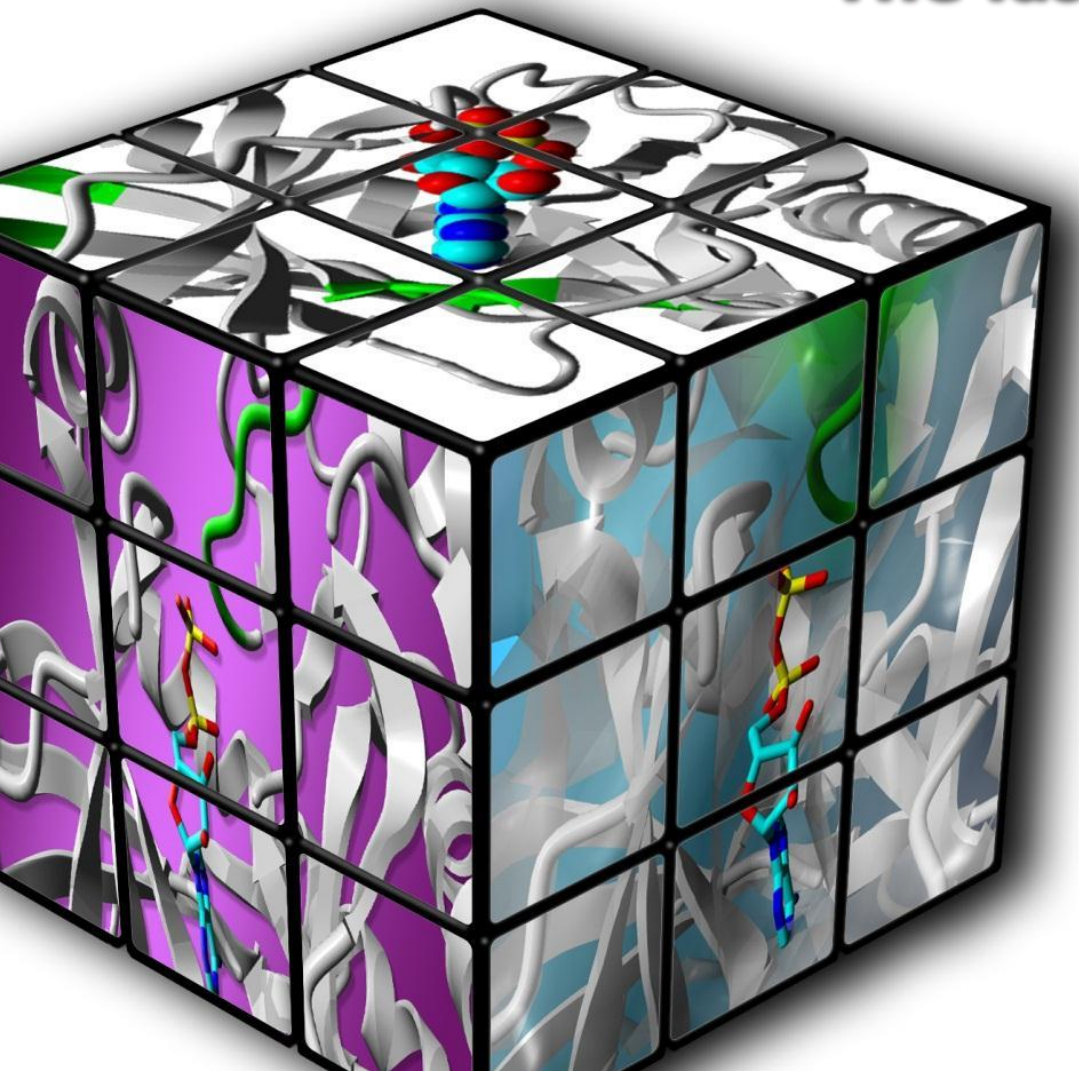
Variant Analysis with HOPE

The last piece of the puzzle



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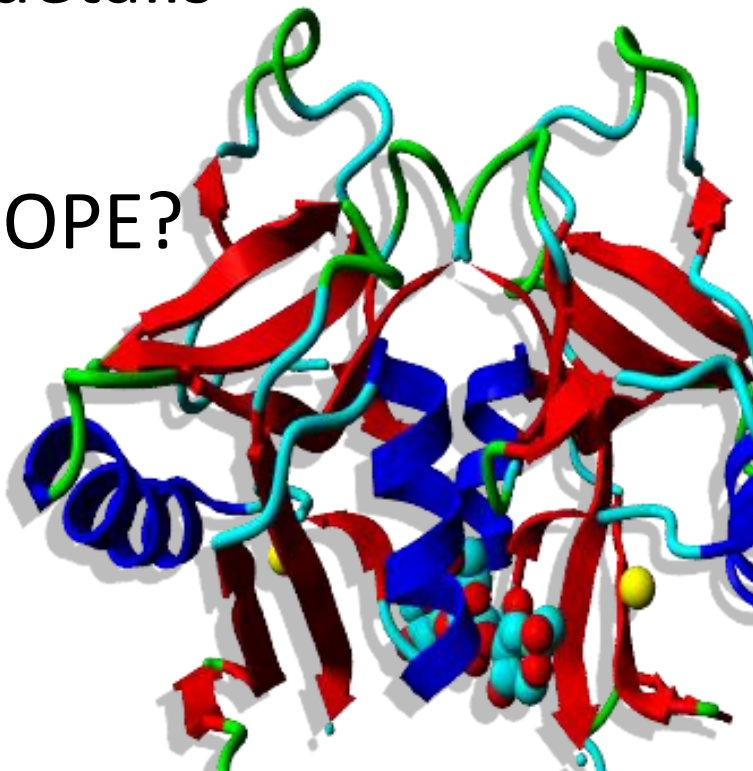
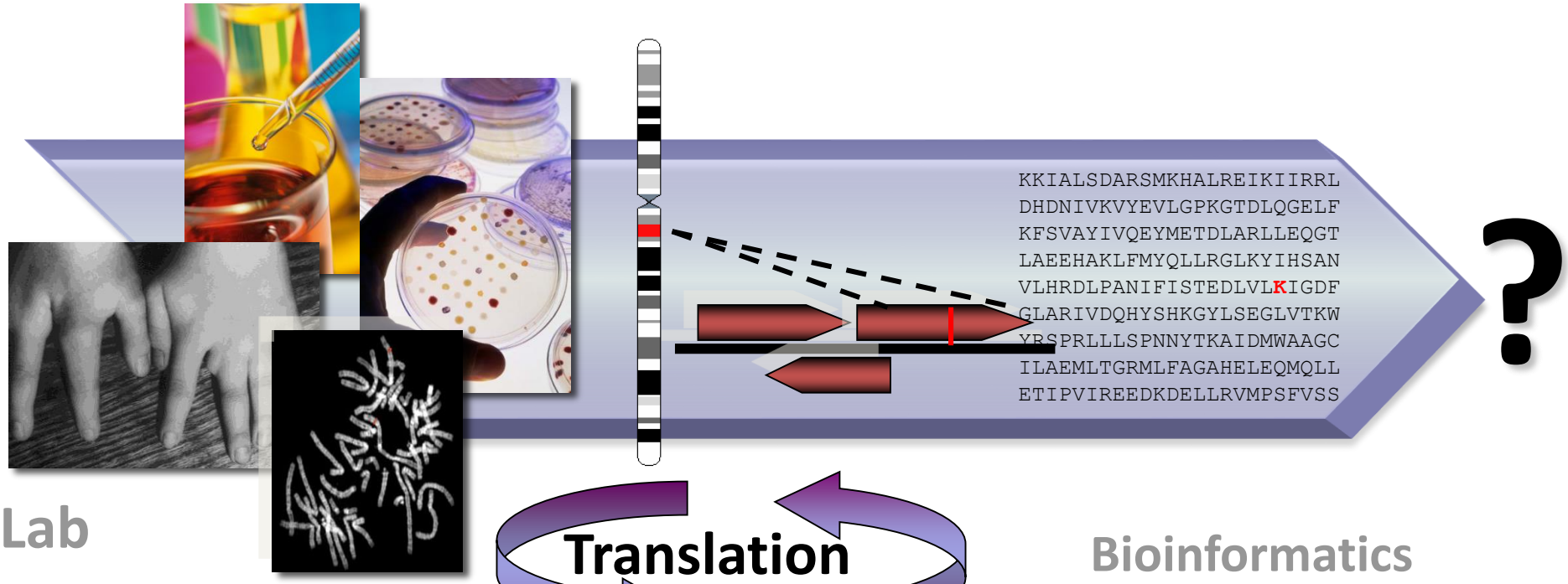
Outline

- Structural bioinformatics at Radboud UMC
- Project HOPE, the technical details
- Practical work, how to use HOPE?



Academic hospital RadboudUMC
Focus on curing patients + understanding disease

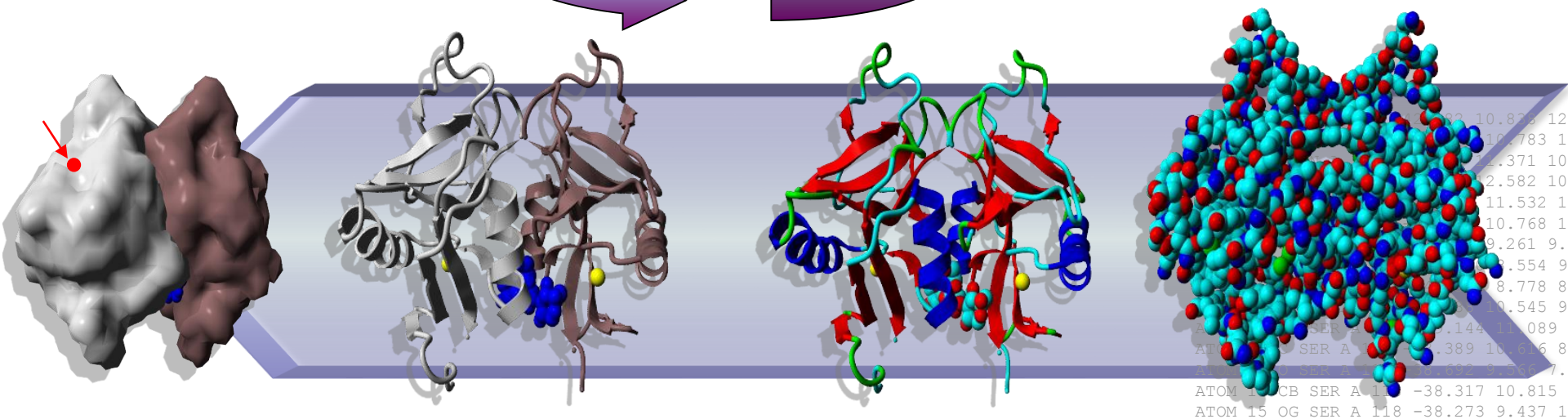
From lab to protein structures ….and back



Lab

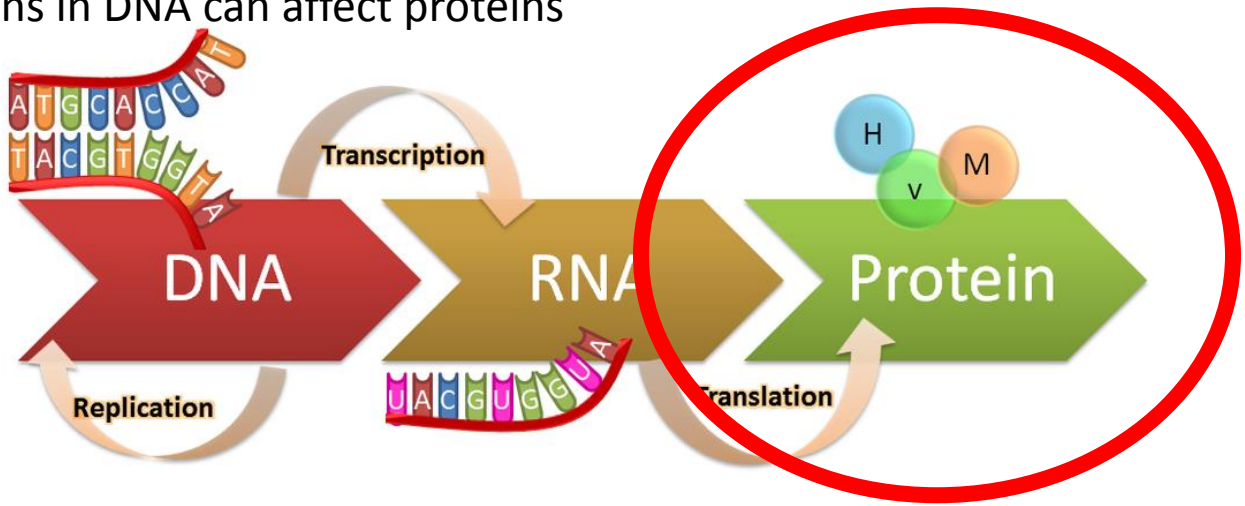
Translation

Bioinformatics

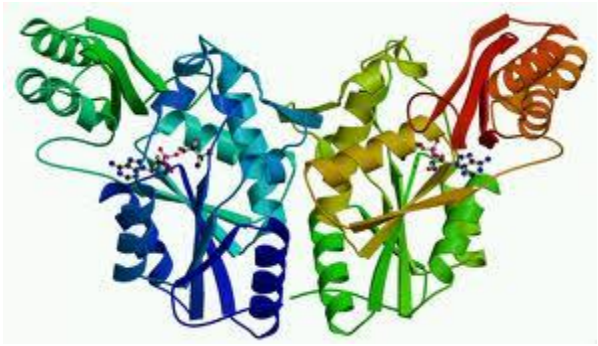


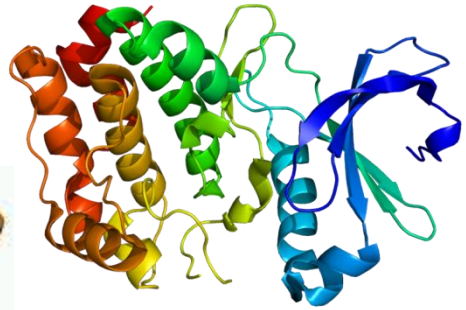
2 things to remember…

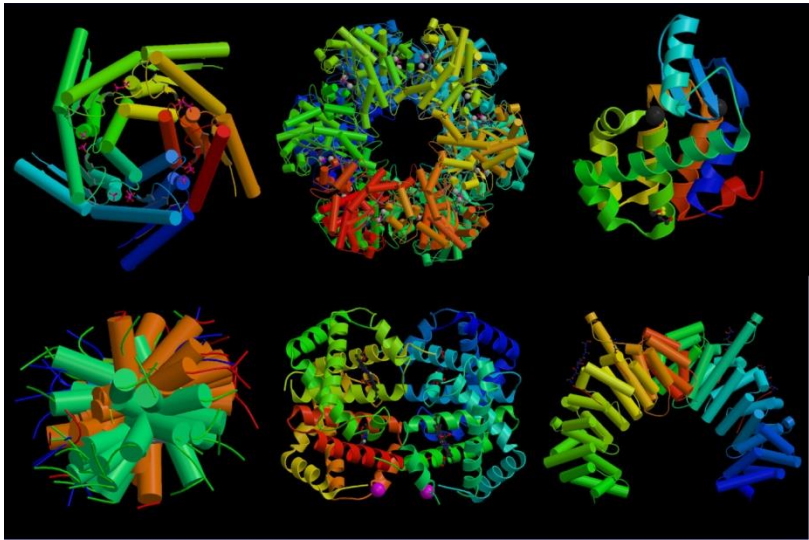
1: Mutations in DNA can affect proteins



2: Each protein has its own structure that defines its function



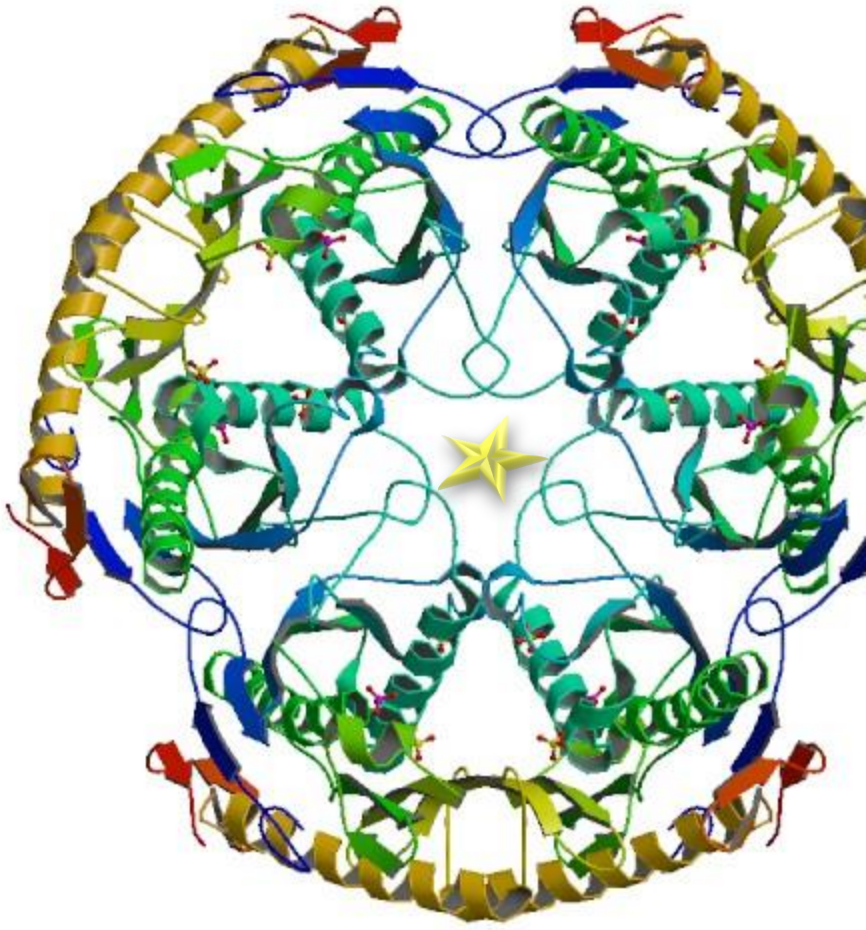




Hidden secrets in protein structures

For example:

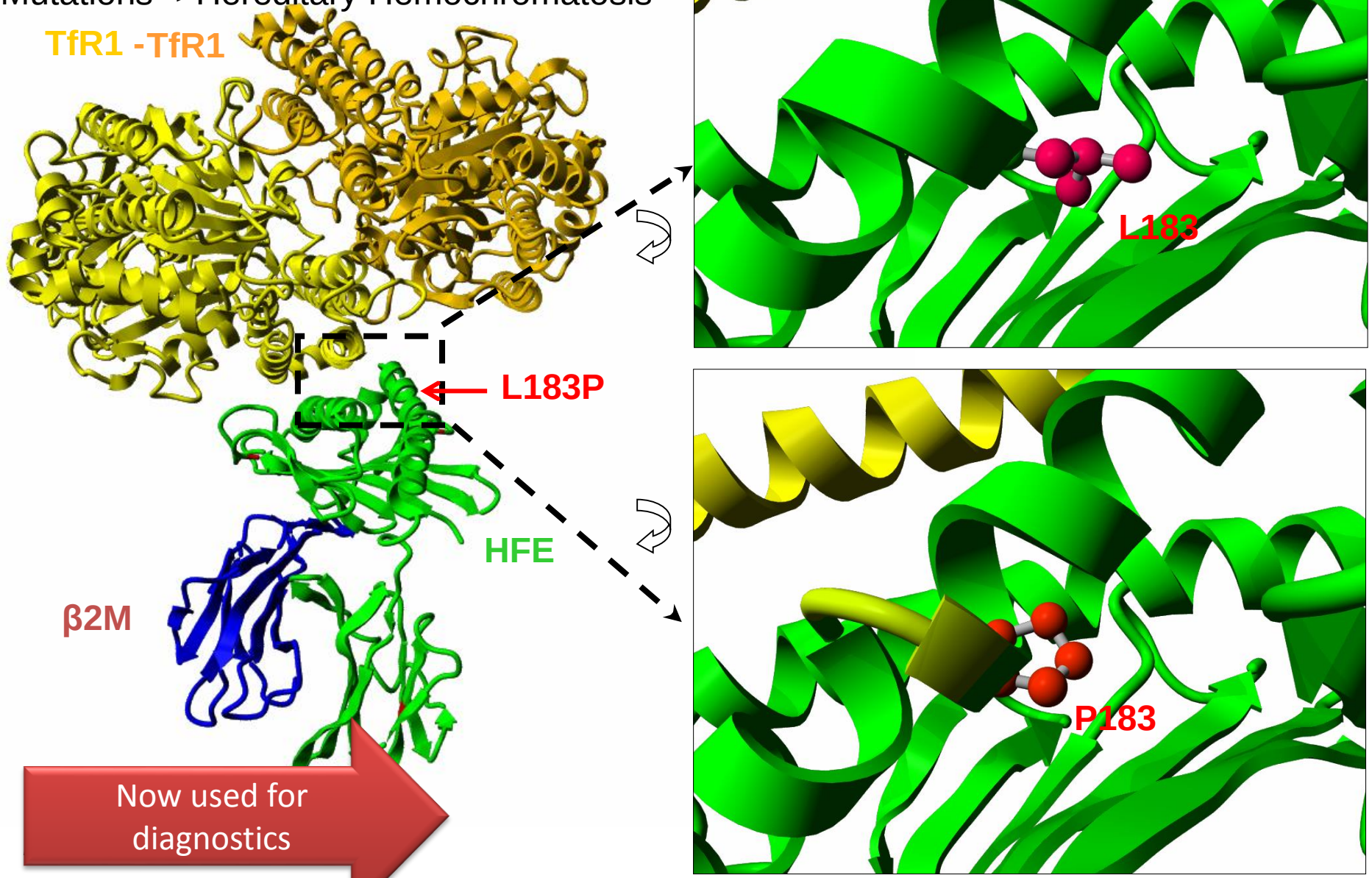
- Active sites
- Ligands / co-factors
- Surface interactions with other molecules
- Stabilizing interactions
- Transmembrane regions
- Flexible domains
- Etc…etc..etc…

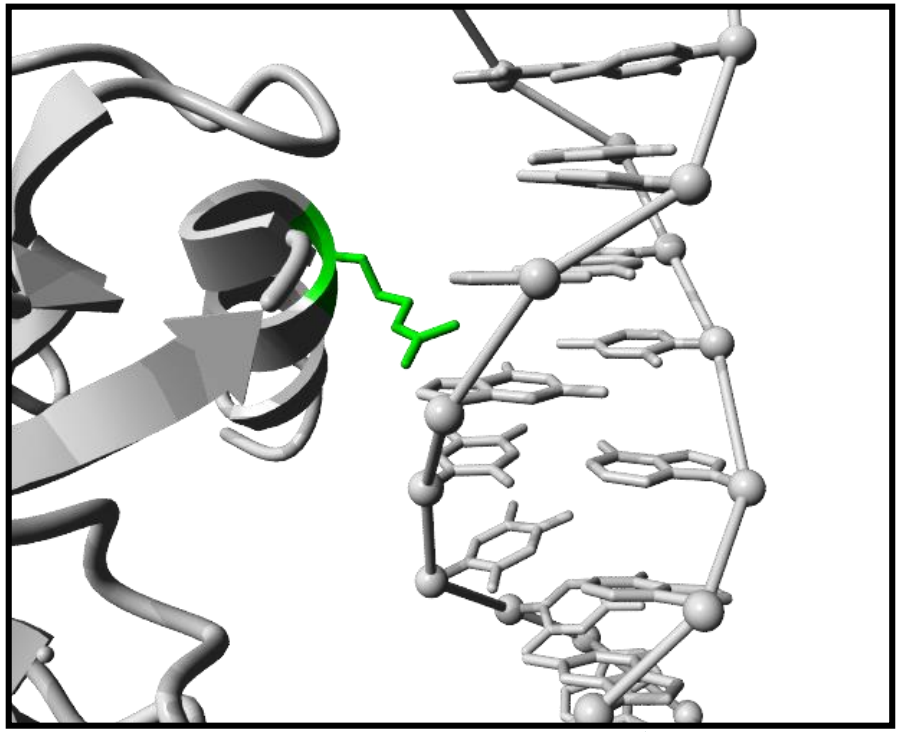


Example: HFE protein

Department of Clinical Chemistry

Mutations ->Hereditary Hemochromatosis



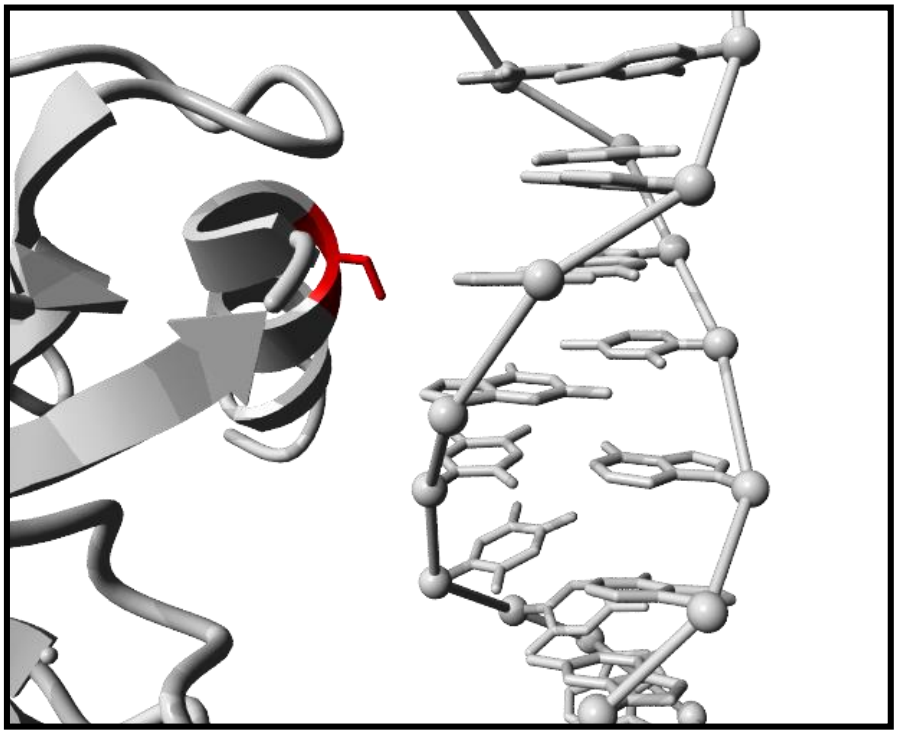


Arginine

- Loss of negative charge
- Loss of interaction with the DNA

Mutation R→S

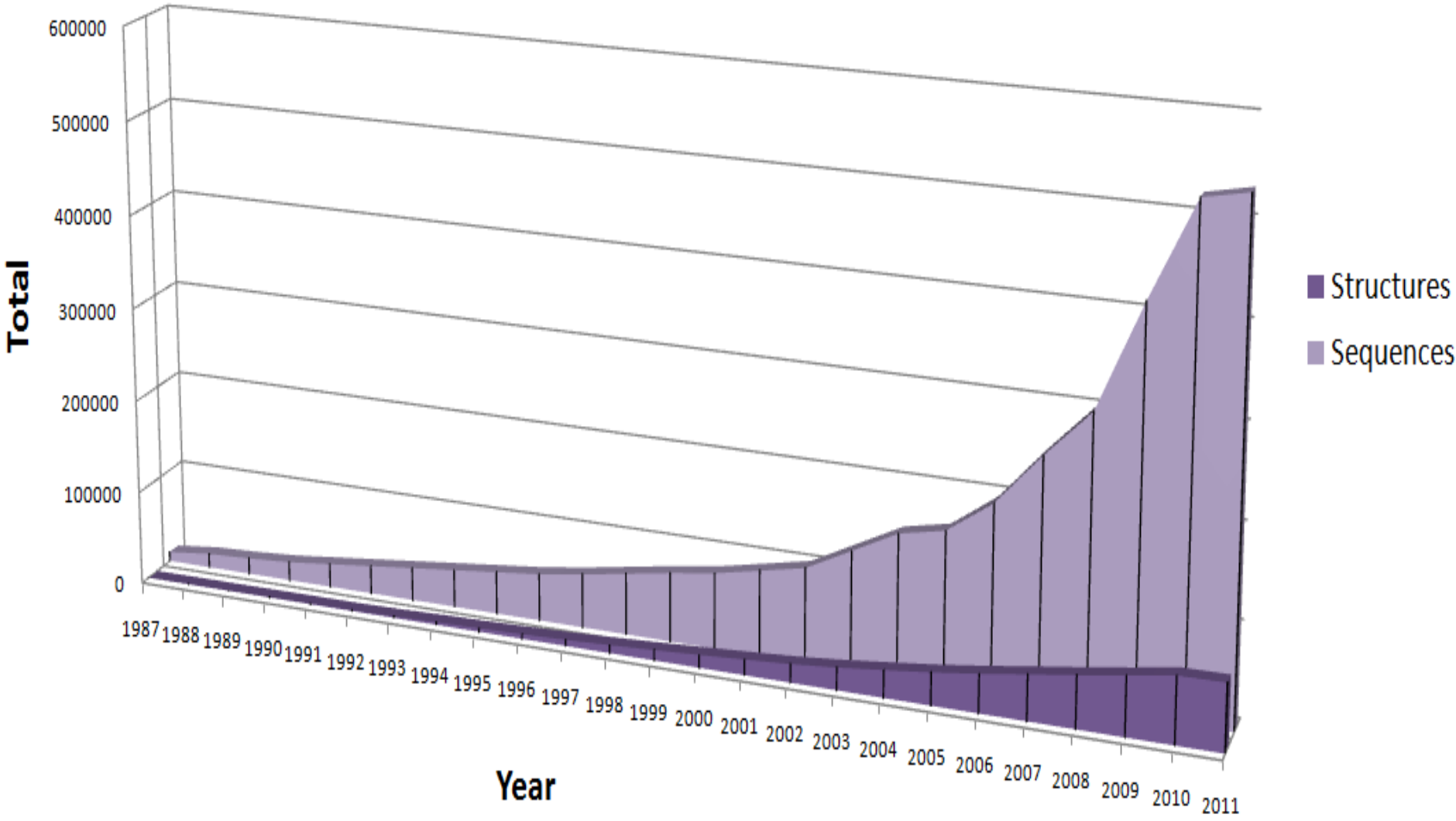
In p63



Serine

Protein structures contain a wealth of information...

...there is just 1 problem....



3D-structures for human proteins

20% Solved 3D-structures

RCSB PDB PROTEIN DATA BANK

30% Modelling templates

YASARA

50% No structural information

Predictions/Annotations

Homology modelling…..

MSASTQTNEFLSPEVFQHIWDFLEQPICSVQP
IDLNFVDEPSEDGATNKIEISMDCIRMQDSDL
SDMWPQYTNLGLLNSMDQQIQNGSSSTSPYNT
DHAQNSVTAPSPYAQPSSTFDALSPSPAIPSN
TDYPGPHSFDVSFQQSSTAKSATWTYSTELKK
LYCQIAKTCPIQIKVMTPPPQGAVIRAMPVYK
KAEHVTEVVKRCPNHELSRREFNEGQIAPPSHL
IRVEGNSHAQYVEDPITGRQSVLVPYEPPQVG
TEFTTVLYNFMCNSSCVGGMNRRPILIIVTLE
TRDGQVLGRRCFEARICACPGRDRKADEDSIR
KQQVSDSTKNGDGTKRPFRQNTHGIQMTSIKK
RRSPDDELLYLPVRGRETYEMLLKIKESLELM
QYLPQHTIETYRRVIDAVRFTLRQTISFPPRD
EWNDFNFDMDARRNKQQRIKEEGE

BLAST against the PDB

SPAIPSNTDYPGPHSFDVSFQQSSTAKSATW
SSSVPSQKTYQGSYGFRLGFLHSGTAKSVTC

GAVIRAMPVYKKAEHVTEVVKRCPN**H**ELSRE
GTRVRAMAIYKQSQHMTEVVRRCPHHERCSD

Known 3D structure

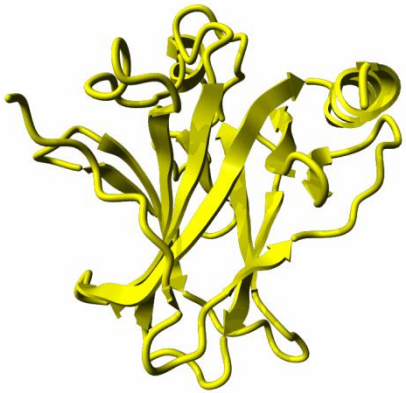
MSASTQTNEFLSPEVFQHIWDFLEQPICSVQP
IDLNFVDEPSEDGATNKIEISMDCIRMQDSDL
SDMWPQYTNLGLLNSMDQQIQNGSSSTSPYNT
DHAQNSVTAPSPYAQPSSTFDALSPSPAIPSN
TDYPGPHSFDVSFQQSSTAKSATWTYSTELKK
LYCQIAKTCPIQIKVMTPPPQGAVIRAMPVYK
KAEHVTEVVKRCPNHELSRREFNEGQIAPPSHL
IRVEGNSHAQYVEDPITGRQSVLVPYEPPQVG
TEFTTVLYNFMCNSSCVGGMNRRPILIIVTLE
TRDGQVLGRRCFEARICACPGRDRKADEDSIR
KQQVSDSTKNGDGTKRPFRQNTHGIQMTSIKK
RRSPDDELLYLPVRGRETYEMLLKIKESLELM
QYLPQHTIETYRRVIDAVRFTLRQTISFPPRD
EWNDFNFDMDARRNKQQRIKEEGE

BLAST against the PDB

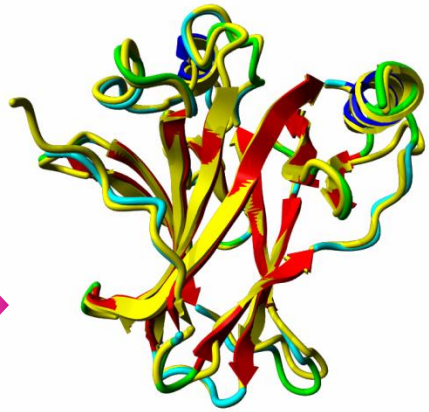
SPAIPSNTDYPGPHSFDVSFQQSSTAKSATW
--------EFLKSSRLTVDS---VDAKATPF

GAVIRAMPVYKKAEHVTEVVKRCPN**H**ELSRE
ALKMRAMP-----EFLCMNWLNSDDMELS--

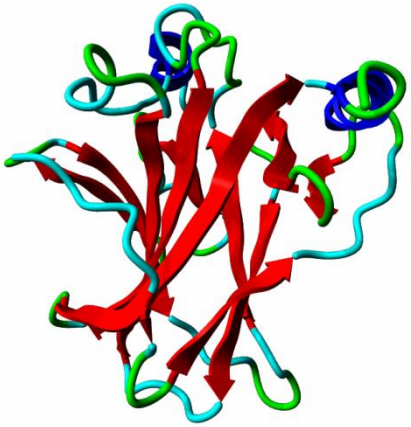
Unknown 3D structure



Homologous 3D structure

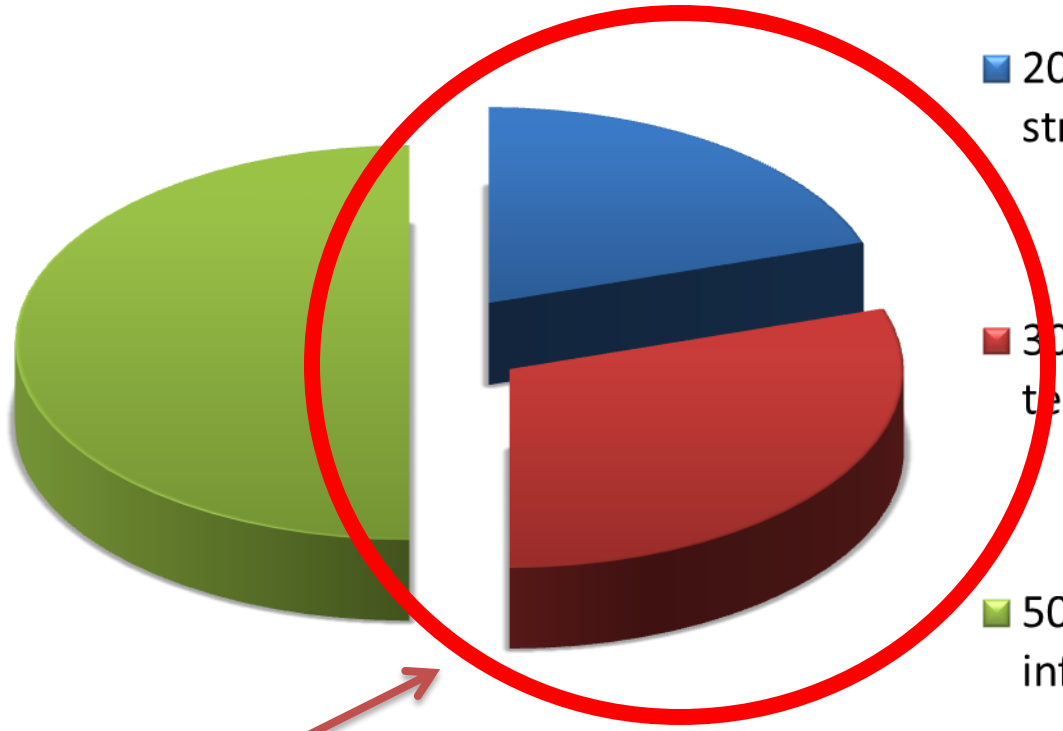


Use the homologous structure to predict the unknown 3D structure



Homology model

3D-structures for human proteins



20% Solved 3D-structures

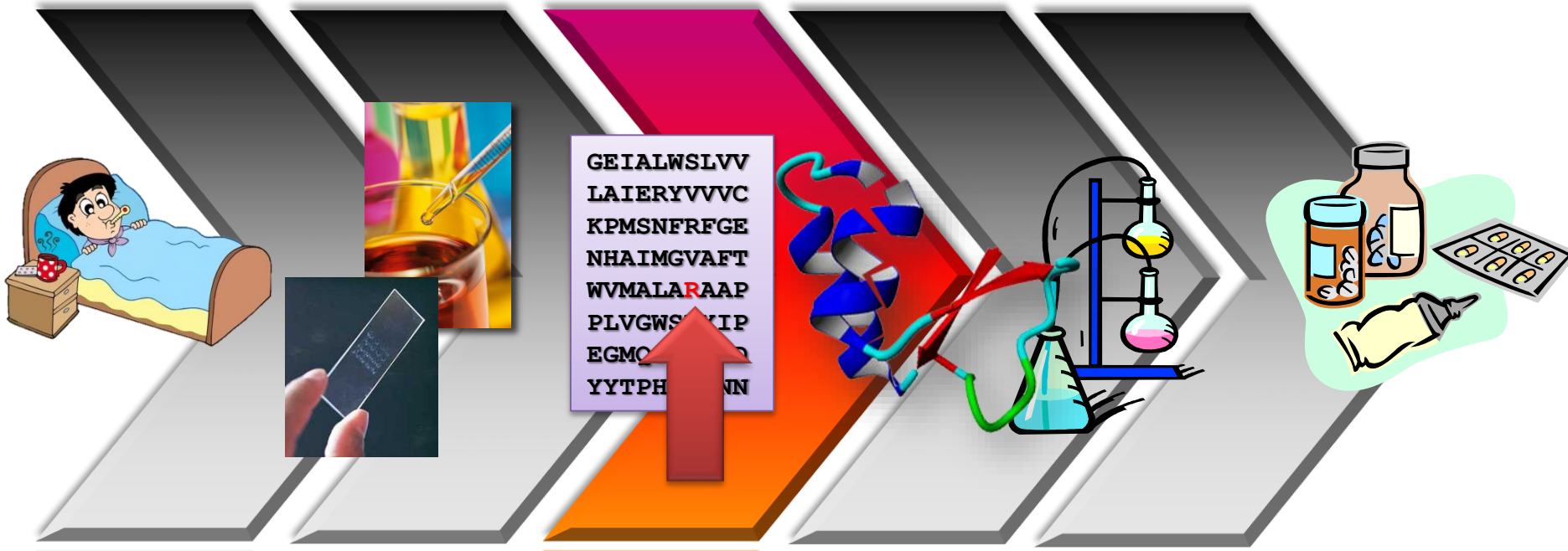
30% Modelling templates

50% No structural information

This is what HOPE will use…

Predictions/Annotations

The Molecular Puzzle: from patient to medicine



Patient in hospital

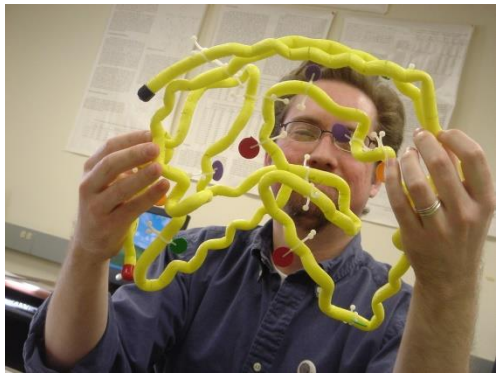
Experiments

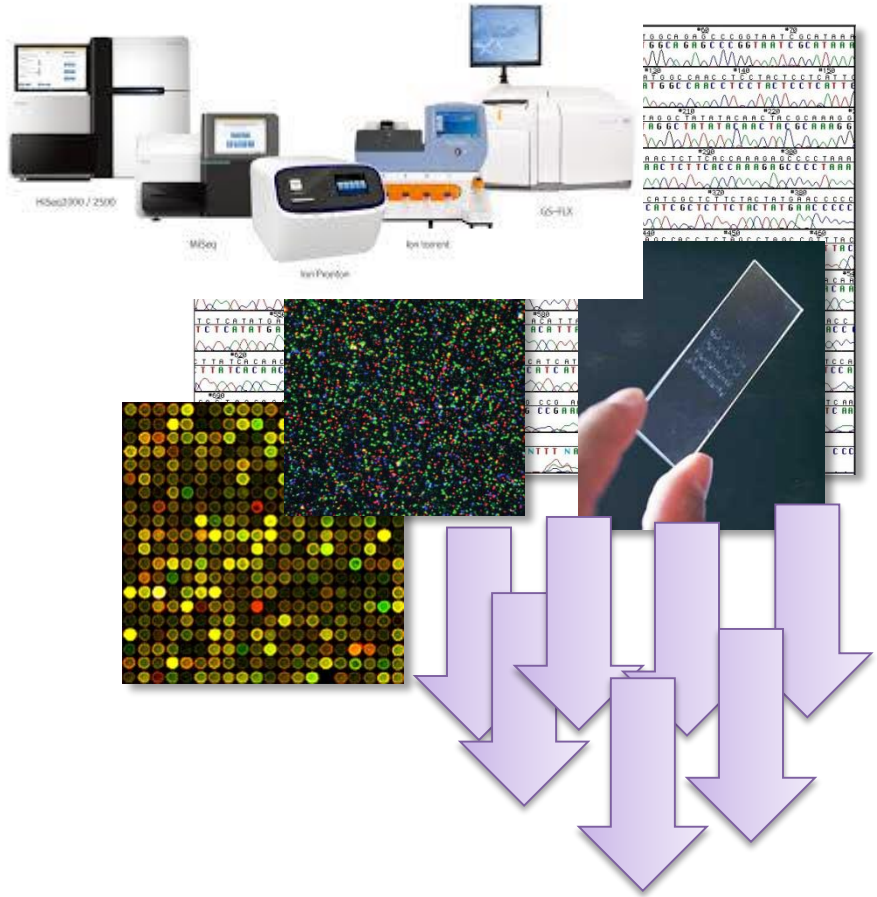
Bioinformatics

New experiments

Drug development

Yesteryear vs Nowadays



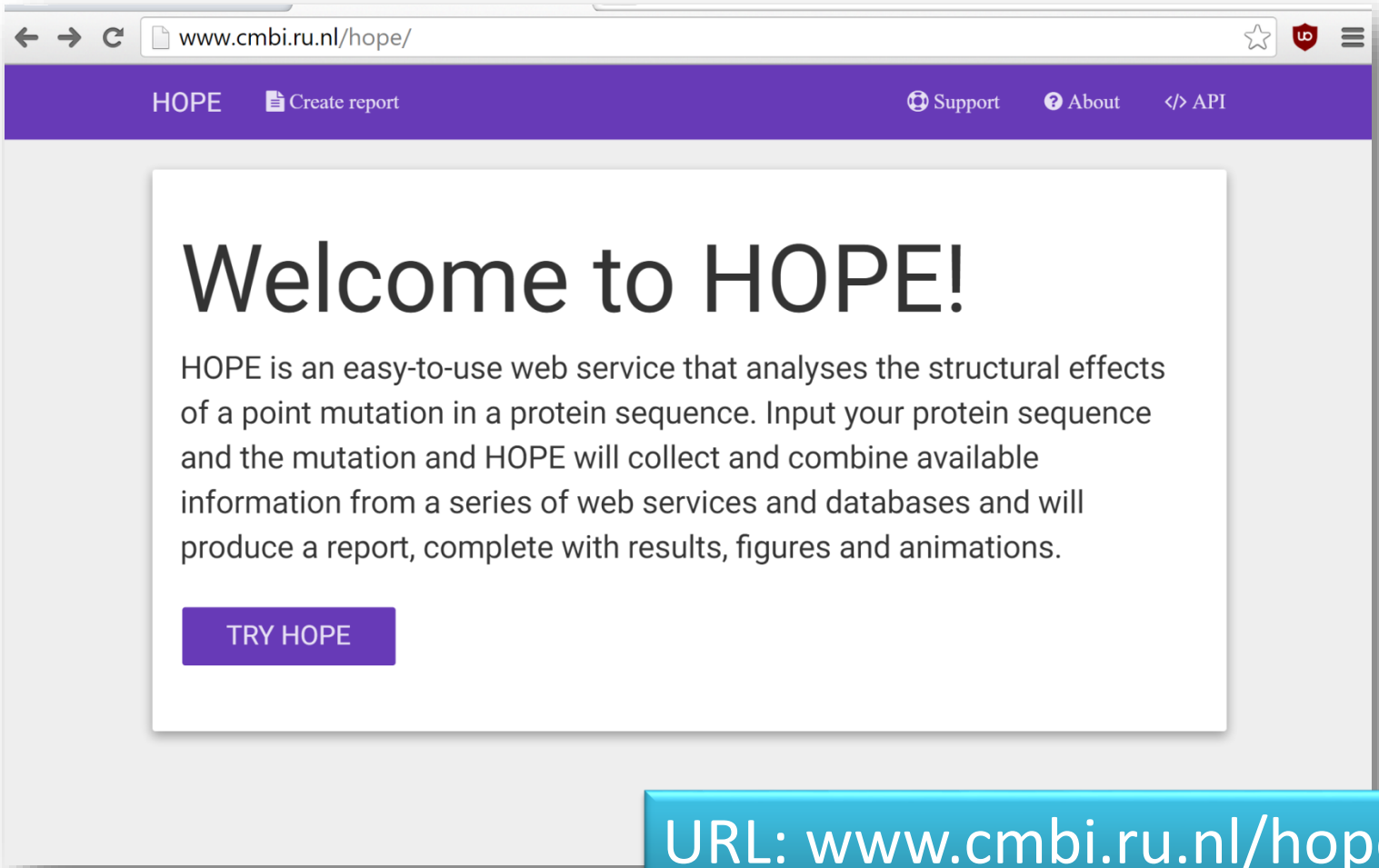


HOPE

So, we developed HOPE….

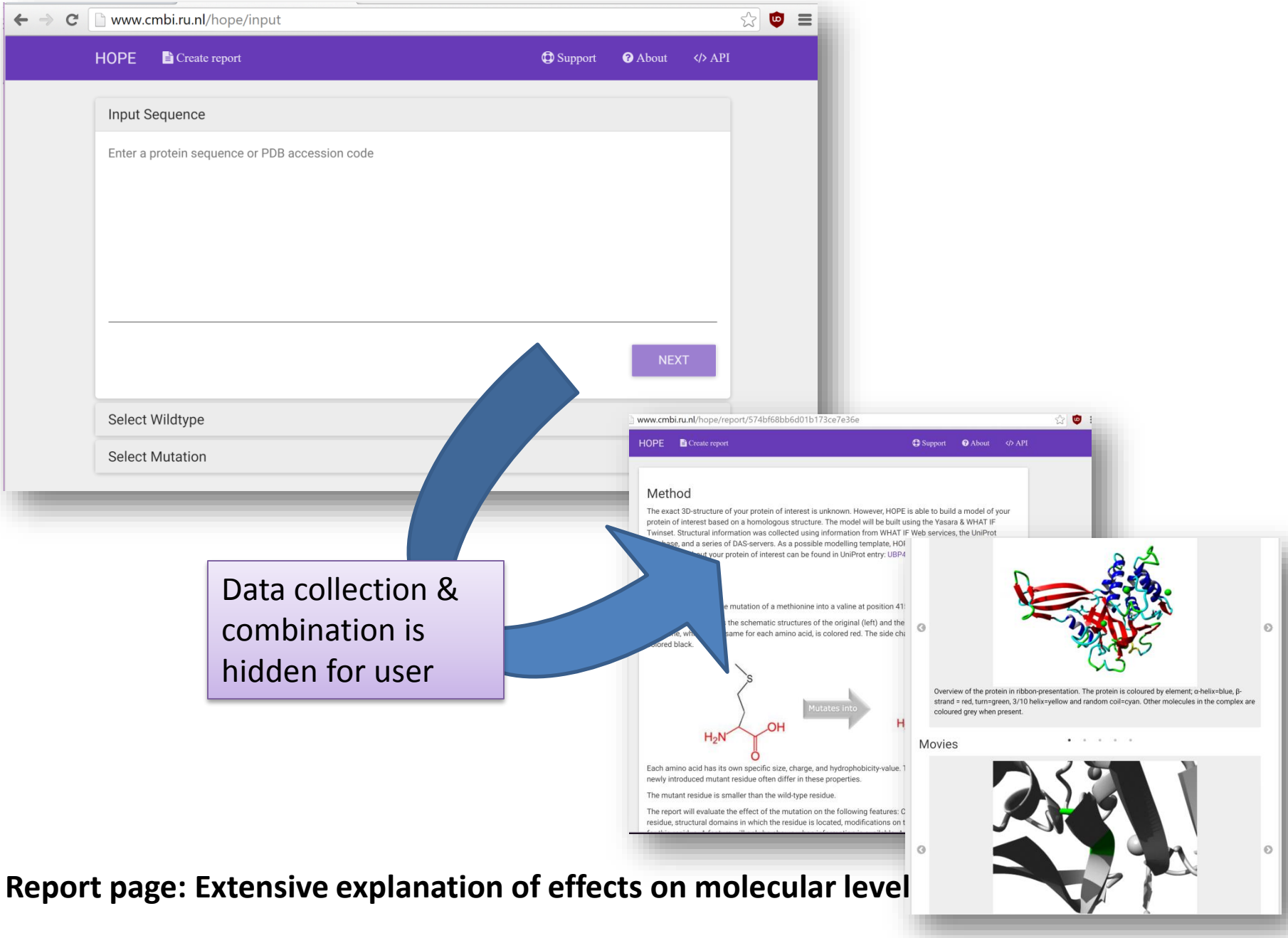
HOPE, an automatic web server that:

Is easy to use for (bio)medical scientists & Provides structural explanation of point mutations

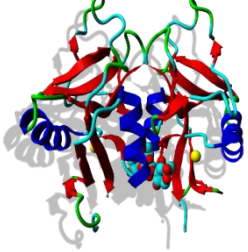


URL: www.cmbi.ru.nl/hope

Input page: Sequence + Mutation



Report page: Extensive explanation of effects on molecular level



Data collection

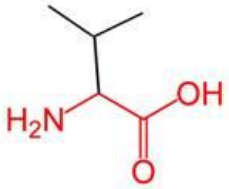
KMAD

HSSP

InterPro
Protein sequence analysis & classification

HOPE

UniProt

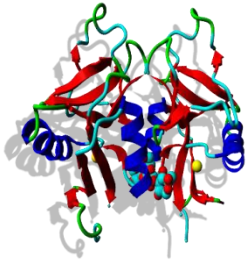


Data storage (in database) and combination/prioritization

Information sources for HOPE:

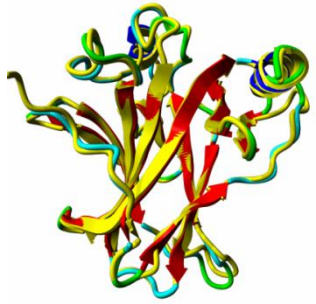
3D Structures

RCSB PDB PROTEIN DATA BANK



3D Structure Homolog

RCSB PDB PROTEIN DA YASARA



UniProt

Annotated SwissProt/ UniProt features

HSSP

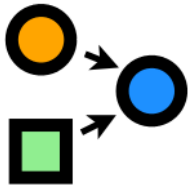
Conservation scores from multiple sequence alignments

InterPro Protein sequence analysis & classification

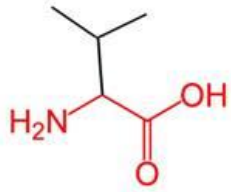
Domain GO-terms

KMAD

Disorder prediction & motifs

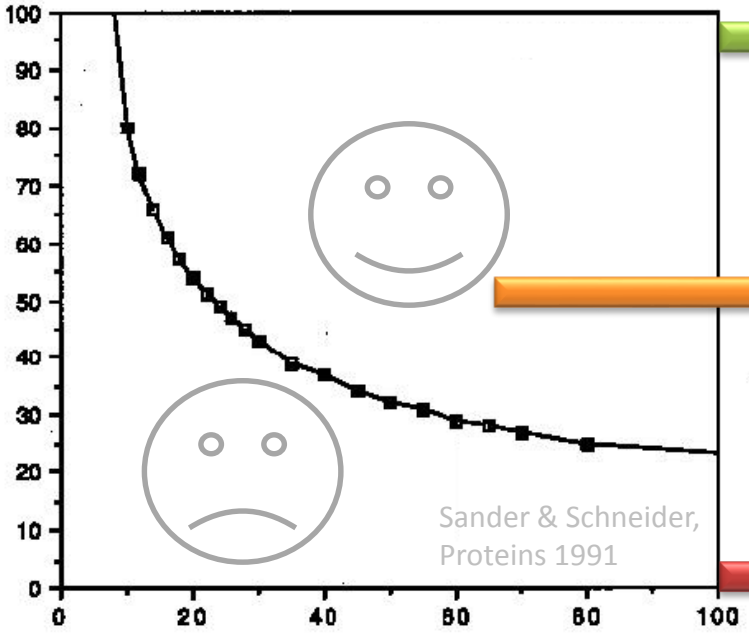


Sequence-based predictions



Amino Acid properties

Data Collection: Structural information



100% BLAST hit, PDB-file = structure
(Decision tree defines best structure based on NMR vs X-ray, resolution, length of the hit)

Enough sequence identity to build model
(Decision tree defines best template based on %identity, length)

No (homologous) structure, use sequence-based predictions

Structure analysis by

Web services.

Examples: metal-contacts, ligand-contacts, hydrogenbonds, ionic interactions, disulfide bridges, torsion angles, secondary structure, accessibility

Data colelction: SwissProt annotations

- Collect the sequence features in the SwissProt record
- Information is annotated, checked and sometimes cross linked

Features

metal	1232	1232	1	Zinc 4.
binding	1142	1142	1	S-adenosyl-L-methionine.
binding	1226	1226	1	S-adenosyl-L-methionine; via amide nitrogen.
mod_res	466	466	1	Phosphoserine.
var_seq	805	825	21	DAEGSTCLHLAAKKGHYEVVQ -> IQKTSKVYTESQETQR SQTIL (in isoform 2). /FTId=VSP_002222.
var_seq	826	1267	442	Missing (in isoform 2). /FTId=VSP_002223.
var_seq	1150	1153	4	DGEV -> ISSA (in isoform 3). /FTId=VSP_002224.

Sequence information

Length: **1267 aa**, molecular weight **138254 Da**, CRC64 checksum **FF221BEAE9022010**

```
MAADEGSAEK QAGEAHMAAD GETNGSCENS DASSHANAAK HTQDSARVNP QDGTNTLTRI
AENGVSERDS EAAKQNHVTA DDFVQTSVIG SNGYILNKPA LQAQPLRTTS TLASSLPGHA
AKTLPGGAGK GRTPSAFPQT PAAPPATLGE GSADTEDRKL PAPGADVKVH RARKTMPKSV
VGLHAASKDP REVREARDHK EPKEEINKNI SDFGRQQLLP PFPSLHQSLP QNQCYMATTK
SQTACLPFVL AAAVSRKKKR RMGTYSLVPK KKTKVLKQRT VIEMFKSITH STVGSKGEKD
LGASSLHVNG ESLEMDSDED DSEELEEDDG HGAEQAAAFP TEDSRTSKES MSEADRAQKM
DGESEEEQES VDTGEEEEGG DESDLSSESS IKKKFLKRKG KTDSPWIKPA RKRRRRSRKK
PSGALGSESY KSSAGSAEQT APGDSTGYME VSLDSLDLRV KGILSSQAEG LANGPDVLET
DGLQEVPLCS CRMETPKSRE ITTLANNQCM ATESVDHELG RCTNSVVKYE LMRPSNKAPL
LVLCEDHRGR MVKHQCCPGC GYFCTAGNFM ECQPESSISH RFHKDCASRV NNASYCPHCG
EESSKAKEVT IAKADTTSTV TPVPGQEKGS ALEGRADTTT GSAAGPPLSE DDKLQGAASH
VPEGFDPTGP AGLGRPTPGL SQGPGKETLE SALIALDSEK PKKLRFHPKQ LYFSARQGEL
```

Example Uniprot-features:
Regions, domains, motifs, mutagenesis sites, variants, active site, signal, glycosylation sites, etc

Data collection: Conservation scores

HSSP

- Calculated from the multiple sequence alignment in HSSP
- Profile gives % occurrence of residue type on that position
- Precalculated (for PDB-files/templates) or newly generated (sequence)

SEQUENCE PROFILE AND ENTROPY

SeqNo	PDBNo	V	L	I	M	F	W	Y	G	A	P	S	T	C	H	R	K	Q	E	N	D	NOCC	NDEL	NINS	ENTROPY	RELENT	WEIGHT
1	126 A	0	0	0	0	0	0	0	0	67	0	33	0	0	0	0	0	0	0	0	0	3	0	0	0.637	21	0.29
2	127 A	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	291	0	0	0.000	0	1.00
3	128 A	7	0	0	0	0	0	0	0	2	1	87	2	0	0	0	0	0	1	0	0	291	0	0	0.554	18	0.74
4	129 A	0	0	1	0	0	0	1	0	0	3	1	14	0	1	0	0	23	0	53	0	293	0	0	1.366	45	0.32
5	130 A	0	0	1	0	0	0	0	0	1	0	5	59	0	0	0	27	0	3	2	2	294	0	0	1.178	39	0.38
6	131 A	0	0	0	0	0	0	0	0	0	0	0	28	0	0	0	1	0	3	3	63	294	0	0	0.995	33	0.47
7	132 A	1	0	0	0	1	0	96	0	0	0	0	0	0	2	0	0	0	0	0	0	295	0	0	0.241	8	0.92
8	133 A	0	0	0	0	0	0	0	0	10	69	0	0	1	4	0	0	14	0	0	0	296	0	0	1.043	34	0.57
9	134 A	0	0	0	0	0	0	0	99	0	0	1	0	0	0	0	0	0	0	0	0	296	0	0	0.079	2	0.98
10	135 A	1	3	1	0	0	0	0	0	3	42	14	4	1	0	0	2	0	8	10	11	297	0	0	1.844	61	0.24
11	136 A	0	5	0	0	2	1	46	0	0	0	0	0	1	46	0	0	0	0	0	0	300	0	0	1.031	34	0.43
12	137 A	1	0	0	0	0	0	0	46	2	0	21	5	0	12	1	0	1	2	5	3	303	0	0	1.673	55	0.37
13	138 A	0	4	0	0	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	309	0	0	0.186	6	0.98
14	139 A	0	0	0	0	0	0	0	0	0	0	1	4	4	7	23	3	9	22	1	26	309	0	0	1.868	62	0.23
15	140 A	44	45	8	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	309	0	0	1.024	34	0.67
16	141 A	0	1	0	0	0	0	0	31	0	0	36	15	0	1	9	0	0	3	2	1	309	0	0	1.623	54	0.32
17	142 A	0	1	0	0	97	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	311	0	0	0.197	6	0.96
18	143 A	0	33	1	0	0	0	0	3	4	2	4	0	0	0	0	0	50	1	1	1	311	3	296	1.347	44	0.18
19	144 A	0	0	0	0	0	0	0	0	0	7	0	0	0	21	0	7	58	4	2	1	308	0	0	1.276	42	0.50
20	145 A	0	0	0	0	0	0	0	0	0	1	93	1	0	0	0	0	0	0	3	1	309	0	0	0.376	12	0.86
21	146 A	0	0	0	0	0	0	0	42	2	1	44	0	0	0	0	8	0	0	2	0	309	2	308	1.182	39	0.43
22	147 A	1	0	0	0	0	0	0	0	86	2	2	9	0	0	0	0	0	0	0	0	309	0	0	0.562	18	0.73
23		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.000	0	1.00
24	149 A	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	98	1	0	0	0	310	0	0	0.121	4	0.95
25	150 A	0	0	0	0	1	0	0	0	0	0	96	1	0	0	0	1	0	0	1	0	311	0	0	0.229	7	0.90
26	151 A	43	0	0	1	0	0	0	0	45	1	0	8	0	0	0	0	1	0	1	0	311	0	0	1.109	37	0.35
27	152 A	0	0	0	6	1	0	0	0	0	2	0	89	0	0	0	0	1	0	0	1	311	0	0	0.503	16	0.77
28	153 A	0	0	0	0	1	56	4	0	0	0	9	0	31	0	0	0	0	0	0	0	311	0	0	1.071	35	0.07
29	154 A	0	0	1	0	0	0	0	0	0	0	0	97	0	0	0	0	0	1	0	0	313	6	179	0.173	5	0.93
30	155 A	0	0	0	0	1	0	98	0	0	0	0	0	0	0	0	0	0	0	0	0	315	0	0	0.111	3	0.99
31	156 A	0	0	0	0	0	0	0	0	0	0	99	0	0	0	1	0	0	0	0	0	321	0	0	0.074	2	0.96
32	157 A	1	0	1	0	0	0	1	0	1	53	1	27	0	0	0	0	0	13	0	2	322	0	0	1.269	42	0.37
33	158 A	2	20	4	2	0	0	0	1	7	4	15	2	1	0	0	4	1	26	0	11	322	0	0	2.136	71	0.14
34	159 A	0	97	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	325	0	0	0.183	6	0.90
35	160 A	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	50	1	0	46	2	325	0	0	0.905	30	0.49
36	161 A	1	0	0	2	0	0	0	0	0	0	0	0	0	0	1	96	0	0	0	0	325	0	0	0.226	7	0.90
37	162 A	2	88	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	327	0	0	0.430	14	0.95

Data collection: Gene Ontology-terms per domain

- InterPro Predicts functional domains
- Annotated with GO-terms, a controlled vocabulary that describes the protein
- Cellular component, molecular function and biological process
- Examples: GO: 0003677 DNA-binding, GO: 0005155 Protein binding,

  GO: 003700 sequence-specific DNA binding transcription factor activity

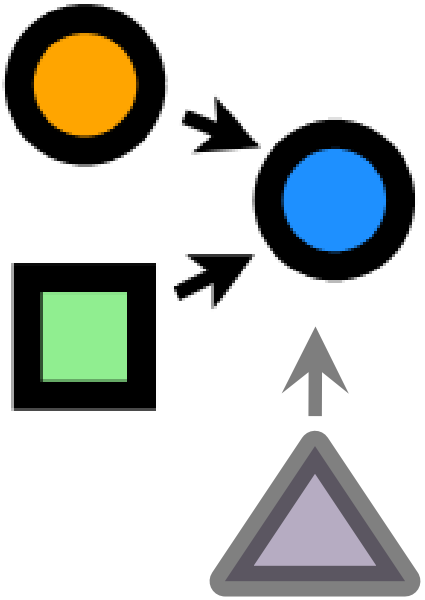
Data collection: Disorder predictions & Motifs

- **K**nowledge Based **M**ultiple Sequence **A**lignment for Intrinsically **D**isordered Proteins
- Predicts % disorder of the protein, and whether the mutation of interest is located in a disordered region
- Uses Short Linear Motifs from ELM to make alignments and therefore it can also predict whether a mutation will disturb such a motif

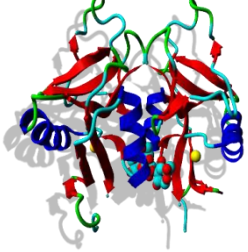
Data Collection: Sequence based predictions

- Prediction servers, based on sequence only
- Accessible using the Uniprot accession code or sequence

Predictions:
transmembrane domains, secondary structure, phosphorylation sites, accessibility



→Other servers/services can be added



Data collection

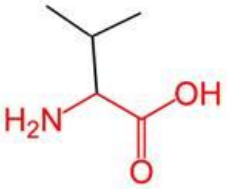
KMAD

HSSP

InterPro
Protein sequence analysis & classification

HOPE

UniProt



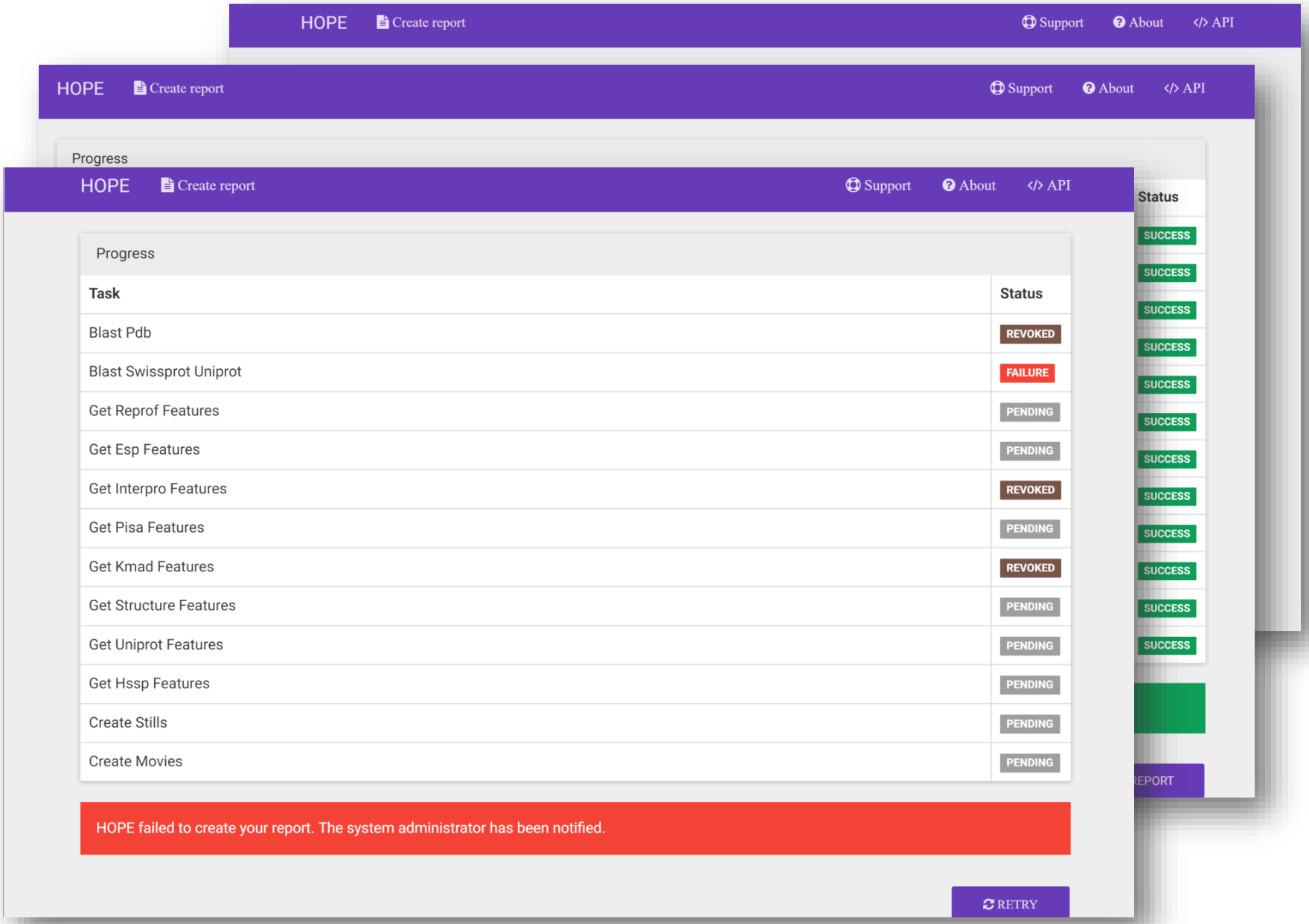
Data storage (in database) and combination/prioritization

Report creation

URL for HOPE

www.cmbi.ru.nl/hope

The gateway page



Method

The exact 3D-structure of your protein of interest is unknown. However, HOPE is able to build a model of your protein of interest based on a homologous structure. The model will be built using the Yasara & WHAT IF Twinset. Structural information was collected using information from WHAT IF Web services, the UniProt database, and a series of DAS-servers. As a possible modelling template, HOPE identified PDB: 2F1Z. More information about your protein of interest can be found in UniProt entry: UBP48_HUMAN. See the method page for more information.

Amino Acids

You are interested in the mutation of a methionine into a valine at position 415.

The figure below shows the schematic structures of the original (left) and the mutant (right) amino acid. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black.

Each amino acid has its own specific size, charge, and hydrophobicity-value. The original wild-type residue and newly introduced mutant residue often differ in these properties.

The mutant residue is smaller than the wild-type residue.

The report will evaluate the effect of the mutation on the following features: Contacts made by the mutated

The report

Contacts

In the 3D-structure can be seen that the wild-type was involved in a metal-ion contact.

The mutant residue is bigger than the wild-type residue.

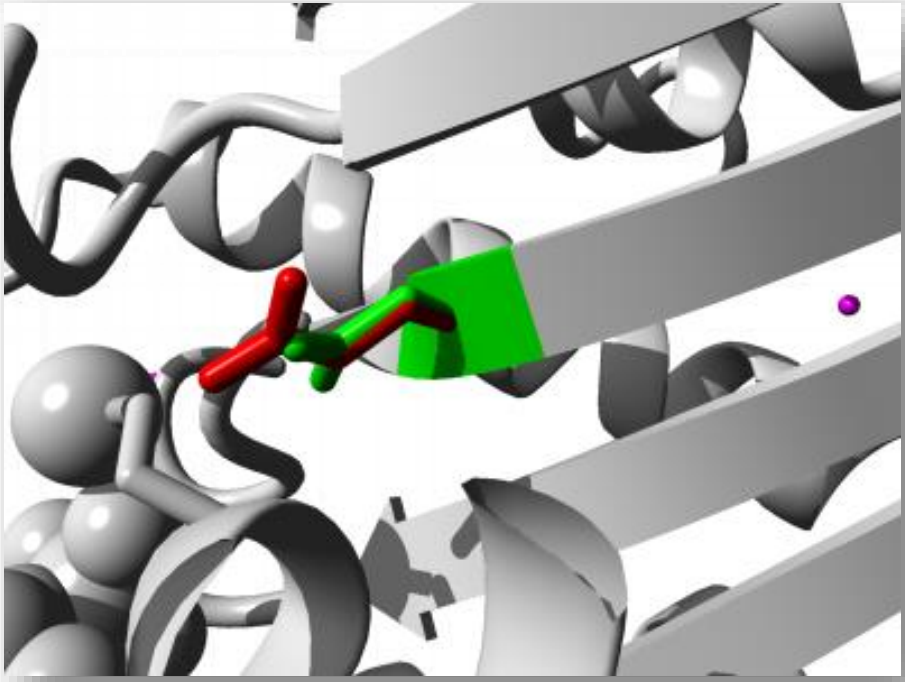
The size differences between the wild-type and mutant residue disturb the interaction with the metal-ion: "MG".

The wild-type residue forms a hydrogen bond with: serine at position 101, serine at position 101,

The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did.

The wild-type residue forms a salt bridge with: lysine at position 100, arginine at position 200,

This paragraph mentions all contacts made by the wildtype residue and the changes caused by the mutation.



The report

Structure

The mutation is located within a domain, annotated in UniProt as:

- `Kinesin motor`

The mutation introduces an amino acid with different properties, which can disturb this domain and abolish its function.

Structure

In the 3D-structure can be seen that the wild-type residue is located in its preferred secondary structure, a turn. The mutant residue prefers to be in another secondary structure, therefore the local conformation will be slightly destabilized.

Structure

The wild-type residue is a glycine, the most flexible of all residues. This flexibility might be necessary for the protein's function. Mutation of this glycine can abolish this function.

This paragraph will mention special structural features such as transmembrane stretches, regions and secondary structure elements

The report

Predicts whether (and how much of) the protein is disordered and whether the mutation occurs in such a disordered region.

Disorder

The protein is predicted to contain disordered regions (disorder percentage: 52%), but the residue of interest is not predicted to be located in a disordered region.

Conservation

The wild-type residue is very conserved, but a few other residue types have been observed at this position too.

Neither your mutant residue nor another residue type with similar properties was observed at this position in other homologous sequences. Based on conservation scores this mutation is probably damaging to the protein.

Your mutant residue is located near a highly conserved position.

Indicates whether the residue is very conserved (=important) or more variable.

The report

Domains

This residue is part of an interpro domain named `Transforming Growth Factor Beta, Conserved Site` (IPR017948).

This domain is annotated with the following Gene-Ontology (GO) terms to indicate its function:

- `Growth Factor Activity` (GO:0008083)

More broadly speaking, these GO annotations indicate the domain has a function in:

- `Protein Binding` (GO:0005515)

This residue is part of an interpro domain named `Transforming Growth Factor-Beta-Related` (IPR015615).

This residue is part of an interpro domain named `Transforming Growth Factor-Beta, C-Terminal` (IPR001839).

This domain is annotated with the following Gene-Ontology (GO) terms to indicate its function:

- `Growth Factor Activity` (GO:0008083)

More broadly speaking, these GO annotations indicate the domain has a function in:

- `Protein Binding` (GO:0005515)

This residue is part of an interpro domain named `Cystine-Knot Cytokine` (IPR029034).

The mutated residue is located in a domain that is important for binding of other molecules. The mutated residue is in contact with residues in another domain. It is possible that the mutation disturbs these contacts.

The mutated residue is located in a domain that is important for binding of other molecules and in contact with residues in a domain that is also important for binding. The mutation might disturb the interaction between these two domains and as such affect the function of the protein.

The mutated residue is located in a domain that is important for binding of other molecules and in contact with residues in a domain that is important for the activity of the protein. The mutation might affect this interaction and thereby disturb signal transfer from binding domain to the activity domain.

Domain paragraph: mentions and links the InterPro domains, their respective GO-terms, and whether 2 domains with an annotated function are in contact.

The report

Predicts whether the residue was located in an annotated motif (taken from ELM database) and whether this mutation changes that motif

Motifs

The following motifs are predicted at this position:

- `Y-based sorting signal` (TRG_ENDOCYTIC_2). The motif is not damaged by the mutation.

Amino Acid Properties

There is a difference in charge between the wild-type and mutant amino acid.

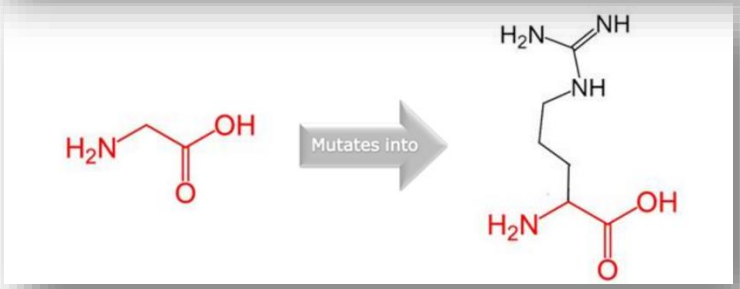
The mutation introduces a charge at this position, this can cause repulsion between the mutant residue and neighboring residues.

The wild-type and mutant amino acids differ in size.

The mutant residue is bigger than the wild-type residue.

The residue is located on the surface of the protein, mutation of this residue can disturb interactions with other molecules or other parts of the protein.

The torsion angles for this residue are unusual. Only glycine is flexible enough to make these torsion angles, mutation into another residue will force the local backbone into an incorrect conformation and will disturb the local structure.



Last remark: the amino acid compared on their properties such as size, hydrophobicity and charge. Accessibility (if availble) is used here.

The report

This will tell you whether the mutation is annotated in dbSNP and Swissprot (Disease vs Polymorphism)

Variants

This mutation matches a previously described variant, with the following description: `In dbSNP:rs11559290.` .

See the ExPASy site about this variant: VAR_062966 .

The variant is annotated with severity: `POLYMORPHISM` .

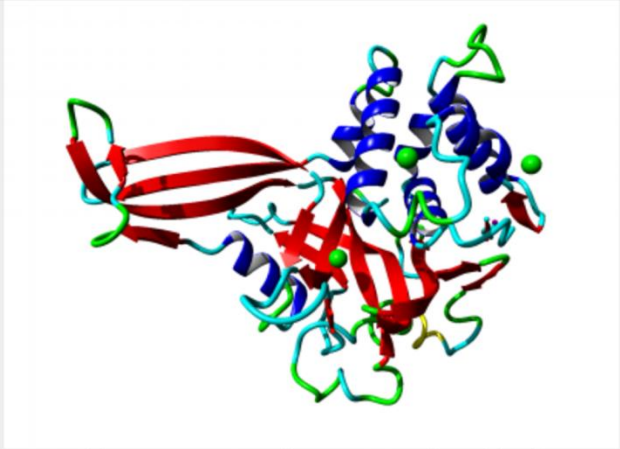
Modifications

The wild-type residue is predicted (by KMAD) to be a phosphorylation site.

Only serine, threonine and tyrosine residues can be phosphorylated, mutation into another residue type will disturb this modification.

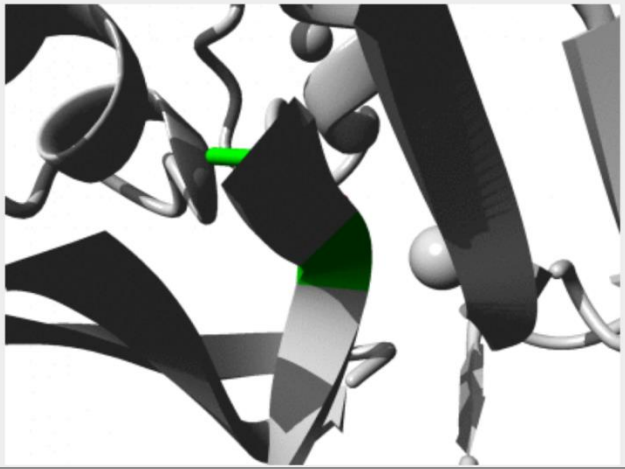
This paragraph will list the annotated/predicted modifications at this position.

The report



Overview of the protein in ribbon-presentation. The protein is coloured by element; α-helix=blue, β-strand = red, turn=green, 3/10 helix=yellow and random coil=cyan. Other molecules in the complex are coloured grey when present.

Movies



Pictures and animated gifs

HOPE → Understanding protein structures and mutations

www.cmbi.ru.nl/hope/report/574bf68bb6d01b173ce7e36e

HOPE | Create report | Support | About | </> API

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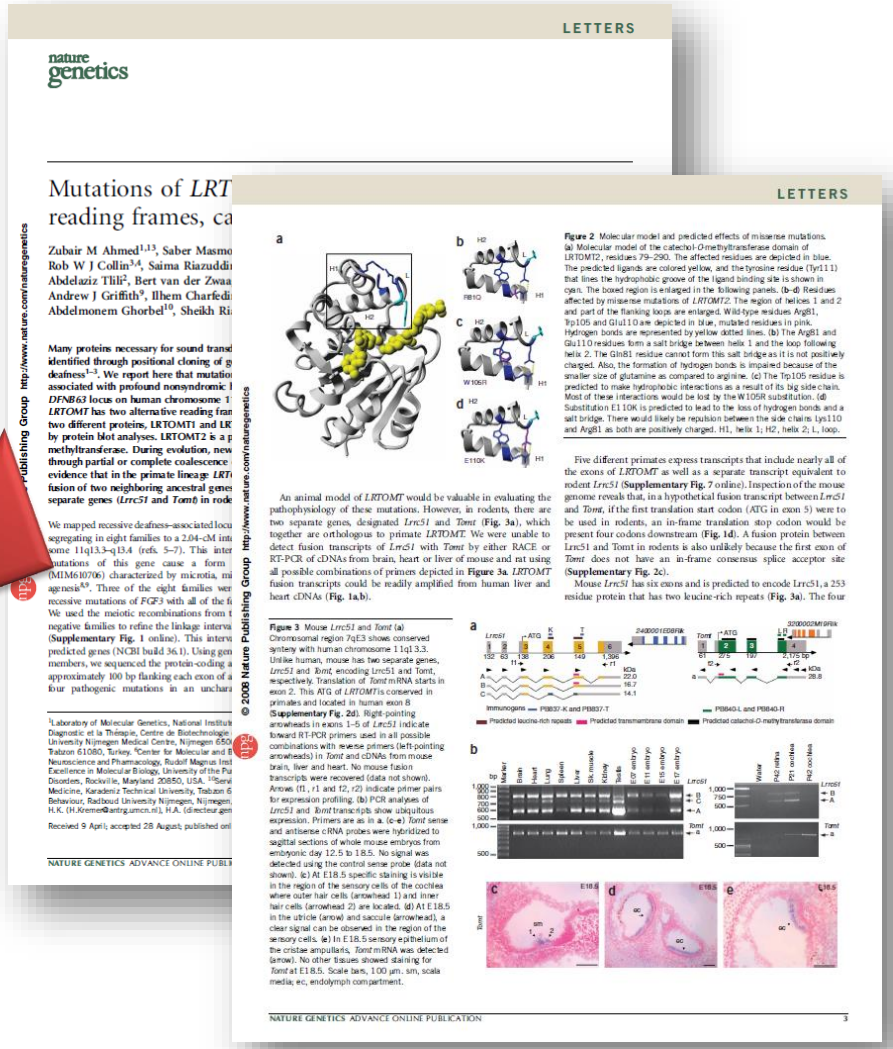
The mutant residue is smaller than the wild-type residue.

The report will evaluate the effect of the mutation on the following features: Contacts made by the mutated residue, structural domains in which the residue is located, modifications on this residue and known variants



Citing HOPE

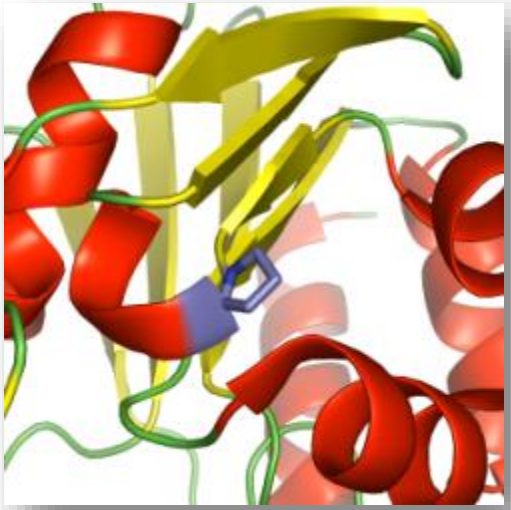
Please use the following citation when referencing the results in your report:

Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces.

BMC Bioinformatics. 2010 Nov 8;11(1):548. DOI: 10.1186/1471-2105-11-548. PubMed: 21059217.

Many mutant analysis tools exist already…. Why HOPE?

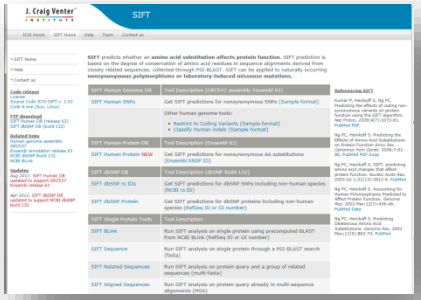
SDM



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Grantham scores

MutPred

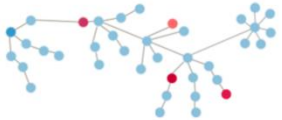


SIFT

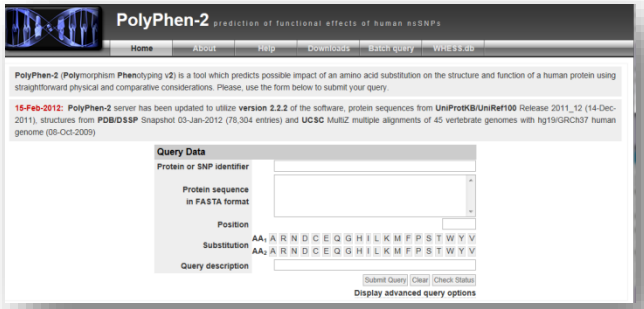
SNAP²

Predicting functional effects of sequence variants

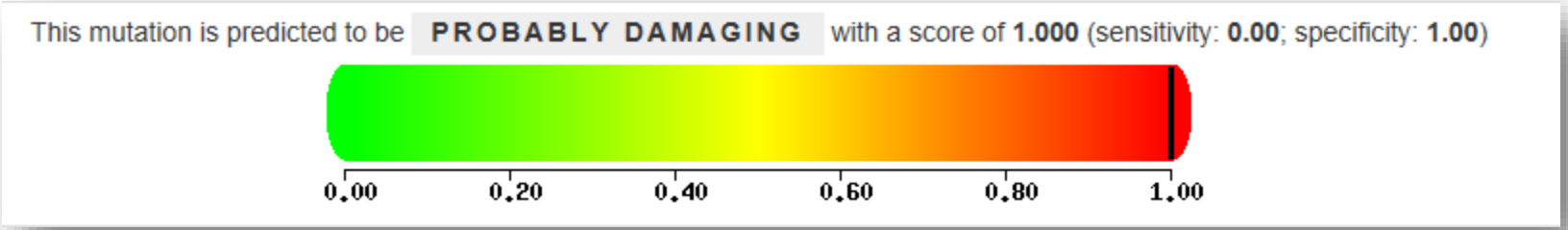
SNPs&GO



PolyPhen

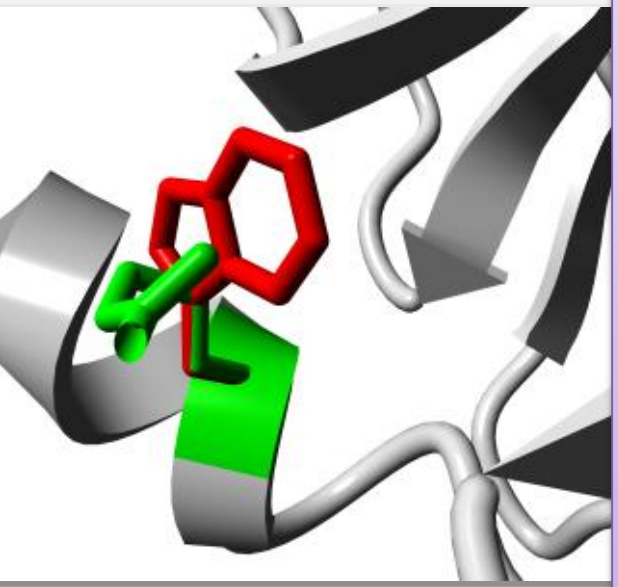


The difference between HOPE and other methods



Method	Result
Grantham-score	89
PolyPhen-2	Probably damaging
SIFT	Not tolerated
SNP&GO	Disease 10
SNAP	Non-neutral 70%
SNPs3D	-1.43
MutPred	0.82
nsSNPanalyzer	Neutral
Panther	-2.93
PHD-SNP	Disease 2

Method	Result
Grantham-score	101
PolyPhen-2	Probably damaging
SIFT	Not tolerated
SNP&GO	Disease 10
SNAP	Non-neutral 93%
SNPs3D	-2,35
MutPred	0.918
nsSNPanalyzer	Disease
Panther	-6,95
PHD-SNP	Disease 9



HOPE report R287W

Contacts

The wildtype residue forms a hydrogen bond with the Leucine on position 271 and with the Tyrosine on position 237. The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogenbond as the original wild-type residue did. The difference in hydrophobicity will affect hydrogenbond formation. The difference in charge will disturb the ionic interaction made by the original, wild-type residue.

Conservation

Only this residue type was found at this position. Mutation of a 100% conserved residue is usually damaging for the protein.

Domains

The mutated residue is buried in a domain that is important for binding of other molecules. The differences between the wild-type and mutant residue might disturb the core structure of this domain and thereby affect the binding properties.

Amino acid properties

There is a difference in charge between the wild-type and mutant amino acid. The charge of the buried wild-type residue is lost by this mutation. The wild-type and mutant amino acids differ in size. The mutant residue is bigger than the wild-type residue. The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit.

Validation experiment: Grantham scores, SIFT, PolyPhen and HOPE

Dataset 1: Mutations selected from publications 115

Dataset 2: Mutations from in-house projects 66

Neutral SNPs 46

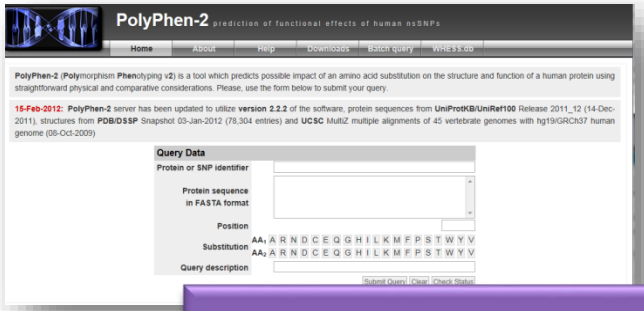
Analysis of these variants by 4 different methods. Results were compared to our own manual analyses.

Grantham scores

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

SIFT

PolyPhen-2

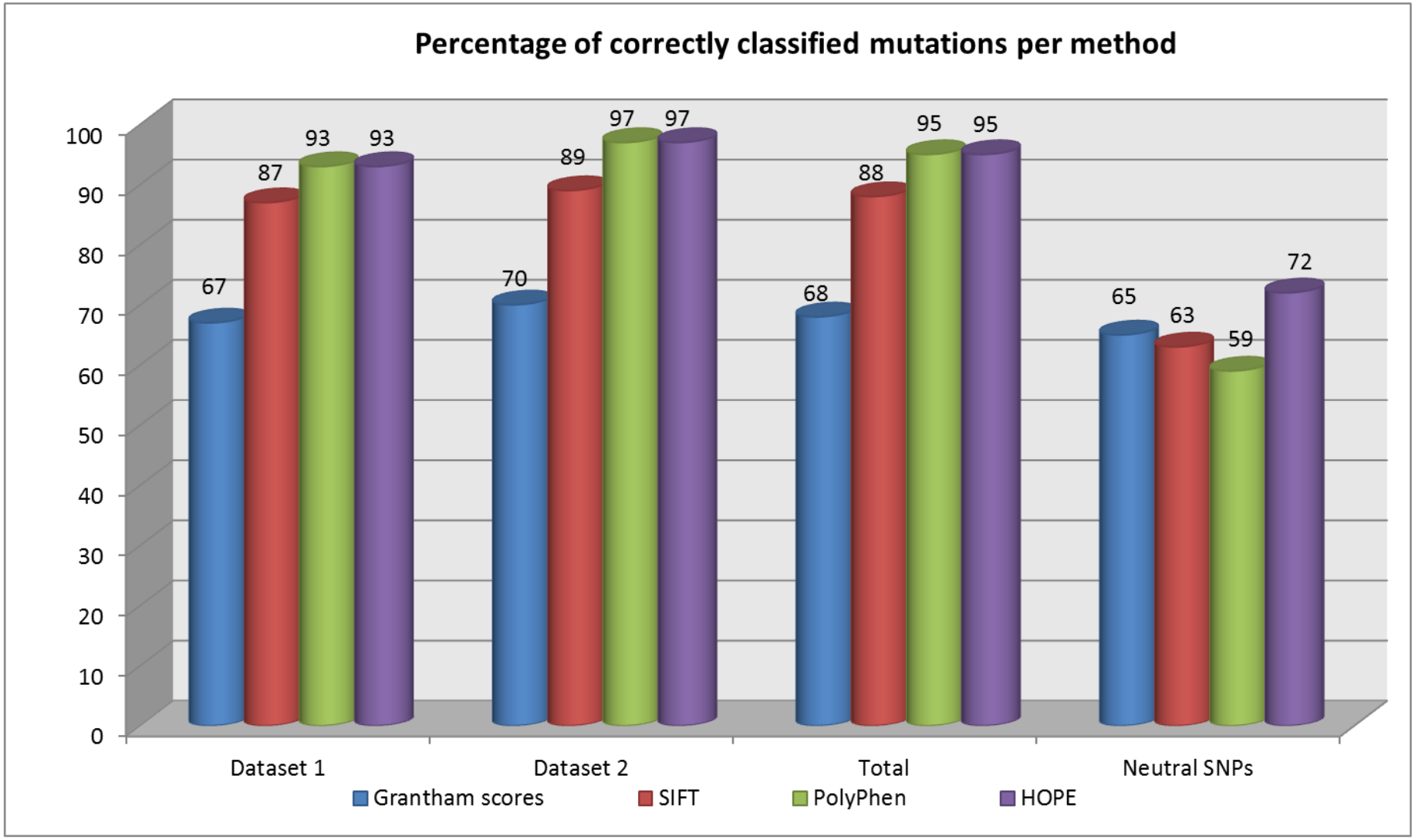


HOPE

Amino Acid / Sequence based

Structure based

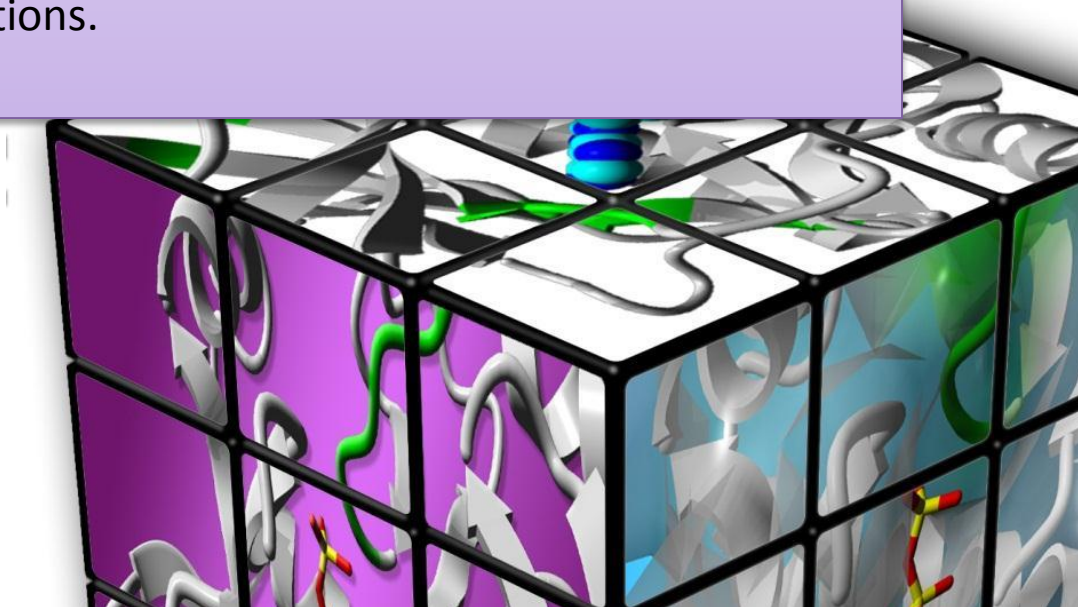
The results: HOPE's score equals PolyPhen2



So, using multiple sequences improves the predictions, using 3D-structure information improves the results even more.

Take home messages….

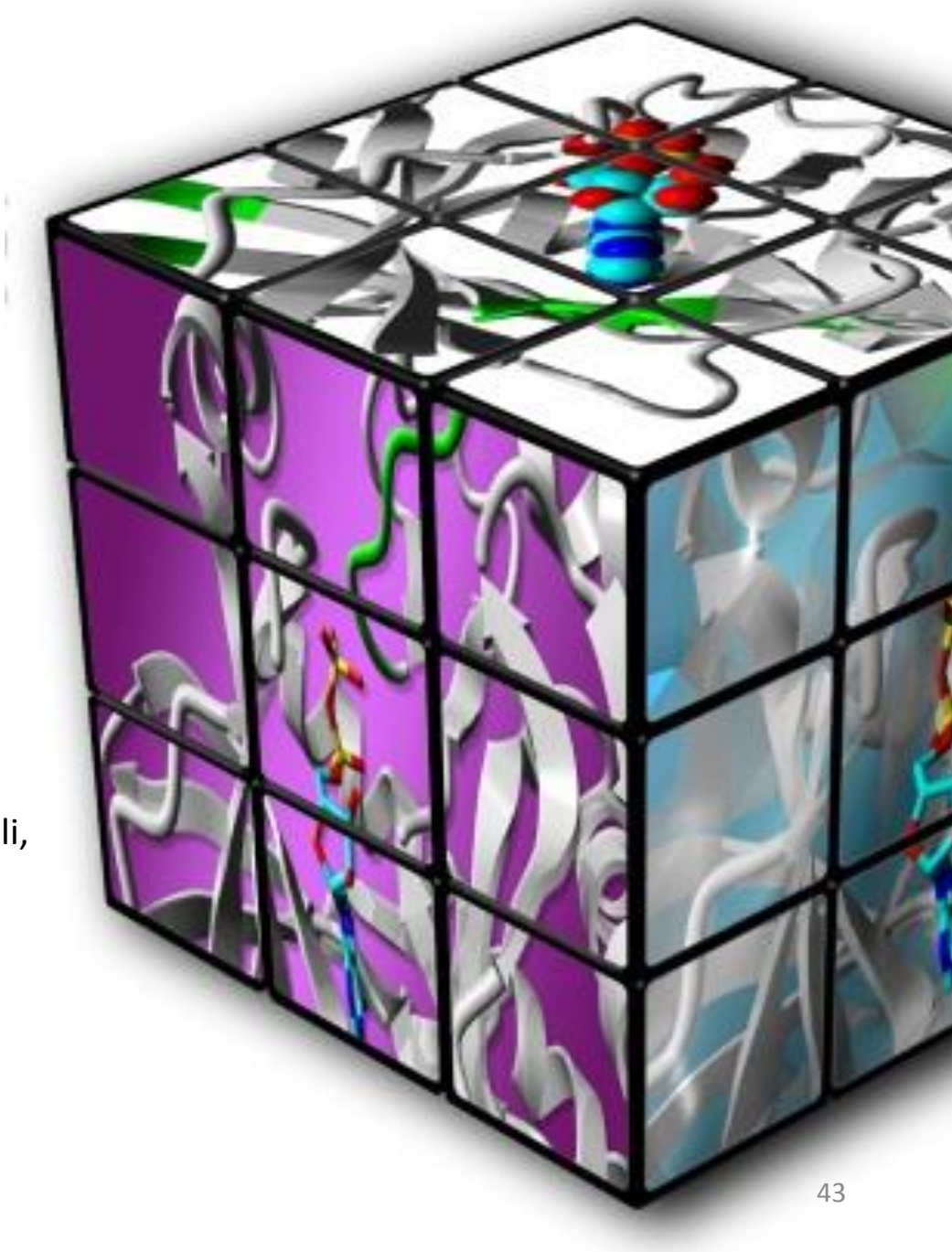
- HOPE can analyze the effect of point mutations on molecular level.
- Use HOPE for those mutations you have identified as possibly harmful, and for which you are missing the last piece of the puzzle to understand structural effects.
- HOPE’s performance is comparable to PolyPhen (and probably to other tools that use structural information).
- HOPE is not a classifier! HOPE provides insight and understanding of the structure and effect of the mutations.



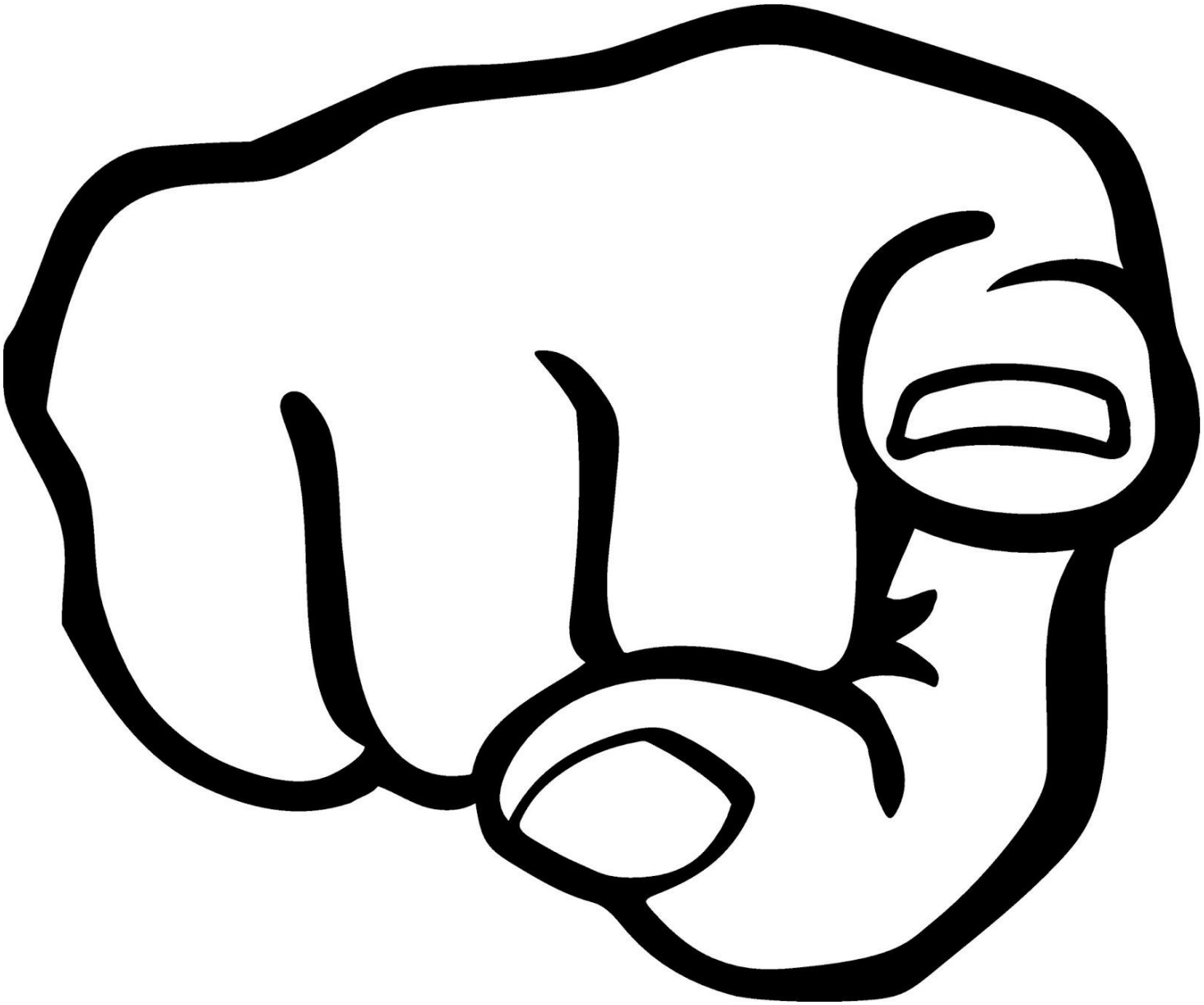
Thanks to…

- Tim te Beek
- Remko Kuipers
- Maarten Hekkelman
- Coos Baakman
- Elmar Krieger
- Jules Kersenmakers
- Jon Black
- Joanna Lange

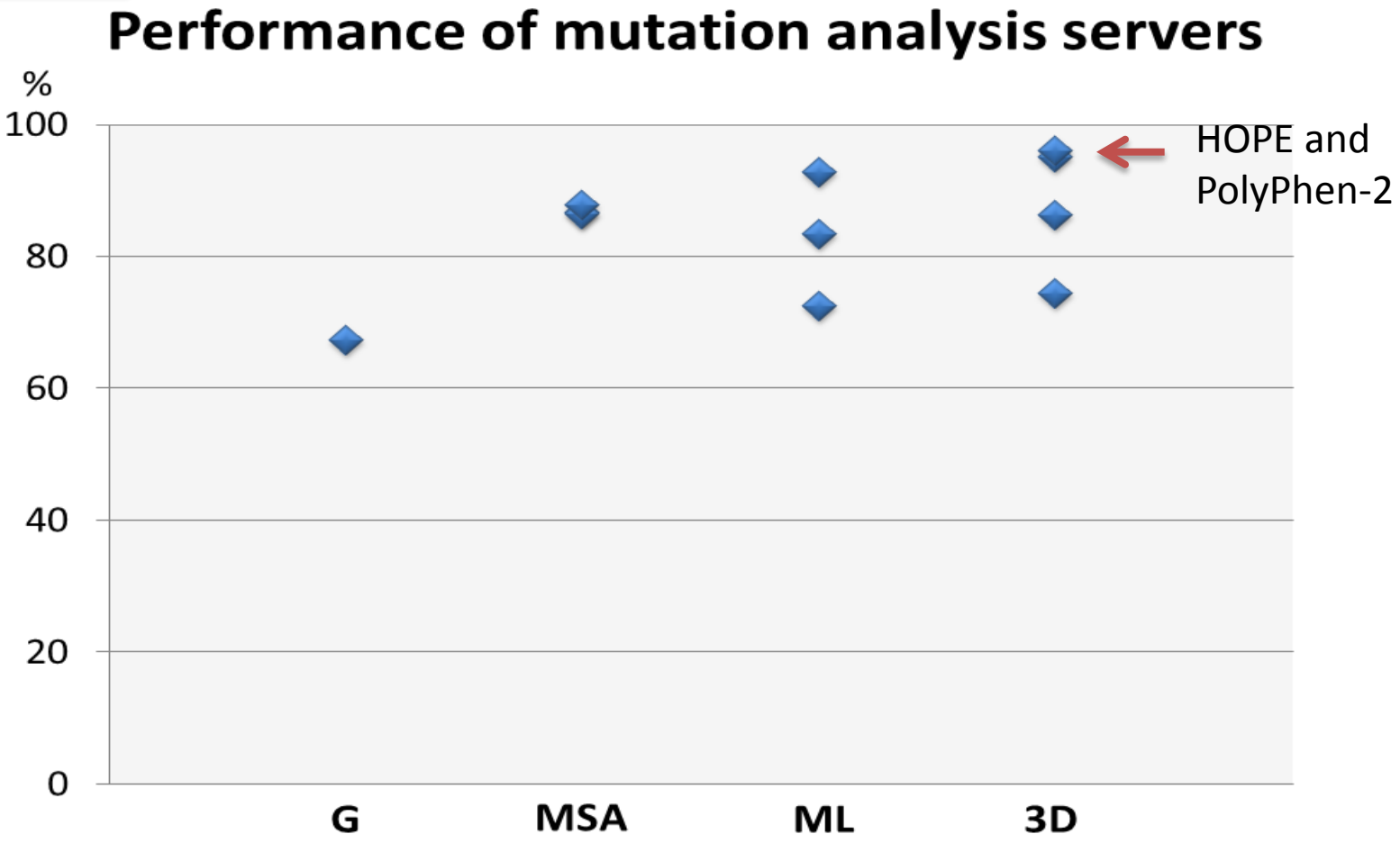
- Students: Annika Borman, Franscesca Camilli, Shimah Golizadeh, Marlou Snelleman
- All the HOPE users, every collaborator, and every scientist in the world who added something to the numerous databases available on the web



Hanka.Venselaar@radboudumc.nl



Extra slide:



- Results improve when we use more datasources
- Multiple sequence alignments largely improve Grantham scores
- Machine learning techniques/more data sources can improve the scores
- HOPE/PolyPhen-2 make the best use of 3D-structures - >best scores

Validation of HOPE, an experiment

Extra slide:

- Dataset of >200 mutations (181 damaging/46 benign) collected from literature
- Mutations were analyzed using 10 online servers and HOPE

Method	Damaging mutations	Benign SNPs
Grantham score	67,4%	65,2%
PhD-SNP	85,6%	73,9%
Panther	86,5%	35,1%
SIFT	87,8%	64,4%
SNPs&GO	72,5%	77,8%
SNAP	83,4%	56,5%
MutPred	92,8%	85,7%
nsSNPanalyzer	74,5%	67,6%
SNPs3D	86,3%	62,8%
PolyPhen-2	95,0%	58,6%
HOPE	96,1%	76,1%

G = differences between amino acids

MSA = Conservation scores from multiple sequence aligments, and amino acid differences

ML = Conservation scores and GO-terms or predictions based on sequence, amino acid differences

3D = 3D-Structural features, Swissprot annotations, conservation scores, amino acid differences

HOPE demo: practical work

The following examples will guide you through HOPE's report pages.

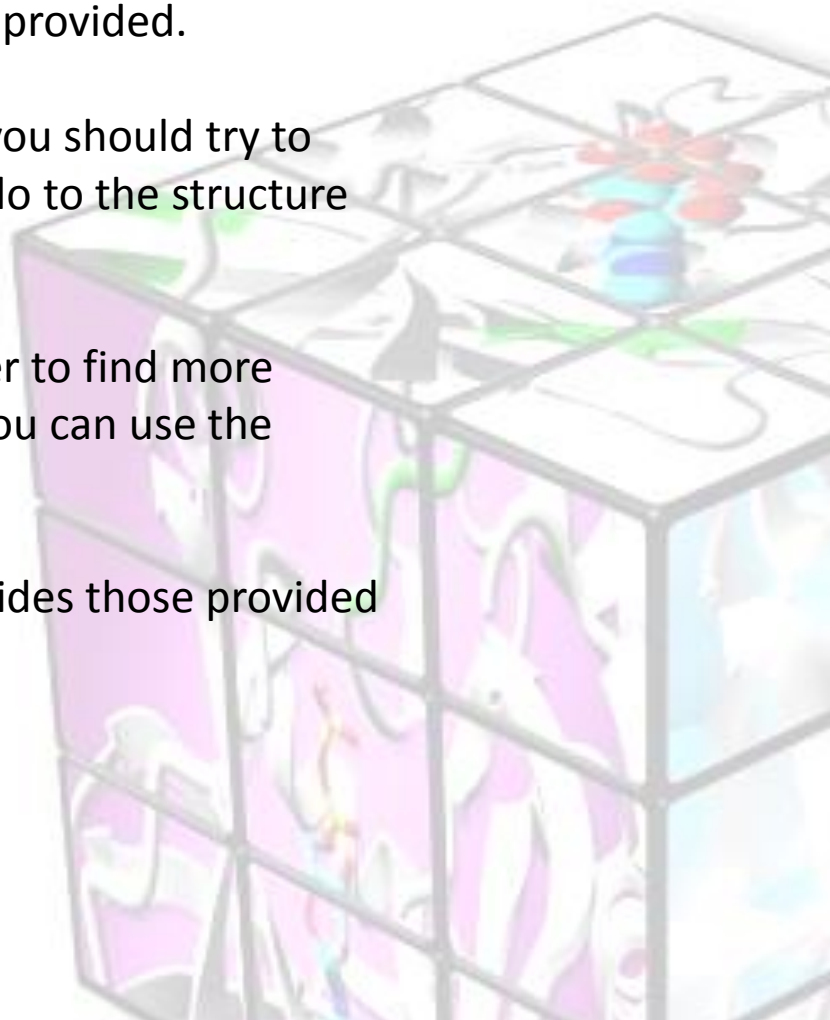
You can simply copy and paste the sequence we provided.

We have added some questions, but in general you should try to answer the question "What does this mutation do to the structure and function of the protein?"

Sometimes you might want to use another server to find more information for the protein of interest, for this you can use the accession codes.

Your are free to try other random mutations besides those provided here….

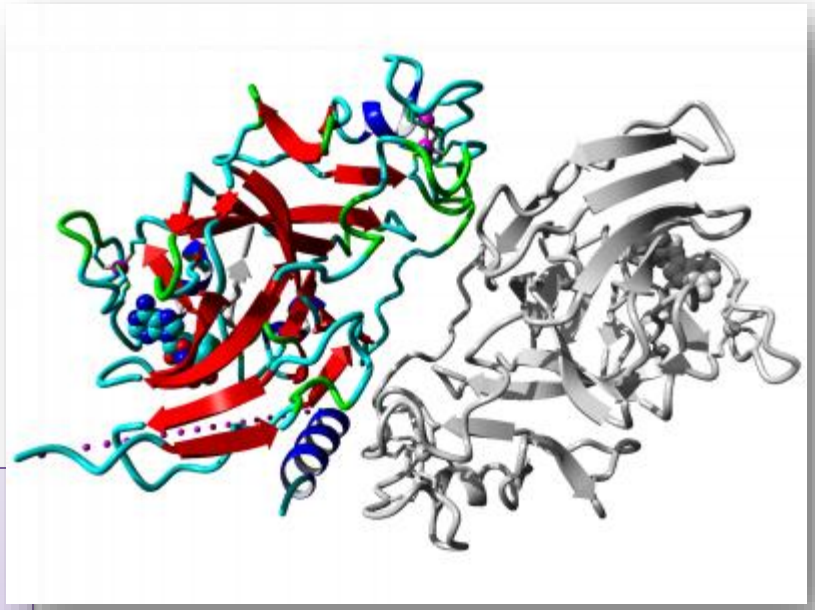
→ If you have questions, let me know!



Example 1: Euchromatic Histone Methyl Transferase
Accession codes: EHMT1_HUMAN **Q9H9B1**

Sequence:

MAAADAEAVP ARGEPQQDCC VKTELLGEET PMAADEGSAE KQAGEAHMAA DGETNGSCEN
SDASSHANAA KHTQDSARVN PQDGTNTLTR IAENGVSERD SEAAKQNHVT ADDFVQTSVI
GSNGYILNKP ALQAQPLRTT STLASSLPGH AAKTLPGGAG KGRTPSAFPQ TPAAPPATLG
EGSADTEDRK LPAPGADVKV HRARKTMPKS VVGLHAASKD PREVREARDH KEPKEEINKN
ISDFGRQQLL PPFPSLHQSL PQNQCYMATT KSQTACLPFV LAAAVSRKKK RRMGTYSLVP
KKKTKVLKQR TVIEMFKSIT HSTVGSKGEK DLGASSLHVN GESLEMDSDE DDSEELEEDD
GHGAEQAAAF PTEDSRTSKE SMSEADRAQK MDGESEEEQE SVDTGEEEEG GDESDLSSES
SIKKKFLKRK GKTDSPWIKP ARKRRRRSRK KPSGALGSES YKSSAGSAEQ TAPGDSTGYM
EVSLDSLDLR VKGILSSQAE GLANGPDVLE TDGLQEVPLC SCRMETPKSR EITTLANNQC
MATESVDHEL GRCTNSVVKY ELMRPSNKAP LLVLCEDHRG RMVKHQCCPG CGYFCTAGNF
MECQPESSIS HRFHKDCASR VNNASYCPHC GEESSKAKEV TIAKADTTST VTPVPGQEKG
SALEGRADTT TGSAAGPPLS EDDKLQGAAS HVPEGFDPTG PAGLGRPTPG LSQGPGKETL
ESALIALDSE KPKKLRFHPK QLYFSARQGE LQKVLLMLVD GIDPNFKMEH QNKRSPLHAA
AEAGHVDICH MLVQAGANID TCSEDQRTPL MEAAENNHLE AVKYLIKAGA LVDPKDAEGS
TCLHLAAKKG HYEVVQYLLS NGQMDVNCQD DGGWTPMIWA TEYKHVDLVK LLLSKGSDIN
IRDNEENICL HWAAFSGCVD IAEILLAAKC DLHAVNIHGD SPLHIAAREN RYDCVVLFLS
RDSDVTLKNK EGETPLQCAS LNSQVWSALQ MSKALQDSAP DRPSPVERIV SRDIARG**Y**ER
IPIPCVNAVD SEPCPSNYKY VSQNCVTSPM NIDRNITHLQ YCVCIDDCSS SNCMCGQLSM
RCWYDKDGRL LPEFNMAEPP LIFE**C**NHACS CWRN**C**RNRVV QNGLRARLQL YRTRDMGWGV
RSLQDIPPGT FVCEYVGELI SDSEADVREE DSYLFDLDNK DGEVYCIDAR FYGNVSRFI**N**
HHCEPNLVPV RVFMAHQDLR FPRIAFFSTR LIEAGEQLGF DYGERFWDIK GKLFSCRCGS
PKCRHSSAAL AQRQASAAQE AQEDGLPDTS SAAAADPL



Mutations to try:

N1200V (what kind of contacts does this residue make, how are they disturbed, how conserved is the residue?)

C1115Y (what kind of contact does the residue make, can you see this in the pictures?)

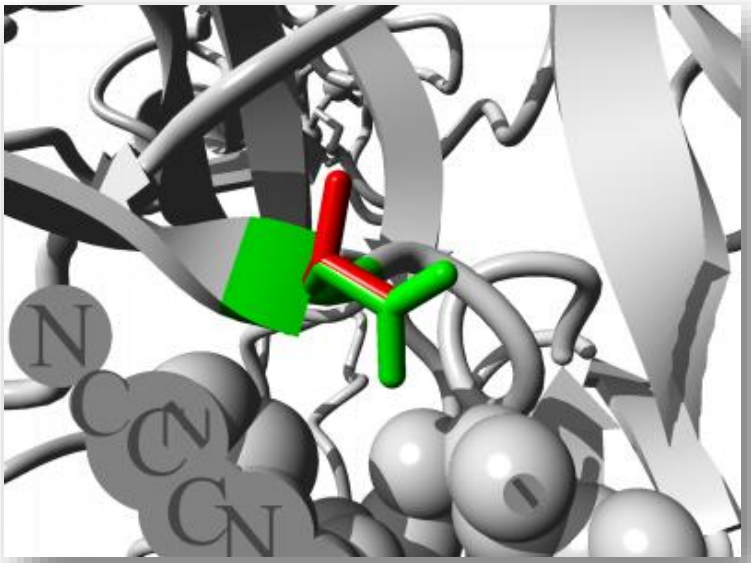
Y1018F (what kind of contact does this residue make, what kind of interactions are now missing?

Mutation N1200V, important notes

First of all: The mutant residue is smaller and more hydrophobic (cannot make hydrogenbonds)

The wildtype made contacts with a ligand (click on the link), and it made hydrogenbonds (which the mutant cannot make). PISA identifies contacts with another molecule, in this case that is the ligand again.

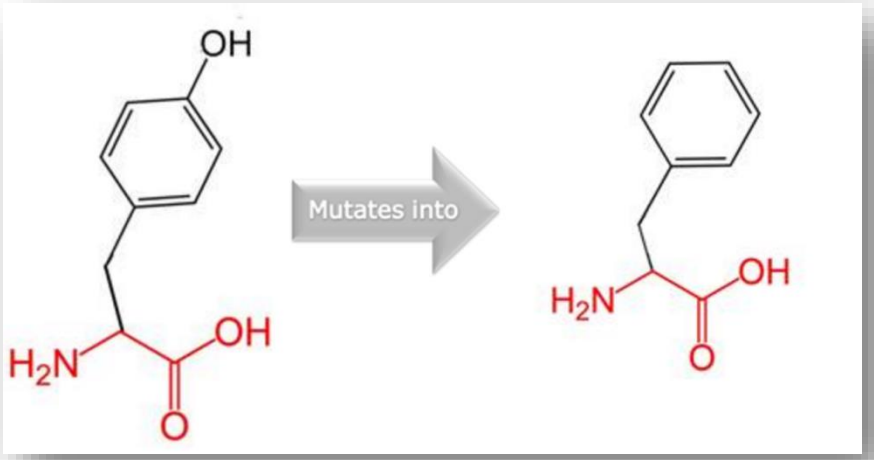
The wildtype and its neighbor are 100% conserved, so they are important! (for ligand binding, something you can also see in the pictures)



Everything tells you that the residue is located in the ligand binding domain and important for interactions with the molecule. Mutation will cause loss of these interactions and therefore cause loss of function.

Mutation Y1018F, important notes

The wildtype and mutant residue differ slightly, the mutant is smaller and missing the –OH, so therefore it cannot make the same hydrogenbonds



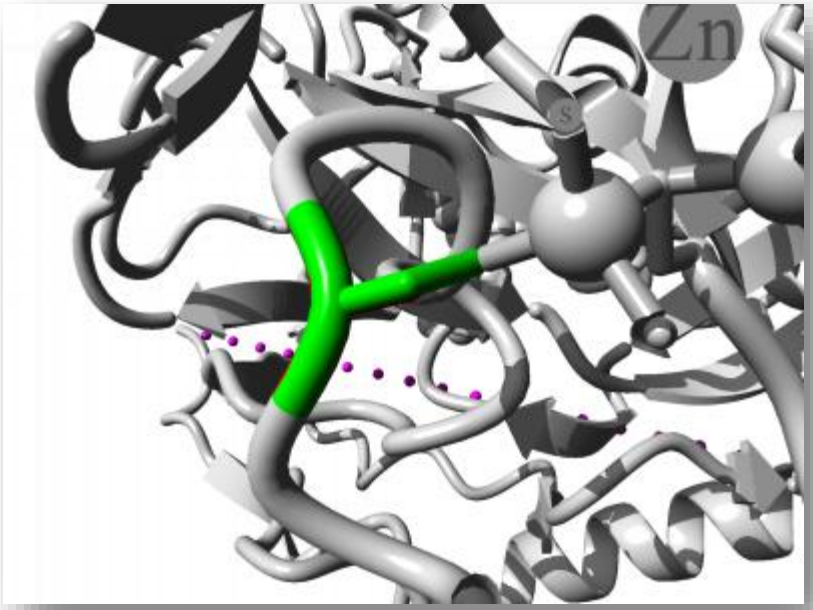
The wildtype residue is located on the surface, where it makes a hydrogenbond. The pictures, and PISA, indicate that this is a hydrogenbond to the other monomer. Losing this bond might mean loss of dimerisation.

However! Conservation scores indicate that the mutant residue was seen at this position in other structures. This makes our conclusion less clear and you might need experimental backup to underline your conclusions.

Mutation C1115A, important notes

The wildtype is a S, often involved in disulfide bonds. Mutant A is smaller and cannot make these bonds.

However, the contacts section doesn't mention a disulfid bond, but a metal contact instead. The pictures show the metal-interaction too.



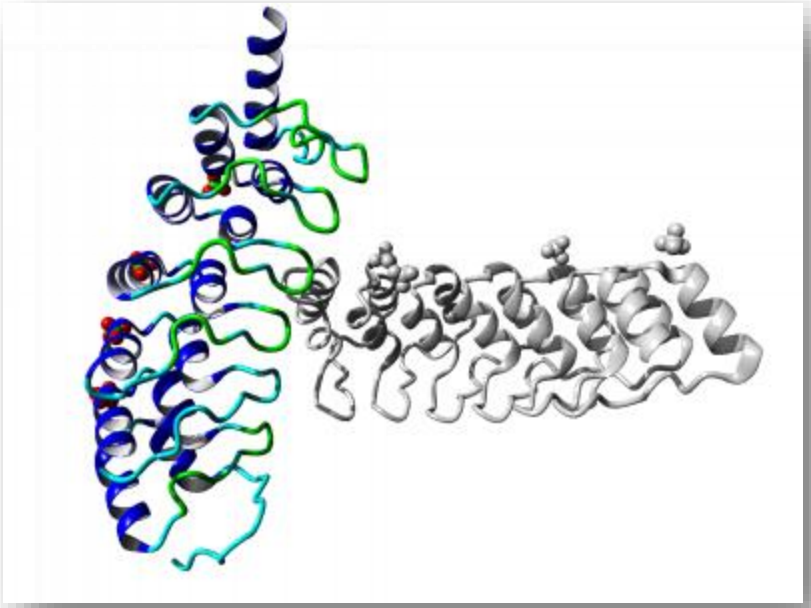
Conservation scores indicate a damaging mutation too… The mutation will cause loss of stability of the SET-domain because A cannot bind metal similar to S.

Continued Example 1: Euchromatic Histone Methyl Transferase

Accession codes: EHMT1_HUMAN **Q9H9B1**

Sequence:

```
MAAADAEAVP ARGEPQQDCC VKTELLGEET PMAADEGSAE KQAGEAHMAA DGETNGSCEN
SDASSHANAA KHTQDSARVN PQDGTNTLTR IAENGVSERD SEAAKQNHVT ADDFVQTSVI
GSNGYILNKP ALQAQPLRTT STLASSLPGH AAKTLPGGAG KGRTPSAFPQ TPAAPPATLG
EGSADTEDRK LPAPGADVKV HRARKTMPKS VVGLHAASKD PREVREARDH KEPKEEINKN
ISDFGRQQLL PPFPSLHQSL PQNQCYMATT KSQTACLPFV LAAAVSRKKK RRMGTYSLVP
KKKTKVLKQR TVIEMFKSIT HSTVGSKGEK DLGASSLHVN GESLEMDSDE DDSEELEEDD
GHGAEQAAAF PTEDSRTSKE SMSEADRAQK MDGESEEEQE SVDTGEEEEG GDESDLSSES
SIKKKFLKRK GKTDSPWIKP ARKRRRRSRK KPSGALGSES YKSSAGSAEQ TAPGDSTGYM
EVSLDSLDLR VKGILSSQAE GLANGPDVLE TDGLQEVPLC SCRMETPKSR EITTLANNQC
MATESVDHEL GRCTNSVVKY ELMRPSNKAP LLVLCEDHRG RMVKHQCCPG CGYFCTAGNF
MECQPESSIS HRFHKDCASR VNNASYCPHC GEESSKAKEV TIAKADTTST VTPVPGQEKG
SALEGRADTT TGSAAGPPLS EDDKLQGAAS HVPEGFDPTG PAGLGRPTPG LSQGPGKETL
ESALIALDSE KPKKLRFHPK QLYFSARQGE LQKVLLMLVD GIDPNFKMEH QNKRSPLHAA
AEAGHVDICH MLVQAGANID TCSEDQRTPL MEAAENNHLE AVKYLIKAGA LVDPKDAEGS
TCLHLAAKKG HYEVVQYLLS NGQMDVNCQD DGGWTPMIWA TEYKHVDLVK LLLSKGSDIN
IRDNEENICL HWAAFSGCVD IAEILLAAKC DLHAVNIHGD SPLHIAAREN RYDCVVLFLS
RDSDVTLKNK EGETPLQCAS LNSQVWSALQ MSKALQDSAP DRPSPVERIV SRDIARGYER
IPIPCVNAVD SEPCPSNYKY VSQNCVTSPM NIDRNITHLQ YCVCIDDCSS SNCMCGQLSM
RCWYDKDGRL LPEFNMAEPP LIFECNHACS CWRNCRNRVV QNGLRARLQL YRTRDMGWGV
RSLQDIPPGT FVCEYVGELI SDSEADVREE DSYLFDLDNK DGEVYCIDAR FYGNVSRFIN
HHCEPNLVPV RVFMAHQDLR FPRIAFFSTR LIEAGEQLGF DYGERFWDIK GKLFSCRCGS
PKCRHSSAAL AQRQASAAQE AQEDGLPDTS SAAAADPL
```



More Mutations to try:

H770A: For this analysis HOPE will use a different structure than for the previous mutations, why? Also check the conservation scores. What do you think of this?

A43V: Is there a structure for this mutation? Is this a known mutation?

Mutation H770AA, important notes

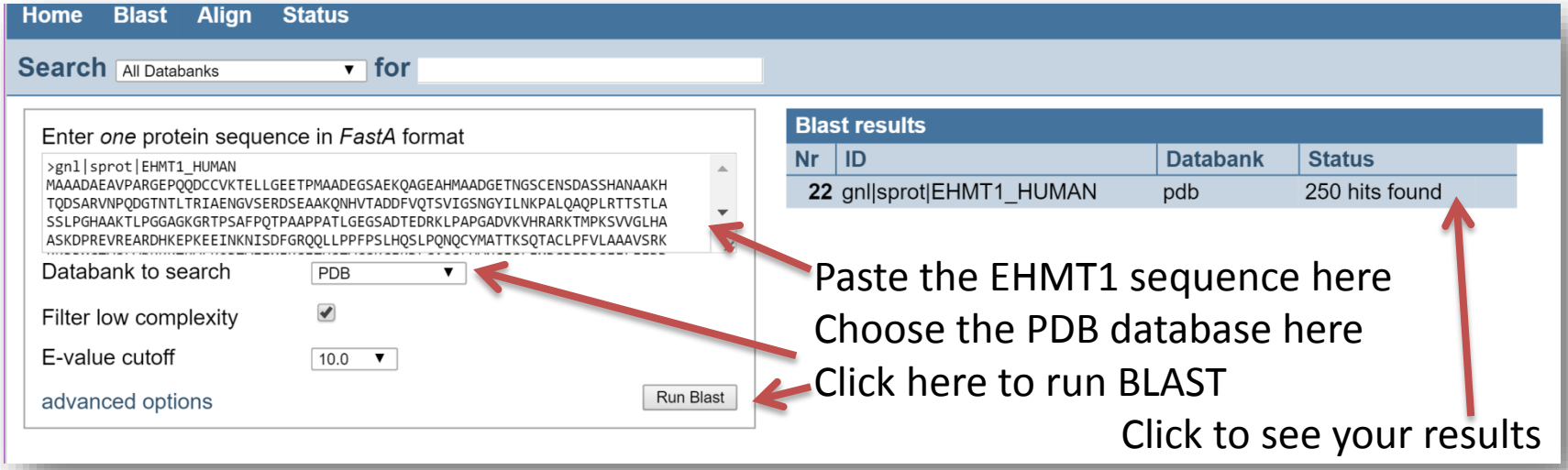
As you can see HOPE uses PDB file 3B7B for its structural analysis. This was PDB file 3HNA for the previous examples. Why?

Method

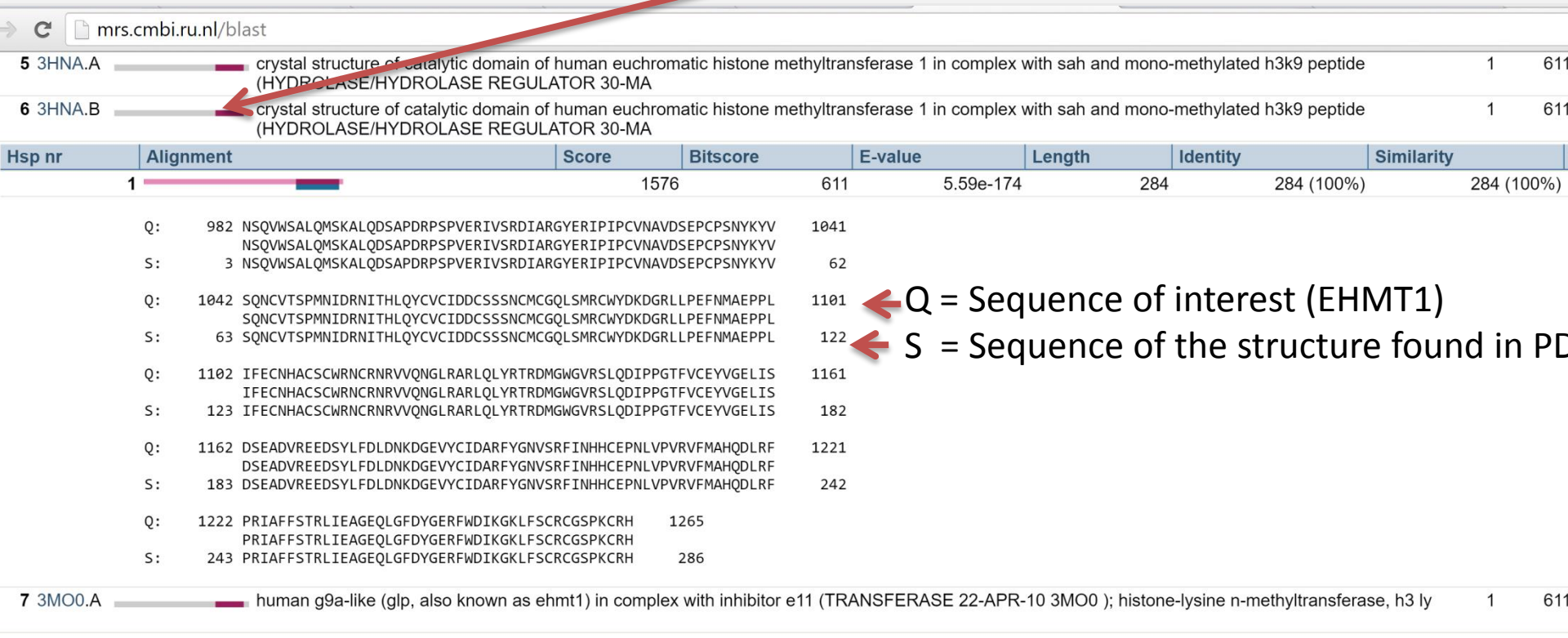
The 3D-structure of your protein of interest is known. Information from this 3D-structure will be obtained using WHAT IF Web services, the UniProt database and a series of DAS-servers. The structural information was obtained from the analysis of PDB: 3B7B. Annotations about this protein were obtained from UniProt entry EHMT1_HUMAN. See the method page for more information.

To figure this out you can perform a BLAST search against the PDB database to see which parts of the EHMT1 protein are experimentally solved.

Go to: http://mrs.cmbi.ru.nl/blast



Your list with BLAST hits will appear. These are all PDB files of which the sequence shows a % identity with your sequence of interest. Somewhere around no 6, you will find a hit for 3HNA, click on the grey/coloured bar to see this:



You will see the alignment of the matching part of your sequence of interest with the structure found in the PDB database.

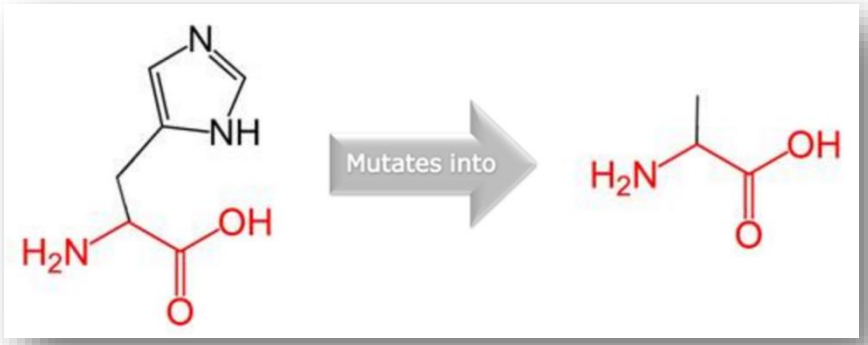
Do you understand the result? Can you see that the structure found contains residue 982-1265 of your sequence of interest?

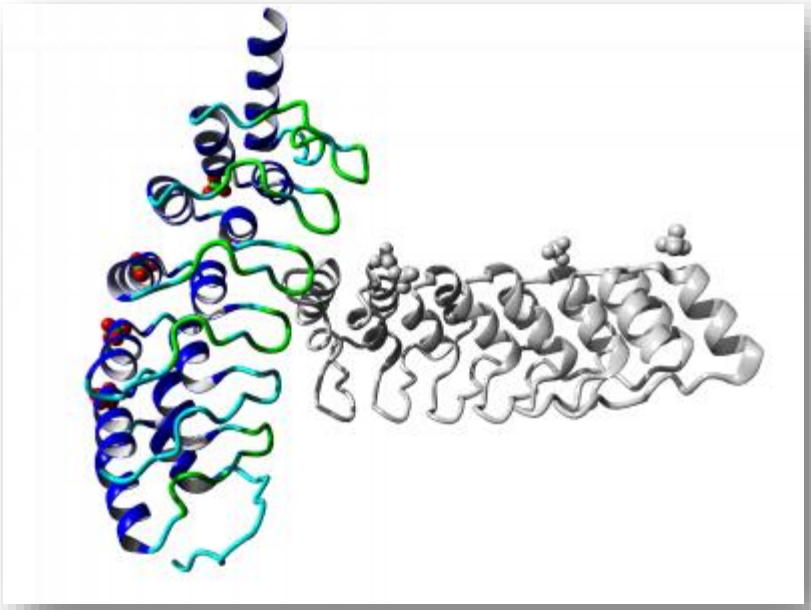
Find BLAST hit 3B7B (somewhere around 25). Do you understand which part of the structure can be found in this PDB file?

Mutation H770A, important notes

So, HOPE uses a different PDB file for structural analysis of mutation H770, simply because 3HNA does not contain this position, and 3B7B does.

The amino acid properties differ again between H and A. But have a look at the conservation score.
What does the conservation tell you?
Does the wildtype have any special contacts? Or special modifications?
Follow the domain link to read more about “Ankyrin repeat containing domains”. Can you find a special function for the domain?





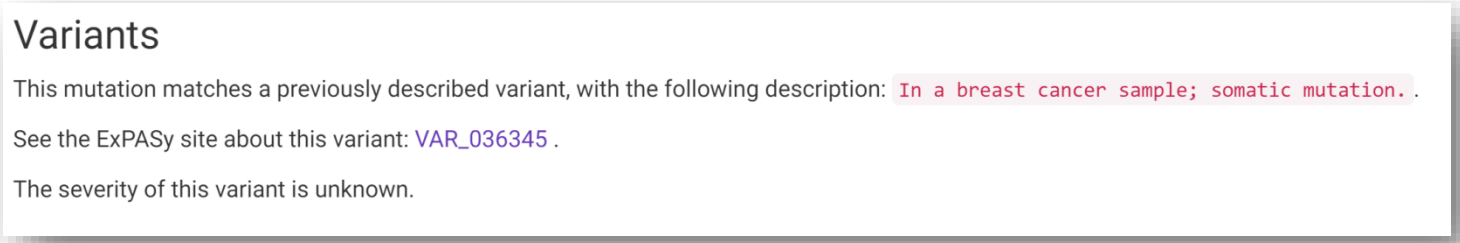
It doesn’t look like this mutation does much damage to the protein, you will need more experimental results.

Mutation A43V, important notes

As you can see HOPE cannot find a source of structural information. You can use the BLAST results from the previous example to see if there is indeed no structure known for this part of the protein.

It doesn't look like there is anything known about the function of the wildtype residue. And the two residue types only differ in size.

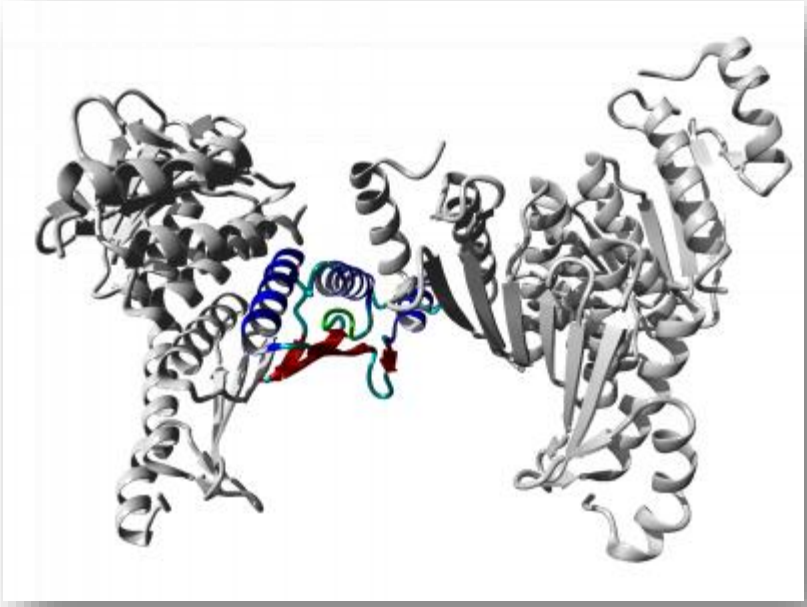
However, the mutation was found before and is known in the Expasy database



You can follow the link to read more about this variant, would you classify this as a damaging mutation?

Example 2: Phenylalanine hydroxylase-stimulating protein

Accession code: PHS_HUMAN **P61457**



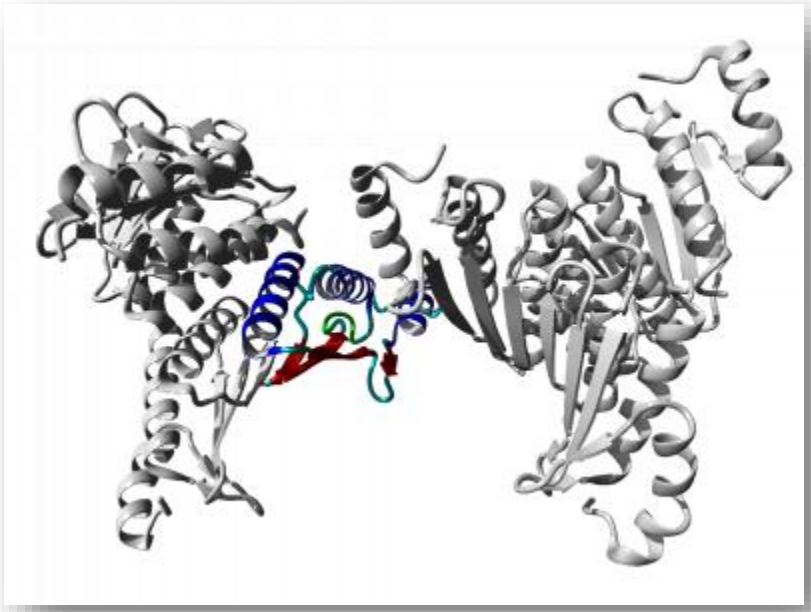
Sequence:

```
MAGKAHRLSA EERDQLLPNL RAVGWNELEG
RDAIFKQFHF KDFNRAFGFM TRVALQAEKL
DHHPEWFNVY NKVHITLSTH ECAGLSERDI
NLASFIEQVA VSMT
```

Mutations to try:
H63S: Look at the interactions with other molecules?
N44P: What happens when a proline is introduced in a helix?

Mutation H63S, important notes

H and S are very different residues and conservation is high.



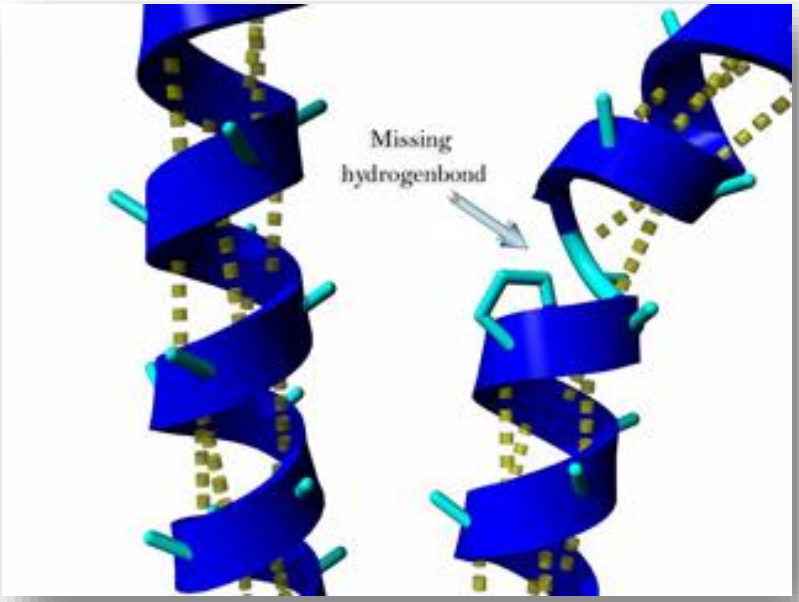
Read the information about contacts indicated by PISA and look at the pictures. Do you agree that this residue is in contact with other molecules?

Does the domain information agree with the fact that this protein needs to function in a complex?

Look at the moving animations, how many mutations can you see? Why? In what kind of complex does this protein function?
If you want you can read more information about the protein here:
http://mrs.cmbi.ru.nl/entry?db=sprot&nr=300243&q=phs_human

Mutation N44P, important notes

N an P have a few differences in amino acid properties. You can see that due to size and hydrophobicity changes a few contacts will be lost.



More important for this protein is the fact that a proline will be introduced in a helix structure, this will cause a kink and destabilization of the structure. The pictures show that the helix is important for interactions with the other subunits in the complex.

Example 3: Transcription factor 7-like 2
Accession code: TF7L2_HUMAN Q9NQB0

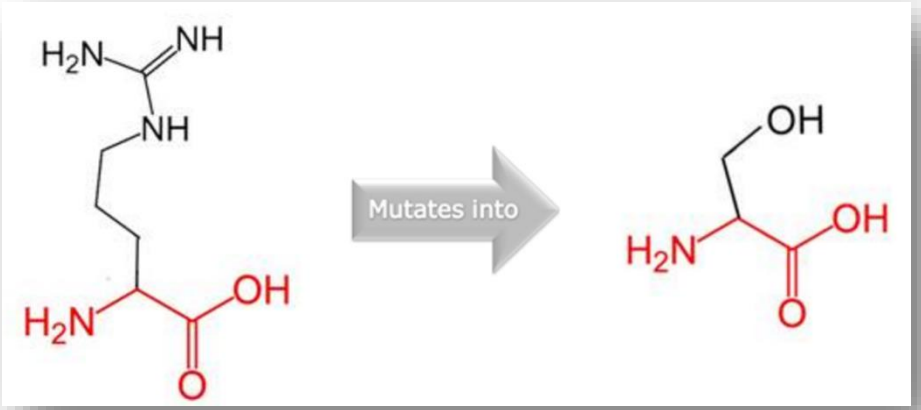
Sequence:

MPQLNGGGGDDLGANDELISFKDEGEQEEKSSENSSAERDLADVKSSLVNESETNQNSSS
DSEAERRPPPRSESFRDKSRESLEEAAKRQDGGLFKGPPYPGYPFIMIPDLTSPYLPNGS
LSPTARTLHFQSGSTHYSAYKTIEHQIAVQYLQMKWPLLDVQAGSLQSRQALKDARSPSP
AHIVSNKVPVVQHPHHVHPLTPLITYSNEHFTPGNPPPHLPADVDPKTGIPRPPHPPDIS
PYYPLSPGTVGQIPHPLGWLVPQQGQPVYPITTGGFRHPYPTALTVNASMSRFPPHMVPP
HHTLHTTGIPHPAIVTPTVKQESSQSDVGSLHSSKHQDSKKEEEKKKPHIKKPLNAFMLY
MKEMRAKVVAECTLKESAAINQILGRRWHALSREEQAKYYELARKERQLHMQLYPGWSAR
DNYGKKKKRKRDKQPGETNEHSECFLNPCLSLPPITDLSAPKKCRARFGLDQQNNWCGPC
RRKKKCVRYIQGEGSCLSPPSSDGSLLDSPPPSPNLLGSPPRDAKSQTEQTQPLSLSLKP
DPLAHLSMMPPPPALLLAEATHKASALCPNGALDLPPAALQPAAPSSSIAQPSTSSLHSH
SSLAGTQPQPLSLVTKSLE

Mutations to try:
R387S: For what function is the wildtype residue required?
T212S: What function does the residue have and will it really be lost by this mutation?

Mutation R387S, important notes

The mutant residue S is much smaller and neutrally charged when compared to wildtype R. This causes a potential loss of many interactions.



The contact paragraph tells us that a residue close to the mutated one had an important function in DNA binding. HOPE will look at important functions of neighboring residues.

Contacts

The mutated residue did not interact with DNA, however, one of its neighbor residues was found to interact with DNA. This interaction might be affected by the mutation.

Many stabilizing interactions will be lost. If the structure destabilizes locally, the residues important for DNA binding will also be affected. Remember the function/name of the protein?

Mutation T212S, important notes

These two residues show very minor differences.

Look at the variant and modification paragraphs. The wildtype residue is phosphorylated. During mutagenesis experiments was found that mutation of this residue to a V (so removal of phosphorylation) has an enhancing effect. Can you understand why?

Variants

Mutagenesis experiments have been performed on this position. Mutation of the wild-type residue into `V` has the following effect: `Reduced phosphorylation by NLK and enhanced DNA-binding; when associated with V-201.`.

This is an example of a mutation that causes the removal of regulation. Sometimes that results in a constitutively active protein.

However, since S can also be phosphorylated the effect of this mutation might be less severe.

Your own examples…..

Now it's time to try your own examples….

1: Find the amino acid sequence of your protein of interest. And submit the sequence in HOPE

Hint: use MRS or Uniprot to search for your protein:
http://mrs.cmbi.ru.nl/ or http://www.uniprot.org/

2: Choose a mutation (if you don't know an interesting mutation, you can try to find an interesting residue in MRS or Uniprot and see whether HOPE finds the same info). Submit this mutation to HOPE.

3: Wait for HOPE to finish the report and read it. See if you understand the results. Write down any questions you have so we can discuss these results.

You can also send any questions to Hanka.Venselaar@radboudumc.nl