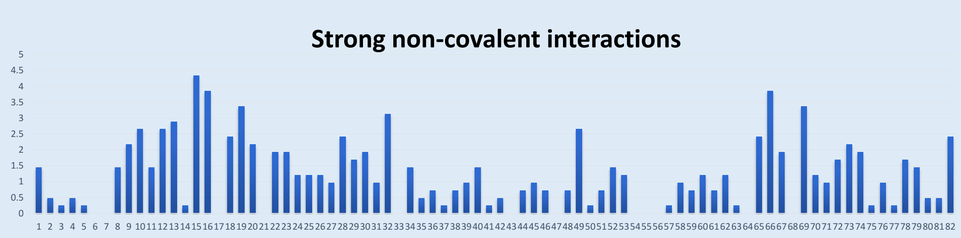

Figure 1: Distribution of amino acid contacts for strong non-covalent interactions. The X axis represents the numbering of all amino acids. The absolute Y values represent the percentage of participation among the EcoGrx3 protein complexes derived from the interactome which was eluted with acid.

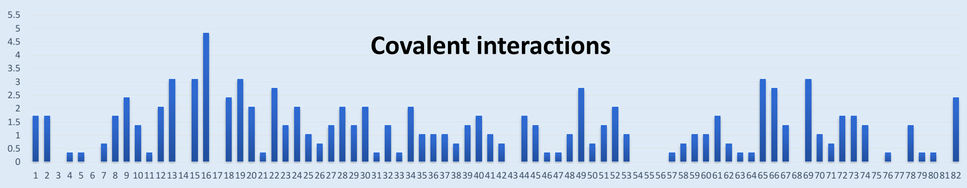

Figure 2: Distribution of amino acid contacts for covalent interactions. The X axis represents the numbering of all amino acids. The absolute Y values represent the percentage of participation among the EcoGrx3 protein complexes derived from the interactome which was eluted with DTT.

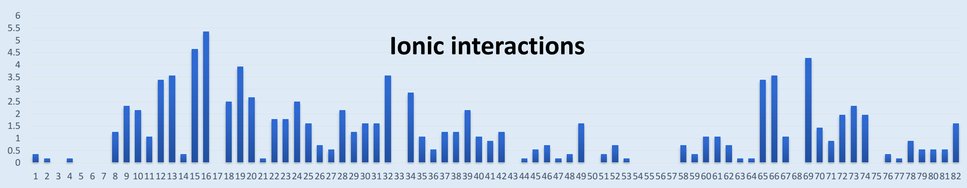

Figure 3: Distribution of amino acid contacts for Ionic interactions. The X axis represents the numbering of all amino acids. The absolute Y values represent the percentage of participation among the EcoGrx3 protein complexes derived from the interactome which was eluted with KCl.

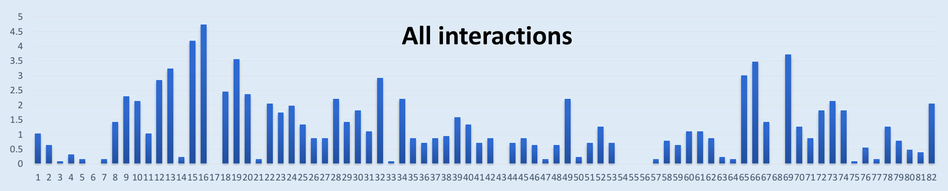

Figure 4: Distribution of amino acid contacts for strong non-covalent, covalent and ionic interactions. The X axis represents the numbering of all amino acids. The absolute Y values represent the percentage of participation for interaction among all EcoGrx3 protein complexes.

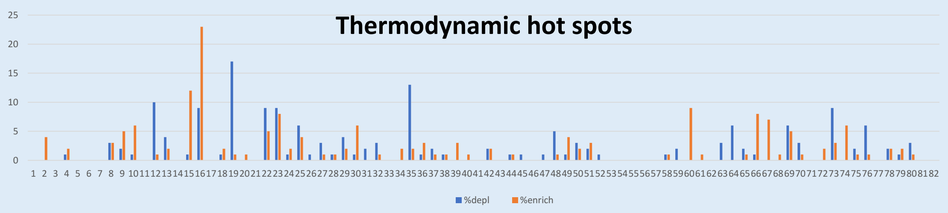

Figure 5: Predicted thermodynamic residues for the interaction of EcoGrx3 with its protein ligands. The X axis represents the numbering of all amino acids. The absolute Y values represent the statistical rate contribution for a predicted thermodynamic hot spot.

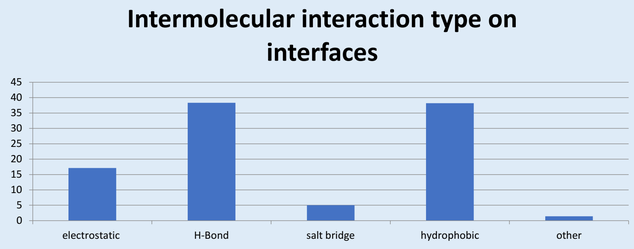

Figure 6: Contribution of intermolecular interaction types of amino acids on the interfaces in complexes of EcoGrx3 and its protein ligands. Results represent percentages of the types of interactions considering all examined complexes of EcoGrx3**.**

2. The favored residues and surfaces of EcoGrx3 for its interactions with protein ligands

Calculation of standard deviation Z for each amino acid of EcoGrx3 Poisson approximation

- Interactions per amino acid are not continuous values but counted events.
- Interactions are independent events because each complex with EcoGrx3 is unique.
- The expected probability of an amino acid participating in an interaction is relatively small: **P = 1/82.**
- The number of events is large **(1266 interactions).**

Π_i: Observed number of interactions
Expected value:
E(X) = λ = A
A = P × 1266 = 1/82 × 1266 ≈ 15.4
Variance: Var(X) = σ^2 = λ = A
Standard deviation: σ = √λ = √A

EcoGrx3 contact hot spots (Z > 1.44 (85 % confidence):

Pro[12], Tyr[13], His[15], Arg[16], Ala[19], Pro[32], Tyr[65], Asp[66], Tyr[69]

$$z_i = \frac{\Pi_i - A}{\sqrt{A}}$$

Table 2: Energy network Neighbors for all contact hot spot residues of EcoGrx3. All residues are presented as numbers. "C" stands for covalent and "sNC" for strong non-covalent interactions. The presented Cys[9] is not considered a critical contact spot (hot spot).

Hot spot	Interaction type	Energy neighbors	all DTT	all ACID	All Ionic	Common
Cys[11]	Active site	7-15	6-8 10-23 30-34 53 64-73	6-8 10-23 30-34 53 64-73	6-8 10-23 30-34 53 64-73	6-8 10-23 30-34 53 64-73
Pro[12]	Acid/DTT/Ionic	10-16				
Tyr[13]	Acid/DTT/ Ionic	11-17, 53, 65				
His[15]	Acid/DTT/ Ionic	11-19				
Arg[16]	Acid/DTT/ Ionic	12-20, 65				
Ala[19]	Acid/DTT/ Ionic	15-23				
Pro[32]	Acid/ DTT/Ionic	6-8, 30-34				
TyrR[65]	Acid/DTT/ Ionic	13,14,16,17, 53, 64-69				
Asp[66]	Acid/DTT/ Ionic	64-70				
Tyr[69]	Acid/DTT/ Ionic	65-73				

Table 3: Parameters for Network Analysis

Network type	C-α
Lower threshold	0 Å
Upper threshold	7 Å
Residue separation	1
Nodes:	215
Eges	868
Diameter	12.00
Radius	7.00
Average degree	8.07
Average shortest path	5.21
Clustering coefficient	0.53

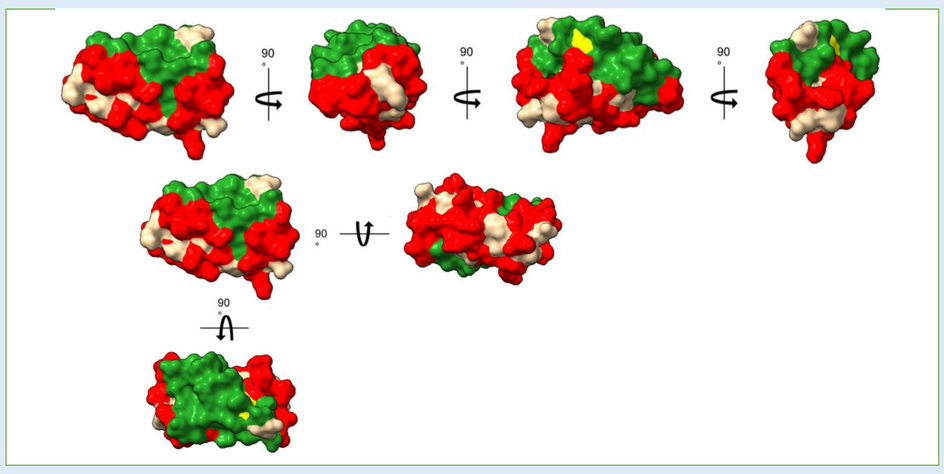

Figure 7: Surface areas of EcoGrx3 involved in protein–ligand interaction. Contact hot spots were common; the potential interaction surfaces (shown in green) are shared among covalent, strong non-covalent, and ionic interactions. The red residues represent the predicted thermodynamic contact spots (35/59) that are not included within the potential interacting areas. The active site Cys[9] is in yellow.

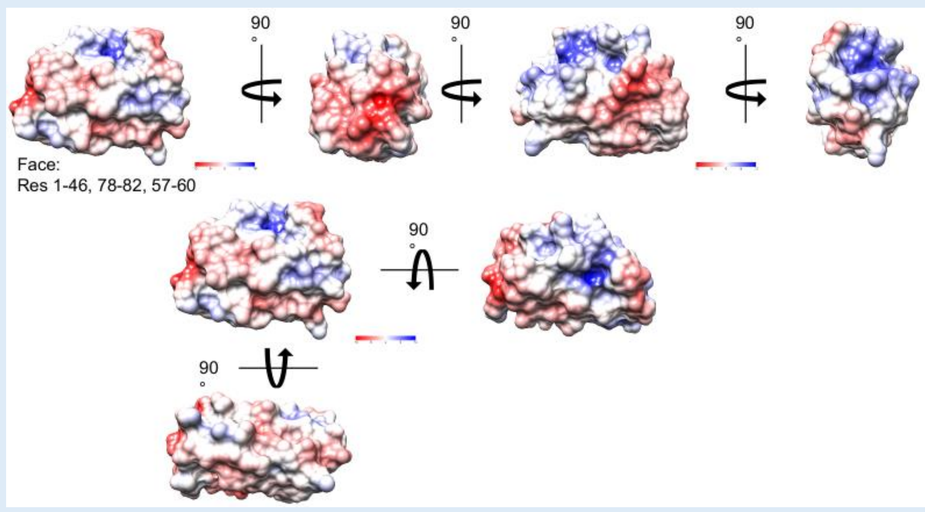

Figure 8: Proposed surfaces presented the charge distribution in the aforementioned areas. Positively charged residues are in red, while negatively charged in blue.

Conclusions

1. 162 verified protein ligands of EcoGrx3 corresponding to 170 interfaces gave successful molecular docking that highlighted the "contact hot spots" and the respective topology of the interacting surfaces of EcoGrx3 with its protein ligands.
2. The contact hot spots combined with their energy neighbors, defined an extended surface area for protein-protein interactions located adjacent to the active site thiol. If the molecule can be envisaged as a rectangle brick, he negatively charged proteins may interact with the positively charged area below and right to the active site.
3. All the contact hot spots on the surface of EcoGrx3 were common for the proteins interacting with covalent, strong non-covalent and ionic interactions. They were placed in an area which was neutral or positively charged.
4. The protein interactions of EcoGrx3 appeared largely driven by hydrophobic interactions and hydrogen bonds.
5. 35 out of the predicted 59 thermodynamic contact residues were within the herein proposed interacting surfaces.

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