

SUMMER INTERNSHIP REPORT

on

*Classification Of Non-Cancer Proteins from Cancer Proteins
Using Random Forest Prediction Model with Amino Acid
Sequence Features*

at

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1. INTRODUCTION:

Researchers' world over has spent over a decade in cancer research and have successfully demonstrated great understanding and advancements in its molecular mechanism. However, in spite of innumerable expert-curations describing the genes/proteins in human system that drive towards cancer, there are several factors having a strong causal impact to cancer that remain functionally uncharacterised. Accurate identification of these genes/proteins is therefore very crucial for investigating into newer targets for cancer therapy.

Probing into the characteristics of proteins, in general, in most mammalian systems, they are mostly found functionally associated with other major building blocks of life such as the nucleic acids, carbohydrates, lipids or even other proteins. Proteins interacting with carbohydrates, known as lectins have shown to aid protein-folding, localization, cell to cell adhesion, proliferation as well as cell death (Gorelik et al., 2001). Recent studies have also evidenced their role in growth and metastasis in malignant cells especially in colon, thyroid and breast cancer (Nangia-Makker et al., 2002) and therefore characterizing these proteins based on their involvement in oncogenesis will help in deeper understanding of cancer causatives.

Other interacting proteins include the nucleic acid binding proteins, the DNA-binding and RNA-binding proteins, known to play vital roles in diverse cellular activities such as the splicing, translation, post-translational modifications are also associated with cancer upon mutation. Structural dysregulation of these genes/proteins result in a multistep process of replicating multiple copies of mutant genes by uncontrolled proliferation of cells (Furney et al., 2006). These cancer-causing genes have been broadly classified as proto-oncogenes and tumor-suppressor genes, depending on gain or loss of function to drive cancer respectively.

Recently, a census of human cancer genes has been compiled in a Cancer Gene Census (CGC) resource which is within the Catalogue of Somatic Mutations in Cancer (COSMIC) describing all those genes with causal effect in human cancer (<https://cancer.sanger.ac.uk/census>). In spite of the list comprising 719 cancer-causing gene entries, it is evidenced that at least 5-10% genes (i.e., ~25000 putative genes) in the human genome are yet to be identified which could be involved in malignant transformations. By unveiling the common features as well differences between the different group of genes/proteins contributing to cancer state, it is possible to uncover the molecular complexity of cancer as a disease en masse, facilitating the further identification of new drug targets (Furney et al., 2006).

Other biomolecule complexes that are vulnerable to cancerous mutations include the protein-protein interactions (PPI). These PPI contain some amino acids that contribute to the binding more than others and are called hotspots.

In general, the characteristics of any protein or peptide primarily depend on the number and sequence of their amino acids. In recent years, techniques such as Machine learning and deep learning have been widely applied for making predictions on the mutability characteristic of different proteins based on analysis of functional properties such as the composition, compressibility (K^0), thermodynamic transfer hydrophobicity (H_t), surrounding hydrophobicity index (H_p), polarity (P), isoelectric point (pH_i), equilibrium constant with reference to the ionization property of COOH group (pK'), molecular weight (M_w), bulkiness (B_1), chromatographic index (R_1), refractive index (μ), normalised consensus hydrophobicity (H_{nc}), short and medium range non-bonded energy (E_{sm}) and more are derived from their primary sequences (Cao et al., 2021).

In this study, we propose a random forest classification strategy to identify gene/proteins are highly likely to be cancer-causing by analysis the cancer properties of carbohydrate, DNA, RNA and protein interacting proteins against the cancer genes curate in CGC, based on the hypothesis that these parameters impact the predisposition of these genes/proteins towards alterations that may lead to a cancer specific phenotype.

2. MATERIALS AND METHODS

2.1. Data Resources

The initial datasets for the study contained 1,130 Carbohydrate-binding, 3,859 DNA-binding and 963 RNA-binding protein fasta sequences, 880 protein binding protein heterodimers collected from the UniProt and 768 CGC protein sequences from the CGC resource in fasta format.

2.2. Data Pre-processing and derivation of properties

The duplicate sequences within each of the datasets were first deleted after which 842 unique DNA-binding sequences, 232 RNA-binding, 1130 Carbohydrate-binding and 647 protein heterodimer sequences were remained. The presence of duplicates was also checked in the CGC protein sequences. Each of these protein sequences were then tested for redundancy against the CGC sequences and the matches were filtered out from each of the protein datasets. Further, the sequence identifiers from each dataset were ID-mapped in Uniprot database in order to obtain complete sequence information and each mapped output sequence was again tested for matches with CGC sequences. These matched sequences were also removed from the protein-binding datasets.

About 130 physiochemical, energetic and conformational properties of the 20 amino acids were considered for the study (**Table 1***).

The average amino acid compositions for each class of proteins and average values other properties in Table 1 for the 20 amino acids in all the datasets were calculated. Further, CD-HIT (v.4.8.1) was used to remove redundant sequences by precluding clusters having identical sequences at different user-set thresholds of 0.7, 0.75, 0.8, 0.85, 0.9 and 0.95. After these removals, the final datasets contained 280 DNA-binding sequences, 163 RNA-binding, 1111 Carbohydrate-binding, 500 protein heterodimer sequences and 693 CGC sequences.

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2.3. Train-test split

From the above datasets, 80% of the property records were randomly selected as the training dataset and while the remaining 20% of the features as the independent test datasets (**Table 2.**)

Table 2: Distribution of training and test datasets

Protein Classes	Training	Test
DNA-binding	778	195
RNA-binding	684	172
Protein heterodimer	954	239
Carbohydrate-binding	1443	361

2.4. Random Forest Classifier

In this study, we created four classifiers for each class labels of the proteins (carbohydrate, DNA, RNA and protein heterodimer) by applying “one-vs-CGC” binary classification technique using random forest classifier in the scikit-learn package, then trained the model using the training data and finally predict the test data under each class labels.

2.5. Hyperparameters tuning and feature selection

The tuning of hyperparameters (bootstrap, max-depth, max_features, min_samples_leaf, min_samples_split and n_estimators) was done placing appropriate search range (**Table 3.**)

Table 3: Hyperparameters search range for the classifiers

Parameters	Tested value range
Bootstrap	True, False
max_depth	2,4
Max_features	Auto, sqrt
Min_samples_split	2,5
n_estimators	10, 25, 40, 55, 71, 86, 101, 116,132, 147, 162, 177, 193, 208, 223, 238, 254, 269, 284, 300

The best parameters were optimized using grid search (**Table 4.**). The random forest classifier was fitted to compute the feature importance using the sci-kit learn packages.

Table 4: Hyperparameter tested-value result for the classifiers

Protein Class	Parameter tested values					
	Bootstrap	max_depth	max_features	min_samples_leaf	Min_samples_split	n_estimators
DNA-binding	True	4	auto	1	5	10
RNA-binding	True	4	auto	1	2	55
Protein heterodimer	True	4	auto	1	2	116
Carbohydrate-binding	False	4	auto	1	5	40

Important features (top 10) were sub-set form the sequences to reduce overfitting problems (**Table 5.**).

Table 5: Class-wise top ten important features selected for model preparation

DNA		Hetero		RNA		Carb	
Feature	Importance	Feature	Importance	Feature	Importance	Feature	Importance
C	0.124264	H	0.067093	S	0.069648161	Q	0.123183

GEIM800108	0.05076	S	0.047136	G	0.048445558	R	0.066823
IV0	0.050154	R	0.041721	Q	0.04632393	P	0.064244
OOBM850105	0.049998	Q	0.034027	C	0.04547044	K	0.062882
RACS770102	0.048675	L	0.031625	H	0.041588343	S	0.06186
MAXF760106	0.047584	T	0.030458	I	0.036775609	E	0.057489
ASAD	0.047545	E	0.030345	N	0.027620529	H	0.055449
Hp	0.044061	P	0.03034	L	0.026219557	NADH010101	0.048027
NI	0.042493	D	0.02723	T	0.025002648	C	0.034995
E	0.029169	V	0.02704	K	0.024318277	ROBB760105	0.026609

2.6. Performance evaluation

To evaluate performance of prediction, the assessment measurements used include accuracy, recall, precision and F1 score (**Table 6**).

Table 6: Definitions of performance measures used in the study

Accuracy	$\frac{TP + TN}{TP + TN + FP + FN}$
Recall	$\frac{TP}{TP + FN}$
Precision	$\frac{TP}{TP + FP}$
F1 score	$\frac{2 * TN}{2 * TP + FP + FN}$

Where, **TP**, **TN**, **FN** and **FP** are true positives, true negatives, false negatives and false positives, respectively.

The accuracy measures the correctly predicted observations against the total observations. Recall indicates accuracy of the positive samples predicted, while precision is the count of correctly predicted positives against total positive observations and F1 score is the weighted average of precision and recall. For all these measures, 1 is the maximum value indicating best performance that is closeness between predicted and actual observations; 0 is minimum value, which indicates the lowest performance that is the predicted observations differ greatly from the actual observations. Also, an AU-ROC curve (area under the receiver operating characteristics

curve) was used to determine the performance of the classification model. An ROC curve features true-positive rates false-positive rates on Y and X-axes respectively, which means greater area under the curve (AUC) is preferred.

3. RESULTS AND DISCUSSION

3.1. Performance of the Random Forest classifier on the test data

The performance of Random Forest classifier on the test data for each protein type was evaluated (**Table 7.**).

Table 7: Evaluation of performance of test data

Protein class	Precision	Recall	F1 score
DNA-binding	0.78	0.73	0.76
RNA-binding	0.90	0.98	0.94
Carbohydrate-binding	0.68	0.64	0.66
Protein-heterodimer	0.85	0.87	0.86

The confusion matrix on test data shown in **Fig 1A.** shows the result of random forest classification on the DNA-binding protein test data collected in the study. It shows that 27 of 195 total sequences of DNA-binding protein were correctly identified as DNA sequences and 102 sequences were identified as CGC proteins (cancer genes). Similarly, **Fig1B.** shows 18 of 172 total RNA-binding proteins were rightly identified as RNA and 136 of these as CGC proteins. Confusion matrix in **Fig 1C.** shows 79 of 239 heterodimer proteins to be correctly identified as heterodimers and 121 as CGC and **Fig 1D.** shows 181 of 361 carbohydrate-binding proteins protein were correctly identified as carbohydrate-binding proteins while 89 of these were correctly identified as CGC proteins.

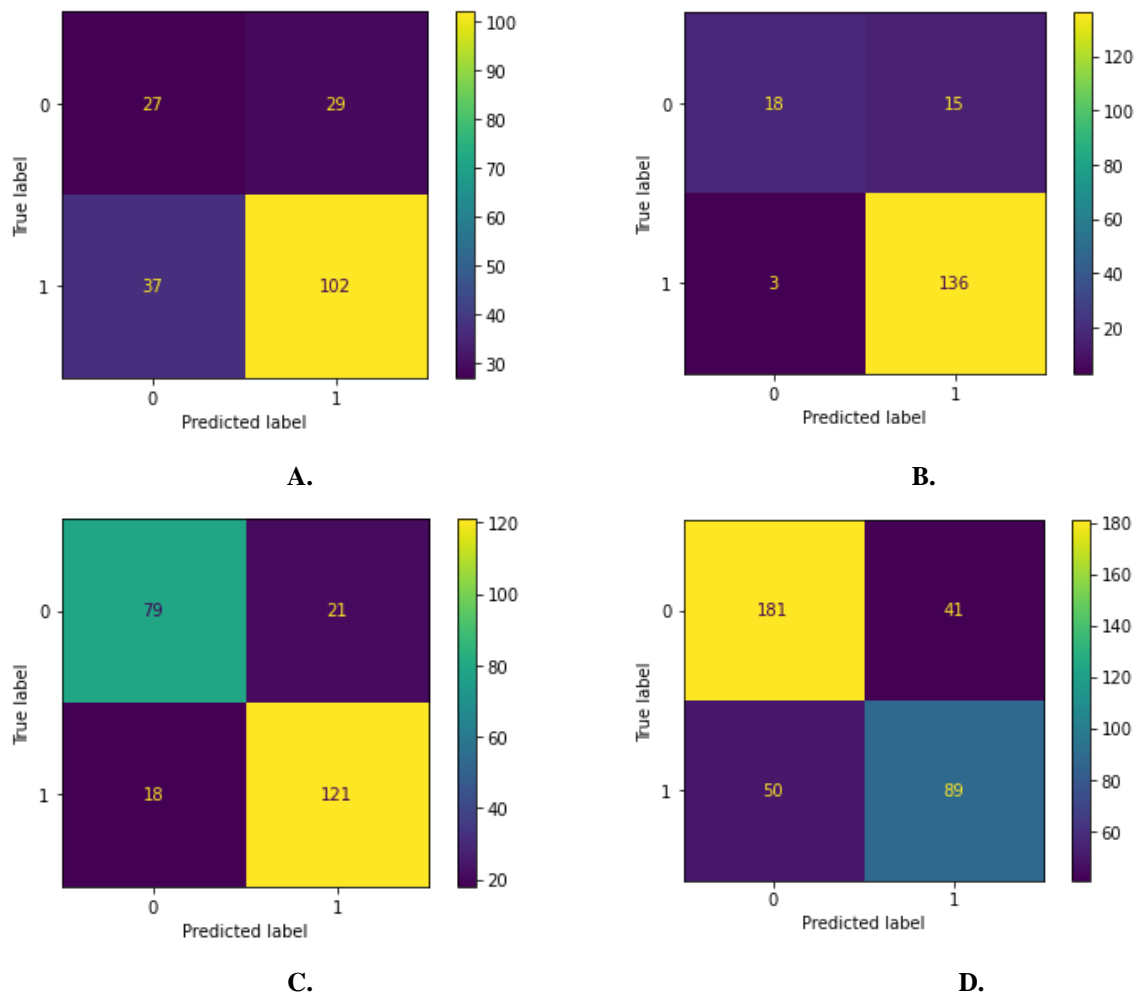


Fig 1. Performance of the Random Forest classifier model on the test datasets of **A.** DNA-binding proteins **B.** RNA-binding proteins **C.** heterodimer proteins and **D.** Carbohydrate-binding proteins tested against the sequences in CGC protein dataset.

The evaluation metrics, including precision, recall, F1 scores as mentioned in **Table 6.** and Accuracy (DNA-binding protein: 0.66, RNA-binding protein: 0.90, Heterodimer protein: 0.84, Carbohydrate-binding protein: 0.75) to access the performance of the Random Forest classifier indicate the evaluation values of DNA, RNA, Carbohydrate and protein-binding proteins are good, especially the classification of RNA-binding proteins.

The ROC-AUC curves of all the test datasets depict the prediction efficiency for the selected features (**Fig 2A-D**).

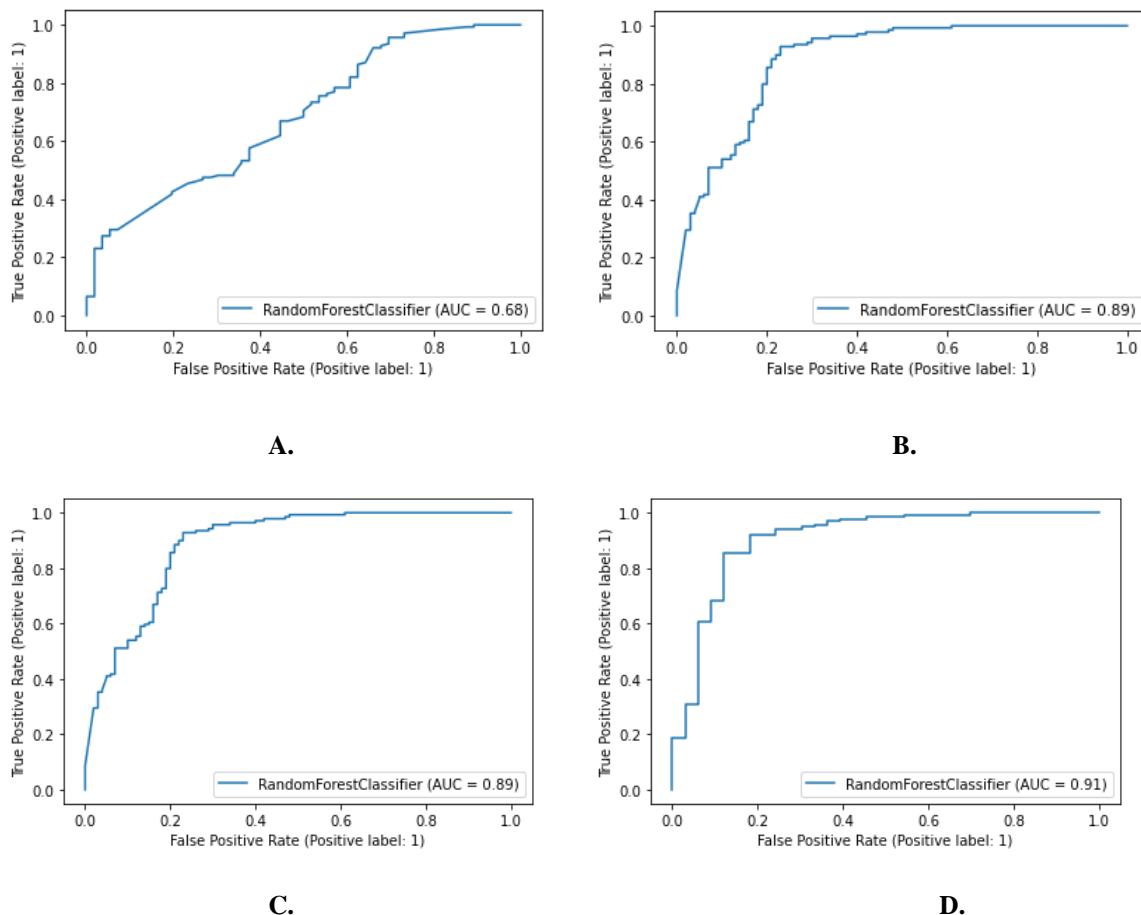


Fig 2. ROC-AUC curves of the Random Forest classifier model on the test datasets of **A.** DNA-binding proteins **B.** RNA-binding proteins **C.** Heterodimer proteins and **D.** Carbohydrate-binding proteins tested against the sequences in CGC protein dataset.

The confusion matrix, ROC-AUC curves and the evaluations indicate that Random Forest classifier achieves good results on the collected test data.

4. CONCLUSION:

This study successfully applies machine learning algorithm to classify cancer genes from the non-cancerous proteins in DNA-binding, RNA-binding, Carbohydrate-binding and protein-binding heterodimer protein datasets. The study can further be extended to classify several other human non-cancer proteins by applying array of machine learning algorithms.

5. References

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Table 1: Physiochemical, energetic and conformational acid properties of the 20 amino acids

Properties	A	D	C	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
K0	0.83	0.24	0.26	0	0.13	0.71	0.34	0.34	0.29	0.34	0.39	0.41	1	0.28	0.74	0.49	0.38	0.43	0.46	0.09
Ht	0.23	0.18	0.4	0.18	0.76	0.03	0.23	0.84	0.44	0.58	0.44	0.02	0.73	0	0.23	0.02	0.02	0.5	1	0.71
Hp	0.47	0.02	0.76	0.09	0.67	0.27	0.33	1	0	0.73	0.6	0.16	0.01	0.18	0.32	0.15	0.26	0.86	0.68	0.59
P	0	0.96	0.03	0.96	0.01	0	0.99	0	0.95	0	0.03	0.07	0.03	0.07	1	0.03	0.03	0	0.04	0.03
pHi	0.4	0	0.29	0.31	0.34	0.4	0.6	0.41	0.87	0.4	0.37	0.33	0.44	0.36	1	0.36	0.36	0.4	0.39	0.36
pK'	0.96	0.64	0.28	0.81	0.52	0.96	0.45	0	0.8	0.98	0.9	0.65	0.62	0.79	0.44	0.83	0.73	0.94	1	0.82
Mw	0.11	0.45	0.36	0.56	0.7	0	0.62	0.43	0.55	0.43	0.57	0.44	0.31	0.55	0.77	0.23	0.34	0.33	1	0.82
Bl	0.44	0.45	0.55	0.56	0.9	0	0.56	0.99	0.68	0.99	0.71	0.52	0.77	0.61	0.6	0.33	0.68	1	1	0.8
Rf	0.44	0	0	0.03	1	0.18	0.34	0.89	0.04	0.93	0.74	0.16	0.75	0.39	0.11	0.26	0.42	0.72	0.89	0.76
Mu	0.34	0.28	0.84	0.41	0.69	0	0.51	0.45	0.5	0.44	0.51	0.31	0.26	0.41	0.63	0.15	0.26	0.33	1	0.74
Hnc	0.81	0.88	0.72	0.46	0.95	0.77	0.54	1	0.26	0.92	0.81	0.45	0.68	0.43	0	0.6	0.63	0.92	0.85	0.71
Esm	1	0.5	0.94	0.5	0.46	0.92	0.63	0.56	0.31	0.83	0.79	0.54	0.67	0.42	0	0.79	0.69	0.69	0.23	0.23
El	0.36	0.09	0.7	0.13	0.79	0.43	0.45	0.87	0	0.66	0.66	0.15	0.3	0.19	0.47	0.28	0.42	0.81	1	0.66
Et	0.79	0.22	1	0.25	0.73	0.79	0.58	0.87	0	0.9	0.88	0.28	0.49	0.22	0.16	0.57	0.6	0.91	0.75	0.48
Pa	0.9	0.47	0.14	1	0.6	0	0.46	0.54	0.63	0.68	0.94	0.11	0	0.57	0.44	0.21	0.28	0.52	0.54	0.13
Pb	0.35	0.13	0.62	0	0.76	0.29	0.38	0.92	0.28	0.7	0.51	0.39	0.14	0.55	0.42	0.29	0.62	1	0.75	0.83
Pt	0.17	0.91	0.66	0.25	0.12	1	0.44	0	0.5	0.11	0.12	1	0.96	0.47	0.44	0.88	0.45	0.03	0.45	0.61
Pc	0.12	0.61	0.59	0.25	0.12	0.91	0.47	0.07	0.39	0.1	0	0.76	1	0.27	0.47	0.74	0.48	0.04	0.17	0.47
Ca	0.15	0.27	0.25	0.42	0.69	0	0.5	0.54	0.69	0.46	0.62	0.31	0.19	0.48	0.88	0.15	0.31	0.42	1	0.69
F	0.57	0.96	0.38	0.81	0	1	0.23	0.15	0.96	0.21	0.19	0.74	1	0.81	0.77	0.94	0.57	0.21	0.17	0.68
Br	0.64	0.19	1	0.09	0.89	0.64	0.51	0.98	0	0.87	0.72	0.21	0.26	0.13	0.06	0.36	0.36	0.83	0.68	0.42
Ra	0.32	0.14	0.21	0.26	0.82	0.23	0.3	1	0	0.69	0.58	0.06	0.06	0.15	0.13	0.11	0.14	0.91	0.76	0.21
Ns	0.39	0.02	1	0.07	0.58	0.43	0.31	0.88	0	0.84	0.51	0.05	0.26	0.19	0.28	0.22	0.31	0.92	0.7	0.62
aN	0.83	0.28	0.17	0.76	0.6	0.28	0.47	0.64	0.59	1	0.65	0.28	0	0.51	0.35	0.37	0.39	0.74	0.7	0.3
aC	0.58	0.97	0.21	0.9	0.34	0.13	0.09	0.16	0.11	0.11	0.19	0.3	1	0.45	0	0.23	0.48	0.13	0.56	0.18
aM	0.55	0.25	0.69	0.57	0.51	0.18	1	0.35	0.74	0.47	0.66	0.42	0	0.73	0.71	0.39	0.21	0.54	0.21	0.23
V0	0.17	0.3	0.24	0.42	0.78	0	0.55	0.64	0.65	0.64	0.62	0.35	0.39	0.5	0.84	0.17	0.33	0.47	1	0.8
Nm	0.83	0.51	0.59	0.81	0.69	0.22	0.69	0.47	0.67	0.92	1	0.55	0	0.75	0.65	0.26	0.26	0.33	0.61	0.37

NI	0.42	0.05	0.99	0	0.63	0.56	0.37	1	0.02	0.65	0.5	0.32	0.3	0.12	0.37	0.36	0.48	0.95	0.74	0.77
Hgm	0.6	0.06	0.97	0	0.62	0.48	0.6	0.95	0.05	0.67	0.6	0.4	0.24	0.3	0.42	0.49	0.32	0.78	1	0.61
ASAD	0.22	0.43	0.43	0.65	0.79	0	0.68	0.72	0.89	0.64	0.85	0.45	0.45	0.67	1	0.28	0.42	0.52	0.99	0.9
ASAN	0.17	0.5	0	0.7	0.17	0.13	0.44	0.12	1	0.15	0.26	0.48	0.48	0.6	0.85	0.34	0.38	0.11	0.24	0.36
dASA	0.22	0.21	0.57	0.29	0.84	0	0.52	0.8	0.35	0.69	0.84	0.24	0.24	0.4	0.58	0.15	0.27	0.58	1	0.82
dGh	0.86	0.48	0.69	0.36	0.78	0.86	0.41	1	0.6	0.99	0.85	0.38	1	0.32	0	0.34	0.64	0.97	0.34	0.05
GhD	0.91	0.5	0.81	0.41	0.86	0.9	0.54	0.99	0.51	0.98	0.9	0.46	1	0.42	0	0.49	0.68	0.97	0.6	0.32
GhN	0.96	0.53	0.93	0.46	0.93	0.94	0.66	0.98	0.73	0.98	0.95	0.54	1	0.52	0	0.63	0.73	0.97	0.84	0.57
dHh	0.92	0.67	0.79	0.55	0.6	1	0.42	0.74	0.61	0.78	0.71	0.54	0.95	0.48	0.03	0.51	0.68	0.82	0.18	0
-TdSh	0.19	0.16	0.21	0.24	0.74	0	0.6	0.76	0.45	0.68	0.62	0.29	0.32	0.33	0.83	0.29	0.36	0.56	1	0.96
dCph	0.29	0	0.17	0.01	0.93	0.05	0.44	1	0.38	0.91	0.74	0.03	0.53	0.03	0.35	0.09	0.34	0.76	0.89	0.71
dGc	0.13	0.47	0.45	0.57	0.52	0.16	0.62	0	0.33	0.01	0.22	0.53	0	0.59	0.81	0.55	0.31	0.03	0.86	1
dHc	0.12	0.26	0.56	0.34	0.81	0	0.49	0.21	0.18	0.19	0.44	0.18	0.06	0.25	0.36	0.35	0.24	0.17	0.93	1
-TdSc	0.78	0.83	0.34	0.79	0	0.98	0.6	0.52	0.84	0.56	0.33	1	0.76	0.95	0.95	0.76	0.72	0.61	0.1	0.09
dG	0.24	0.22	0.62	0.2	1	0.28	0.44	0.22	0.14	0.22	0.43	0.14	0.23	0.17	0	0.11	0.16	0.23	0.87	0.56
dH	0.44	0.41	0.86	0.4	1	0.37	0.46	0.41	0.26	0.41	0.65	0.21	0.39	0.24	0	0.38	0.4	0.42	0.79	0.72
-TdS	0.49	0.53	0.06	0.54	0	0.6	0.53	0.53	0.69	0.53	0.28	0.77	0.55	0.74	1	0.53	0.52	0.51	0.24	0.22
v	0.1	0.4	0.2	0.5	0.7	0	0.6	0.4	0.5	0.4	0.4	0.4	0.3	0.5	0.7	0.2	0.3	0.3	1	0.8
s	0	0.4	0	0.6	0.4	0	0.4	0.2	0	0.4	0	0.4	0	0.6	1	0	0.2	0.2	0.4	0.4
f	0	0.4	0.2	0.6	0.4	0	0.4	0.4	0.8	0.4	0.6	0.4	0	0.6	1	0.2	0.2	0.2	0.4	0.4
Pf-s	0.12	0.5	0.18	0.15	0.1	0.68	0.53	0.01	0.32	0.07	0.1	1	0	0.27	0.28	0.21	0.01	0.02	0.12	0.18
GEIM800105	0.84	1.27	0.59	0.57	1.15	0.94	0.81	1.29	0.86	1.1	0.88	0.66	0.8	1.02	1.04	1.05	1.2	1.56	1.15	1.39
GEIM800108	0.91	0.93	1.4	0.97	0.72	1.51	0.9	0.65	0.82	0.59	0.58	1.64	1.66	0.94	1	1.23	1.04	0.6	0.67	0.92
BIOV880102	44	90	-91	-139	148	-8	47	100	-188	108	121	-72	-36	-117	-68	-60	-54	117	163	22
GRAR740102	8.1	5.5	13	12.3	5.2	9	10.4	5.2	11.3	4.9	5.7	11.6	8	10.5	10.5	9.2	8.6	5.9	5.4	6.2
GRAR740103	31	55	54	83	132	3	96	111	119	111	105	56	32.5	85	124	32	61	84	170	136
HOPA770101	1	0.1	6.5	6.2	1.4	1.1	2.8	0.8	5.3	0.8	0.7	2.2	0.9	2.1	2.3	1.7	1.5	0.9	1.9	2.1
ISOY800102	0.86	1.39	0.69	0.66	1.16	0.7	1.06	1.31	0.77	1.01	1.06	0.74	1.16	0.89	0.98	1.09	1.24	1.4	1.17	1.28
ISOY800103	0.78	0.6	1.5	0.97	0.67	1.73	0.83	0.4	1.01	0.57	0.3	1.56	1.55	0.78	1.06	1.19	1.09	0.44	0.74	1.14
ISOY800104	1.09	0.5	0.77	0.92	0.5	1.25	0.67	0.66	1.25	0.44	0.45	1.14	2.96	0.83	0.97	1.21	1.33	0.56	0.62	0.94

JOND750101	0.87	1.52	0.66	0.67	2.87	0.1	0.87	3.15	1.64	2.17	1.67	0.09	2.77	0	0.85	0.07	0.07	1.87	3.77	2.67
JUKT750101	5.3	1.3	3.6	3.3	2.3	4.8	1.4	3.1	4.1	4.7	1.1	3	2.5	2.4	2.6	4.5	3.7	4.2	0.8	2.3
KANM800102	0.81	1.17	0.71	0.53	1.2	0.88	0.92	1.48	0.77	1.24	1.05	0.62	0.61	0.98	0.85	0.92	1.18	1.66	1.18	1.23
KANM800103	1.45	0.7	0.91	1.29	1.2	0.53	1.13	1.23	1.27	1.56	1.83	0.64	0.21	1.14	1.15	0.48	0.77	1.1	1.17	0.74
KARP850101	1.041	0.96	1.033	1.094	0.93	1.142	0.982	1.002	1.093	0.967	0.947	1.117	1.055	1.165	1.038	1.169	1.073	0.982	0.925	0.961
KRIW710101	4.6	-1	5.7	5.6	3.2	7.6	4.5	2.6	7.9	3.25	1.4	5.9	7	6.1	6.5	5.25	4.8	3.4	4	4.35
KRIW790101	4.32	1.73	6.04	6.17	2.59	6.09	5.66	2.31	7.92	3.93	2.44	6.24	7.19	6.13	6.55	5.37	5.16	3.31	2.78	3.58
LAW840101	-0.48	-0.32	-0.75	-0.71	1.03	0	-0.51	0.81	-0.09	1.02	0.81	-0.87	2.03	-0.32	-0.06	0.05	-0.35	0.56	0.66	1.24
LEVM760101	-0.5	-1	2.5	2.5	-2.5	0	-0.5	-1.8	3	-1.8	-1.3	0.2	-1.4	0.2	3	0.3	-0.4	-1.5	-3.4	-2.3
LIFS790101	0.92	1.16	0.48	0.61	1.25	0.61	0.93	1.81	0.7	1.3	1.19	0.6	0.4	0.95	0.93	0.82	1.12	1.81	1.54	1.53
LIFS790103	0.9	1.24	0.47	0.62	1.23	0.56	1.12	1.54	0.74	1.26	1.09	0.62	0.42	1.18	1.02	0.87	1.3	1.53	1.75	1.68
MANP780101	12.97	14.63	10.85	11.89	14	12.43	12.16	15.67	11.36	14.9	14.39	11.42	11.37	11.76	11.72	11.23	11.69	15.71	13.93	13.42
MAXF760102	0.86	1.17	0.72	0.62	1.16	0.97	1.06	1.24	0.79	0.98	1.08	0.74	1.22	0.89	0.94	1.04	1.18	1.33	1.07	1.25
MAXF760106	1	1.09	1.39	1.04	0.65	0.46	0.71	0.68	1.05	1.01	0.36	0.87	1.95	1.13	1.18	1.56	1.23	0.58	1.1	0.87
MIYS850101	2.36	3.36	1.67	1.74	4.37	2.06	2.41	4.17	1.23	3.93	4.22	1.7	1.89	1.75	1.92	1.81	2.04	3.49	3.82	2.91
NAGK730102	0.96	1.13	0.9	0.33	1.37	0.9	0.87	1.54	0.81	1.26	1.29	0.72	0.75	1.18	0.67	0.77	1.23	1.41	1.13	1.07
NAGK730103	0.72	1.01	1.04	0.75	0.58	1.35	0.76	0.8	0.84	0.63	0.62	1.38	1.43	0.81	1.33	1.34	1.03	0.83	0.87	1.35
BURA740102	0.288	0.533	0.271	0.262	0.318	0.312	0.2	0.411	0.265	0.4	0.375	0.229	0.34	0.327	0.362	0.354	0.388	0.495	0.231	0.429
ARGP820101	0.61	1.07	0.46	0.47	2.02	0.07	0.61	2.22	1.15	1.53	1.18	0.06	1.95	0	0.6	0.05	0.05	1.32	2.65	1.88
NISK860101	-0.22	4.66	-4.12	-3.64	5.27	-1.62	1.28	5.58	-4.18	5.01	3.51	-2.65	-3.03	-2.76	-0.93	-2.84	-1.2	4.45	5.2	2.15
OOBM770103	0.491	-0.67	-0.356	-0.371	-0.729	0.534	-0.54	-0.762	-0.3	-0.65	-0.659	-0.382	-0.463	-0.405	0.554	-0.455	-0.515	-0.728	-0.839	-0.656
OOBM850103	0.46	0.2	-0.33	0.48	0.52	0.64	-1.31	3.28	-1.71	0.43	0.15	1.31	-0.58	-1.12	-1.54	-0.83	-1.52	0.54	1.25	-2.21
CHAM820101	0.046	0.128	0.105	0.151	0.29	0	0.23	0.186	0.219	0.186	0.221	0.134	0.131	0.18	0.291	0.062	0.108	0.14	0.409	0.298
OOBM850105	4.55	-0.78	2.85	5.16	4.37	9.14	4.48	2.1	10.68	3.24	2.18	5.56	5.14	4.15	5.97	6.78	8.6	3.81	1.97	2.4
PONP800102	7.62	10.93	6.18	6.38	8.99	7.31	7.85	9.99	5.72	9.37	9.83	6.17	6.64	6.67	6.81	6.93	7.08	10.38	8.41	8.53
PONP800103	2.63	3.36	2.29	2.31	3.02	2.55	2.57	3.08	2.12	2.98	3.18	2.27	2.46	2.45	2.45	2.6	2.55	3.21	2.85	2.79
PONP800107	3.7	3.03	2.6	3.3	6.6	3.13	3.57	7.69	1.79	5.88	5.21	2.12	2.12	2.7	2.53	2.43	2.6	7.14	6.25	3.03
PRAM900104	0.78	0.8	1.41	1	0.58	1.64	0.69	0.51	0.96	0.59	0.39	1.28	1.91	0.97	0.88	1.33	1.03	0.47	0.75	1.05
RACS770101	0.934	0.9	0.994	0.986	0.773	1.015	0.882	0.766	1.04	0.825	0.804	0.986	1.047	1.047	0.962	1.056	1.008	0.825	0.848	0.931

RACS770102	0.941	0.866	1.071	1.1	0.723	1.055	0.911	0.742	1.232	0.798	0.781	1.038	1.093	1.15	1.112	1.082	1.043	0.817	0.867	1.05
RACS820113	17.05	28.84	19.27	20.12	16.26	38.14	23.07	16.66	16.46	10.89	20.61	34.81	23.94	15.42	21.25	19.95	18.92	17.06	23.36	26.49
CHOC760101	115	135	150	190	210	75	195	175	200	170	185	160	145	180	225	115	140	155	255	230
ROBB760105	-2.3	4.4	-4.4	-5	2.6	-4.2	-2.5	6.7	-3.3	2.3	2.3	-4.1	-1.8	1.2	0.4	-1.7	1.3	6.8	-1	4
ROSM880102	-0.67	-2	1.57	1.78	-3.24	0	1.09	-3.02	2.46	-3.02	-1.67	2.27	-1.75	2.12	3.89	0.1	-0.42	-2.18	-2.86	0.98
SIMZ760101	0.73	0.7	0.54	0.55	2.65	0	1.1	2.97	1.5	2.49	1.3	-0.01	2.6	-0.1	0.73	0.04	0.44	1.69	3	2.97
WOEC730101	7	5.5	13	12.5	5	7.9	8.4	4.9	10.1	4.9	5.3	10	6.6	8.6	9.1	7.5	6.6	5.6	5.3	5.7
CHOP780202	0.83	1.19	0.54	0.37	1.38	0.75	0.87	1.6	0.74	1.3	1.05	0.89	0.55	1.1	0.93	0.75	1.19	1.7	1.37	1.47
YUTK870102	6.8	8.3	7	4.9	8.3	6.4	9.2	10	7.5	12.2	8.4	6.2	6.9	8.5	0	8	7	9.4	5.7	6.8
ZIMJ680101	0.83	1.48	0.64	0.65	2.75	0.1	1.1	3.07	1.6	2.52	1.4	0.09	2.7	0	0.83	0.14	0.54	1.79	0.31	2.97
ZIMJ680102	11.5	13.46	11.68	13.57	19.8	3.4	13.69	21.4	15.71	21.4	16.25	12.82	17.43	14.45	14.28	9.47	15.77	21.57	21.67	18.03
ZIMJ680105	9.9	2.8	2.8	3.2	18.8	5.6	8.2	17.1	3.5	17.6	14.9	5.4	14.8	9	4.6	6.9	9.5	14.3	17.1	15
CHOP780204	1.29	0.66	2.02	2.44	0.61	0.76	0.73	0.67	0.66	0.58	0.71	0.81	2.01	1.22	0.44	0.74	1.08	0.61	1.47	0.68
ONEK900102	-0.77	-0.23	-0.15	-0.27	-0.41	0	-0.06	-0.23	-0.65	-0.62	-0.5	-0.07	3	-0.33	-0.68	-0.35	-0.11	-0.14	-0.45	-0.17
VINM940101	0.984	0.906	1.068	1.094	0.915	1.031	0.95	0.927	1.102	0.935	0.952	1.048	1.049	1.037	1.008	1.046	0.997	0.931	0.904	0.929
NADH010101	58	116	-97	-131	92	-11	-73	107	-24	95	78	-93	-79	-139	-184	-34	-7	100	59	-11
NADH010102	51	137	-78	-115	108	-13	-55	106	-205	103	73	-84	-79	-128	-144	-26	-3	108	69	11
NADH010104	32	182	-29	-74	132	-22	-25	106	-124	104	82	-73	-82	-95	-95	-34	20	113	118	44
NADH010105	24	194	0	-57	131	-28	-31	102	-9	103	90	-76	-85	-87	-79	-36	34	111	116	43
FUKS010102	6.77	0.31	8.57	12.93	1.92	7.95	2.8	2.72	10.2	4.43	1.87	5.5	4.79	5.24	6.87	5.41	5.36	3.57	0.54	2.26
FUKS010103	7.43	0.42	8.71	5.86	1.18	9.4	1.49	1.76	9.67	2.74	0.6	9.12	5.6	5.42	4.51	9.6	8.95	3.1	1.18	3.26
KUHL950101	0.78	0.55	1.35	1.45	0.47	0.68	0.99	0.47	1.1	0.56	0.66	1.2	0.69	1.19	1.58	1	1.05	0.51	0.7	1
ZHOH040101	2.18	3.89	1.75	1.89	5.88	1.17	2.51	4.5	2.12	4.71	3.63	1.85	2.09	2.16	2.71	1.66	2.18	3.77	6.46	5.01
ZHOH040102	1.79	2.22	2.33	2.52	4.84	0.7	3.06	4.59	2.5	4.72	3.91	2.83	2.45	2.37	3.2	1.82	2.45	3.67	5.64	4.46
ZHOH040103	13.4	22.6	8.2	7.3	23.9	7	11.3	20.3	6.1	20.8	15.7	7.6	9.9	8.5	8.5	8.2	10.3	19.5	24.5	19.5
PONJ960101	91.5	114.4	135.2	154.6	198.8	67.5	163.2	162.6	162.5	163.4	165.9	138.3	123.4	156.4	196.1	102	126	138.4	209.8	237.2
WOLR790101	1.12	0.59	-0.83	-0.92	0.67	1.2	-0.93	1.16	-0.8	1.18	0.55	-0.83	0.54	-0.78	-2.55	-0.05	-0.02	1.13	-0.19	-0.23
OLSK800101	1.38	1.43	0.52	0.71	1.72	1.34	0.66	2.32	0.15	1.47	1.78	0.37	0.85	0.22	0	0.86	0.89	1.99	0.82	0.47
KIDA850101	-0.27	-1.05	0.81	1.17	-1.43	-0.16	0.28	-0.77	1.7	-1.1	-0.73	0.81	-0.75	1.1	1.87	0.42	0.63	-0.4	-1.57	-0.56

CORJ870102	0.414	0.162	-1.31	-1.218	1.938	0.684	-0.63	1.237	-0.67	1.215	1.02	-0.916	-0.503	-0.905	0.584	-0.563	-0.289	0.899	0.514	1.699
CORJ870104	-0.26	0.83	-1.3	-0.73	1.09	-0.4	-0.18	1.1	-1.01	1.52	1.09	-0.46	-0.62	-0.83	0.08	-0.55	-0.71	1.15	-0.13	0.69
CORJ870106	-1.35	4.37	-11.88	-4.56	11.35	-5.82	6.54	10.93	-11.92	9.88	7.47	-10.96	-10.86	-1.34	-3.89	-6.21	-4.83	8.2	1.8	7.61
CORJ870108	1.37	-4.47	8.93	4.04	-7.96	3.39	-1.65	-7.92	7.7	-8.68	-7.13	6.29	6.25	3.88	1.33	4.08	4.02	-6.94	0.79	-4.73
MIYS990101	-0.02	-0.96	0.72	0.74	-2.22	0.38	0	-1.89	1.01	-2.29	-1.36	0.63	0.47	0.56	0.44	0.55	0.25	-1.34	-1.28	-0.88
FASG890101	-0.21	-6.04	1.36	2.3	-4.65	0	-1.23	-4.81	3.88	-4.68	-3.66	0.96	0.75	1.52	2.11	1.74	0.78	-3.5	-3.32	-1.01
DAYM780201	100	20	106	102	41	49	66	96	56	40	94	134	56	93	65	120	97	74	18	41
EISD860101	0.67	0.38	-1.2	-0.76	2.3	0	0.64	1.9	-0.57	1.9	2.4	-0.6	1.2	-0.22	-2.1	0.01	0.52	1.5	2.6	1.6
FASG760101	89.09	121.15	133.1	147.13	165.19	75.07	155.16	131.17	146.19	131.17	149.21	132.12	115.13	146.15	174.2	105.09	119.12	117.15	204.24	181.19
FASG760102	297	178	270	249	284	290	277	284	224	337	283	236	222	185	238	228	253	293	282	344
FAUJ830101	0.31	1.54	-0.77	-0.64	1.79	0	0.13	1.8	-0.99	1.7	1.23	-0.6	0.72	-0.22	-1.01	-0.04	0.26	1.22	2.25	0.96
FAUJ880101	1.28	1.77	1.6	1.56	2.94	0	2.99	4.19	1.89	2.59	2.35	1.6	2.67	1.56	2.34	1.31	3.03	3.67	3.21	2.94
FAUJ880106	2.04	3.41	3.78	3.31	6.02	1	5.66	3.49	4.87	4.45	4.8	4.37	4.31	3.53	6.24	2.7	3.17	3.17	5.9	6.72
BIGC670101	52.6	68.3	68.4	84.7	113.9	36.3	91.9	102	105.1	102	97.7	75.7	73.6	89.7	109.1	54.9	71.2	85.1	135.4	116.2

Abbreviations: K_0 = compressibility; H_t = thermodynamic transfer hydrophobicity; H_p = surrounding hydrophobicity; P = polarity; pH_i = isoelectric point; pKV = equilibrium constant with reference to the ionization property of COOH group; M_w = molecular weight; B_1 = bulkiness; R_1 = chromatographic index; l = refractive index; H_{nc} = normalized consensus hydrophobicity; E_{sm} = short and medium range non-bonded energy; E_l = long range non-bonded energy; E_t = total non-bonded energy ($E_{sm} + E_l$); P_a , P_h , P_t and P_c = respectively, α -helical, h-structure, turn and coil tendencies; C_a = helical contact area; F = mean rms fluctuational displacement; B_r = buriedness; R_a = solvent accessible reduction ratio; N_s = average number of surrounding residues; a_n , a_c and a_m = respectively, power to be at the N-terminal, C-terminal and middle of α -helix; V_0 = partial-specific volume; N_m and N_l = respectively, average medium and long-range contacts; H_{gm} = combined surrounding hydrophobicity (globular and membrane); ASA_D , ASA_N and $DASA$ = respectively, solvent accessible surface area for denatured, native and unfolding; DG_h , G_hD and G_hN = Respectively, Gibbs free energy change of hydration for unfolding, denatured and native protein; DH_h = unfolding enthalpy change of hydration; $_TDS_h$ = unfolding entropy change of hydration; DC_{ph} = Unfolding hydration heat capacity change; DG_c , DH_c and $_TDS_c$ = respectively, unfolding Gibbs free energy, unfolding enthalpy and unfolding entropy changes of chain; DG , DH , and $_TDS$ = respectively, unfolding Gibbs free energy change, unfolding enthalpy change and unfolding entropy change; v = volume (number of non-hydrogen side chain atoms); s = shape (position of branch point in a side-chain); f = flexibility (number of side-chain dihedral angles). K_0 in $m^3/mol/Pa$ ($\times 10^{-15}$); H_t , H_p , H_{nc} , H_{gm} , DG_h , G_hD , G_hN , DH_h , $_TDS_h$, DG_c , DH_c , $_TDS_c$, DG , DH and $_TDS$ in kcal/mol; P in Debye; pH_i and pKV in pH units; E_{sm} , E_l and E_t in kcal/mol/atom; B_1 , C_a , ASA_D , ASA_N and $DASA$ in \AA^2 ; F in \AA^2 ; V_0 in m^3/mol ($\times 10^{-6}$); DC_{ph} in cal/mol/K and the rest are dimensionless quantities (Sony et al., 2006);

Amino acid abbreviations: A = Alanine, D: Aspartic acid, E: Glutamic acid, F: Phenylalanine, G: Glycine, H: Histidine, I: Isoleucine, K: Lysine, M: Methionine, N: Asparagine, P: Proline, Q: Glutamine, R: Arginine, S: Serine, T: Threonine, V: Valine, W: Tryptophan, Y: Tyrosine