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Using SuSPect to Predict the Phenotypic Effects of Missense Variants

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Outline

- SAVs and Disease
- Development of SuSPect
  - Features included
  - Feature selection
  - Performance
- Web-Server & Availability
  - Usage
  - Example results

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Background

- 10-15,000 single amino acid variants (SAVs) per exome.

- Many variants are tolerated, but some SAVs cause disease.
  - Glu6Val in HBB causes sickle cell anæmia.

- Many mechanisms by which SAVs can impair function.
  - Decrease stability,
  - Change active site,
  - Protein-protein interaction.

- Need methods for predicting SAV effects
  - Sequence- and structure-based.

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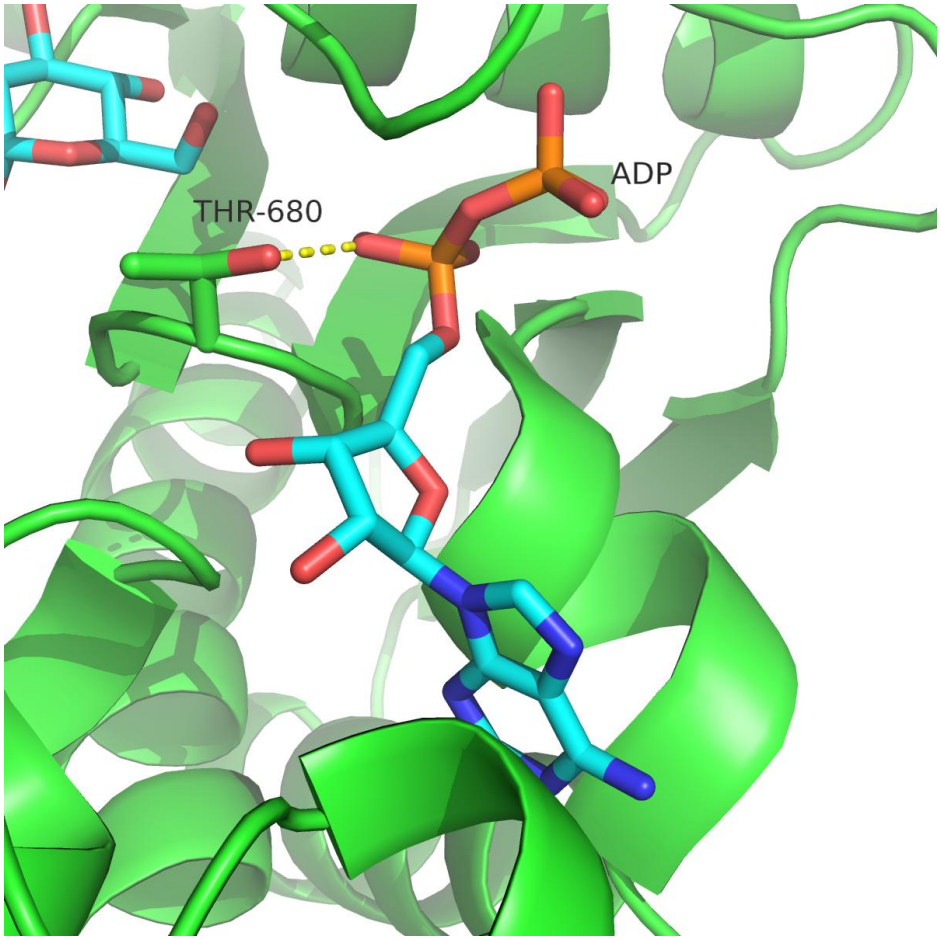
Hexokinase



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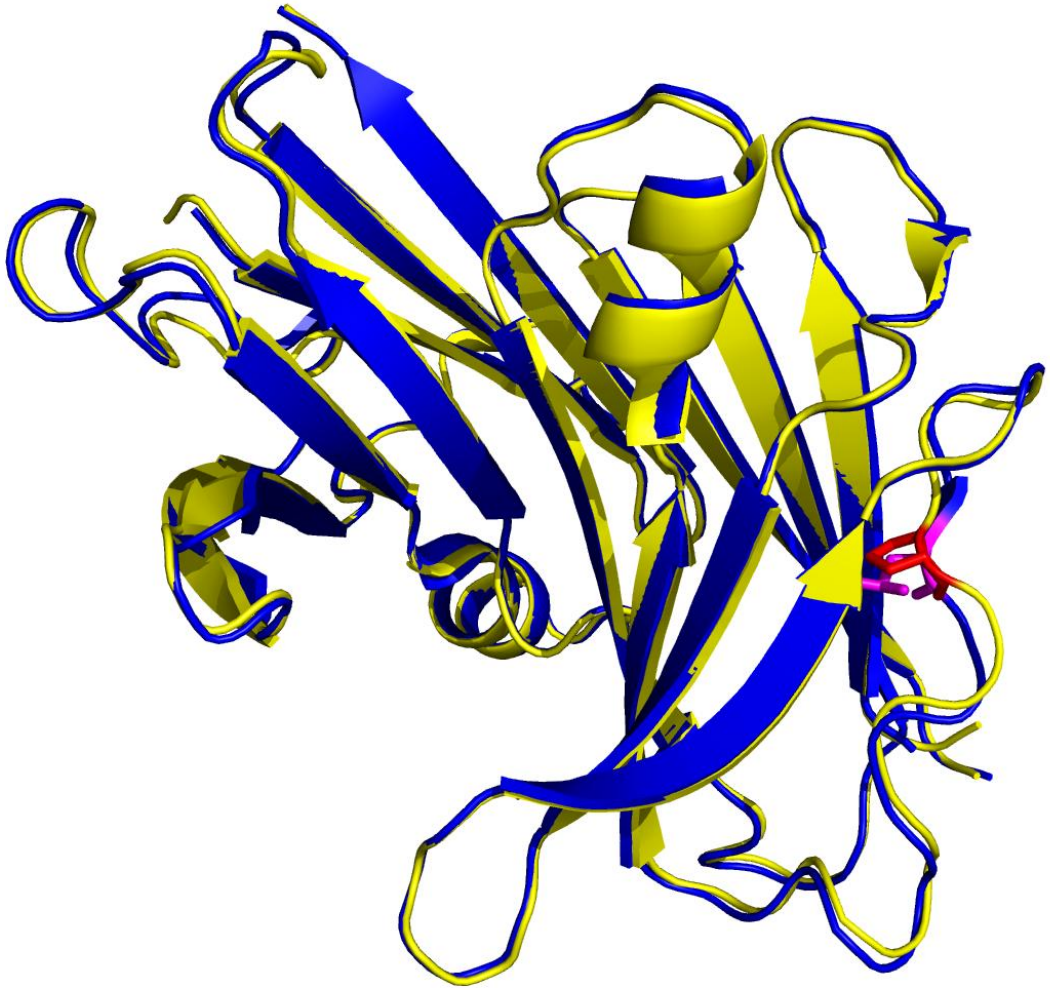
Transthyretin



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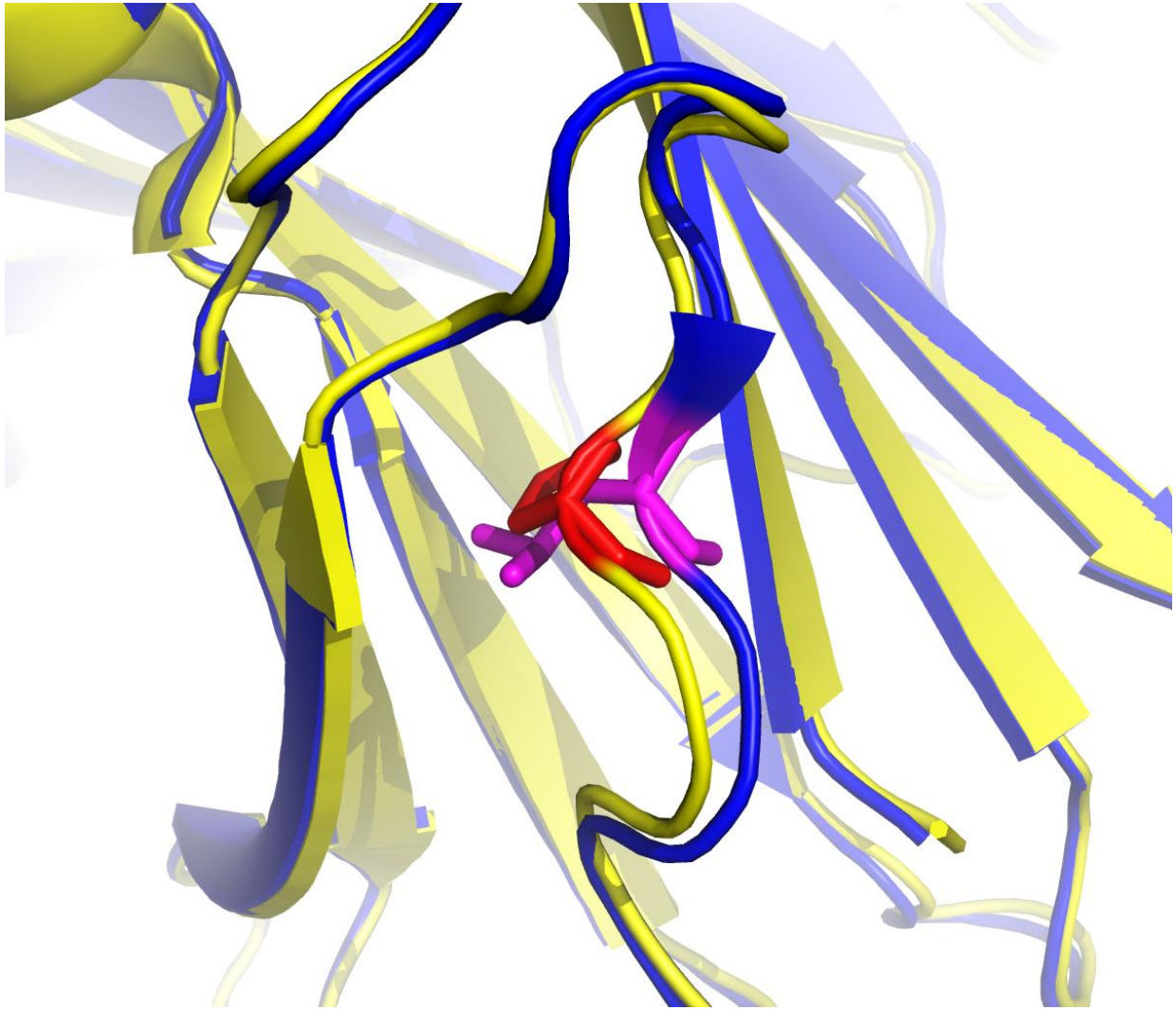

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Features

Sequence conservation

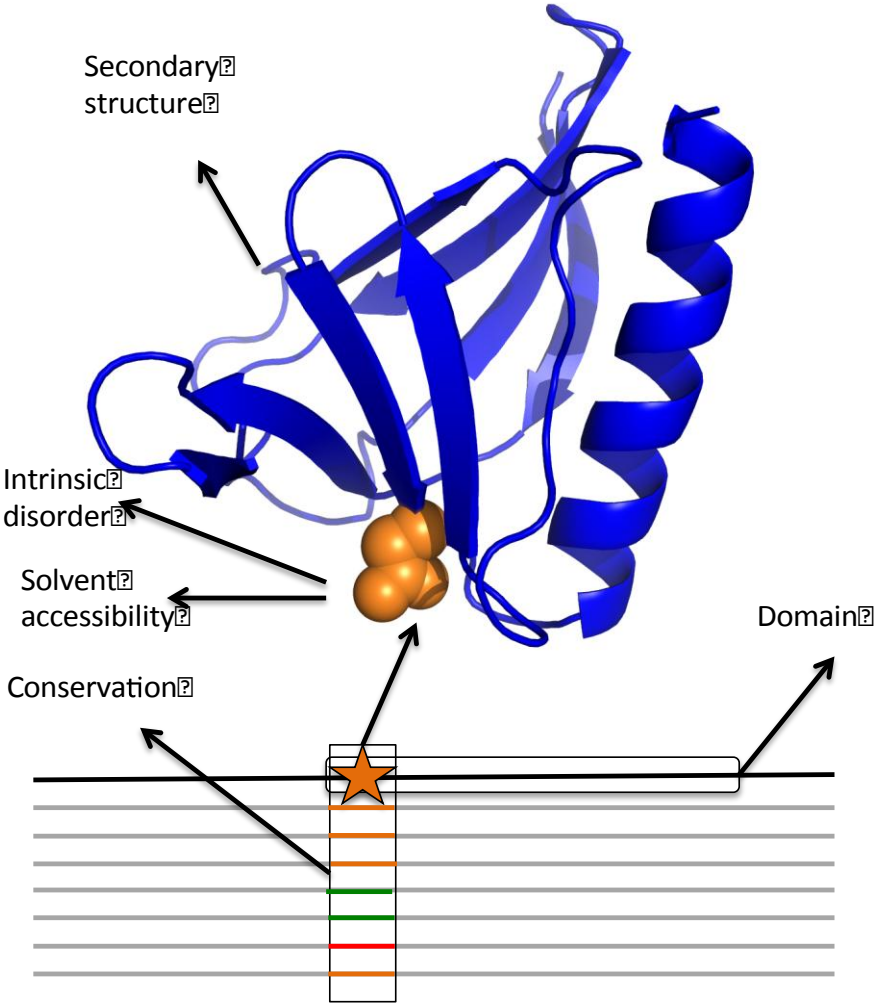
- Position-specific scoring matrix (PSI-BLAST)
- Pfam domain
- Jensen-Shannon divergence

Structural features

- From PDB or Phyre2 homology models where available.
- Secondary structure
- Solvent accessibility

Network features

- Protein-protein interaction (PPI)
- Domain-domain interaction (DDI)
- Domain bigram



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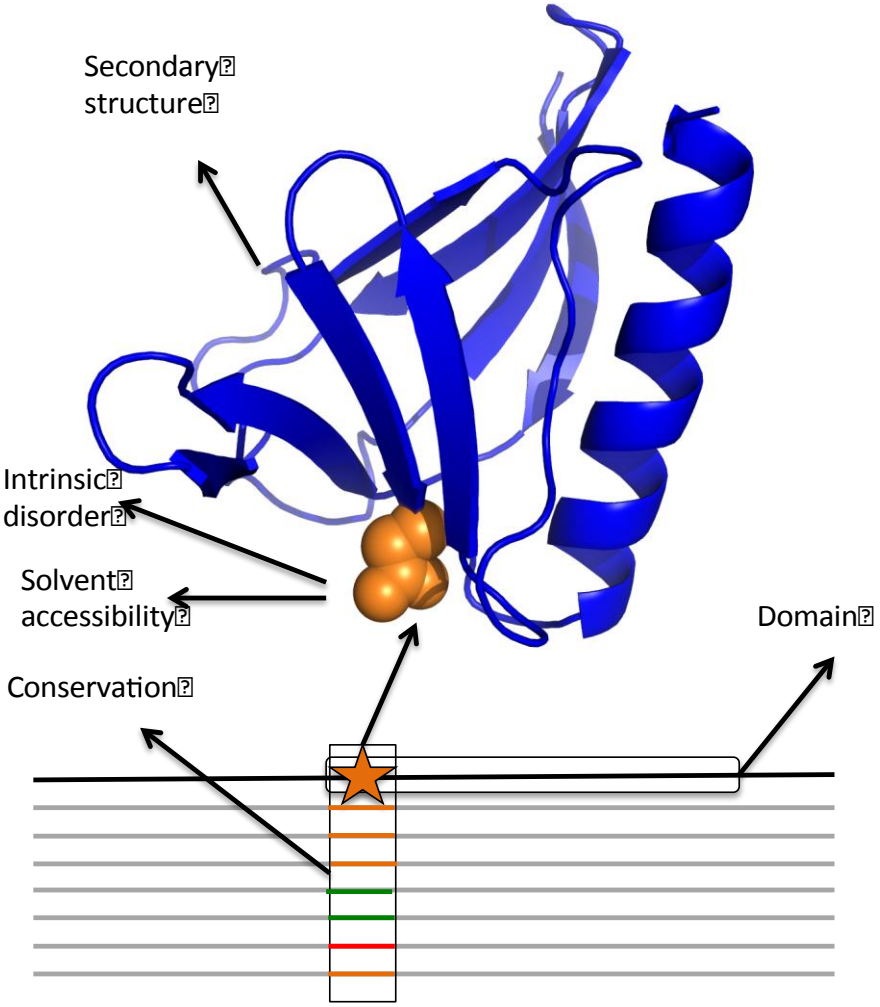

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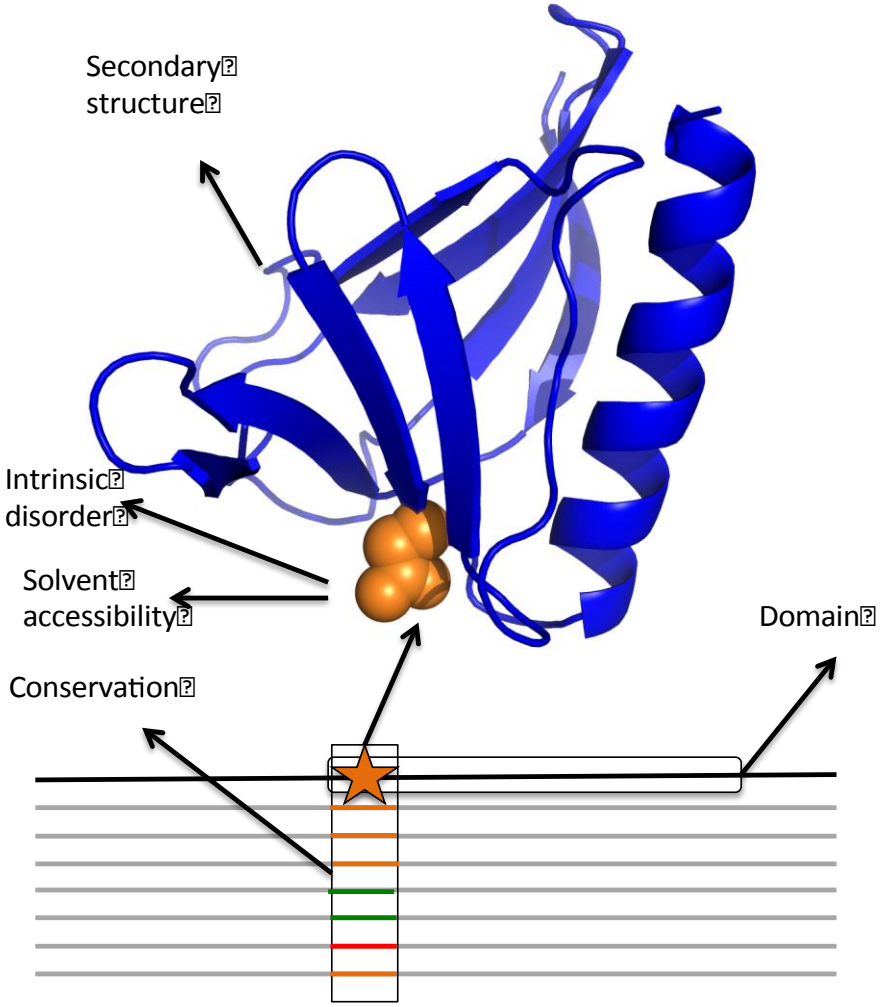

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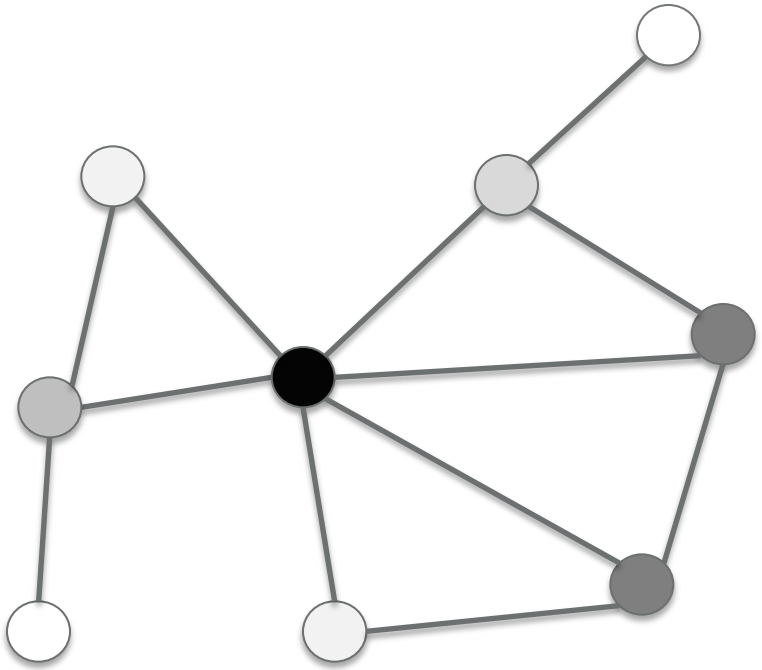
Network Features

Change in protein function is not the same as causing disease.

More ‘important’ proteins are more likely to be involved in disease.

Centrality of a protein within a protein-protein interaction network can be used to measure ‘importance’.



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VariBench

Neutral and Pathogenic datasets obtained from VariBench (Thusberg *et al.* 2011).

Neutral SAVs from dbSNP version 131, filtered by allele frequency (>0.01) and chromosome count (>49).

- SAVs present in OMIM removed.

Pathogenic SAVs from PhenCode (2009).

VariBench datasets were filtered to remove any SAVs present in training data.

13,236	Neutral
5,397	Pathogenic

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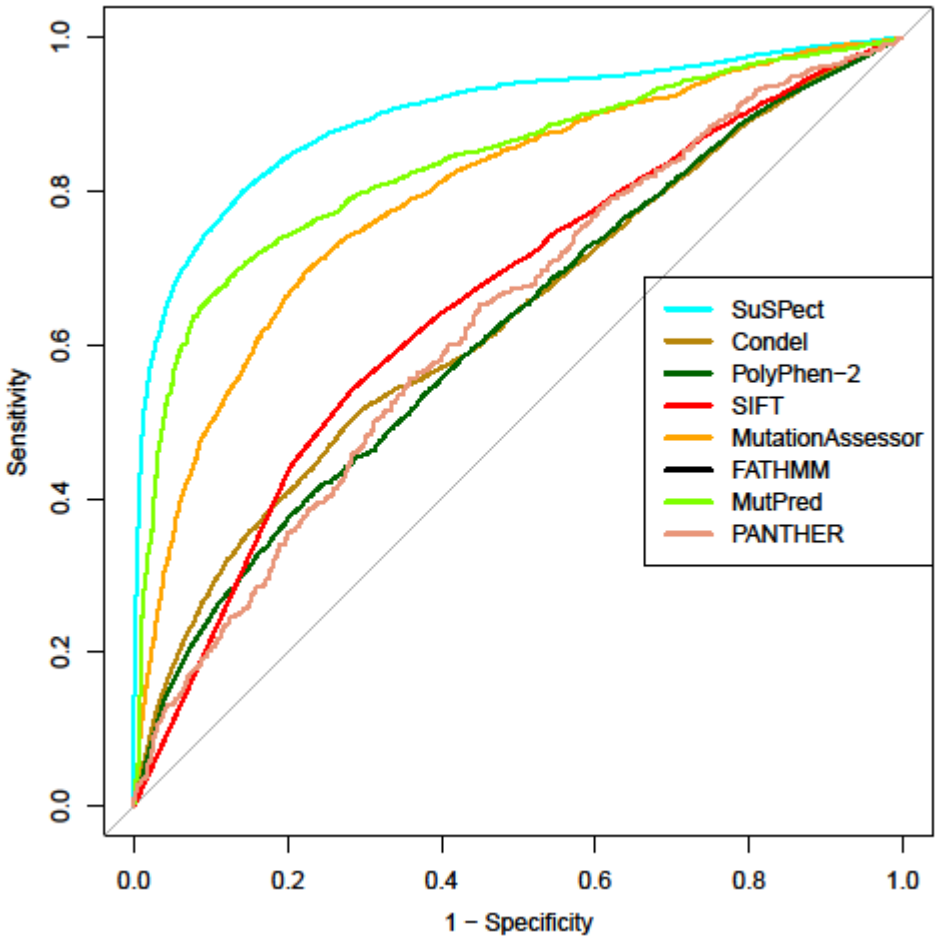
VariBench



Method	AUC	Balanced Accuracy
SuSPect	**0.90**	**0.82**
MutPred	0.84	0.75
MutationAssessor	0.79	0.70
SIFT	0.65	0.63
FATHMM	0.63	0.63
Condel	0.63	0.61
PANTHER	0.63	0.59
PolyPhen-2	0.62	0.58

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Results – Take home messages

Feature selection improves performance

- Top 9 features selected.
  - Predicted relative solvent accessibility;
  - WT and Variant scores in PSSM, and their difference;
  - Number of UniProt annotations;
  - Difference in Pfam scores;
  - PPI network degree centrality;
  - Jensen-Shannon divergence;
  - Sequence identity with best-matching sequence to lack WT amino acid.

Network features are important

- Removal of network features drops AUC from 0.88 to 0.78.
- Removal of PPI centrality from SuSPect-FS gives drop from 0.90 to 0.74.
- Network centrality helps show the difference between variants affecting protein function and leading to disease.

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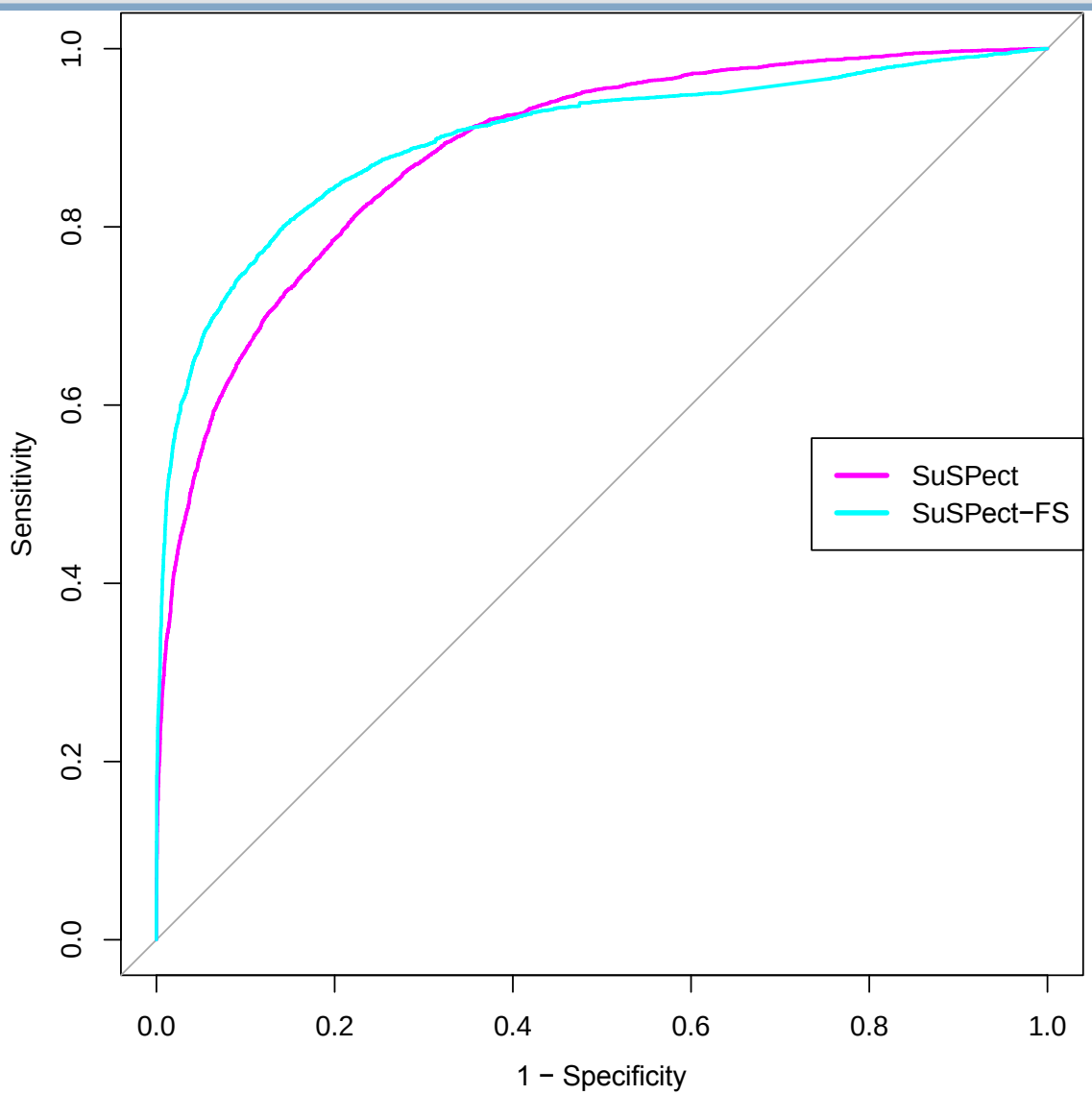
Results – Feature Selection



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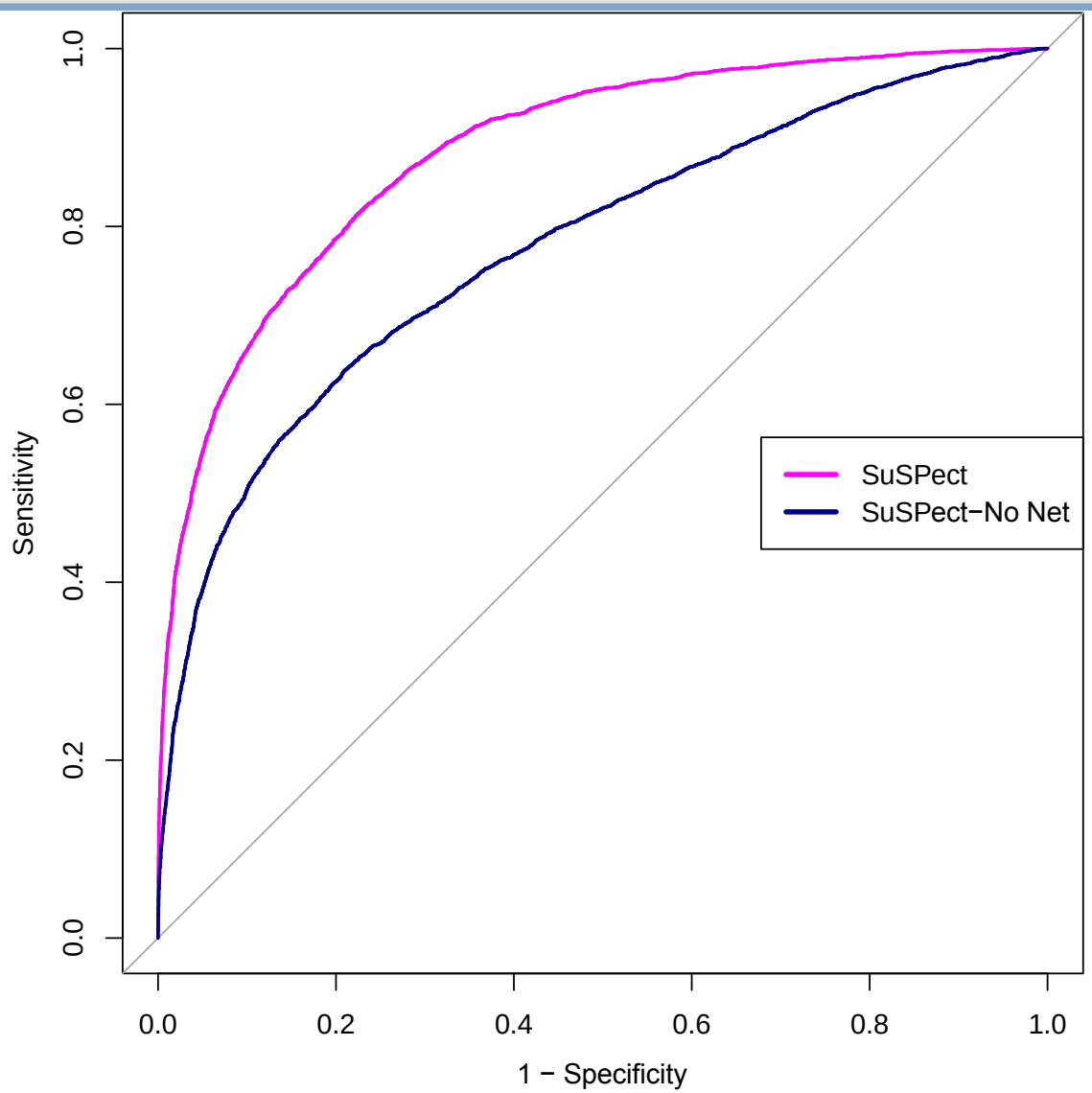
Results – No Network Features



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Results - Prokaryotic Mutations

HIV-1 protease – Loeb *et al.* (1989)

- 225 deleterious
- 111 neutral

LacI repressor – Suckow *et al.* (1996)

- 1,774 deleterious
- 2,267 neutral

T4 lysozyme – Rennel *et al.* (1991)

- 638 deleterious
- 1,377 neutral

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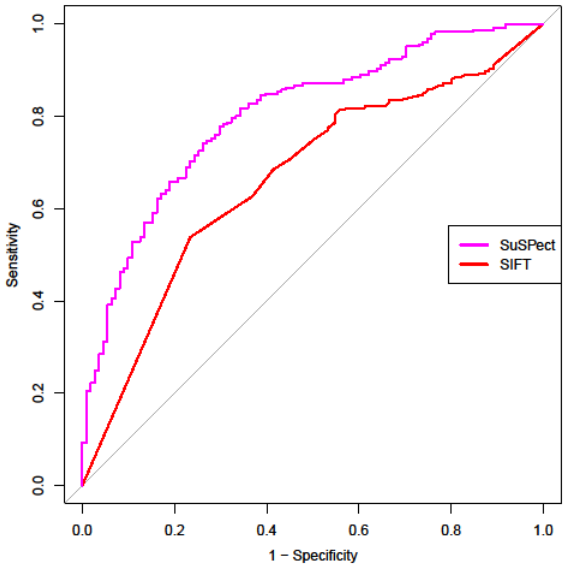
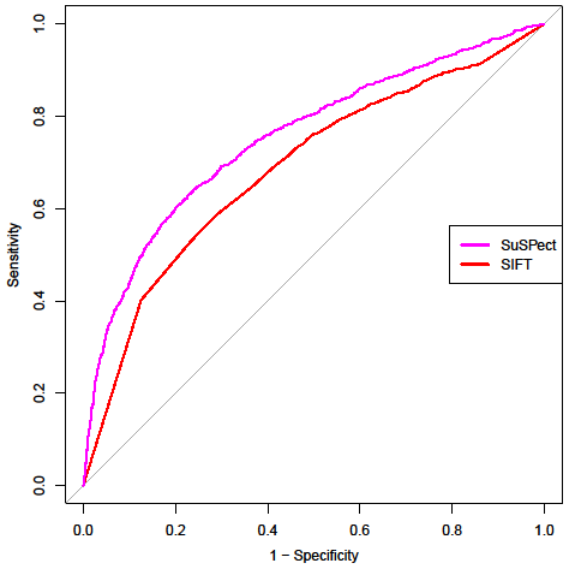
Results - Prokaryotic Mutations

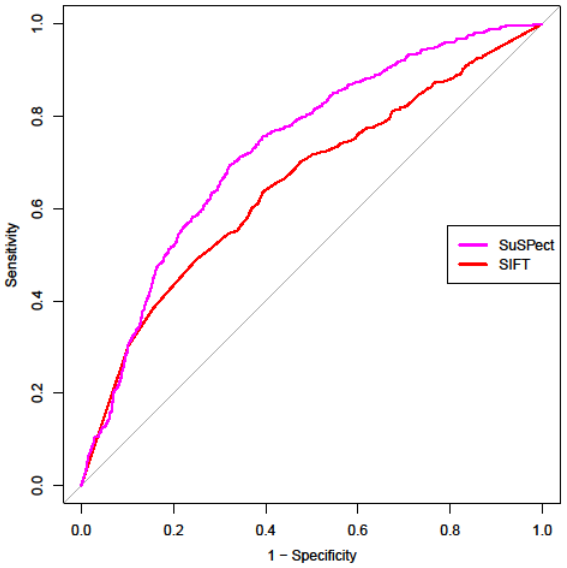
HIV-1 Protease



E. coli LacI repressor



T4 Lysozyme



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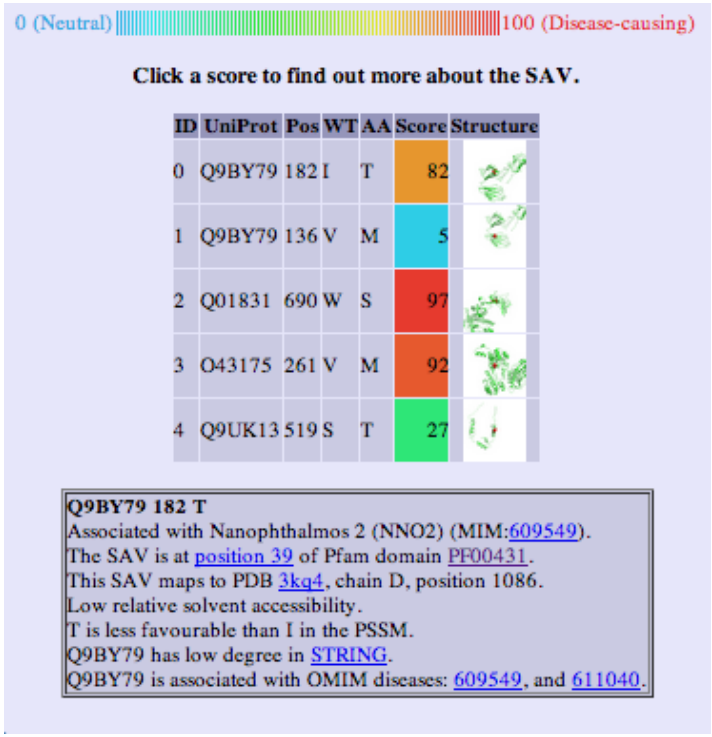
Web-Server & Download

Available at www.sbg.bio.ic.ac.uk/suspect

Upload list of SAVs or VCF file to obtain scores for human missense variants

- In addition to score, gives easily interpretable descriptions.
- Sequence conservation, structure, active site, and much more.
- Useful for interpretation of how variants can have their effects.

SuSPect Package – downloadable database of pre-calculated scores for all possible human missense variants.



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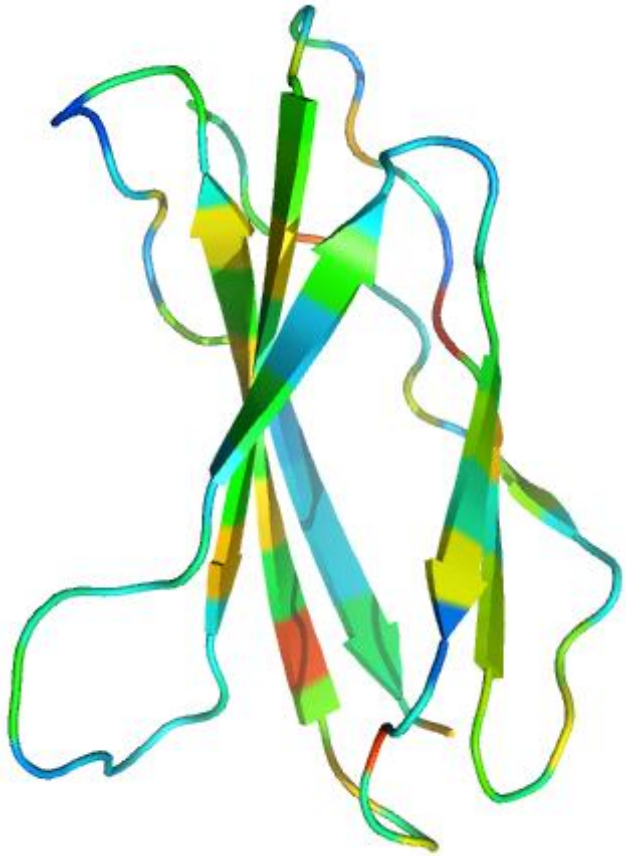
Web-Server & Download

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
11	D	17	34	4	14	24	20	26	32	22	32	35	13	18	21	18	16	20	22	44	28	D
12	V	40	63	53	45	63	61	62	32	52	39	48	52	47	49	40	42	41	26	65	63	V
13	T	62	82	51	57	79	43	76	73	61	67	66	41	66	57	54	37	4	68	80	72	T
14	D	24	56	5	17	47	31	29	46	30	41	45	27	18	33	37	18	25	42	39	38	D
15	T	52	64	27	45	60	53	43	53	42	62	66	31	54	48	41	26	4	57	75	52	T
16	T	45	75	52	45	65	49	57	65	52	65	66	51	54	49	54	18	29	48	72	58	T
17	A	31	73	79	53	39	54	73	20	69	19	26	64	56	67	66	56	54	22	79	53	A
18	L	29	42	30	20	29	39	24	30	27	22	29	23	36	24	19	18	14	25	40	29	L
19	I	61	80	88	83	56	82	87	4	84	30	65	86	79	84	70	77	63	25	87	76	I
20	T	33	65	49	29	66	36	34	48	38	47	43	33	53	34	30	21	28	43	59	52	T
21	W	97	98	97	97	87	96	97	95	98	92	97	98	95	98	97	97	96	92	27	90	W
22	F	12	22	6	6	4	13	12	14	9	12	16	8	12	8	12	5	5	13	31	14	F
23	K	18	52	34	25	43	31	25	28	4	28	41	23	9	19	20	24	27	23	46	41	K
24	P	56	86	74	60	81	72	74	78	69	53	76	78	25	67	76	53	67	61	88	84	P

Launch JSmol | Generate Image | Clear All



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Web-Server & Download

Human Proteins

• Scores have been pre-calculated for the Mar-2013 release of UniProt.

• If human variants or proteins are uploaded (either as sequence, structure or ID), these pre-calculated scores are used.

•These scores are calculated using SuSPect-FS, which is quicker and shows better performance than the full version.

Other Organisms

• For non-human proteins, scores are calculated on-the-fly, using a version of SuSPect including all features except the PPI network information and UniProt annotations.

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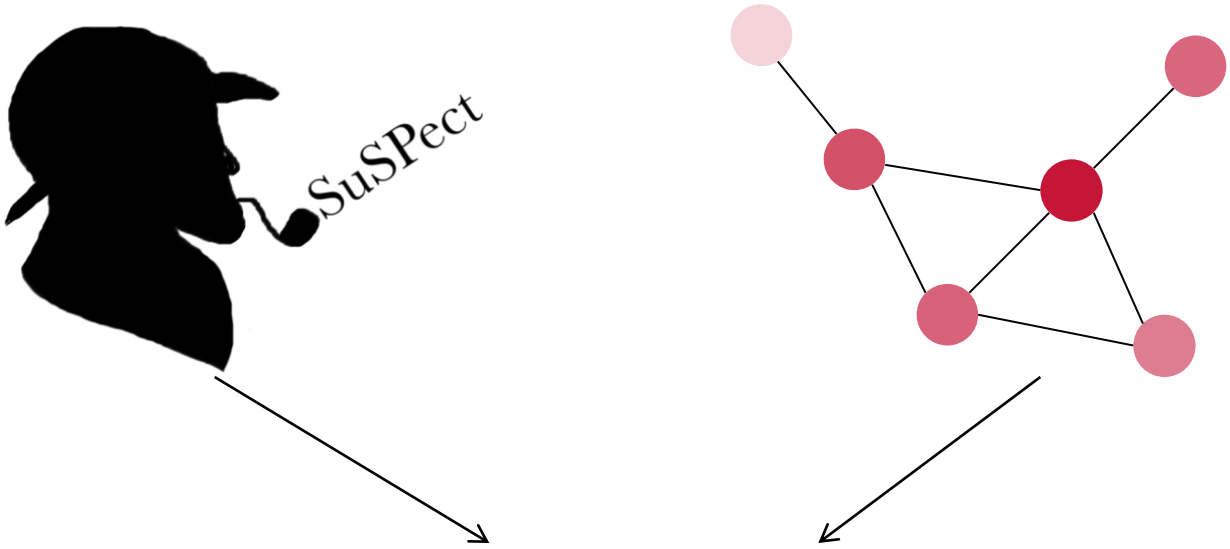
SuSPectP



Disease-specific scores associating SAVs with disease

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SuSPectP

Home | About | Contact

SuSPect-P is a new method giving disease-specific scores to rank variants for the specific disease you are interested in. Try SuSPect-P

Download scores

0 (Neutral) 100 (Disease-associated)

Click a score to find out more about the SAV.

ID	UniProt	Pos	WT	AA	Score
0	P04217	52	H	R	13
1	E5RG08	39	Q	P	22
1	Q9NQG7	44	Q	P	27

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SuSPectP

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SuSPect-P

Using SuSPect for **P**rioritisation

-SuSPect-P is an extension to SuSPect that ranks single amino acid variants according to how likely they are to be involved in a specific disease of interest.
- In simulations on whole exomes, SuSPect-P identifies the true causative variant around 50 times more often than using SuSPect alone.

Disease:

Filter by MAF (<0.02)

Run SuSPect-P

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SuSPectP

Protein	Variant	Score
P48382	R197Q	0.80
P13569	R75Q	0.41
-----**Confident** above here-----		
P51168	M51I	0.31
Q30201	C282Y	0.24
P15848	S384N	0.24
Q9ULD9	P1017S	0.20
O14836	V220A	0.18
P35670	V149M	0.18
P19438	R121Q	0.18
P30613	R510Q	0.15
P17813	E41K	0.14
P52333	P151R	0.13
-----**Possible** above here-----		
P06865	I436V	0.11
P11509	L160H	0.11
Q07021	T130M	0.10
Q9NQR1	L373P	0.10

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Acknowledgements & References

- Prof. Michael Sternberg
- Dr Ioannis Filippis
- Dr Lawrence Kelley
- Dr Suhail Islam

- Yates CM & Sternberg MJE (2013) Proteins and domains vary in their tolerance of non-synonymous single nucleotide polymorphisms. *J. Mol. Biol.*, **425**:1274-86
- Yates CM *et al.* (2014) SuSPect: enhanced prediction of single amino acid variant (SAV) phenotype using network features. *J. Mol. Biol.*, **426**:2692-701

MRC Medical Research Council

Cross-Validation

	Precision	Recall	MCC	Balanced Accuracy
SAV	0.81	0.75	0.66	0.83
Protein	0.80	0.72	0.64	0.81
Feature Selection	1.00	0.63	0.72	0.82

$$Precision = \frac{TP}{TP + FP} \qquad Recall = \frac{TP}{TP + FN} \qquad BA = \frac{0.5 \times TP}{TP + FN} + \frac{0.5 \times TN}{TN + FP}$$

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FP)(TP + FN)(TN + FP)(TN + FN)}}$$

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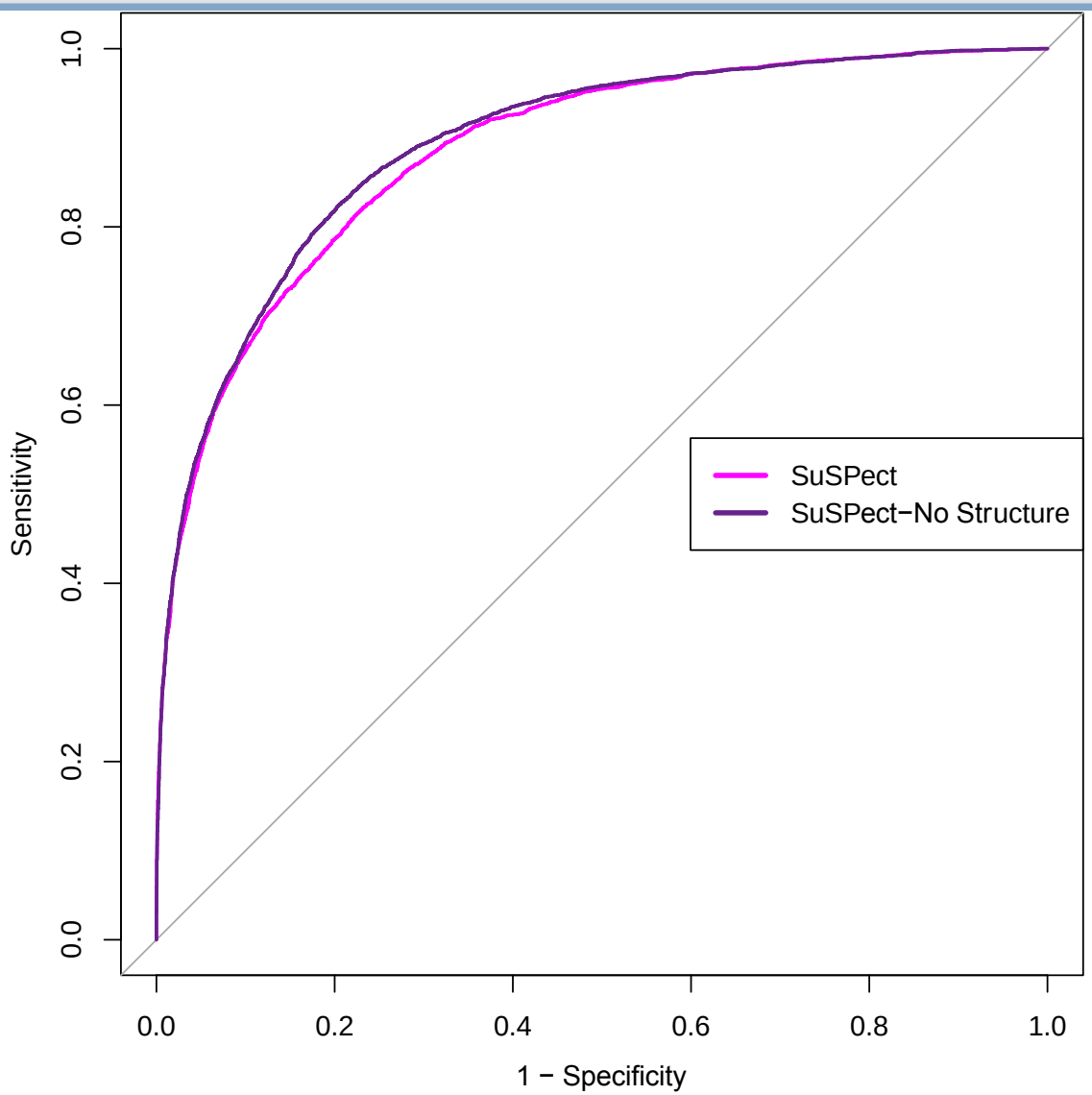
Results – No Structural Features



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Results – No Network Features