Supplementary Data

Molecular Dynamics Simulation Protocol for Example Application: Identifying Functional Residues in Trypsin Structure Using gRINN.

The trypsin structure to be simulated was taken from Protein Data Bank (PDB) accession number 3OTJ. The PDB file included both the trypsin enzyme from bovine and an inhibitor (Bovine Pancreatic Trysin Inhibitor, BPTI). Inhibitor atoms and all other water molecules were removed from the file. The system was solvated using TIP3P water molecules with at least 10 Å padding distance to the periodic boundary box boundary in each direction. Following solvation, the total charge of the system was neutralized by replacing randomly selected water molecules with appropriate amounts of sodium and/or chloride ions. Total ion concentration was set to 0.15 nM.

Following solvation and ionization, the system was subjected to a 10000 steps of enegy minimization. Then, the system was simulated in the NPT ensemble for 50 nanoseconds. The temperature and pressure were held constant at 310 K and 1 bar, respectively. Simulations were conducted using NAMD 2.12 simulation engine. The time-step of simulation was 2 fs. Coordinated were saved every 2 ps of simulation time. This results in 50000 frames for a 50 ns long simulation.

Equilibrium Assessment

It is more sensible to quantify pairwise residue interaction energies in an equilibriated simulation trajectory. Therefore, we assessed equilibrium by calculating the Root Mean Square Deviation (RMSD) for all frames included in the trajectory to the reference (initial) frame (Figure S1). The portion of simulation between 25 and 50 nanoseconds was then selected for interaction energy calculation. This portion was saved to another DCD file and used as input to gRINN.

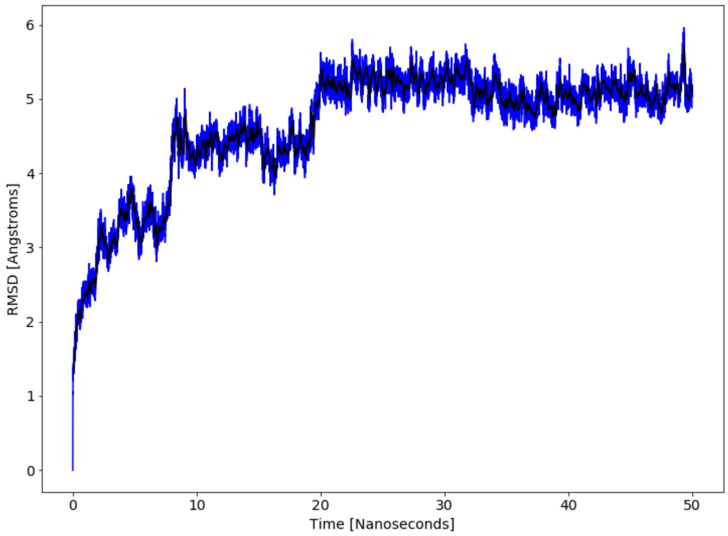

Figure S1 – RMSD of frames included in MD simulation with respect to the initial frame

Degree, Betweenness-centrality and Closeness-centrality of Trypsin Residues in the Protein Energy Network Constructed by gRINN

Note that due to the usage of residue insertion codes in chain E (trypsin) of PDB entry 3OTJ (GLY184A, GLY188A, ALA221A), residue numbering was changed to be able to take inserted residues into protein topology in MD simulation. This results in differences between residues numbers in the original PDB file and those reported by gRINN. The following table (Table S1) includes both (Residue (gRINN): Reported by gRINN, Residue (3OTJ): as in 3OTJ). The numbering in 3OTJ was used in the Main Text.

Table S1 – Degree, Betweenness-centrality and Closeness-centrality of trypsin residues in the constructed PEN

Residue (gRINN)	Residue (3OTJ)	Degree	Betweenness-centrality	Closeness-centrality
EILE16	EILE16	36	0.0862174392	0.5759809417
EVAL17	EVAL17	19	0.0009783539	0.4346153976
EGLY18	EGLY18	12	0.0011414129	0.4079503652
EGLY19	EGLY19	15	0.0016305899	0.4221127703
ETYR20	ETYR20	16	0.0011414129	0.4248255776
ETHR21	ETHR21	17	0.002894297	0.4333552397
ECYS22	ECYS22	16	0.0004076475	0.4049245434
EGLY23	EGLY23	12	0.0030981207	0.4009774831
EALA24	EALA24	15	0.0025274143	0.4092084671
EASN25	EASN25	22	0.0033019445	0.4557159338
ETHR26	ETHR26	17	0.0008560597	0.4120749323
EVAL27	EVAL27	24	0.0069707717	0.4729698334
EPRO28	EPRO28	21	0.0068484774	0.4523812702
ETYR29	ETYR29	30	0.0204639028	0.5054754787
EGLN30	EGLN30	31	0.0184256655	0.499506353
EVAL31	EVAL31	32	0.0214830215	0.5046121158
ESER32	ESER32	29	0.0112103053	0.4803699786
ELEU33	ELEU33	25	0.0108026579	0.4891956884
EASN34	EASN34	25	0.0067261832	0.4310701748
ESER37	ESER37	12	0.0001222942	0.4291074829
EGLY38	EGLY38	9	0.0003668827	0.3625758657
ETYR39	ETYR39	16	0.0026904733	0.3982943773
EHSD40	EHSD40	24	0.0123517182	0.4792797831
EPHE41	EPHE41	21	0.003057356	0.4622470133
ECYS42	ECYS42	24	0.0071745954	0.4960620984
EGLY43	EGLY43	21	0.0126370715	0.4806929722
EGLY44	EGLY44	26	0.0114141291	0.4794344717

ESER45	ESER45	21	0.0030981207	0.4523277557
ELEU46	ELEU46	23	0.0081937141	0.4812224747
EILE47	EILE47	22	0.0065223595	0.4416440178
EASN48	EASN48	20	0.0044433574	0.4302991856
ESER49	ESER49	17	0.0015898251	0.411074142
EGLN50	EGLN50	16	0.0023643553	0.3923492916
ETRP51	ETRP51	27	0.0126370715	0.4576860457
EVAL52	EVAL52	26	0.0106803636	0.4938986178
EVAL53	EVAL53	26	0.0101911867	0.4964493024
ESER54	ESER54	26	0.0254779667	0.5009303706
EALA55	EALA55	22	0.0097835392	0.4826751184
EALA56	EALA56	17	0.0041172394	0.4511396405
EHSD57	EHSD57	17	0.0089274795	0.453060186
ECYS58	ECYS58	19	0.0037095919	0.461771266
ETYR59	ETYR59	16	0.0028535323	0.414016159
ELYS60	ELYS60	23	0.0089274795	0.4602697011
ESER61	ESER61	8	0.0004076475	0.3722726396
EGLY62	EGLY62	5	0.0004484122	0.3683919412
EILE63	EILE63	19	0.008682891	0.4584790945
EGLN64	EGLN64	23	0.0058293588	0.4194278239
EVAL65	EVAL65	24	0.0074191839	0.4711231094
EARG66	EARG66	33	0.0233174351	0.4946184819
ELEU67	ELEU67	22	0.0041580042	0.4394355249
EGLY69	EGLY69	19	0.0022012963	0.4174576837
EGLU70	EGLU70	29	0.0328563858	0.5034990129
EASP71	EASP71	34	0.0259263789	0.4853355706
EASN72	EASN72	20	0.0015490604	0.4097326061
EILE73	EILE73	13	0.0020382373	0.4283875151
EASN74	EASN74	11	0.0005707065	0.3868934994
EVAL75	EVAL75	14	0.0006522359	0.3794852137
EVAL76	EVAL76	8	0.0003668827	0.3751315189
EGLU77	EGLU77	21	0.0083160083	0.4498926739
EGLY78	EGLY78	6	0	0.3636005217
EASN79	EASN79	14	0.0006114712	0.3901575892
EGLU80	EGLU80	21	0.0101911867	0.4378075788
EGLN81	EGLN81	19	0.002894297	0.4249653317
EPHE82	EPHE82	16	0.0008152949	0.4045428496
EILE83	EILE83	22	0.0041987689	0.4166006417
ESER84	ESER84	19	0.0015898251	0.391233151

EALA85	EALA85	12	0.0014675309	0.3827979572
ESER86	ESER86	11	0.0011414129	0.3644027607
ELYS87	ELYS87	17	0.0183033712	0.4134899329
ESER88	ESER88	19	0.0048917696	0.4165591868
EILE89	EILE89	10	0.0021197668	0.4059765509
EVAL90	EVAL90	17	0.003383474	0.4140525736
EHSD91	EHSD91	18	0.0165504871	0.4259249086
EPRO92	EPRO92	9	0.0010191187	0.3836596966
ESER93	ESER93	9	0.0017936489	0.3708941887
ETYR94	ETYR94	12	0.0061554767	0.3992908149
EASN95	EASN95	10	0.008682891	0.3894859966
ESER96	ESER96	6	0	0.3272912911
EASN97	EASN97	4	4.07647466471E-05	0.2952603874
ETHR98	ETHR98	8	0.0034650035	0.3475811032
ELEU99	ELEU99	9	0.0039541804	0.388858558
EASN100	EASN100	10	0.0021197668	0.3662163142
EASN101	EASN101	21	0.0164689576	0.4461901137
EASP102	EASP102	21	0.0452896335	0.5080244488
EILE103	EILE103	20	0.0112510701	0.4776559646
EMET104	EMET104	18	0.0031388855	0.4290550689
ELEU105	ELEU105	20	0.0076637724	0.4400023883
EILE106	EILE106	23	0.0105988341	0.4844087171
ELYS107	ELYS107	14	0.0014675309	0.4045255415
ELEU108	ELEU108	20	0.003546533	0.4237900902
ELYS109	ELYS109	18	0.0011414129	0.4122813944
ESER110	ESER110	15	0.0024866495	0.3884453022
EALA111	EALA111	14	0.0010191187	0.3911082011
EALA112	EALA112	11	0.0003668827	0.4010467455
ESER113	ESER113	13	0.0010191187	0.3969318237
ELEU114	ELEU114	13	0.0014267661	0.3774065863
EASN115	EASN115	10	0.0015490604	0.3812204933
ESER116	ESER116	8	0.000489177	0.384805906
EARG117	EARG117	22	0.018018018	0.4537298531
EVAL118	EVAL118	18	0.0046064164	0.4259436145
EALA119	EALA119	19	0.0051363581	0.4418408941
ESER120	ESER120	12	0.0033427092	0.4202702966
EILE121	EILE121	22	0.0128816599	0.4414069186
ESER122	ESER122	17	0.0098243039	0.4340165601
ELEU123	ELEU123	10	0.0012229424	0.3988053692

EPRO124	EPRO124	7	0.0004484122	0.3492747008
ETHR125	ETHR125	5	0.0010191187	0.3538725586
ESER127	ESER127	8	0.0047694754	0.3711242205
ECYS128	ECYS128	9	0.0041172394	0.3750337572
EALA129	EALA129	7	0.0033019445	0.3722233338
ESER130	ESER130	6	0.0007745302	0.3346551664
EALA132	EALA132	10	0.0014267661	0.3612336251
EGLY133	EGLY133	7	0.0033427092	0.3767538448
ETHR134	ETHR134	8	0.0011414129	0.3563194245
EGLN135	EGLN135	12	0.0015082956	0.3798374587
ECYS136	ECYS136	15	0.012392483	0.4578805823
ELEU137	ELEU137	15	0.0081121846	0.4482467818
EILE138	EILE138	19	0.0086013615	0.467891639
ESER139	ESER139	28	0.0094574212	0.491568892
EGLY140	EGLY140	20	0.0060331825	0.4688339532
ETRP141	ETRP141	32	0.012066365	0.4880562365
EGLY142	EGLY142	15	0.0005707065	0.4353006632
EASN143	EASN143	15	0.000489177	0.4313257258
ETHR144	ETHR144	16	0.0014267661	0.4284827869
ELYS145	ELYS145	13	0.0006930007	0.4104985277
ESER146	ESER146	10	0.0004076475	0.4134067278
ESER147	ESER147	6	0.0002038237	0.3816643241
EGLY148	EGLY148	5	4.07647466471E-05	0.4021441287
ETHR149	ETHR149	8	0.0006930007	0.3898126407
ESER150	ESER150	11	0.0017528841	0.4167704313
ETYR151	ETYR151	15	0.0014675309	0.4039781488
EPRO152	EPRO152	13	0.000326118	0.4245280143
EASP153	EASP153	22	0.0049325343	0.4480257294
EVAL154	EVAL154	18	0.0037503567	0.451293677
ELEU155	ELEU155	25	0.0031388855	0.4655201521
ELYS156	ELYS156	21	0.0097020097	0.470161052
ECYS157	ECYS157	19	0.0019567078	0.4511508143
ELEU158	ELEU158	23	0.0040357099	0.4610156961
ELYS159	ELYS159	22	0.0133300722	0.4684544195
EALA160	EALA160	14	0.0043618279	0.4169493581
EPRO161	EPRO161	12	0.0013044719	0.3700241257
EILE162	EILE162	16	0.0046064164	0.3891943745
ELEU163	ELEU163	11	0.0008560597	0.3658164798
ESER164	ESER164	12	0.0026497085	0.3708978355

EASP165	EASP165	23	0.0285760874	0.4069136933
ESER166	ESER166	8	0.0009375892	0.3515791203
ESER167	ESER167	11	0.0053401818	0.3677069553
ECYS168	ECYS168	15	0.0016305899	0.3630902238
ELYS169	ELYS169	11	0.0016305899	0.3691049438
ESER170	ESER170	9	0.0008968244	0.3264512136
EALA171	EALA171	8	0.0006522359	0.3381866782
ETYR172	ETYR172	12	0.0026904733	0.3690710096
EPRO173	EPRO173	7	0	0.2858809631
EGLY174	EGLY174	6	0.0011414129	0.3086776772
EGLN175	EGLN175	11	0.0054217113	0.3547351491
EILE176	EILE176	10	0.0006522359	0.3573221512
ETHR177	ETHR177	12	0.0013452366	0.3478110537
ESER178	ESER178	7	0.0013860014	0.3380250652
EASN179	EASN179	18	0.0152052505	0.4045702854
EMET180	EMET180	18	0.0125147772	0.4125220358
EPHE181	EPHE181	18	0.0172027231	0.4683163646
ECYS182	ECYS182	17	0.0120256003	0.4467259807
EALA183	EALA183	14	0.0013044719	0.4425290411
EGLY184	EGLY184A	14	0.003546533	0.4382080874
ETYR185	ETYR184	17	0.0057478293	0.4006062452
ELEU186	ELEU185	11	0.0048102401	0.4020639897
EGLU187	EGLU186	22	0.0165912519	0.4597855115
EGLY188	EGLY187	13	0.0027720028	0.437138847
EGLY189	EGLY188A	16	0.0049325343	0.4574720698
ELYS190	ELYS188	43	0.1653010477	0.6079771644
EASP191	EASP189	21	0.0986506869	0.5765715334
ESER192	ESER190	13	0.0006522359	0.4295098543
ECYS193	ECYS191	9	0.000326118	0.4132802781
EGLN194	EGLN192	27	0.0096204802	0.4870543597
EGLY195	EGLY193	15	0.0021605316	0.4724157507
EASP196	EASP194	27	0.0875626758	0.605795525
ESER197	ESER195	21	0.0136154254	0.502142046
EGLY198	EGLY196	16	0.0087236558	0.4848135302
EGLY199	EGLY197	13	0.0089274795	0.4646964941
EPRO200	EPRO198	17	0.0038318862	0.4658783776
EVAL201	EVAL199	17	0.0105173046	0.4799535591
EVAL202	EVAL200	12	0.0072968896	0.4270355688
ECYS203	ECYS201	16	0.0077453019	0.4378886534

ESER204	ESER202	15	0.0048510049	0.4322532426
EGLY205	EGLY203	16	0.0068484774	0.4396189554
ELYS206	ELYS204	19	0.0185887245	0.4577237057
ELEU209	ELEU209	18	0.0269047328	0.4852908072
EGLN210	EGLN210	19	0.0062370062	0.4165074145
EGLY211	EGLY211	12	0.009498186	0.4632279494
EILE212	EILE212	16	0.0084383026	0.4704706916
EVAL213	EVAL213	14	0.0166320166	0.4571322038
ESER214	ESER214	11	0.0044841221	0.4481142584
ETRP215	ETRP215	12	0.0024051201	0.3865605326
EGLY216	EGLY216	11	0.0054217113	0.425044202
ESER217	ESER217	5	0.0002853532	0.3515579994
EGLY219	EGLY219	10	0.0010191187	0.4176717719
ECYS220	ECYS220	9	0.0002853532	0.4157252802
EALA221	EALA221A	12	0.0002445885	0.4258070098
EGLN222	EGLN221	15	0.0012637071	0.4203089238
ELYS223	ELYS222	18	0.0099465982	0.4658259585
EASN224	EASN223	18	0.0013860014	0.427699507
ELYS225	ELYS224	26	0.0476947536	0.4775998772
EPRO226	EPRO225	18	0.0079898903	0.4440177828
EGLY227	EGLY226	13	0.0080306551	0.4587706207
EVAL228	EVAL227	21	0.0166320166	0.4452055308
ETYR229	ETYR228	20	0.0143899556	0.4431591118
ETHR230	ETHR229	17	0.0125147772	0.4282162447
ELYS231	ELYS230	27	0.0438628674	0.4672170576
EVAL232	EVAL231	9	0.002731238	0.3950734832
ECYS233	ECYS232	10	0.0016305899	0.3723787818
EASN234	EASN233	15	0.0059924178	0.3957393963
ETYR235	ETYR234	12	0.0028535323	0.3489826034
EVAL236	EVAL235	10	0.0017121194	0.3455347985
ESER237	ESER236	13	0.0055032408	0.3848534622
ETRP238	ETRP237	10	0.0021605316	0.3541914675
EILE239	EILE238	9	0.0009375892	0.3497070587
ELYS240	ELYS239	10	0.0020790021	0.3470172112
EGLN241	EGLN240	12	0.0023235906	0.3699079247
ETHR242	ETHR241	8	4.07647466471E-05	0.3265835014
EILE243	EILE242	6	0.000326118	0.3215168712
EALA244	EALA243	7	0.0013860014	0.3357888193
ESER245	ESER244	8	0.0039949452	0.3345647929

EASN246	EASN245	5	0.0028535323	0.3422353982